Agilent WinGPC Software

for GPC/SEC Data Acquisition and Analysis

User Guide

Notices

Manual Part Number

D0029603 Rev. A

Edition 07/2023

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Software Revision

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1 About Agilent WinGPC Software

Agilent WinGPC Software is the first complete macromolecular chromatography data system (MCDS) for all aspects of modern polymer chromatography including state of the art data acquisition and support of all data processing methods. This MCDS uses the latest database technologies to ensure reliable data tracking and ultimate compliance for any laboratory. WinGPC Software is completely validated, ISO certified and supports all regulations. WinGPC Software can be used as a stand-alone software on a single PC and it can be vertically upgraded to a Client/Server system which can be accessed from any point using any local PC as a terminal (even pocket PC and Unix/Linux terminals). WinGPC Software is based on the well-received features of the previous PSS WinGPC versions. We tried to incorporate current data analysis methods and support new instruments and further improve the usability. The software's philosophy and its look and feel is kept similar which will minimize the amount of training needed. In order to accommodate the new features and improved usability the data file structure had to be changed. Previous data can be read, but WinGPC Software will use a modified file format to save data. Therefore, it is not recommended to share WinGPC Software project databases with users of previous PSS WinGPC versions.

The WinGPC Software will continue to incorporate new analytical techniques, instrumentation and processes in order to be a supreme product in any laboratory.

2 Using the Agilent WinGPC Software User Guide

This guide shall assist in making the complete functionality of the WinGPC Software available in an easy and straight-forward way. WinGPC Software offers many optional software modules which upgrade the functionality of the product according to the changing needs of the laboratory. All software modules are described in this guide even if the local software license does not include them. This guide and the extensive online help system of WinGPC Software shall answer any upcoming questions. It can also be used to learn about additional features which might be useful for the current work.

This guide also highlights *Notes* and *TIPS* which offer additional insight, useful features and applications which might be missed otherwise.

Contents of the Agilent WinGPC Software User Guide

This User Guide will cover the description of all tasks and data evaluation processes as well as an in-depth description of all windows, menus and dialog boxes. A table of contents and a subject index allow to search for keywords in order to find the necessary information quickly.

Chapter "Basic Theory and Application of GPC/SEC" on page 15 describes the basics of GPC methodology and theoretical background on GPC separation, calibration and detection. It discusses techniques, concepts and terms used in GPC, analyzes and introduces important parameters which might influence results. It also presents the basic equations of GPC data processing and the calculation of molar mass distribution and average molar masses. Here the reader can also find important references which will assist in understanding more about the GPC technique.

Chapter "Introduction to Agilent WinGPC Software Features and Familiarization" on page 63 offers novice users in-depth familiarization with WinGPC Software. It describes the user interface, working with the menus, sub windows and axes. It also elucidates on data transfer, printing options and copying WinGPC Software graphs and reports to other applications. This chapter also contains information on the WinGPC Software use in regulated environments and explains WinGPC Software's approach to comply to the guidelines of regulating agencies.

In Chapter "First Steps" on page 103 you find an in-depth "How To" guide which teaches the first necessary steps of doing successful GPC analyzes, calibrations and data evaluations. It contains detailed hands-on instructions and "how-to" guides for all major tasks when working with a macromolecular data system. Separate Step-by-step instructions are available as a WinGPC Quick Reference Guide in the "Documentation" folder of the installation medium and the WinGPC program folder.

Chapter "Description of Menus and Options" on page 154 explains all menu commands and sub-windows for conventional GPC data capture and analysis in full detail. The calibration methods and their use are described in Chapter "WinGPC Software Calibration Window" on page 297, which also covers all details on calibration menu commands, options and calibration dialogs.

WinGPC Software also allows to quantify peaks based on HPLC-type data processing which is useful for determination of e.g., oligomer content, the amount of residual monomers or solvents. The HPLC analysis functionality of WinGPC Software is described separately in Chapter "Application of the HPLC Mode" on page 324.

The major features and use of the ReportDesigner option are described in Chapter "WinGPC Software ReportDesigner" on page 333. More details on the powerful ReportDesigner can be found in the online help. Step-by-step instructions are available in ReportDesigner Quick Reference Guide (**Documentation** folder on installation medium and WinGPC program folder).

All aspects of molar mass detection methods in GPC like light scattering and online viscometry and their use with WinGPC Software are fully explained in Chapter "The Viscosity Module" on page 362, Chapter "Light Scattering Module" on page 382. A major advantage of WinGPC Software software (hence the name) is that it handles all molar mass sensitive detection techniques and detectors from any vendor similarly. This makes using the software with different methods extremely easy since only one software has to be used for any detector from any vendor. The same is true for detector combinations and so-called Triple Detection data analysis, which is described in Chapter "Triple Detection" on page 412.

Chapter "2-Dimensional Chromatography" on page 415, Chapter "Copolymer Analysis Software Module" on page 429 and Chapter "Chemical Heterogeneity Module" on page 433 cover 2-dimensional chromatography and different methods to determine the chemical composition of macromolecules. In Chapter "Copolymer Analysis Software Module" on page 429 the compositional analysis is described which is based on GPC separations using the calibration of different concentration detectors to measure the composition in each analytical fraction. Alternatively, interaction chromatography can also be used to measure average compositions and composition distributions. This chemical heterogeneity HPLC technique is described in Chapter "Chemical Heterogeneity Module" on page 433 and relies on a composition calibration of the retention axis. Moreover, WinGPC Software also supports 2-dimensional chromatography which is described in Chapter "2-Dimensional Chromatography" on page 415. The determination of polymer functionalities and the absolute molar mass calibration based on Endgroup signals, as employed in the Endgroup Analysis WinGPC Software software module, is described in Chapter "Endgroup / Heparin Analysis" on page 438.

Chapter "3D Spectra Module" on page 450 covers the 3D visualization of spectra which were acquired time dependent, e.g., using a DAD.

LC-MS data evaluation is described in Chapter "Mass Spectrometry Module" on page 461. True molar masses of complex samples may be determined via automated data processing.

Instrument control by ChromPilot which can manage various GPC systems is described in Chapter "ChromPilot System Control" on page 466. Connectivity details are available in the ChromPilot Control documentation ("Documentation" folder of the WinGPC program folder).

The Compliance Edition for complete traceability and conformity to virtually all regulations is described in Chapter "WinGPC Compliance " on page 507. Background information on compliance issues and how WinGPC Software covers them can be found in Chapter "Working in Regulated Environments: Agilent WinGPC Software Compliance Edition" on page 71.

The "Appendix" on page 530 shows reference documents, lists background information and discussed hardware related issues.

NOTE

Instructions for software and hardware installation and hints on how to update easily from a previous PSS WinGPC UniChrom or Unity version is described in the Agilent WinGPC Software Installation Instructions.

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Conventions

This guide uses several typing conventions to make reading and working with the Agilent WinGPC Software User Guide as easy and understandable as possible:

- Menu Items will be marked with brackets [ ]
- Functions will be presented in bold italic letters
- User Input will be presented in italic letters
- Window names and soft keys will be presented in italic letters in font Arial
- The Compliance Edition symbol indicates functions that are only available or react different when the Compliance Edition is present. WinGPC Software and Compliance Edition functions can be restricted depending on defined user levels. If a user lacks of sufficient rights, some menu options will not be listed or displayed on grey background (deactivated).

The creation of a manual is always a walk on the edge between information as complete and understandable as possible on one hand and a documentation to a reasonable extent on the other hand. We do hope that with the current manual we have found a good compromise. Any user comments for the improvement of the Agilent WinGPC Software User Guide are always welcome.

3 Basic Theory and Application of GPC/SEC

This chapter covers the basic theory of GPC/SEC. If you are a GPC/SEC novice you should carefully read the first sections in order to get used to these analytical techniques. The sections “Molar Mass Sensitive Detection” on page 41 and “Determination of Chemical Heterogeneity” on page 52 are meant for advanced users which are interested in highly specific detection techniques and chromatographic methods.

Introduction to GPC/SEC

The interest in gel permeation chromatography (GPC), which is also well-known as size exclusion chromatography (SEC), has dramatically increased since its introduction by Moore[1] and others as a powerful characterization tool for bio- and synthetic macromolecules. Quality assurance and control, even during the production and processing stage increase in significance, to retain international competitive ability. Also, in academic and industrial polymer research, the exact knowledge of molecular weights and their distribution are of great importance.

However, support for users by evaluation of the GPC/SEC data has not found the necessary interest in the past. The improvement of the GPC/SEC Software has not kept in step with the improvement and re-development of GPC/HPLC components. Therefore, it still happens to this day, that GPC/SEC elugrams have to be evaluated manually. Through the increasing automation of polymer analytics, GPC/SEC Software today should be flexible and powerful at the same time and simplify the workload of the user, thereby making effective use if the powerful and expensive hardware. GPC/SEC is characterized by a series of specific marginal conditions, which partly differentiate considerably from the conditions of liquid chromatography (LC):

- completely different calibration procedure for transformation of elution volume in molar masses
- completely different separation mechanism: diffusion-controlled exclusion chromatography instead of adsorption equilibration
- basically, different requirements to the system parameters which determine the reproducibility, measurement and evaluation accuracy
- varied time bases: Measurement time, peak width etc.
- varied of analysis goals: Molecular mass specification instead of qualitative or quantitative analysis
- multi-detection operation in the GPC suggests parallel evaluation of all used detectors
- evaluation procedure should automatically process volume correction between the different detectors, so that only one calibration relation will be needed for all connected detectors

Basics of GPC Analysis

In contrast to gas chromatography (GC) and high pressure liquid chromatography (HPLC) the separation mechanism of gel permeation chromatography (GPC) is not based on a distribution equilibration, but presents a volume exclusion chromatography[2]. The separation results in GPC/SEC due to the hydrodynamic volume (V_h) of the sample molecule[3] which can be determined from the quasi-elastic light scattering by measurement of the diffusion coefficients. The separation in the GPC is also based on the molecular *size* and not on the molar mass of the sample molecule.

Macro porous polymer gels are mostly used as column material in GPC/SEC. The diffusion of molecule between mobile phase and pore is the basis for separation mechanism and performance. Since for smaller molecules more pores are accessible, these will be more retarded and consequently elute later than the higher molecular fractions. Unfortunately the GPC/SEC does not present an absolute method; i.e. the retention times (in GPC/SEC terminology called elution volume, V_e) have no direct relation to molecular mass of the examined substance and depend on the measurement conditions (type of polymer, columns, solutions, etc.).

Therefore, an accurate calibration is necessarily within the used analytical conditions. Under optimum conditions it is possible to determine molecular weights quickly, economically, and reliable, with an accuracy that does not differ from other absolute methods (osmosis, light scattering). In contrast to these, GPC/SEC does not have high demands on the sample preparation. Additionally, to the molecular mass GPC also yields the molecular weight distribution, which influences many physical and physico-chemical properties and therefore is of great interest.

Calculation of Molecular Weight averages and their Distributions in GPC/SEC

This section contains detailed information on the calculation of molecular weight averages and their distribution in GPC/SEC.

Molecular Weight Averages and Molecular Weight Distributions in GPC/SEC

The calculation of the molecular weight averages nowadays uses the slice method. Hereby the eluted peak is separated into several equidistant volume slices, which generally are identical with the width. Through calibration, the elution volume is then transformed into the molecular mass. In calculating the molecular averages, the slice concentrations c_i must be corrected with the slope of the calibration curve. This is necessary, because the data recording occurs linear in the time, the molecular mass however does not increase in a linear fashion. Objectively this means, that with the same concentration the number of polymer chains with a defined molecular weight on the high molecular part of the elugram is much smaller than on the low molecular part. In many programs, this correction is not processed. The errors caused through this will increase, the broader the sample is distributed, and the smaller the data recording frequency. Only with strictly linear calibration curves, the correction is not needed.

The molecular weight distribution w(M), which can be calculated of the detector signal, S(V) is most important for the properties of polymers. In contrast the molecular weight averages describe only average properties of the sample. E.g., the molecular weight average of two samples can be identical, although the molar mass distribution is different.

The differential distribution, w(M), of the molar mass M is defined as:

$$w(M) = \frac{d\,m}{d\,M}$$

By simple transformation w(M) can be expressed by measured quantities:

$$w(M) \propto \frac{S(V_e)}{M(V_e) \cdot \sigma(V_e)}$$

with:

$S(V_e)$ the detector signal

$\sigma(V_e)$ the slope of calibration curve

The above qualitative introduced correction by the gradient of the calibration curve can now be allocated by the mathematical derivative of the calibration curve.

The integral distribution I(M) will be used as normalization condition resulting from:

$$I(M) = \int_0^M w(M')\,d\,M'$$

The molecular weight averages can be calculated from the moments, μ_i, of the molar mass distribution:

$$\mu_i = \int_0^\infty M^i \cdot w(M)\,d\,M$$

with: μ_i the *i*-th moment of the molar mass distribution

The molar mass averages are defined and calculated in WinGPC Software by:

Number average molecular weight:

$$M_n = \frac{\sum h(M) \cdot M}{\sum h(M)} = \frac{\sum w(M)}{\sum w(M)/M} = \frac{\mu_0}{\mu_{-1}}$$

Weight average molecular weight:

$$M_w = \frac{\sum h(M) \cdot M^2}{\sum h(M) \cdot M} = \frac{\sum w(M) \cdot M}{\sum w(M)} = \frac{\mu_1}{\mu_0}$$

z-average molecular weight:

$$M_z = \frac{\sum h(M) \cdot M^3}{\sum h(M) \cdot M^2} = \frac{\sum w(M) \cdot M^2}{\sum w(M) \cdot M} = \frac{\mu_2}{\mu_1}$$

Viscosity average molecular weight:

$$M_v = \left(\frac{\sum w(M) \cdot M^a}{\sum w(M)} \right)^{1/a} = \left(\frac{\mu_v}{\mu_1} \right)^{1/a}$$

The width of the molar mass distribution can be described by the polydispersity index D or the (formerly used) non-uniformity:

$$D = \frac{M_w}{M_n} \quad \text{and} \quad U = D - 1$$

respectively.

Calibration

Only by proper calibration the elution volume can be transformed into molar masses. The calibration can be carried out in various ways:

Calibration with Narrow Polymer Standards

The use of polymer standards with narrow molecular weight distribution is the simplest and most accurate way to assign molecular weight to elution volume. For this it is best to assign M_p values (molar mass on the peak maximum), as this value is the only molecular weight that can clearly be identified in the elugram. If weight average molecular weights, M_w, are used, then the calibration function should be iterated until the used M_w values will be received again by re-calculation.

Calibration using Universal Calibration

Since for some polymers no polymer standards are available, very early possibilities were studied to convert existing calibrations to other types of polymers. This method developed by Benoit[4] assumes, that the property which determines the elution behavior in GPC is the hydrodynamic volume, V_h, of the polymer under investigation. Since V_h should be proportional to the product of intrinsic viscosity, [η], and molecular weight, M, it follows that for two polymers eluting at the same elution volume:

$$[\eta]_1 M_1 = [\eta]_2 M_2$$

valid at the same elution volume

Using the Mark-Houwink relation

$$[\eta] = K \cdot M^a$$

the molar mass of the polymer type (1) can be converted into the molecular weight of polymer type (2):

$$\lg M_2 = \frac{1}{1+a_2} \lg \frac{K_1}{K_2} + \frac{1+a_1}{1+a_2} \lg M_1$$

It is apparent, that the Mark-Houwink constants must be known for both polymers in the respective eluent under separation conditions.

Unfortunately, this is not the case for frequently used GPC eluents (e.g., THF, DMF). For the determination of the intrinsic viscosities solution viscometry measurements are necessary. However, the determination of intrinsic viscosities for some polymers is difficult in some solvents (e.g., PMMA in THF).

In such cases a trick may help. Instead of doing viscosity measurements of polymer standards with known weight average molecular weight (M_w) GPC characterizations are performed. Using the known Mark-Houwink constants [η] $M(V_p)$ is calculated from the molar mass calibration curve of the polymer. Thus, it is possible to determine intrinsic viscosities of polymers by GPC. Plotting log [η] vs. log M of the polymers investigated by GPC the Mark-Houwink coefficients can be calculated from slope and intercept.

If there are no narrow standards for the polymer type, the samples have to be fractionated, if you want to use this universal calibration method. In addition, the weight average molecular weight from light scattering measurement must also be

determined. The viscosity measurement for the specification of the intrinsic viscosity can be renounced, if the here presented method for calculation of the intrinsic viscosity is used.

NOTE

The Mark-Houwink relation is valid only above a certain molecular weight, which depend on polymer type (approx. 10 000 to 20 000 Da). Below this limit, the Mark-Houwink coefficients dependent on the degree of polymerization. In this case the log [η] vs log M plot deviates from the straight line to higher viscosities. The reason for this behavior is based in a change of the structure which usually changes from a wormlike to a Gaussian coil behavior.

The universal calibration presents a very useful calibration method; its validity should be checked for the used polymer type (literature, combination of direct calibration with universal calibrated samples). Special attention should also be paid in the molar mass section below approx. 20 000 Da.

Calibration with Broad Standards

The calibration procedure used by the WinGPC is based on papers of Mahabadi[5], Weiss[6] and Mori[7], which use the dependence of the GPC separation on hydrodynamic volume, to calibrate reliable and flexible with broad standards. The procedure described here has no limitations (e.g., only linear calibration function, calibrations only with (inaccurate) M_n and M_w) and even permits deduction of the Mark-Houwink coefficients of the polymers under investigation. Because this calibration method is based on the universal calibration, their requirements have to be considered as well. Requirements for processing of such a calibration are:

- Existence of a base calibration curve (Index: 1), which will be used for the characterization of the pore size distribution of the column set used.
- One or more broad polymer samples (Index: 2) with known average molecular weight values (M_n and/or M_w and/or [η]), which must be clearly known. (Valid for all broad calibration procedures)

Theoretical Background:

According to the theory of universal calibration for each elution volume and independent of the type of polymer it is necessary that all samples have the same hydrodynamic volume. The molecular weight on the other hand is different; however, a transformation can be carried out (see universal calibration in chapter "Calibration Curve Creation by Mark-Houwink Transformation" on page 313), which depends on the stiffness of the main chains of the considered polymer.

For each elution volume the following equation holds true:

$M2 = A \cdot M_1^B$

where A and B are constants, which must be optimized through the mean values of the broad samples.

In order to do so, A and B are varied and the molecular weight averages are calculated from the elugrams and the calibration curve. These molecular weight averages are compared with the reference values. This process will be optimized with a Simplex Algorithm until the calculated and reference molecular weights agree sufficiently exact.

The calculated A and B values relate to the hydrodynamic parameters as follows:

$$A = \left(\frac{K_1}{K_2}\right)^{\frac{1}{1+a_2}} \text{ and } B = \frac{1+a_1}{1+a_2}$$

where A and B are constants, which must be optimized through the mean values of the broad samples.

K, a: Mark-Houwink constants for base polymer (1) and broad sample (2)

If the Mark-Houwink coefficients of polymer (1) are known at the analytical conditions used, the Mark-Houwink parameters for the unknown sample (2) can be calculated.

Independent of the used procedure, the user always has to fit optimally the measured or calculated calibration points with a function. This can be a big problem, which many users are unaware of. Since the calibration function in GPC is not linear or only slightly curved as usual for GC or HPLC calibrations but shows a strong S-shaped curvature. It may be difficult to fit this dependance by a simple function. WinGPC Software assists with specialized GPC calibration fit (PSS Poly fits) functions which take the sigmoidal shape into account.

Flow Correction and Internal Standard

Beside the calibration, the reproducibility of the analysis conditions and its relation to the calibration play an important role. Although the now available GPC instruments achieve very good reproducibility, the GPC requires special needs to data recording and processing. The accuracy of the elution volume is determined at first through the quality of the constant flow pump, if the measurement data recording is not controlled by a drop counter (a flow-driven data acquisition version of the WinGPC Software system is also available). Again, the GPC method shows deviations from the well-known LC conditions. Because the separation of the GPC occurs through a diffusion-controlled process in porous polymer gels, the thermodynamic condition of the gel in the column also highly influences the reproducibility of the separation.

Each user has already observed this effect: the equilibration of the GPC columns takes much longer than the time needed by the pump to produce a constant flow; analysis in this phase clearly yields different results than the analysis under complete equilibration of GPC columns.

Both factors can be easily logged by use of an internal standard, which should be added to the sample solvents. Often lower molecular substances are used as internal standard, which are specifically distinguished through their characteristics (absorption, refraction etc.).

A modification of the gel condition and the flow is reflected in the displacement of the internal standard. Upon correcting the experimental elution volume by aid of the volume of the internal standard for analysis and calibration, such effects can be balanced. Using this procedure, it is easier to use calibration libraries and save the time-consuming calibrations prior to each sample series. The concept of the usage of the internal standards requires a continuous change of the gel condition and/or of the flow; erratic changes can naturally not be corrected with this approach.

The corrected elution volume is calculated by:

$$V_e^{korr} = V_e^{mess} \cdot \frac{V_{ref}^{cal}}{V_{ref}^{mess}}$$

The use of this methods also permits to define GPC molecular weights exactly and reproducible. Considering the requirements of light scattering measurements or other absolute methods like membrane osmosis or ultra centrifugation, the GPC is very qualified for routine type characterization of polymers.

In order to demonstrate the influence of the correction with the internal standard, the molecular weight distribution of a poly(o-chloro styrene) is compared in the next Figure, which has been evaluated with (solid curve) and without correction (dotted curve) for the internal standard (BHT; measurement in THF by room temperature with 1.0 ml/min.). The change of the weight average, M_w, is more than 10 %; however, the difference in elution volume between reference and experimental elution volume is less than 1% (20.37 ml vs. 20.20 ml). These deviations become even more pronounced if the slope of the calibration curve steeper.

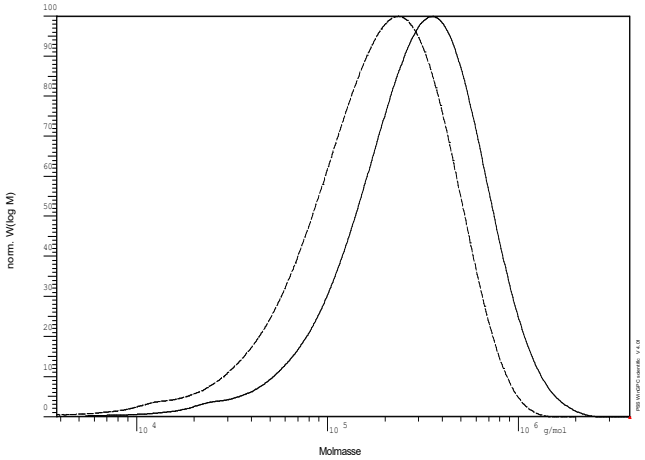


Figure 1 Influence of volume correction by the internal standard to the molecular weight distribution

How much this fluctuation of the flow and/or modifications of the gel condition influence the molecular weights shows following table:

Table 1 Fluctuation influence

Internal Standard in ml	Deviation in %	Mw in Da	Deviation in%
21.80	+ 2.01	46,500	+ 36.8
21.65	+ 1.31	41,900	+ 23.2
21.50	+ 0.61	37,500	+ 10.3
21.00	-	34,000	-
21.20	- 0.80	29,800	- 12.4
21.05	- 1.50	26,400	- 22.4
20.90	- 2.20	23,300	- 31.5

These deviations not only show up in the molecular weight distribution, but also in the elution curves, however not so clear. The values mentioned in the table generally depend on the used columns combination and the position of the peaks in the calibration curve. For linear (mixed bed) columns or columns with higher particle size (> 10 µm), but same column length a higher deviation must be taken into consideration. Also, the preferred overlay of elugrams and molecular weight distributions will be more evident by use of an internal standard.

Determination of Detector Delay

WinGPC Software enables the simultaneous recording and evaluation of several detectors. Therefore, it is not necessary to create an own calibration curve for each detector since the chromatographic delay between the detectors is corrected on-line. Beside a common calibration curve for various concentration detectors the correct allocation of concentration signal to viscometer and/or light scattering signal is an indispensable requirement for the specification of molecular weight distribution by GPC with light scattering or viscosity detection.

To define the delay between the detectors, define a delay of 0 ml in the WinGPC Software method window for all detectors. Inject a monodisperse substance, which yields sufficient signal intensities in the detectors used. Evaluate the sample in all channels. In the mass distribution window you can read the V_P values for the elution volumes at peak maximum for the different detectors. The difference of

$$\Delta V_i = V_p(det\_1) - V_p(det\_i)$$

is the required delay for the i-th. detector. According to this definition the first detector always has a delay volume of 0 ml.

Separation Efficiency, Resolution, Plate Count

A simple method for controlling the performance of a GPC instrument is the specification of the plate count. Therefore, a lower molecular substance (acetone, BHT, etc.) will be injected. The details stated in WinGPC Software conform to the DIN 55672 resp. ISO 13885.

The calculation of the theoretical plate count per meter, $N_{th.}$, uses the peak position and the peak width at half peak height according to

$$N_{th.} = \left(\frac{V_P}{\sigma}\right)^2 = \frac{554}{L[cm]}\left(\frac{V_P}{W_{1/2}}\right)^2 \qquad \left[\frac{1}{m}\right]$$

where σ is the variance, which can be estimated by the half-height method, $w_{1/2}$, and *L* is the column length in cm.

Appropriate conditions: injection volume < 20 µl, flow rate 1.0 ml/min., conc. approx. 50 ppm.

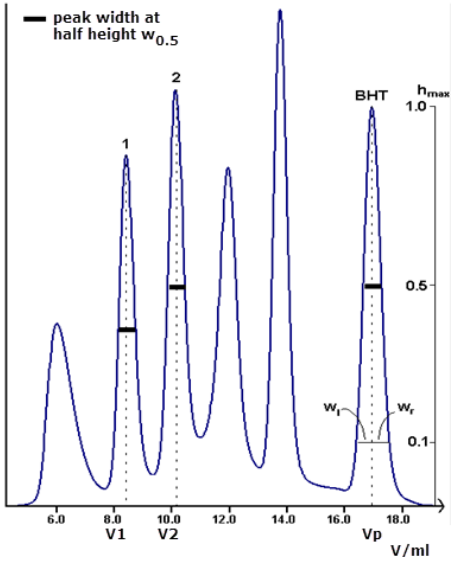


Figure 2 Definition of parameters for the calculation of plate count, N, R_{sp}, and asymmetry

The peak asymmetry in DIN 56672 and ISO/EN 13885 is defined as $A = w_l / w_r$,

where w_l and w_r are the peak widths on the left and right side of the peak maximum (measured in 10 % of the peak height).

NOTE

In ASTM and in most HPLC software packages, asymmetry is defined as $A' = w_r / w_l$, thus, $A' = 1/A$.

In general, the resolution is more important since it directly yields information about the performance of the column separation. Therefore, a mixture of polymer standards will be injected. The resolution is calculated according to the following equation (*D* is the slope of the calibration curve):

$$R_s = \frac{V_2 - V_1}{2 \cdot (\sigma_1 + \sigma_2)} = \frac{\lg(M_1 / M_2)}{2 \cdot D \cdot (\sigma_1 + \sigma_2)}$$

The resolution defined in this way depends apparently on the selection of molecular weights used. The specific solution R_{sp} can be defined as it specifies the quality of resolution of two peaks, whose molecular weight differs by one order in magnitude:

$$R_{sp} = \frac{R_s}{\lg(M_1 / M_2)} = \frac{0.579}{\sigma \cdot D}$$

Within DIN 55672 and ISO 13885, it is required that near the peak maximum of the sample the following conditions for the separation efficiency, T, applies:

$$\frac{V_e(M) - V_e(10 \cdot M)}{A} > 6$$

where A is the cross-section area of the column in cm^2.

That means that the separation distance between the elution positions per molar mass decade around the peak position of the sample must be a minimum of 6 cm.

Accuracy and Precision of GPC Results

Introduction

GPC results and their validity are crucial in many applications, e.g., as part of a QC/QA test for a product release. Obviously, the accuracy and precision of the GPC results are an important issue in such a context. This chapter describes the fundamentals how WinGPC Software performs comprehensive tests to quantify the influence of experimental factors on final results in GPC tests.

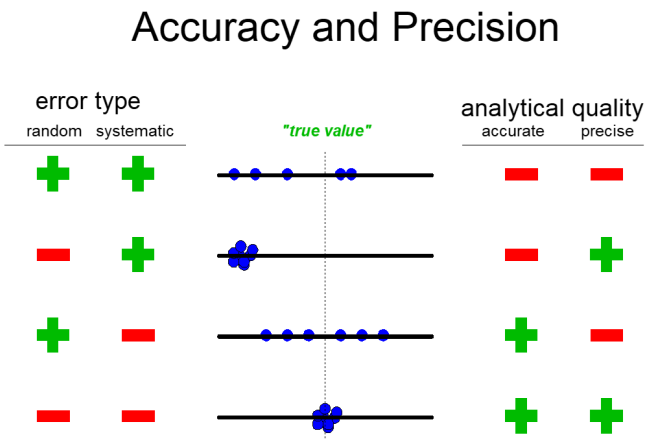


Figure 3 Definition of analytical quality and trueness of results

Every chromatography user knows from own experience that many methodological aspects and experimental details can influence a result and the final analytical quality of an experiment[8]. There are always various systematic and random contributions to the accuracy and precision of the final result. High analytical quality is only achieved if systematic and random error are avoided (Figure 3). The good news is that statistical models are very well suited to quantify random result deviations from the true (or generally accepted) value[9].

Typical systematic errors in GPC experiments might be:

- leak in GPC system
- inappropriate method (wrong column set, eluent, temperature)
- improper dissolution of sample
- miscalculation of sample concentration
- molar mass calculation based on outdated calibration or incorrect sample parameters
- wrong injection volume
- improper use of DPT sensitivity factor in viscometry detection
- wrong or unknown dn/dc values in light scattering detection
- incorrect or outdated instrument calibration factors in viscometry and/or light scattering setups

Typical contributions to statistical (random) error are among others:

- pump flow fluctuation
- old (noisy) UV lamp
- unpurged RI detector
- insufficient degassing of eluent
- improper calibration fit
- large variations in MALLS detector normalization

Obviously, any software can only deal with random errors and has no control over systematic errors which are specific to the user environment. Since many sources of errors contribute to the overall deviation of the measured result from its true value, advanced error propagation calculations have to be performed to get a reliable estimate of the final result uncertainty[10].

Baseline quality, noise and drift of detector signals, calibration type and quality among others are factors which contribute to GPC/SEC result uncertainty. Therefore, WinGPC Software will allow to determine with better confidence and reliability e.g., mass fractions < 500 Da.

WinGPC Software employs the most advanced technologies and methodologies to determine the uncertainty for all kinds of GPC results, such as molar mass averages, mass fractions, peak areas, peak positions, viscosities, radii, Mark-Houwink constants, independent of the applied calibration method (conventional, universal, viscosity or light scattering detection).

WinGPC Software reports the GPC result in the following form:

result value ± result uncertainty

(at a confidence level of 1 standard deviation)

This means that the result G of an analysis lies within G - ΔG and G + ΔG with a probability of 68 %.

Higher result certainties can easily be obtained by using higher orders of significance which can be obtained by using a factor >1 for the uncertainty value. In general, the result is given by:

G ± k ΔG with k: 1, 2, 3, ...

The defaults value for the result uncertainty given in WinGPC Software is k = 1 which corresponds to a confidence level of 68 % based on Gaussian statistics. This is the generally accepted level for statistical errors. However, higher significance can be obtained by using larger k-factors; e.g., a confidence level of 96 % is obtained for k = 2 and confidence levels of 99.7 % are reached for k = 3. Higher confidence levels can be calculated automatically with the WinGPC Software ReportDesigner option. All standard WinGPC Software reports will show result uncertainties obtained with 68 % significance. The WinGPC Software ReportDesigner also allows to report absolute uncertainty values instead of the per cent values.

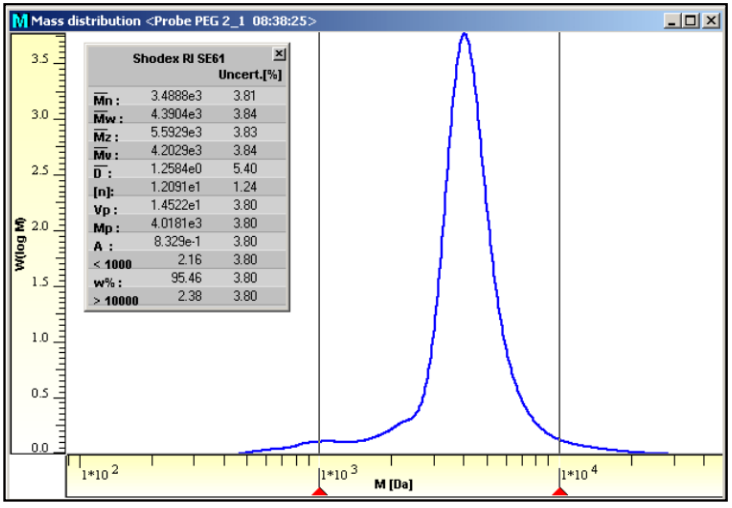


Figure 4 WinGPC Software screen shot with GPC result uncertainties

The figure shows a WinGPC Software screen shot of the molar mass distribution window with precision calculation enabled. The result table (information window) shows the result for different properties (in the rows) in column 2 and the respective relative result uncertainty in column 3 in per cent. Additional detector signals will be presented similarly.

In this example the weight average molar mass M_w has a value of 4304 Da with an uncertainty of 3.84% which relates to 165 Da. Consequently, the true M_w value for this sample will be between 4139 Da and 4469 Da with a confidence level of about 70 %. In order to achieve (practically) 100 % confidence for a result the error has to be multiplied by 3 (k = 3), which means the true weight average molar mass M_w will be within 3808 Da and 4800 Da. These result uncertainties also mean that the results of independent experiments are identical with a with a validity of 68 % (99.7 %) if the individual results are within the confidence limits of 4139 Da and 4469 Da (3808 Da and 4800 Da).

The availability of result uncertainty tests has always been required in many applications, but was very difficult to assess for end users themselves. In cases where result precision is less important, this option can permanently be switched off.

Parameters Contributing to GPC Result Uncertainty

There are many parameters which are affected by statistical error and will influence the final quality of GPC results. WinGPC Software supervises instrumental parameters, raw signal quality, method requirements and data processing settings and evaluates how these factors influence the results depending on the selected data processing specification.

For each sample and for each signal of every detector acquired the quality and variation of the signal with time is checked. The adjacent Figure shows a simplified example for illustration purposes.

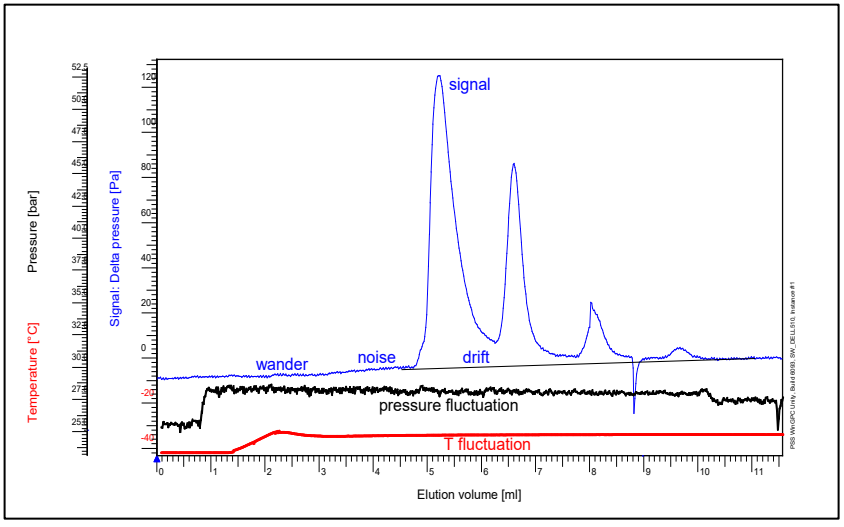
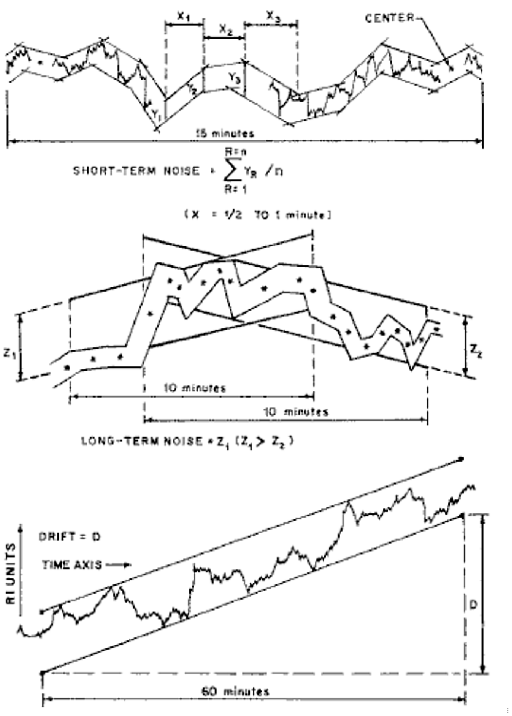


Figure 5 System and signal contributions to overal result uncertainty

The following list exemplifies a number of system properties which are continuously monitored to ensure a proper and comprehensive estimation of result precision:

- flow stability
- pressure fluctuation
- temperature stability
- injection reproducibility
- signal noise, drift and wander
- calibrated sample range
- calibration quality
- precision of viscosity data
- precision of light scattering data

The determination of system suitability test results is performed by WinGPC Software according to international and national standards (ISO 13885, ISO 16014, ASTM D5296). Signal noise, baseline drift and baseline wander are determined as described in ASTM E1657 in 0.5 min intervals as shown in the adjacent Figure.



Determination of GPC Result Uncertainty

Different end results will be influenced in different ways by system properties, which will be taken into account when calculating the overall result uncertainty.

The uncertainty of a given parameter, *x*, contributing to the overall results is calculated from its standard deviation σ_x.

Temperature stability for example is calculated by measuring the average temperature and its standard deviation during the analytical run. The results for temperature stability are calculated according to the equations given below and will be reported in the following way:

$T = \langle T \rangle \pm \Delta T$

with:

<T>: average temperature

ΔT: standard deviation, σT

Calculation of property average:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

In the case of weighted averages the following formula is used:

$$\bar{x} = \frac{\sum_{k} x_k \cdot n_k}{n}$$

Calculation of property variance:

$$\sigma_x^2 = \frac{1}{n}\sum_{i=1}^{n}(x_i - \mu)^2$$

Calculation of property standard deviation:

$$\sigma_x = \sqrt{\sigma_x^2}$$

Since many factors contribute to the overall result uncertainty WinGPC Software employs modern error propagation methods for the complete parameter set in the mathematically most rigorous way:

$$\sigma_f = \sqrt{\left\{\frac{\partial f(\bar{x}_1, \bar{x}_2, \ldots\ldots, \bar{x}_n)}{\partial x_1}\right\}^2 \sigma_{x_1}^2 + \ldots\ldots + \left\{\frac{\partial f(\bar{x}_1, \bar{x}_2, \ldots\ldots, \bar{x}_n)}{\partial x_n}\right\}^2 \sigma_{x_n}^2}$$

Linear regression results are calculated in the general form by the least squares method including standard error of the slope and the standard error of the intercept. The general equation of a linear function y in the independent variable x with individual data points (x_i, y_i) is:

$$y = f(x) = a + b\,x$$

The least squares methods minimizes the residuals D(a,b) according to:

$$D(a,b) = \sum_{i=1}^{n} (y_i - a - bx_i)^2$$

The intercept a_0 and slope b_0 of the optimized linear regression line are then given as:

$$a_0 = \frac{\sum y_i \sum x_i^2 - \sum x_i \sum x_i y_i}{n\sum x_i^2 - (\sum x_i)^2}$$

and

$$b_0 = \frac{n\sum x_i y_i - \sum x_i \sum y_i}{n\sum x_i^2 - (\sum x_i)^2}$$

The uncertainties of the intercept a_0 and the slope b_0 can be calculated as the standard deviations σ_{ma} and σ_{mb}, respectively:

$$\sigma_{ma} = \sqrt{\frac{D}{n-2}} \sqrt{\frac{\sum x_i^2}{n\sum x_i^2 - (\sum x_i)^2}}$$

and

$$\sigma_{mb} = \sqrt{\frac{D}{n-2}} \sqrt{\frac{n}{n\sum x_i^2 - (\sum x_i)^2}}$$

Reviewing GPC Result Uncertainty

If the calculation of result uncertainty is switched on (menu **Definition > Calculate > Precision** enabled in the Method Window) then each analytical result will be shown with its precision value (see Figure 6). WinGPC Software allows to assess the contributions for each GPC run so users can take action to improve the precision of their GPC results and the analytical quality of the setup (see Figure 7).

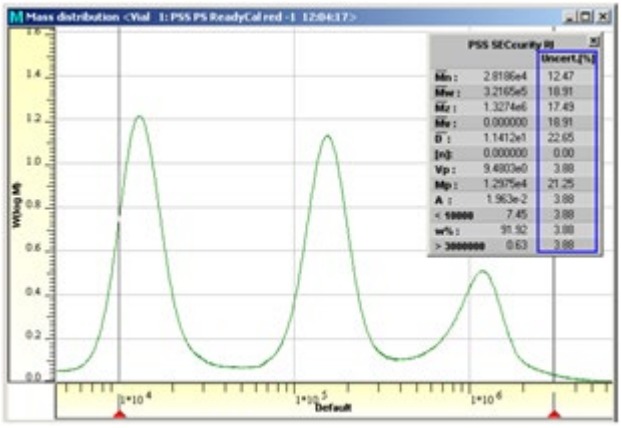


Figure 6 GPC MWD with results showing uncertainty of measurement

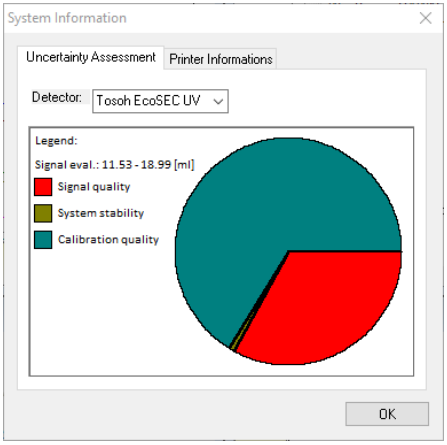


Figure 7 Overview of contributions to result precision

The analytical quality for each analysis can easily be judged based on the uncertainty assessment accessible via the information icon  located in the WinGPC Software icon bar. It summarizes the major contributions to the result uncertainty for the selected detector and the elution volume range which is used for the determination of signal quality.

In the example shown above the GPC results based on the RI signal show molar mass uncertainty between about 10 % and 20 % (Figure 6). The reason for this is shown in the pie chart (Figure 7). The major contribution to result precision is the quality of the calibration and to a much lesser extend signal quality. In oder to improve result quality, the user can first optimize the calibration (being conventional, universal, or light scattering). In a second step results will be improved even further if the user will repeat the measurement with better signal quality (stabilize detector signal, optimize injection volume and/or injection concentration). Further information on signal quality can be obtained by running a system suitability test (see chapter "System Suitability and Performance Tests" on page 247), which will show if signal wander is a major contribution or the signal/noise ratio is too low.

Molar Mass Sensitive Detection

Molar mass sensitive detectors are very useful in GPC, because they yield the molar mass of each fraction of a polymer peak independent on the architecture of the molecule. Since the response of such detectors depends on both concentration and molar mass, they have to be combined with a concentration-sensitive detector. It allows the direct measurement of molar mass in each analytical fraction and no longer relies on a calibration curve generated from reference polymer standards. This can be done by using molar mass-sensitive detectors based on Rayleigh light scattering or intrinsic viscosity measurements.

The detector response, *I*, of all molar mass sensitive detectors for a mixture of samples, *i*, is given by:

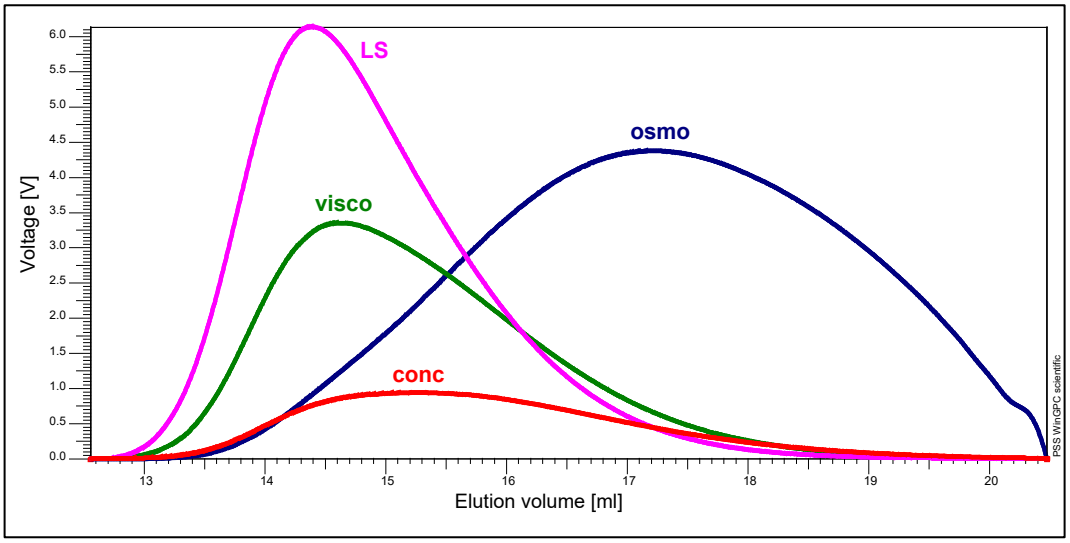
$$I = K_{det} \cdot \sum (k_{sample,\,i} \cdot c_{sample,\,i} \cdot M_i^x)$$

where:

K_{det} detector calibration constant

K_{sample} sample property (dn/dc, absorption coefficient, etc)

The exponent *x* is zero for concentration detectors (no molar mass dependence), it is "1" for all light scattering detectors, "-1" for osmometers and equal to the Mark Houwink exponent *a* in the case of viscosity detectors.



All molar mass sensitive detectors are supported by WinGPC Software. The following types of molar mass-sensitive detectors are used frequently:

- multi-angle laser light scattering detector (MALLS)
- low-angle laser light scattering detector (LALLS)
- right-angle laser light scattering detector (RALLS)
- differential viscometer (DV)

The information which can be obtained from such a detector is somewhat different. From light scattering detection, the absolute MMD can be determined directly. With LALLS (measuring the scattering intensity at a single low angle), no information is obtained on polymer conformation. Using more than three angles as in real MALLS instruments, it is possible to obtain the radius of gyration and determined the molecular conformation.

On the other hand, SEC with viscosity detection yields the intrinsic viscosity distribution (IVD). The MMD is, however, determined indirectly (through the universal calibration), and is thus subject to retention errors. Therefore, it makes sense to combine a light scattering detector with a viscometer detector (so-called triple detection *plus*). In such a detector combination, reliable information on branching, aggregation and other structural information can be obtained.

Light Scattering Detection

Online light scattering measurements overcome the calibration dilemma in GPC analyses by direct determination of molar masses independent of the nature of the sample or its architecture. In such setups there is no longer a need for using reference polymer standards as calibrants to relate molecular size to molar mass. This information is the most accurate such a detector will provide (primary information). In multi-angle setups, additional information can be obtained like molecular size, R_g, and structural information (secondary information).

Table 2 Detector specifications

Type	Method	Application	Limitation	Requirements*
LALLS low angle laser light scattering	molar mass measurement without extrapolation	• MMD • high molar mass samples	• "spikes" • high maintenance • no Rg	extremely clean system (no particles, dust, etc.)
RALLS right angle laser light scattering	molar mass measurement without angular correction	• only for low molar mass samples • comparative analysis	• no angular correction • no R_g	• M <200000 Da • sample properties must be known
TALLS two/three angle laser light scattering	molar mass measurement with 2(3)-point extrapolation	• MMD • high and low molecular weight samples	• spikes at 15° • limited angular correction • R_g inaccurate	coil statistics should be known
MALLS multi angle laser light scattering (e.g. Agilent 1260 Infinity II Multi-Angle Light Scattering Detector)	M and R_g measurement with zero angle extrapolation	• accurate MMD • reliable Rg • branching • structure	-	-

*) general condition for all GPC light scattering methods:

- the value of the refraction index increment (dn/dc) has to be known very accurately[8],a because its square value is used for the determination of the molecular weight. It is important, that dn/dc corresponds to the measuring conditions (solution, wavelength, etc.)[8],b
- use of good SEC columns without particle shedding in order to avoid "spikes"
  flexible and simple software for an extensive analysis of the data, calculation and graph of the results so that the number of different data systems in a lab can be reduced

A light scattering detector measures the scattered light of a laser beam passing through the detector cell at various observation angles. The (excess) intensity R(θ) of the scattered light at an angle q is related to the weight-average of molar mass M_w:

$$K^*c / R(\theta) = [1/M_w P(\theta)] + 2A_2c$$

where:

c	sample concentration,
A_2	second virial coefficient, and
P(θ)	form factor

The optical constant, K*, is given below:

$$K^* = 4\pi^2 n_0^2 (dn/dc)^2 / (\lambda_0^4 N_A)$$

where:

N_A	Avogadrós number,
λ_0	wavelength of light source,
n_0	solvent refractive index, and
dn/dc	sample refractive index increment

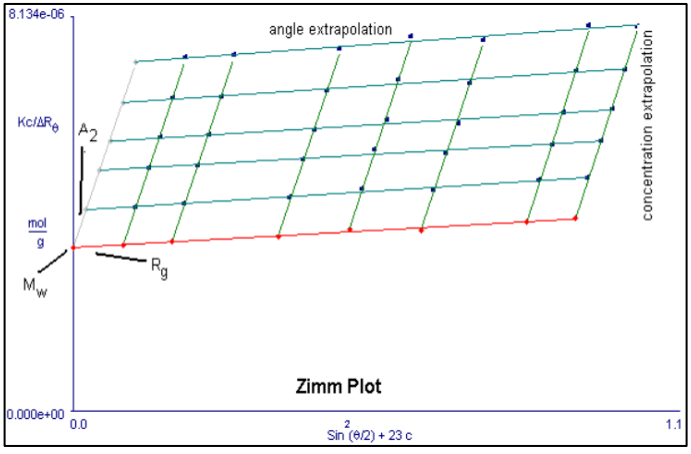


Figure 8 Determination of light scattering results from Zimm plot

In a plot of $K^*c/R(\theta)$ vs. $\sin^2(\theta/2)$, M_w can be obtained from the intercept and the radius of gyration, R_g, from the slope at small scattering angles. This is shown in the adjacent figure. A multi-angle GPC-LS measurement can provide additional information on molecular size, structure, conformation, aggregation state, etc.

Viscosity Detection

Another very useful approach to molar mass information of complex polymers is the coupling of GPC to a viscosity detector. The viscosity of a polymer solution is closely related to the molar mass (and architecture) of the polymer molecules. The product of polymer intrinsic viscosity, [η], and molar mass, M, is proportional to the size of the polymer molecule (the hydrodynamic volume). Based on the Einstein-Stokes theory the molar mass, M, of an unknown sample can be determined directly in a GPC experiment from the measured intrinsic viscosity [η] and relating it to the known molar mass, M_{std}, of a polymer standard and its intrinsic viscosity, $[\eta]_{std}$, independent of sample type or architecture:

$$M = [\eta]_{std} M_{std} / [\eta]$$

This behavior is generally referred to as "universal calibration". In conventional calibrations, the logarithm of molar mass is plotted vs. elution volume; different samples yield different calibration curves. In a universal calibration the logarithm of molar mass times intrinsic viscosity is plotted vs. elution volume. In this plot the calibration curves of very different samples all fall on a single line (so-called universal calibration curve, see Figure 9).

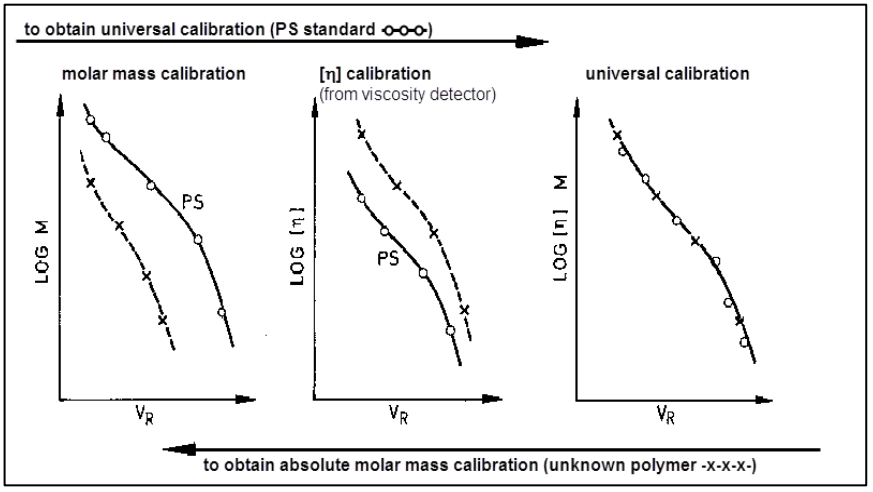


Figure 9 Comparison of conventional and universal calibration curves and how molar mass and intrinsic viscosities depend on molecular structure

Viscosity measurements in GPC can be performed by measuring the pressure drop across a capillary, which is proportional to the viscosity η of the flowing liquid (the viscosity of the pure mobile phase is denoted as η_0). The relevant parameter, intrinsic viscosity [η], is defined as the limiting value of the ratio of specific viscosity ($\eta sp = (\eta - \eta_0)/\eta_0$) and vanishing concentration c:

$$[\eta] = \lim (\eta - \eta_0)/\eta_0 c = \lim \eta_{sp}/c$$

for c ->0

Thus, the concept of universal calibration provides an appropriate calibration also for polymers for which no calibration standards exist.

Various experimental designs of online viscometers have been investigated since the late 60-ies. The most useful viscometry detection technique is based on a balanced or non-balanced 4- capillary bridge design. Signal artifacts and sometimes strong dependence on flow rate changes make other experimental configurations less attractive for general use.

Due to the problems encountered with GPC-LS and GPC-viscometry, a combination of these techniques has been developed, where three on-line detectors are incorporated into a single GPC system. In addition to the concentration detector, an on-line viscometer and a LS instrument are used in the GPC (so-called triple detection). This allows for the absolute molar mass determination for polymers that are very different in chemical composition and molecular conformation. The usefulness of this approach has been demonstrated in a number of applications.

Determination of Slice Concentration

Molar mass sensitive detectors like on-line light scattering or viscometer detectors require a concentration detector for the determination of the molar mass distribution and the molar mass averages. The concentration detector can be e.g., a UV or a refractive index (RI) detector. The concentration detector can be selected in the conc. detector field of the *light scattering/-viscometry window*.

The signal(s) of the molar mass sensitive detector and the measured slice concentration (from the concentration detector) must be combined to get the desired information

- for light scattering detectors: the molar mass for every slice
- for viscometers: the intrinsic viscosity for every slice and therefore the molar mass when a universal calibration curve is available.

There are four different approaches to determine the slice concentration. All methods are based on the same principle which relates a detector signal *U* to the concentration *c*.

The response of e.g., a differential refractive index detector is given by:

$$U_{RI} = F \cdot \left(\frac{dn}{dc} \right) \cdot c$$

Integration over the total elution volume yields to:

$$A_{RI} = F \cdot \left(\frac{dn}{dc} \right) \cdot m_{inj} = F \cdot \left(\frac{dn}{dc} \right) \cdot V_{inj} \cdot c_{inj}$$

U_{RI} and A_{RI} are the voltage and detector areas produced by the respective detector. (dn/dc) is the specific refractive index increment of the polymer in the solvent, *c* and c_{inj}. are the concentrations in the detector resp. the injected sample concentration. m_{inj} and V_{inj} are the injected sample mass and the injected sample volume. Finally, *F* is an concentration detector instrument calibration factor.

NOTE

If a UV detector is used, the substance specific constant will be the exctinction coefficient dA/dc. The respective concentration determination methods will be Fact.*dA/dc and Conc.*dA/dc.

Injected Mass Method

The easiest concentration determination method is to weigh in the samples precisely and to calculate the sample concentration. This concentration is entered in the sample editor (Raw Data Window **> Editor > Samples**). The injection volume has to be entered here too. WinGPC Software offers in the Method Window the possibility to enter a default injection volume, but in the sample editor this default value can be overwritten for every sample if necessary. The injected mass method can be chosen from the method field (second select bar) in the light scattering and/or viscometry window.

This method has the advantage that no detector calibration is needed. The concentration detector factor in the Method Window and the entered refractive index increment of the polymer in the sample editor have no influence on the calculation of the slice concentrations. However, the sample concentration and the injected volume must be exactly known. It is assumed, that the *complete* sample mass, and *only this*, elutes in the elution volume defined by the baseline. The slice concentration is calculated as:

$$C = m_{inj} \cdot \frac{U_{RI}}{A_{RI}} = c_{inj} \cdot V_{inj} \cdot \frac{U_{RI}}{A_{RI}}$$

The overall concentration displayed in the column **calculation** of the Viscosity or Lightscattering windows is calculated by:

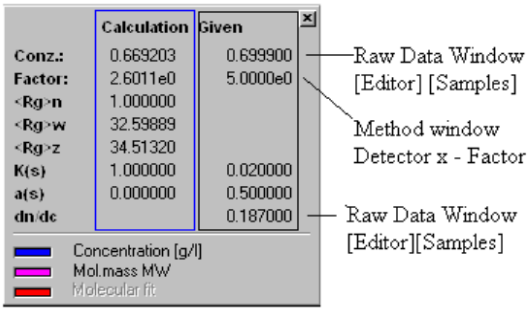
$$c = c_{inj} \cdot x_F$$

where x_F is the detector area fraction within the integration limits.

Fact.*dn/dc Method

In this concentration determination method the slice concentration is calculated using the concentration detector constant (*F*) and the refractive index increment (dn/dc) of the injected sample. The concentration detector constant is entered in the Method Window in the instrument layout view in the Factor field of the used concentration detector, the dn/dc is entered in the sample editor (Raw Data Window > **Editor > Samples**).

To get the detector constant a substance with precisely known injected mass and dn/dc is needed. The detector can be calibrated by injecting different concentrations and by plotting the concentration *vs*. the area underneath the detector signal. Linear regression gives the slope, which is dn/dc * *F*.



A more simple and fast, but less accurate way to determine the concentration detector factor is to use the **Factor** value displayed in the **Calculation** column of the information box in the **Light Scattering/Viscometry** window (one-point calibration). This factor has to be entered in the **factor** field of the used concentration detector.

In this case, the concentration detector factor, *F*, defined in the equation above is used. The concentration of the unknown sample is then calculated using the known refractive index increment.

NOTE

You can also work without knowledge of the refractive index increment. In this case, the calculated instrument factor is only applicable for this polymer type.

The concentration given in the column **Calculation** in the information box in the Light scattering/Viscosity window is calculated according to:

$$C = \frac{A_{RI} \cdot x_F}{V_{inj} \cdot \left(\frac{dn}{dc}\right) \cdot F}$$

where A_{RI} x_F is the area calculated in the mass distribution window.

It is obvious from the equation above that this calculation is independent on the knowledge of the injected mass.

Fact*conc. Method

Here the slice concentration is calculated using the bulk sample concentration and the detector factor *F*. The detector constant is entered in the Method window in the instrument layout view at **factor**, the concentration is entered in the sample editor (Raw Data Window > **Editor > Samples**). Please make sure that you also entered the correct injection volume, because WinGPC Software automatically calculates the injected mass from the concentration and the injection volume.

To get the detector constant a substance with known dn/dc is needed. The detector can be calibrated by injecting different known concentrations of that sample and by plotting the concentration vs. the area below the detector signal. Linear regression gives the slope, which is dn/dc * *F*. A more simple and fast, but less accurate way to determine the concentration detector factor is to use the **Factor** value displayed in the **Calculation** column of the information box in the Light Scattering/Viscometry window (one-point calibration).This value has to be entered in the **factor** field of the used concentration detector in the Method window.

WinGPC Software automatically calculates and displays the dn/dc of the sample in the information box in the **Light Scattering** Window. This value is automatically used for the determination of the light scattering molar masses.

Conc.*dn/dc Method

Here the slice concentration is calculated using the concentration and the refractive index increment (dn/dc). Both values are entered in the sample editor (Raw Data Window > **Editor > Samples**). Please make sure that you also entered the correct injection volume, because WinGPC Software automatically calculates the injected mass from the concentration and the injection volume.

WinGPC Software calculates and displays the concentration detector constant F in the information box in the Light Scattering/Viscometry Window. However, this value is not automatically used for the evaluation. The concentration detector constant has to be entered manually in the Method Window in the instrument layout view **factor** field of the used concentration detector.

Determination of Chemical Heterogeneity

WinGPC Software supports both characterization methods for the determination of chemical composition distributions (CCD) in macromolecules:

- GPC separation with composition determination based on multiple concentration detectors with different detector response factors (software option: copolymer); from the CCD the average composition and the copolymer molar masses can be obtained;

and

- HPLC separation with composition determination based on the elution volume position, which has to be calibrated with copolymer standards of known composition (software option: chemical heterogeneity); this methods give the CCD, average and width of the chemical composition and its skew factor.

Copolymer GPC Analysis by Multiple Detection

Conventional GPC data processing is unable to determine other important polymer properties like copolymer composition or copolymer molar mass. The reason is that the GPC separation is based on hydrodynamic volume rather than the molar mass of the polymer and that molar mass calibration data are only valid for polymers of identical structure. This means that polymer topology (e.g., linear, star-shaped, comb, ring or branched polymers), copolymer composition and chain conformation (isomerization, tacticity, etc.) determine the *apparent* molecular weight. The main problem of copolymer analysis is the calibration of the SEC instrument for copolymers with varying comonomer compositions. But even if the bulk composition is constant, second order chemical heterogeneity has to be taken into account, i.e. composition will vary for a given chain length in general.

Several attempts have been made to solve the calibration dilemma. Some are based on the universal calibration concept which has been extended for copolymers another approach to copolymer calibration is multiple detection. The advantage of multiple detection can be seen in its flexibility and yielding the composition distribution as well as molar masses for the copolymer under investigation. This method requires the molar mass calibration and an additional detector response calibration to determine chemical composition at each point of the elution profile. No other kind of information, parameters or special equipment are necessary to do this kind of analysis and calculate compositional drift, bulk composition and copolymer molar mass.

Determination of Comonomer Concentration

In order to characterize the composition of a copolymer of *k* comonomers the same number of independent detector signals *d* are necessary in the GPC experiment; e.g., in the case of a binary copolymer two independent detectors (e.g., UV and RI) are required to calculate the composition distribution $w_k(M)$ and the overall (bulk) composition w_k. The detector output U_d of each detector *d* is the superposition of all individual responses from all comonomers present in the detector cell at a given elution volume *V*. Therefore,

$$U_d(V) = \sum_d f_{dk} \cdot c_k(V)$$

with f_{dk} being the response factor of comonomer *k* in detector *d* and c_k the true concentration of comonomer k in the detector cell at elution volume *V*. The detector response factors are determined in the usual way by injecting homo polymers for each comonomer of known concentration and correlating that with the area of the

corresponding peak. If no homo polymers are available model compounds have been used to estimate the detector response factors.

In the case of a binary copolymer the weight fraction, w_A, of comonomer A is then given by:

$$W_A(V) = \left[1 + \frac{\left[U_1(V) - \frac{f_{1B}}{f_{2B}} \cdot U_2(V) \right] \left[f_{1A} - \frac{f_{1B}}{f_{2B}} \cdot f_{2A} \right]}{\left[U_1(V) - \frac{f_{1A}}{f_{2A}} \cdot U_2(V) \right] \left[f_{1B} - \frac{f_{1A}}{f_{2A}} \cdot f_{2B} \right]} \right]^{-1}$$

Obviously, the sum of all comonomer weight fractions is unity. The accurate copolymer concentration and the distribution of the comonomers across the chromatogram can be calculated from the apparent chromatogram and the individual comonomer concentrations.

The accuracy of the compositional information is not affected by the polymer architecture. Deviations from the true comonomer ratios are only possible if the detected property is dependent on the local environment. This is the case if neighbor-group effects will exist. The possibility of electronic interactions causing such deviations is very low, because there are too many chemical bonds between two different monomer units. Other types of interactions especially those which proceed across space (e.g., charge-transfer interactions) may influence composition accuracy.

Determination of Copolymer Molar Mass Averages

The major difficulty in the determination of the copolymer molar mass distribution is the fact, that the GPC separation is based on the molecular size of the copolymer chain. Its hydrodynamic radius, however, is dependent on the type of the comonomers incorporated into the macromolecule and their placement (sequence distribution). Consequently, there can be a coelution of species possessing different chain length *and* chemical composition. The influence of different comonomers copolymerized into the macromolecule on the chain size can be measured by the GPC elution of homo polymer standards of this comonomer. Unfortunately, the influence of the comonomer sequence distribution on the hydrodynamic radius cannot be described explicitly by any theory at present. However, there are limiting cases which can be discussed to evaluate the influence of the comonomer placement in a macromolecular chain.

From a GPC point-of-view the simplest copolymer is an alternating copolymer $(AB)_n$, which can be treated exactly like a homo polymer with a repeating unit (AB). The next simple copolymer architecture is an AB block copolymer, where a sequence of comonomer A is followed by a block of B units. The only hetero-contact in this chain is the A-B link, which can influence the size of the macromolecule. The A segment and the B segment of the AB block copolymer will hydrodynamically behave like a pure homo polymer of the same chain length. In the case of long A and B segments in the AB block copolymer the only A-B link acts as a defect position and will not change the overall hydrodynamic behavior of the AB block copolymer chain. Consequently, the molar mass of the copolymer chain can be approximated by the molar masses of the respective segments. Similar considerations are true for ABA, ABC, and other types of block structures and for comb-shaped copolymers with low side-chain densities.

In such cases the copolymer molar mass M_c can be determined from the interpolation of homo polymer calibration curves $M_k(V)$ and the weight fractions w_k of the comonomers *k* according to

$$\lg M_c(V) = \sum_k w_k(V) \cdot \lg M_k(V)$$

The calculation of copolymer molar mass averages $M_{n,c}$, $M_{w,c}$, etc. and copolymer polydispersity D_c is done as in conventional GPC calculations using the copolymer molar mass calculated from the equation above.

In cases where the number of hetero-contacts can no longer be neglected, this simplified reasoning breaks down and copolymer molar masses cannot be measured accurately by GPC alone. This is the case with statistical copolymers, polymers with only short comonomer sequences and high side chain densities. In such cases more powerful and universal methods have to be employed, e.g., 2D separations (see chapter "2-Dimensional Combination of Separation Techniques" on page 59).

Characterization of Segmented Copolymers

Block copolymers are an important class of polymers used in many applications from thermoplastic elastomers to polymer blend stabilizers. Block copolymer properties strongly depend, e.g., on the exact chemical composition, block molar mass and block yield. These parameters can be evaluated in a single experiment using copolymer GPC with multiple detection.

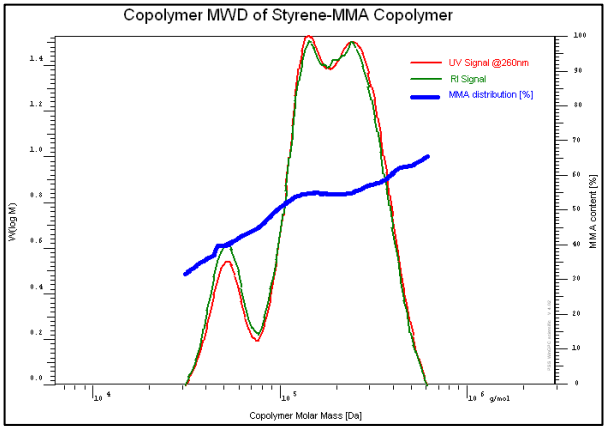


Figure 10 Molar mass distribution with overlaid chemical composition distribution of a styrene/MMA block copolymer with poor block formation

The figure above shows the measured molar mass distribution of an styrene/MMA block copolymer using RI and UV detection. The RI responds to the styrene and MMA units, whereas the UV tuned to 260 nm predominantly picks up the presence of styrene in the copolymer. After detector calibration the styrene and MMA content in each fraction can be measured. The MMA content distribution (blue line) is super-imposed to the MMD of the product. It is obvious that the MMA content is not constant throughout the MMD, but continuously increases with the molar mass. The trimodal MMD itself only shows the presence of three different species. The MMA content information clearly reveals that the copolymerization process was not producing block structure, but that the MMA was added to chains of different styrene molar mass.

Copolymer HPLC Analysis

Alternatively, copolymers and blends of polymers can be separated by interactive chromatography. This characterization technique offers another route to study copolymers and blends and analyze their composition distribution and blend ratios. This HPLC method is based on the differences in the adsorptivity of the different polymer segments with regard to the stationary phase. In contrast to the previous method (GPC) which relies on differences in the detection of compounds, this HPLC technique requires the calibration of the retention axis with standards of known chemical composition. In this respect the determination of copolymer composition by interaction chromatography can be compared to the determination of the molar mass distribution of homo polymers by calibration of the retention axis with polymer standards of known molar mass.

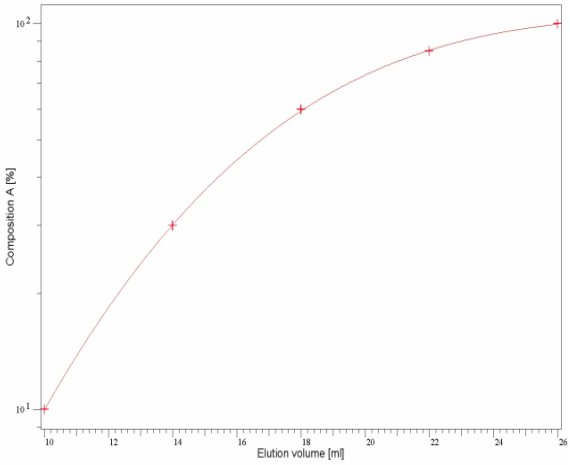


Figure 11 Calibration of chemical heterogeneity in HPLC mode by WinGPC Software

Similar to the calculation of molar mass distributions the chemical composition distribution (CCD) and its mean values can be determined.

The mean chemical composition, G, is calculated according to:

$$\overline{G} = \mu_1(G) = \frac{\sum c_i \cdot G_i}{\sum c_i}$$

The width of the distribution, dG, is given by:

$$dG = \sqrt{\mu_2(G) - (\mu_1(G))^2} = \sqrt{\frac{\sum c_i (G_i - \overline{G})^2}{\sum c_i}}$$

with:

μ_i the *i*-th moment of the concentration distribution

c_i the mass concentration in the *i*-th slice

The skew, S, of the distribution is defined by:

$$S = \frac{\frac{\sum c_i (G_i - G)^3}{\sum c_i}}{\left(\frac{\sum c_i (G_i - \overline{G})^2}{\sum c_i} \right)^{3/2}}$$

The value of the skew parameter is zero, if the composition distribution is symmetrical.

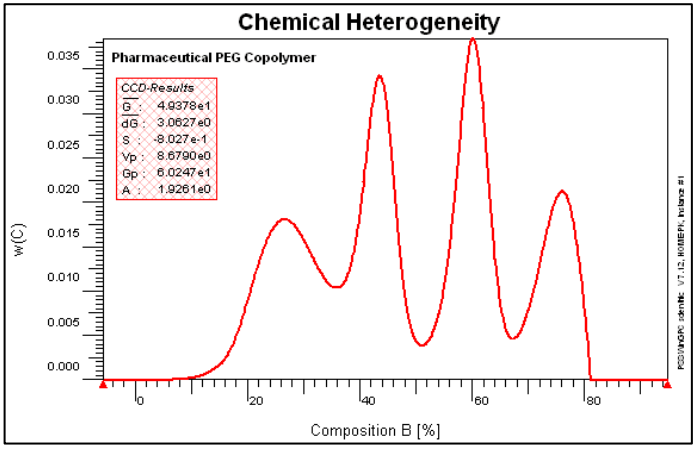


Figure 12 Chemical composition distribution and results derived from WinGPC Software

The slice concentration relates to the “true” concentration of the species in the HPLC fraction, not the apparent concentration measured by the detector. The true concentration can be measured by entering the detector response factors of the components in the WinGPC Software sequence manager / sample editor. These values are obtained from a detector response calibration.

2-Dimensional Combination of Separation Techniques

Complex polymer topologies, polymer blends and multi-component formulations require a different approach to perform a proper molecular characterization. In 2-dimensional chromatography different separation techniques are used to avoid coelution of species and to measure molar mass and chemical composition in a truly independent way.

It is obvious that *n* independent molecular properties require n-dimensional methods for accurate (independent) characterization of all parameters. Additionally, the separation efficiency of any single separation method is limited by the efficiency and selectivity of this separation mode, i.e., the plate count *N* of the column and the phase system selected. Adding more columns will not overcome the need to identify more components in a complex sample, due to the limitation of peak capacities, *n*. The corresponding peak capacity in a *n*-dimensional separation is substantially higher due to the fact that each dimension contributes to the total peak capacity as a factor and not as an additive term for single dimension methods:

e.g., for a 2D system:

where n_{total} represents the total peak capacity, n_i the peak capacity in dimension *i* and ϑ_i is the "angle" between two dimensions; for orthogonal separations this angle will be 90° and the peak capacity will be maximized. The angle between dimensions is determined by the independence of the methods; a 90 degree angle is obtained by two methods, which are completely independent of each other and will e.g., separate two properties solely on a single parameter without influencing themselves.

In 2D chromatography separations an aliquot from a first column (method) is transferred into the next separation method in a sequential and repetitive manner using automated sample transfer valves which are equipped with one or more sample loops. Alternatively, as a simpler and less useful transfer technique, "heart cuts" from peaks in the first separation mode can be manually injected into the next separation column (2^{nd} dimension).

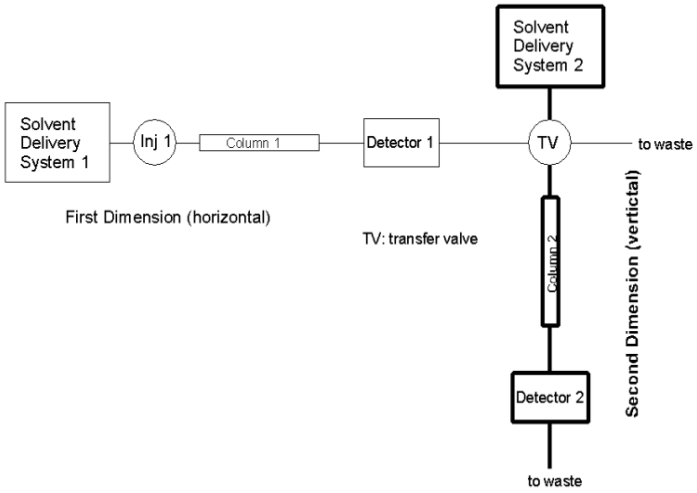


Figure 13 General experimental setup of comprehensive 2D separations

The use of different modes of liquid chromatography facilitates the separation of complex samples selectively with respect to different properties like hydrodynamic volume, molar mass, chemical composition or functionality. Using these techniques in combination, multi-dimensional information on different aspects of molecular heterogeneity can be obtained. If, for example, two different chromatographic techniques are combined in a "cross-fractionation" mode, information on chemical composition distribution and molar mass distribution can be obtained. Reviews on different techniques and applications involving the combination of GPC and various LC methods can be found in the literature.

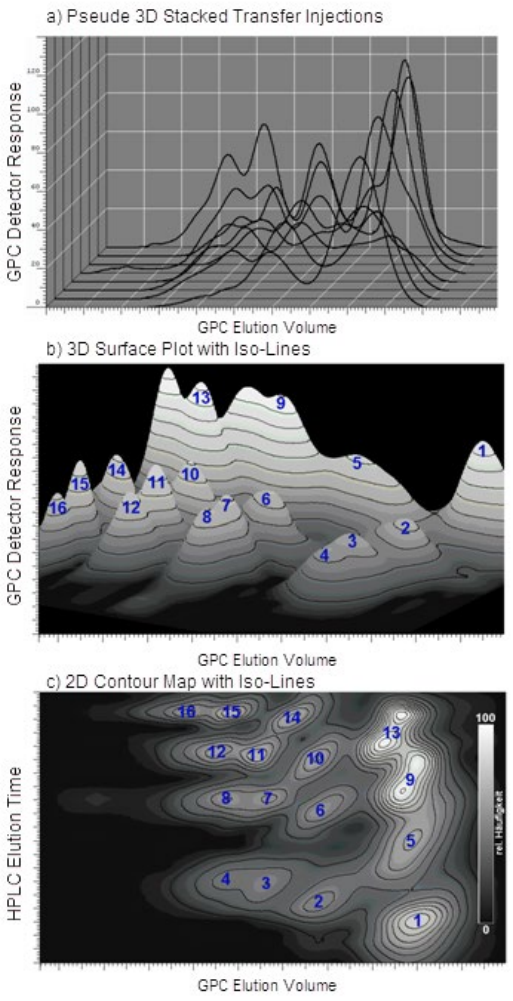
The potential of 2-dimensional separations can be explained best by a 16-component blend of a styrene/butadiene star block copolymer. The copolymer mixture consisted of 4 different molar masses (M, 2M, 3M and 4M reflecting the 1-, 2-, 3- and 4-arm star molecules) and 4 styrene compositions (20 %, 40 %, 60 % and 80 %) for each arm. Molar mass and composition were very thoroughly controlled by a special anionic poly-merization technique to ensure macromolecular architecture [(7)]. Gradient HPLC analysis only showed a broad elution profile, while high resolution GPC just separated out the molar masses of the 4 arms. The GPC result gave no indication of additional peaks with identical molecular weight but different composition hiding behind the detected peaks. The online combination of both techniques under otherwise identical conditions allowed for the separation of all 16 species in the mixture. The separation was not completely orthogonal, because the gradient HPLC separation was partially dependent on molar mass (cf. contour map in figure below).

Further data analysis allows the determination of molar mass, chemical composition and relative concentration for each component.

2D chromatography data can be displayed in various forms:

a Stacked or waterfall presentation showing the individual traces transferred into the second dimension;
b surface plot allowing to view the 3D surface from different angles;
c 2D contour maps, which are most useful for data analysis and interpretation.

Other data views can be also calculated from the 3D data set: cuts in any direction of properties (e.g., composition or molar mass), true projections (accumulation) of data to create virtual chromatograms for each separation dimension.



Various data presentation views in 2D chromatography showing the 2D analysis of a 16-component star block copolymer

Literature on GPC/SEC Theory

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[8] a) D. Held, P. Kilz; Qualification of GPC/GFC/SEC Data and Results, in: Quantification in LC and GC, S. Kromidas, H.-J. Kuss (eds.), Wiley-VCH, Weinheim, 2009

b) ISO Guide to the Expression of Uncertainty in Measurement, International Organisation for Standardisation, Geneva, 1995

c) ISO 5725: Accuracy of measurement methods and results, Geneva, 1997

d) EURACHEM/CITAC Guide: Quantifying Uncertainty in Analytical Measurement; S. Ellison, M. Rosslein, A. Williams, (eds.), London, 1995

e) EURACHEM/CITAC Guide: Use of Uncertainty Information in Compliance Assessment; A. Williams, S. Ellison (eds.), London, 2007

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[9] P.R. Bevington, D.K. Robinson; Data Reduction and Error Analysis for the Physical Science, Mc.Graw-Hill, New York, 1992

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4 Introduction to Agilent WinGPC Software Features and Familiarization

WinGPC Software is a modular designed program which offers highest quality GPC software for all kinds of GPC users according to their special needs. Therefore, you only pay for the software parts you really need. When another GPC instrument is added or an existing instrument is upgraded, the modular design of the software allows you to continue to work seamlessly with the familiar software as well as with the any existing data without losing previous investment in money, time, information, and training. The added software functionality is made available without changing the software or re-installation and therefore no re-validation is necessary.

Agilent follows new developments in the chromatographic sector and implements them to be able to continuously provide a product which will fully support all current technical possibilities. Our software modules for viscosity, light scattering and copolymer, chemical heterogeneity, Endgroup studies as well as our 2D chromatography software are perfect examples for this. Therefore, do not hesitate to inform us of new developments in the sector of GPC or submit improvement suggestions to us. Only through the active help of the user can we provide to you exactly what you need.

WinGPC Software allows the data acquisition of up to 64 independent chromatographic instruments with up to 192 detectors. WinGPC Software is a truly scalable application which can be installed on a single computer and can migrate seamlessly to networked data capture and full client/server capabilities.

Further options offered by Agilent are copolymer evaluation by multi detections, data recording and evaluation of viscometers, data acquisition and evaluation for single-angle and multi-angle light scattering instruments, evaluation of the combination of light scattering detection with viscometry (so-called triple detection).

The WinGPC Software integrates data acquisition, data evaluation and calibration in a single package. The Software Verification tool **WinGPC IQ** performs installation qualification tests. The separate KeyViewer tool offers to read the license number and prevents uncontrolled data access and data manipulation for WinGPC G7890AA.

WinGPC Software has passed rigorous software test cycles and compliance tests. Agilent guarantees that its software fully complies with international and national GPC standards. The software itself is developed and produced based on the life-cycle approach and the ISO 9001 quality system. WinGPC Software is fully validated; software validation can be performed at the end-user site to ensure accurate and valid results also in the local environment. It complies to 21CFR11 and GxP requirements (WinGPC Compliance Editions G7890AB and G7890AD) and international and national GPC standards, e.g., ISO 13885, ASTM D5296-05, DIN 55672, GB/T 36214 among others.

Launching WinGPC Software and Start-up Options

WinGPC Software starts up with an user authentication dialog.

WinGPC Software uses by default the Windows Logon name as the WinGPC Software username. The Windows or network username is passed on to WinGPC Software without any modification. If another WinGPC Software operator name shall be used, it can be entered in the WinGPC Software **Login** screen. This ensures that a username is always logged as the WinGPC Software operator. Subsequent changes of the WinGPC Software Login name in the Method window using the Operator option are still possible.

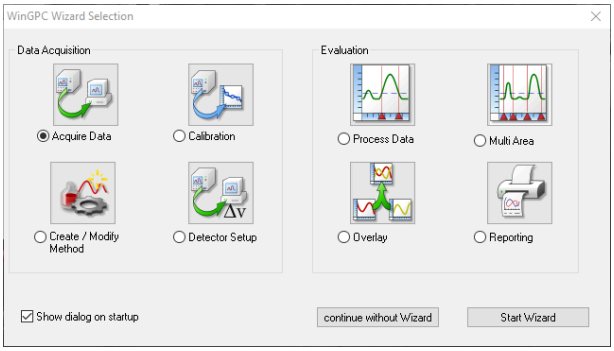
If a common password has been assigned to WinGPC Software with the KeyViewer utility (possible for licenses without Compliance Edition), it has to be entered now to launch the software.

WinGPC Software with Compliance Edition always requires a registered username and correct password to start up.

The option **Show Login Screen** can be checked to open the login dialog. This option is only available if the field **Password** is activated (by mouse-click).

If you use WinGPC Software with Compliance Edition, the **Show Login Screen** option is only available for users with **Administrator** or **Advanced User** level. All other users skip the login screen automatically and cannot change the software configuration. If the login screen has been skipped, WinGPC Software starts with the last used configuration.

If the tick mark for **Show dialog on startup** is set for WinGPC Software wizards, the optional **Wizard Selection** dialog will appear after the authentication screen (see chapter "WinGPC Software Wizards" on page 104).



The next dialog will be the login screen (if **Show Login Screen** was activated in the authentication dialog).

The WinGPC Software Login dialog summarizes all details about the software license, the available software options (modules) and the data capture capabilities. The WinGPC Software license information (license number, customer identification) is shown in the center of the login screen.

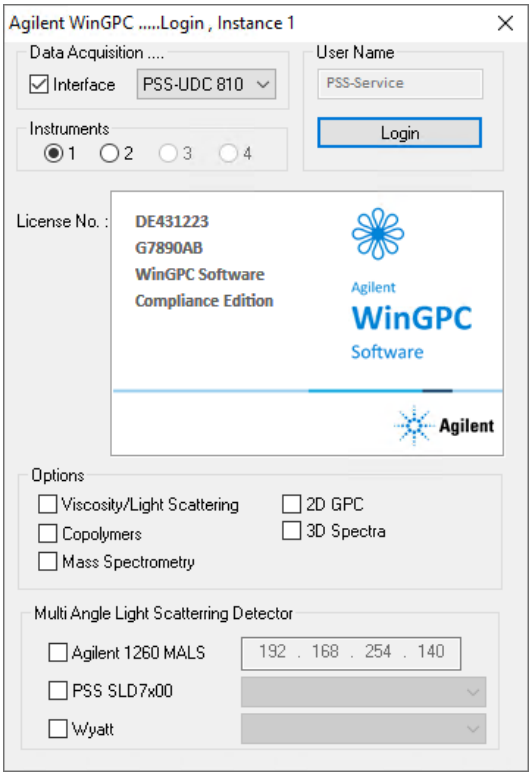


Figure 14 WinGPC Software Login Window

The **User Name** shows the user authentication taken from the authentication dialog. The **Data Acquisition** section shows the data capture parameters for acquiring data. These are:

PSS-UDC810: Default setting. Used for digital data acquisition and WinGPC Software users who update from legacy WinGPC versions with a physical PSS-UDC810 device. In latter case the UDC communication port is automatically detected depending on the cable connections. The user can select the UDC location and UDC port (if more than a single cable is connected) in this dialog.

COMx: Specifies the serial port from which PSS WinCHROM Interfaces, the PSS eta-100x/201x or the WGE products send data to the local PC.

NetConnect: Identifies the optional network capabilities when using PSS networked data capture products (e.g., NetConnect Server, Cubes, WinGPC Software Client/Server Edition). Details can be found in the respective PSS product documentation.

NOTE

If no data capture method is selected, WinGPC Software will start in **Reprocessing Mode**.

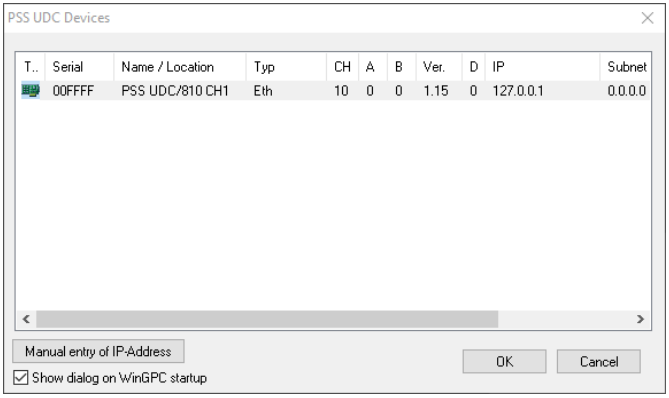
The **Instruments** section shows the maximum number of simultaneous time bases (instruments with simultaneous data capture) being served by the WinGPC Software license. The radio buttons allow to limit the number of simultaneously captured instruments to the selected number.

The **Options** section summarizes available software modules and those which have been licensed (selectable). There is no limitation in combining these options and modules in a given configuration.

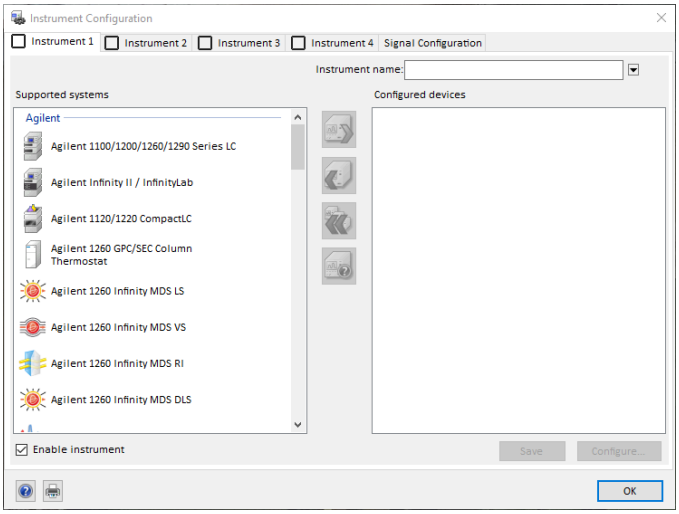
The multi-angle light scattering options specify digital data capture (without analog output and A/D converters) from the PSS SLD7x00/BI-MwA, the Agilent 1260 MALS, and/or the Wyatt DAWN products (DAWN DSP, EOS, miniDAWN). Please select the proper option for the attached detector.

Pressing the **Login** button will launch WinGPC Software with the selected options and settings specified below.

The next (optional) dialog is the UDC Device Connection dialog which is used to select the correct UDC data acquisition device. A physical UDC can be connected via COM, LAN or USB. The digital UDC used for digital data acquisition will be displayed with the serial no. **00FFFF** and the connection type **Eth**. All currently available UDCs will be listed in the dialog. If WinGPC Software shall always connect to the same UDC automatically, the tick mark **Show dialog on WinGPC Software startup** can be removed. If the default UDC cannot be connected (e.g., because it is not switched on or it just doesn´t answer fast enough), the dialog will appear even if the tick mark was removed before. The dialog can be activated again within the WinGPC Software **Method** Window (see **Interface > Information...** dialog).



The last optional dialog before launching WinGPC Software is the ChromPilot **Instrument Configuration** window. For more details, refer to the chapter „ChromPilot Configuration Manager“ on page 469.



Using Networked Data Capture

NOTE This option is only available for legacy WinGPC licenses.

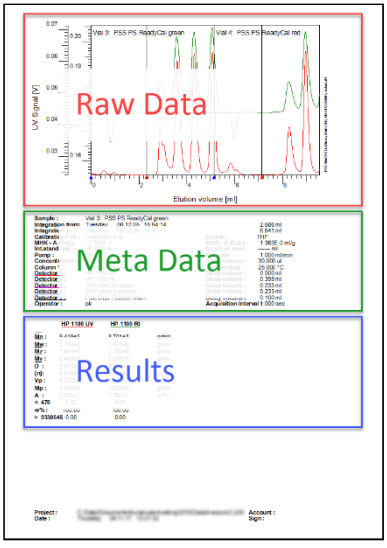
Logon to the optional PSS NetConnect Server is done in the common WinGPC Software login screen. In the **Communication Port** section, select **PSS NetConnect** from the drop-down list. Please note that this option is only available if a PSS NetConnect client option is licensed or the Client/Server option of the WinGPC Software has been purchased. The PSS NetConnect connection dialog opens after the WinGPC Software login to allow specification and selection of different system parameters. The parameters and options are described in the menu **Interface > NetConnect settings** in the Method window.

Working in Regulated Environments: Agilent WinGPC Software Compliance Edition

WinGPC Software is a macromolecular chromatography data system (MCDS) used for liquid chromatography of macromolecules. It can

- record data from several different detectors
- evaluate the data
- print results (reports).

According to the GAMP guideline, WinGPC Software is a “commercial on the shelf” standard software package and a “closed system”. In a regulated environment, a “closed system” is used with restricted user access and clearly defined responsibilities for data.



WinGPC Software differentiates three types of data:

- raw data: measurement data, recorded at a defined time
- meta data: method, user, sample number, calibration data,...
- results/reports: generated from the raw data by using meta data

A standard software package used in chromatography labs of organizations as, for instance, the FDA (US Food and Drug Administration) has to meet specific requirements:

- requirement 1: data safety
- requirement 2: accuracy
- requirement 3: user authorization
- requirement 4: complete traceability
- requirement 5: electronic signature.

Basic requirements such as data safety, accuracy, software development and documentation are completely fulfilled by WinGPC Software. Additional features like advanced traceability, audit trails, user authentication and electronic signature require the WinGPC Software Compliance Edition software module. The Compliance Edition is completely integrated in the WinGPC Software, it can be added easily and works with all systems and software modules licensed.

The Compliance Edition adds the following major features to WinGPC Software (further details can be found in chapter "WinGPC Compliance " on page 507):

- WinGPC Software User Administration:

  A separate tool to create and administrate WinGPC Software user accounts. Administrators can choose from four pre-defined user levels and add WinGPC Software specific rights to individual users. The administration software can be accessed from the Authentication window by pressing **Administration**.

- WinGPC Software User Access Control:

  If the Compliance Edition option is installed only authorized users have access to WinGPC Software. The authentication by WinGPC Software uses the policies set by the organization in the Windows environment (in general in the Windows domain).

- Traceability, audit trails and electronic signatures:

  The Compliance Edition allows to log automatically all system and user activities in Audit Trails and adds **Sample** and **Session** audit trail buttons to the status bar as well as the icon for electronic signatures to the tool bar. User Administration and ChromPilot activities are monitored and logged in their respective audit trails. All audit trails are encrypted and human readable only within WinGPC Software.

- WinGPC Software User Levels and User Rights

  The Compliance Edition allows to control the functionality of WinGPC Software by assigning pre-defined user levels and setting individual user rights. This will lead to limited functionality for general WinGPC Software users which are by default e.g., not allowed to create/modify methods or sign results by an electronic signature. In cases where access is restricted, a button or a menu item will be grayed out and not accessible by a non-authorized user.

- Qualification Workbook:

  Comprehensive guidebook with documents and forms for all phases of instrument and software qualification during the validation of the complete GPC system. Includes preventive maintenance plans, instrument service checklists and comprehensive standard operating procedures (SOPs).

NOTE

The activation of options depends on the rights of the corresponding user level, to be assigned in the Administrator Dialog.

Compliance Edition functionality is highlighted in the user documentation with the adjacent symbol.

WinGPC Software Concepts to Implement the Requirements of a Regulated Environment

Requirement 1: Data Safety

Raw data must not be overwritten, changed or deleted. Relevant data has to be archived and to be kept ready for eventual inspection within the relevant time span.

WinGPC Software concept for data integrity:

- It's impossible to change WinGPC Software raw data; only copies are used for processing.
- Raw data will be stored along with the meta data in a database. Results are always generated from the respective raw and meta data and are not saved in separate file(s).
- In the database all data is stored binary and cannot be read, edited or changed outside WinGPC Software.
- Copies of the database files can be used to archive the data. It's not necessary to copy and archive method or calibration files as well, because all meta data is included in the database already.
- It's not possible to overwrite sample data unintendedly, because every sample measurement is labeled with an unambiguous timestamp that correlates with the injection time.

Requirement 2: Validation of the Software Installation and System Operation

This requirement includes 4 separate validation steps with different responsibilities:

DQ: Design Qualification. In case of standard software packages, the manufacturer is responsible (PSS GmbH - A part of Agilent) for all aspects of software design and implementation.

IQ: Installation Qualification. If the manufacturer's validation service is not used, the customer is responsible for the IQ. The manufacturer can provide supporting software tools.

OQ: Operational Qualification. Cooperation between manufacturer and customer is often required in this type of validation. The manufacturer can provide the general OQ procedures, but the customer is ultimately responsible for the qualification of the application if the manufacturer's validation service is not used. Software OQ can be supported by software tools provided by the manufacturer.

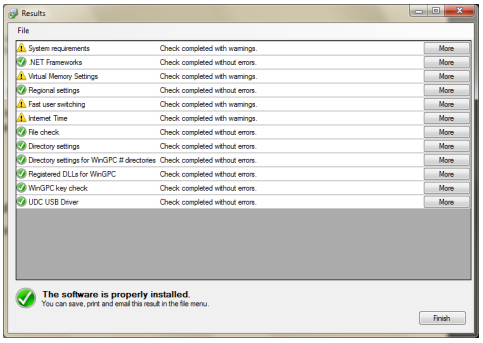
PQ: Performance Qualification. The customer should regularly run system checks to document and prove the system performance.

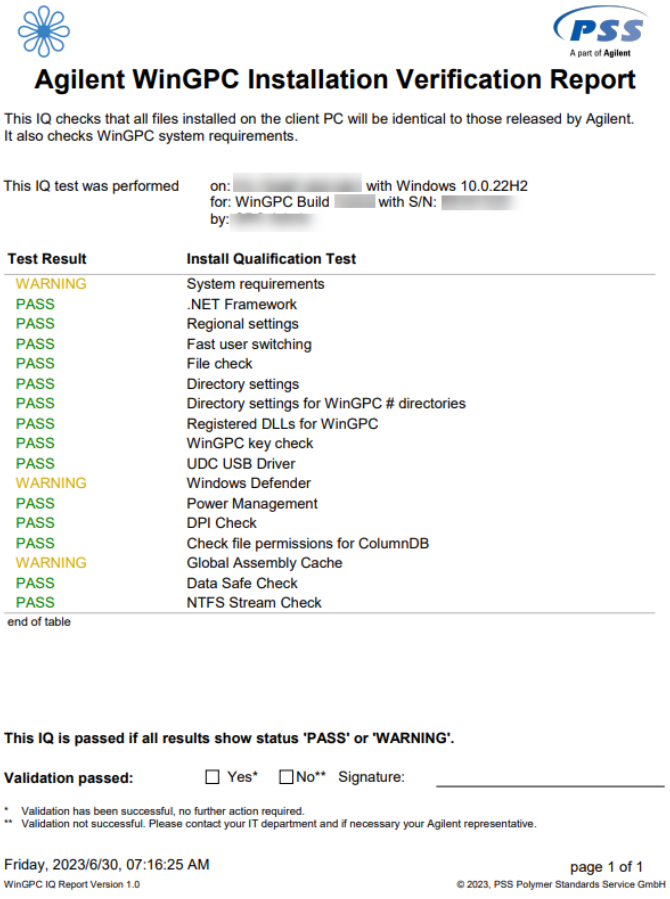
If validation is handled very strictly, the manufacturer and the customer should cooperate. The customer should provide application information and the manufacturer should provide all data and procedures of the system.

WinGPC Software concept for accuracy:

- Software Win GPC IQ:

  The installation verification tool verifies if all required files are present, have the correct size and are identical with those files tested, validated and that have passed all QC procedures of the manufacturer´s ISO 9001 requirements. This installation verification routine is part of all WinGPC software versions. An enhanced installation verification will check for the correct hardware and OS requirements as well as the settings of all affected folders. The installation verification may automatically be executed (and printed) after installation and repeated any time necessary afterwards.





Agilent WinGPC Installation Verification Report

This IQ checks that all files installed on the client PC will be identical to those released by Agilent.
It also checks WinGPC system requirements.

This IQ test was performed on: [redacted] with Windows 10.0.22H2
for: WinGPC Build [redacted] with S/N: [redacted]
by: [redacted]

Test Result	Install Qualification Test
WARNING	System requirements
PASS	.NET Framework
PASS	Regional settings
PASS	Fast user switching
PASS	File check
PASS	Directory settings
PASS	Directory settings for WinGPC # directories
PASS	Registered DLLs for WinGPC
PASS	WinGPC key check
PASS	UDC USB Driver
WARNING	Windows Defender
PASS	Power Management
PASS	DPI Check
PASS	Check file permissions for ColumnDB
WARNING	Global Assembly Cache
PASS	Data Safe Check
PASS	NTFS Stream Check

end of table

This IQ is passed if all results show status 'PASS' or 'WARNING'.

Validation passed: ☐ Yes* ☐ No** Signature: _____

* Validation has been successful, no further action required.
** Validation not successful. Please contact your IT department and if necessary your Agilent representative.

Friday, 2023/6/30, 07:16:25 AM page 1 of 1
WinGPC IQ Report Version 1.0 © 2023, PSS Polymer Standards Service GmbH

Figure 15 WinGPC installation verification, screen view and printout

- Software OQ/PQ:

  The WinGPC Software system verification checks all operational and numerical procedures on the PC used for WinGPC Software data processing. Raw data from theoretical molar mass distributions with exactly known results are processed in exactly the same way as any unknown sample to compare the calculated results with the theoretical molecular weight references. The system verification routine is part of WinGPC Software and should be executed and printed right after first launch of WinGPC Software (see example printout in chapter "Reference Printout of System Verification" on page 531).

Requirement 3: User Authorization

Only authorized users should be able to access and to process analytical data.

WinGPC Software's concept for authorized user access:

Depending on the WinGPC Software configuration, user access will be verified as follows.

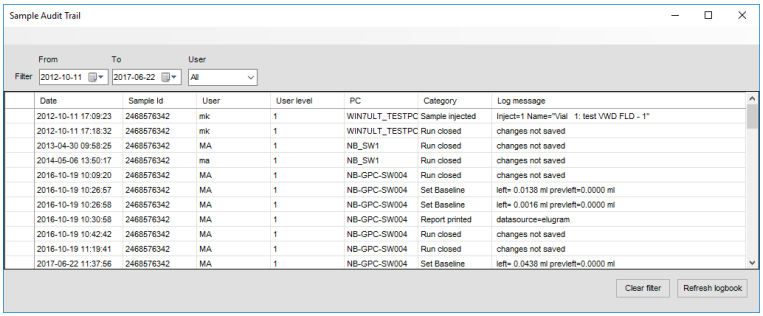
- Agilent WinGPC Software: Simple access control with the same password for all users (optional).
- Agilent WinGPC SW Client/Server: Access control with username and password.
- Agilent WinGPC SW Compliance Editions: Access control with user name and password according to the user authentication policy of the organization enforced by the Windows domain. WinGPC Software users can have one of the following four user levels: **Administrator**, **Advanced User**, **User** or **Guest**. The access to WinGPC Software features and functionality is correlated with the user level of the logged-in user.

Requirement 4: Traceability and Audit Trails

Results are determined not only by the raw data but also by the meta data. All evaluations should always be traceable, and all modifications should be documented in automatically generated audit trails. These audit trails should be human-readable within the GPC software but impossible to hamper or modify from outside the native application.

WinGPC Software concept for traceability and audit trails:

- A timestamp is added automatically to all injects and raw data points. Since raw data cannot be changed, documentation within the project database is comprehensive.
- All meta data operations are logged and documented in the sample audit trail if the Compliance Edition option is licensed. Additional "reason for change" comments can be enforced when modifying meta data (optional as set by WinGPC administrator).



- Besides the sample audit trail, the session audit trail logs all activities by every user during WinGPC Software sessions.
- The WinGPC Software session history is available only for WinGPC Software Compliance Editions.

- If the ChromPilot is used, an instrument audit trail is always generated for each controlled GPC system. It logs all instrument activities, error messages and maintenance information.
- The WinGPC SW Compliance Edition also keeps track of all activities regarding user rights and user level as set in the WinGPC User Administration Console.

- All audit trails are encrypted and cross-referenced to each other. All entries are automatically entered with time stamps, username, PC name, etc, and cannot be manipulated by any user or from outside the application. Audit trails are logged monthly and must be archived separately except the sample audit trail which is an integral part of the project database.

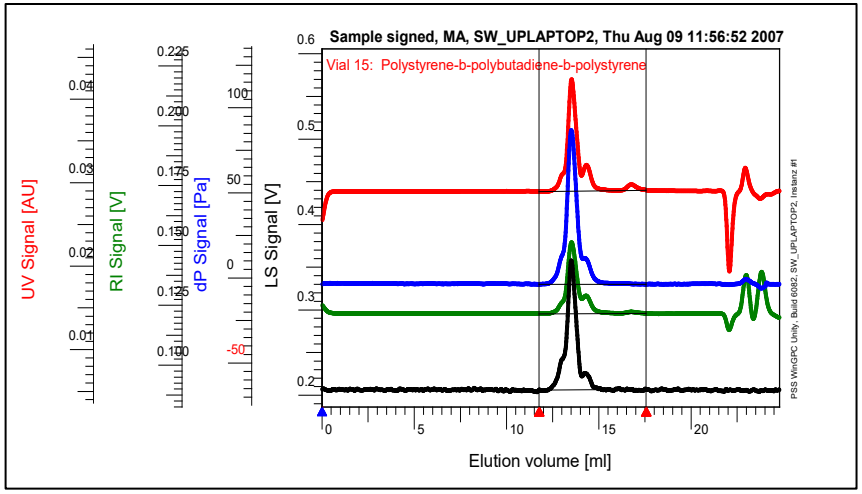
Requirement 5: Electronic Signature

The 21 CFR 11 regulations defines a digital signature as an electronic signature based upon cryptographic methods of originator authentication, computed by using a set of rules and a set of parameters such that the identity of the signer and the integrity of the data can be verified.

WinGPC Software implementation of electronic signatures (Compliance Edition only):

- WinGPC Software results with all related meta data can be signed electronically. The meta data status will be locked to prevent any type of intended or unintended result modification. If electronically signed data or results will be printed (on paper or electronically) or exported, the locked meta data will be restored before printing or data export if they have been under temporary review.

- The electronic signature is automatically added to each WinGPC Software printout.



Synopsis of WinGPC Software Standard and Compliance Edition

Table 3 WinGPC Software synopsis

Requirement	WinGPC Software	WinGPC Software Compliance Edition
Data safety	• Sample database with timestamp for each inject and each raw data point • Saving data possible on any (network) drive	• Sample database with timestamp for each inject and data point • Saving data possible on any (network) drive; archive SOP available
Access control	• Simple access control (same password for all users) • WinGPC Software Client/Server Edition: access control with username and individual password	• Controlled access with username and password according to company policy; domain controller can be used • WinGPC Software Client/Server: access control with username and individual password
User administration	Not available	Account and user level administration in the administration software tool: • WinGPC administrator • WinGPC advanced user • WinGPC user • WinGPC guest
Audit trails	• Session audit trail for current month • Instrument audit trail if system controlled by the ChromPilot	• Session audit trail, including complete history • Sample audit trail: additional meta data documentation and reporting for each sample • Instrument audit trail if system controlled by the ChromPilot • Administration audit trail for user account and user level administration • All audit trails are cross-referenced to each other
Electronic signature	Not available	• Users with respective rights can set/remove electronic signatures • Meta data status and results locked by e-signature • E-signature information is automatically added to each printout

Table 3 WinGPC Software synopsis

Requirement	WinGPC Software	WinGPC Software Compliance Edition
Accuracy: DQ	• Life cycle management • Software tests • Implementations tests • Documentation	• Life cycle management • Software tests • Implementations tests • Documentation
Accuracy: IQ	WinGPC Software installation verification	WinGPC Software installation verification
Accuracy: OQ	• WinGPC Software system verification • UDC810 verification • EasyValid tool (optional system suitability test)	• WinGPC Software system verification • UDC810 verification • EasyValid tool (optional system suitability test)
Accuracy: PQ	• WinGPC Software system verification for software PQ • System and columns test • Overlay mode for monitoring longtime performance	• WinGPC Software system verification for software PQ • system and columns test • overlay mode for monitoring longtime performance

The WinGPC Software User Interface

The WinGPC Software user interface is based on an integrated database concept which automatically links all related information to the raw data, which cannot be modified in any way. Only copies of raw data are used for baseline and flow corrections, data smoothing, etc. All user interactions are saved in the WinGPC Software database. They are retrieved with the raw data for re-calculation of results on the fly. This means that there are no results files which can easily be modified and all calculation parameters are incorporated in the WinGPC Software database and used by the software as required.

After WinGPC Software is launched it opens up full screen and places four sub-windows inside its program window (see Figure below). In general, each conventional GPC measurement in the WinGPC Software software is associated with 4 sub-windows. These are: the **Method** window, the **Raw data** (or instrument no. for real-time data acquisition) window, the **Elugram** window and the **Mass distribution** window. They always refer to the currently selected sample data which is shown in the Sample field in the status bar.

NOTE

It is possible to show several **Raw data** windows or *Instrument No.* windows simultaneously disabling the **Window > Single Raw Data Window** menu.

The Method window contains the parameters of the data acquisition. The data acquisition or Raw data window contains the baseline data of a completed measurement or a current run. The Elugram window contains the baseline and elution volume corrected data (cf. internal standard procedure in chapter "X-Axis Context Menu" on page 200), which will be used for further calculations. In the Mass distribution window you finally receive the molar mass distribution as well as the calculated molar mass averages which are obtained from the elugram data and the calibration curve.

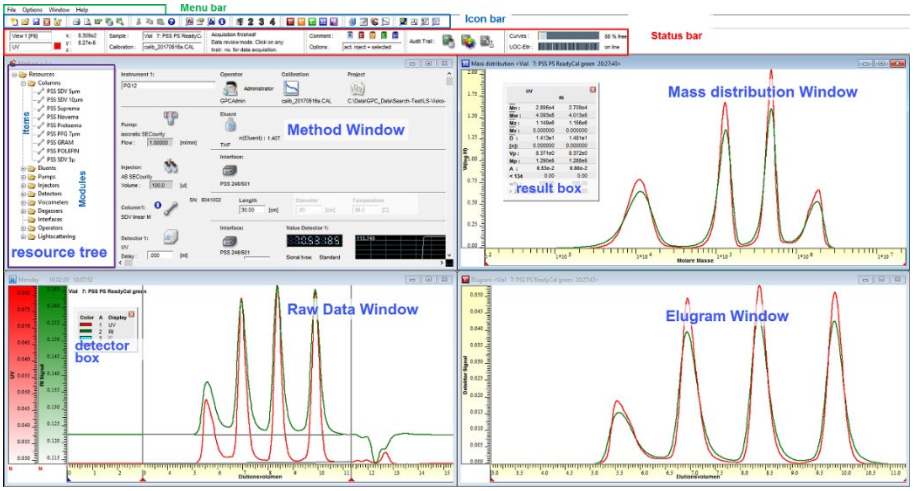


Figure 16 Bird-Eye-View in WinGPC Software shows sub-windows and information boxes for direct access

For some sub-windows you can get detailed information using the *information boxes*, which can be moved and re-sized individually. The information box of the mass distribution window displays the calculation results for all shown detector signals. The information box of the raw data window allows to assign detector signal display settings (color, Y-axis selection, signal display on/off, etc).

Description of the WinGPC Software Window Design and Layout

WinGPC Software allows the fine-tuning of all axis parameters (scaling, properties) and labels (axis name, unit, color, font, size, etc) which are saved in the WinGPC Software project database for each injection/sample individually. Any WinGPC Software method can also contain the calibration file and the file with the multi-area settings. Specifying a WinGPC Software method will load all these parameters and files automatically without any other user interaction. WinGPC Software methods and data files save also the instrument number on which the run is or was done.

Working with axes and detector signals:

Each WinGPC Software window which shows chromatographic information consists of four major objects which are present in all sub-windows and are used in the same way (see Figure 16):

- Title bar: identifies the window contents (with optional sample information)
- Major part: contains the graphical view of the information; can be zoomed, etc.
- Information box: can be switched on/off using **Window > Information**; information content can be selected using the options
- X-axis: context menus allow the selection of parameters/properties, setting the axis scale, and the appearance of the axis in the **Properties** dialog
- Y-axis: one or more axes to display signal or result information which can be selected and scaled individually.

Windows can be zoomed (magnifier effect) for better inspection of certain sections. Click into the window and start by pressing the left mouse button where the zoom area should start. Keep the button pressed and drag the appearing rectangle until it encloses the section which should be magnified. After releasing the left mouse button the magnified section is displayed in full scale. To undo one or all zoom actions (unzoom) click with the right mouse button inside the window.

The axes which are assigned to the various detector signals in the Raw data window are located on the left edge of the raw data window. Because WinGPC Software can process several detectors simultaneously, the selection of an active curve is necessary to make peak searches and similar features unique. The active detector signal is always displayed in the status bar. By clicking on the detector name, another detector can be selected from the pop-up list of available detectors (see Figure 16).

The raw data signal shown in the Raw data window can be selected and its properties set from its *information box*.

- Clicking on the color field opens the color selector box for assigning signal color.
- Clicking on the axis number will assign an axis number (1…4) to the signal.
- Clicking on the signal/detector name will show (black)/hide (gray) the signal.

There are several ways to change the Y-axis scale.

When the mouse cursor is in the top left position of any Y-axis, the interactive scale buttons will appear (please note that these interactive scale buttons will only appear if this window is active):

- The arrow up (down) button is used to increase (decrease) the gain of the detector signal interactively. If the Y-axis was in normalized state, pressing any arrow button will change that to the manual scale state indicated by an **M** below this axis.
- The square button in the middle toggles between normalized (**N**) and manual (**M**) or standard (**S**) scaling. The normalized scale will use the largest and smallest detector signal value within the windows to be scaled automatically to the minimum and maximum vertical window positions. If the signal is in normalized state, an **N** will appear below the axis. Alternatively, the Y-axis context menu **Norm**. can be used to toggle between display options.
- A standard scale can be used with each sample injection for every Y-axis. After entering the default settings, using the Y-axis context menu item **Set Standard Scale**, **Standard Scale** becomes available that toggles between standard scaling (**S**) and normalized view (**N**).

The possibilities for the scaling of the X-axis are:

When the mouse cursor is in the bottom right position of the X-axis, the interactive scale buttons will become visible. The right (left) arrow button is used to increase (decrease) the elution volume (or time) displayed interactively. (Please note that these interactive scale buttons will only appear if this window is active.) Alternatively, manual scaling by typing in minimum and maximum values for the X-axis is available from the X-axis context menu. When a manual scale has been set, the X-axis context menu item **Standard Scaling** becomes available and will reset any X-axis scaling to the standard parameters defined by the **Set Standard Scale** values.

Axis properties can also be set from the context menu. It allows setting axes properties individually. For example, text attributes (font, size, color etc) and background properties of the axis can be defined. It is also possible to switch from elution volume to elution time representation for X-axis properties. The axis properties will be saved for each axis individually.

Data Handling and Sample Management

WinGPC Software uses a modern and up-to-date sample and project management. The raw data and the sample parameters as well as all method, processing, and calibration parameters are stored in sample databases assuring complete documentation and data integrity.

The database approach has many advantages:

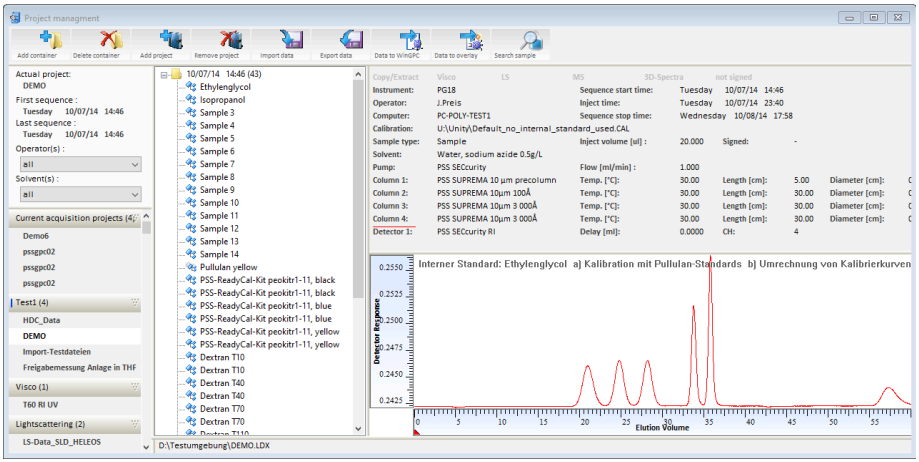
- All information and parameters necessary for sample evaluation are stored with the corresponding sample. E.g., method and calibration information are available any time, even when the original *.MET or *.CAL files used for evaluation are not present as separate files any more.
- Samples are identified not only by their name (and therefore a resulting file name) but also by the injection time and date and the sample name. This protects from accidental overwrite of samples with the same name or slightly different names.
- Databases can be searched with different restrictions. Therefore, fast sample retrieval is possible even when only a part of the sample name is known or only method or date information are available (see "Sample Search" on page 281).
- Samples can be easily recorded using the overlaid injection feature to save up to 30 % time and solvent per sample.

WinGPC Software distinguishes between **projects**, **logins** and **injects**.

- Every **project** is a sample database that consists of minimum five different files with the same name but different extensions: *.LDX, *.MDX, *.FSX, *.INX and *.SAX. Depending on the licensed modules additional files might be present and necessary to display the data (e.g., *.ATX, *.SPX or *.MSX). The complete project is created automatically by assigning a project file name: a right mouse click on the symbol with the three discs in the instrument layout view of the **Method** window opens a window where a new project name can be assigned or where an existing project can be loaded to add new logins.
- Every project can have several **logins** (maximum 2048). A login is created automatically every time data acquisition is started and stopped (e.g., using the **Sequence Manager** or manually with **Record** or **Start Baseline** and the according stop functions.

- A login can have up to 256 **injects** meaning that up to 256 samples, each assigned to a trigger signal from the autosampler or manual injector, can be present in the same login.

Loading data/samples:



Projects can be loaded in the **Project management** window (button). You can use this window to organize several projects in project containers (see chapter "The Project Management Window" on page 269). The projects of the current methods will automatically be displayed in the container **Current acquisition projects**. If you select a project, all logins are listed with their distinct start date and time. Click on **+** in front of a login to expand it and display the individual samples (injects). If you select a sample, a preview will be shown in the right part of the project management window. This preview contains a summary of the sample information and the raw data. A double-click or a click on the button **Data to WinGPC** will transfer the data to the raw data window for further processing.

Please note that always the complete login will be loaded with all sub windows described in the WinGPC Software user interface section. The selected sample from the list becomes active sample and is automatically displayed.

Several samples (injects), also located in different logins and/or projects, can be open simultaneously in WinGPC Software. The list with all available instruments (ready to record new data) and all open logins is displayed in the WinGPC Software status bar with a left mouse click into the **Sample** field. The status bar offers the fastest way to toggle between open logins and samples as well as the available instruments.

The **search sample** option is also available in the status bar login list as well as in the project management window. It opens a window where different search restrictions can be entered to perform an overall search (project independent) for a specific entry (see chapter "Sample Search" on page 209). Samples can also be loaded directly from the **search sample** dialog.

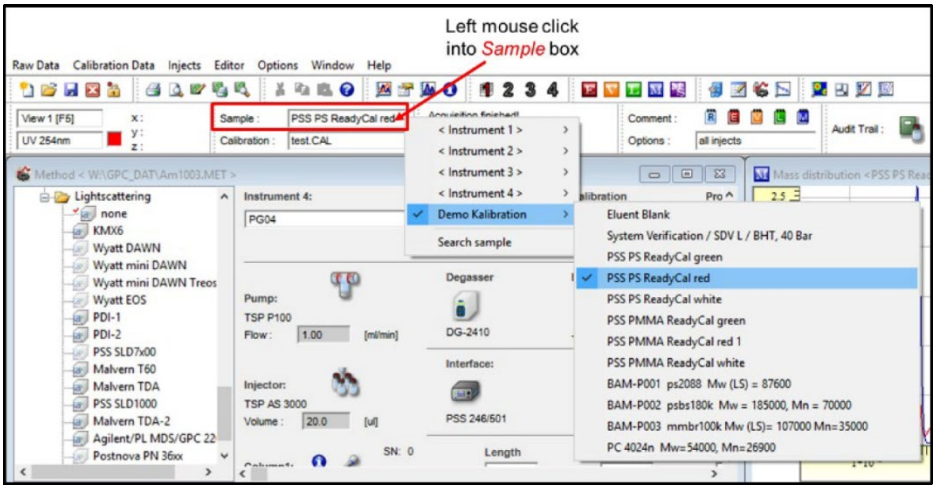


Figure 17 Status bar shows the login list and injection sequence for all opened runs

Examples for different project approaches:

Depending on the analytical needs and structures in the laboratories itself the approaches for managing the samples in databases are very different:

All samples can be measured into the same or different projects: for example, projects can be organized by year and month, year, instrument number, solvent, operator, account, product or product group and many other parameters.

It is not possible to merge projects or logins from different projects. For safety reasons it is only possible to add samples/logins to a project by measuring them into this project. However, it is possible to extract certain measurements from an existing project using the **Export data** button in the **Project management** window. The logins to be exported may be selected and saved with a new and unique project name. All necessary files will be created automatically.

Method Handling

WinGPC Software distinguishes between several method levels:

- A default method for every available instrument:

  WinGPC Software will automatically launch with the default method(s). The default method for an instrument will be updated every time a measurement is started and stopped or the menu item **Method > Save method** is used.

- Method files (*.met):

  Method files can be created, modified, or loaded in the method window prior to a measurement overwriting the default method. They can not be loaded to already existing logins/runs or edited while data acquisition is active.

- Method information saved with the sample (login):

  The method information of the method file or the default method is saved with every sample in the database. All analysis or display options that can, but must not, be saved with the method file may also be entered during data capture or after the run is completed. The instrument layout view (e.g., detector name, flow rate) can only be changed in the WinGPC Software supervisor mode.

In some cases, e.g when performing measurements with more than one detector, it is necessary to do a setup measurement. After the setup run is completed, the method fine-tuning can be done (e.g., the inter-detector delay can be determined). In that case, it is important to change the corresponding values not only in the sub windows of the sample but also to save the method separately with the updated values!

Agilent recommends using method files and load these files prior to every run!

Creating method files for fast and easy data analysis

Method files are created, modified and saved in the method window. WinGPC Software will automatically show **Instrument 1** with all its sub windows after launching it. The desired instrument can also be chosen by pressing its button in the WinGPC Software icon bar. If the **Method** window of the instrument is not automatically displayed, click the method button to bring it to the foreground.

The resource tree contains and manages all resources from all available SEC/GPC instruments. New resources to be defined in the resource tree. A right mouse-click on the resource entry opens the context menu with the options **Add** and **Edit**. The resources can be named in the corresponding resource dialog box and assigned with parameters like e.g., serial number for columns or refractive index for solvents.

NOTE

Molar mass sensitive detectors (viscometers and light scattering devices) can only be edited. They cannot be added by the user since these detectors require special data acquisition or detector dependent calculations. Please contact your Agilent representative if problems of identifying the viscometer/light scattering device occur.

NOTE

Changing the resource settings (e.g. the refractive index or the name) in the tree does not mean that the resources in existing or displayed methods are also automatically updated! The update has to be done manually by selecting the resource in the instrument layout view again.

After entering all resources for all instruments these components can be used to create a new method. A new method consists of three parts outlined below:

- Add items to the instrument layout view
- File definitions
- Data processing and presentation

Add Items to the Instrument Layout View

Use drag & drop of the resources to add them from the tree to the instrument layout view.

NOTE

For columns and detectors, it is important where you drop the resource. If they are just dropped somewhere in the layout view, an additional column or detector is added to the instrument layout view. To replace an item please drop the resource directly on the column symbol or detector symbol respectively or click directly with the left mouse button on the symbol and choose the resource from the appearing pop-up list.

NOTE

The number of detectors and/or columns can be reduced using the menu item **Definition > Number of columns** or **Number of detectors**.

- Molar mass sensitive detectors are removed with drag & drop of “none” from the resource tree directly on the detector symbol in the instrument layout view.
- Please add the detectors in the order they appear in the resource tree. Light scattering detectors with digital data acquisition (vendors: Agilent, PSS, BIC or Wyatt) must be the last detector in the instrument layout view.

For recording data the channel numbers of each detector must be entered in the instrument layout view as described in chapter “Instrument Layout View” on page 170.

File Definitions

The calibration curve and the project, in which the data should be saved, are also part of the method. These settings can be changed by a left mouse click on the symbol and by selecting a project/calibration curve or by selecting/creating a new project.

Data Processing and Presentation

Display and analysis options can also be saved with the WinGPC Software method.

- Settings (color, font, axis name,...) of all axes in all windows
- Axis numbers and color of each detector signal are saved. The information box of the **Raw data** window displays the signal color, the axis number (counted from left hand side of the window) and the detector name. Items can be modified by a mouse-click.

  Maximum four Y-axes are available. Agilent recommends displaying only detectors with the same unit and range on the same Y-axis.

  Example: A useful setup for a system with UV, RI, viscometer and MALLS detector would be: UV detector on Y-axis 1, RI detector on Y-axis 2, delta and inlet pressure viscometer both Y-axis 3, all signals from the MALLS detector Y-axis 4. In that case it is also recommended to switch off the trace of the inlet pressure signal and (if measured) the trace of the reference beam for the light scattering detector.

NOTE

A switched off signal in the raw data window (detector name is grayed out in the information box) does only mean that the signal is not displayed. The data are recorded even when the signal is not displayed!

WinGPC Software offers also a variety of data processing options, that can be pre-set in the method:

Raw data window:	Analysis of positive/negative peaks, baseline type, multi area data evaluation, several display/scale options
Elugram window:	Signal smoothing, HPLC mode, HPLC reference list, HPLC peaklist sort for, several display/scale options
Light scattering window:	Normalization coefficients, weight function, concentration detector, method, MW fit, plot, theta fit, used angles
Viscosity window:	Weight function, concentration detector, method, [n] fit
Mass distribution window:	Display/scale options, method for molar mass evaluation: calibration curve, light scattering, viscometry, triple detection.

NOTE

The available evaluation options depend on the WinGPC Software configuration. If the module is not available the option will not be offered, e.g., if there is no viscosity module and no viscometer in the method the mass distribution window will not offer viscometry and triple detection data processing.

After establishing the method it can be saved as *.met file using the **Method > Save method as...** dialog.

If a method file is used to record data, the method information itself is saved with the sample in the sample database.

NOTE

Please remember that a method file can not be loaded to a finished login. Some changes in the Method window of the finished run can for safety reasons only be done in the supervisor mode. However, the evaluation options, calibration curve and axis settings for the samples can be selected manually even after the measurement has been finished.

Improving Reproducibility using Flow Markers

Since GPC analyses are in general based on calibration the reproducibility of analytical conditions (flow stability, column condition, etc) is important for precise and accurate results. The major source of deviation arises when the. calibration (curve) was done in (slightly) different conditions as compared to a (later) measurement of the unknowns. WinGPC Software offers an (optional) internal standard correction (flow correction) to avoid the creation of a calibration curve before every single analysis and to ensure identical constant pump flow. The mathematical background is explained in detail in section "Calibration" on page 21.

Internal standards (flow markers) are low molecular weight compounds that are added to the solvent used to prepare the sample solutions. They should be easy to detect and they should not co-elute with other peaks.

Typical examples for flow markers are:

- in organic systems: BHT (2,6-di-tert.-butyl-4-methyl phenol), acetone or toluene
- in aqueous systems: ethylene glycol

The concentration of the flow marker should kept constant and adjusted to the detector response.

When the solvent with the internal standard is injected as a sample, a peak in the low molecular weight region will appear. The elution volume of this peak will be used for internal standard correction.

Three simple steps to better GPC results:

- Determining the reference value of the internal standard
- Entering the reference value for the internal standard position
- Assigning the reference value to unknown samples

Determining the Reference Value of the Internal Standard

Inject and measure the solvent with the internal standard as a sample. The elution volume of the flow marker can be easily determined by evaluating the peak, meaning by setting the baseline in the **Raw data** window. The volume can then be read in the **Elugram** window. It is shown in the **Add to calibration** dialog after selecting **find minimum/maximum** from the X-axis context menu.

NOTE

Make sure that the correct detector signal is used in multi detector systems (the active curve can be changed in the status bar).

Entering the Reference Value for the Internal Standard Position

The reference elution volume of the internal standard is saved in the calibration file. It can be entered in the **Calibration** window when the calibration file is loaded or open. The menu item **Calibration > Parameters...** opens the dialog **Calibration Settings** where the **Int. Standard** can be named and the elution volume can be entered (**at [ml]**).

Assigning the Reference Value to Unknown Samples

When a calibration curve with a reference value for the internal standard is loaded to a sample run, the reference internal standard position is automatically assigned to that run. A dark green triangle appears in the **Raw data** window at the position where the internal standard peak is expected. The position of the internal standard of the sample is correlated to the reference internal standard position (of the calibration) by an interactive or automatic internal standard *search* for every sample.

NOTE

If the calibration curve, that has been used before, had no reference internal standard or a different position for the internal standard, a warning message will appear. It reminds the user to update the internal standard correction for every sample.

The determination of the internal standard for peaks with limited number of data points can be improved by changing the interpolation for the curve from **linear** to **spline** (has to be set in the **Elugram** window, but will be used for internal standard calculations in the **Raw data** Window as well).

There are different ways to search for an internal standard:

Table 4 Internal standard search

Search ...	Window	Description
Interactive	**Raw data window** X-Axis context menu: **Int. standard search maximum/minimum**	Searches from the mouse cursor position where the function has been selected to the next relative maximum/minimum in the active curve and sets the internal standard to this position. When the minimum/maximum is found, an information window opens, where you can compare the value found with the reference value stored in the active calibration file. This needs to be done for every sample in the run.
Automatic, data acquisition still active	**Method window** menu item: **Definition > Automation properties**	Automatic internal standard search during runtime for all samples of the run. Any events or errors during the internal standard search will be documented in the logbook, which can be inspected by pressing the button of the WinGPC Session Logbook (), part of the Tool window Audit Trails.
automatic, finished runs	**Raw data window** menu item: **Options > Analysis > quick analysis**	Automatic internal standard search for finished runs. This action can be performed inject dependent: for the actual sample, for the actual samples and following, for a list of samples and for all samples of that run. Any events or errors during the internal standard search will be documented in the logbook, which can be inspected by pressing the button of the WinGPC Session Logbook (), part of the Tool window Audit Trails.

After setting the internal standard, the dark green triangle marker changes to light green indicating that the internal standards correction has been done for this sample.

The status of the internal standard correction for a sample can also be checked at the **Calibration > Information** menu.

NOTE

The flow corrected data are only shown in the elugram window. WinGPC Software keeps the original raw data as measured in the Raw data window and never changes them. Therefore, the positions of peak maxima in the Raw data Window and in the Elugram window need not to be identical.

WinGPC Software Reporting

The Standard Report in WinGPC Software

The WinGPC Software standard report prints a pre-formatted page of the currently active sub window which contains the most useful information as shown on screen (WYSIWYG). Printing in portrait orientation contains the graphics as formatted on screen, the method details and the result table of the mass distribution window. In landscape orientation only the graphics are printed.

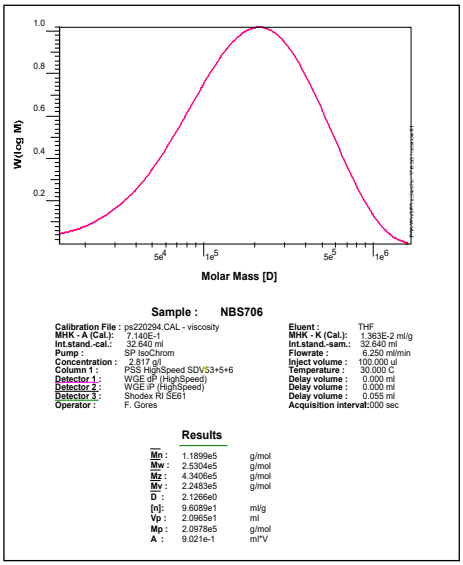


Figure 18 Standard WinGPC Software MWD Report

Issuing the print command from a menu (e.g., from the Raw data or File menu) brings up a dialog box, which allows the selection and configuration of all printers installed on the local computer. The number of WinGPC Software report copies can be set before printing. If the print command is issued from the printer icon on the icon bar, no printer selection is possible and printer output is directly sent to the Windows default printer (including its configuration). This twofold approach gives the user the flexibility and the ease-of-use of an one-click report.

The same functionality is present in all other print commands using the WinGPC Software menu or the icon (e.g., with the ReportDesigner option; see chapter "WinGPC Software ReportDesigner" on page 333 for details).

The printout contents depend on:

- the active sub window from which the command is issued.
- the current presentation of the chromatogram on screen.
- the paper orientation.

All WinGPC Software printouts now use the axis label settings (axis name, axis dimension) saved with the raw data. The axis labels on each chromatogram consists of the axis caption and the unit as specified in the axis properties settings. Please note that only the axis label for the first detector (which is shown in the chromatogram) is printed.

Example: The loaded run has UV (curve 1) and RI (curve 2) traces and the Y-axes in the raw data window are set to **UV Signal** with unit **[AU]** and **RI Signal** with unit **[V]**. If both curves are displayed, the printout only shows **UV Signal [AU]** for the Y-axis, because this is the first detector. If the first curve is not displayed, WinGPC Software automatically uses the second detector label **RI Signal [V]**.

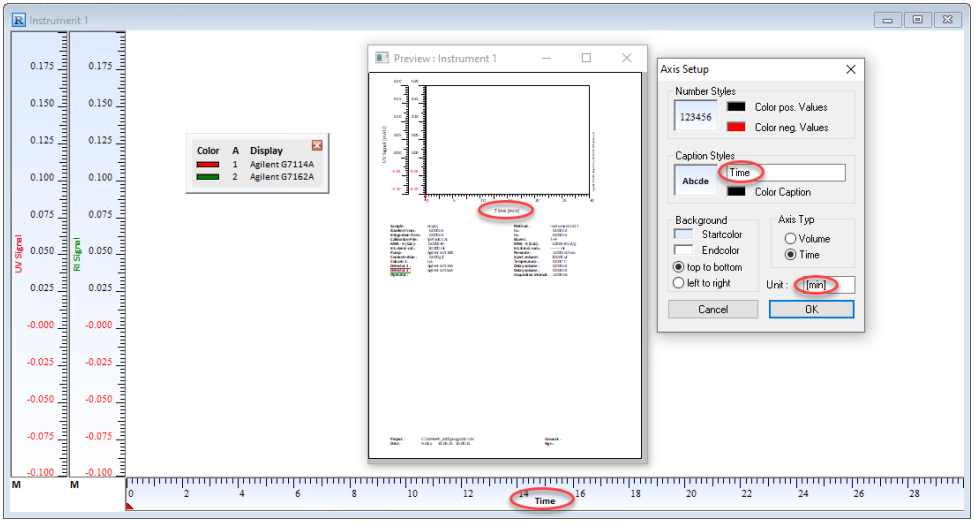


Figure 19 Definition and display of axis labels/dimensions

Previous WinGPC Software versions had default axis labels for printing independent from the user settings which were only shown on screen. Printing of font type, font size, font color is not supported; WinGPC Software will always use Arial font in black.

WinGPC Software automatically uses the sample name (or the name of the calibration file in the calibration window) as file name. This makes identification of files on the file systems much easier and saves time because the filenames need not to be edited before saving to disk. This feature is also available in the Windows print spooler, which identifies each print job by its sample (or file) name.

Custom Reporting

Powerful and flexible custom reports can be created using the WinGPC Software ReportDesigner which is described in more detail in chapter "WinGPC Software ReportDesigner" on page 333.

The WinGPC Software ReportDesigner is unique as results and parameters can be checked during printing. This allows for result dependent printing, e.g., automatic incorporation of a **QC passed** tag or a personal signature if user set conditions are met for this sample injection.

ChromEdit

If no standard reporting features are needed but a report or chromatogram annotation tool, then the WinGPC Software add-on **ChromEdit** is the best way to go. It allows to place text, graphics objects, arrows, etc. everywhere on a report and also allows to change the colors, font, font size, etc. of the report objects with a breeze. This tool is essential for creating poster graphics which contain WinGPC Software results. An example report annotated by ChromEdit is shown in Figure 20.

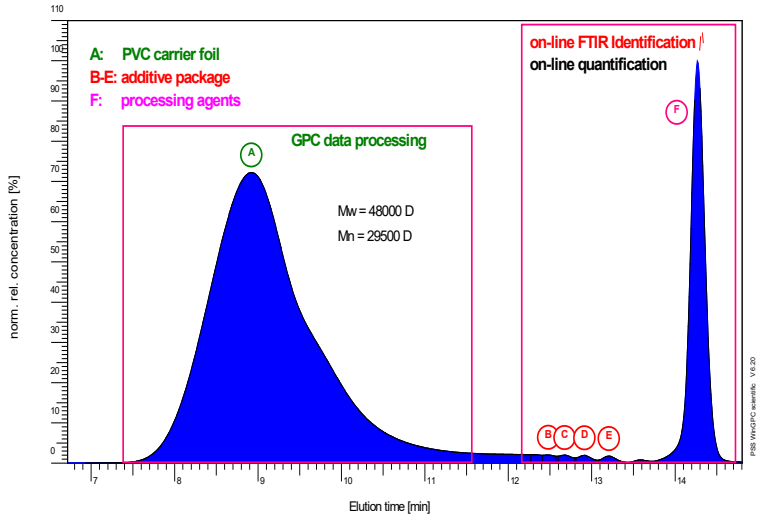


Figure 20 Annotation of WinGPC Software created graph by the ChromEdit add-on

Using the Windows Clipboard

WinGPC Software supports *copy and paste* of WinGPC Software information using the Windows clipboard. The windows clipboard is updated every time the Page Preview command is issued in WinGPC Software. After this command the clipboard contents can be pasted into every Windows target application using the **Edit > Paste Special** command in the target application. All WinGPC Software information is transferred in vector format which allows scaling without loss of information.

The information copied to the clipboard depends on the printer settings:

- Selecting landscape mode in the printer settings will only copy the graphics information of a WinGPC Software sub window.
- Selecting portrait mode in the printer settings will copy the WinGPC Software standard report to the clipboard, which contains graphics, method, and result information.

Every time WinGPC Software fills the clipboard, it creates a temporary EMF file which can also be used to transfer. Such files can be imported in many applications (e.g., word processing, graphics and presentation software) as an alternative method for information transfer.

NOTE

The page preview command is context sensitive and allows to preview the information in the currently active WinGPC Software sub window.

5 First Steps

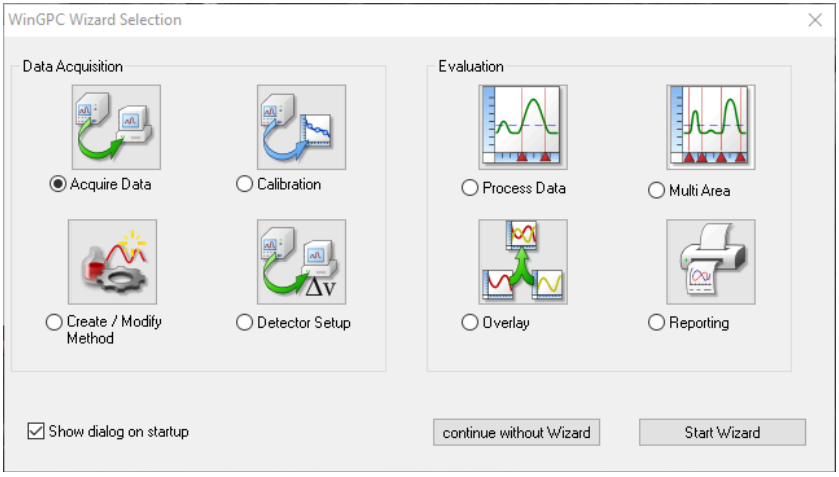
This short tutorial provides an easy access to the handling of the WinGPC Software. For didactical reasons not all options of the software can be explained. If in some cases the instructions should turn out to be insufficient, you will find further information in the detailed description of latter parts of this guide, in the enclosed WinGPC Software Quick Reference Guide available as PDF in the "Documentation" folder of the installation medium or the WinGPC menu item **Help > Step-by-Step**.

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter “WinGPC Compliance ” on page 507 of this manual.

WinGPC Software Wizards

The WinGPC Software wizards offer assistance for several functions within WinGPC Software. These wizards help advanced users functioning in terms of saving “clicks” and support beginners or casual users as a step-by-step guide for certain tasks.

On WinGPC Software startup the WinGPC Software wizard selection dialog will open automatically as the second dialog after the authentication dialog. You can deactivate it by unchecking **Show dialog on startup** on the lower left side of the dialog. You can open it again any time during a WinGPC Software session via the method window menu item **method > WinGPC Wizard**.

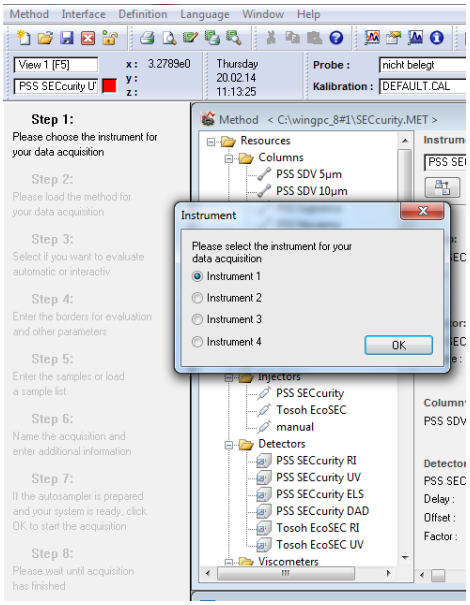


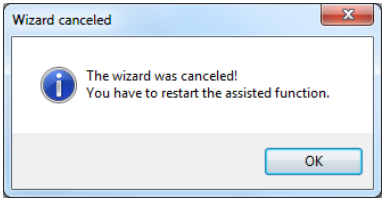
If you choose one of the wizards, the WinGPC Software UI (sub window arrangement) will automatically be optimized for the selected task. If you press **continue without Wizard**, WinGPC Software will open with the UI of your last session.

The steps needed are listed on the left side of the **Method** window. Already executed steps and the current step are displayed in black font, whereas pending steps are displayed in grey.

Example:

The wizard shown in the figure below (wizard **Acquire Data**) prompts for the instrument, which is to be used for data acquisition. Steps 2 to 8 are still greyed out. It is possible to stop the wizard at any stage (by pressing **Cancel**), as a confirmation you will get a corresponding message.





In addition to the wizards described in this section WinGPC Software offers assistance with other menu guides as well:

- *Detector Setup* (formerly **Lightscattering Setup**): determine all necessary detector and system parameters (see chapter "Detector Setup" on page 183). This may be executed with already existing data.
- *2D valve setup*: guided 2D valve setup and control (see chapter „Guided 2D Valve Setup" on page 187).
- *Guided broad/integral calibration* (see chapter "Calibration Curve Creation by Broad Standard Calibration" on page 314 and "Calibration Curve Creation by Integral Calibration" on page 318).

You can also save time using the following functions, which are used by the WinGPC Software wizards, but can be executed separately as well:

- *Automation*: automated data evaluation (needs to be defined and activated prior to sequence start), including optional new calibration (see chapter "Performing a GPC Measurement with Full Automation" on page 127).
- *Sample Wizard*: automated sequence generation (see chapter „Sample Wizard" on page 497) .

Acquire Data

You can use the **Acquire data** wizard to measure and optionally analyze your already prepared samples.

NOTE

Prior to executing this wizard some general WinGPC Software parameters need to be set (see "Requirements").

WinGPC Software Subwindow UI:

Method, Raw Data, Elugram, MWD.

Requirements:

- In ChromPilot: all modules (hardware) of the GPC system are connected and ready to be controlled.
- An existing WinGPC Software method, saved as *.MET file (e.g., using the wizard Create/modify method).
- Controlled instruments (ChromPilot) require an instrument method (*.SPM file), minimum requirement: open instrument manager in order to check all the settings, this action will generate a default method for the given instrument (wingpc_8#1\ CurrentMethodSettingsInstrumentn.spm) at the time the wizard is started.

Steps:

1 Choose the instrument for your data acquisition.

Message: **Select the instrument for your data acquisition**.

=> Select instrument.

2 Load the method for your data acquisition.

Message: Select a method and load it into WinGPC Software!

=> select *.MET file (e.g., as described in chapter "Create/modify method" on page 116)

3 Choose if you want to evaluate data automatically or interactively.

Message: **Select, either manual or automatic data evaluation**.

4 Enter the borders for data evaluation and other parameters.

=> **Automatic** (Step 3) opens the automation dialog (see chapter "Performing a GPC Measurement with Full Automation" on page 127). This step will be skipped if manual evaluation is selected.

5 Enter the samples or load a sample list.

Message: **Load an instrument method and then enter the samples you want to acquire or load a sample list**.

=> Prompts for an instrument method (*.spm), which can be saved in the **Instrument manager**. The current settings may be loaded as „CurrentMethodSettingsInstrumentn.spm" (with n = Instrument no.) in the folder C:\wingpc_8#1. In order to create/load a sequence, the **Sequence manager** will open.

6 Name the acquisition and enter additional information.

=> The information will be added automatically if ChromPilot is used; if the instrument is not controlled, additional information regarding login name and wait time after last inject need to be entered.

7 If the autosampler is prepared and your system is ready, click **OK** to start the acquisition.

Message **Prepare your autosampler and click OK to start data acquisition**.

8 Wait until acquisition has finished.

=> As soon as the sequence is finished, data acquisition will stop and the wizard will close.

Calibration

You can use the **Calibration** wizard to measure calibration standards and automatically create calibration curves.

NOTE

Prior to executing this wizard some general WinGPC Software parameters need to be set (see "Requirements").

WinGPC Software Subwindow UI:

Method, Raw Data, Elugram, MWD.

Requirements:

See wizard „Acquire Data" on page 106, the wizards differ only in step 3 and 4, the calibration is performed using the WinGPC Software automation (see "Performing a GPC Measurement with Full Automation" on page 127).

Steps:

1 Choose the instrument for your data acquisition.

Message: **Select the instrument for your data acquisition**.

2 Load the method for your data acquisition.

Message: **Select a method and load it to WinGPC Software**.

=> Select *.MET file (e.g., as described in chapter "Create/modify method" on page 116).

3 Do you want the calibration to be shown after the acquisition stops?

Message: **Select a file and folder for your calibration**, check box **Open calibration after data acquisition finished**.

=> Will prompt for the name, fit function and display options.

4 Enter the borders for the data evaluation and other parameters.

=> **Automatic** (Step 3) opens automation dialog (see chapter "Performing a GPC Measurement with Full Automation" on page 127).

5 Enter the samples or load a sample list.

Message: **Load an instrument method and then enter the samples you want to acquire or load a sample list-**.

=> Prompts for an instrument method (*.spm), which can be saved in the **Instrument manager**. The current settings may be loaded as „CurrentMethodSettingsInstrumentn.spm“ (with n = Instrument no.) in the folder **C:\wingpc_8#1**. To create/load a sequence, the **Sequence manager** will open.

6 Name the acquisition and enter additional information.

=> the information will be added automatically if ChromPilot is used; if the instrument is not controlled, additional information regarding login name and wait time after the last injection need to be entered.

7 If the autosampler is prepared and your system is ready, click **OK** to start the acquisition.

Message: **Prepare your autosampler and click OK to start data acquisition**.

8 Wait until acquisition has finished.

Process Data

You can use **Process Data** for a quick analysis of already existing data, including optional printouts.

WinGPC Software Subwindow UI:

Elugram, Raw Data and MWD.

Requirements:

A finished GPC run.

Steps:

1 Select an acquisition and load it into WinGPC Software.

Message: **Please select an acquisition and load it to WinGPC**.

=> Opens dialog **Project management**, choose a project and a login from a project container.

2 Select the samples you want to process.

=> Opens dialog **Inject options**: one or more samples may be selected.

3 Set the borders for evaluation and the options for the internal standard.

=> Opens dialog quick analysis.

4 Decide, if you want to print the results.

=> If **No**, step 5 will be skipped.

5 Select the samples you want to print.

=> Opens dialog print multiple injects (see chapter "Raw Data Window" on page 195, samples and output format can be selected).

6 Select if your changes should be stored.

=> If **No**", changes (new evaluation) will not be saved and closed.

Overlay

The **Overlay** wizard is used to overlay data of multiple samples. If you have already created an overlay and want to keep this information, you should save the current overlay prior to starting the wizard. The first step of the wizard deletes all data, which may already be loaded into the overlay.

WinGPC Software Subwindow UI:

Project management, Elugram and MWD.

Requirements:

Finished and analyzed GPC runs.

Steps:

1 An existing overlay will be deleted.

=> Deletes all entries of the current overlay.

2 Choose an acquisition and load it into the overlay.

Message: **Select a data acquisition and press the button ‚Data to overlay'**.

=> Opens window project management; select a sample and add it to the overlay using the button **Data to overlay**.

Message: **Select an additional data acquisition and load it into the overlay**.

=> Repeat until all required samples are added to the overlay.

Reporting

You can choose to print one or more samples using the **Print Multiple Injects** option either with a default printout or using a user defined print report layout (if you have a ReportDesigner license).

WinGPC Software Subwindow UI:

Raw Data, Elugram and MWD

Requirements:

Finished and analyzed GPC runs.

Steps:

1 Select an acquisition and load it into WinGPC Software.

Message: **Please select an acquisition and load it to WinGPC**.

=> Opens window project management.

2 Select the samples you want to print.

Message: **Select the samples you want to print**.

=> Opens dialog print multiple injects (see chapter “Raw Data Window” on page 195, samples and output format can be selected).

Multi Area

The **Multi Area** wizard assists to either create multi area settings or to perform a multi area analyis.

WinGPC Software Subwindow UI:

Elugram and MWD.

Requirements:

A finished GPC run and, as appropriate, existing Multi Area settings.

Steps:

1 Select if you want to calculate multi area settings or evaluate a sample

=> Opens a selection dialog (**Calculate multi area settings** or **evaluate multi area for sample**). The second option should only be selected if you have already created/loaded multi area settings before.

2 Select a run (and a suitable sample) and load it into WinGPC Software.

Message: **Please select an acquisition and load it to WinGPC**.

=> Opens the window **Project management** to select a sample.

In case of option **Calculate multi area settings**:

3 Set the evaluation borders and options for the internal standard.

=> Opens dialog **Quick analysis** in order to enter/acknowledge the settings.

4 Edit the calculated settings and store the settings to a file.

Message: **Calculate Multi area settings. Edit and save the calculated settings as required**.

=> Opens window multi area evaluation to check, edit and save the settings.

In case of option **Evaluate multi area for sample**:

3 Select the samples you want to process.

Message: **Select the samples you want to process**.

=> Opens dialog **Inject options**: one or more samples may be selected.

Set the evaluation borders and options for the internal standard.

=> opens dialog **Quick analysis** to enter/acknowledge the settings.

4 Select and load the multi area settings from a file or use the existing settings.

Message: **Load the multi area settings**.

=> Opens multi area evaluation window to load existing settings, all selected samples will be evaluated using these settings.

Detector Setup

You can perform first measurements using the Detector Setup wizard and automatically determine the inter detector delay for a multiple detector setup.

NOTE

Prior to executing this wizard some general WinGPC Software parameters need to be set (see "Requirements").

WinGPC Software Subwindow UI:

Method, Elugram, MWD.

Requirements:

- Within ChromPilot: all modules (hardware) of the GPC instrument are connected and ready to be controlled.
- A WinGPC Software method, saved as *.MET file (e.g., using the wizard **Create/modify method**).
- Controlled instruments (ChromPilot) require an instrument method (*.SPM file), minimum: open the instrument manager in order to check all settings, this action will generate a default method for the given instrument (wingpc_8#1\CurrentMethodSettingsInstrumentn.spm) when the wizard is started.

Steps:

1 Choose the instrument for your data acquisition.

Message: **Select the instrument for your data acquisition**.

=> Select instrument.

2 Load the method for your data acquisition.

Message: **Select a method and load it into WinGPC Software!**

=> Select *.MET file (e.g., as described in chapter “Create/modify method” on page 116)

3 Choose if you want to evaluate data automatically or interactively.

Message: **Select, either manual or automatic data evaluation**.

4 Enter the borders for data evaluation and other parameters.

=> **Automatic** (Step 3) opens the automation dialog (see chapter “Performing a GPC Measurement with Full Automation” on page 127). This step will be skipped if manual evaluation is selected.

5 Enter the samples or load a sample list.

Message: **Load an instrument method and then enter the samples you want to acquire or load a sample list**.

=> Prompts for an instrument method (*.spm), which can be saved in the instrument manager. The current settings may be loaded as „CurrentMethodSettingsInstrumentn.spm“ (with n = Instrument no.) in the folder **C:\wingpc_8#1**: In order to create/load a sequence, the sequence manager will open

6 Name the acquisition and enter additional information.

=> the information will be added automatically if ChromPilot is used; if the instrument is not controlled, additional information regarding login name and wait time after last inject need to be entered.

7 If the autosampler is prepared and your system is ready, click **OK** to start the acquisition.

Message **Prepare your autosampler and click OK to start data acquisition**.

8 Wait until acquisition has finished.

=> As soon as the sequence is finished, data acquisition will stop and the wizard will close.

9 Data acquisition will be loaded to process the detector setup.

=> Data will be reloaded and detector setup will be executed automatically, detector delay will be calculated relative to the detector with the first maximum within the baseline limits.

Create/modify method

After installing WinGPC Software or after reconfiguring your GPC system, you can use the **Create/modify** method wizard to create/modify a WinGPC Software method (*.MET).

A proper WinGPC Software method is a prerequisite for a trouble-free date acquisition. If you use a multi detector setup we recommend running the **Detector setup** wizard afterwards.

WinGPC Software Subwindow UI:

Method

Requirements:

- All components should be entered in the resource tree (e.g., check columns incl. serial numbers, concentration detectors and operators).
- Digital data acquisition: all available signals should be checked in **Signal configuration** (tab in the ChromPilot **Configuration manager**).

Steps:

1 Choose the instrument for your data acquisition.

Message: **Select the instrument for your data acquisition**.

=> Select instrument.

2 Load the method you want to modify or cancel if you wish to create a new method.

Message: **Select a method to modify and load it into WinGPC Software. If you cancel you will create a new method**.

=> It might be wise to just modify existing methods.

3 Choose a project to store your acquisition data.

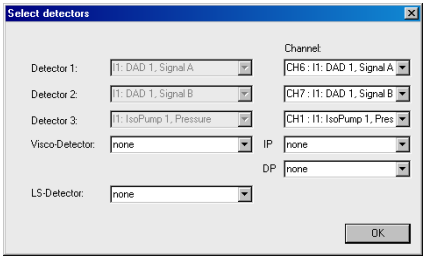
Message: **Select a project to store your data during future acquisitions**.

=> You can either select an existing project or create a new one by entering a new name.

4 Choose the detectors for your future acquisitions.

Message: **Select the detectors for your future acquisitions. Use 'Signal Configuration' in the configuration manager in order to assign the available signals**.

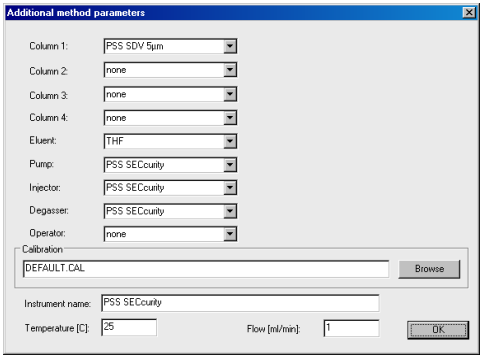
=> Description detectors 1 to 3: concentration detectors entered in the resource tree, please check the correct channel for the detectors; digital data acquisition: the description will be automatically assigned with the channel. This may be edited after method creation by clicking onto the detector symbol in the **Method** window



5 Enter additional parameters for this method.

Message: **Select further parameters for your method**.

=> Entries will be chosen using existing components of the resource tree



6 Save your new method.

Performing a Simple GPC Measurement

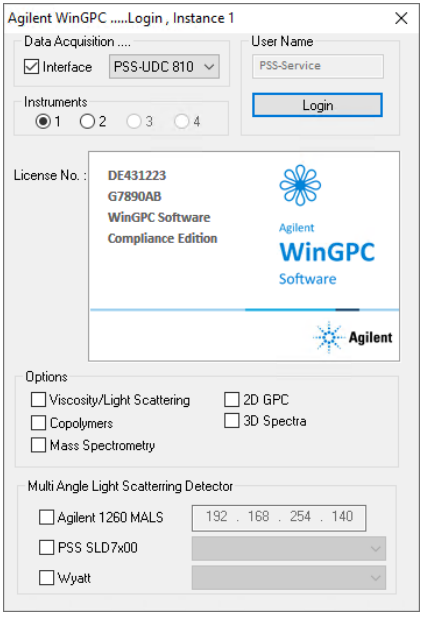


Figure 21 WinGPC Software Login Window

Starting the Software

Start the WinGPC Software by double-clicking on the WinGPC Software icon on the desktop or in the start menu.

An authentication dialog will open. Depending on the license, you can go on without entering a password (password mandatory for licenses with Compliance Edition). The following dialogs are optional, these are:

- WinGPC Software Wizard selection (details see chapter “WinGPC Software Wizards” on page 104)
- Login screen (details see chapter “Launching WinGPC Software and Start-up Options” on page 65)
- UDC Device Connection dialog
- ChromPilot Configuration Manager

After acknowleding above mentioned dialogs (or skipping them because **Show on startup** or **Show login screen** was not activated), WinGPC Software opens up and the 4 sub-windows for instrument 1 are displayed. If necessary, change to another instrument by clicking on the respective icon (Instrument No.) on the icon bar. Now switch to the window Method.

Creating a Method

In the Method window, you will recognize the instrument layout view of a GPC instrument consisting of solvent, pump, injector, columns, detectors, etc. Each item can be named according to your GPC instrument. This will ensure that the data acquisition conditions and all used equipment will be stored with the raw data and can be accessed and used at any time.

Define the number of columns and detectors used for this data acquisition using the **Definition** menu. The GPC instrument layout now should show the respective number of components.

Click on the respective icon to assign names to the individual items. A pop-up list of items appears, in which you can select the respective one. If the required item is not entered yet or no pop-up list appears, it can be added in the resource tree. Right-click on the resource module you would like to modify and select **Add** from the context menu. Enter the item name in the edit box (top line). Repeat this until all components of the instrument have been named.

To enter numerical data e.g., flow and detector parameters, click in the respective field with the left mouse button and enter the value using the keyboard. Now press **Enter** to confirm. In the field **Channel** you must enter the channel number of the Data Interface to which the detector is connected via the fiber optical cable.

You can choose up to 4 different, individually scalable y-axes for your detector signals in the raw data window. This offers always optimum settings for detectors with different sensitivity.

After start of the data acquisition the user entries in the window **Method** cannot be changed.

Starting Data Acquisition

After assigning names to all modules and entering the necessary numerical inputs, the data recording can now be started. Click on the **Sequence** Button (or **Record** for uncontrolled systems) button of the Toolwindow **Start/Stop**.

The data may be viewed and evaluated in real time in the **Instrument No.** window using the respective icon of the icon bar or the **Window** menu.

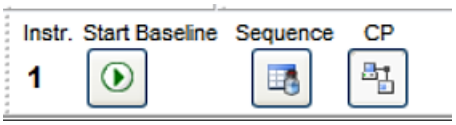


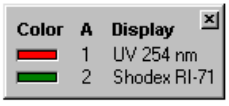
Figure 22 Toolwindow Start/Stop for controlled Systems

Figure 23 Toolwindow Start/Stop for uncontrolled Systems

NOTE

The Toolwindow **Start/Stop** will show three buttons **Start Baseline** (to start a baseline record), **Sequence** (to open the **Sequence manager**) and **CP** (to open ChromPilot the **Instrument manager**), if the selected instrument is controlled by WinGPC Software. In that case, the data acquisition will be started by pressing the **Start Sequence** button in the **Sequence manager** window.

Scale the X- and Y-axes according to demand (cf. chapter "Raw Data Window" on page 195). On the X-axis, arrow keys at the lower right corner will appear when the mouse cursor is close. The Y-axes can be scaled automatically, so that the highest data point is on the upper and the lowest data point is on the lower window edge (standardized representation). To do so click on the middle scale button, which appears when moving the mouse to the top left part of the window. This scale button can be toggled from standard scaling (**S**) to normalized scaling (**N**). Alternatively, you can scale the X- and Y-axes manually by right clicking on the axis with the mouse and selecting **Manual scale**.



The *information box* allows to switch on and off the different curves by clicking on the detector name. Furthermore, you can alter the curve's color upon clicking on the color panel. The information box will be closed by clicking on its **Close** button. It may be reopened using **Window > Information**.

NOTE

The Information Boxes of individual WinGPC Software windows are free scalable and may be closed by clicking the respective icon in the upper right corner. Use the menu item **Window > Information** to open the information box again (will always show the information box of the active sub window).

Entering Sample Information

While data acquisition is going on you could enter your sample names into the sample editor (menu item **Editor > Samples**). A dialog box appears, in which the name of the sample and sample parameters like concentration, molecular weight, etc. can be entered. Especially for the creation of the calibration curves it is recommended to enter the molecular weights of the samples, because the creation of the calibration table will be considerably simplified. Inputs for concentration, response factors (only for copolymer module), inject volume, dn/dc and A_2 value are not necessary for standard GPC measurements, but will be used by the modules for copolymer analysis, light scattering and viscosity evaluation (optional). The sample names and all other entered parameters will be saved at the end of the run together with the acquired data and therefore permit useful documentation of the raw data. The input in the sample editor can also be edited after completion of data recording.

Data capture will be stopped by clicking on the **Stop** button of the Toolwindow **Start/Stop**. If the selected instrument is controlled by WinGPC Software, the data acquisition will be stopped by pressing the **Stop Sequence** button in the **Sequence manager** window.

Data Processing

Evaluation of your data can be done during data acquisition or after completion of the run. To evaluate your samples first load your calibration curve (**Calibration Data > Load**). Select the sample for evaluation (menu item **Inject**). Now WinGPC Software is searching for the position of the inject of the selected sample, and places it on the left edge of the window. Furthermore, the elution volume will be counted from the injection point of the sample. The inject marker will be displayed by a blue triangle on the lower left edge of the window. A subsequent re-scaling of the window (e.g., by scaling or moving of the window image section with the scroll bar) has no influence on the allocation of the inject time to the raw data. Further injection marks may appear in the window, if another sample has been injected within the displayed time window.

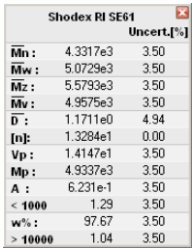
The location of the internal standard and the baseline will be set in the **Raw Data** window (see chapter "Raw Data Window" on page 195 for further information). To set the baseline click on the blue injection marker and drag the first baseline marker out of the blue injection marker to the position where the baseline shall end. This position will be marked with a red triangle. To define the starting position of the baseline, drag the second baseline marker from the injection marker in the same way. To modify the baseline settings simply click on the red triangles and drag them to the new position. Please note that in the **Elugram** window only the chromatogram within the baseline is displayed.

To define the position of the internal standard right-click with the mouse on the volume axis underneath the peak for the internal standard and select **Int. standard search max/min**.

NOTE

The internal standard will always be searched in the active curve. In the status bar on the top left side you can see which curve is presently active.

A pop-up box appears in which you can search for the internal standard (cf. internal standard search max./min. in chapter "X-Axis Context Menu" on page 200). WinGPC Software will look for the next peak maximum/ minimum starting from the mouse cursor position where the internal standard search command was invoked. A dialog box appears, which displays the position found for the internal standard as well as the reference value which is stored in the current calibration curve. After the internal standard is defined, the color of the respective triangle changes from dark to light green. Please change to the **Elugram** window after baseline and internal standard have been set.



Shodex RI SE61		Uncert.[%]
$\overline{Mn}$:	4.3317e3	3.50
$\overline{Mw}$:	5.0729e3	3.50
$\overline{Mz}$:	5.5793e3	3.50
$\overline{Mv}$:	4.9575e3	3.50
$\overline{D}$:	1.1711e0	4.94
[n]:	1.3284e1	0.00
Vp :	1.4147e1	3.50
Mp :	4.9337e3	3.50
A :	6.231e-1	3.50
< 1000	1.29	3.50
w% :	97.67	3.50
> 10000	1.04	3.50

In the **Elugram** window you can see the baseline corrected data (see chapter "Elugram Window" on page 224 for details). The volume axis may be converted to match the calibration conditions (i.e. if the data have been corrected using the internal standard, the volume might be different in the raw data and the elugram window). In this window the integration limits can be set for the subsequent calculation of molecular weight distributions and molecular weight averages. To set integration limits move the red triangles on the left and the right edge of the window to the position, in which the molar mass calculation shall be done. Alternatively, the integration limits can be defined by pressing the right mouse button underneath the peak on the volume axis. The function **Manual borders** allows to enter the integration limits either in elution volume or molecular weight. Now change to the window **Mass Distribution**.

The **Mass Distribution** window shows the calculated molar mass distribution and the different molecular weight averages in an information box (see chapter "Mass Distribution Window" on page 261).

Creation of a Conventional Calibration Curve

In order to create a conventional calibration curve, the elution volumes, molar masses and statistical weights have to be entered in the calibration table of the **Calibration** window. This can be done using the **Find minimum/maximum** dialog in the X-axis context menu of the **Elugram** window (cf. chapter "Elugram Window" on page 224 for details). The **Find minimum/maximum** menu item opens a dialog with the volume of the actual minimum/maximum and the preset molar masses which are taken from sample editor / sequence entries (if those were entered together with the sample information). Press **Add to calibration** in order to add the data point to the active calibration table and the calibration plot of the calibration window.

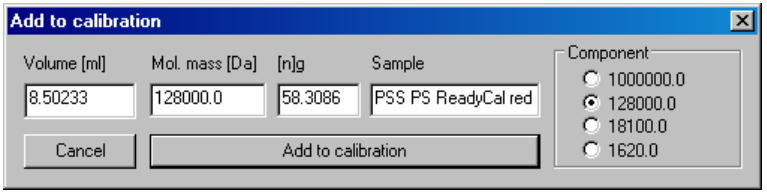


Figure 24 Peak search dialog to ease calibration table creation

NOTE

If the **Add to calibration** button is grayed out, no calibration curve is available to accept new data points. Use the **File > New** menu in the **Calibration** window to create an empty calibration table.

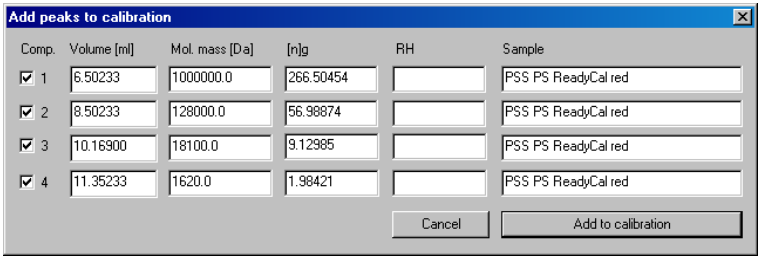
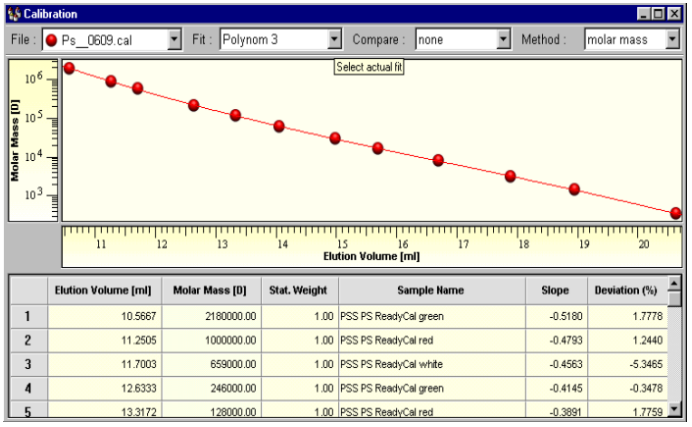


Figure 25 Add peaks to calibration dialog

NOTE

With WinGPC Software a new context menu item was added to the **Elugram** window. A right mouse click on the elution volume axis offers **Add peaks to calibration** in order to add multiple peaks to the calibration table with just one click (e.g., using a PSS ReadyCal). This can be used for standard as well as for universal calibration (peaks are detected and integrated automatically).

Immediately after input of a calibration point it will be displayed in the graphics section of the calibration window. The calibration molar masses can be selected from the list, if they have been entered previously in the sample editor. Make sure that you have selected **Molar Mass** in the *Method* selector to show the conventional plot of log M vs. elution volume. The individual calibration points will be displayed when entered to the calibration table.



	Elution Volume [ml]	Molar Mass [D]	Stat. Weight	Sample Name	Slope	Deviation (%)
1	10.5667	2180000.00	1.00	PSS PS ReadyCal green	-0.5180	1.7778
2	11.2505	1000000.00	1.00	PSS PS ReadyCal red	-0.4793	1.2440
3	11.7003	659000.00	1.00	PSS PS ReadyCal white	-0.4563	-5.3465
4	12.6333	246000.00	1.00	PSS PS ReadyCal green	-0.4145	-0.3478
5	13.3172	128000.00	1.00	PSS PS ReadyCal red	-0.3891	1.7759

Figure 26 The WinGPC Software Calibration window displays calibration data in graphical and tabular form

When all calibration points have been entered into the calibration table section, you can select a suitable calibration function from the *Fit* selector drop-down list. (WinGPC Software supports linear and polynomial regression models as well as three specific calibration functions (PSS Poly3, PSS Poly5 and PSS Poly7). The software performs a least squares regression calculation and shows the resulting curve fit in the graph section of the calibration window. Residuals and Slope of the calibration curve are calculated as well and (if activated with **Compare**) displayed in table and graphics section. Note that the statistical weight assigned to the data point defines whether the calibration point will be used for the creation of the conventional calibration curve. If the statistical weight is zero, the data point is shown, but not used in the regression calculation.

Unfortunately, no analytical function exists which describes the shape of a calibration curve for all cases. The user must rely on knowledge when selecting the

calibration curve for the calibration data. The quality of the adjustment can be determined by 3 criteria:

1 The deviation between calibration points and calibration curve should be low.

2 The distribution of residuals along the volume axis should be random.

3 The slope of the calibration curve should be physically meaningful, i.e. should not have inflection points.

In order to get a better understanding about the quality of the calibration curve, you can add the information for residuals or the slope of the calibration curve to the graph. This is done by selecting the appropriate entry in the **Compare** selector drop-down list.

It is highly recommended to try different regression models to find the best calibration curve.

Select **Calibration > Parameters** and add the values for internal standard, etc. Save your calibration curve using the options in the **File** menu.

For a detailed work list for performing calibrations with narrow standards consult the WinGPC Software Quick Reference Guide. Step-by-step instructions are also available in the WinGPC Software online help menu (**Help > Step-by-Step**].

Performing a GPC Measurement with Full Automation

WinGPC Software is able to perform data acquisition, data processing, calibration, recalibration and data reporting during a run automatically. The WinGPC Session Logbook will record all events for subsequent inspection. The logbook can be accessed from the Toolwindow **Audit Trails** by pressing () for the WinGPC Session Logbook, which is part of the *Status Bar*.

GPC Runs with AutoProcessing

This is the fastest and easiest way to evaluate routine GPC samples with an established method. Results can be directed either to the Windows default printer or screen. The selection of the default printer can be done in the printer settings (**Start > Settings > Printers**).

At first load an existing data acquisition method or create a new one. Then select with highlighted **Method** window > **Definition > Automation properties** from the menu and the Automation dialog **WinGPC Software Batch Processing Settings** will pop-up (see Figure 27) and can be edited.

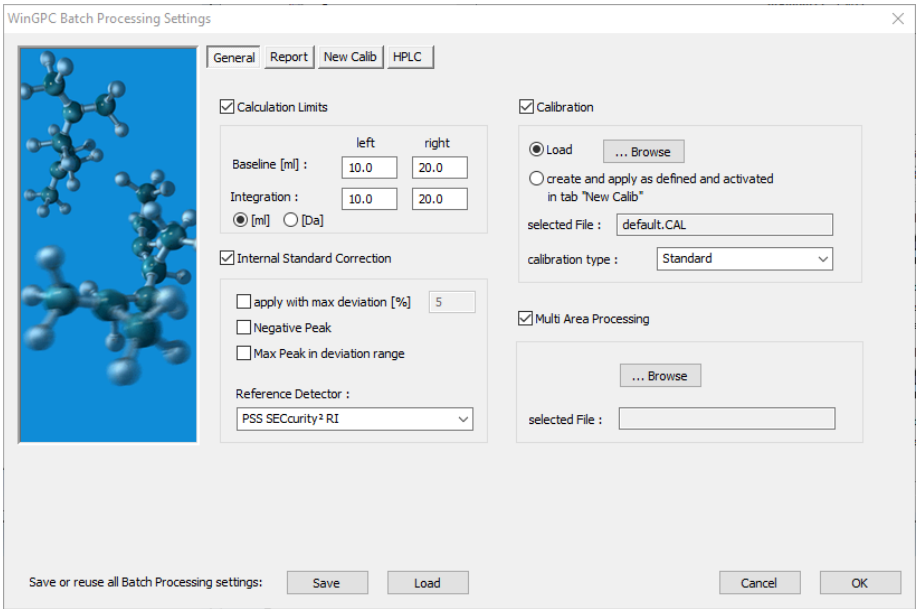


Figure 27 Setting WinGPC Software automation properties

The **General** tab (Figure 28, 1) shows an overview of automation parameters. Specific parameters can be selected and edited by setting the tick mark in the respective check box (e.g., **Calculation Limits**, **Calibration**, etc.). Items of unchecked boxes are grey out (deactivated) and can not be edited.

In the activated **Calculation Limits** section (Figure 28, 1), enter the start and end position of the baseline and integration range. The integration limits can be given either in the unit [mL] for a specific retention volume range or given in the unit [Da] for a specific mass range (requires an existing calibration curve). This can be done best after running the respective samples a few times in interactive mode.

In the activated **Calibration** section (Figure 28, 1), specify the calibration curve which shall be used for molar mass calculations. Therefore, use the **Load** option and **…Browse** button to select an existing calibration curve. The drop-down menu of the **Calibration type** allows to specify which of the four different calibration types (i.e. Standard, Light Scattering, Viscometry or Triple Detection) shall be executed. In combination with the **New Calib** tab the calibration section of the **General** tab (Figure 28, 1) enables an additional option (create and use **New Calib**) to automatically create a new calibration curve, which might be directly used to analyse the samples of an automation run. The necessary adjustments have to be performed in the **New Calib** tab (see section below for further information).

If an internal standards correction is required, activate it by a click into the check box for the **Internal Standard Correction**, select the signal which will be used to search for the internal standard, specify the search window and if the internal standards peak will be negative. The **Max. Deviation** parameter specifies the search window for internal standard and recalibration.

In the activated **Multi Area Processing** section (Figure 28, 1), specify details on the desired multi area evaluation of the chromatograms by selecting an already created *.MAS file, which shall be used as basis for the multi area report of the current samples. Details are described in chapter “Multi Area Data Analysis” on page 220.

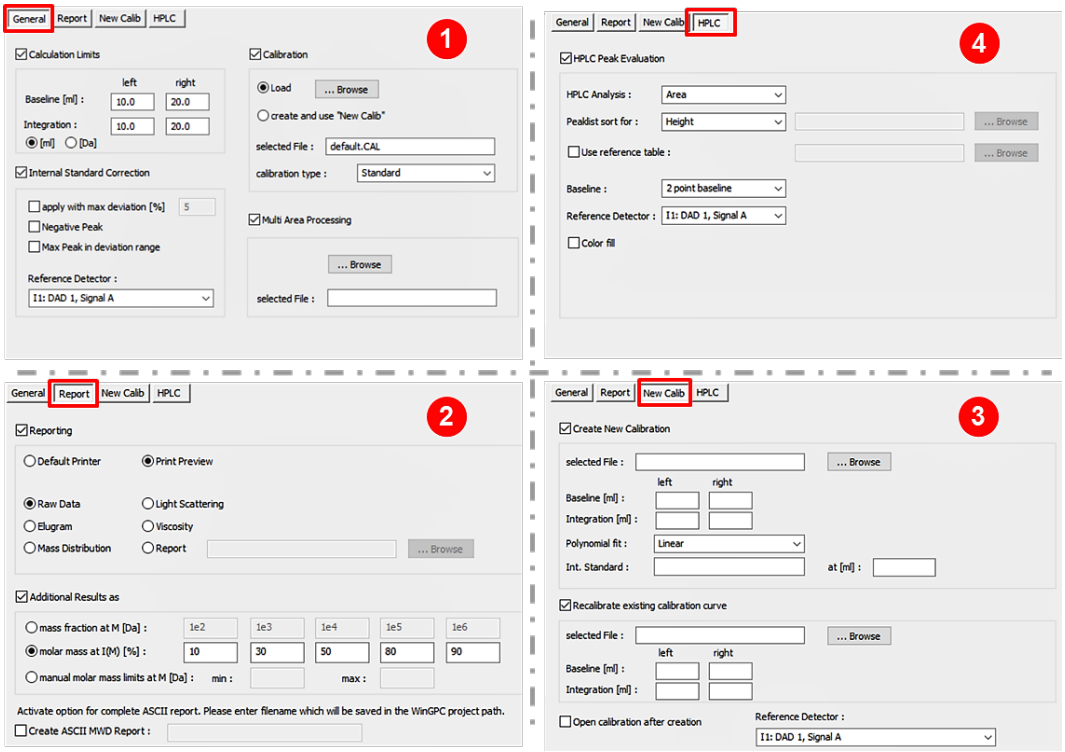


Figure 28 The four different tabs of the WinGPC Software automation dialog

The **Report** tab (Figure 28, 2) gives an overview of reporting options and necessary adjustments can be done by setting the tick mark in the respective check box to activate the desired section. In the activated **Reporting** section (Figure 28, 2), specify if the results should be sent either to the default Windows printer or directly to the screen (Print Preview mode), which will be replaced automatically as soon as next sample is evaluated. Furthermore, the reporting section allows to determine from which WinGPC Software window the data and results shall be printed. If desired, it is also possible to select a specific report layout. Therefore, use the **…Browse** button to select and load an existing report for later use.

In the **Additional Results as** section (Figure 28, 2), choose the type of subset information which is useful and specify the required inputs. The option **Create ASCII MWD Report** will automatically create an ASCII file for each sample with all method parameters, results and graphics (the output is identical to the **Save ASCII Report** function documented in the mass distribution window section (cf. chapter "Mass Distribution Window" on page 261). You can enter a descriptive template name for the text output; the software will add a sample identifier to this template (e.g., a template name "instrument1.txt" will generate the ASCII file

"001_instrument1.txt" for injection #1). The files will be located in the project file folder.

The **New Calib** tab (Figure 28, 3) includes different functions concerning the creation of a new calibration curve or the recalibration of an existing calibration curve. If a new calibration curve shall be created, activate the section by a click into the **Create New Calibration** check box, select the Reference Detector which will be used for the analysis of the calibration standards (bottom right), enter the start and end position of the baseline and integration range, select the Polynomial fit and if required, an internal standard coorection can be carried out.

If a recalibration of an existing calibration curve shall be performed, activate the section by a click into the respective check box, select the desired calibration file (use **…Browse** button) and enter the start and end position of the baseline and integration range. When the calibration/ recalibration is done and the option **Open calibration after creation** was activated the new calibration curve opens automatically (calibration window) and is ready for deeper inspection.

The **HPLC** tab (Figure 28, 4) allows to simultaneously quantify the chromatograms by HPLC parameters. If required, the **HPLC Peak Evaluation** section can be activated by setting the tick mark into the respective check box. Here you can specify if the HPLC analysis of the peaks shall be performed based on area or peak height. Furtermore, the option **Peaklist sort for** enables sorting by four different parameters (i.a. Height, Width, Area and Time) and in addition the **from File** function of the drop-down menu makes it possible to load an existing peak list, which will serve as a basis for the peak evaluation. In a similar way an existing reference table can be loaded as evaluation basis (both ways are interesting features for routine applications and standard quality control requirements). As well it is important once again to select the Reference Detector which shall be used for the evaluation of the peaks and to specify the type of baseline, if a two point baseline or a vertical drop shall be applied.

NOTE

All performed settings in the automation dialog (including all tabs) can be saved and loaded again for future applications. Therefore, use the button **Save** or **Load** of the option **Save or reuse all Batch Processing settings** located in the lower left corner of the automation dialog window.

When finished, leave the **Automation properties** dialog with **OK**. The autoprocessing will become active only after checking **Automation activated** from the **Definition** menu of the **Method** window. Now switch to the corresponding Instrument No. window and enter the sample information in the sample editor at **Editor > Samples**. In case of a controlled instrument, the Sequence Manager will pop-up instead of the Sample Editor. Use **Samples** as the sample type keyword for processing of unknowns and use **Calibration** for processing the calibration

standards, which is necessary for the full automation process involving the evaluation of the new calibration curve, if the option has been selected. When finished with sample editing, leave the editor with **OK**.

After entering all data acquisition and processing parameters start data capture by pressing the **Start** button in the Toolwindow **Start/Stop**. If the selected instrument is controlled by WinGPC Software, the data acquisition will be started by pressing the **Start Sequence** button in the **Sequence manager** window.

NOTE

Any events or errors will be documented in the logbook, which is accessible by pressing the respective button of the Toolwindow **Audit Trails**.

Automated Creation of Calibration Curves

A fully automated creation of calibration curves requires parameter input in the **Method**, **Instrument No**. and **Calibration** windows. Each calibration injection can consist of up to four components. Please note that molar masses have to be entered for components 1 through 4 in decreasing order (as samples elute from the column); i.e. component 1 has to be assigned to the highest molar mass.

Create an empty calibration curve in the **Calibration** Window by clicking on **File > New** and select the calibration fit function to be used in the Fit drop-down box. Save the (empty) calibration file with **File > Save As**.

NOTE

Please remember to enter the name and reference position of the internal standard in the **Calibration > Parameters...** dialog at **Int.Standard** and **at** respectively.

Switch to the **Method** window and load an existing data acquisition method or create a new one. Then select **Definition > Automation properties** from the menu. In the activated **Calibration** section of the **General** tab choose the option **create and use New Calib** and afterwards switch to the **New Calib** tab. Here, the check box **Create New Calibration** has to be activated by setting the tick mark and specify all necessary parameters. Use the **...Browse** button to load the just created (empty) calibration file which shall be used to add the calibration standards. If an internal standards correction is required, click on the tick mark for internal standard correction in the **General** tab, select the signal which will be used to search for the internal standard and specify the search window and if the internal standards peak will be negative.

In the **Calculation Limits** section of the **General** tab enter the start and end position of the baseline and integration range (entered limits should match with the limits specified in the respective section of the **New Calib** tab).

NOTE

The integration limits should only include the peaks of the calibration sample and not internal standard or solvent peaks. Otherwise, the molar mass assignment might go wrong.

When finished leave the **Automation properties** dialog with **OK**. The autoprocessing will become active only after checking **Automation activated** from the **Definition** menu.

Now switch to the corresponding **Instrument No.** window window and enter the sample information in the sample editor at **Editor > Samples**. In case of a controlled instrument by the WinGPC Software ChromPilot, the **Sequence manager** will pop-up instead of the Sample Editor. Use **Calibration** as the sample type keyword for processing polymer standards. For each calibration standard enter the molecular weights of all components in the calibration mixture. The number of components will be calculated by the software and need not be specified.

NOTE

If you work with PSS ReadyCal standard vials, there is no need to enter the information manually. In the *sample editor* or *sequence manager* click on import and select the kind of PSS ReadyCal vial you would like to append from the file open dialog.

When finished with sample editing, leave the editor with **OK**.

After entering all data acquisition and processing parameters start data capture by pressing the Start button in the Toolwindow **Start/Stop**. If the selected instrument is controlled by WinGPC Software, the data acquisition will be started by pressing the **Start Sequence** button in the **Sequence manager** window.

Background Information:

For each calibration standard or standard mixture the software will perform the baseline and integration limits processing. Optionally the elution volume will be corrected by the internal standard position in the current chromatogram. The software searches for the tallest peaks and assigns the molar masses entered for that sample in the data editor to these peaks in order of increasing elution volume. Peak position, molar mass and sample name will be entered automatically in the calibration table after the injection has been processed.

NOTE

Any events or errors will be documented in the logbook, which is accessible by pressing the respective button of the Toolwindow **Audit Trails**.

Automated Recalibration of Calibration Files

This process allows the automated recalibration of existing calibration files without the need to manually enter peak position, molar masses etc. The process will update the elution volume column automatically for the specified calibration sample (identical sample names are required).

A fully automated recalibration (peak position update) of an existing calibration curve requires parameter input in the **Method** and the **Instrument No.** windows. Each recalibration injection can consist of up to four components. Please note that molar masses have to be entered for components 1 through 4 in decreasing order (as samples elute from the column); i.e. component 1 has to be assigned to the highest molar mass.

In the **Method** window load an existing data acquisition method or create a new one. Then select **Definition > Automation properties** from the menu.

In the activated **Calibration** section of the **General** tab choose the option **create and use New Calib** and afterwards switch to the **New Calib** tab. Here, the check box **Recalibrate existing calibration curve** has to be activated by setting the tick mark and specify all necessary parameters. Use the **…Browse** button to load the respective calibration file which shall be used for the recalibration process. If an internal standards correction is required, click on the tick mark for internal standard correction in the **General** tab, select the signal which will be used to search for the internal standard and specify the search window and if the internal standards peak will be negative.

In the **Calculation Limits** section of the **General** tab enter the start and end position of the baseline and integration range (entered limits should match with the limits specified in the respective section of the **New Calib** tab).

NOTE

The integration limits should only include the peaks of the calibration sample and not internal standard or solvent peaks. Otherwise, the molar mass assignment might go wrong.

When finished leave the **Automation properties** dialog with **OK**. The autoprocessing will become active only after checking **Automation activated** from the **Definition** menu.

Now switch to the corresponding Instrument No. window and enter the sample information in the sample editor at **Editor > Samples**. In case of a controlled instrument by the WinGPC Software ChromPilot, the **Sequence manager** will pop-up instead of the Sample Editor. Select **Recalibration Replace** or **Recalibration Average** as the sample type keyword for processing the polymer standards. For each recalibration standard enter the molecular weights of all components in the calibration mixture. The number of components will be calculated by the software and need not be specified.

Recalibration replace:	Already existing data points will be overwritten (replaced).
Recalibration average:	Calculates the arthmetic average of old and new data point. **Recalibration average** can be executed more than once. The average calculation will then take into account, that the last value was already an average. The number of averages will be shown in the calibration window data editor as **Average**. If the column is not displayed, it can be activated using **Table > Columns…**.

NOTE

If you work with PSS ReadyCal standard vials, there is no need to enter the information manually. In the sample editor click on import and select the kind of PSS ReadyCal vial you would like to append from the file open dialog.

When finished with sample editing, leave the editor with **OK**.

After entering all data acquisition and processing parameters start data capture by clicking on the **Start** button in the Toolwindow **Start/Stop**. If the selected instrument is controlled by WinGPC Software, the data acquisition will be started by pressing the **Start Sequence** button in the **Sequence manager** window. In the calibration file that was specified for recalibration the elution volume column will be updated directly after each injection has been processed. However, this will only occur, if the peak position is found within the specified search window and the sample name of the recalibration standard is identical to that one in the calibration file which shall be recalibrated.

After the recalibration run has been finished, please inspect the modified calibration file. If there are discrepancies, please inspect the logbook, which will give details on data processing errors.

The logbook is accessible by pressing the respective button of the Toolwindow **Audit Trails**.

Performing a GPC Light Scattering Measurement

Starting the software

Start the WinGPC Software by double-clicking on the WinGPC Software icon on the desktop or in the start menu.

An authentication dialog will open. Activate the tick mark for **Show Login screen**. In order to do this, you need to set the cursor into the password field before. Depending on the license, you can go on without entering a password (password mandatory for licenses with Compliance Edition).

The next dialog is optional: WinGPC Software Wizard selection (details see chapter "WinGPC Software Wizards" on page 104).

In the login screen select the **Light scattering** option and make sure that the correct interface types (Data Interface, PSS or Wyatt LS instruments) are selected for data capture (for details on the login screen see chapter "Launching WinGPC Software and Start-up Options" on page 65).

The following dialogs are optional again, these are:

- UDC Device Connection dialog
- ChromPilot Configuration Manager

After acknowleding above mentioned dialogs (or skipping them because **Show on startup** or **Show login screen** was not activated), WinGPC Software opens and the 4 sub windows for instrument 1 are displayed. If necessary, change to another instrument by clicking on the respective icon (Instrument No.) on the icon bar. Now switch to the window **Method**.

Creating a Light Scattering Method

The **Method** window depicts a flow diagram of a GPC instrument consisting of solvent, pump, injector, columns, detectors etc. Each item can be named according to the GPC instrument. This will ensure that the data acquisition conditions and all used equipment will be stored with the raw data and can be accessed and used at any time later.

Define the number of columns and *concentration detectors* using the **Definition** menu. The GPC instrument layout now should feature the respective number of components.

To assign names to the individual items click on the respective icon. A pop-up list of items appears, in which you can click onto the respective one. If a respective item is not yet entered in the pop-up list, it can be added in the resource tree. Right-click on the resource entry to be modified and select **Add** from the context menu. Enter the item name in the edit box (top line) and all necessary parameters like e.g., refractive index of the solvent. Repeat naming and assigning until all components of the instrument have been named.

Then add the light scattering device (already predefined in the resource tree) as the *last detector* in the method by dragging it from the resource tree to the instrument layout view. A right click on the light scattering instrument in the resource tree opens the properties window to edit/review its properties.

NOTE

Molar mass sensitive detectors like light scattering detectors or viscometers can be removed with drag & drop of **none** from the resource tree directly on the detector symbol in the instrument layout view.

To enter numerical data e.g., flow and detector parameters, click in the respective field with the left mouse button and enter the value through the keyboard. Now press **Enter** to confirm.

In the field **Channel** you must select the correct channel, to which the detector is connected. A context menu is available after a right mouse click in the *CH No.*: field:

- For multi angle light scattering devices the channel is automatically set
- For other detectors the correct channel number has to be assigned:

  If the detector is connected to a PSS UDC810 or WinCHROM Interface the channel number is the number of the optical input at the interface.

  If the detector is connected to a light scattering detector select the correct channel from the context menu ("AUX1"/"AUX2" for Wyatt detectors, “AUX1-4” for Agilent 1260 MALS,, or "BI-MwA analog *n*" in the case of the PSS SLD 7x00 light scattering detector).

Starting Data Acquisition

After assigning names to all modules and entering the necessary numerical inputs, the data recording can now be started. Click on the **Sequence** Button (or **Record** for uncontrolled systems) button of the Toolwindow **Start/Stop**.

The data may be viewed and evaluated in real time in the **Instrument No.** window using the respective icon of the icon bar or the **Window** menu.

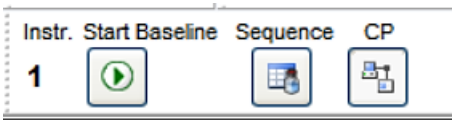


Figure 29 Toolwindow Start/Stop for controlled Systems

Figure 30 Toolwindow Start/Stop for uncontrolled Systems

NOTE

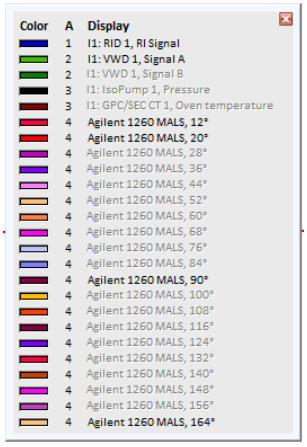
The Toolwindow **Start/Stop** will show three buttons **Start Baseline** (to start a baseline record), **Sequence** (to open the **Sequence manager**) and **CP** (to open ChromPilot the **Instrument manager**), if the selected instrument is controlled by WinGPC Software. In that case, the data acquisition will be started by pressing the **Start Sequence** button in the **Sequence manager** window.

Scale the X- and Y-axis according to the requirements (cf. Raw Data Window in chapter "Raw Data Window" on page 195). On the X-axis, arrow keys at the lower right corner will appear when the mouse cursor is close. The Y-axes can be scaled automatically, so that the highest data point is on the upper and the lowest data point is on the lower window edge (standardized representation). To do so click on the middle scale button, which appears when moving the mouse to the top left part of the window. This scale button can be toggled from standard scaling (**S**) to normalized scaling (**N**). Alternatively, you can scale the x- and y-axes manually by right-clicking on the axis with the mouse and selecting **Manual scale**.

NOTE

The Information Boxes of individual WinGPC Software windows are free scalable and may be closed by clicking the respective icon in the upper right corner. Use the menu item **Window > Information** to open the information box again (will always show the information box of the active sub window).

The *information box* of the **Raw data** window displays the signal color, the axis number (counted from left hand side of the window) and the detector name. Items can be modified by a mouse-click. Curves can be switched on or off the by clicking on the detector name. Maximum four Y-axes are available. Agilent recommends to display only detectors with the same unit and range on the same Y-axis. The information box will be closed by clicking on its close button. It may be reopened using **Window > Information**.



Example:

A useful setup for a system with UV, RI, viscometer and MALLS detector would be: UV detector on Y-axis 1, RI detector on Y-axis 2, delta and inlet pressure viscometer both Y-axis 3, all signals from the MALLS detector Y-axis 4. In that case it is also recommended to switch off the trace of the inlet pressure signal and (if measured) the trace of the reference beam for the light scattering detector.

Entering Sample Information

While data acquisition is going on you could enter your sample names into the sample editor (menu item **Editor > Samples**). A dialog box appears, in which the name of the sample and sample parameters like concentration, molecular weight etc. can be entered. Enter correct values for concentration, inject volume, dn/dc and (only if available) A_2 since they are required for light scattering data processing. The sample names and all other entered parameters will be saved together with the acquired data. The input in the sample editor can also be edited after completion of data recording.

NOTE

For measurements with molar mass sensitive detectors Agilent recommends to weight in the samples as precisely as possible. WinGPC Software offers the possibility to measure the concentration, when a calibrated concentration detector is used, but determining the mass recovery is always useful.

Data capture will be stopped by clicking on the **Stop** button of the Toolwindow **Start/Stop**. If the selected instrument is controlled by WinGPC Software, the data acquisition will be stopped by pressing the **Stop Sequence** button in the **Sequence manager** window.

Setting up light scattering parameters

Besides the sample information several parameters are necessary for the precise analysis of GPC/SEC-light scattering data:

- the inter detector delay. WinGPC Software allows the input of a detector delay for every detector.
- the instrument constant of the light scattering detector.
- if all concentration methods should be available: the factor for the concentration detector (compare).
- in case of multi angle light scattering: the normalization coefficients for the different angles.

If known, these parameters can be entered prior to the run or after completion.

If the parameters are not known WinGPC Software allows to determine them by using the **Guided Detector Setup**. The guided detector setup should only be performed with a well characterized polymer standard with

- narrow molecular weight distribution: polydispersity D< 1.1
- known concentration: about 1-2 mg/ml depending on injection volume and number of separation columns
- known dn/dc
- known M_w

Since an isotropic scatterer is needed for the determination of the normalization coefficients Agilent recommends using a polymer standard with an M_w in the range of 50 000 to 100 000 g/mol.

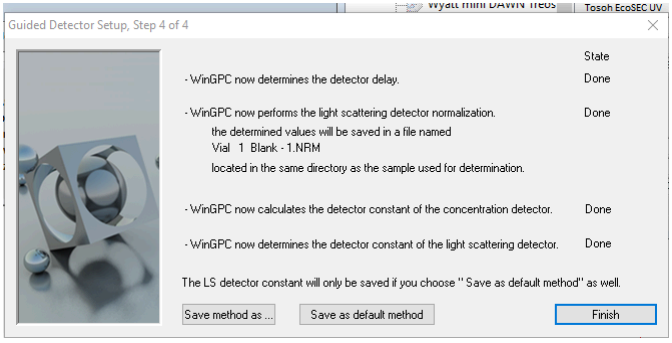


Figure 31 Guided detector setup dialog with progress information

To use the guided detector setup the well characterized polymer standard should be weight in precisely and measured according to the routine described above. After stopping the run, the data can be loaded from the Raw data window with **Raw data > Load** and selecting the sample from the login. The location of the internal standard (optional) and the baseline will be set in the Raw Data window (see chapter “Raw Data Window” on page 195 for further information). To set the baseline click on the blue injection marker and drag the first baseline marker out of the blue injection marker to the position where the baseline shall end. This position will be marked with a red triangle. To define the starting position of the baseline drag the second baseline marker from the injection marker in the same way. To modify the baseline settings simply click on the red triangles and drag them to the new position.

NOTE

For the light scattering setup it is necessary to set the baseline directly around the polymer peak excluding all system or solvent peaks.

After setting the baseline the setup can be started in the **Method** window with **Method > Guided Detector Setup**. During the setup all parameters that are necessary for the setup have to be confirmed by the user. Please follow the 4 setup steps until the setup is finished.

Save method as... opens a dialog that allows to save the method with all determined parameters as a *.met file. Please use this fine tuned method for the analysis of the samples lines. This requires loading the method file in the **Method** window before starting data capture.

Save as default method should only be used if the setup was run on the same PC that is used for data acquisition. It updates the default method containing the light scattering device (see chapter "Detector Setup" on page 183 for details).

NOTE

Method files can not be loaded to finished sample runs. However, the determined values can be entered manually to already existing logins.

Data Processing

Evaluation of your data can be done during data acquisition or after completion of the data acquisition. First the settings in the **Raw Data** window have to be done, therefore this window has to be highlighted. Enter the sample information like sample name, concentration, injection volume and refractive index increment dn/dc (**Raw data** window: **Editor > Samples**).

The location of the internal standard (optional) and the baseline will be set there, too (see chapter "Raw Data Window" on page 195 for further information). To set the baseline click on the blue injection marker and drag the first baseline marker out of the blue injection marker to the position where the baseline shall end. This position will be marked with a red triangle. To define the starting position of the baseline, drag the second baseline marker from the injection marker in the same way. To modify the baseline settings simply click on the red triangles and drag them to the new position.

To define the position of the internal standard right-click with the mouse on the volume axis underneath the peak for the internal standard and select **Int. standard search max/min**.

NOTE

The internal standard will always be searched in the active curve. In the status bar on the top left side you can see which curve is presently active.

A pop-up box appears in which you can search for the internal standard (cf. internal standard search max./min. in chapter "X-Axis Context Menu" on page 200). WinGPC Software will look for the next peak maximum/minimum starting from the mouse cursor position where the internal standard search command was invoked. A dialog box appears, which displays the position found for the internal standard as well as the reference value which is stored in the current calibration curve. After the internal standard is defined, the color of the respective triangle changes from dark to light green.

Please change to the **Elugram** window after baseline and internal standard have been set.

In the **Elugram** window you can see the baseline corrected data (see chapter "Elugram Window" on page 224 for details). The volume axis may be converted to match the calibration conditions (i.e. if the data have been corrected using the internal standard, the volume might be different in the raw data and the elugram window). In this window the integration limits can be set for the subsequent calculation of molecular weight distributions and molecular weight averages. To set integration limits move the red triangles on the left and the right edge of the window to the position, in which the molar mass calculation shall be done. Alternatively, the integration limits can be defined by pressing the right mouse button underneath the peak on the volume axis. The function **Manual borders** allows to enter the integration limits either in elution volume or molecular weight. Now change to the window **Mass Distribution**.

The **Mass Distribution** window shows the calculated molar mass distribution and the different molecular weight averages in an information box (see chapter "Mass Distribution Window" on page 261 for details). WinGPC Software is able to calculate the molar mass distribution using calibration curves established with narrow molecular weight standards as well as using the results from light scattering.

The setting of the X-Axes of the Mass Distribution window defines what evaluation method is used:

- **Calib.Standard** displays the results from the calibration curve that can be loaded in the **Raw Data** window with **Calibration Data > Load...**.
- **Calib. Lightscattering** displays the results from on-line light scattering

A right mouse click on the X-Axis allows to switch between the different evaluation methods.

The **Calibration** field in the status bar shows which method is used:

- if the name of the calibration curve is displayed, **Calib.Standard** is active
- if Lightscattering is displayed, **Calib. Lightscattering** is active

The calculation of the different molecular weight averages is explained in the theoretical part (see chapter "Molecular Weight Averages and Molecular Weight Distributions in GPC/SEC" on page 18). Moreover, the intrinsic viscosity [h] and viscosity average molar mass M_v will be calculated, based on the Mark-Houwink constants of the calibration curve. M_p and V_p are the molecular weight and the elution volume at the peak maximum. **A** is the peak area below the respective curve within the integration limits.

Fractions within the mass distribution can be defined using the red markers on the lower margins of the window, e.g., to calculate what percentage of the distribution that is below or above the set limits. These data appear in the lower three lines of the information window. Further options of this window are explained in chapter "Mass Distribution Window" on page 261.

A detailed light scattering data analysis is shown in the Light Scattering window. This window allows to select the detector used for determining the slice concentration, the concentration detection method (compare page) and the fit function for fitting the measured molecular weight. For multi angle light scattering the yellow arrow button on the top right corner allows to toggle between the view with or without the angular dependence plot. If the angular dependence plot is shown the plot type and the plot fit function can be selected using the selector bars. All settings here can also be saved with the method.

NOTE

A printout of the mass distribution window prints the content of this window with molar masses from light scattering, if selected. The printout of the light scattering window shows the light scattering results and parameters, as well as the molar mass averages. With the optional WinGPC Software ReportDesigner it is possible to combine these two printouts as well as printing light scattering and conventional results on one page.

Performing a GPC Viscometry Measurement

Starting the Software

Start the WinGPC Software by double-clicking on the WinGPC Software icon on the desktop or in the start menu.

An authentication dialog will open. Activate the tick mark for **Show Login screen**. In order to do this, you need to set the cursor into the password field before. Depending on the license, you can go on without entering a password (password mandatory for licenses with Compliance Edition).

The next dialog is optional: WinGPC Software Wizard selection (details see chapter "WinGPC Software Wizards" on page 104).

In the login screen select the **Viscosity** option and make sure that the correct interface type (UDC, COMx or NetConnect) is selected for data capture (for details on the login screen see chapter "Launching WinGPC Software and Start-up Options" on page 65).

The following dialogs are optional again, these are:

- UDC Device Connection dialog
- ChromPilot Configuration Manager

After acknowleding above mentioned dialogs (or skipping them because **Show on startup** or **Show login screen** was not activated), WinGPC Software opens and the 4 sub windows for instrument 1 are displayed. If necessary, change to another instrument by clicking on the respective icon (Instrument No.) on the icon bar. Now switch to the window **Method**.

Creating a Viscometry Method

The **Method** window depicts a flow diagram of a GPC instrument consisting of solvent, pump, injector, columns, detectors etc. Each item can be named according to the GPC instrument. This will ensure that the data acquisition conditions and all used equipment will be stored with the raw data and can be accessed and used at any time later.

Define the number of columns and concentration detectors using the **Definition** menu. The GPC instrument layout now should feature the respective number of components. Click on the respective icon to assign names to the individual items. A pop-up list of items appears, in which you can click onto the respective one. If an item is not yet entered in the pop-up list, it can be added in the resource tree. Right click on the resource module to be modified and select **Add** from the context menu. Enter the item name in the edit box (top line). Repeat this until all components of the instrument have been named.

Add the viscometer device (already predefined in the resource tree) behind the concentration detectors by dragging it from the resource tree to the instrument layout view. Depending on the type of viscometer one or two detectors will be added to the layout view: detectors that measure the inlet pressure as well as the differential pressure are automatically inserted with their two detector signals.

NOTE

Molar mass sensitive detectors like light scattering detectors or viscometers can be removed with drag & drop of **none** from the resource tree directly on the detector symbol in the instrument layout view. The viscometer can be edited just like the other items in the tree. Factor, offset and channel number (compare page) can be saved with the detector.

To enter numerical data e.g., flow and detector parameters, click in the respective field with the left mouse button and enter the value through the keyboard. Now press **Enter** to confirm.

In the field **Channel** you must select the correct channel number to which the detector is connected. A context menu is available after a right mouse click in the *CH No.:* field.

If the detector is connected to a PSS UDC810 or WinCHROM Interface via the fiber optical cable then the channel number is the number of the optical input at the interface, in the case of WGE viscometer detector the channel numbers are documented in the viscometer manual or see section “Window Description” on page 165.

After start of the data acquisition, the user entries in the window **Method** cannot be changed.

Starting Data Acquisition

After assigning names to all modules and entering the necessary numerical inputs, the data recording can now be started. Click on the **Sequence** Button (or **Record** for uncontrolled systems) button of the Toolwindow **Start/Stop**.

The data may be viewed and evaluated in real time in the **Instrument No.** window using the respective icon of the icon bar or the **Window** menu.

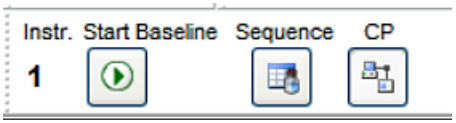


Figure 32 Toolwindow Start/Stop for controlled Systems

Figure 33 Toolwindow Start/Stop for uncontrolled Systems

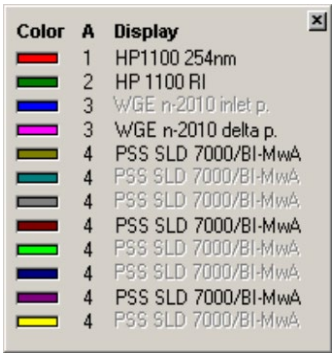
NOTE

The Toolwindow **Start/Stop** will show three buttons **Start Baseline** (to start a baseline record), **Sequence** (to open the **Sequence manager**) and **CP** (to open ChromPilot the **Instrument manager**), if the selected instrument is controlled by WinGPC Software. In that case, the data acquisition will be started by pressing the **Start Sequence** button in the **Sequence manager** window.

Scale the X- and Y-axis according to the requirements (cf. **Raw Data** window in chapter "Raw Data Window" on page 195). On the X-axis, arrow keys at the lower right corner will appear when the mouse cursor is close. The Y-axes can be scaled automatically, so that the highest data point is on the upper and the lowest data point is on the lower window edge (standardized representation). To do so, click on the middle scale button, which appears when moving the mouse to the top left part of the window. This scale button can be toggled from standard scaling (**S**) to normalized scaling (**N**). Alternatively, you can scale the x- and y-axes manually by right clicking on the axis with the mouse and selecting **Manual scale**.

The *information box* of the **Raw data** window displays the signal color, the axis number (counted from left hand side of the window) and the detector name. Items can be modified by a mouse click. Curves can be switched on or off the by clicking on the detector name. Maximum four Y-axes are available. Agilent recommends displaying only detectors with the same unit and range on the same Y-axis.

The information box will be closed by clicking on its **Close** button. It may be reopened using **Window > Information**.



Example:

A useful setup for a system with UV, RI, viscometer and MALLS detector would be: UV detector on Y-axis 1, RI detector on Y-axis 2, delta and inlet pressure viscometer both Y-axis 3, all signals from the MALLS detector Y-axis 4. In that case it is also recommended to switch off the trace of the inlet pressure signal and (if measured) the trace of the reference beam for the light scattering detector.

Entering Sample Information

While data acquisition is going on you have the opportunity to enter your sample names into the sample editor (menu item **Editor > Samples**). A dialog box appears, in which the name of the sample and sample parameters like concentration, molecular weight etc. can be entered. Enter correct values for concentration, inject volume and/or if the concentration determination method **factor*dn/dc** should be used the correct dn/dc. The sample names and all other entered parameters will be saved at the end of the run together with the acquired data and therefore permit useful documentation of the raw data. The input in the sample editor can also be edited after completion of data recording.

NOTE

For measurements with molar mass sensitive detectors Agilent recommends weighing in the samples as precisely as possible. WinGPC Software offers the possibility to measure the concentration, when a calibrated concentration detector is used, but determining the mass recovery is always useful.

Data capture will be stopped by clicking on the **Stop** button of the Toolwindow **Start/Stop**. If the selected instrument is controlled by WinGPC Software, the data acquisition will be stopped by pressing the **Stop Sequence** button in the **Sequence manager** window or will be automatically stopped at the end of a sequence.

Data Processing

Evaluation of your data can be done during data acquisition or after completion of the run. To evaluate your samples first load your universal calibration curve (**Calibration Data > Load** – see below how to establish a universal calibration curve). Select the sample for evaluation (menu item **Inject**). Now WinGPC Software is searching for the position of the inject of the selected sample, and places it on the left edge of the window. Furthermore, the elution volume will be counted from the injection point of the sample. The inject marker will be displayed by a blue triangle on the lower left edge of the window. A subsequent re-scaling of the window (e.g., by scaling or moving of the window image section with the scroll bar) has no influence on the allocation of the inject time to the raw data. Further injection marks may appear in the window, if another sample has been injected within the displayed time window.

The location of the internal standard and the baseline will be set in the **Raw data** window (see chapter "Raw Data Window" on page 195 for further information). To set the baseline, click on the blue injection marker and drag the first baseline marker out of the blue injection marker to the position where the baseline shall end. This position will be marked with a red triangle. To define the starting position of the baseline, drag the second baseline marker from the injection marker in the same way. To modify the baseline settings simply click on the red triangles and drag them to the new position. Please note that in the **Elugram** window only the chromatogram between the baseline margins is displayed.

To define the position of the internal standard right click with the mouse on the volume axis underneath the peak for the internal standard and select **Int. standard search max/min**.

NOTE

The internal standard will always be searched in the active curve. In the status bar on the top left side you can see which curve is presently active.

A pop-up box appears in which you can search for the internal standard (cf. internal standard search max./min. in chapter "X-Axis Context Menu" on page 200). WinGPC Software will look for the next peak maximum/ minimum starting from the mouse cursor position where the internal standard search command was invoked. A dialog box appears, which displays the position found for the internal standard as well as the reference value which is stored in the current calibration curve. After the internal standard is defined, the color of the respective triangle changes from dark to light green. Please change to the **Elugram** window after baseline and internal standard have been set.

In the **Elugram** window you can see the baseline corrected data (see chapter "Elugram Window" on page 224 for details). The volume axis may be converted to match the calibration conditions (i.e. if the data have been corrected using the internal standard, the volume might be different in the raw data and the **Elugram** window). In this window, the integration limits can be set for the subsequent calculation of molecular weight distributions and molecular weight averages. To set integration limits move the red triangles on the left and the right edge of the window to the position, in which the molar mass calculation shall be done. Alternatively, the integration limits can be defined by pressing the right mouse button underneath the peak on the volume axis. The function **Manual borders** allows to enter the integration limits either in elution volume or molecular weight. Now change to the window **Mass Distribution**.

The **Mass Distribution** window shows the calculated molar mass distribution and the different molecular weight averages in an information box (see chapter "Mass Distribution Window" on page 261 for details). WinGPC Software is able to calculate the molar mass distribution using conventional calibration curves as well as universal calibration curves.

The setting of the X-Axes of the **Mass Distribution** window defines what evaluation method is used:

- **Calib.Standard** displays the results with the conventional calibration curve
- **Calib. Viscometry** displays the results with the universal calibration curve

A right mouse click on the X-Axis allows to switch between the different evaluation methods.

The **Calibration** field in the status bar shows which method is used:

- If the name of the calibration curve is displayed, **Calib.Standard** is active
- if Viscometry is displayed, **Calib. Viscometry** is active

The calculation of the different molecular weight averages is explained in the theoretical part (see chapter "Molecular Weight Averages and Molecular Weight Distributions in GPC/SEC" on page 18). Moreover, the intrinsic viscosity [h] and viscosity average molar mass M_v will be calculated, based on the Mark-Houwink constants of the calibration curve. M_p and V_p are the molecular weight and the elution volume at the peak maximum. **A** is the peak area below the respective curve within the integration limits.

Fractions within the mass distribution can be defined using the red markers on the lower margins of the window, e.g., to calculate what percentage of the distribution that is below or above the set limits. These data appear in the lower three lines of the information window. Further options of this window are explained in chapter "Mass Distribution Window" on page 261.

A detailed viscometry analysis is shown in the **Viscosity** window. This window allows to select the detector used for determining the slice concentration, the concentration detection method, and the fit function for fitting the measured intrinsic viscosity. All settings here can also be saved with the method.

NOTE

A printout of the mass distribution window prints the content of this window with molar masses from viscometry, if selected. The printout of the Viscosity window shows the viscometry results and parameters, as well as the molar mass averages. With the optional WinGPC Software ReportDesigner it is possible to combine these two printouts as well as printing universal and conventional results on one page.

Creation of a Universal Calibration Curve

NOTE

Agilent recommends measuring at least 12 molar mass calibration standards for establishing the calibration curve. It is possible to measure sample mixtures of up to 4 standards, but the peaks should be baseline separated and the concentration for each component should be known. The performance of the viscometer can be checked separately using viscometry standards.

In order to create a universal calibration curve, the elution volumes, molar masses, intrinsic viscosities and statistical weights have to be entered in the calibration table of the **Calibration** window. This can be done by using the **Set Peak Integration** and **Find minimum/maximum** dialog in the X-axis context menu of the Elugram window (cf. chapter "Elugram Window" on page 224 for details).

NOTE

If the **Add to calibration** button is grayed out, no calibration file is available to accept new data points. Use the **File > New** menu in the **Calibration** window to create an empty calibration table.

Immediately after input of a calibration point it will be displayed in the graphics section of the calibration window. The calibration molar masses can be selected from the list, if they have been entered previously in the sample editor, the fields elution volume and intrinsic viscosity are already filled with the measured values.

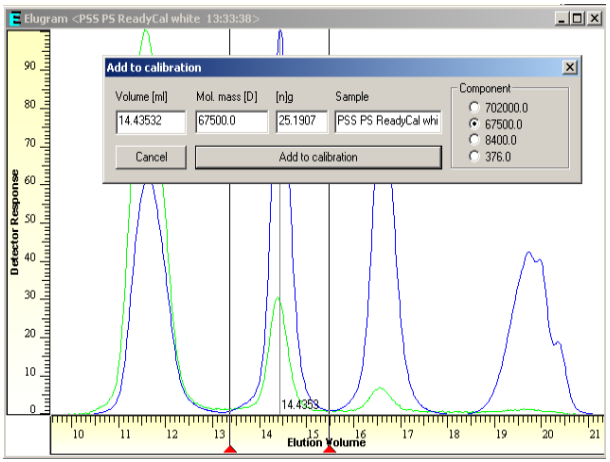


Figure 34 Creating calibration table with viscometry results

When all calibration points have been entered into the calibration table section, suitable calibration functions from the *Fit* selector drop-down list can be chosen. WinGPC Software supports linear and polynomial regression models as well as three specific calibration functions (PSS Poly3, PSS Poly5 and PSS Poly7). The software performs a least squares regression calculation and shows the resulting curve fit in the graph section of the calibration window. Note that the statistical weight assigned to the data point defines whether the calibration point will be used for the creation of the conventional calibration curve. If the statistical weight is zero, the data point is shown, but not used in the regression calculation.

WinGPC Software will save a conventional (log M vs. Elution volume) and a universal calibration curve (log([n]*M vs. elution volume) using the same file name. To use both calibration methods in WinGPC Software it is necessary to choose the fit function for the method **Molar Mass** and for the method **mass*[n]g**. Which calibration curve is shows is selected in the **Method** selector box.

Unfortunately, no analytical function exists which describes the shape of a calibration curve for all cases. The user must rely on knowledge when selecting the calibration curve for the calibration data. The quality of the adjustment can be determined by 3 criteria:

1. The deviation between calibration points and calibration curve should be low.
2. The distribution of residuals along the volume axis should be random.
3. The slope of the calibration curve should be physically meaningful, i.e. should not have inflection points.

In order to get a better understanding about the quality of the calibration curve, you can add the information for residuals or the slope of the calibration curve to the graph. This is done by selecting the appropriate entry in the **Compare** selector drop-down list.

It is highly recommended to try different regression models to find the best calibration curve.

Select **Calibration > Parameters** and add the values for internal standard, etc. Save your calibration curve using the options in the **File** menu.

For a detailed work list for performing calibrations with narrow standards consult the WinGPC Software Quick Reference Guide. Step-by-step instructions are also available in the WinGPC Software online help menu (**Help > Step-by-Step**).

6 Description of Menus and Options

This chapter will explain in detail the commands, functions, and options of each WinGPC Software window. It will also deal with the user interface and give tips and tricks for additional ease of use.

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter “WinGPC Compliance ” on page 507 of this user guide.

General Description of the Screen Design

The menu bar is always shown at the upper end of the display just below the program title bar. The menu items which are displayed will depend on which sub-window is currently active. That means that each window has its own menu items. Only the menu items **Window** and **Help** are identical for all sub-windows and will be explained below.

The icon bar (see Figure 35) summarizes the most often used commands and is located right below the menu bar. The icon bar will not change icons when changing the active sub-window.

Figure 35 WinGPC Software icon bar with frequently used icons (explanation see table below)

The icon bar icons and their use are explained below. The icons possess different functionality in the calibration context (i.e. calibration window is active) or in GPC data capture (method window) and data processing mode (i.e. any other sub-window is active):

Table 5 Icons

Icon	Data Capture/Data Processing Mode	Calibration Mode
	Create new method	Create new calibration
	Opens method or project file	Opens calibration file
	Saves active method file	Saves active calibration curve
	Closes active raw data run (window)	Closes active calibration curve
	Create electronic sample signature (only if Compliance Edition is licensed, otherwise gray)	
	Prints report of active window	Prints calibration report
	Page preview and copies to clipboard	Page preview
	Create or modify ReportDesigner report layouts	

Table 5 Icons

Icon	Data Capture/Data Processing Mode	Calibration Mode
	Prints default ReportDesigner report (QuickReport) as specified in the **Raw Data** menu	
	Shows preview of default ReportDesigner report as specified in the **Raw Data** menu	
	Currently not used	Cuts calibration points to clipboard
	Currently not used	Copies calibration points to clipboard
	Currently not used	Pastes calibration points from clipboard
	Opens online help system	Opens online help system
	Copies chromatogram to overlay, toggles overlay mode on/off	Toggles overlay mode on/off
	Currently not used	Opens default settings dialog
	Toggles HPLC mode on/off	Toggles HPLC mode on/off
	Displays uncertainty assessment (precision) background information and printer properties	
	Switches between instrument windows	Switches between instrument windows
	Toggles elugram window on/off	Toggles elugram window on/off
	Toggles viscosity window on/off	Toggles viscosity window on/off
	Toggles light scattering window on/off	Toggles light scattering window on/off
	Toggles mass distribution window on/off	Toggles mass distribution window on/off
	Opens ProjectManager window	Opens ProjectManager window
	Toggles data editor window on/off	Toggles data editor window on/off
	Toggles method window on/off	Toggles method window on/off
	Toggles calibration window on/off	Toggles calibration window on/off
	Toggles 2D window on/off	Toggles 2D window on/off
	Toggles mass spectrometry window on/off	Toggles mass spectrometry window on/off

Electronic signatures will be assigned by clicking on the padlock icon ( ). If a sample is already signed, a closed padlock will be displayed (– yellow if only signed, red if signature was approved as well). Signatures can be removed by pressing the closed padlock. Setting as well as removing an electronic signature requires authentification with username and password. The user rights to sign, approve and/or remove electronic signatures can be set in the WinGPC Software administration database.

The section below the icon bar always shows the **Status Bar**, which summarizes important status information when processing data.

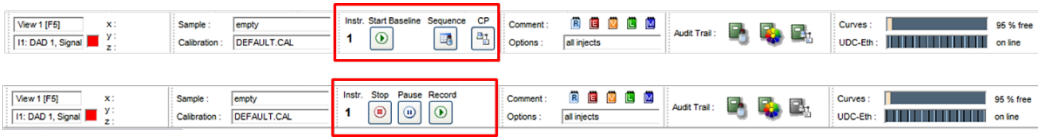


Figure 36 WinGPC Software Status Bar for a (top) controlled and (bottom) not controlled instrument with data evaluation control fields. The red marked area (Toolwindow Start/stop) differs for controlled and not controlled instruments

White data fields in the status bar indicate the availability of pop-up dialog boxes, where special functions can be picked from a list. The following toolwindows are located in separate sections in the status bar:

If the WinGPC Software Compliance Edition module has been licensed, an additional Status Bar control (Audit Trail) will be visible that allows inter alia quick access to the Sample audit trail (for details refer to chapter "WinGPC Compliance " on page 507). If the Compliance Edition module is not licensed the Sample Audit Trail button ( ) will be grey out (not accessible).

Detector and View

The field *View* on the top left on the icon bar displays the currently used sub-windows arrangement (view). The active view can be either switched by a mouse click in the white field or by pressing a function key (**F5** though **F8**). Different views can be created by arranging the sub-windows inside WinGPC Software. The new view will be saved automatically when switching from that view to another one. Four different views are supported by WinGPC Software.

The field *Detector* shows the name of the active curve (detector selection field) and its assigned color (field *Color*). The active curve in comparison to others is distinguished by the fact that all peak search functions relate to it. The active curve can be changed by a mouse click on the *detector* selection field of the active curve. To change the detector color of the active curve click on the *detector color* field, and chose the color from the color selector panel. Next to this, the x-coordinate of the mouse pointer and the detector response at that position (y-coordinate) of the active curve is shown.

Date and Time

In the middle part of the status bar the current day, date and time are displayed. In the default settings the Toolwindow **Date and Time** is not activated. If it is required, please activate the Toolwindow by selecting **Window > Toolwindows > Date and Time**.

Sample and Calibration

In the central section of the **Status Bar**, the name (field *Sample*) of the sample, which is currently in evaluation, as well as the currently used calibration curve (field *Calibration*) are displayed. Upon clicking the field *Sample*, you can change to any inject of any loaded or ongoing data acquisition or you can enter directly the sample search window, whose functions are explained in chapter “Sample Search” on page 281.

Similarly, clicking in the field *Calibration* allows to change the currently active calibration curve for the currently displayed run sequence. The calibration curve is selected from a calibration index file which allows previewing the calibration before loading it. The calibration index is created by clicking on the **new search** button in the dialog box for the whole drive. The drive selection can be changed by clicking on **path** and selecting the desired drive (including network drives) from the pop-up list.

NOTE

The index must be re-created after the creation of new calibration curve or if calibration files have been moved from the indexed path.

Alternatively, calibration file selection from the menu is also possible. This requires however to activate the raw data window and selecting **Calibration Data > Load...** from the menu. The *Calibration* field in the status bar is independent of the activated sub-window.

Start/Stop

The icons of the **Start/Stop** Toolwindow allow to start (stop) data acquisition, pause data acquisition and/or record baseline. This toolwindow differs for controlled and not controlled insturments. In case of a controlled instrument the **Start/Stop** Toolwindow shows three buttons: one for start baseline record (), one for opening the **Sequence Manager** () and the third one for opening the ChromPilot () (**Instrument Manager**).

Audit Trails

The icons of the **Audit Trail** Toolwindow allow to open the desired Audit Trail. A left mouse click on the first icon from left () opens the sample audit trail, the second icon from left () opens the WinGPC Session Logbook (Session Audit Trail) and the third icon () opens the instrument logbook (ChromPilot).
Important: If the respective instrument is not controlled by the WinGPC Software ChromPilot, the button for the instrument logbook will be grey out. If the WinGPC Software is installed without the Compliance Edition module, the button for the sample audit trail will be grey out as well.

Comment and Options

The comment icons of the **Comment and Options** Toolwindow allow to add notes for each sample in the sequence separately for documentation and information sharing. The comment fields can contain up to 1024 characters to describe details in sample preparation, data processing, etc. and can be added for every sub window separately. Comment printing is possible with the ReportDesigner software module. The letter on the comment icon indicates the sub window to add the comment to. If the icon shows an open notepad (), comments have been added otherwise the notepad is closed ().

WinGPC Software offers different batch analysis tasks. Several settings, e.g., loading a calibration curve, choosing the baseline type or quick analysis can be done:

- only for the actual inject/sample
- for the actual inject/sample and all following samples
- for the actual inject/sample and a free selection of samples of the same login
- for all samples/the complete login.

Upon clicking the field *Options* one of the options mentioned above can be chosen. WinGPC Software applies then the selected analysis task depending on the option.

NOTE

For novice users WinGPC Software offers the window **Inject options** after an analysis task, that allows inject dependent settings, has been chosen. This window shows again the options and offers the complete injection list to select samples. Experienced users can deactivate this window using **Do not display dialog**, with the exception that the window will always appear if **actual inject and selectable** is active.

Curves and Communication

NOTE

This chapter is addressed to the legacy PSS data acquisition hardware.

On the right-hand side of the **Status Bar**, the field *Curves* displays the free program resources to handle sub-windows and signals. The maximum number of sub-windows is 14. The field below *Curves* identifies the data transfer connection between the PSS Data Interface and the host computer (see table).

Table 6 Data transfer connection

Connection	Data interface connection
COM*n*	*Serial*, *n* port number
UDC-COM *UDC-USB* *UDC-Eth*	*serial UDC810 data link* *UDC810 with USB link* *networked UDC810*
Pipe	*NetConnect (Cube)*

The turquoise lines indicate a packet reception from the host computer (PC). They should appear steadily if the communication port is available all the time. If the port is busy or the data transmission fails, the interface will re-send the data until transmission is successful. A high number of turquoise lines in the information field either indicates a high frequency transmission or a problem with data transfer

between interface and PC. Details of the data transmission can be investigated in the Method window under **Interface > Information**.

WinGPC Software menus are context sensitive and will change depending on the currently activated sub-window. These menu items will be described in the respective sub-window sections. The permanent menus, which will be shown in every context, are described below.

Relay

In the right part of the status bar the Relay Toolwindow is displayed. Here the user can controll the output relays, which can be activatied/deactivated manually by the user or in an automated fashion (for details refer to chapter "Digital I/Os Pin Assignment" on page 536). In the default settings the Toolwindow **Relay** is not activated. If it is required, please activate the Toolwindow by selecting **Window > Toolwindows > Relay**.

Description of the Window Menu

The Window Menu allows to switch to other windows or changes the arrangement of the windows.

Table 7 Windows

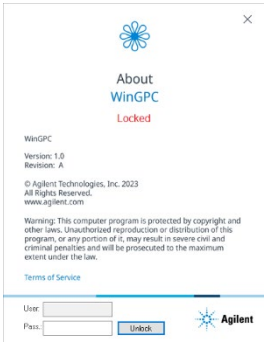
Window	Description
Information:	Toggles on/off the information box in the raw data, mass distribution, light scattering or viscosity window.
Lock WinGPC Software: 	Prevents that a different user can access a WinGPC Software session of a colleague without interfering with ongoing data capture and automatic processing. A WinGPC Software "LOCKED" splash screen is displayed on top of all applications as long as user interaction is prohibited. Access to WinGPC Software is regained by typing in the password of the user who originally launched WinGPC Software. The login name of the user that launched WinGPC Software is shown. This functionality is only available for WinGPC Software licenses with the Compliance Edition option. 
Tile:	The standard setup arranges the sub-windows according to a scheme given by the operating system. After the software is started the sub-windows are arranged as follows: **method** window at top left, raw data window at bottom left, elugram window at bottom right and the mass distribution window at top right. In this setup the data flow is counterclockwise. Another useful representation for the evaluation of collected data can be created, if only one raw data window with the respective elugram and mass distribution windows are open. After selecting **Window > Tile** the window will be arranged in such a way that the raw data window occupies the lower part of the screen, while elugram and mass distribution share the upper part of the display. With this you will obtain direct control during the evaluation how a correction in one window will influence the results of other windows. The order of the sub-window arrangement is determined by the order the sub-windows were activated before. If you want to get above mentioned pattern, you need to click first onto the **Elugram**, then **MWD**, **Raw Data** and last onto the **Method** Window (= bottom right, top right, bottom left and top left).

Table 7 Windows

Window	Description
Single Raw Data Window:	With multiple instrument versions or multiple loaded data sets in switched-on single raw data window mode (identified by the tick mark) only one raw data window will be displayed at a time. Other loaded raw data sequences or real-time display of other instruments will be listed in the *Sample* dialog in the **Status Bar**. By switching-off the single raw data window mode several raw data windows can be displayed at the same time.
Toolbars:	Allows the selection of icon displayed in the icon bar. Bottons can be positioned according to Windows user interface standards.
Toolwindow:	Allows the selection of information shown in the status bar. Information sections can be positioned according to Windows user interface standards. The current toolwindow settings will be saved and used in subsequent WinGPC Software sessions.

Description of the Help Menu

Table 8 Help menu

Window	Description
Contents:	This opens the on-line help system for WinGPC Software. It opens with a table of contents where you can look for the major topics easily. Alternatively, you can use the search index to look for special key words; click on the index tab for that. A list of search results will be displayed, and you can browse through them one by one. If even more detailed information is needed, a full text search option is implemented also. Click on the search tab and create the full text database. This has to be done only once.
Step by step:	This help option directs you step-by-step through many tasks which can be done with the software. For each task step-by-step instructions are available on-line. The step-by-step help is an extended version of the printed WinGPC Software Quick Reference Guide. It can be very useful for training new users on the job. For most of the topics "how to" videos with audio comment are available as well.
ReportDesigner:	The WinGPC Software ReportDesigner is an optional software module; see chapter "WinGPC Software ReportDesigner" on page 333 for details. The on-line help contents are always available and can be used to evaluate the potential and features of the WinGPC Software ReportDesigner. Please contact your Agilent representative for a quotation.
Step by step report:	This help option opens step-by-step instructions on WinGPC Software ReportDesigner use and covers the most important aspects of the product like creating layouts, formatting results and report printing. This step-by-step help is an extended version of the printed WinGPC Software ReportDesigner Quick Reference Guide. It can be very useful for training new users on the job.
About:	Shows the release version and build number of the WinGPC Software as well as copyright and contact information.

The Method Window

A WinGPC Software method includes all data acquisition parameters such as flow rates, inter-detector delay, component names, axes scaling, calibration curves, etc. In the case of autoprocessing the WinGPC Software method also contains all autoprocessing parameters. The complete method will be stored with the acquired data and serves to document all data capture and data processing parameters (meta data). Methods may be created, stored, and loaded in this window. WinGPC Software saves the current method settings during data acquisition, not only the method name. This guarantees that the configuration during the data acquisition is stored, even if a method will be modified and saved with an already existing method name. WinGPC Software will load at start-up the settings for each instrument which were active at successful completion of the previous data acquisition.

Window Description

The **method** window serves to display and control the data acquisition parameters. It is also used to program the data interface with a graphical user interface. This ensures data acquisition even if the host computer (PC) might be occupied otherwise or even crash. After the software is restarted the missing captured data during downtime will be added to the run automatically without loss of information.

The **method** window shows a schematic representation of a GPC instrument on the right-hand side of the window. The left-hand side contains the resources (resource tree) available for GPC data acquisition. A single resource list is used for all instruments which are connected to this computer.

NOTE

If you control your GPC instrument with the ChromPilot module, the method window will show an additional button (ChromPilot) below the instrument name and a modified Toolwindow Start/Stop. For details refer to the ChromPilot documentation in chapter “ChromPilot System Control” on page 466).

Resource Tree

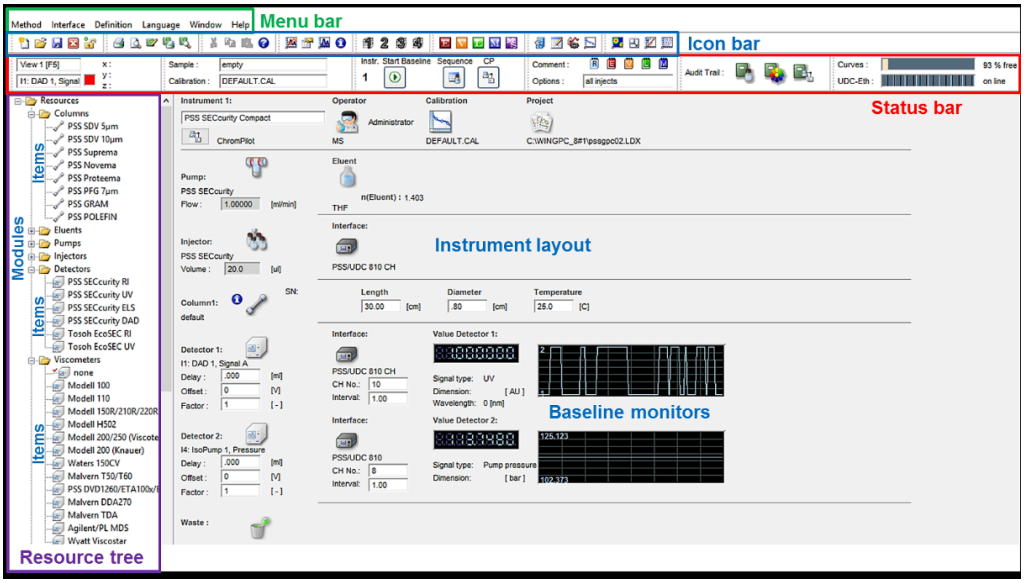
The list of GPC resources displays all GPC modules available for the number of instruments connected to the PC via LAN (digital data acquisition) or a legacy PSS Data Interface (analog data acquisition). The item names and their properties will be saved in an independent file. The item list file WinGPC_8.BTL is stored in the \wingpc_8#1 folder.

Please note that the resource tree is not visible if a user is logged in with **User** or **Guest** status in case of the Compliance Edition is installed. For further information consult the Compliance Edition chapter in this user guide.

NOTE

The item list can be copied to any different computers, thus you can eliminate the necessity for re-entering of the GPC components.

The resource tree (see next Figure) sections can be expanded to display individual items or collapsed to show just the main modules just like folders in the Windows Explorer. Items for each object can be added or modified any time using a right mouse click and selecting the desired option from the pop-up dialog box. The **edit object** dialog box will vary from object to object to reflect the object specific configuration needs; e.g., the edit column dialog will ask for column dimensions. These will be stored with the raw data and used to calculate theoretical plate numbers, resolution and efficiency factors (see chapter “Options Menu” on page 234, **Elugram** menu item **Options > System test**).



The *edit columns* dialog will ask for length, diameter and serial number. The entries **Length** and **Diameter** are used for the system test, but can be corrected in the *System Test* dialog as well.

The *edit eluents* dialog will ask for refractive index. This entry is essential for light scattering measurements, for all other measurements this entry is optional.

The *edit detector* dialog will ask for factor and offset settings of the A/D converter modules.

The following parameters are used to get a voltage reading from the detector output:

Table 9 Parameters (legacy products)

A/D Converter Model	Power LED Color	Voltage Range [V]	Offset [V]	Factor [1/V]
PSS ADC 401	yellow	-2.5 to +7.5	-0.5	5
PSS ADC 401-HR	blue	-0.5 to +1.5	-0.5	1
PSS UDC810	internal A/D	-2.5 to +7.5	-0.5	5
PSS ADC+	n/a	-0.01 to +10	-1	1
PSS ADC±	n/a	-2.5 to +7.5	-2.5	1

When the internal A/D converters of digitally controlled detectors like the PSS SLD 7x00, Agilent 1260 MALS, the BI-MwA, and the Wyatt DAWNs are used, in

each case “0" (zero) has to be entered as Offset and “1" as Factor values. These are the default parameters in WinGPC Software.

The *edit viscometer* dialog will ask for channel number, offset, factor and DPT sense.

When a PSS/WGE eta100x/201x online viscometer is used for data capture and its internal A/D capabilities are used the following parameters have to be entered:

Table 10 Parameters

Signal name	Channel No.	Signal Type	Offset	Factor	Unit
Inlet pressure	1	Inlet pressure	-8.3861	24000	Pa
Differential pressure	2	Delta pressure	-8.3861	1000	Pa
Cell temperature	3	Temperature	-8.3861	25	°C
Ext. 1	4	Standard	-8.3861	250	V
Ext. 2	5	Standard	-8.3861	250	V

Otherwise, the channel number is the number of the optical input (fibre optical cable) at the PSS Data Interface. Offset and factor have to be determined as described below.

When a PSS SLD7x00 or BI-MwA is used for data acquisition and its internal A/D capabilities are used the following parameters have to be entered to get signal readings in Volts [V]:

Offset: 0, Factor: 1

If a PSS/WGE eta100x/201x online viscometer is connected to a PSS SLD7x00 or BI-MwA the following default configuration can be used:

Table 11 Parameters

Signal name	Channel No.	Signal Type	Offset	Factor	Unit
Ext. 1	1	Inlet pressure	0	21000	Pa
Ext. 2	2	Delta pressure	0	1000	Pa

A concentration signal should always be connected to channel “Ext.4".

The PSS A/D converters are calibrated voltage meters. They can be used to measure any property (e.g., absolute concentration, pressure drops etc.) by converting the primary voltage signal to the required property. The conversion can be done by correlating the output voltage U to the property Y using the following equation:

$$Y = (U - O) F$$

E.g., to convert the voltage into pressure values for your viscometer, enter at the beginning a factor of 1 and an offset of 0.

Record pressure/signal pairs (y/U) and carry out a linear regression (y=ax+b), by which you receive the slope a and the intercept b. The slope a of the linear regression is equal to the factor F, while the offset, O, results as ratio if intercept and slope (O = b/a).

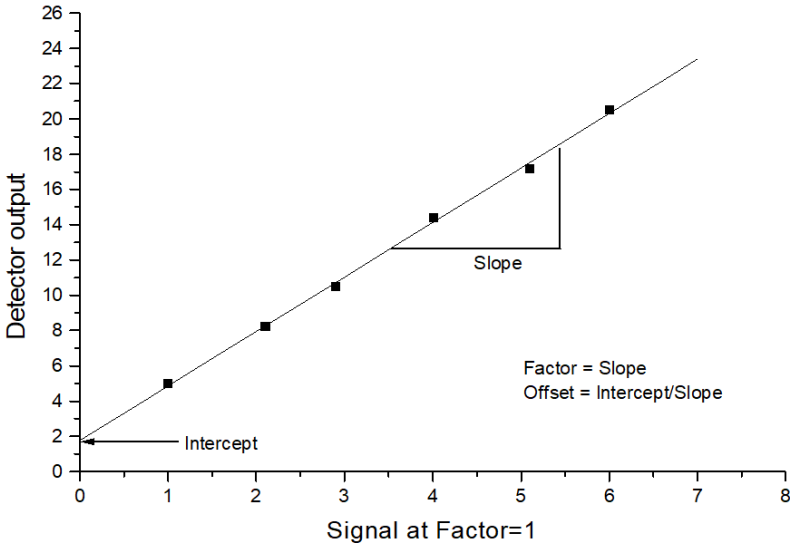


Figure 37 Calculation of Factor and Offset for non-standard detectors

WinGPC Software supports all viscometry detectors on the market (vendor independence). Viscometers in the resource tree section can only be edited and not added since they need certain model dependent calculation routines.

The DPT-Sense of the viscometer can be measured using a viscometry standard. This factor enables a correction of the calculated values for the specific viscosity, if the pressure values displayed on the viscometer do not correspond to the actual pressures (e.g. through aging of the pressure transducer). The value of specific viscosity measured by the pressure signals will be multiplied with the value of DPT-Sense.

The edit lightscattering dialog will ask for several parameters depending on the light scattering device and for the *instrument constant*. If the dialog asks for an Interface channel enter the number of the optical input (fibre optical cable) at the PSS Data Interface. The instrument constant has to be determined using a narrow molecular weight standard. It can be automatically measured using **Method > Guided Detector Setup** (see chapter "Detector Setup" on page 183), that is available for finished runs only.

WinGPC Software supports all light scattering detectors on the market (vendor independence). Light scattering detectors in the resource tree section can only be edited and not added.

NOTE

None in the viscometer and light scattering section is used to remove viscometers and/or light scattering devices from the instrument layout view.

Instrument Layout View

Assigning names to GPC modules can be done in two ways: either by clicking onto the respective icon and selecting the correct name from the pop-up list or by drag and drop from the resource tree to the instrument layout. In the case of columns and detectors, dropping the item to the (grey) background will add another column or detector to the instrument configuration (because there can be more than only one). If an existing column or detector shall be re-named, the resource icon of the item has to be dropped on the icon in the instrument layout.

The data of WinGPC Software are managed in projects. Each project may contain various measurement series (logins), which in turn may contain various injections of one or several samples. Projects can be arbitrarily created and data acquisitions can be written into the projects. A special sample search module (query editor) allows to search for samples by their names or other criteria. This search can be processed within a specified project but can also proceed in all projects of a hard disk. Visually you can perceive a project like an index card file. The single measurement series (login) correspond to the index cards, which in turn register the sample names of the injections.

Starting point and possibilities for project organization is e.g., the organization by months. For service groups which often carry out series of measurement for a

specific customer, organization by customer name can be useful. For users of multiple GPC stations e.g., with different eluents organization by the solvent names can be a good choice. In chapter "Sample Search" on page 281 you will find further detailed information how to search and find samples.

You can create a new project by clicking on the item *Project* (icon diskettes) and selecting a new project name. Alternatively, you can open an already existing project to append new data to it. The new data acquisition will be appended to the existing project database.

A default value for the injected sample volume can be entered in the field *injection volume*. However, each individual injection (sample) can be assigned its own injection volume. Volume information entered in the ChromPilot Sequence Manager (or the sample editor of the Raw Data Window for non controlled instruments, see **Editor > Samples** in chapter "WinGPC Software Sample Editor" on page 211). In case of a controlled instrument by ChromPilot module the **Sequence Manager** will be used to assign the specific injection volume for each individual sample.

The flow rate of the pump must be entered in the field *flow* to convert time into volume units.

The fields for *column length* and *diameter* (see resource tree section above) also may be edited manually and have a higher priority than the entries in the item list. These values will be used for the system test (calculation of plate counts and separation efficiency).

For the different detectors enter the respective *Delay* volumes. Please note that only positive delay volumes are permitted. The detector delay volume is the chromatographic volume difference between the first detector and the considered detector. The eluate which at the time t (or elution volume V) is measured in detector 1, passes through the next detector cell at the time Δt (or volume ΔV) later. Therefore, its own calibration curve (M(t), or M(V)) must be created for each detector. The correct delay input allows the evaluation of any detectors with respect to the same calibration curve. This saves a lot of time and effort in data processing and maintaining GPC data. The software automatically corrects the time (or volume) shift between the detectors using the given values for delay and flow rate. The physical first detector of an instrument has by definition the delay volume of 0 mL. Within the software a positive delay can be entered for the first detector in the instrument layout, this is useful e.g., if the first detector is not necessary for the measurement and no data shall be recorded, however, the calibration curve was created with respect to this detector. The determination of the detector delay is explained in chapters "Determination of Detector Delay" on page 28 and "Molar Mass Sensitive Detection" on page 41.

Offset and **Factor** for the detectors (cf. chapter "The Method Window" on page 165 for details) can be entered in the fields provided and have a higher priority than the

values stored in the item list. In general, these parameters should be entered by selecting the appropriate item from the items list.

The *channel number* (CH No) specifies from which source the detector data should be recorded. A source can be the optical input of the PSS Interface (UDC810, WinChrom or LAN) or the analog in of a light scattering or viscometer device.

The channel number can be typed into the field or selected from the pop-up list on a right mouse click into the channel number field (green marked area, Figure below).

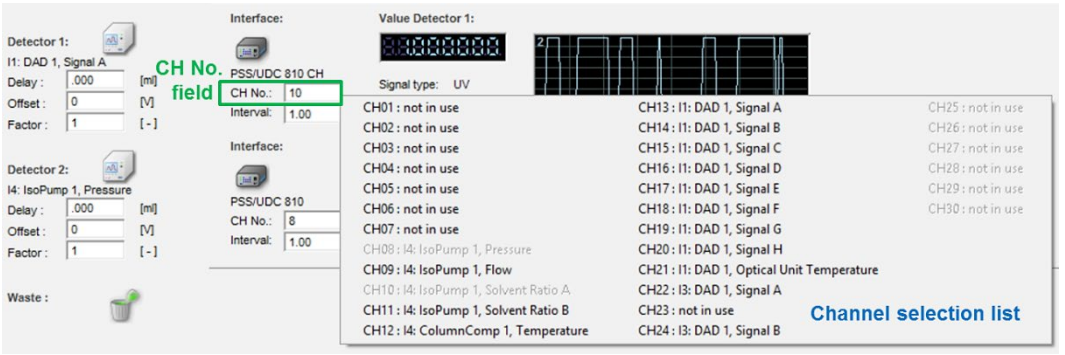


Figure 38 Selection of NetConnect channels

PSS Interface channels which are already assigned in the method are greyed out. The allowed channel range is 1... 10 for the UDC810 (1... 6 for the WinCHROM) interface. The selection of NetConnect detector channels is done accordingly. The pop-up window shows a list of channels (detectors) as they have been identified in the NetConnect.

If multi angle light scattering devices are present in the instrument layout view their analog channels are added to the list automatically.

NOTE

For multi angle light scattering instruments the channel selection only effects the content of the baseline monitor. All light scattering signals will be recorded independent from the chosen channel number.

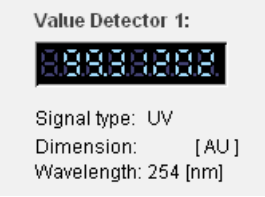
The field interval specifies the integration time of the detector connected to the A/D Converter and thereby the density of data points. In principal each detector will be read with the same frequency, which means that the data acquisition frequency must be defined for only one channel, all others will be set to the same data frequency automatically. Detector dependent intervals can be obtained using the menu **Definition > Data Interval**. Detector dependent data intervals are useful e.g., if oven temperature or pump pressure is to be recorded beside the chromatographic data.

Table 12 Definition and Use of Signal Types:

Signal type	Unit	Type No.*	Comment
Standard	V	0	for conventional signals
UV	V	1	endgroup number signal, dA/dc
RI	V	2	endgroup mass signal
Concentration	g/l	24	conc. calc. using factor F
Column temperature	°C	64	no baseline subtractation
Temperature	°C	65	no baseline subtractation
Pump pressure	Bar	66	no baseline subtractation
Inlet pressure	Pa	71	no baseline subtractation
Difference pressure	Pa	72	baseline subtraction
Light scattering	V	27	baseline subtraction
R_h signal	V	28	
Other	V	100	

* required for user defined signal display in ReportDesigner layouts

The field *value detector no.* shows the current value of the detector output. The signal types and dimension can be modified upon clicking onto the *field value detector No.* For "normal" GPC measurements with concentration proportional signals the signal type **standard** can be set. If a UV detector is used and controlled via ChromPilot, the wavelength will automatically be displayed in the instrument layout if signal type **UV** is selected.

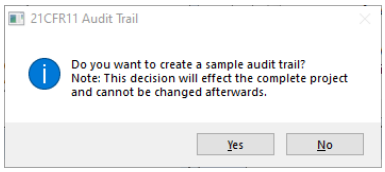


The signal types “UV” and “RI” need to be selected if an endgroup evaluation for heparins shall be performed (see “Endgroup / Heparin Analysis” on page 438).

Other mathematical procedures will be used, if other signal types are selected e.g., in light scattering or viscosity detection. They are used to correlate the detector signal and physical unit and how the signal will be processed (e.g., subtraction of baseline etc.). E.g., the pressure signal of the viscometer requires other mathematical calculations than a RI signal.

The graphic display shows the current detector data and acts as a baseline monitor. Only the last 4 minutes are displayed on a first-in first-out basis. The Y-axis is autoscaled to reveal details of the baseline.

If you have the right to decide whether an audit trail will be created or not, you will get following message each time you create a new project:

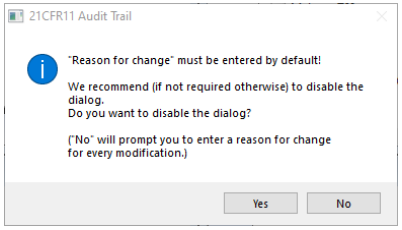


If you answer **Yes**, a sample audit trail will be created, **No** will deactivate the audit trail for the complete project.

NOTE

The sample audit trail cannot be activated/ deactivated later on. It has to be defined during the creation of the project.

If you have the right to decide whether a reason for change has to be entered (on each modification of the GPC meta data), a second dialog will open:



'Reason for change' must be entered by default! Do you want to disable the dialog? – **Yes** means you will not be asked to enter a reason for change.

No will activate the request for a **Reason for change**. You need to assign a reason for each action that modifies the meta data (e.g., baseline settings). The last 10 entries will be saved in a list box, so you can save time and clicks in answering the reason by selecting it from the list:

Recommended setting:

Agilent recommends creating a sample audit trail and to deactivate the reason for change dialog (if not necessary due to company compliance requirements). This will guarantee traceability of all actions and prevent from unnecesary extra work.

The user rights for these actions will be defined in the administration console.

Instrument Status Informations

The Toolbar **Instruments** (available by **Window > Toolbar > Instruments**) displays the status of all currently opened instruments and in combination with the Toolwindow **Start/Stop** (available by **Window > Toolwindow > Start/Stop**) a fast manual control of data acquisition and instrument state is given. For detailed informations about the current sequence step (in case of a controlled instrument) open the **Sequence Manager** ( ) to inspect the status bar of the Sequence window.

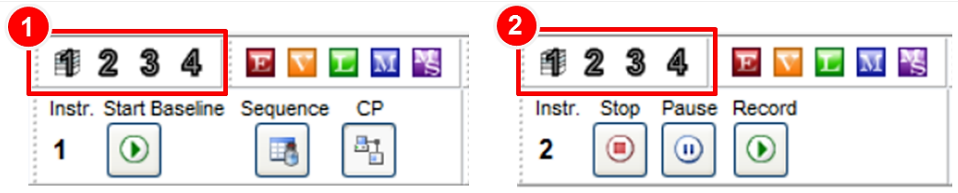


Figure 39 (1) Toolbar Instruments (red area) and Toolwindow Start/Stop for the controlled Instrument 1 and (2) Toolbar Instruments (red area) and Toolwindow Start/Stop for the not controlled Instrument 2

NOTE

The previous WinGPC Software version 8.2 combines the toolwindows **Start/Stop**, **Audit Trails** and **Relay** in the Instrument *Information Box* of the **Method** window. The Toolwindow **Start/Stop** differs for controlled and not controlled instruments (see Figure 39). *Important*: Starting with the WinGPC Software version 8.3 the *Information Box* of the **Method** window is no longer available and instead of that the new toolwindows can be used.

For each instrument (specific Instr. Number; max. up to 4 per WinGPC Software instance) the status can be seen by the following run parameters:

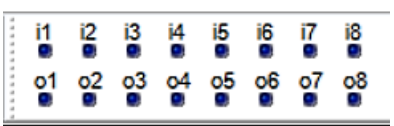
- "Color" of icon symbolizes:
  - no data capture, instrument in ready state ( ) – grey instrument number
  - pausing data capture and/or instrument not in ready state ( ) – yellow instrument number
  - data acquisition on, sample analysis running ( ) – green instrument number
  - no data acquisition, instrument in error state ( ) – red instrument number
- "Type" of icon symbolizes:
  - controlled instrument ( ) – instrument number on top of a device is shown
  - not controlled instrument ( ) – only instrument number is shown

If there is more than one instrument configured and/or controlled by WinGPC Software (depending on the Licence Key) a left-mouse click on the respective instrument icon in the Toolbar **Instruments** allows to switch between the instruments. When a mixed configuration of controlled and not controlled instruments is used, the Toolwindow **Start/Stop** differs in the button arrangement (see Figure 39). The buttons will manually start (**Record**), stop (**Stop**) or temporarily interrupt (**Pause**) data acquisition for the selected instrument. Data capture start and stop conditions are defined in the **Definition > Start Condition** or **Definition > Stop Condition** menus. The status can be viewed as tooltip for each instrument by placing the mouse-cursor on top of the respective instrument icon.

NOTE

Stop and **Record** buttons are only available if the instrument is not digitally controlled by WinGPC Software ChromPilot. In case of a controlled Instrument use **Start Baseline**, **Sequence** and **CP** (ChromPilot) buttons. To start data acquisition, open the **Sequence Manager** window and press the **Start Sequence** button (top right).

To inspect the status of the relays of the PSS Data Interface click on **Window > Toolwindows > Relay**, which will open the Toolwindow **Relays** (first toolwindow from right within the WinGPC Software status bar).



In the top line the i# indicates the input relays (status of the injectors for instrument #), the o# below refers to the output relays which can be programmed in the **Definition > Timed Events** menu. Active (closed) relays are indicated by the relay button changing to red.

Method Menu

Table 13 Method menu

Menu	Description
New:	Removes all items from the instrument layout view to initialize a new data acquisition method with minimum requirements.
Load:	Loads an existing data acquisition method.
Save Method:	Saves the current contents of the **method** window as default method for the next WinGPC Software session in the file *Instrumenti.set* (i = instrument number) in the \wingpc_8#1 folder.
Save Method as:	Saves the current method in a method file (file extension Met). These pre-defined methods can be saved and loaded for different applications.
Printer Setup:	Allows the adjustment of parameters of the default printer. However, the default printer itself must be defined in the Windows Control Panel. *Landscape* format prints the graphics on a full page. *Portrait* format prints the graphics, full method documentation and results by default. The printed information in the portrait layout can be selected for automated runs in the **Definition > Automation Settings** dialog box. The exact information of the portrait format printout depends on which window will be printed. For color printers you can select color or monochrome printing depending on the printer driver options. The color of the curves in monochrome printouts are mapped automatically to a line style to avoid unreadable black and white prints. The correlation between curve color and line style in monochrome print is listed in "Curve Colors and Line Styles in Monochrome Printing" on page 534.

Table 13 Method menu

Menu	Description
WinGPC Software Wizard	The WinGPC Software Wizard will optionally be shown at WinGPC Software start (set tick mark for **Show wizard selection on startup**) or if started using the menu item. Following procedures that are important for GPC data acquisition and evaluation are available as guided step by step wizards (see chapter "WinGPC Software Wizards" on page 104): • Acquire Data • Calibration • Process Data • Overlay • Reporting • Multi Area • Detector Setup • Create / modify method
Print:	Prints the contents of the **Method** window on the default printer.
SystemVerification:	This command is intended to validate the data station. It will check the correct processing of raw data on the local installation (i.e. computer processors, WinGPC software and printer output). This is done by reading in raw data which are derived from well-known theoretical molar mass distribution data, which are processed in the same way all other raw data will be treated by WinGPC Software. The system verification passes, if the printed results are within 0.5 % of the reference information, which is given in the "Appendix" on page 530. For more information, refer to Agilent WinGPC Software Installation Instructions. Please note, that the information is printed directly to the default printer. No other output is possible to avoid hampering with the data.
Guided Detector Setup: 	The menu item is available for finished runs that include two or more detector signals. During the setup all parameters needed for evaluation are determined using the actual sample. For more details see chapters "Detector Setup" on page 183 and (especially for light scattering) "Setting up light scattering parameters" on page 140. The setup will not be started, if electronic signatures are set within the login. Changes of system parameters like delay or detector factors would affect the results of the electronically signed samples. In order to execute the setup, all signatures need to be removed first.
Show ChromPilot on Startup:	This toggle allows to hide ChromPilot Connection Manager at WinGPC Software launch. This option is only active if WinGPC Software ChromPilot is licensed.
ChromPilot Configuration:	Allows to change the instrument control setup during a WinGPC Software session and (re-)configuration of systems. All changes will be active immediately.
Options:	The options dialog allows to save a default inject dependent option (see status bar) with the method. The selection in the status bar has a higher priority so that, if needed, the inject dependent evaluation can be activated later.

Interface Menu

Table 14 Interface menu

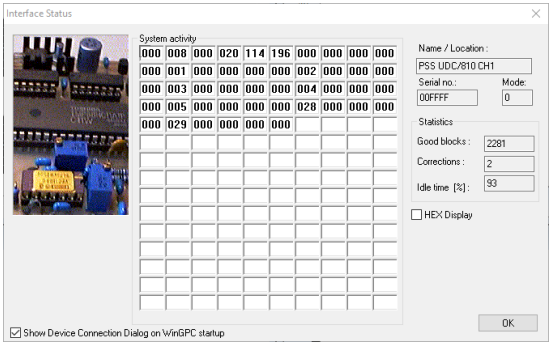
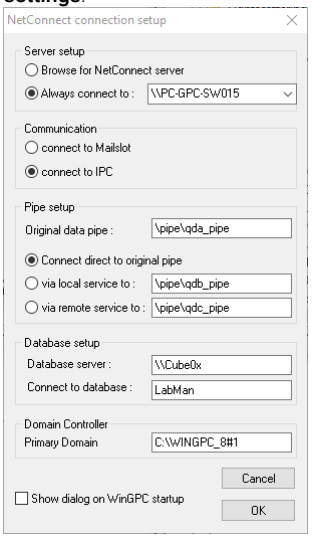
Menu	Description
Information:	Retrieves type and serial number from the PSS data interface firmware and requests an update of the method settings to the Data Interface. This is useful e.g., if the number of detectors or channels to be used have been changed. This dialog can also be used to activate/deactivate the display of the UDC device connection dialog on WinGPC Software startup. 
UDC Verification:	Starts the automatic verification of PSS UDC810 Universal Data Center verification to check the performance of the interface and data transfer to the host PC. Please use the PSS UDC verification plug for advanced hardware testing. Test will take about 5 minutes and UDC hardware test report will be printed automatically to the default Windows printer. No data processing and no user interaction is allowed during the tests.
UDC Training Mode:	Supported in future release

Table 14 Interface menu

Menu	Description
LAN Interface / NetConnect settings: 	This dialog opens when NetConnect option is selected in the WinGPC Software Login screen. Additionally, it can be accessed in this menu during a WinGPC Software session to allow specification and selection of different system parameters. The LAN connection dialog box can set to open with every start of WinGPC Software or can be configured only once. This is accomplished by the check box *Show dialog...*. The **Server Setup** section allows to specify if a connection should always be established to the same NetConnect Server. This dialog/option is only available if licensed with a predecessor of Agilent WinGPC. The dialog opens if the user should be allowed to select the appropriate NetConnect himself in the network context when login into WinGPC Software. No changes should be done in the section **Pipe Setup**; the default value original pipe should be used. The other options are used by administrators for improved flexibility in huge networks. In such cases contact your Agilent representatives before making any changes to the default value.
Reset...:	Initializes the PSS Data Interface. This is comparable to a power off/power on and deletes any buffered data.

Definition Menu

Table 15 Definition menu

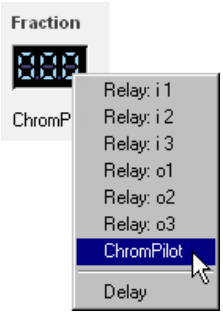
Menu	Description
Number Columns / Number Detectors:	Defines how many columns/concentration detectors will be used in the method. In the instrument layout view the respective number of detector or column symbols, which all can be named, appear. WinGPC Software can process up to 6 independent detector signals per instrument.
Automation properties:	Opens the dialog for setting the parameters for automated data processing and reporting. See chapter "GPC Runs with AutoProcessing" on page 104 for details.
Automation activated:	Activates the WinGPC Software automation features as specified in the **Automation properties** dialog box. A tick mark (✓) shows if automated data processing is active.
Instrument Scheduler:	*Allows to activate instruments automatically at user-defined times and start sequences when instrument ready-conditions are met (temporarily deactivated in WinGPC Software).*
Degasser:	Inserts a degasser icon in the instrument layout view to visualize the use of a degasser for the mobile phase. Alternatively, the icon can be inserted by drag and drop from the resource tree.
Drop Counter:	A drop counter allows for flow-driven data acquisition; i.e. the data capture will stop as the pump flow stops and resumes as the flow is resumed. This command inserts a drop counter icon in the instrument layout view and makes drop counter properties available. This option needs the flow-driven data acquisition module for the WinGPC Software.
Fraction collector: 	Creates a fraction number display box in the instrument layout view. Upon mouse click onto the **fraction** field you can select which relay should be used to control the fraction markers. The fraction positions are marked in the raw data and elugram window with pink triangles on the X-axis and annotated with F# in the chromatogram. Also, the number of the current fraction will be displayed in the Fraction number field of the instrument layout. Since WinGPC Software version 8.2, ChromPilot valve control can be used to configure valves to collect fractions (activate fraction collection in ChromPilot Instrument Manager as well as in your WinGPC Software method as described in this section). Tip: If your fraction collector is connected to a PSS UDC810 Interface, you can use the output relays of the Interface to control a fraction collector.
Chem Heterogeneity:	Only available if the software module is present and activated during login. See chapter "Chemical Heterogeneity Module" on page 433 for details.

Table 15 Definition menu

Menu	Description
Calculate Precision:	Activates/deactivates the automatic determination and reporting of GPC result uncertainty (cf. chapter "Accuracy and Precision of GPC Results" on page 31); major contributions to the uncertainty assessment can be found by clicking on the information icon . From this analysis information on how to improve analytical quality and results will less uncertainty can be obtained.
Start condition / Stop condition:	Allows the automated start/stop of data acquisition at a certain date/time or by specifying an injection number. If a fraction collector is defined data acquisition start/stop can also be controlled by given fraction numbers. To start or stop the data recording at a defined time/inject click the corresponding option. Enter the respective parameters in the dialog box. To start the measurement at a determined time or by a certain injection you must initialize data acquisition. This is done by clicking on the **Pause** button in the Toolwindow **Start/Stop** for not controlled instruments. Do not press the **Record** button. When the start condition has been met, the data acquisition will automatically begin. The option Start/Stop conditions is not available for controlled instruments.
Timed events:	WinGPC Software can control up to 8 output relays in the PSS Data Interface. These relays can be used to e.g., control pumps or to switch off detectors after completion of a measurement. Click on the respective relay and set the parameters and specifications. Specific tips for connecting and controlling the relays can be found in the Appendix in chapter "Relay Control" on page 537. The Guided 2D valve setup is only available if the 2D module is present, activated during login and if no data are presently recorded. See chapter „Guided 2D Valve Setup" on page 187 for details.
Data interval:	This option allows to determine whether all detectors should be recorded with the same data frequency or if different detectors should be recorded with different frequencies. This is useful e.g., if e.g., the pump pressure or temperature should be registered during a run.

Procedure for method repetitions (please note that only identical number of samples per series (repeat) are possible):

At **Definition > Start conditions** select the option **Inject** #1. Select at **Definition > Stop conditions** the menu item **Inject** and enter the number of injects which are to be written into a sequence (login) (e.g., 5). Now enter a useful delay time (e.g., 30 min). Once again change to **Definition > Start conditions**. Now the option **Repeats** can be selected. Enter the number of repeats (e.g., 2). Now initialize the measurement with **Pause**, but do not start it (data recording is triggered by the injector start signal). Enter your sample names for all samples into the window instrument #.

On receiving an inject signal the first series will be started. After the software received the 5th inject signal, it acquires data for 30 more minutes and closes the first series (this series can now be processed even on another computer). With the 6th injection the second data acquisition series starts. After reception of the 10th inject signal and the 30 min. delay time, the second series will be closed and the whole sample sequence is finally completed. Please note, that generally with this procedure the duration between two injections must be longer than the used delay time specified in the **Definition > Stop conditions**.

Language Menu

The language menu allows to switch between different language localizations of the WinGPC Software. Currently *English* and *German* languages are implemented and can be used interactively. The change of the program language is done by clicking on the language name and will affect the user interface.

Detector Setup

If more than one detector is used for GPC measurements, it is necessary to determine with a first measurement the inter detector delay. If one of the detectors is a light scattering detector it is also necessary to determine the instrument constant of the light scattering detector and useful to measure the instrument constant of the concentration detector. Besides these parameters, for multi angle light scattering detectors also a detector normalization is needed.

All these parameters can be measured with one injection using an isotropic scatterer with known concentration (injected mass), known refractive index increment dn/dc and known M_w. For most organic solvents a polystyrene molecular weight standard with M_w around 100.000 Da and a narrow molecular weight distribution is a good choice. For aqueous solvents a pullulan molecular weight standard with M_w around 100.000 Da and a narrow molecular weight distribution should be used.

The setup sample is measured using a method established as described in "Performing a GPC Light Scattering Measurement". After the setup sample has been measured it should be loaded to perform the guided setup.

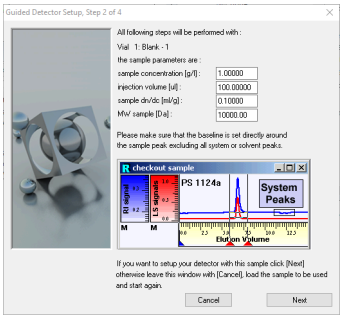


Figure 40 Detector Setup step 2: sample parameter verification

NOTE

This menu item **Method > Detector Setup** in the **Method** window menu is only available for finished measurements. Since the elution volume needs to be corrected according to the detector delay this setup is not allowed during active data acquisition.

After loading the sample, the baseline has to be set so that only polymer peak is inside the baseline borders; all system peaks, solvent peaks or salt peaks should be excluded. It is not necessary to set the integration limits.

The sample parameters like

- concentration
- injection volume
- refractive index increment dn/dc

can be entered in the sample editor (**Raw data** window **Editor > Samples**).

The setup can be started after activating the method window by selecting **Method > Detector Setup**.

The first step of the setup summarizes the prerequisites and describes when the setup has to be done. **Cancel** stops the setup while **Next** leads to step 2 of the setup, where the sample parameters need to be verified.

Wrong entries can be directly changed in the edit fields. Changes that are done here lead to automatic correction of the parameter in the sample editor.

Cancel stops the setup while **Next** leads to step 3 of the setup, where the method parameters need to be verified.

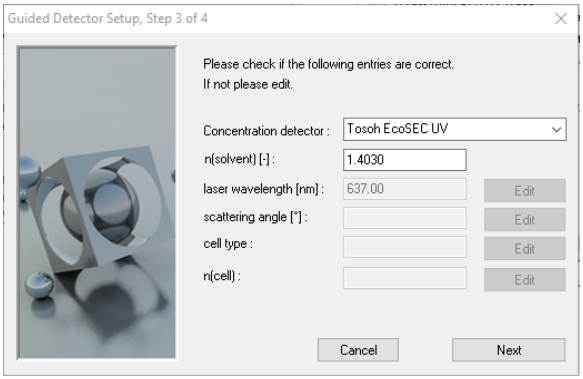


Figure 41 Detector Setup step 3: method parameter

In step 3 all method parameters can be changed if needed. The availability of edit fields depends on the light scattering detector used. The concentration detector and the refractive index of the solvent (n(solvent [-]) can be changed for any detector. The laser wavelength can only be changed for detectors where it is not directly read from the data stream. For the Agilent 1260 MALS detector and legacy PSS SLD2020/9000/7x00, and the BI-MwA this edit field is not useable since the wavelength is directly accessible for WinGPC Software. The same is valid for the scattering angle. Please note that this edit field is not used for multi angle light scattering detectors; it is only used for RALLS and LALLS detectors. Cell type and refractive index of the cell (n(cell)) are only accessible when using Wyatt detectors that require a correction of the scattering angle dependent on the used cell and solvent. For detectors of other vendors this option is not needed. Changes done in step 3 will automatically lead to changes of the method.

Cancel stops the setup while **Next** leads to step 4 of the setup. Please note that this is the last chance to cancel the setup. Step 4 will automatically start the setup without the possibility to stop. The only chance to discard the changes then is to close the measurement without saving the changes.

Step 4 of the setup is different for multi angle light scattering detectors and for detectors that support only one angle. In case of MALLS detectors the detector normalization is also done automatically during the setup. Since this is not necessary for detectors with one angle, step 4 contains for them only the determination of three parameters instead of four.

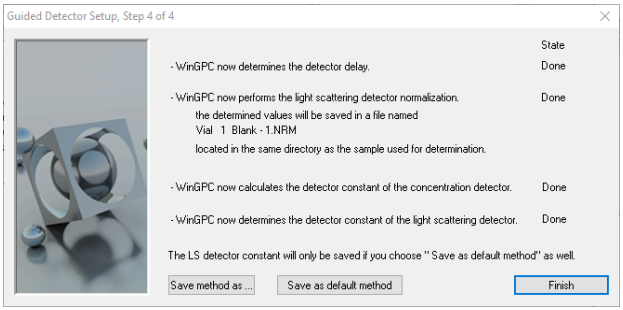


Figure 42 Detector setup step 4 during runtime: two parameters have already been determined while two are still to be processed. For one angle light scattering detectors point 2 is missing.

During the last step of the setup, first the inter detector delay for all detectors is determined. After that for MALLS detectors the normalization is done. The normalization factors are additionally saved in a file (Samplename.nrm) located in the project folder. After that the factor for the concentration detector is determined. This is needed, if the concentration determination methods “Fact.*dn/dc”or “Fact.*conc.” should be used and/or if the mass recovery should be measured. Last but not least the instrument constant of the light scattering detector is measured using the recommended evaluation conditions for light scattering (weight function: ci*ci, MW Fit: linear, Method: injected mass, Calculation limits: 1 %).

After the setup has been finished the buttons **Save method as...**, **Save as default method** and **Finish** become available.

Save method as... allows to save the fine-tuned method for further use. All determined parameters are automatically saved with the new method while settings different from the recommended evaluation options named above are reset to their original setting. If the recommended settings should also be used, the method has to be loaded, the settings have to be selected manually and the method has to be saved again.

Save as default Method should only be used on the PC where the light scattering detector is connected to e.g., where the measurement has been performed with. It updates the settings for the instrument including the light scattering device settings and all temporary files.

Finish allows to leave the dialog. Please note that the changes are only affecting the actual measurement. If the parameters should also be used for other already finished measurements they need to be manually entered. New data, recorded with the methods saved above, are automatically assigned with the determined parameters.

NOTE

All parameters determined during the setup can also be determined by the user itself. For determination of the detector delay refer to chapters "Determination of Detector Delay" on page 28 and "Molar Mass Sensitive Detection" on page 41; for normalization see the description of the light scattering menu item **Options > Light scattering > Normalize**, for the concentration detector factor compare and for the light scattering detector instrument constant calculate: Mw(expected)/ Mw(measured with instrument constant 1).

NOTE

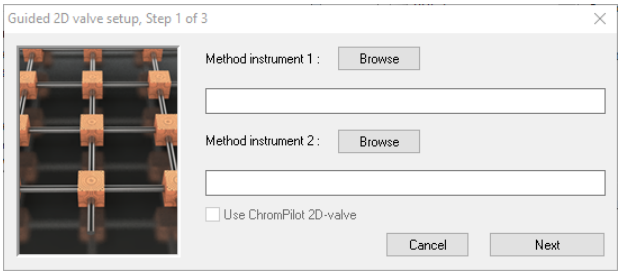
If some parameters are already measured using software packages of other vendors check the Tips & Tricks to see what values can be used in WinGPC Software and if correction factors due to constants using different units are necessary.

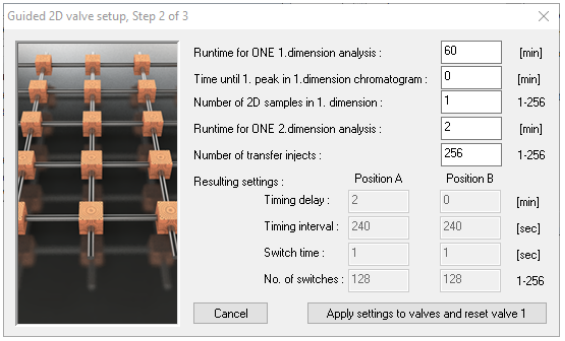
Guided 2D Valve Setup

WinGPC Software allows to perform automated 2-dimensional measurements where chromatographic methods (like e.g HPLC and GPC) can be coupled. The PSS Data Interface can be used to control a transfer valve for the automated sample transfer from one dimension to the other. For the transfer two output relays of the Interface have to be programmed. The Guided 2D valve setup supports the easy setup of the output relay settings and initializes both instruments, so that after that only an injection signal is required for automatic 2D data acquisition. It is only applicable with transfer valves provided by PSS/Agilent, for user supplied 2D valves the transfer valve configuration has to be done manually as described in section.

The setup is located in the **Method** window menu. To open the setup, instrument 1 should be active. The menu item **Definition > Timed Events > Guided 2D valve setup** is only accessible

- during Login at least two instruments are selected
- during Login the option 2D-GPC is activated and
- when no measurements are currently running on instrument 1 and 2 (the instruments used for the 2D measurements)





After entering the setup in the first step methods for instrument 1 and 2 have to be loaded. The **Browse** button allows to browse for methods.

Cancel stops the setup while **Next** leads to step 2 of the setup, where method related parameters have to be entered.

The method related parameters are defined as follows:

Table 16 Method-related parameters

Parameter	Explanation
Runtime for ONE 1. dimension analysis (Time 1st D) [min]	Total runtime for one 2D sample = analysis time for the first dimension
Time until 1st peak in 1st dim. chromatogram (delay) [min]	Delay for the first dimension = delay time: after that time the transfer to the second dimension and the data acquisition for the 2nd dimension starts
Number of 2D samples in 1. dimension	Number of 2D samples to be measured in both dimensions
Runtime for ONE 2. dimension analysis (Time 2nd D) [min]	Analysis time for the second dimension = time until the next fraction is injected into the 2nd dimension
Number of transfer injects	Total number of transfer injects = number of fractions taken from the 1st dimension to inject into the 2nd dimension

The number of transfer injects depends on the settings of the two dimensions. The maximum number of transfer injections is 256 (= maximum number of injects per login) or calculated by

- Max. number of transfer injections = (Time 1st D - delay)/Time 2nd D

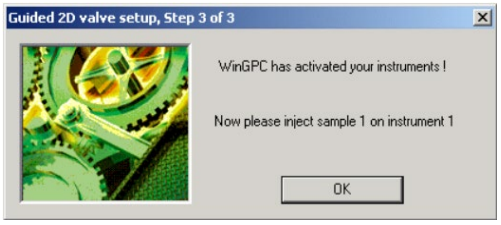
The settings are activated using **Apply settings to valves and reset valve 1**. When the communication between hard- and software works, this will prepare the PSS Data Interface and the switching valve for the 2D run:

- methods for instrument 1 and 2 will be loaded
- **Definition**, **Timed Events**, **Relay o1** and **Relay o2** will be automatically set

Definition, **Start Condition** and **Stop Condition** will be automatically filled for both instruments.

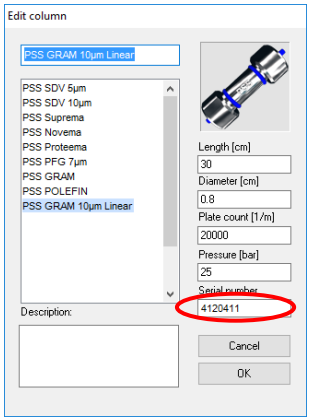
- **Definition**, Start Condition, **Repeats** will be automatically filled
- the valve will automatically be switched to Start position
- the measurements for instrument 1 and 2 will be automatically set to **Pause**

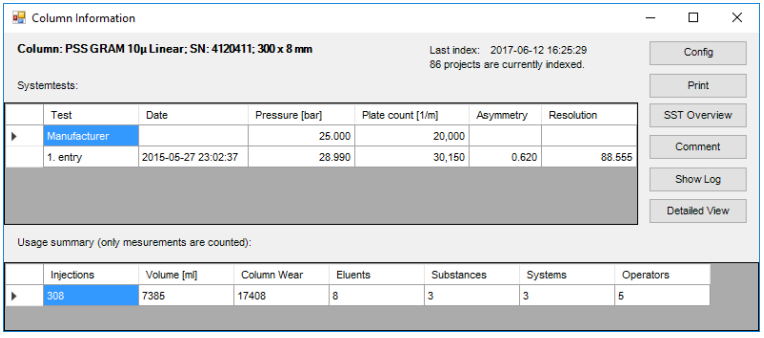
In the following window, step 3, you will be asked to leave the setup with **OK** and to inject the first sample into the first dimension. The trigger signal will then start data acquisition for both dimensions. All 2D samples will be automatically measured.



Column Database

The WinGPC Software column database can be accessed via the symbol next to the column within the instrument layout. The column database provides a summary and detailed history of column usage (including information about sample types, eluents, and column combinations). As a prerequisite you need to enter the serial number of your columns before you acquire data (WinGPC Software ressource tree).





Below the current column name and serial number SST information are shown.

The first entry is taken from the information entered in the ressource tree (e.g., if available taken from the column certificate delivered with the column). The next rows are filled if an SST was executed for this column. As soon as the **System Test**

dialog is acknowledged with **OK**, the results are added to the column database. By default, the first and the last system test will be displayed.

On the right side of the dialog different buttons will lead to additional information:

Table 17 Information buttons

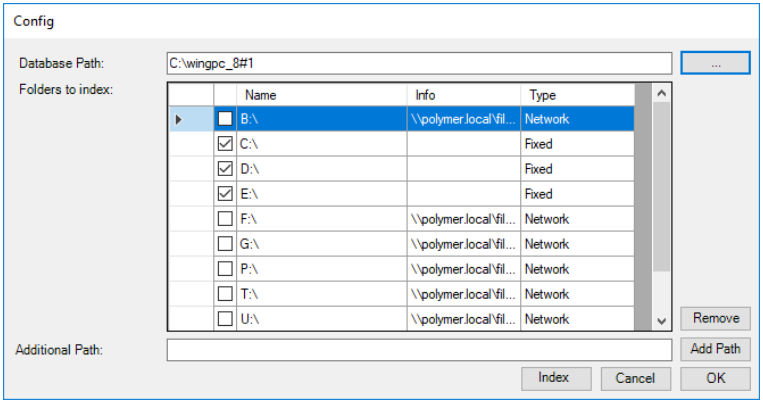
Button	Description
Config 	The database updates automatically, a manual index needs only to be created once (or if new drives with already existing WinGPC Software data will be added to the network). Please note that this dialog might be deactivated (greyed out) due to certain user level restrictions. For further information consult the chapter "WinGPC Compliance " on page 507 of this user guide. The dialog will show the currently selected path for the column database. On networked installations with more than one user or WinGPC Software client, it is recommended to use the same (network) path for all WinGPC Software installations. Thus, all users will have access to the entire information (e.g., including system tests and comments added by any user to the column database).  The Config dialog also allows to select drives (local or networked) for the index and even enter additional (UNC) paths to be indexed (**Add path**). The list will show all drives currently mapped on the PC and any path information added manually. The **Remove** button will remove selected rows from the displayed list. Selecting/deselecting certain rows for index will be achieved by activating/removing the tick mark in front of the row. A (re-)index will be performed by pressing **Index**. You can select **Update** or **Full**. In doubt (e.g., for initial index or after changing drive structures) select **Full**. Depending on the size of the drives and the number of already existing WinGPC Software data the initial index may take some time (recommended to run it over the weekend). The index can run as a background job and if WinGPC Software is closed during the indexing process, it will automatically proceed after WinGPC Software restart.
Print	Prints the column status (overview on systemtests). Printable sections are systemtests data and graphs, a print preview is possible.

Table 17 Information buttons

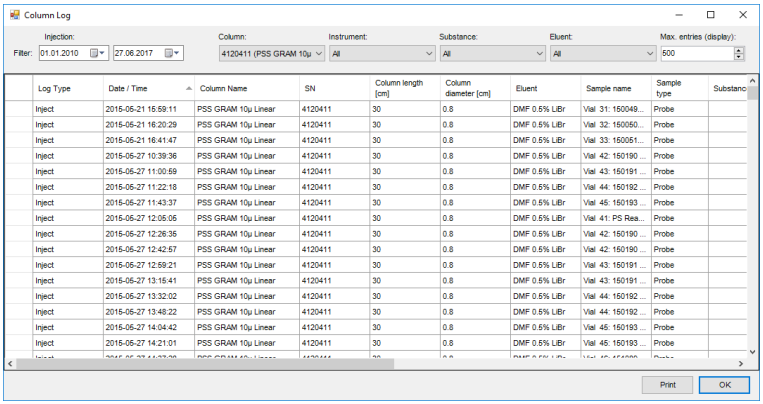
Button	Description
SST Overview	Switches the table on the left-hand side to a list with all system tests for this colum (default display is just the first and the last system test).
Comment	Option to add a comment for the current column, the comment may be displayed in the logbook (see Show Log).
Show Log	 Logbook overview with flexible filter options. Filters are available for: date, column serial number, instrument, substance, and eluent. The drop-down lists automatically provide all entries of the current column database. To change the columns displayed in this dialog right mouse click on the table header.
Detailed View	**Detailed View** opens a segmented dialog. On the left-hand side all columns of the database are listed (sorting according to serial number, column name or column dimensions possible by clicking on the respective table header). After highlighting a specific column, the right-hand side of the dialog will show:

Table 17 Information buttons

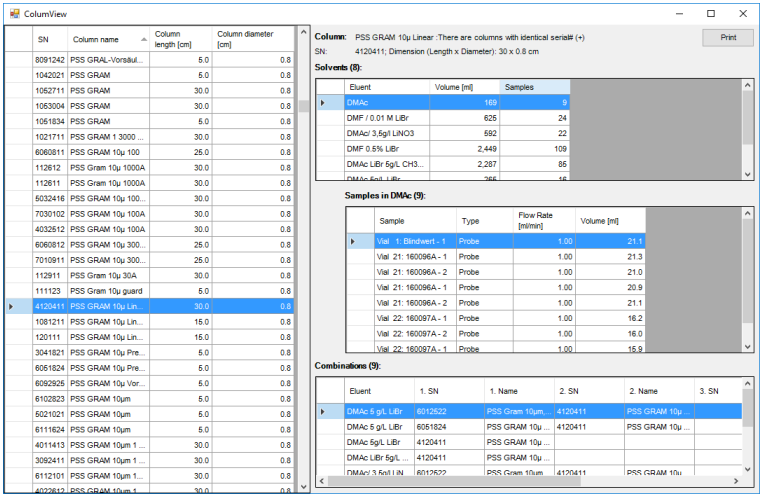
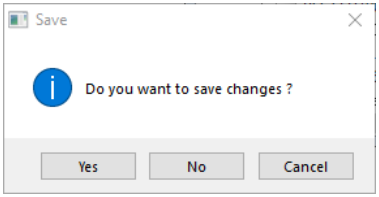
Button	Description
	 Column name and serial number: If the same serial number was registered more than once (e.g., a SDV 10 µm column was entered as “PSS SDV 10 µm 1000Å” and a second time as “SDV 10 µ 10^3 Å”), then this will be mentioned and multiple entries will be displayed by clicking on (+). If multiple entries are available, the newest one will be used as the default column name. Solvents: Lists all solvents used with this serial number. To view more details, select one of the solvents, then the other tables (Samples and Combinations) will show information about this specific solvent. Samples in... : Sample details for the selected column and solvent. Combinations... : Lists the column combinations for this serial number in the selected eluent.
The lower part displays a usage summary containing:	
Injections:	Volume which was pumped through the column during the measurements (note that the total solvent volume which was pumped through the columns is higher, since only the amount during data acquisition can be calculated)
Volume [ml]:	Indicator for the wear of a column, considering the pressure during injects, flow rate and analysis time (note that the real column wear can be higher since only the amount during data acquisition can be calculated, requires inject pressure information – only available for controlled systems)
Column Wear:	Number of different eluents used with this column, taken from WinGPC Software methods
Eluents:	Number of different substances used with this column, taken from the substance

Table 17 Information buttons

Button	Description
	selected in the **Sequence Manager** (or **Sample Editor**), note that the substance list contains not only specific polymers but also (since WinGPC Software version 8.3) substance classes to support more comprehensive sample information
Substance:	Number of different GPC systems used with this column. The name is taken from the entry visible in the **Method** Window below the instrument number (for controlled systems taken from the name entered in the **Configuration Manager**).
Systems:	Number of different operators as saved within the **Method** Window (default: username that logged in to WinGPC; Software if a WinGPC Software method was loaded: name saved within the WinGPC Software method or loaded within the sequence)
Operators:	Volume which was pumped through the column during the measurements (note that the total solvent volume which was pumped through the columns is higher, since only the amount during data acquisition can be calculated)

Raw Data Window

The raw data window shows the real-time view of raw data capture or a completed and loaded runs from disk. In this window calibration files can be loaded and sample names can be entered. Finally, the internal standard will be set and the baseline defined in the **Raw Data** window.



WinGPC Software updates the database every time a run is closed or when invoking the **Raw Data > Save Analysis** command. However, sometimes it is useful *not* to save changes in data processing parameters if they do not improve the results. The raw data close dialog in WinGPC Software allows to save the changes (**Yes**), discard the changes (**No**) or cancel out of the close dialog. These dialog options are available when using the **Close** button in the **Raw Data** window or when using the close icon in the icon bar. When runs are closed automatically on exiting the WinGPC Software session, then all changes are automatically saved to the database without user interaction. Alternatively, runs could be opened as **read-only**, but this required a prior knowledge which cannot be assumed in experimental labs.

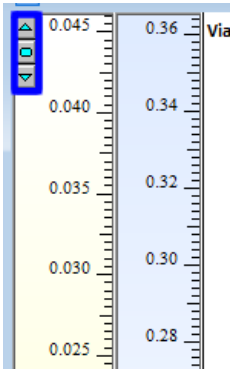
NOTE

If you close the WinGPC Software session without closing the **Raw Data** windows separately first, then WinGPC Software assumes the user wants to save the changes and does not ask for user input.

General Features

The axes which are assigned to the various detector signals are located on the left edge of the raw data window. Because WinGPC Software can process several detectors simultaneously, the selection of an active curve is necessary to make peak searches and similar features unique. The active detector signal is always displayed in the status bar. By clicking on the detector name, another detector can be selected from the pop-up list of available detectors.

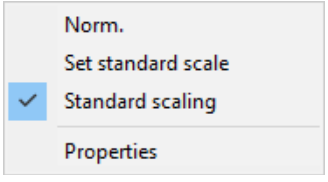
There are several ways to change the Y-axis scale:



When the mouse cursor is in the top left position of any Y-axis, the interactive scale buttons (see Figure on the left) will appear. (Please note that these interactive scale buttons will only appear if this window is active.)

The arrow up (down) button is used to increase (decrease) the gain of the detector signal interactively. If the Y-axis was in normalized state, pressing any arrow button will change that to the manual scale state indicated by an **M** below this axis.

The square button in the middle toggles between normalized (autoscale) (**N**) and manual (**M**) scaling. The normalized scale will use the largest and smallest detector signal value within the windows to be scaled automatically to the minimum and maximum vertical window positions. If the signal is in normalized state, an **N** will appear below the axis. Alternatively, the Y-axis context menu **Norm.** can be used to toggle between both display options.

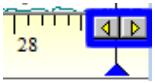


Alternatively, the context menu of any Y-axis can be used to toggle between manual and normalized scaling (command: **Norm.**). The context menu also allows to define (**Set standard scale**) and use (Standard scaling) a preset Y-axis scale. If the standard scale is in use, the menu item shows a tick mark and an **S** designator will appear below this axis.

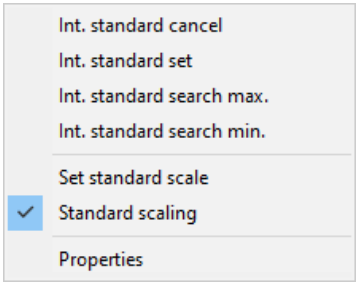
NOTE

If the curve disappears from of the window through manual scaling or by using the scroll bars, a retrieval may be difficult. Change to standardized representation. The curve will immediately appear again in the window.

The possibilities for the scaling of the X-axis are:



When the mouse cursor is in the bottom right position of the X-axis, the interactive scale buttons (see Figure above) will become visible. The right (left) arrow button is used to increase (decrease) the elution volume (or time) displayed interactively. (Please note that these interactive scale buttons will only appear if this window is active.)



Alternatively, manual scaling is available from the X-axis context menu by typing in minimum and maximum values in the **Set standard scale** dialog. If the standard scale is in use, the menu item shows a tick mark.

NOTE

The method file also saves the scaling settings of the various axes. These will be loaded when the method file is loaded.

Windows can be zoomed (magnifier effect) for better inspection of certain chromatogram sections. Click into the window and start by pressing the left mouse button where the zoom area should start. Keep the button pressed and drag the appearing rectangle until it encloses the section which should be magnified. After releasing the left mouse button, the magnified section is displayed in full scale. To undo one or all zoom actions (unzoom) click with the right mouse button inside the window.

Colored triangles with line cursors in the window can be seen underneath the X-axis (see next figure). Blue triangles are injection positions, red triangles define start and end positions of the baseline (only visible after defining the baseline), green triangles define the position of the internal standard. The triangle for the internal standard is dark green, if the reference position defined in the calibration file is use for this analysis. After setting the internal standard it will be shown in light green to indicate the difference. The purple marks define different fractions (only for runs with fraction collector operation).

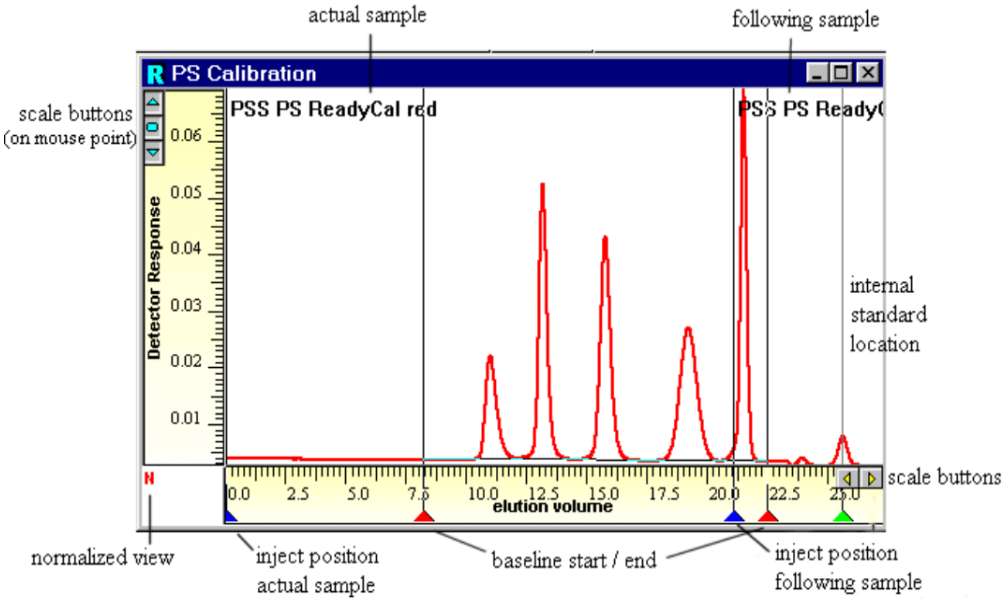


Figure 43 WinGPC Software Raw Data Window with interactive control of display and processing parameters

Table 18 Color explanation

Color of triangle	Explanation
Red	Baseline start/end
Blue	Inject position
Dark green	Location of the internal standards (not set yet)
Light green	Location of the internal standards (if already set)
Purple	Fraction markers

Baseline Settings

First select the inject that should be evaluated either by selecting the respective sample name under **Inject** or through the field *Sample* on the status bar. The injection marker for the sample (blue triangle) is now located on the lower left edge of the chromatogram. Click onto the injection marker and drag the first baseline marker (identified by a red triangle and the baseline). The baseline marker will be set at the x-position where the mouse button was released. Set the second baseline marker in the same way. Corrections of the baseline can be carried out by dragging the corresponding baseline marker and dropping it at the desired position.

NOTE

In the normalized scale the baseline might look horizontal. Switch to the manual scale to set the baseline exactly. Switching back to normalized view shows the complete chromatogram. Use the **Norm** function in the Y-axis context menu to toggle between both views.

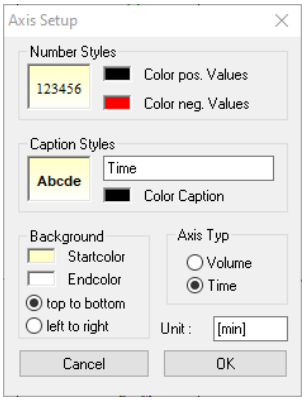
X-Axis Context Menu

These functions are accessible from the X-axis context menu, if you click on the x-axis scale with the right mouse button. A popup menu appears in which the following functions can be selected:

Table 19 X-axis context menu

Function	Description
Internal standard cancel:	Deletes the value for the internal standard of the sample.
Internal standard set:	Opens a dialog box in which the value for the internal standard for this sample can be set. (Deviations of more than 20 % from the position specified in the calibration file will generate an error message. The software assumes that the chromatographic system has changed too much to be compensated by the internal standard and the command is ignored). The x-value is the x-position of the mouse cursor where the function has been selected. After setting the internal standard, the marker changes from dark to light green to indicate that the internal standards correction has been used for this sample. The status of the internal standard correction for this sample can be checked also at the **Raw Data > Information** menu.
Int. standard search maximum/minimum:	Searches from the mouse cursor position where the function has been selected to the next relative maximum/minimum in the active curve and sets the internal standard to this position. (Deviations of more than 20 % from the position specified in the calibration file will generate an error message. The software assumes that the chromatographic system has changed too much to be compensated by the internal standard and the command is ignored). The x-value is the x-position of the mouse cursor where the function has been selected. After setting the internal standard, the marker changes from dark to light green to indicate that the internal standards correction has been used for this sample. When the minimum/maximum is found an information window opens, where you can compare the value found with the reference value stored in the active calibration file. The status of the internal standard correction for this sample can be checked also at the **Calibration > Information** menu.
Set standard scale:	Allows the manual setting of the displayed X-axis range of the chromatogram and sets the default standard scale.
Standard scaling:	Restores the scale of the last manual scaling (e.g., after editing the scaling by the arrow keys or the scroll bar of the X-axis). A tick mark in the context menu indicates, if the standard scale is in use.

Table 19 X-axis context menu

Function	Description
Properties:	This context menu is available on each axis in the WinGPC Software. It allows setting axes properties individually. For example, text attributes (font, size, color etc) and background properties of the axis can be defined. It is also possible to switch from elution volume to elution time representation for X-axis properties. The axis properties will be saved for each sequence individually. 

Y-Axis Context Menu

These functions are available from the context menu, if you click on the Y-axis scale with the right mouse button. A popup menu appears in which the following functions can be selected:

Table 20 Y-axis Context menu

Functions	Description
Norm.:	Toggles between normalized and manually scaled view. This status is indicated by the **N** (normalized view) or **M** (manual scale) below the corresponding Y-axis.
Set standard scale:	Allows the manual setting of the displayed Y-range of the chromatogram and sets the default standard scale.
Standard scaling:	Use preset Y-axis scale as defined in **Set standard scale**. If the standard scale is in use, this command shows a tick mark, and a **S** designator appears below the axis.
Tip: The method file also saves the scaling settings of the various axes. These will be loaded when the method file is retrieved from the file system.	
Properties:	This context menu is available on each axis in the WinGPC Software. It allows setting axes properties individually. For example, axis labels, text attributes (font, size, color etc) and background properties of the axis can be defined.
Tip: It can be very useful to assign the Y-axis caption in the same color as the detector signal and give the axis a descriptive name (e.g., UV signal).	

Raw Data Menu

Table 21 Raw data menu

Function	Description
Load:	Changes into the window **Project management** from where raw data or projects for evaluation can be loaded.
Save:	Forces writing of unsaved data to disk during the data acquisition. For safety reasons WinGPC Software saves the data independently as well in pre-defined intervals. Normally this command does not have to be executed manually.
Save analysis:	Saves the current state of evaluation and updates the entries in the project database (*.FSX, *.INX). When closing a data set WinGPC Software saves the current state of all evaluations in the sequence.
Name raw data:	Allows to name a raw data series. The raw data window will be named with a free form text (max. 32 characters). In the window **Project management**, the label appears on the right-hand side and helps to identify runs more easily.
Edit Comment:	Allows to enter a raw data text (comments, hints for data treatment, sample preparation details, observations, etc.). Up to 1024 characters can be entered for each injection (sample) separately. Alternatively, the  icon in the status bar can be used to open the comment dialog box. This icon is gray if no comment has been entered for this sample, if text has been entered it is highlighted in green.
Merge JCAMP:	Imports data from any JCAMP FTIR file prior to version 5 into the WinGPC Software project database. Choose which of the available detectors should be overwritten with the imported FTIR data after using the [Raw Data] [Save Analysis] menu to permanently modify the project database. WARNING The data which have been previously acquired on this channel will be overwritten. For safety reasons a dummy detector should always be defined in the method when FTIR data shall be imported later.

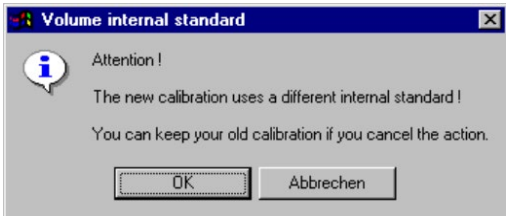
Printer Setup:	Allows definition of parameters of the active printer. However, the active printer must be defined in the Windows Control Panel. Landscape format prints the graphics on a full page. Portrait format prints the graphics, full method documentation and results by default. The printed information in the portrait layout can be selected for automated runs in the **Definition > Automation Settings** dialog box. The exact information of the portrait format printout depends on which window will be printed. For color printers you can select color or monochrome printing depending on the printer driver options. The color of the curves in monochrome printouts are mapped automatically to a line style to avoid unreadable black and white prints. The correlation between curve color and line style in monochrome print is listed in "Curve Colors and Line Styles in Monochrome Printing" on page 534.
Print:	Prints the current contents of the raw data window to the default printer. The graphics are always printed in WYSIWYG mode, i.e. the current window display will be printed identically as shown.
Print multiple injects:	Opens a batch print dialog which allows to print a selectable list of samples to the Windows default printer. The type of printed information (which window) and the list of injections can be selected. Printed reports are identical to the individual reports which would be printed from the standard print report. User defined reports are available with the WinGPC Software ReportDesigner option which allow full report customization (see chapter "WinGPC Software ReportDesigner" on page 333). User selectable reports can be selected using the **report** option in the **Serial print options** dialog. Please make sure that the samples have been processed prior to printing. Please note that this option will be grayed for data recorded with former WinGPC Software versions that have not been converted to the new data format.
Page Preview:	Shows a print preview in a separate print-preview window, which can be sized and moved. At the same time this command copies the preview contents to the Windows clipboard. When printing in portrait format, the graphics and the text for the measurements will shown, while landscape format will print only the graphics.
Print width options:	Allows the adjustment of font size on the print out. As an option a barcode can be printed on each printout. Using the "prim.color"/"prim. black_white" command will force printouts in color/black&white independent on any printer driver information. This can be useful if Postscript color or PDF printers are used, whose printer drivers sometimes report only black&white capabilities.
ASCII save captured data:	Saves the displayed raw data as text file. Baseline correction and correction of the volume axis are not being considered. Such data can be exported from the elugram window.

ASCII save injects:	Saves the injects of the loaded data set as text file. Output will be: Inject number, elution volume since inject number 1, date and time of inject, sample name.
	Example:
	`1: 0.000 ml, Monday 21.02.94 16:02:49:12, BHT`
	`2: 44.348 ml, Monday 21.02.94 16:47:10:01, PS-Mix1`
	`3: 76.478 ml, Monday 21.02.94 17:19:17:79, PS-Mix2`
	`4:124.072 ml, Monday 21.02.94 18:06:53:45, PS-Mix3`
	`5:160.015 ml, Monday 21.02.94 18:42:50:04, NBS706`

Edit Report:	Opens the WinGPC Software ReportDesigner for creation and modification of report templates. The WinGPC Software ReportDesigner is an option, which has to be purchased separately. See chapter "WinGPC Software ReportDesigner" on page 333 and the separate WinGPC Software ReportDesigner documentation for details.
Print Report:	Prints a customized report which was created in the WinGPC Software ReportDesigner (see chapter "WinGPC Software ReportDesigner" on page 333 for details). Report template files can be selected before printing. Report output can be either send to the printer, to screen, to disk or published as HTML.
Information:	Opens a status window in which the name of measurement, number of data points, use of internal standard etc. will be displayed. This information can be useful for troubleshooting.

Calibration Data Menu

Table 22 Calibration data menu

Function	Definition
Load:	Loads a calibration file (*.CAL) for evaluation of the active raw data file. This calibration file is valid for all samples of the series until another calibration file is loaded. The last loaded calibration file is saved together with the baseline data when closing the raw data window. If samples within a measurement series are evaluated with various calibration files, then either various calibration files must be loaded or the calibration curve must be converted internally through universal calibration (see command **Universal calibration** below). WinGPC Software checks the internal standard position of the current and future calibration file and displays a warning if the reference positions of the two calibrations are different. In such cases the internal standard positions of the samples cannot be used any longer since WinGPC Software cannot predict the consequences of an automatic update. Until now WinGPC Software disabled internal standard corrections automatically for all samples, if a new calibration file with a different reference position was loaded and displayed a warning dialog. Canceling the action was not possible. The current WinGPC Software version still shows the warning dialog, but also allows to undo the loading of a calibration file with different internal standard position.  *Canceling loading calibration file with different internal standard position*
Load Copo sec.:	Loads a second calibration file which is needed for data processing of copolymers using multiple concentration detectors (see chapter "Copolymer Analysis Software Module" for details). Please note that this option will be grayed out if the WinGPC Software Copolymer data analysis option is not activated the WinGPC Software login screen.

Send to calibration:	Allows to send the calibration curve of a (finished or current) run to the Calibration window including all data points and calibration parameters. A new calibration table will be created in the **Calibration** Window, even if it is not open or in the foreground. In such a case the **Calibration** window has to be opened or brought on top by clicking on the calibration icon on the icon bar or using the menu **Window > Calibration**. The temporary calibration is identified in the **Calibration** window by the name "Memoryfile .###" and can be saved as a conventional *.CAL file with **File > Save as**. It then acts exactly as a calibration done from a calibration run. Please note that this feature only works with calibration and raw data files that have been created by the current WinGPC Software version; earlier file versions do not contain the necessary information and the menu item is grayed out.

Tip: This feature is very useful if a "lost" calibration file should be restored from a finished run or to compare different versions of a calibration which have been retrieved from raw data files to check system variations.

Tip: This new function enables the temporary change of a calibration curve in the raw data window and its consequences on molar mass results. This is accomplished by temporarily saving the file "Memoryfile .###" in the Calibration window with **File > Save** (do not use **Save As...** for that purpose). This will send the calibration information to the **Raw Data** window and automatically update the results and graphics in the **Mass distribution** window.

Universal calibration:	Calculates a calibration curve for each sample using universal calibration. The sample specific calibration curve is calculated from the loaded calibration curve and it's Mark-Houwink Parameters. Furthermore, the Mark-Houwink parameters entered in the sample editor (**Editor > Samples**) will be used for the unknown sample. Activation of the **Universal Calibration** option is recognizable in the status bar in the field **Calibration** by describing the calibration file as "UE→Calibration file".
Information:	Opens a dialog box in which the name of the calibration curve, elution volume of first and last point of the calibration curve, the reference value for the internal standard of the calibration curve and the sample under investigation as well as the MH Parameters of calibration curve, is shown

Injects Menu

The menu **Inject** serves to move from sample injection to sample injection within a run sequence. **Inject > Beginning** shows the raw data from the start of the data recording, while **Inject >** *No. "Sample name"* defines the injection time of the selected sample as chromatogram time zero. Simultaneously the injection marker, will be placed at the left edge of the raw data window. Alternatively, the list of injection can be accessed from the status bar using the Sample field (see section "Sample and Calibration" on page 158).

NOTE

A right mouse click on the title bar of the **Raw data** window opens a dialog displaying the list of injections as a shortcut.

NOTE

Changing the active inject is also possible without the **Inject** menu. There are 2 possibilities. With the arrow keys (←→) it is possible to move forward or backward by one inject. This shortcut is available for all windows, while the same window remains active. Another method is to use the field Sample in the status line, from where any Sample of any opened or running measurement can be selected.

Editor Menu

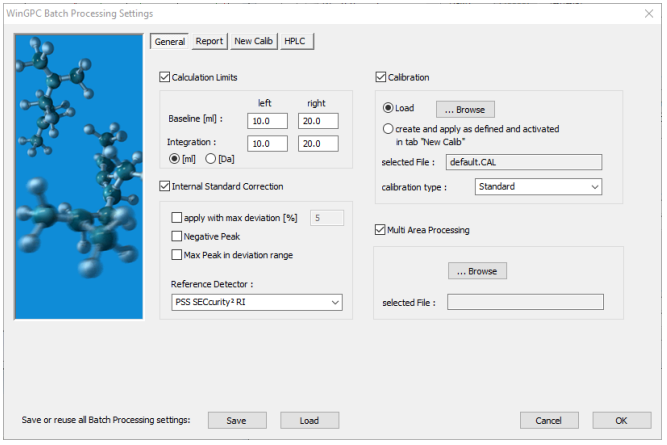
Table 23 Editor menu

Function	Definition
Samples:	The **Editor > Samples** command allows to assign sample names to the injections and add further sample related information. Sample names and parameters can be edited before, during and after data acquisition. Upon clicking on an entry with the label "Sample#" the selected entry is copied into the edit line (first line). The sample name can be edited there. Sample parameters can then be entered. Details can be found in chapter "WinGPC Software Sample Editor" on page 211. If the GPC system is digitally controlled by the WinGPC Software ChromPilot the SequenceManager is opened instead of the sample editor. See chapter "ChromPilot System Control" on page 466 for details.
Slice data:	The **Editor > Slice data** command copies the data of the raw data window to the data editor window. In the data editor window the data table will be displayed and can be processed and exported.

Options Menu

Table 24 Options Menu

Function	Description
Analysis:	
• **Positive/ Negative Peaks**	Determines which peaks will be included in the evaluation. Evaluation of negative peaks will result in mirroring these at the baseline for inclusion in the elugram window. Thus, negative peaks will appear as positive peaks in the elugram.
• **At one/two mark(s)**	Determines if the data processing will be performed after the first baseline marker is set or when both baseline markers are set. The option **with one mark** can save time and effort in the case of file imports **File > Import from...** from HPLC data file, which are generally acquired from the injection point to a stop time.
• **Quick analysis:**	This option opens a dialog window in which automation parameters can be entered (see Figure below). The quick analysis complements the automation possibilities within the WinGPC Software and represents the offline approach. Consequently, this option can only be used for already measured and recorded data. Thus, e.g., the position of baseline and integration limits for the current and all following samples (depends on inject option, see details below) of a series can be entered. The dialog box will use the baseline and integration settings of the current injection as the default parameters, if already set. Existing evaluations of samples will be overwritten by this command!



The WinGPC Software inject options (available by Toolwindow **Comment and options**) can be used to direct the batch process (for details see chapter "Comment and Options" on page 159). Thus, different batch analysis tasks can be selected for the offline automation process.

Table 24 Options Menu

Function	Description
	Note: With highlighted **Raw Data** window, the menu item **Options > Analysis > Quick analysis** will open the same dialog window as for the automation properties (**Definition > Automation properties**) and desired settings can be adjusted in the same way (refer to chapter "GPC Runs with AutoProcessing" on page 128 for details). In previous WinGPC Software versions the quick analysis window is limited in terms of selectable options and parameter settings. Full description of all details to the sections and tabs of the new quick analysis dialog window (implemented with WinGPC Software version 8.3) can be consulted in chapter "GPC Runs with AutoProcessing" on page 128.
TIP: Delete evaluation of several samples: The deletion of baselines and integration limits for all samples of a measurement series from a defined sample, can be reached by selecting the selected sample and use **quick analysis** with 0 as start and end volume for the baseline. Thereby, deleting all evaluation limits also of the following samples.	
• **Multi area create:**	This option uses the current sample to automatically create areas based on a HPLC evaluation. Each recognized peak will handeled as a separate area. The properties may be edited and saved in the multi area settings dialog. *Note:* This option overwrites all existing multi area settings (use **Multi area settings** to edit the parameters).
• **Multi area settings:**	This option allows to define several parts (areas) of a chromatogram and process them simultaneously under identical conditions. It is also possible to combine separate chromatogram ranges and process them together. For further flexibility, the user can specify which results are needed for which part of the chromatogram in the multi-area report; the result parameter list is the same as for conventional data processing with WinGPC Software. Details are described in chapter "Multi Area Data Analysis" on page 220.
• **Multi area activate:**	This toggle switch is used to switch on/off multi-area processing. The multi-area data analysis is not active by default. If the multi-area analysis mode is active, the WinGPC Software standard report will consist of a second page showing the result table of the multi-area analysis for all concentrations detectors which are displayed in the raw data window.
• **Multi area results:**	The results of a multi-area analysis can be displayed interactively on-screen, printed or saved to a file. A two-page report is printed when using the standard print command; the first page is identical to the conventional report for this window, the second page contains the multi-area results as specified in the settings. Alternatively, the WinGPC Software ReportDesigner (optional) to design a specific multi-area report, select additional data and enhance the report layout (e.g., table grid, shading) in desktop publishing quality.
• **Disregard:**	Deletes the baseline and integration limits of the selected sample.

Baseline:	Besides the standard baseline (linear connection between the baseline markers and the curve) WinGPC Software allows the use of curved baselines as well as the definition of baseline endpoints, which are not placed on the curve. Free definable baselines will be defined by two or more points, which have the same color as the active curve. These points can be moved to the desired positions by dragging them with the mouse. The non-standard baseline is displayed thicker than the normal baseline. The non-standard baselines have to be defined for each curve separately, such that the baseline form as well as its start and end points for various detectors can be set differently.
Color Scheme:	Allows the selection of window background color. **Palette** allows to activate a self defined color background. The creation and selection of the color background is done in the elugram window (see **Options > Palette** in chapter "Elugram Window" on page 224). It is also possible to load a bitmap as background (bitmaps and colored backgrounds are not printed; however, this can be done using screenshots). Different colored backgrounds e.g., are useful to distinguish several instruments, thereby making it immediately obvious in which real-time presentation the data of which **Raw data** window is displayed.
Grids:	Allows the representation of different grids in the graphics windows.
Lines:	Selection of line thickness for all signals (traces) in the current run.

WinGPC Software Sample Editor

If the GPC system is digitally controlled by the WinGPC Software ChromPilot the **Sequence Manager** is opened instead of the sample editor. See chapter "ChromPilot System Control" on page 466 for details how autosamplers are controlled. If a GPC system is not digitally controlled by WinGPC Software, or a finished run is reviewed then the Sample Editor is used to manage the sample list and sample parameters.

The **Editor > Samples** option in the **Raw data** window menu allows to assign sample names to the injections made and to add sample related information. Sample names and parameters can be edited before, during and after data acquisition. Up to 256 injects/samples can be measured into one login and therefore named in the sample editor.

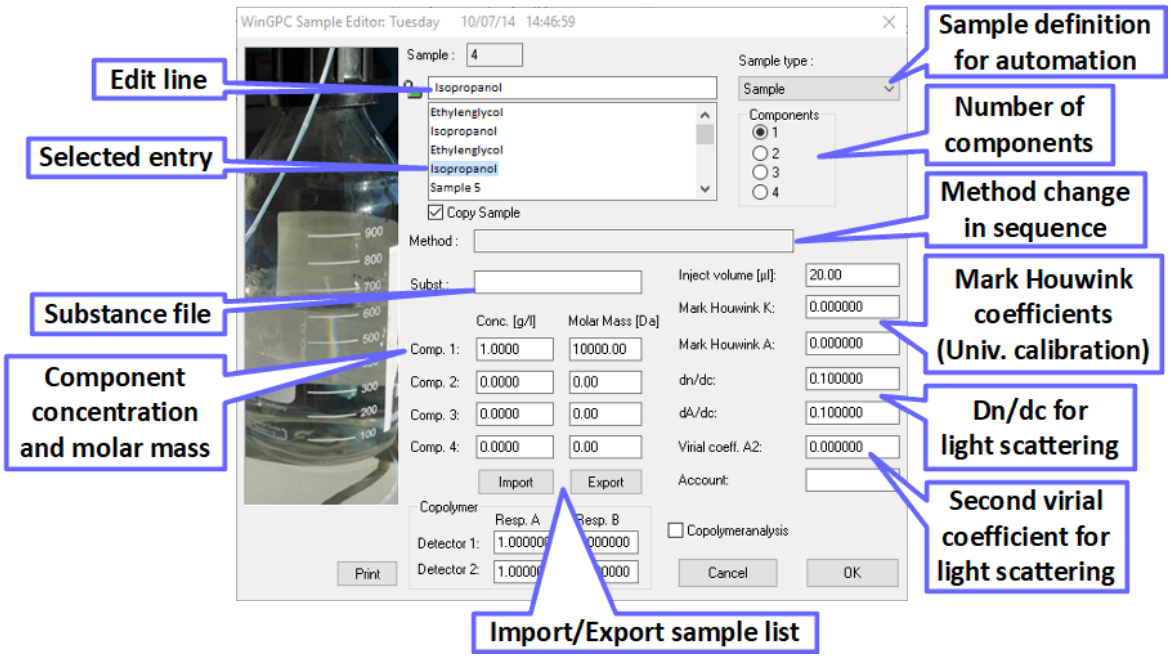


Figure 44 The Sample Editor summarizes all sample dependent information for documentation and processing

The selected entry is copied into the edit line (first line) by clicking on an entry with the label **Sample#**. The sample name can be edited in the edit line not in the sequence list below.

The editor is designed that up to 4 different components can be entered (e.g., calibration mixtures with 4 different molecular weight standards). For each (calibration) component its concentration and molar mass can be entered separately.

A correct concentration entry is necessary for GPC light scattering and/or GPC viscometry measurements if the concentration determination methods **injected mass**, **Fact.*Conc**. or **Conc.*dn/dc** should be used. For conventional GPC measurements the concentration is not required for evaluytion but can be entered for documentation. The entered molar mass values are used for interactive or automatic calibration/recalibration but not for data evaluation.

When changing the default entries for concentration (1.0000 g/l) and/or molar mass (10000.00 Da) of components, the component counter automatically adjusts to the number of components.

The Inject volume entered for the individual sample will overwrite the default value entered in the **method** window. This is useful if variable injection volumes are used

in a run and takes care of autosamplers with variable injection volume capability. A correct entry of the injection volume is necessary for GPC-light scattering and/or GPC-viscometry measurements where the concentration determination methods **injected mass**, **Fact.*Conc**. or **Conc.*dn/dc** should be used.

The Mark Houwink parameters K and A of the sample can be entered to allow the creation of calibration curves by universal calibration for each sample (see **Calibration > Universal Calibration**).

The refractive index increment (dn/dc) can be entered, if absolute concentrations must be calculated from the refractometer signal (e.g., light scattering or viscometer evaluation) to account for samples with different response factors. It is required for the analysis of on-line light scattering detector signals. WinGPC Software can determine the dn/dc online for unknowns, but for higher precession Agilent recommends measuring this value independent of GPC measurements.

If an UV detector is used, the extinction coefficient dA/dc may be entered as well. It is required to define the signal type as **UV** in the **Method** window (see chapter "Instrument Layout View" on page 170). Otherwise, the dA/dc value will not be used for further calculations.

For frequently used polymers, substance specific parameters like the Mark-Houwink constants and the refractive index increment can be saved in a text file **Substanz.ACC** in the program directory. To read in the data from this file, click on the name **Subst** next to the field Substance name. A list of substances saved in the substance file appears and the respective polymer type can be selected. The fields **MH coefficient**, **MH exponent** and **dn/dc** will be filled in with the stored values automatically.

The substance list provided during installation is extended by a list of various substance classes, so the substance information can be used to create more comprehensive statistics, even if no MH parameters or dn/dc values are available.

The substance file **Substanz.ACC** is an ASCII file with following structure:

```
"SUBST:","Substance name"
"MHSPRE :", MHCoefficient K
"MHSEXP :", MHExponent A
"DNDC:", Refractive index increment
"DADC:", Extinction coefficient dA/dc
"A2:", 2. Virial coefficient A2
"EOF"
```

An example of a substance file with values for polystyrene and PMMA in THF is shown in the following. Not all information is required for proper interpretation of the data set (minimum: **SUBST:** and **EOF**):

```
"DATCHG:",#1996-02-29 17:56:06#
"PRNAME:",""
"CUSTOMR:","PSS"
"DATE:",#1995-07-25 11:05:23#
"SUBST :","Polystyrene/THF/30°C at 633nm"
"SOLVENT:",THF
"CONC :",
"DNDC:",0.187
"ETA   :",0.0
"MHSPRE :",.01363
"MHSEXP:",0.714
"PH:",#NULL#
"EOF "

"DATCHG:",#1996-02-29 17:56:06#
"PRNAME:",""
"CUSTOMR:","PSS"
"DATE:",#1995-07-25 11:05:23#
"SUBST :","PMMA/THF/30°C at 633nm"
"SOLVENT:",THF
"CONC :",
"DNDC:",0.087
"ETA   :",0.0
"MHSPRE :",.01298
"MHSEXP:",0.688
"PH:",#NULL#
"EOF "
```

If known, the second virial coefficient (Virial coeff. A2) can be entered for every sample to improve data evaluation for high molar mass species in GPC-LS experiments. If the A2 value is not known, the default value ("0") should be used.

The field Account serves for the documentation of whom to bill for the analysis.

NOTE

The search sample query form allows to search for the account so that statistics can be done using the WinGPC Software search options.

The **Print** button allows to print complete sequence lists. This option is only available for finished runs and not during data capture. A pre-formatted report layout is used to print the sequence list. This report layout can be edited if the WinGPC Software ReportDesigner option is licensed.

If the copolymer module is active the sample editor is expanded with edit fields for the response factors for the components A and B. Each component has a response factor for Detector 1 and 2, so this results in 4 additional edit fields. The option “copolymeranalysis” needs to be checked if the sample should be evaluated with the copolymer option (see chapter “Copolymer Analysis Software Module” on page 429 for details).

Entering Sample Sequences

To assign the sample names and parameters to injection marks open the sample editor and click with the left mouse button on **Sample#**. This copies the selected entry into the edit field. The sample name can then be entered in the edit line. All other sample parameters can now be entered for this sample.

If another sample should be named, click on the next entry **Sample#+1** to transfer it to the edit field. By activating the **copy sample** option before, the sample entries of the last sample will be copied and inserted for the new sample to be transferred to the edit line.

NOTE

This will only be done, if this new sample still holds the standard sample name (which is Sample #)).

The button **Delete** permits the deletion of the marked sample from the sample editor. The button **Insert** permits the insertion of sample names in front of the marked sample.

NOTE

After inserting and/or deleting samples the number for the standard sample names (e.g., "Sample 5") is no longer identical with the number of injects. The correct inject number will be displayed in the upper left corner of the sample editor.

Example:

Insertion of a new sample after the samples sequence has already been entered.

A new sample for the inject no. 15 should be entered (**0815**) into an existing sample queue. Consequently, the default sample name **sample 15** is assigned to inject no. 16, the default sample name **sample16** to inject no. 17 etc.

Procedure:

Select sample 15 with the left mouse button from the list field (edit line shows **Sample 15**), click on the **Insert** button, overwrite in the editor line **Sample 15** with the new sample name **0815**, accept input (**OK**, or transfer another sample to the edit panel).

After all sample names and parameters have been entered, leave the sample editor with **OK** or with the Return key. The entered values will then be accepted. **Cancel** is leaving the sample editor without acceptance of the processed changes.

NOTE

The buttons **Import** and **Export** permit to save the input of a sample or a sample sequence (export) and to load it/them later (import). This is useful for calibrations standards or reference samples as well as for samples or sequences that have to be measured again. When the **Export** button is pressed WinGPC Software asks for the sample range to be exported. Enter the sample number of the first and last sample in the **Export Samples...** dialog box. It is important to enter the sample numbers (number in the Sample field (top left) and not the number from the default sample name (sample no. n) since this number will become different when samples are inserted or deleted in the sample editor. **Import** reads in all injects from an exported list, including all sample parameters.

NOTE

Please pay attention that sample list import will overwrite existing entries in the sample editor. If a subsequent sample should be kept, please insert an equal number of new samples before clicking on the “Import” button. The inserted (empty samples) will be automatically replaced by the imported sample list.

The sample list import file is an ASCII file and has the following format:

```
define sample {No}
Probenname = PSS PS ReadyCal red
Probentyp = 1
Substanzbezeichnung = Poly(styrene) in THF at 30°C
Injektvolumen (ul) = 20
Konz. Komp. 1 (g/l) = 0.5
Konz. Komp. 2 (g/l) = 1
Konz. Komp. 3 (g/l) = 1
Konz. Komp. 4 (g/l) = 1
Molmasse Komp. 1 = 1.09e+006
Molmasse Komp. 2 = 130000
Molmasse Komp. 3 = 17800
Molmasse Komp. 4 = 1620
Mark-Houwink K = 0.01363
Mark-Houwink A = 0.714
Brechungsink. dn/dc = 0
Absorption dA/dc      = 0.1
Verduennungsfaktor = 0
Virialkoeffizient_A2 = 0
g_cDepartment = Demo1234
end define sample {No}
```

This ASCII file can also be created using any text editor or an external program.

The sample type (**Probentyp** in the file) defines how the sample will be processed in automation mode (for details refer to chapter "Performing a GPC Measurement with Full Automation" on page 127 in the Agilent WinGPC Software User Guide).

It is coded according to:

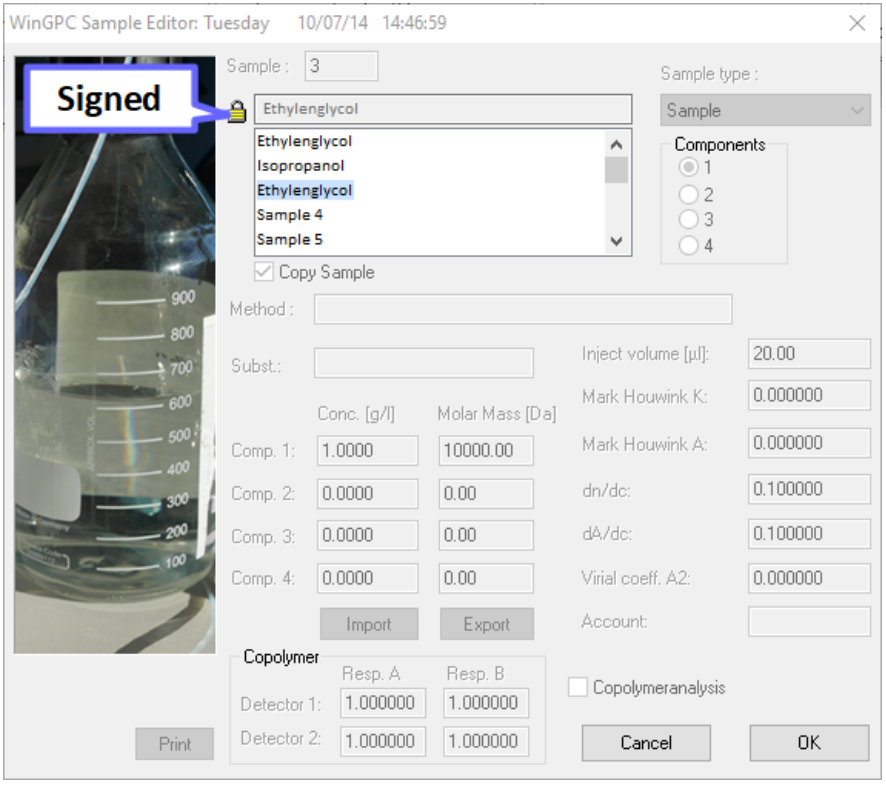
0: Sample (unknown sample): process as an unknown sample

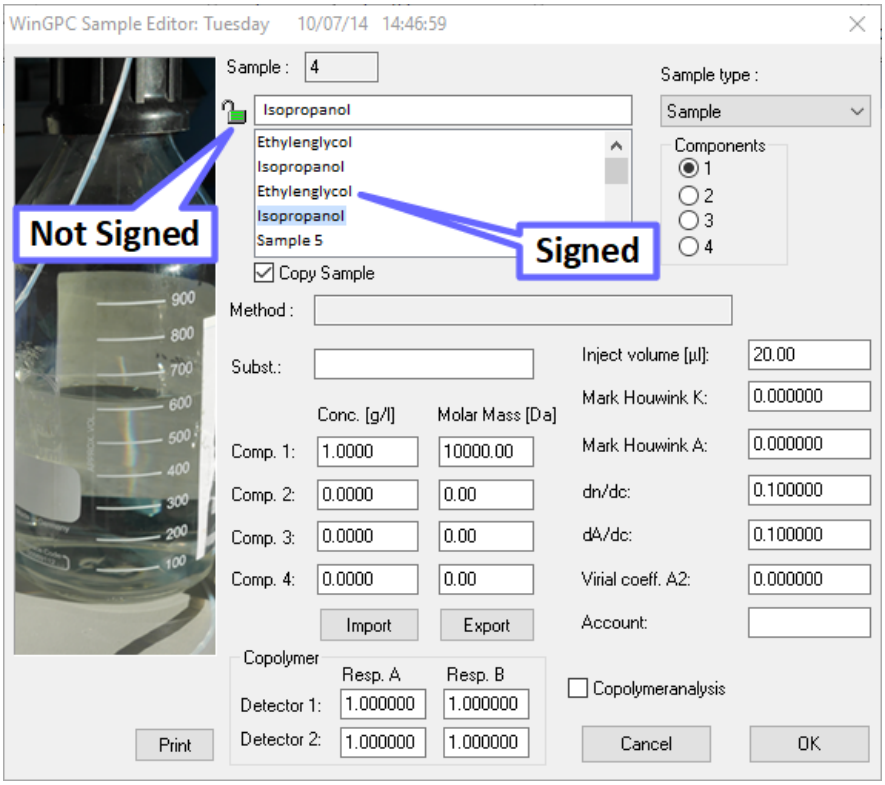
1: Calibration: process as a narrow standard, add the peak position to the calibration file

2: Recalibration replace: process as a calibrant, replace the peak position in the calibration

3: Recalibration average: process as a calibrant, average the peak position in the calibration

Sample information of samples with electronic signatures cannot be modified. According to the display in the status bar, a closed padlock symbol will indicate if a sample is signed. To perform any changes, the signature has to be removed at least temporarily. For this purpose, you need to leave the sample editor, because signatures can only be set/ approved or removed using the respective symbol in the status bar.

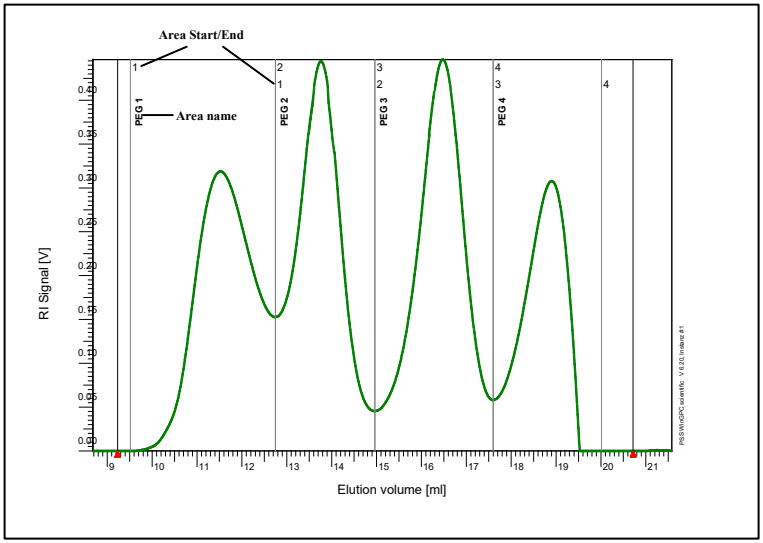




Changes to not-signed samples (open padlock symbol) are still possible. The only restriciton is that the buttons **Insert**, **Delete**, **Import** and **Export** cannot be used if any signed samples exist after the current sample in the same sequence.

Multi Area Data Analysis

WinGPC Software allows the simultaneous analysis of up to eight different evaluation regions under identical conditions. These regions can either be just one of the eight free selectable areas or a combination of some of the eight free selectable areas.



The multi area settings (area and evaluation definition) are entered in a **Raw data** window dialog box available from the menu **Options > Analysis... > Multi area settings**. Once defined these settings can be saved for later use (*.MAS files). The *.MAS files are part of the WinGPC Software data acquisition method (*.MET files). This makes multiarea data processing very easy, because only the method has to be opened to prepare the run.

NOTE

The multi area wizard (see chapter “Multi Area” on page 113) is a step-by-step guide through this function.

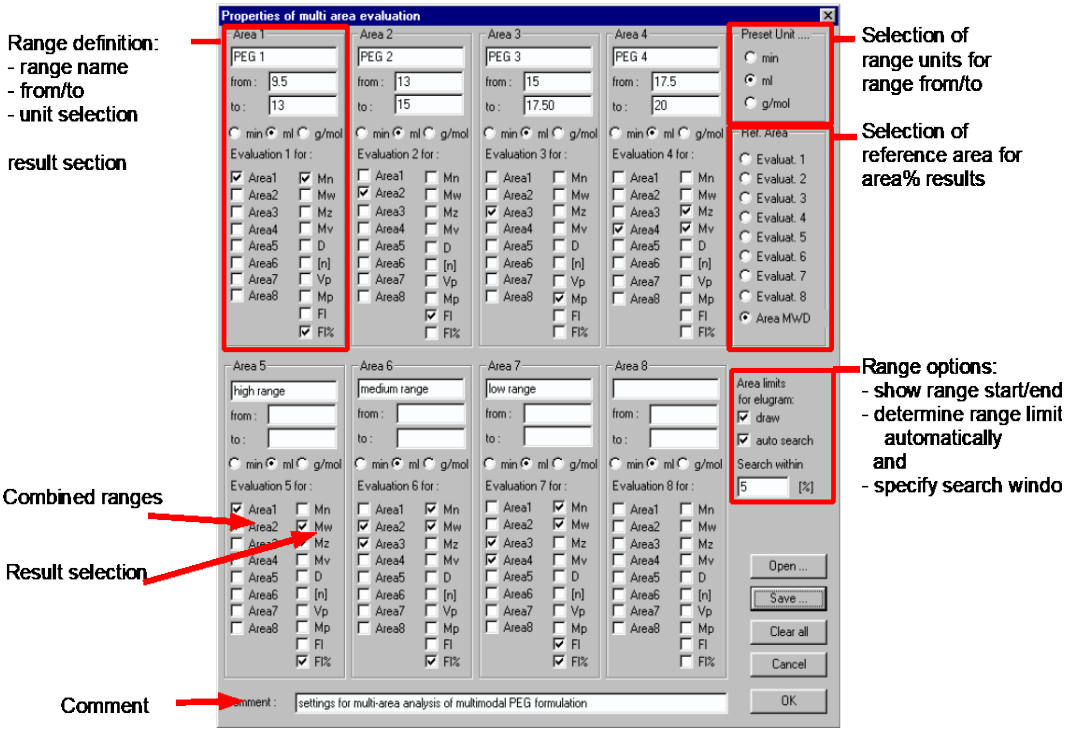
Multi area processing is also possible during automated runs. All parameters, multiarea results and chromatogram slice lists are included either in the ASCII report file or in the automation “text file”, if this option is selected (e.g., for LIMS transfers, macro programming).

WinGPC Software will remember multiarea parameters in finished runs by default. The toggle switch **Options > Analysis... > Multi area activate** can be used to switch on/off multiarea processing. If the multiarea analysis mode is active, the WinGPC

Software standard report will consist of a second page showing the result table of the multiarea analysis for all concentrations detectors which are displayed in the raw data window.

After data acquisition with multi area analysis has been terminated WinGPC Software will offer the previous multiarea configuration for additional ease of use. WinGPC Software will prompt for multiarea analysis activation when the settings dialog box is left with **OK**.

Definition of Areas



Options > Analysis... > Multi area settings from the **Raw data** window menu opens the **Properties of multi area evaluation** dialog. Chromatograms can be sub-divided into 8 individual parts (areas); each area can be denoted by an area name. The area borders are entered in the **from**/**to** fields in either time, volume, or molar mass units for each area (user selectable by specifying the appropriate option in **Preset units...**). The default preset unit can be changed separately for each individual area. Please note, that the (re-)selection of the default preset unit will cause to overwrite individual unit selections.

The menu item **Options > Analysis… > Multi area create** generates the areas based on an HPLC analysis of the actual sample. These automatically created areas just need to be named and edited if necessary.

The software sub-divides the chromatogram by vertical drops into the different parts. Area borders are displayed and printed in the elugram if **draw** in the range options is activated. The individual areas are represented in the **Elugram** window by vertical lines and the area start/end is identified by its area number (shown at the top of the elugram graphics). The area name is additionally shown at the area start line.

Instead of using fixed area limits it is possible to use local minima for sub-dividing the chromatogram. This is done when the **auto search** option is activated, and the search window has been specified in the **search within** % field. In that case the area limits **from**/**to** are used to start searching for the first local minimum within the **search within** window.

The reference area for the calculation of the area% results of each chromatogram part can be set in the **Ref.Area** section by specifying an individual evaluation area or the area of the total chromatogram (**Area.MWD**).

Selection of results/evaluations:

Multiarea results (evaluations) can refer to just one area of a chromatogram or to several combined areas. If “Area x” should be evaluated, only “Areax” should be marked with a tick mark in the result section of **Evaluation {n} for**. If the evaluation should be done for combined areas, then the tick marks should be set for all areas to be included into evaluation {n}.

Besides selecting areas for the evaluation, it is also possible in the **Evaluation {n} for** section to specify which results should be calculated and printed. This is done by ticking off the respective result parameter in the result list. When new multiarea settings are created all result parameters are by default not marked.

After defining all areas and evaluations a comment can be entered and the settings can be saved as *.MAS file using **Save...**. *.MAS files can also be loaded in empty Raw data windows of instruments so that they can be saved with methods in the *.MET file.

Display of Results

The results of a multiarea analysis can be displayed interactively on-screen, printed, or saved to a file. A two-page report is printed when using the standard print command; the first page is identical to the conventional report for this window, the second page contains the multiarea results as specified in the settings. All multi area parameters are also available in the ReportDesigner list of variables, so that it is possible to create a customized one page report with all the details needed.

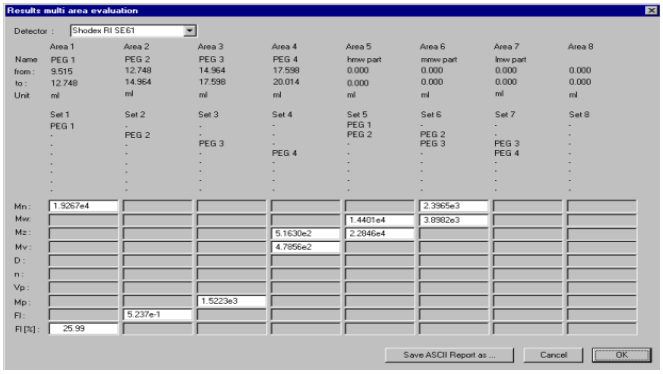


Figure 45 On-screen result table of multiarea analysis

Options > Analysis... > Multi area results from the **Raw data** window menu opens a window where the selected results for the defined evaluations are displayed. The multiarea results are calculated for all concentration detectors in the method, but only the results for the active detector are reported by default. The detector can be selected from the **Detector** drop-down list.

The **Save ASCII Report** command in the **File** menu of the Mass distribution window will contain all results and parameters of the multiarea analysis, if it has been activated before; otherwise, these results are not part of the ASCII report file.

Elugram Window

In the elugram window the baseline corrected raw data are presented. The volume axis may have been corrected by the internal standard, if an internal standard has been used. Because the volume axis is corrected for the internal standard, the positions of peak maxima in the raw data window and in the elugram window need not to be identical. The presented volume range corresponds to the range between the baseline markers of the raw data window.

In the elugram window the integration limits for the calculation of the molar mass distribution and molar mass averages have to be set. Furthermore, different injections can be overlaid in the elugram. Curves which have been overlaid in the elugram window can also be viewed as overlay in the mass distribution. Creation of calibration tables for calibration purposes is done in the elugram window as well.

If the HPLC Mode is active (cf. chapter "Application of the HPLC Mode" on page 324), HPLC evaluations can be processed in the **Elugram** window. Thus, peaks can be searched for and identified based on their elution volume. Furthermore, quantitative determinations can be performed using external standard calculations and response factors.

General Features

In the elugram window only one Y-axis exists. The data are presented with their real detector output values (in physical units, normally V), or in a normalized representation by which the maximum of each curve will be set to 100% (recognizable by the Y-axis label 100%). Toggle between both options by clicking the left mouse button on the middle scaling button or select "norm." from the Y-axis context menu.

The X-axis can be scaled in several ways:

When the mouse cursor is in the bottom right position of the X-axis, the interactive scale buttons will become visible. The right (left) arrow button is used to increase (decrease) the elution volume (or time) displayed interactively. (Please note that these interactive scale buttons will only appear if this window is active). Alternatively, manual scaling by typing in minimum and maximum values for the X-

axis is available from the X-axis context menu. When a manual scaling has been done, then the X-axis context menu item **Standard Scaling** becomes available and will reset any X-axis scaling to the standard parameters defined by the **manual scaling** values.

NOTE

The method file also saves the settings of the various axes. These will be loaded when the method file is retrieved from the file system.

NOTE

If large (e.g., viscometry) and small (e.g., concentration) signals are used in the method, only the largest signal will show up in the elugram window. Selecting the normalized scale will display all signals independent on their absolute size.

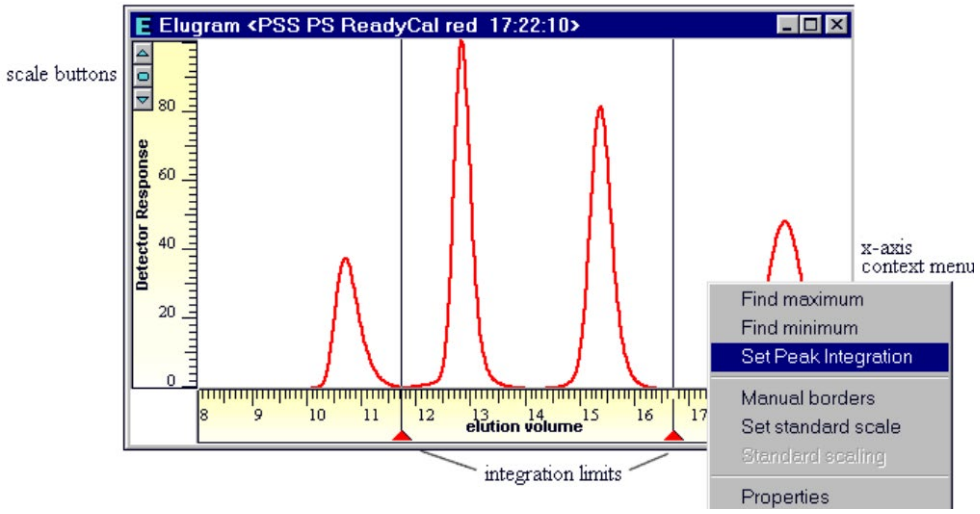


Figure 46 Setting advanced processing options in the WinGPC Software Elugram Window

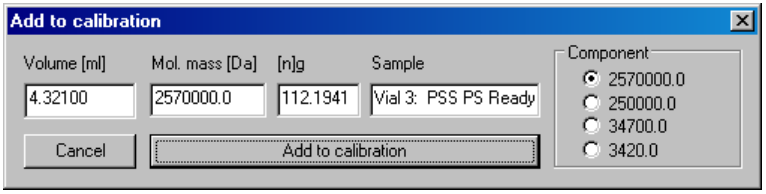
Windows can be zoomed (magnifier effect) for better inspection of certain elugram sections. Click into the window and start pressing the left mouse button where the zoom area should start. Keep the button pressed and drag the appearing rectangle until it encloses the section which should be magnified. After releasing the left mouse button, the magnified section is displayed in full scale. To undo one or all zoom actions (unzoom) click with the right mouse button inside the window.

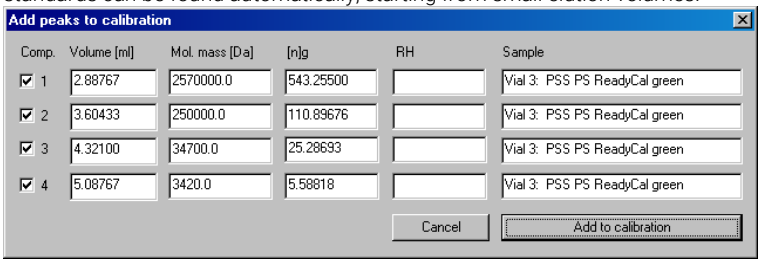
To set the integration limits, click with the left mouse button on the red markers on the right and left window edge and pull these while keeping the mouse button pressed. Release the mouse button at the required positions in the elugram. The influence on the molecular weight distribution becomes visible in the mass distribution window. Alternatively, the integration limits can be entered numerically using the functions of the X-axis.

X-Axis Context Menu

These functions are accessible from the X-axis context menu, if you click on the X-axis scale with the right mouse button. A pop-up menu appears in which the following commands can be selected:

Table 25 X-axis context menu

Function	Description
Find maximum / minimum:	Searches from the mouse cursor position to the next relative maximum/minimum in the active curve from where the command has been invoked. When the next relative minimum/ maximum is found an dialog box opens, where peak position (V_p), molar mass and sample name for the creation of calibration tables are displayed. If the detector signal is noisy use the **Curves >** *{curve letter}...* menu option for signal smoothing. When using a viscosity detector, the intrinsic viscosity from the calculation column of the viscosity information box will be shown. These can be edited and molar masses can be selected from the radio button list on the right. The calibration molar masses displayed there must be entered in **Editor > Samples**. A temporary cursor is displayed at the determined x-position of the extremum in the **Elugram** window when searching for minima or maxima. This is very handy when many peaks are close by and identification of the accurate peak position by visual inspection is not simple. The **Add to calibration** button appends data set to the active calibration table. If no calibration table is loaded in the **Calibration** Window, this button is grayed out. Please remember to use the **File > New** command in the calibration window to create a new calibration curve; otherwise, data sets will be appended to the one currently displayed in the calibration window. **Cancel** will close this dialog without any changes. 

Add peaks to calibration:	Determines peak maxima automatically and relates reference molar masses (as entered in the sample information) for transfer to the currently active calibration table. The maxima are temporarily marked in the elugram and listed in the dialog **Add peaks to calibration** to acknowledge the peak assignment. A maximum of 4 standards can be found automatically, starting from small elution volumes.  A calibration table is required to send the data to the calibration window (e.g., create a new one with **File > New**), otherwise the button **Add to calibration** is greyed out.
Set peak integration:	Performs a peak search in all displayed curves. The integration limits are placed around the found peak; the first local minimum to the left or right of the mouse pointer position will be used. If the detector signal is noisy use the **Curves >** *{curve letter}*... menu option for signal smoothing. The current WinGPC Software version introduces another improvement for modifying baselines and integration limits. Up to now each change in baseline settings in the **Raw Data** window caused the integration limits in the **Elugram** window to be reset. With WinGPC Software, integration limits will be updated automatically only, if an integration limit will move outside of the elution volume range defined by the new baseline limits. A small modification (optimization) of the baseline will in general not change the integration limit. This can save lots of time when working on the optimization of data processing parameters.
Manual borders:	Allows to set the integration limits manually in form of predefined molecular weights or elution volumes. Any parameter can be entered and the other one will be calculated from the currently active calibration curve. The TAB key can be used to update the parameters.
Set standard scale:	Allows the manual setting of the displayed X-axis range of the elugram and sets the default standard scale.
Standard scaling:	Restores the scale of the last manual scaling (e.g., after editing the scaling by the arrow keys or the scroll bar of the X-axis.). A tick mark in the context menu indicates, if the standard scale is in use.
Properties:	This context menu is available on each axis in the WinGPC Software. It allows setting axes properties individually. For example, text attributes (font, size, color etc) and background properties of the axis can be defined. It is also possible to switch from elution volume to elution time representation for X-axis properties. The axis properties will be saved for each instrument individually.

Y-Axis Context Menu

These functions are available from the Y-axis context menu, if you click on the Y-axis scale with the right mouse button. A popup menu appears in which the following functions can be selected:

Table 26 Y-axis context menu

Function	Description
Norm.:	Toggles between normalized and manually scaled view. This status is indicated by the **N** (normalized view) or **M** (manual scale) below the corresponding Y-axis.
Set standard scale:	Allows the manual setting of the displayed Y-range of the chromatogram and sets the default standard scale.
Standard scaling:	Use preset Y-axis scale as defined in **Set standard scale**. If the standard scale is in use, this command shows a tick mark, and a **S** designator appears below the axis.
Tip: The method file also saves the scaling settings of the various axes. These will be loaded when the method file is retrieved from the file system.	
Properties:	This context menu is available on each axis in the WinGPC Software. It allows setting axes properties individually. For example, text attributes (font, size, color etc) and background properties of the axis can be defined. It is also possible to switch from elution volume to elution time representation for X-axis properties. The axis properties will be saved for each instrument individually.

NOTE

It can be very useful to assign the Y-axis caption in the same color as the detector signal and give the axis a descriptive name (e.g., UV signal).

File Menu

Table 27 File menu

Function	Description
Import ASCII:	Allows to import ASCII files (*.txt) (format: volume, detector- signal 1, detector-signal 2, etc.) into the elugram window (floating point data should have decimal points with comma or tabulator as separators). When importing ASCII Data into the elugram the overlay mode is automatically activated. Several ASCII Files can be imported to overlay various imported samples. Calibration curves can be assigned to imported data to calculate the mass distribution and the molecular weight averages. To do this select the corresponding curve under the option **curves** of the overlay mode and enter the path for the calibration curve (see also chapter "Overlay Mode" on page 237).
Tip: Some computers have problems to load ASCII Data directly into the raw data or elugram window. In this case, first import the data into the WinGPC Software data editor. Export the data again as ASCII. The data now have a format which allows reading them into the elugram or raw data window.	
Export ASCII:	Exports the ASCII Data of the curves presented in the window. The file will contain the following columns: `volume, detector signal 1, detector signal 2, etc.`
...to 2D Graphic:	Transfers all evaluated injects into the 2D graphic window of the WinGPC Software for analyzing 2-dimensional chromatography data. Please note that this menu item is grayed out unless the optional WinGPC Software 2D add-on has been purchased.
...to 3D Spectra:	This menu item is only accessible, if the 3D spectra module is licensed and the current sample contains spectra information (this can vary from sample to sample even within the same sequence). If 3D data are available, those may be transferred tot he 3D spectra module (see chapter „3D Spectra Module" on page 450).
Printer Setup:	Allows definition of parameters of the active printer. However, the active printer must be defined in the Windows Control Panel. Landscape format prints the graphics on a full page. Portrait format prints the graphics, full method documentation and results by default. The printed information in the portrait layout can be selected for automated runs in the **Definition > Automation Settings** dialog box. The exact information of the portrait format printout depends on which window will be printed.
Print:	Prints the current contents of the elugram window to the default printer. The graphics are always printed in WYSIWYG mode, i.e. the current window display will be printed identically as shown.
Page Preview:	Shows a print preview in a separate print-preview window, which can be sized and moved. At the same time this command copies the preview contents to the Windows clipboard. When printing in portrait format, the graphics and the text for the measurements will shown, while landscape format will print only the graphics.
Print Annotation:	Opens a dialog box to enter a header for the standard report. This header line will be printed directly above the elugram on the WinGPC Software elugram report; other printouts may have different text. A total of 80 characters can be entered in this line.

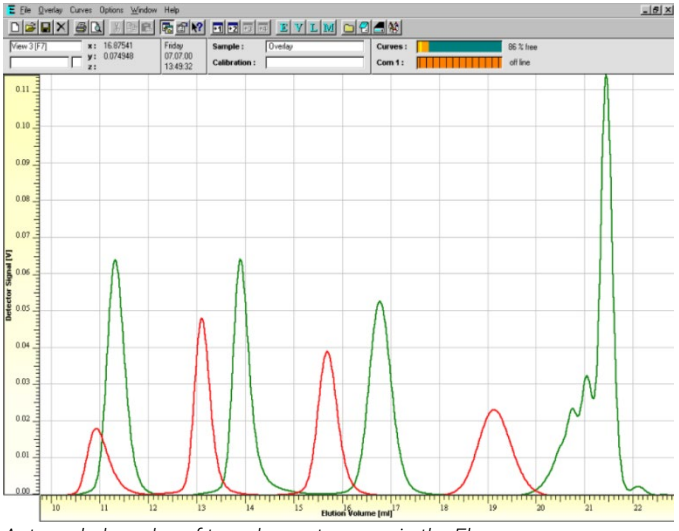
Table 27 File menu

Function	Description
Edit Comment:	Allows to enter an elugram related text (comments, hints for data treatment, integration, or calibration details, observations, etc.). Up to 1024 characters can be entered for each injection (sample) separately. Alternatively the icon in the status bar (see "Comment and Options" on page 159) can be used to open the comment dialog box. This icon is gray if no comment has been entered for this sample, if text has been entered it is highlighted in green.

Overlay Menu

Table 28 Overlay menu

Function	Description
Include Curve:	This command copies the curves presented within the integration limits into the overlay buffer to view and evaluate the curve in the overlay mode (see chapter "Overlay Mode" on page 237). Alternatively, the overlay icon on the icon bar (which can be accessed independently from the active window) can be used to include a sample to the overlay. If the selected injection is already included in the overlay, the icon toggles between normal and overlay mode. This means that different processing options (of the same injection) cannot be overlaid with the overlay icon; use this menu item instead.



Autoscaled overlay of two chromatograms in the Elugram

Table 28 Overlay menu

Function	Description
Overlay:	Toggles between normal and overlay mode (recognizable by the hook or in the status line of the Sample field, or by the overlay icon on the icon bar). The selection of another injection by the filed sample on the status bar or by the arrow keys (←→) switches from overlay mode to the normal mode. In the overlay mode samples of various runs can be compared visually with each other or calculation operations can be processed with the curves. Overlays are always done for elugram (baseline corrected) data and molar mass distributions at the same time (if a calibration has been loaded for the chromatogram).
Tip: If raw data shall be overlaid, use the **Raw Data > ASCII save captured data** menu and import the raw data directly in the elugram with **File > Import ASCII**.	
Delete all curves:	Deletes all curves in the overlay mode and automatically switches back to the normal mode of the window. This menu item is greyed out if the overlay doesn´t contain any entries.
Calculate sieve curve(s):	Opens the dialog to determine the molar mass cut off, pore dimension, etc of membranes from filtration experiments; see "Determination of Sieve Curves" on page 239 for details. These current values will be saved automatically and will be used as default parameters when this dialog is opened again. This command is only available if the overlay mode is active.
Retention [%]:	Allows setting the parameters for the characteristic points of sieve curves and to relate molar mass and pore diameter; see 1 for details. These most current parameters will automatically be saved and used as default values when the user opens the dialog again. This command is only available if the overlay mode is active.
Save as:	Saves the window contents as overlay file (*.ADD). This file can be distributed to others and can be retrieved later without the need to access the raw data itself. Saved overlay files can also be used to create calibration curves using broad standard calibration methods.
TIP: To trace the column performance save the chromatogram of a test mixture after receiving your new columns (**Overlay > Save As**). Measure this test mixtures again at regular intervals. Change to the elugram window and load the overlay file (**Overlay > Load**) to retrieve the measurement with the new columns. The software automatically changes into the overlay mode. Leave the overlay mode (**Overlay > Overlay**) and evaluate the new measurement. In the elugram window select **Overlay > Include Curve**, This transfers the new run to the overlay buffer for curve comparison. Now switch to the overlay view to see the actual chromatogram overlaid with the chromatogram, which was obtained with new columns.	
Load:	Loads a saved overlay file from the file system and switches to the overlay mode. Now further curves from WinGPC Software measurements can be added to the overlay (see **Overlay > Include Curve** in the elugram window). Please note, that overlay files can only be loaded, if the overlay buffer is empty. They cannot be added to a collection of data.

Table 28 Overlay menu

Function	Description
Properties:	Shows a summary of samples/signals which are currently in the overlay. It allows to edit all entries in a single dialog (as compared to the **Curves** menu where individual traces can be edited). The following sample/signal properties can be edited in this dialog: add comment, display/hide trace and trace color (see also chapter "Overlay Mode" on page 237). In this dialog an overlay color scheme can be defined by selecting a color sequence which will overwrite the signal color in the elugram. This setting is saved for all users with the **Save as default** button.
Information:	Opens an information window, which displays the number of transferred samples to the overlay buffer and the total number of the curves.

Curves Menu

Table 29 Curves menu

Function	Definition
Calibration Curve:	Overlays the conventional calibration curve in the elugram window with the sample elugram on a separate axis. Red dots mark the first and last calibration point (highest and lowest molar mass). This allows easily to check whether the presented sample elutes completely in the calibrated section or partially outside the calibrated section of the chromatogram. Please note that calibrations which are directly measured as from light scattering detectors are not available here.
Curve A B..:	Opens the curve property dialog box, which allows smoothing, interpolation or specific fit routines like Fourier transformation. The option Interpolation defines how two consecutive points will be connected to each other. By default, the data points are connected linear with each other. Spline permits curved connections, which can improve e.g., peak position determination when the data interval is too wide. *Smoothing* uses the moving average algorithm e.g., to remove noise or bumps. The larger the number of data points the larger the smoothing effect and the peak shape change will be. However, the peak area will not be affected by any smoothing method. The special fit *Fourier* transformation opens a window in which the Fourier coefficients, which are necessary for the description of the curves, will be presented. The number of harmonics define the number of terms used for the synthesis of the signal. The smaller the number of used coefficients, the higher the smoothing effect, but at the same time the general form of the synthetic curve will be more different from the original one. Upon clicking on **Synthesis,** the calculated (red) and measured curve (blue) is presented. The special fit *Despike* uses a special smoothing routine which removes short time detector noise (spikes). The despike routine defines 3 data point sections. A data point section in which the viewed data is located and two further data sections to its left or right side. Each section will be averaged separately. The mean value of both outside sections will be connected by a straight line. If the mean value of the inner section deviates by more than a given tolerance from the connecting line of the outside section, this point will be identified as a spike and ignored. The three parameters which have to be entered are therefore *Width 1* (the number of data points of the outside section), *Width 2* (the number of data points of the center section) and the *Tolerance*.

NOTE

If you overlay data from detectors with multiple signals (like a multi-angle light scattering detectors), the operation for a single signal will be applied to all similar signals of the same detector (e.g., smoothing).

Options Menu

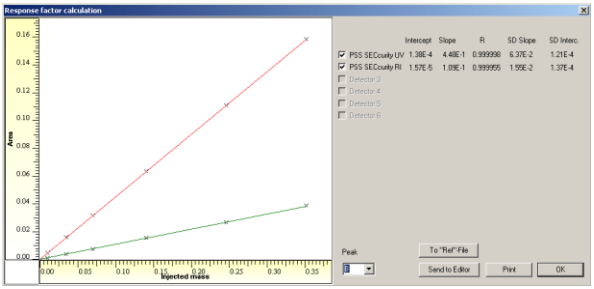
Table 30 Options menu

Function	Description
Component:	Allows the selection of the component from the sample editor, e.g., to assign a concentration or a molecular weight to a peak (see also **Editor > Sample** in chapter "Editor Menu" on page 208). This option is important for the creation of calibration curves and for light scattering and/or viscosity measurements runs, where is correct concentration must be assigned to the evaluated peak area.
Editor Slice Data:	Transfers the presented data to the data editor where it can be edited and organized in columns of the structure volume, molecular weight, detector 1, detector 2, etc. For a description of the data editor see chapter "Data Editor Window" on page 286.
System Test:	Performs a comprehensive system suitability test according to different standards and regulations. User-defined requirements can also be set. Details can be found in chapter "System Suitability Tests" on page 247.
Performance Tests:	Allows to check pump flow accuracy as well as detector and injector performance for linearity and reproducibility. These tests can also be used for instrument qualification. Further details can be found in chapter "Instrument Performance Tests" on page 251.
Endgroup Analysis	Allows the creation of a calibration curve from an Endgroup containing compound, which can be detected by a combination of a universal (e.g., RI) and specific (e.g., UV) detector. The required response factor can be entered optionally as the molar mass (Heparin module, optional). The number average molecular weight (M_n) of the sample must be entered into the sample editor if no response factor is available. More detailed information to **Endgroup analysis** can be found in chapter "Endgroup / Heparin Analysis" on page 438. Then the calibration table can be created automatically by using the **Endgroup analysis > Create Calibration** command. This will transfer 100 calibration points from the elugram into the calibration editor.
Dextran Monograph:	Will be used if a dextran analysis based on USP or EP will be performed. For more details and instructions see chapter "Performing Dextran Analysis based on USP / EP" on page 254. Available menu items (only accessible if prerequisits are met): **Calibrate**, **Evaluate**
Stacked Plot:	Creates a pseudo-3-dimensional representation of elugram data. The curves will be stacked at a fixed angle of 45 degrees in the order they have been assigned by the axis number or entered into the overlay buffer. This type of representation is also available in the overlay mode.
HPLC Analysis:	This option allows to switch on the WinGPC Software HPLC mode to quantify components simultaneously with GPC data processing. The menu offers peak area or peak height HPLC calculations. Details on the HPLC mode can be found in chapter "Application of the HPLC Mode" on page 324.

Table 30 Options menu

Function	Description
Peaklist sort for:	This menu is only active if the HPLC mode is activated. It performs a peak search within the integration. Peaks are marked with letters by default. The peak limits will be marked by turquoise (right peak limits) and pink triangles (left peak limits) on the X-axis. Moving of peak limits is possible upon clicking and pulling with the right mouse button for right (turquoise) marks and left mouse button for left (pink) peak marks. A mouseclick on the letter of the peak maximum displays HPLC results like volume at peak maximum (V_p), peak height (Y_p), molecular weight at the peak maximum (M_p), peak area and concentration. If a reference table was loaded in advance and if the peak can be assigned by elution volume to a substance from the reference table, the peak will be provided with compound name, and the concentration will be calculated using the specific response factor for this compound. A right mouse click on the peak letter will delete this peak from the peaklist. Details on the HPLC mode can be found in chapter "Application of the HPLC Mode" on page 324.
Peaklist send to:	**Editor**: Copies the current list of peak data from all found peaks to the data editor. This menu is grayed out if the HPLC mode is not activated. **Multi area settings**: Uses the HPLC peak information to create new multi area settings. The information can be viewed and edited in the multi area settings dialog.
Reference table:	Allows loading, creating, deleting of reference tables and the (multi-level) determination of response factors for quantification. If the HPLC mode is not activated only the create sub-menu is available. The found peaks will be assigned according to their retention times to the data in the reference table, if a reference file has been loaded and the measured retention time lies within the limits of identification (max. deviation). The compound concentration will then be calculated from the determined peak area and the response factor from the reference table for that compound. The reference table is a text file which can be created with any text editor. The creation is simple when using an example chromatogram. In order to create a reference table use **Options > Peaklist sort for**. A peaklist will be created. Use **Options > Reference table... > create** to transfer the peak data to the data editor. Then switch the **data editor** window. The columns of the data editor contain consecutively: detector number (axis number), volume at peak maximum, acceptable deviation of the retention volume in %, response factor and peak name. Lines can be added and edited as necessary. Afterwards save the reference table within the data editor with **File > HPLC Ref. export**. The **Option > Delete reference table** deletes the reference table from the program memory (not from the harddisk) and disables the use of the HPLC reference file information for this analysis. The **Options > Reference table... > Response factors** command allows to calculate for multiple peaks the corresponding response factors for all concentration detectors simultaneously and transfer them to an existing HPLC reference table. This option is only available if HPLC peaks of known concentrations have been entered in the overlay mode (external standard method).

Table 30 Options menu

Function	Description
	 Single and multi-level (injections with multiple concentrations are overlaid) calculations are supported. The dialog shows the response functions and results of the linear regression for each detector and all compounds in the overlay (**Peak** A...Z). A discussion of result uncertainty can be found in chapter "Accuracy and Precision of GPC Results" on page 31. Further details on how the dialog is used can be found in chapter "Instrument Performance Tests" on page 251. The send "to **Ref." file** button can be used to update a user selected HPLC reference file with the calculation results. After selecting a *.REF file from the file selection dialog, a backup copy of the original HPLC reference file is always created (*.RBK). The **Send to Editor** button copies the results as displayed in the dialog for the selected **Peak** to the WinGPC Software Data Editor.
Baseline:	WinGPC Software provides for a valley-to-valley integration as well as vertical drop definitions in the HPLC Mode. Without the **Option > Baseline** (no tick mark) the peak area is defined in the peak limits are defined by vertical drop. By changing to **Option > Baseline** (tick mark in the menu option) valley-to-valley integration will be performed. Generally, for polymers and oligomers the vertical drop method is the better choice, because long tailings do not occur in GPC elugrams.

Note: The change of the integration method will only influence the peak area definitions of the HPLC evaluation. There will be no effect on the distribution window, since for the distribution window only the red integration limits are of concern.

Note: Please note that the compound concentrations in the sample editor will be assigned in ascending elution order to the peaks for HPLC response factor calculation; e.g., concentration **comp. 1:** will be assigned to **Peak A**, etc.

Compare with list:	Forces a new comparison with the reference table (see **Options > Reference Table**), if e.g., a peak has been deleted manually.
Colorfill off:	In HPLC mode the peak area will be color-filled by default to distinguish visually from a conventional GPC data evaluation.
Palette:	Allows the selection and creation of a self-defined color background. The palette color is activated as background color in the **Raw Data** window within **Options > Color scheme**.

Overlay Mode

The overlay mode allows to overlay chromatogram and results of different samples simultaneously. It also enables a multitude of mathematical operations with curves. The transfer of curves into the overlay mode is done in the elugram window (**Overlay > Include Curve** or with the overlay icon on the icon bar). For viewing an overlay, the menu item **Overlay** in the **Overlay** menu must be activated.

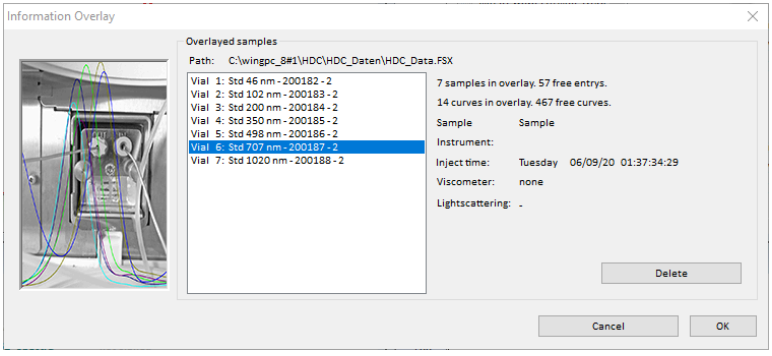
NOTE

If the overlay icon in the icon bar is pressed, the inject options are active and can be set to add multiple samples (from currently loaded run) at once

The activated overlay mode is identified by the tick mark on the Menu item **Overlay**, the pressed overlay icon on the icon bar, by the name in the elugram (mass distribution) title bar <Elugram Overlay> (<Mass Distribution Overlay>) or by the sample name **Overlay** in the **Sample** field in the status bar). The activation of the overlay mode forces all subordinate windows (mass distribution window, viscosity window, light scattering window) also to display the results of the overlay data.

NOTE

Samples can also be overlayed directly from the ProjectManager without loading a run by selecting a sample from the sequence and clicking on the **send to overlay** button



Information on the current overlay contents can be obtained from the **Overlay > Information** menu as seen in the adjacent figure.

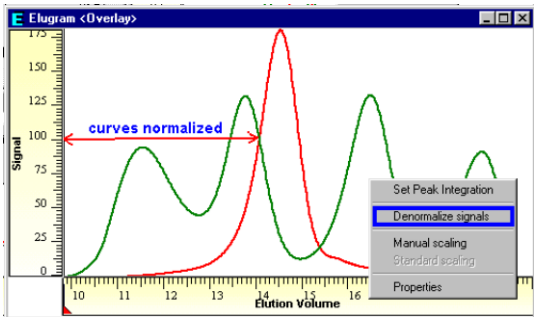
The menu items of the overlay window are not different from those in the elugram window, only the menu **Curves** has a slightly different meaning.

Selecting a curve letter from the **Curves** menu opens a dialog box which is similar to the curve menu of the elugram window. It displays smoothing, fit and interpolation options and the curve color can be selected by clicking on the color

selector panel. The **Trace** option permits to show/hide the current curve. A comment can be entered in the **Comment** field, which appears in the printout of the elugram overlay. In the **Calibration** field any calibration file can be assigned to the current curve for the evaluation of the chromatogram. By default, the active calibration file is used, which was loaded at the time of adding the curve to the overlay. To simplify curve comparisons, the software normalizes any presented curves after processing a mathematical operation. To display the non-normalized (physical) values, change scaling using the scaling field or scale the Y-axis manually (see chapter "General Features" on page 224).

NOTE

Curve properties are retained in the overlay mode and retrieved if an overlay has been saved. This means that if a signal has been e.g., smoothed prior to overlaying this sample, the full information on smoothing is available (and will be shown in the option) in the overlay mode. This is also true if an overlay file (*.ADD) was opened and contained pre-processed signals.



Please note that in the overlay mode the X-axis context menu contains an additional option **normalize signals**, which allows to set all signals to 100%. The software uses the x-position of the mouse cursor when this command is invoked (cf. figure). This function can be used to highlight differences between different injections or samples; e.g., if two samples contain the same amount of a compound and the differences of other components should be shown based on this "internal standard".

It can be helpful to zoom into the desired peak area before using this command or to use one of the **set peak integration** cursors (by default sitting at the left and right end of the X-axis) as a means to find the x-position.

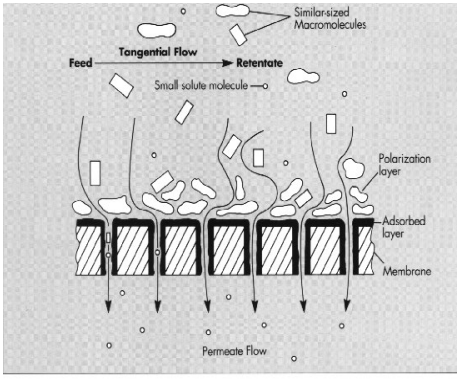
The entry **free** in the **Curves** menu allows the creation of synthetic curves from existing chromatograms using mathematical operations listed in the table below. Please use brackets extensively to ensure that the correct mathematical operation is used.

Table 31 Mathematical Overlay Calculation Functions in the Synthetic Curve Menu

Symbol	Function	Example
+	addition	A+B; add curve A to curve B
-	subtraction	A-B, subtract curve B from curve A
*	multiplication	A*B; multiply curve A by curve B
/	division	B/5; divide curve B by 5
ln	nat. logarithm	ln(A); create nat. logarithm of curve A
**	power	B**2; square the values of curve B
'	1st derivative	A'; differentiate Curve A
"	2nd derivative	A''; differentiate Curve A'
(and)	brackets	advisable for complex equations, e.g., (A+B)/(A+B+C+D)
curve.m	molar mass of curve	A*A.m multiply curve A by it's calibration curve molar mass

Determination of Sieve Curves

In this section the characterization of the porosity of various kinds of membranes by GPC methods is described.



Since GPC is a reliable, simple and fast characterization method it can be used with great benefit to investigate the separation behavior of membranes (planar or fiber alike). A known sample (stock solution) is separated by the membrane and filtrate and retentate solutions can be injected into the GPC instrument for the determination of concentrations, retention and molar masses (see adjacent figure). Permeation and retention behavior of the membranes across the whole pore size range can be derived from 2 injections. The resulting sieve curves are directly generated by the software. This membrane characterization method is a vast improvement in time and cost as compared to the traditional methods of membrane characterization.

Every calibration method can be used to determine the molar masses for cutoff measurements.

The pore radius is calculated from the molar mass is based on the following scaling law:

$$r = Q\,M^{a}$$

The pore diameter, dp, is simply related to 2·r. The “Q” and “a” parameters are known for many well investigated polymer/solvent systems and can be found in the literature.

The GPC method described below has a number of unique advantages:

- it is not necessary to achieve 100% filtration at any point;
- the shape of the sieve curve can be measured accurately even at low signal strength;
- the sieve curve calculation is based on elugram data and is therefore directly comparable to ASTM E1343-90(2001) and many in-house calculations.

Background and Calculation of Results

In every filtration experiment the mass of the stock (feed) solution is conserved and distributed between filtrate (permeate) and retentate solutions. This means that at any point in the chromatogram the following relation is valid (see Figure 47):

$$m_S = m_F + m_R$$

Please note that this relation is not true for concentrations since filtration conditions can cause additional dilution.

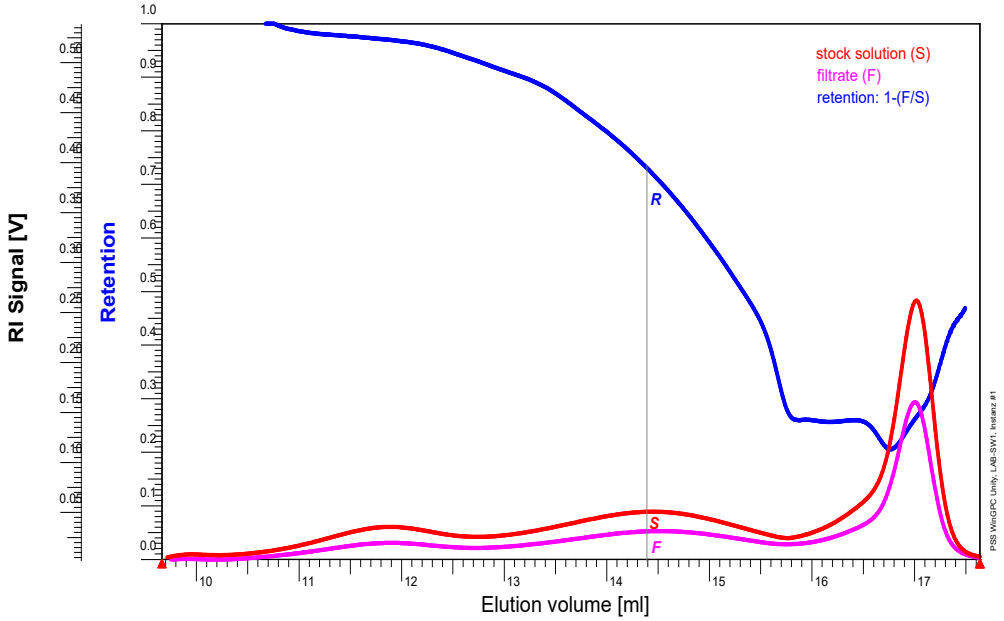


Figure 47 GPC chromatogram of stock solution (red), filtrate (pink) and resulting sieve curve (blue) as calculated by WinGPC Software using concentrations indicated by gray line

The retention behavior, *R*, of a synthetic or natural membrane can be described by the amount and size (molar mass) of the solutes which are retained for each size (or elution volume as measured by GPC):

$$R_i = c_i / c_{0,i}$$

At each position in the GPC chromatogram, the molar mass of the retained species is known from molar mass calibration.

Sieve curve, S:

It describes the filtration behavior of the membrane which is directly related to its average pore size and pore size distribution. It can be calculated in different ways (user selectable) and can be expressed in terms of pore diameter, molar mass or elution volume. An example of a sieve curve calculation using the stock solution (S), the filtrate (F) and the retentate (R)) is shown here:

$$S(M) = 1 - (c_F / c_S)$$

Average Pore Size, d_p:

Pore diameter at the inflection point of the sieve curve

$$\mathrm{dp} = \frac{d^2 S(d)}{d \cdot d^2}$$

Membrane Selectivity, D:

typically determined at 25% and 75% retention

$$\mathrm{D} = \frac{M(S(M) = 0.75)}{M(S(M) = 0.25)}$$

ideal selectivity: D = 1

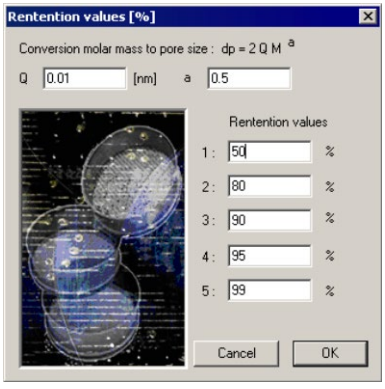
no selectivity: D = ∞

The cutoff molar mass is directly obtained from the sieve curve at the set value of retention (e.g., 90%). Up to 5 molar mass values are calculated automatically.

Please note that only WinGPC Software Scientific supports this kind of data treatment.

Performing Sieve Curve Calculations

All calculations are done in the overlay mode (cf. previous chapter "Overlay Mode" on page 237) of the WinGPC Software which has to be active to prevent that the menu items are grayed out. At least a single stock and filtrate solution has to be injected and overlaid to proceed with the membrane characterization. Only concentration signal can be used for membrane data analysis (but molar mass sensitive detectors can be used for calibration to measure molar masses directly).

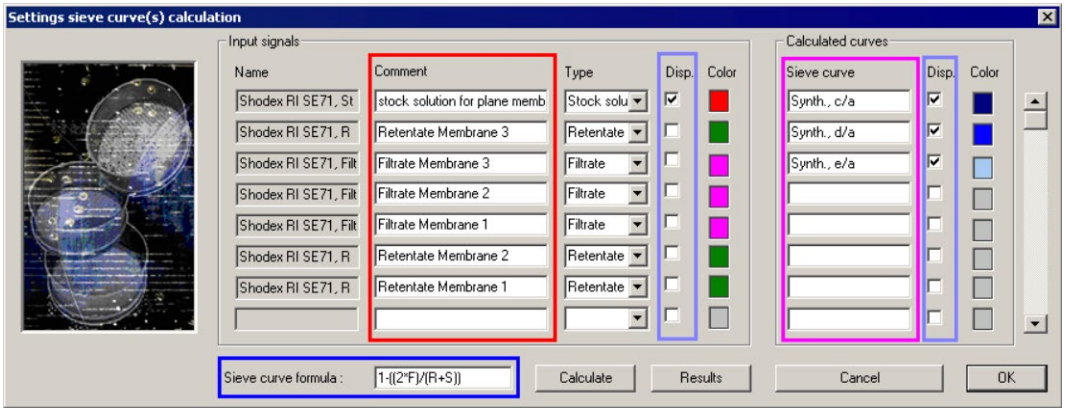


The parameters for molar mass cutoff results and their related pore dimensions are entered in the **Retention %** dialog (cf. adjacent figure) in the **Overlay** menu of the **Elugram** window. These most current parameters will automatically be saved and will be used as default values when the user opens the dialog again.

The Q and a parameters to calculate pore diameter from molar massed are entered at the top of the form, while the positions to calculate the molar mass cutoff and dp values are entered in the edit fields on the right. Q and a parameters can be found for many well-investigated polymer/solvent systems in the literature.

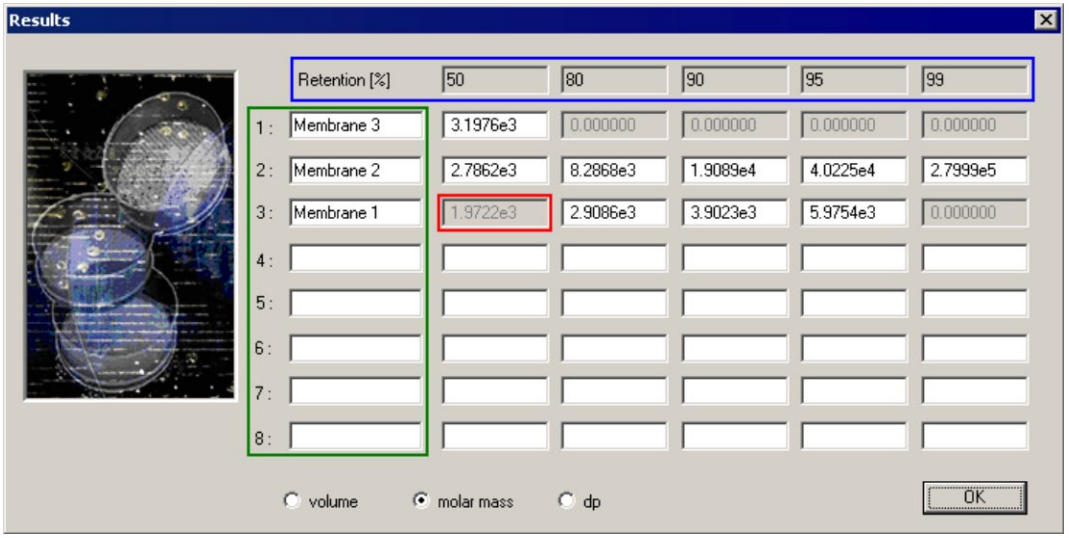
The calculation of the sieve curve is based on a sieve curve equation which can be entered in the **Overlay > Calculate sieve curve(s)** menu in the **Elugram** window (cf. Figure below). This equation can be entered by the user (blue box in figure below) but must contain exactly one curve representing a stock solution (S). The same mathematical operations and identical notation as described in the previous (overlay) chapter can be used. It may contain a number of filtrate (F) and retentate (R) samples from different membrane based on the same stock solution. This allows for the simple comparison of different membranes with minimal user input. Comments can be entered in the **Comment** fields for the raw data (analyzed samples) and in the edit fields for the calculated sieve curves (by default the parsed equation is displayed here) (cf. red and pink boxes in figure below). Each signal can be shown or hidden in the graphs depending on the state of the **Disp.** check box (see light blue boxes in figure below). The curve color can be selected by clicking in the usual way on the **Color** field of the respective signal.

The most current sieve curve equation will automatically be saved and will be used as default equation when the user opens this dialog again. WinGPC Software parses the equation and automatically assigns the related curves using the sample type information.



For the characterization of series of membranes, it is possible to process the filtrates (and/or retentates) of different membranes automatically using the same stock solution using the same sieve curve equation. The overlay of several sieve curve is also possible. The curve assignment is based on the sample type of the injection (sample), which can be entered in the WinGPC Software sample editor (or during sample processing in the sieve curve dialog).

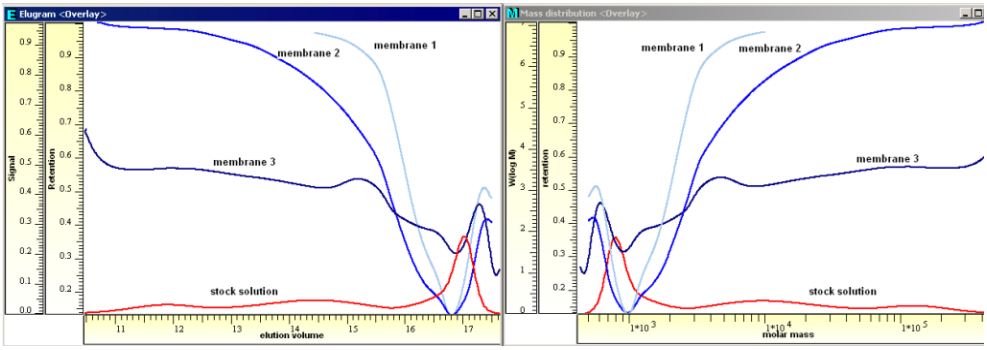
The numeric description of the sieving characteristics of a membrane is based on its retention ratios and their relation to molar masses and pore dimensions (see Figure below). The retention values shown as table headers (blue rectangle in figure below) are those entered in the **Retention %** dialog box. The names in the left column (green box in figure below) are the names of the sieve curves as entered in the **Calculate sieve curve(s)** dialog above. The results can be represented in elution volumes (volume), molar mass (which is the default setting) or pore size (dp) of the membrane. Results views can be selected by clicking on any of the radio button at the bottom of the dialog box below. Please note that all results can be reported on a single printout with the WinGPC Software ReportDesigner. Please see chapter "WinGPC Software ReportDesigner" on page 333 for an overview of sieve curve related variables.



NOTE

- A result "0" means that this retention value has not been reached during filtration and its value is grayed out (e.g., result row 1 for membrane 3 which only reached R=50%).
- If the fields show results with a gray background (e.g like in red rectangle in the figure below) then this result is not unique and represents the first finding in multiple values at the given cutoff property.
- The retention results are calculated from the elugram data starting from the center and searching to high and low elution volumes. Only the first findings are reported if multiple results are found. This method is most robust and often prevents reporting of results which are cause by baseline noise.

The graphical display of a sieve curve analysis is shown in the following Figure. It shows the stock solution (red) and the sieve curves of three different membranes (dark blue, blue, light blue) which were obtained with a single data analysis. The raw data are shown on the left (elugram) the molar mass view is shown on the right-hand side. Each of these windows has two independent Y-axis. The first (from the left-hand side) corresponds to the raw signal(s) of the analyzed samples, the second Y-axis in both windows show the filtration (retention) behavior of the membranes as determined from the sieve curve equation entered by the operator.



Sieve Curve Analyses – Step by Step

- Run new samples in WinGPC Software as usual or open a finished run from the database.
- Edit the inject/sample list in **Editor > Samples** and assign the correct sample type for each sample by clicking on **Sample type** drop down box and selecting the correct entry.
- Overlay in the usual way a single stock solution and up to 5 filtrate and/or retentate samples.
- Changes the settings for the cutoff values and/or the equation to relate molar masses to pore dimensions can be done in the **Overlay > Retention %** menu.
- Select **Calculate sieve curve(s)** from the **Overlay** menu to enter the sieve curve equation; please use the following symbols in the sieve curve equation:
  - S: stock solution
  - F: filtrate
  - R: retentate

Please use brackets extensively to enter complex equations (cf. chapter "Overlay Mode" on page 237 for details on parsing equations in WinGPC Software), e.g.: 1 - ((2*F)/(R+S)).

- In this dialog window click on the **Calculate** button, to determine the sieve curve(s) according to the equation shown to its left.
- In the same dialog click on the **Result** button to display sieve curve results on screen.
- Comprehensive result reports in desktop publishing quality can be created/modified using the WinGPC Software ReportDesigner. Make sure that the overlay mode is activated and that the sieve curves are shown on screen. Now select from the menu **Raw Data > Print Report** and select desired report template for printing.

Hints for data analysis:

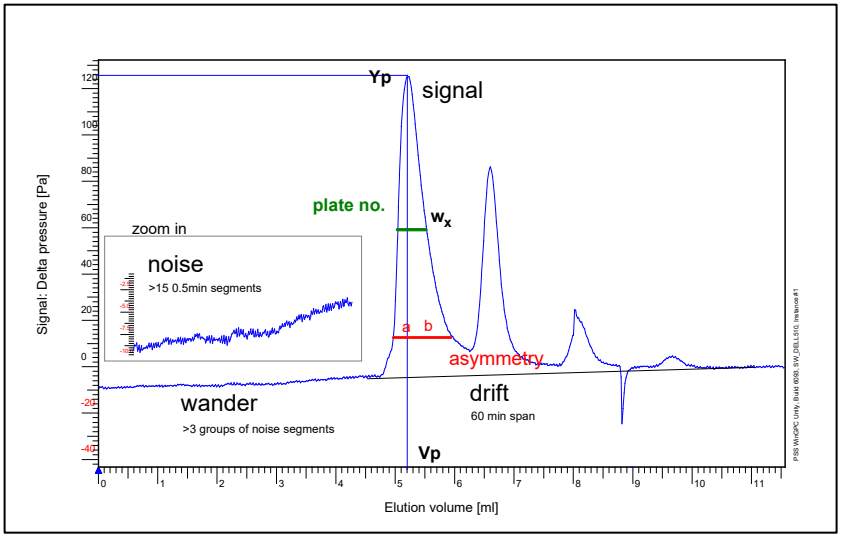
- Stable input signals will ensure accurate and unambiguous results. Please make sure to include only significant data in the overlay. Signal ratioing is sensitive to noise which may result in noisy sieve curves.
- Extra signal noise, non reproducible injections (flow variation), etc. can cause fluctuations in the sieve curve (non-monotonous sieve curve) and lead to wrong retention results.
- It is best to have narrow (close to peak start and end) integration limit settings prior to adding to the overlay.

System Suitability and Performance Tests

WinGPC Software allows for comprehensive tests to prove system suitability under user operating conditions. These tests are performed according to various international and national GPC standards (ISO 13855, ISO 16014, ASTM D5296, DIN 55672, GB/T 36214) as well as reference compliance guidelines (e.g., CFR 21 Part 11) and regulations (e.g., USP, EP, JP, DAB). These tests can e.g., be used to perform qualifications and comprise:

- system suitability tests
- complete sample/method related parameter set like resolution, theoretical plates, peak asymmetry, signal/noise ratio
- performance tests
- checking of instrument related performance parameters like flow accuracy, detector linearity, injection reproducibility

System Suitability Tests



WinGPC Software tests sample/method related parameters according to different GPC standards and pharmacopeias to get test results which can directly be submitted to government agencies, arbitration institutions or for litigation. Measured test results are compared to requirements specified in various guidelines, regulations and all current GPC specific standards and automatically

flagged for conformity or test fail. Moreover, users can define their own test rules and limits.

The following system test variables are determined in the WinGPC Software system suitability test; the calculation details are given in the next table for the different standards and guidelines. See also the adjacent Figure for a graphical explanation of the parameters and their calculation.

Table 32 Summary of system suitability parameters and their determination

Parameter	Guideline				
	ISO 13885 DIN 55672	**ISO 16014 Jp. Pharmacopeia**	**ASTM D5296**	**US Pharmacopeia**	**EU Pharma-copeia, DAB**
theoretical plates, Nth [1/m]	$554(V_p/w_{0.5})/L$	$555(V_p/w_{0.5})/L$	$16(V_p/w_{4\sigma})/L$	$16(V_p/w_{4\sigma})/L$	$554(V_p/w_{0.5})/L$
asymmetry, A	a/b @ $0.1Y_p$	a+b/2a @ $0.1Y_p$	a/b @ $0.05Y_p$	a+b/2a @ $0.05Y_p$	a+b/2a @ $0.05Y_p$
resolution, R_{sp}	$R_{sp} = \frac{R_s}{lg\, M_1 / M_2} = \frac{0.579}{\sigma \cdot D}$				
efficiency, T [cm]	$T = \frac{V_e(M) - V_e(10 \cdot M)}{A} > 6$				
baseline drift	$(Y_2\text{-}Y_1) / (V_2\text{-}V_1)$				
baseline noise, N	$\sqrt{\frac{\sum(Y_{max} - Y_{min})}{n-1}}$				
signal/noise, S/N	$2Y_p / N$				
wander, W	$\frac{\sum(<Y>_{max} - <Y>_{min})}{m}$				
pump pressure fluctuation	$\sigma_P = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(P_i - \bar{P})}$				
column temperature fluctuation	$\sigma_T = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(T_i - \bar{T})}$				

Definitions:

a front volume segment as defined in Figure above

b tail volume segment as defined in Figure above

V_p elution volume at peak apex

Y_p signal height at peak apex

L: total column length

$w_{0.5}$ peak width at 50% of peak height

$w_{4\sigma}$ peak width at 4σ of peak

The system test can be performed for evaluated samples via the **Options > System test** menu. The tallest peak in each detector signal within the integration limits is used for the calculations. If a specific peak in a complex chromatogram shall be used, the integration limits should be set accordingly. A conventional calibration and known column dimensions are also necessary for the calculation of SST results. These data can also be assigned post-run.

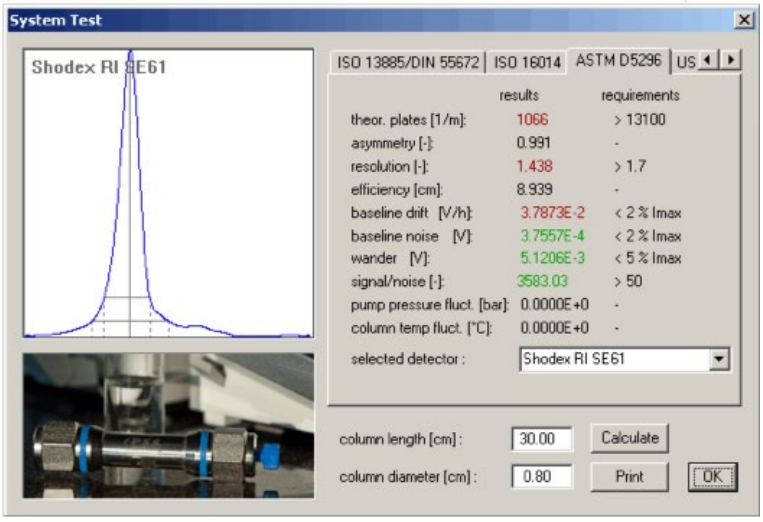


Figure 48 System suitability test according to GPC standard ASTM D5295; plate number, resolution and baseline drift are not met

If the requirements in those standards are met, results will be reported in *green*; otherwise, they are shown in *red*. If no performance limit (requirement) is specified, those results are reported in black (see Figure 48).

As soon as the dialog is acknowledged with **OK**, the results of the system test will automatically be added to the column database.

If pump pressure and column temperature have been recorded, their stability will be shown and their contribution to the result uncertainty will also be evaluated (these signals have to be of the **pump pressure** and **columntemp.** signal types).

Since not everybody has to work according to external regulations, internal standards with method dependent requirements can be defined. This allows to reflect different best practice approaches for different eluents, columns and detectors in local environments and increases productivity and analytical quality. They will appear as a separate SST tab in the system test dialog.

A new SST definition can be added by clicking on the SST tab **New**. This opens a settings dialog for system test specifications set by the user. Enter a name for the new system test definition. This name will be shown in the SST TAB. Plate number and asymmetry calculations can be selected independently from a list of standards. Enter user-defined limits into the parameter fields in the requirements section of the dialog.

NOTE

Baseline noise and wander are determined according to ASTM E1657 for up to 15 segments (0.5 min each) for each signal from baseline start to sample injection.

Please note that overlaid injections may show peaks from the previous injection between injection time and baseline start that may affect noise and wander results; details can be found in the **uncertainty assessment** tab of the information icon in the icon bar.

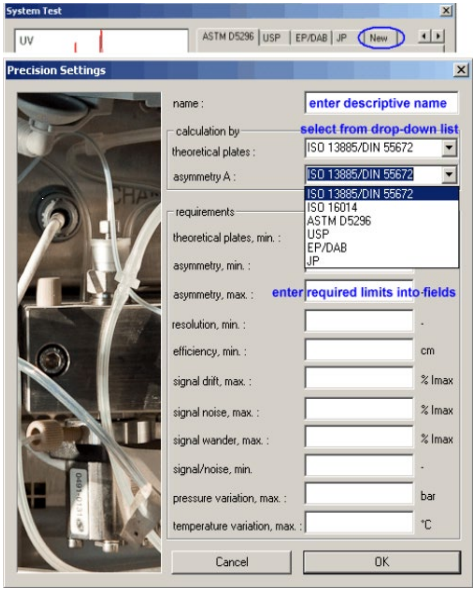


Figure 49 Dialog for user-defined SST limits

Instrument Performance Tests

Proper instrument performance is a must for any type of chromatography. Pump performance (accurate flow rate) is especially for GPC applications as the absolute peak position in the chromatogram is used to determine the molar mass in conventional GPC. In GPC setups with molar mass sensitive detectors the injector performance is critical as absolute samples amounts have to be injected. WinGPC Software allows to check system components for proper performance. The following tests can be accessed from the **Options > Performance Tests** menu in the **Elugram** Window:

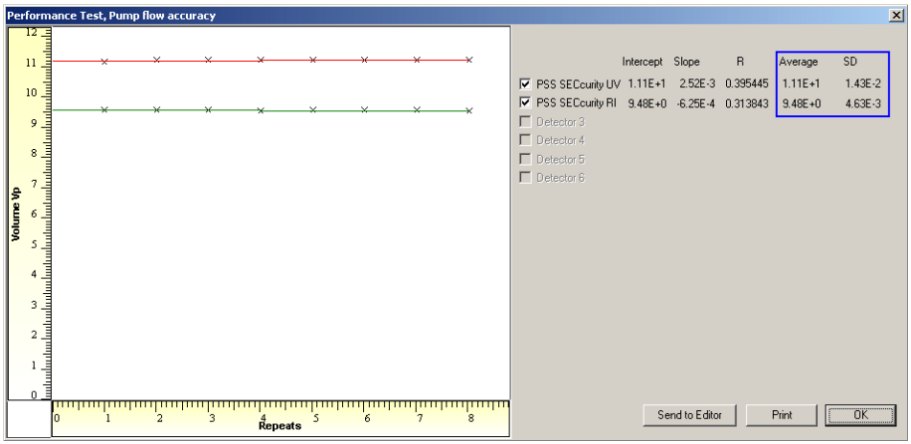
- pump flow accuracy
- injector reproducibility
- injector linearity
- detector reproducibility
- detector linearity

The performance tests are based on multiple injections which have to be overlaid for processing; the performance tests menu will be grayed if the overlay mode is not activated.

Each instrument performance test can be done with a minimum of two and a maximum of 32 injections. The figure below shows results of a flow accuracy test for each configured detector signal (here: UV (red) and RI (green) signals).

The results of the flow accuracy check are:

- number of repeats
- average peak position
- standard deviation (SD) of
- peak position
- difference between average and intercept
- slope of the repeat function



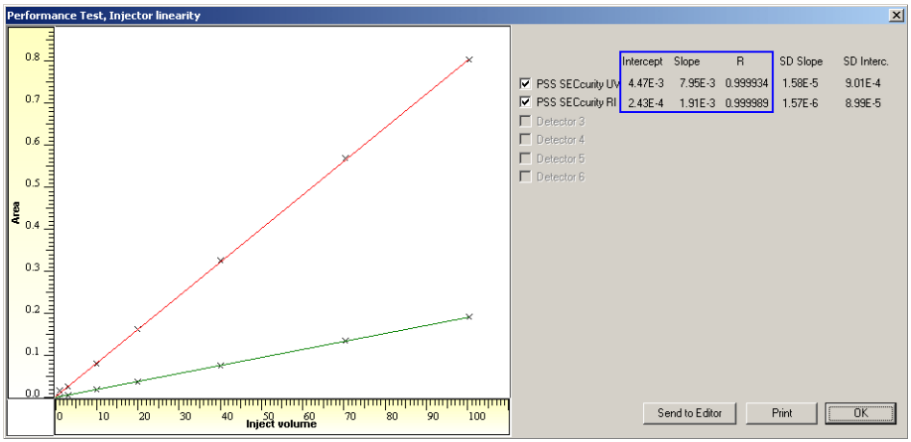
The result of this flow accuracy test is for the RI signal:

Peak position: V_p = (9.47798 ± 0.00463) ml, 0.05% RSD, based on 8 repeats

Since a complete linear regression analysis is made additional details for the estimation of analytical quality are available, e.g., the slope of the repetitions can be indicative of a systematic pump or eluent problem. Small slopes as in the example shown above are normal as they indicate just the randomness of the peak position itself.

Similar checks are performed for the reproducibility of injections and detector response.

There are also checks for the linearity of the injector (injection volumes) and detector responses over a given concentration range. These tests allow to verify that the analytical method is sound, and results are trustworthy. The figure below shows the result of a injector linearity test performed for injections from 1 µl to 100 µl and analyzed simultaneously for UV (red) and RI (green) detector signals.



The results of the injector linearity check are:

- number and range of injection volumes
- slope of the injection increase
- standard deviation of injection increase (SD slope)
- regression coefficient R for the linear regression
- intercept of the linear regression (should be close to zero)
- standard deviation of the intercept expressed as **SD Interc.** in the result dialog

All performance test results can be printed using default printout (by pressing the **Print** button in the dialog) or using the ReportDesigner (optional software module) which allows to meet specific in-house requirements and styles for performance testing.

NOTE

The performance tests can be easily used to run the extended OQ/PQ tests specified in the PSS EasyValid Kits. This product allows for a comprehensive and FDA compliant qualification of instruments. Results are printed according to FDA specifications and auditor expectations. The ReportDesigner option enables users to define outputs meeting their own company requirements.

Performing Dextran Analysis based on USP / EP

Size-exclusion chromatography is a standard technique in each pharmacopoeia to determine molecular weights and molecular weight distribution (MWD) in pharmaceutical testing (e.g., USP <621>, Pharm. Eur. 2.2.30 and similar as the British, Chinese and Japanese monographs). Such experiments can be performed by a combination of Agilent GPC/SEC systems with matching consumables and services to achive highest performance, productivity, and reliability.

This chapter describes the implementation of the USP and EP Dextran Monographs in an optimized and hassle-free WinGPC Software workflow comprising data capture, data analysis and compliant reporting. Regulated laboratories should opt for the WinGPC Software Compliance Edition for 21CFR11 support.

The basis for Dextran workflow implementation are the following monographs:

Table 33 Monographs

	USP monograph	EP monograph
SEC method	USP <621>	Pharm.Eur. 2.2.30
Dextran analysis	USP 40	Pharm.Eur. 2.2.39

The chapters below guide users through all steps to create dextran calibrations and process unknowns based on the USP or EP monograph. Please also refer to the online step-by-step help accessible from the WinGPC Software **Help** menu.

Performing Calibrations According to the Dextran Monographs

1. Run new (refer to Chapter "Acquire Data" on page 106) or load existing the dextran samples (standard solutions) from the **Project Manager** in the WinGPC Software icon bar by a double mouse click on an sample injection entry in one of the listed sequences (see Figure in next chapter for details).
2. Set baselines in the **Raw Data** window by selecting **Options > Quick Analysis** from the menu when analyzing several samples. Alternatively set baselines interactively by dragging the red baseline start (or end) marker from the blue injection marker while pressing the left mouse button (see Figure in next chapter for details).

3 Specify data integration limits in the **Raw Data** window by selecting **Options > Quick Analysis** from the menu when analyzing several samples or set integration limits interactively in the **Elugram** window by dragging the left (right) integration marker (red triangle) at the left (right) window edge while pressing the left mouse button.

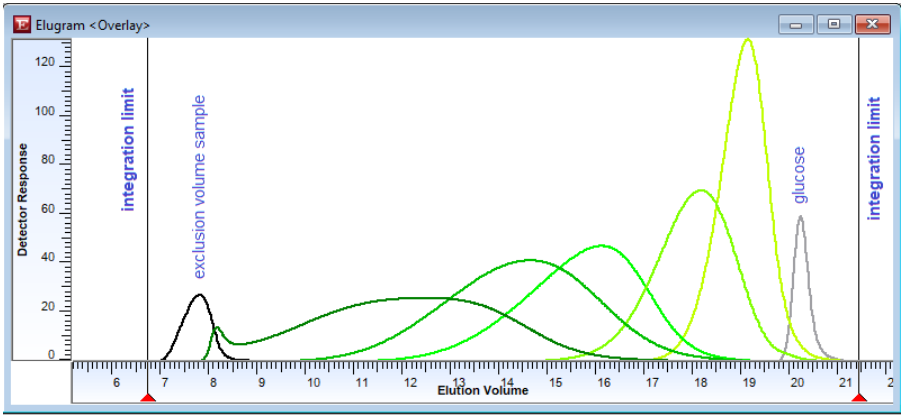
4 Overlay all dextran calibrants which shall be used for establishing the dextran calibration by selecting the dextran calibration sample from the **Inject** menu in the **Raw Data** window followed by a mouse click on the **Overlay** Icon in the icon bar. Agilent recommends setting the **Inject Options** in the WinGPC Software Status Bar to **Actual inject + selectable** to overlay several samples in a single step.

   The monographs require to process 5 calibrant samples plus an exclusion limit and glucose reference material to establish the calibration.

5 Select the V_0 and V_t sample from the **Inject** menu in the **Raw Data** window and set separate integration limits interactively starting with the V_0 peak in the **Elugram** window by dragging the left (right) integration marker (red triangle) at the left (right) window edge while pressing the left mouse button and dropping it before and after the V_0 peak. Click on the **Overlay** icon in the Icon Bar to add the V_0 peak to the dextran calibrant collection.

6 Then drag the integration limits from the V_0 peak (set above) to the V_t peak and click on the menu **Overlay > Include curve** to add the V_t peak to the overlay.

   This procedure ensures that the exclusion limit (black) and glucose (gray) peaks are added to the calibration as separate curves in the dextran calibrant collection as shown in the Figure below.

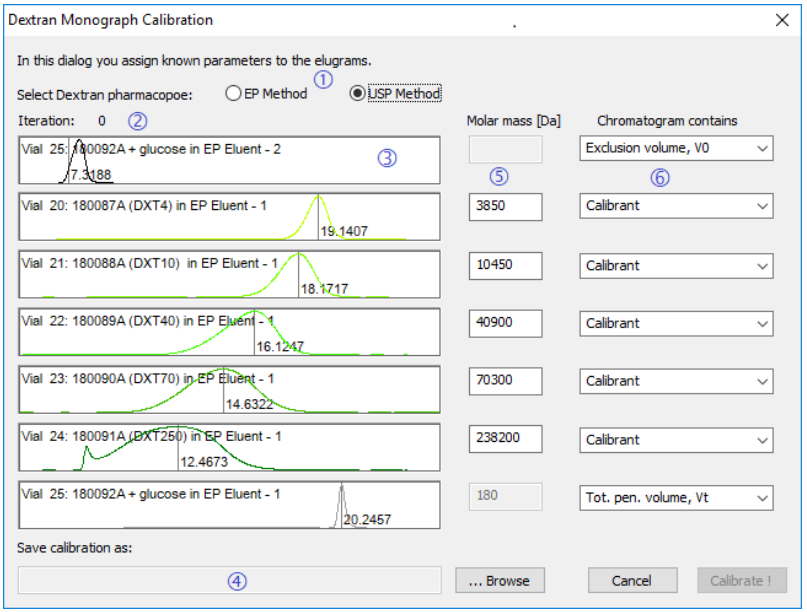


7 Start the dextran calibration process by selecting **Options > Dextran Monograph > Calibrate** from the **Elugram** window menu, select USP or EP monograph and enter the molar mass reference values, which will automatically filled in if they had already been entered with the sample properties, e.g., in the **Sequence Manager**).

NOTE

The [Calibrate] button will stay grayed until the overlay contains exactly 7 samples and a calibration file name has been specified.

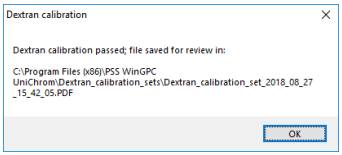
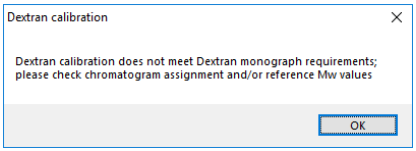
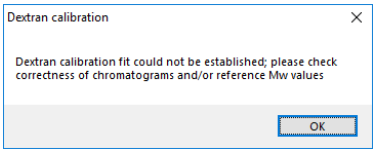
8 If all information is correctly entered in the Dextran Calibration dialog shown below, click on the **… Browse** button to enter a calibration file name and start the calibration process by clicking on the **Calibrate** button.



1 selector for dextran monograph
2 calibration iteration counter
3 sample in overlay with calibration position; deviations will be shown during iteration in red (fail) or green (met)
4 calibration file name selected by **… Browse** button
5 molar mass reference value from USP/EP calibrant
6 sample type used in calibration process; pre-assigned by WinGPC Software

The iteration process may take a few moments depending on the quality of the raw data. At the end of the process a message box is shown indicating the calibration result.

Table 34 Calibration results

Calibration successful	Acceptance limits not met	Calibration failed
		
Calibration file successful created meeting requirements.	Monograph acceptance limits not reached; please follow hints in message box to resolve.	Calibration failed; incorrect input most probable cause. Correct and retry.

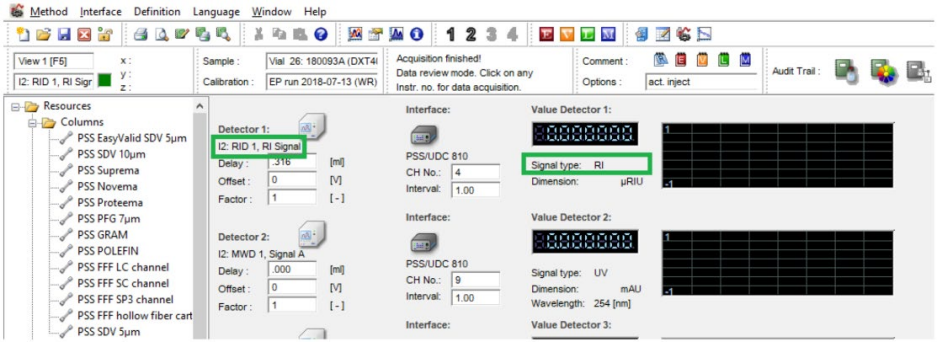
If the calibration is created, a monograph-compliant PDF report is saved together with the calibration file and a copy of the source overlay in the folder ...**\Dextran_calibration_sets**. Follow the on-screen instructions, if the calibration does not meet the monograph acceptance limits.

NOTE

A complete history of the created calibrations is available in the WinGPC Software subfolder ...**\Dextran_calibration_sets** as backup and for review. Each calibration source overlay file (*.ADD) and the related calibration file (*.CAL) is saved in this folder. Each file is date/time stamped, e.g., as "Dextran_calibration_set_2018_08_29_10_53_21.CAL" for revision history.

Performing Dextran Data Analysis

The dextran acquisition method (as displayed in the WinGPC Software **Method** window) requires a refractive index detector with its **Signal type** defined as **RI**. Otherwise, no molecular weight results will be reported automatically to prevent data mismatch between multiple detectors in an acquisition method.

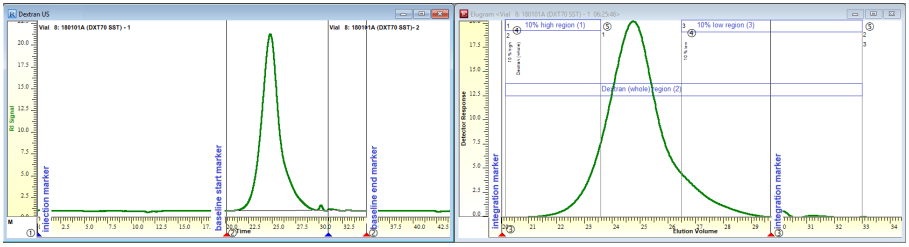


The Dextran data processing method requires a Dextran calibration as outlined in the previous chapter for proper evaluation. If a calibration file is assigned to a sample to be processed according to a Dextran monograph which has not been created accordingly, the menu **Options > Dextran Monograph > Evaluate** is not accessible (grayed out).

1 Run new (refer to Chapter "Acquire Data" on page 106) or load existing the dextran samples (SST or unknowns) from the **Project Manager** in the WinGPC Software icon bar by a double mouse click on an sample injection entry in one of the listed sequences.

2 Set baselines in the **Raw Data** window by selecting **Options > Quick Analysis** from the menu when analyzing several samples. Alternatively, set baselines interactively by dragging the baseline start (or end) marker from the injection marker while pressing the left mouse button.

3 Specify data integration limits in the Raw Data window by selecting **Options > Quick Analysis** from the menu when analyzing several samples or set integration limits interactively in the **Elugram** window by dragging the left (right) integration marker (red triangle) at the left (right) window edge while pressing the left mouse button results as shown in the Figure below.



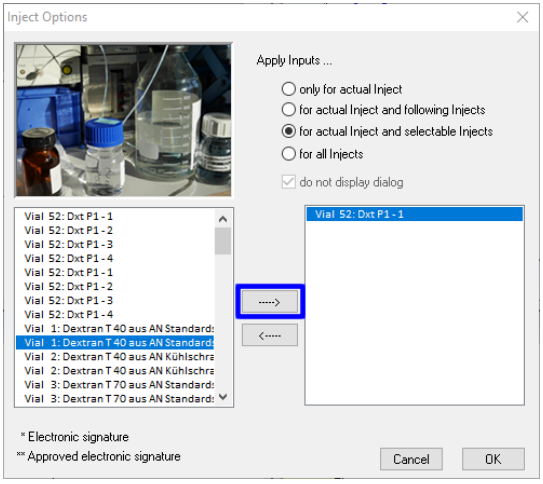
1 injection marker for selected sample injection in raw data window
2 baseline markers (start/end) for selected sample injection in Raw data window
3 integration markers (start/end) for selected sample injection in Elugram window
4 molar mass region (e.g., 10% high) start markers in Elugram window
5 molar mass region (e.g., 10% low) end markers in Elugram window

4 Assign a matching Dextran calibration to the related data set(s) selected in step no. 1 above:

Highlight the WinGPC Software **Raw Data** window with a mouse click, select **Calibration Data > Load…** and choose the related calibration file from the file list.

5 Click into the WinGPC Software **Elugram** window and select **Options > Dextran Monograph > Evaluate** and select a Report layout matching the sample(s) for result generation (e.g., Dextran 70 USP SST.LST for a USP SST test of the USP Dextran 70 SST sample).

Depending on user preferences, an **Inject Options** dialog might be shown which allows users to process several (repeat) samples of the same kind (SST, unknown samples for certification) as shown in the Figure below.



6 Result report output can be directed to printers, preview, PDF, Excel and many more.

NOTE

- If the data acquisition method does not contain a RI detector with signal type **RI** the report will show an error message and no molecular weight results. This property can be changed by a left mouse click into the detector value digital display box below **Value Detector {no.}**, if the user has proper privileges.
- Dextran SST and certification reports currently only support RI detectors configured as **Detector 1** through **Detector 3** in the result tables.

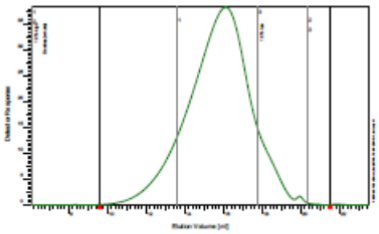
WinGPC Dextran 40 SST Report

PSS
A part of Agilent

This system suitablity report tests the acceptance limits for Dextran 40 for EP verification.

SST was executed for SEC-System:
on: PC-GPC-SW015 with Windows 10 Enterprise (Ver. 2009, Build: 19045),
with Agilent WinGPC, Build 10422 S/N: 432001
by acquisition operator: wr
at (Year-Month-Day): 2018-8-30, 08:17:15
with method: C:\Program Files (x86)\PSS WinGPC UniChrom\Methods\usp dextran.SET
last modified: at:

Elugram with high/low limits



Processing parameters

Injection:	date: 14.07.2018, time: 04:59:39
Project:	C:\Program Files (x86)\PSS WinGPC UniChrom\Projekte\dextran USP EP all LDX
Calibration:	[illegible]
created:	2018-08-30 at: 08:12
last modified:	2018-08-30 at: 08:13
Baseline from:	6,08 ml
to:	23,49 ml
Integration from:	9,61 ml
to:	21,50 ml
Solvent:	EP Dextran Eluent
Flow rate:	1,00 ml/min
Injection volume:	50 µl
Detector(s):	I2: RID 1, RI Signal I2: MWD 1, Signal A I2: MWD 1, Signal B

System Suitability Test results
based on acceptance limits as defined in EP monograph

for sample: Vial 26: 180093A (DXT40 SST) in EP Eluent - 2

	Test parameter	*Allowed range*	*Test result*	*Status*
Results for detector:	*I2: RID 1, RI Signal*			
Mw	Mw range	41000 ... 47000	42827	passed
Mw(high 10%)	Mw result	111000 ... 130000	121076	passed
Mw(low10%)	Mw result	6000 ... 8500	6909	passed
				End of Table

This Dextran EP verification is passed when all results are within the allowed range.

Verification passed: ☐ Yes* ☐ No**

Signature: _____ Date (Month, Day, Year): _____

* Verification passed, no further action required.
** Verification failed. Please check that correct raw data are overlayed and reference values entered in Dextran Calibration dialog.

Print date (month-day-year): 05-16-2023 Page 1 of 1

WinGPC Dextran System Suitability Report Version 1.0 (c) 2023, PSS Polymer Standards Service GmbH

Mass Distribution Window

The mass distribution window shows the molar mass distribution of the sample within the integration limits. The calculated molar mass averages and other information are listed in the information box for the sample identified in the **Sample** field in the status bar. The molecular weight averages always relate to the peaks within the integration limits, which were set in the elugram window (cf. chapter "Elugram Window" on page 224). The type of calibration or the name of the calibration curve are shown in the **Calibration** field. The molar mass determination method can be selected at any time from the X-axis context menu.

The following molar mass calculation options are available:

Table 35 Mass distribution window

X-axes menu selection	Calibration field in status bar	Comment
Calib. Standard	{calibrationfilename}.cal	Conventional calibration with narrow or broad standards (cf. chapter "Interactive Creation of Conventional Calibration Curves" on page 311)
Calib. Standard	UE -> {calibrationfilename}.cal	Conventional calibration with Mark-Houwink parameter correction (cf. chapter "Calibration Curve Creation by Mark-Houwink Transformation" on page 313)
Calib. Lightscattering	Lightscattering	Molar masses directly measured by LS detector (cf. chapter "Light Scattering Window" on page 401)
Calib. Viscometry	Viscometry	Molar masses measured by online viscosity detector (cf. chapter "Universal Calibration Curve Creation with Viscosity Detectors" on page 321)
Calib. Triple Detection	Triple Detection	Molar masses based on LS and viscometry detection (cf. chapter "Triple Detection" on page 412)

NOTE

When a calibration filename is shown in the **Calibration** field in the status bar either direct calibration or universal calibration using Mark-Houwink parameters is possible. If you do not use the **Calibration Data > Universal calib.** option in the Raw Data Window but create a separate calibration file, give a descriptive filename to identify a calibration file generated from Mark-Houwink parameters.

NOTE

The internal WinGPC Software report does not explicitly mention molar mass calculations based on light scattering and/or viscometry detection. The WinGPC Software ReportDesigner option allows to fine-tune reports and also allows to report the results of a sample with all methods simultaneously e.g., on a single page.

The method how the molar mass distribution and the molar mass averages are calculated by the software is described in detail in chapter "Molecular Weight Averages and Molecular Weight Distributions in GPC/SEC" on page 18. Further details and background information can be found in good textbooks on polymer characterization.

The mass fraction limits are defined by two line cursors with red triangles at the lower end in the mass distribution window. They are located on the outer left and right window edge by default. The triangles can be dragged manually to the desired position. Alternatively, molar mass values for both limits can be entered using the X-axis context menu. The report subset settings in the mass distribution window have no effect on the molecular weight calculation.

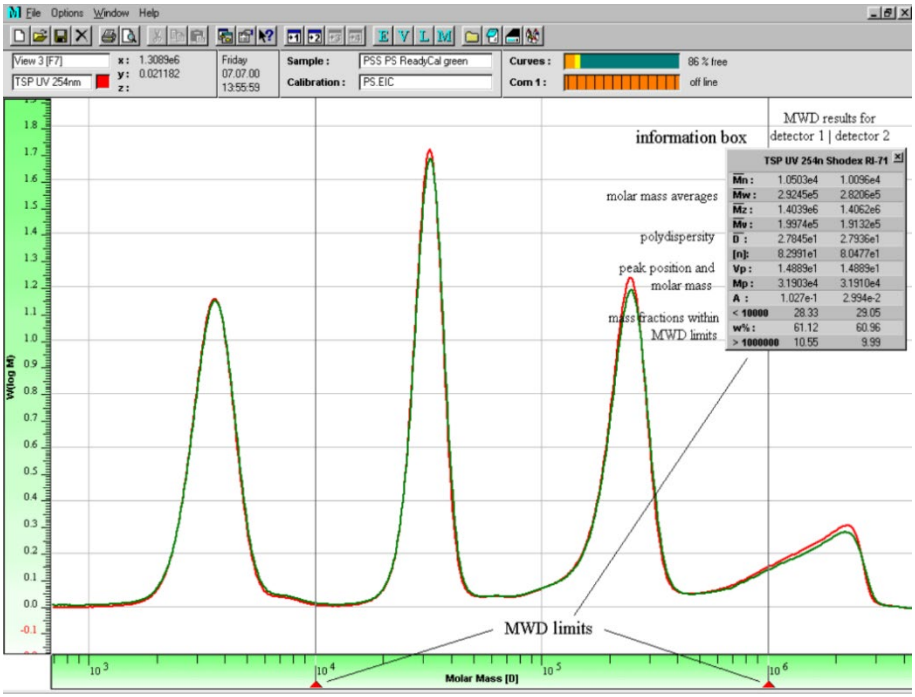


Figure 50 WinGPC Software Mass Distribution Window with advanced processing settings and result box

Table: Description of parameters in the information box are (also see chapter "Molecular Weight Averages and Molecular Weight Distributions in GPC/SEC" on page 18)

Table 36 Description of parameters in the information box

Item	Description
M_n	Number average molecular weight
M_w	Weight average molecular weight
M_z	Z-average molecular weight
M_v	Viscosity average molecular weight; either determined from viscosity detection or calculated from Mark-Houwink parameters in the selected calibration curve; please note that this value will be 0 if either no Mark-Houwink parameters are entered in the selected calibration curve or if the measured Mark-Houwink a is negative
D	Polydispersity; i.e. Mw/Mn

Table 36 Description of parameters in the information box

Item	Description
[η]	Intrinsic viscosity either determined from viscosity detection or calculated from Mark-Houwink parameters in the selected calibration curve; please note that this value will be 0 if either no Mark-Houwink parameters are entered in the selected calibration curve or if the measured Mark-Houwink a is negative
V_p	Volume at the peak maximum of the elugram
M_p	Molecular weight at peak maximum in the elugram
A	Peak area within the integration limits
With standard options:	
<	mass fraction with molecular weight M < M (left MMD limit) (peak area up to the left line cursor)
w%	mass fraction between the limits (between both line cursors)
>	mass fraction with molecular weight M > M (right MMD limit) (peak area above the right line cursor)
Alternatively with **Options > Fixed Cum > Display** activated (5 lines shown with)	
nn %	Molar mass at *nn*% of the cumulated mass fraction

X-Axis Context Menu

These functions are accessible from the context menu, if you click on the X-axis scale with the right mouse button. A popup menu appears in which the following functions can be selected:

Table 37 X-axis context menu

Function	Description
Set peak integration:	Searches around the mouse position for the next peak and sets the mass fraction limits accordingly.
Calib. Standard:	Calculates molar mass averages and distribution based on narrow, broad or universal calibration using Mark-Houwink coefficients.
Calib. Lightscattering:	Molar mass averages and distribution are calculated from direct measurement of molar masses from light scattering detector.
Calib. Viscometry:	Molar mass averages and distribution are calculated from viscosity detector based on the universal calibration method.

Table 37 X-axis context menu

Function	Description
Calib. Triple Detection:	Calculates molar mass averages and distribution based on a com-bination of 90°-light scattering and viscometry measurements.
Manual borders:	Allows the manual setting of mass fraction limits. This enables entering exact molar mass values (e.g., 1000 instead of 1003), while the other options above rely on drag&drop or minimum determination.
Set standard scale:	Allows the manual setting of the displayed X-axis range of the molar mass distribution and sets the default standard scale.
Standard scaling:	Restores the scale of the last manual scaling (e.g., after editing the scaling by the arrow keys or the scroll bar of the X-axis.). A tick mark in the context menu indicates if the standard scale is in use.
Properties:	This context menu is available on each axis in the WinGPC Software. It allows setting axes properties individually. For example, text attributes (font, size, color etc) and background properties of the axis can be defined. It is also possible to switch from elution volume to elution time representation for X-axis properties. The axis properties will be saved for each instrument individually.

Y-Axis Context Menu

These functions are available from the Y-axis context menu, if you click on the Y-axis scale with the right mouse button. A popup menu appears in which the following functions can be selected:

Table 38 Y-axis context menu

Function	Definition
w(logM):	This is the default Y-axes, which shows molar mass distribution area normalized. The physical meaning of the ordinate values relate to weight fractions of eluted mass in a given logarithmic molar mass increment.
norm. w(logM):	This Y-axis display option normalizes each signal to 100% scale. It allows to see all detector signals independent of their strength.
rel. w(logM):	In this Y-axis mode the detector signals are displayed relative to their detector output. This allows to see a weak and a strong detector signal in their relative positions, without changing the detector output by any normalization factor.
Set standard scale:	Allows the manual setting of the displayed Y-range of the chromatogram and sets the default standard scale. Individual Y-axis settings are available for each representation of the molar mass distribution.

Table 38 Y-axis context menu

Function	Definition
Standard scaling:	Use preset Y-axis scales for each Y-axis display option as defined in **Set standard scale**. A tick mark in the context menu indicates if the standard scale is in use.
Properties:	This context menu is available on each axis in the WinGPC Software. It allows setting axes properties individually. For example, axis labels, text attributes (font, size, color etc) and background properties of the axis can be defined.

NOTE

The standard Y-axis settings for the molar mass distribution are always the same independent of the type of distribution calculation (number or weight) as selected from the **Options > Number Distribution** menu.

File Menu

Table 39 File menu

Function	Description
Printer Setup:	Allows definition of parameters of the active printer. However, the active printer must be defined in the Windows Control Panel. Landscape format prints the graphics on a full page. Portrait format prints the graphics, full method documentation and results by default. The printed information in the portrait layout can be selected for automated runs in the **Definition > Automation Settings** dialog box. The exact information of the portrait format printout depends on which window will be printed. For color printers you can select color or monochrome printing depending on the printer driver options. The color of the curves in monochrome printouts are mapped automatically to a line style to avoid unreadable black and white prints. The correlation between curve color and line style in monochrome print is listed in "Curve Colors and Line Styles in Monochrome Printing" on page 534.
Print:	Prints the current contents of the mass distribution window on the default printer.
Page Preview:	Shows a print preview in a separate print-preview window, which can be sized and moved. At the same time this command copies the preview contents to the Windows clipboard. When printing in portrait format, the graphics and the text for the measurements will shown, while landscape format will print only the graphics.
Print Annotation:	Opens a dialog box to enter a header for the standard report. This header line will be printed directly above the mass distribution on the WinGPC Software mass distribution report; other printouts may have different text. A total of 80 characters can be entered in this line.

Table 39 File menu

Function	Description
Save ASCII Report	Creates an ASCII file which contains all results, sample specific parameters, analysis conditions and the displayed data in all graphical windows. This allows for easy and flexible creation of individual reports using a simple macro language on any target computer (including Unix systems). Since 8.2: Includes HPLC results, calibration parameters, additional meta data an Mz+1 values.
FTP Report:	This option can be used for GPC result transfer to external databases (LIMS etc) via FTP transfer. Please contact Agilent about proper configuration of the FTP protocol (cf. **Help > About** for contact information).
Edit Comment:	Allows to enter a mass distribution related text (comments, hints for data treatment, observations, etc.). Up to 1024 characters can be entered for each injection (sample) separately. Alternatively, the  icon in the status bar can be used to open the comment dialog box. This icon is gray if no comment has been entered for this sample, if text has been entered it is highlighted in green.

Options Menu

Table 40 Options menu

Function	Description
Cum. Distribution:	Activates/Deactivates the display of the integral (cumulative) distribution for all curves on a separate Y-axis. The cumulative distribution is overlaid to the differential distribution.
Edit Comment:	Allows to enter a mass distribution related text (comments, hints for data treatment, observations, etc.). Up to 1024 characters can be entered for each injection (sample) separately. Alternatively, the  icon in the status bar can be used to open the comment dialog box. This icon is gray if no comment has been entered for this sample, if text has been entered it is highlighted in green.
Number Distribution	Converts MMD results from mass into number distribution.
Visco Distribution:	Shows Mark-Houwink relation from online viscosity measurements (only available with viscosity module).
Editor Slice Data:	Copies MMD data of all shown detector signals to the data editor. Molar mass, selected Y-axis option, I%(log(M)) is tabulated there for all displayed signals. This list can be exported as ASCII file from the Data Editor Window.
Show Maxima/Minima:	Determines and marks the maxima/minima of all detector signals.

Table 40 Options menu

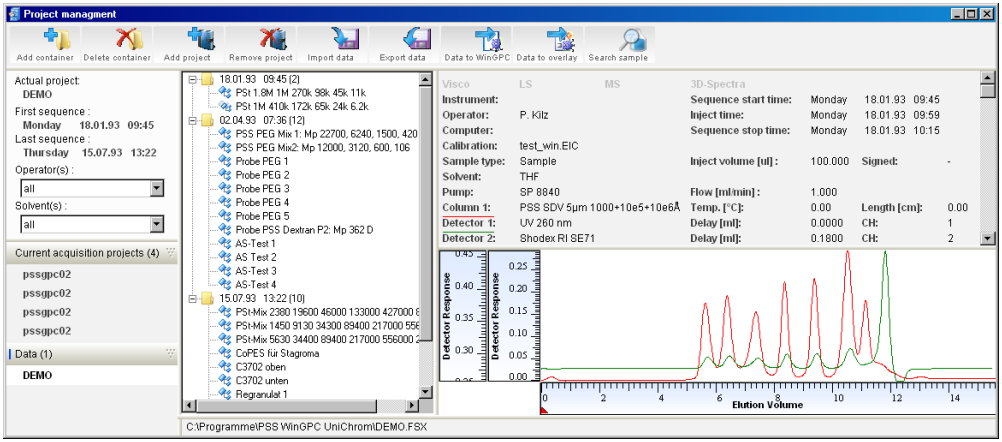
Function	Description
Show Inflection Points:	Determines and marks the inflection points of all detector signals. This can be a useful feature for kinetic studies and calculation (e.g., for pulsed laser polymerizations).
Copy to DataEditor:	Copies the data of shown minima, maxima, and inflection points to the data editor, where they can be exported as an ASCII file.
Fixed Cum%:	• edit: Allows to edit a list of report subsets. The molecular weights are calculated at which the cum. MMD reaches the predefined percentage values. The MMD option integrates by ascending molecular weight, the elugram integrates in elution volume units, i.e. in descending molecular weight. • display: Displays the fixed cum% list in the mass distribution information box.

NOTE

The definition of w% weight fractions is different in the interactive and automated WinGPC Software data processing modes. In interactive data processing three subsets are available while 5 are selectable in automated mode.

The Project Management Window

The window **Project management** is the central point for organizing the samples of the individual data capture projects. From here projects and raw data can be organized in containers. Furthermore, here you can load or search specific logins (Injects) and send them to WinGPC Software for data processing.



The window **Project management** shows the defined container as well as the Login List of the selected project (list of injection series or sequences stored in the project).

The **Project management** window is devided into three areas. The left side shows the currently selected project as well as its first and last sequence (login) with date and time. Two filter options (**Operator** and **Solvent**) offer to list only those logins, which match with the activated filters. The project container that are used to organize the projects, are displayed below.

You can choose any name for the containers, and it is possible to add several projects to one container. The currently active container is highlighted by a blue marker in front of the container name. You can add an additional project to this container by clicking on **Add project** button or with a right mouse click onto the container name and choose **Add project**. Note that only the *.LDX file of each project is displayed in the WinGPC Software file browser, but all files belonging to the project will be loaded together automatically. The project name (which is also the file name) of the current project is always saved in the current method.

NOTE

The container **Current acquisition projects** will be created automatically and always contains the projects which are saved in the current methods. It is not possible to add projects to this container manually. If you want to view data of already existing projects, you need to create at least one additional container.

In the center of the Project management window all Logins with the start and stop time for data acquistion as well as the size of data series (number of injects) will be shown. If the raw data was named (with highlighted **Raw Data** window **Raw data > Name Raw data**, see chapter "Raw Data Window" on page 195 for details) you will also find the name in the center of the window (white area) with all listed *logins* located in the selected container. If there are different injects per *login* shown by the plus symbol, you can click on it and the full list of all injects (sample names) of the selected login will be opened. For *Logins* without an inject ("(0)" next to the login description, no plus symbol shown), e.g., created by pressing only the **Record** and **Stop** button, the recorded raw data can only be displayed by clicking on the button **Data to WinGPC**.

If a certain inject is selected (left mouse click on the sample name, will highlight the sample), sample details are displayed in the preview on the right side of the **Project management** window. On top of the preview selected details about the method, which was used for data recording, are presented.

How to load raw data for processing with WinGPC Software? – To load a data series (login with all injects) highlight the desired login entry by a left mouse click and press the **Data to WinGPC Software** button. The injection series will be opened, and the chromatographic zero point will be set to the injection time of the first inject. A double-click on a specific sample within the login (left click on plus symbol and choose specific entry) will open the desired entry and the chromatographic zero point is related to the injection time of the selected sample.

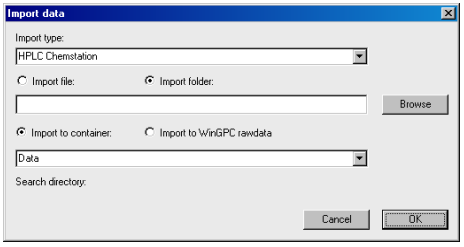
Functions of the **Project management** button bar:

Table 41 Project management buttons

Button	Function
Add container	Adds a new container
Delete container	Deletes the container, the project (project files) are not affected
Add project	Adds a project to the current container
Remove project	Removes a project from a container, the project data (project files are not affected
Import data	Imports data from other applications (see chapter „Import“ on page 271)
Export data	Exports selected logins to a new project (see chapter „Export“ on page 281)
Data to WinGPC Software	Transfers the selected login to the WinGPC Software raw data window
Data to overlay	Adds the current inject (displayed in the preview) to the overlay without loading the complete login
Search sample	Sample search dialog (see chapter „Sample Search“ on page 281)

Import

Imports a multitude of other raw data file formats (PSS-GPC 16 and 24 bit (Atari), Standard-ASCII, EZ Chrom ASCII/CSV, TSP-CDIF, HPLC Chemstation, HPLC Spectra, Jasco MD-910, Therm. FFF, D-7000 Chrom AiA/AnDI, Chromeleon, OpenLab AiA).



After choosing the import format it is possible to import just one file or a whole folder (batch import) to a WinGPC Software container. Alternatively, a file can be

sent to the raw data window directly by choosing the **Import to WinGPC Software raw data** function.

It fully supports version 2 of the international standard for analytical data interchange. This standard was issued first by the Analytical Instrument Association (AIA) based on an Analytical Data Interchange Protocol (AnDI) and is now under the auspices of the ASTM. The AnDI version implemented in WinGPC Software is version 2, because this version is much more stable and allows easier exchange of raw data. Earlier AnDI versions can only be imported if the vendor writing the data file is using a subset of features supported by version 1 and 2.

NOTE The import of Unicode characters is not supported.

The files must have a *.CDF extension to identify them automatically by WinGPC Software as AIA/AnDI files. WinGPC Software imports all data capture parameters in the AIA/AnDI files (as long as they have been saved). When a new AIA/AnDI files has been imported by WinGPC Software data processing parameters are missing (because the AIA/AnDI format does not support GPC methodology explicitly) and data have to be processed interactively. However, all processing parameters will be saved by WinGPC Software in the corresponding FSX file for future use. Please note, that different LC software vendors implement the AnDI standard protocol quite differently in their software programs. It is possible therefore, that not all parameters are available and might be missing in the WinGPC Software method. If a parameter is missing in the AiA/AnDI file, WinGPC Software will document that with **n/a** in the method. In such a case, please contact the producer of the LC software for further information.

The handling of imported raw data is identical to data evaluation from WinGPC Software data capture. WinGPC Software automatically creates a parameter file Filename.FSX when closing the raw data window which contained the imported data. This parameter file contains sample specific parameters (like sample name, concentration etc.), method information (if available), the calibration file as well as evaluation parameters like baseline setting etc. When these data and re-imported later, these parameters will be read automatically for convenience and to generate the similar look and feels as if the data would be read from the chromatogram database (WinGPC Software project).

If WinGPC Software cannot find a valid FSX file for standard ASCII files (which cannot contain any method information), the software prompts to load a related method file. **OK** opens the method **Load** menu, while **Cancel** will use a default method for this import. Since no method documentation is available from ASCII files, the WinGPC Software **method** window will only show **n.a.** (not available) for the modules and defaults to 1.0 ml/min flow rate and 20 µl injection volume.

When reading ASCII Data, the first column is always interpreted as time in minutes and the X-axis will be displayed in units of seconds. The correct conversion to elution volume will be obtained by a qualified method (see above) or by converting the first column in the ASCII file to seconds prior to import. This can be done conveniently in the WinGPC Software **Data Editor** (cf. chapter "Data Editor Window" on page 286).

Importing Chromeleon Data

The WinGPC Software (starting from WinGPC Software 8.3, SR1) supports the import of Chromeleon 7 data with extended functionality. This enables to harness the full functionality of WinGPC Software including access to advanced data processing, which may be part of optional WinGPC Software modules (e.g., Heparin, Copolymer analysis or 2-Dimensional chromatography).

As WinGPC Software is designed for multi-detector data analysis all detector signals (concentration) will be evaluated and their results reported simultaneously.

The WinGPC Software import connects directly to a Chromeleon 7 data vault (database) using the Thermo Chromeleon 7 SDK. Chromeleon 7 has to be installed (with a valid license) on the WinGPC Software PC used for Chromeleon data import.

Otherwise, the Chromeleon Import option **Import Data**  is not shown in the WinGPC Software **Project Manager** dialog.

Complete Chromeleon sample sequences will be imported into a WinGPC Software project structure which can only hold one sequence at a time.

NOTE

Multiple Chromeleon imports (various sequences) per WinGPC Software project are not supported.

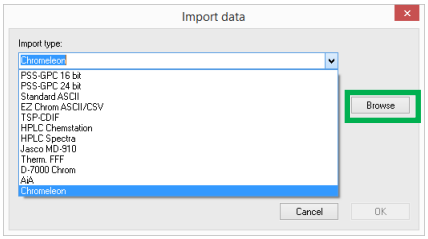
Following sections will guide users in a step-by-step manner through the:

- Chromeleon data import, chapter "Performing an Import" on page 274
- "Performing Heparin Data Analysis based on USP Methods" on page 443
- "Performing Heparin Data Analysis based on EP Method" on page 445

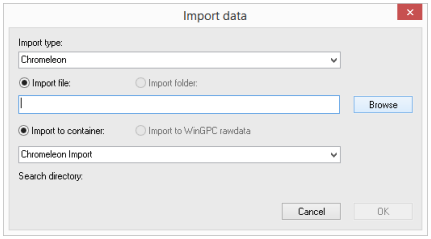
Performing an Import

1 Access the WinGPC Software Project Manager from the button bar ( ) and create a new Project Manager Container or select an existing one for better organizing data sets in WinGPC Software.

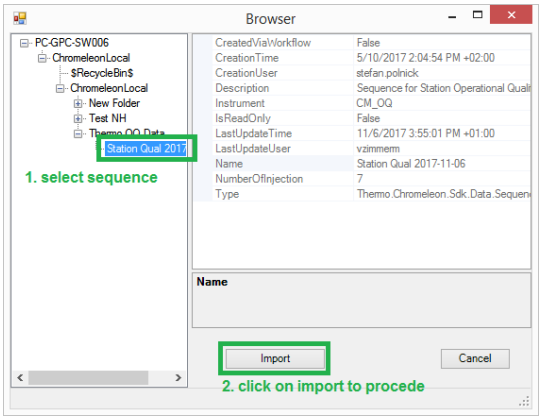
2 Click on **Import Data** and select **Chromeleon** at the bottom of the **Import type** list (see adjacent Figure). If Chromeleon is not listed, it is not installed on *this* WinGPC Software workstation.



3 Click on the **Browse** button to access the Chromeleon data. This will launch the Chromeleon data vault access as shown below.

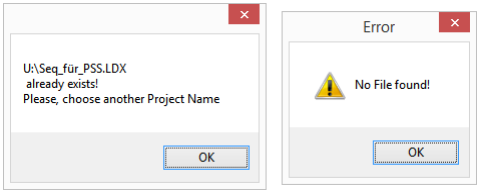


4 Navigate through the Chromeleon data tree and select a sequence in the tree as shown in the following Figure. Sequence details will be shown in the right panel.



5 If ready, click on the **Import** button to proceed with the import of the selected Chromeleon sequence into WinGPC Software GPC/SEC for data processing.

6 Create a new WinGPC Software project at the desired file location by entering a file name (e.g., the Chromeleon sequence name) in the Windows file browser.

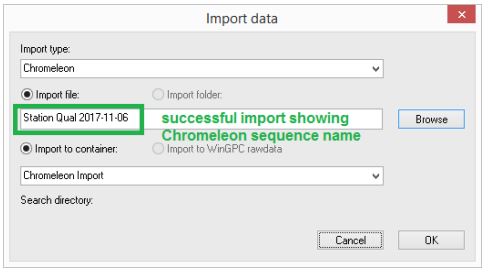


NOTE

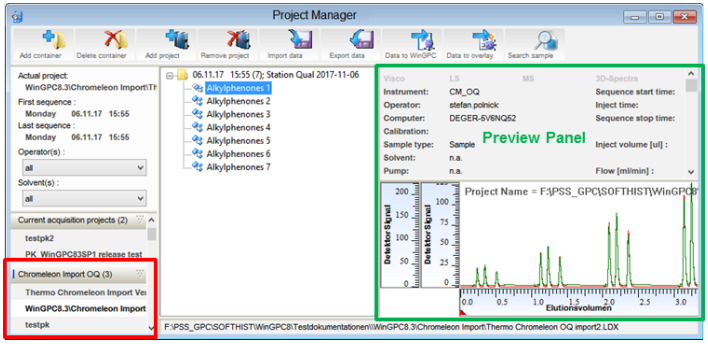
If a WinGPC Software project with identical name exists already in the selected folder, an error message (see Figure above) will pop up and the import will terminate.

Restart the import process with step 4 and select a unique WinGPC Software project file name.

7 The Chromeleon data import is successful when the WinGPC Software Project Manager shows the Chromeleon sequence name in the **Import file** field (see next Figure).



8 The imported sequence is shown in the Project Manager tree (red marked area) and in its preview panel (green marked area in Figure below).

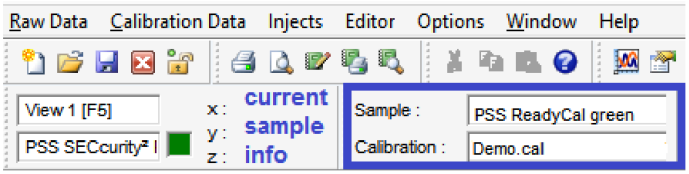


NOTE

If a certain method information is not available (e.g., flow rate) or cannot be imported as it is not available in the 3rd party CDS data structure or method, Agilent recommends to accept the WinGPC Software method adjustment option. A pre-defined WinGPC Software method allows to relate all required information to the imported data set.

9 Click on any entry in the injection list to open the complete sequence for GPC/SEC data processing. The classical WinGPC Software bird-eye-view will open, showing method information (top left), raw data (bottom left), elugram data (or HPLC or overlay data) (bottom right) and molar mass distribution and results (top right).

The selected sample (injection) for data processing is shown in the WinGPC Software Status bar, as illustrated in the following Figure.

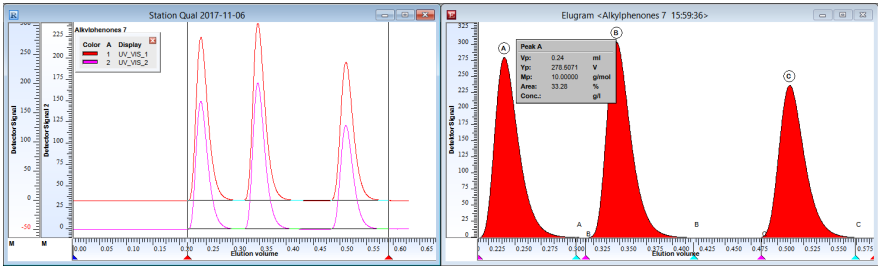


10 WinGPC Software sets the baseline automatically for all signals in each injection of the sequence based on Chromeleon peak results. Baseline start (end) is the baseline start (end) of the first (last) peak found by Chromeleon.

NOTE

If no peaks are available in the Chromeleon data, no baseline are assigned in the WinGPC Software raw data window. Manual baseline setting should be done. Click on **Help > Step-by-step** and select **Evaluating or Reprocessing Data**.

11 Use the **Help > Step by step** menu to guide you through the required steps for data processing, calibration, and reporting. Alternatively, wizards are available from the Method Window using the menu item **Method > WinGPC Wizard** (see chapter "WinGPC Software Wizards" on page 104 for details). A detailed description of how to calibrate, process data and report results is available in chapter "First Steps" on page 103.



The further procedure depends on the method that shall be used for heparin analysis. They are described in detail in following chapters:

- "Performing Heparin Data Analysis based on USP Methods" on page 443
- "Performing Heparin Data Analysis based on EP Method" on page 445

Optimizing/Adding Information to the WinGPC Software Method

As Chromeleon data files do not contain GPC/SEC related information (e.g., sample properties, calibration molecular weight references) nor does it know about related data processing requirements, the WinGPC Software method is not complete when importing data sets from 3rd party data systems.

Advanced data processing functionality of WinGPC Software might require method adjustment after data import from Chromeleon, as Chromeleon does not have GPC/SEC features or related parameters, which would allow importing all system configuration information as required for these advanced features.

Some contents of the WinGPC Software method are only for documentation purposes and is not important for correct data processing (please refer to chapter "Method Handling" on page 90 for details). Other settings and parameters, however, are determining how data are processed and/or influencing results directly.

Therefore, WinGPC Software offers to select a pre-defined WinGPC Software to establish a completely functional method for GPC/SEC data analysis easily. Agilent recommends using the guided method creation in the WinGPC Software **Method** Window by selecting the menu item **Method > New**.

Alternatively, method modification must be performed manually as outlined below:

Ensure that the following method content is correct when importing data from a 3rd party CDS:

- Detector **Signal type**
- Detector delay, if more than a single concentration signal is processed
- Imported data should show detector **Factor** as 1.0 and **Offset** as 0.0

Troubleshooting

Table 42 Troubleshooting

Observation or Problem	Action or Resolution
No Chromeleon data listed in **Browse** dialog	No valid or expired Chromeleon license. Launch Chromeleon and follow hints.
WinGPC Software Import Data dialog does not list Chromeleon	Chromeleon is not installed on this PC. Install Chromeleon with a valid license.
No baseline shown in the WinGPC Software raw data window	No peaks are available in the Chromeleon data. Evaluate data as described in WinGPC Software **Help > Step by Step**, section **Evaluating or Reprocessing Data**
No results shown/reported	Ensure that the detector signal is set to a standard or concentration signal in the WinGPC Software method.
The EP calibration functionality is missing	The EP method requires the optional Heparin software module for WinGPC Software. Enable **Show Login Screen** on WinGPC start and ensure the **Heparin** option is selected. If this option is greyed out, purchase heparin option from Agilent representative.
Incorrect results shown/reported in USP method	Wrong or incorrect calibration file assigned Check or reassign correct calibration file in Raw Data Window via menu **Calibration Data > Load**.
Incorrect results shown/reported in EP method	Missing or wrong EP CRS reference value in Raw Data Window **Editor > Sample**. Wrong or incorrect calibration file assigned. Check or reassign correct calibration file via menu **Calibration Data > Load**.

Imported Information

The following information is imported from the Chromeleon data vault:

Primary information	
Chromeleon Sequence Name	
Chromeleon Instrument Name	
Sample name(s)	
Inject Type	
Sample Data rate	
Inject Volume	
Inject Weight	needs to be converted into concentration
Flow Rate	
Supporting information	
Peak Start/end	translated into GPC/SEC relevant information (baseline start: Peak Start retention time of first peak in peak list, baseline start: Peak end of last peak in peak list)
Signal Name	
Wavelength	when a signal description is available in the Chromeleon method this value will be imported into the WinGPC Software method
Signal unit	when a signal unit is available in the Chromeleon method this value will be imported into the WinGPC Software method
Optional	
Molar Mass	variable can be set by the user for each inject and is used for the known molar mass of the inject
Detector Device	specified in the Chromeleon data set as **detector device type**

Optimizing the WinGPC Software Method

As Chromeleon data files do not contain GPC/SEC related information (e.g., sample properties, molar mass sensitive signals) nor does it know about related data processing requirements, WinGPC Software method modification might be required for advanced data processing.

Export

A copy of the highlighted runs (*logins*) and the respective data is saved as a different project file. A selection is only possible within a loaded project. No merges between different projects are possible for database integrity reasons.

Raw data runs (*logins*) can be highlighted by a left mouse click and copied (exported) into separate (empty) projects by pressing the **Export Data** button. In the corresponding popup dialog mark the desired *logins*, which need to be exported, and use the arrow button 

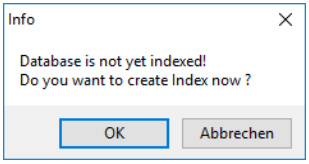
to add them to the export list. All *logins* on the right side (**Sequences to export**) are exported and copied into a new project by pressing the **OK** button after you have provided a specific data path and new and unique project name using the **Browse** button function.

For security reasons you cannot delete raw data (*logins*) within the project files. Furthermore, you cannot merge *logins* of different projects for data integrity reasons.

Sample Search

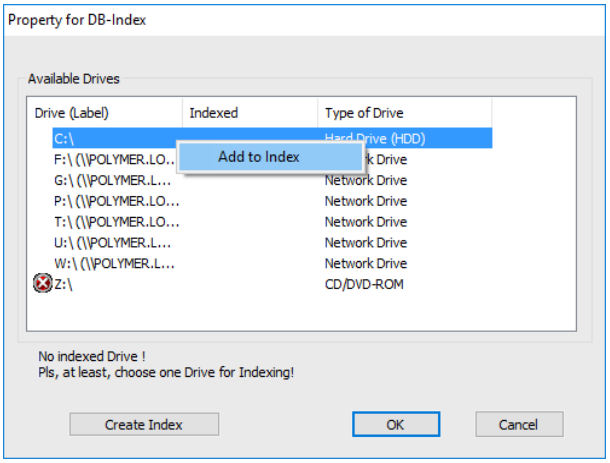
Opens a window, which can be used for a comprehensive search on all WinGPC Software projects on defined (network) drives. Alternatively, the sample search is also available by clicking on the fields **Samples** on the status bar.

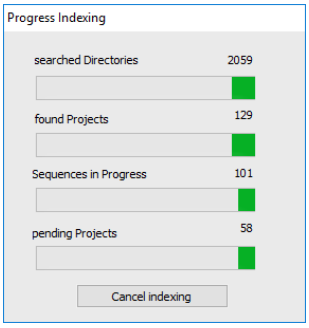
The **Search samples** window uses a sample database filled with all sample information in the WinGPC Software projects of indexed drives. To use the database, it needs to be initialized once.



If you press **OK**, the index dialog appears. All available drives are listed. To select one ore more drives for indexing, click on the specific entry. Below the header Index the hint **Marked for Indexing** will be given. Already indexed drives will be shown with the date of the last index. When you selected all necessary drives for index, press **Create Index** to start the indexing process. This procedure needs only to be done once or if you want to add/remove complete drives from index. After the initial

index the database will automatically update with changes in a background process.

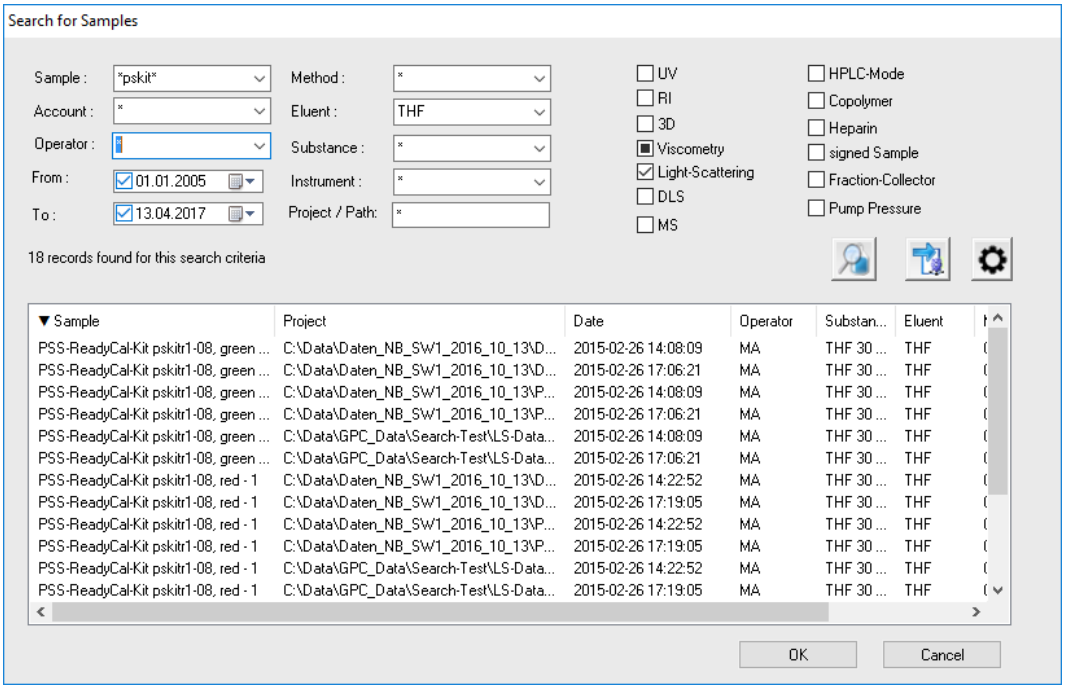




NOTE

Note that you should not perform data acquisition during the initial indexing process. The process takes (depending on drive size and on number of samples to be indexed) a couple of minutes up to (on large company networks) some hours. If you observe that it might take too long, you can cancel the process and re-start it later to perform it (e.g., over night).

Once the index is finished, you can use the **Search samples** window to refine your search critera.



The wildcard character * can be used in all fields (***pskit*** in the field **Sample** means all samples beginning with any character(s), having **pskit** as string within the sample name and end with any number of characters). Capital and small letters will not be differentiated. Samples can be searched for within a specified project (enter complete project path or project name under **Project**) or across all projects of a specific directory (enter path*.LDX). All search fields are generated as drop-down lists containing all entries of the index. If they shall be used to refine the search, they can be selected from the drop-down list (e.g., Operator or Eluent) or just typed in with "*" as a wildcard option.

Additional search options are given as three-state options. In the given example it is not of interest, if the samples were e.g., measured with a signal defined as UV, but the must contain lightscattering data. Samples with viscosity data are not allowed (negative search criterion). The status of these options can be changed by clicking onto the item once, twice or three times.

Available buttons are:

Search button: After entering all search criteria the search is started with this button.

Load button: Loads a selected result (sample) into WinGPC Software.

Settings: Opens the above-described dialog to add/change drives for index

All samples which obey the search criteria (maximum 2048) will be shown in the lower window section. Sort functions are available by clicking on the column name (in the given example: Sorted by sample name).

Backing Up Data

WinGPC Software organizes chromatogram data (raw data, sample specific information, processing parameters and results) in a convenient database, which allows easy access and retrieval of data. However, it is most important to stress that raw data project files should be backed up regularly. WinGPC Software does not provide its own backup software, because most network administrators have their own tools for doing that and will not allow multiple backup strategies in their network. On the other hand, all MS Windows Versions come with integrated backup software which can be used for that purpose without any problem.

In general, two backup options exist:

- copying of raw data files to another medium (diskette, harddisk, USB cruiser, removable drives, CD-ROM etc). The big advantage of this strategy is that all raw data are available as they have been (no compression or other file changes that will exempt them from direct read access from within WinGPC Software).
- backup creation using conventional backup software. The big advantage is the specialized help in managing backups professionally. The major draw-back is, however, that most backup software has its own (compressed) file format which requires a restore before data can be accessed from within any application program including WinGPC Software.

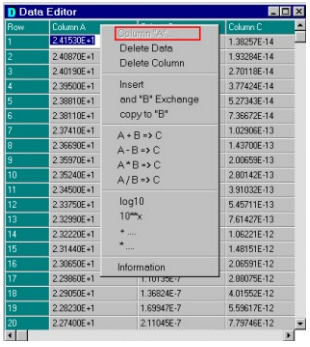
Independent of any backup strategy which will be used, it is important to copy the following data files to a save medium:

- project data files (minimum *.LDX, *.MDX, *.FSX, *.INX, *.SAX and *.ATX (last one for Compliance Edition licenses), in some cases also *.SPX or *.MSX) (these are the most essential files to backup in any case)
- WinGPC Software method files (*.MET)
- calibration files (*.CAL)
- multi area setting files (*.MAS)
- overlay files (*.ADD)
- sequence files (*.sps)
- instrument method files (*.spm)

It may be useful to backup or retain a recent copy of the WinGPC Software cache directory, which is located on the first partition of the first physical harddisk drive on the local computer (typically drive C:). The folder name is always \WinGPC_8#n; the “n” stands for the “n-th” instance of the software. In this folder default methods, module names, configuration files etc are stored. They are not essential for running the software, because they will be created automatically at runtime if missing. However, in order to retain a special configuration or module list, a current copy of that folder can be very useful (e.g., when moving the installation from one computer to a newer one).

Data Editor Window

The data editor allows viewing and editing slice data, which have been transferred from other program windows to the data editor. Furthermore, tables for the control of output relays and reference tables for the HPLC can be created in WinGPC Software's data editor. The data editor also allows loading and saving of ASCII files with full control of column and line separators which can be selected from a simple menu.



Mathematical operations can be performed on any numerical column to allow easy conversion of complete data columns. This is done by clicking on the column header, which will open a pop-up box with various mathematical options. The field Information displays the number of data points in the column, as well as the sum and the integral of all data in the respective column for further mathematical treatment. The first column of the data editor is always used as X-axis.

NOTE

The integration of the data editor always interprets the first column as X-axis, while the selected column is interpreted as f(x).

Column Context Menu

These functions are available from the column context menu, if you right click on the respective column header with the mouse. A popup menu appears in which the following functions can be selected:

Delete Data: deletes all data in this column; the column itself remains but it contains empty cells.

Delete Column: removes the column with all data completely without any questions asked. All columns right to the deleted column will move one column to the left; e.g., if a file contains columns A through D and column B will be deleted, then columns C and D will be moved to column B and C after the deletion of column B.

Delete Column: inserts a new empty column in front of the column from which the command was issued. All columns right to the inserted column will move one column to the right.

and {column} Exchange: swaps the column from which the command was issued with the next column to the right. E.g., if the command is issued from column "C" header, columns C and D will be interchanged. This command can be very handy when reorganizing data in a certain order.

copy to {column}: copies the content of the current column to the column to the right without asking for overwrite permission if the target column already contains data.

math between columns: allows to perform mathematical calculations with adjacent columns and write the result to the second next column. The following operations are supported:

```
column {letter} + column {letter+1}=> column {letter+2}
column {letter} -  column {letter+1}=> column {letter+2}
column {letter} * column {letter+1}=> column {letter+2}
column {letter} /  column {letter+1}=> column {letter+2}
```

math inside column: allows to perform mathematical calculations in the column from which the command has been issued. The following mathematical operations are supported:
"log 10": apply decadic logarithm to each column cell
"10**x": transform each cell value to the power of 10
"+...": add entered value to each column cell (offset)
"*...": multiply entered value to each column cell (factor)

NOTE

Enter a negative value in the dialog box to subtract data from column values. Enter the inverted value in the dialog box to divide column values with the entered value.

Information: displays the number of data points in the column from which the command was issued, and the sum and the integral of all data in that column for further mathematical treatment.

Row Context Menu

These functions are available from the row context menu, if you right click on the respective row number (first column) with the mouse. A popup menu appears in which the following functions can be selected:

Row # Delete: deletes the contents of the row from which the command has been issued

Row # Delete To...: deletes the contents of all rows entered in the dialog box. This function is useful to delete header information from imported files or eliminate baseline information from large files.

Delete every 2.line: deletes every second row starting from the current line from which this command has been issued. This function can be used to reduce the number of data points evenly if a target application cannot accept a very high number of data points. Use the column **Information** command to check the current number of lines in the data editor.

Insert Before Row# inserts an empty row before the row from which the command has be issued.

Insert After Row#: inserts an empty row after the current row from which the command has be issued.

File Menu

ASCII Import...: Loads an ASCII file (*.TXT) from the file system. This import works best with numerical data organized in columns. The ASCII file filter automatically converts decimal commas to decimal points, which are the internal WinGPC Software format to process floating point data. The import filter will try to delete all header (plain text) information before loading the file into the editor. However, this might not be successful in all cases. If the imported data do not look as expected, editing the ASCII file in a conventional ASCII editor (e.g., Windows Notepad) will improve the situation. It is possible to specify column and line separators before file import using the respective menus.

NOTE

This file filter is more powerful than the **Import from... > Standard ASCII** file filter in the Project management Window. It can be useful to import ASCII files first into the data editor and exporting them, when a direct **Import from... > Standard ASCII** command fails to import raw data directly.

HPLC Ref.Import..: Loads a HPLC reference table (*.REF) with the following file structure: Detector number, peak maximum, max. deviation [%], detector response factor, compound name. The HPLC reference table is loaded here for editing and will not be used for the HPLC evaluation. In order to use it for HPLC evaluations it must to be loaded into the elugram window using **Options > Reference table > Load**.

Relay table import.: Imports a relay table (*.REL) for the control of timed events. The file structure is: time of contact closure (min.), time interval for contact closure (sec.). The time of contact closure determines the time, at which the standard condition of the relay will be inverted, the contact closure interval defines the time period of the inverted relay condition.

ASCII Export...: Exports the displayed data in the editor as ASCII file (*.TXT). It is possible to specify column and line separators before file import using the respective menus. Also, the decimal separator can be specified. This can important for spreadsheets data imports when the spreadsheet software is set to a different language or country.

HPLC Ref. export.: Saves the contents of the data editor as reference table (*.REF). The data format must be as follows: Detector number, peak maximum, max. deviation [%], detector response factor, compound name.

Relay table export: Exports the data of the editor as relay table. The data therefore must be available as time of contact closure (min.), length of contact closure (sec.).

Printer Setup: Allows definition of parameters of the active printer. However, the active printer must be defined in the Windows Control Panel.
Landscape format prints the graphics on a full page. Portrait format prints the graphics, full method documentation and results by default. The printed information in the portrait layout can be selected for automated runs in the **Definition > Automation Settings** dialog box. The exact information of the portrait format printout depends on which window will be printed.
For color printers you can select color or monochrome printing depending on the printer driver options. The color of the curves in monochrome printouts are mapped automatically to a line style to avoid unreadable black and white prints. The correlation between curve color and line style in monochrome print is listed in "Curve Colors and Line Styles in Monochrome Printing" on page 534.

Print: Prints the current contents of data editor window to the printer selected in the windows printer selection dialog.

Page Preview: Shows a print preview in a separate print-preview window, which can be sized and moved. At the same time this command copies the preview contents to the Windows clipboard. When printing in portrait format, the graphics and the text for the measurements will shown, while landscape format will print only the graphics.

Print width options: Allows the adjustment of font size on the print out. 8 pt to 12 pt font size con be directly selected from the menu; other sizes can be entered in the **Special...** dialog box. Use this option to print more columns on a sheet of paper.

Columns Menu

Separation by: Allows the selection of the column separators for importing and exporting ASCII files. The selection of tabulator, blank, comma and semicolon separators are supported. This parameter is also used in the **File > Save ASCII Report** in the Mass Distribution window, which outputs all results and parameters as an ASCII file for further processing.

Decimal Sign: Allows the selection of the decimal sign for exporting. The selection of decimal point or decimal comma are supported. This parameter is also used in the **File > Save ASCII Report** in the Mass Distribution window, which outputs all results and parameters as an ASCII file for further processing.

Lines Menu

Separation by: Allows the selection of the line (row) separator for importing and exporting ASCII files. The selection of CR (carriage return), LF (line feed), CR and LF or LF and CR separators are supported. The proper selection of line separators is determined by the target computer system. Please check the corresponding documentation.

Messages in WinGPC Software

In some cases, messages will inform you about options or other issues during WinGPC Software operation. This chapter gives an overview on those messages that offer additional explanations via a help button () within the message box.

Message: Acquisition Canceled

Data acquisition was canceled. Probably the necessary prerequisits weren´t met. Check if all settings in your method are correct and check if your GPC instrumentation is ready. For more information regarding data acquisition refer to the section “Starting Data Acquisition” on page 120.

Message: Timed Events

For more information regarding relay control, following sections could be helpful:

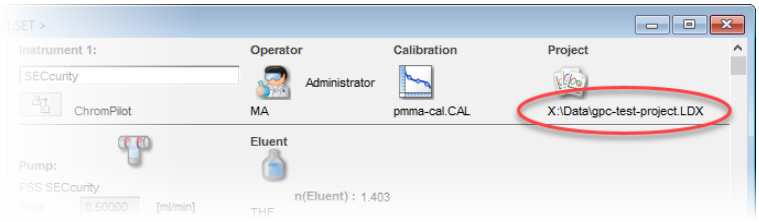
- Timed events in “Definition Menu” on page 181
- “Transfer Valve Setup for 2D Runs without Guided 2D Valve Setup” on page 427
- “Relay Control” on page 537

Message: Project for Acquisition Failed to Load

If the default WinGPC Software method is linked with a project that cannot be loaded for data acquisition, this message appears. Possible reasons are:

- Path is not available (e.g., network drive, currently not connected)
- User rights to write on selected path are not sufficient (check WinGPC Session Logbook if **missing rights** is logged)
- Project doesn’t exist in this path (e.g., because the project was moved to another location)

Check which of the above-mentioned problems applies and correct project information (e.g., select / create project by clicking on project path in WinGPC Software **Method** Window, see figure below).



Message: Volume Internal Standard

If internal standard search is activated (either manual or via automation settings), WinGPC Software will check for a local maximum (or minimum if defined) in a region within the given limits (e.g., +/- 5%) of the value entered in the calibration curve (**Calibration > Parameters...**).

Your internal standard was not found within these limits. Check if the correct calibration curve is used and the internal standard marker (green triangle) is visible in the active signal.

For more information regarding the internal standard, following sections could be helpful:

- "Flow Correction and Internal Standard" on page 25 (Theory)
- "Flow Correction and Internal Standard" on page 25
- Performing a Simple GPC Measurement, "Data Processing" on page 142
- "GPC Runs with AutoProcessing" on page 128
- Toolwindow "Detector and View" on page 158 (assigning active curve)
- "X-Axis Context Menu" on page 377

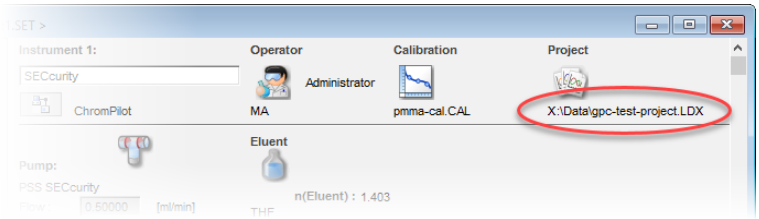
Message: OS Info – OS Message

This message was not created by WinGPC Software itself, but by your operating system. If it appears repeatedly, provide your Agilent representative with the exact message text as well as with the procedure how to reproduce it.

Message: Maximum Number of Logins

The maximum number of logins for your project (2048) is exceeded. You need to generate a new project. In order to prevent this problem, we recommend creating the projects for example on a yearly basis.

To create a new project, click on the project path in WinGPC Software Method Window (see figure below).



Message: Overwrite

You will overwrite an existing file. If you don´t want to overwrite the file, press **Cancel**. Then repeat the save/export procedure, but make sure to enter a new file name.

Message: Properties of Multi Area Settings

The properties of multi area settings are saved. You will find more information about multi area settings in following sections:

- "GPC Runs with AutoProcessing" on page 128
- **Raw Data** Window, activation of multi area: "Options Menu" on page 234
- "Multi Area Data Analysis" on page 220
- Multi area creation using HPLC mode: "Options Menu" on page 234

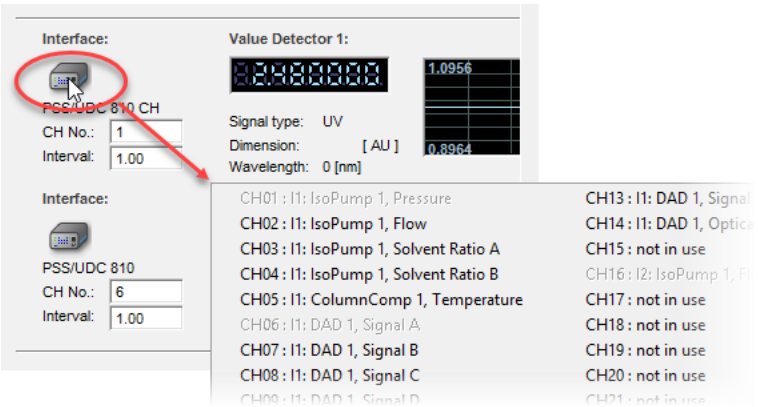
Message: Channel Mismatch

Two or more of your detectors are configured with the same channel number. This can happen if a wrong/old method was loaded.

You can check/correct your channel settings by clicking on the interface icon above **CH No.:** for each detector in your WinGPC Software method(s) (see figure below). You will get a list with all channels (in case of digital data acquisition or NetConnect including channel description, e.g., "I1: DAD 1, Signal A" for Signal A of the DAD 1 of the 1st instrument).

Channels already in use are displayed in grey, not yet configured signals are displayed in black. In this example CH01 and CH06 are already in use by the shown method, CH16 is also in use, but probably in the method of the second instrument.

Duplicates are not allowed!



Message: Missing Signal

On attempt to start data acquisition (e.g., by pressing **Start sequence**, **Record** or **Start Baseline**), WinGPC Software checks if each channel defined in the WinGPC Software method (see also section "Message: Channel Mismatch" on page 294) delivers a signal. If this signal is zero or near zero, this message appears (just once per session). You can cancel and check within your method if the correct signal is configured and if values displayed next to the channel number in the **Method Window** change. If you don´t see any changes you can force WinGPC Software to update the signal by selecting the menu item **Interface > Information...** of the **Method** Window. A dialog will open which displays all data channels coming in. By closing this dialog, the signals displayed in your **Method** Window will be updated as well.

Message: Wizard Canceled

A wizard was canceled. If it was stopped because one or more prerequisits weren´t met, you can change the prerequisits and restart the wizard using the **Method** Window menu item **Method > WinGPC Wizard**. You will find more information about the wizards in chapter "WinGPC Software Wizards" on page 104.

Message: Data Integrity

The data integrity for one or more logins (sequences) within the project could not be verified. Check if all files of the project are available and equipped with read, write, and change rights. We strongly recommend not to add new data to this project, but to create a new project for data acquisition. Check if you have a backup of the project which produces this error message.

Message: Calibrated Range

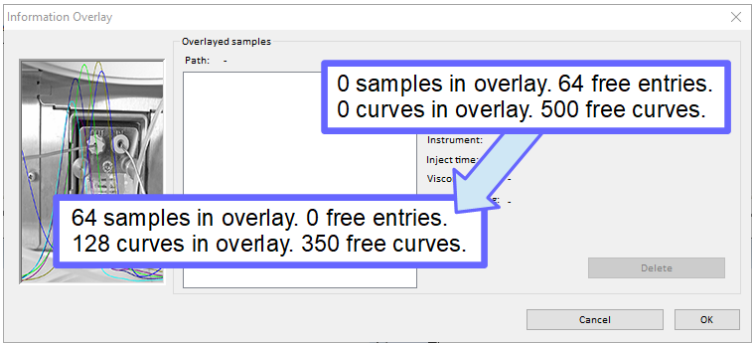
The integration limits set in the **Elugram** Window don't match the range which is covered by the currently selected calibration curve. Check, if you have a more current calibration curve or if you need to adapt the integration limits. Otherwise WinGPC Software will not be able to calculate reliable molar mass information for the non-calibrated range.

Message: Overlay – Maximum Reached

An overlay may contain the curves of up to 64 injections. The maximum number of curves within an overlay depends on different factors (e.g., on how many curves are currently shown in other WinGPC Software windows).

If this message appears, check the number of injections and curves currently added to the overlay using the information box **Overlay > Information**. It shows, how many injects and curves are already occupied (or still available). In order to reset the overlay, you can remove all curves with **Overlay > Delete all curves**. If you require many curves in the overlay and reach the limit, then check if you can reduce the number of occupied curves by closing not needed runs which are open for (re-) processing.

The figure below shows the dialog which will be shown in a new WinGPC Software session (0 curves in overlay, 500 curves free) and with an overlay loaded, that contains 64 samples. Then the message appears, although 350 curves are still free. In an alternative scenario the message may appear if 201 curves are already occupied in (non overlay) windows of WinGPC Software and a new overlay is created with only 30 samples, but altogether 300 curves (i.e. 10 curves per inject).



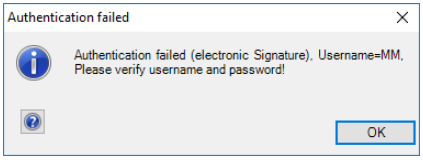
Message: Function Blocked by e-Signature

This message appears for instance if the menu item **Guided Detector Setup** is selected while the current run contains electronically signed samples. Functions that automatically influence the evaluation of all samples in a run cannot be executed in this case.

To execute this function, you need to remove all existing signatures of the login and repeat the procedure. Please note that the results of the previously signed samples might alter due to the execution of the function.

Message: Authentication Failed

The message signals an authentication failure (e.g., during the attempt to set an electronic signature. The username displayed in the dialog does not necessarily represent the username of the incorrect authentication attempt, but the user name of the user who is currently logged in to WinGPC Software.



7 WinGPC Software Calibration Window

The WinGPC Software calibration functionality is an integral part of the WinGPC Software package. It is activated by opening the **Calibration** Window using the **Window > Calibration** menu or by a click on the calibration icon on the icon bar. It allows to perform all types of SEC/GPC and polymer HPLC calibration to calculate molar mass distributions (MMD) or chemical composition distributions (CCD).

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter "WinGPC Compliance " on page 507 of this user guide.

General Features

The calibration software supports the creation of conventional calibration curves by using polymer standards with narrow molar mass distribution and their transformation by use of the Mark-Houwink parameters. Calibrations are also possible with broad standards and using the integral (cumulative) molar mass distribution of reference samples. Besides establishing conventional calibration curves (plotting log(M) vs. V_e) the WinGPC Software calibration software also allows to create universal calibration curves (plotting (log([η]·M) vs. V_e), which will be used in combination with an online viscometer detector. Composition calibrations which are used in the chemical heterogeneity module (optional, see chapters "Copolymer HPLC Analysis" on page 57 and "Chemical Heterogeneity Module" on page 433) can also be created. All calibration curves can be overlaid for easy comparison and inspection.

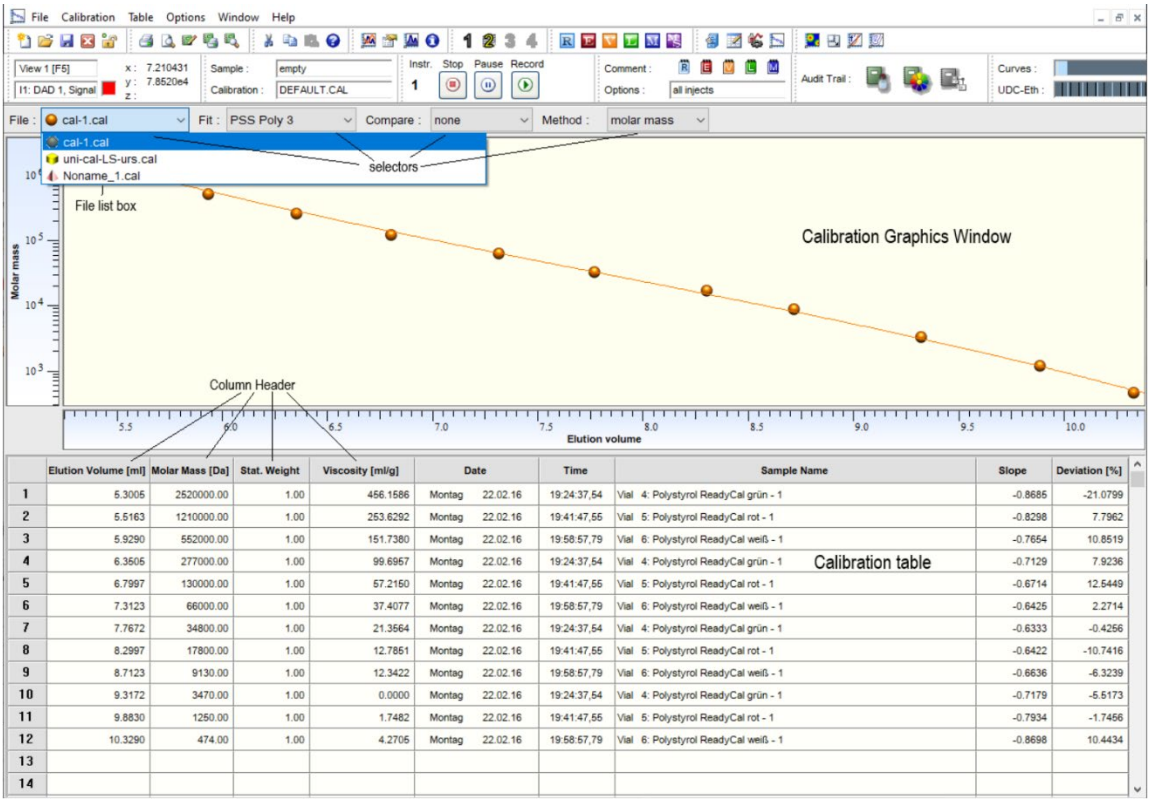
The calibration software uses wizards to guide the users through calibration methods which are not so frequently used by most customers, i.e. broad standard calibration and calibration using the integral (cumulative) molar mass distributions.

If different calibration procedures are used, the calibration software supports easy combination of calibration data by copying and pasting them into a different calibration table. Removal or deletion of data points can be simply done in the graphics part by the cutting tool (on the icon bar), or by using the appropriate tools of the calibration table menu.

Description of the Calibration Window

The **Calibration** window uses the WinGPC Software menu and icon bars. However, it has its on selector bar, which allows fast and easy access to calibration files (*File* selector), calibration fits (*Fit* selector), data comparison views (*Compare* selector) and the calibration method view (*Method* selector). At the same time the selector bar acts as a calibration status bar, because it displays the current conditions of the calibration data displayed.

Please note, that the column headings, axis labels and the number of displayed columns might differ slightly on your computer, since they are user defined.



	Elution Volume [ml]	Molar Mass [Da]	Stat. Weight	Viscosity [ml/g]	Date	Time	Sample Name	Slope	Deviation [%]
1	5.3005	2520000.00	1.00	456.1586	Montag 22.02.16	19:24:37,54	Vial 4: Polystyrol ReadyCal grün - 1	-0.8685	-21.0799
2	5.5163	1210000.00	1.00	253.6292	Montag 22.02.16	19:41:47,55	Vial 5: Polystyrol ReadyCal rot - 1	-0.8298	7.7962
3	5.9290	552000.00	1.00	151.7380	Montag 22.02.16	19:58:57,79	Vial 6: Polystyrol ReadyCal weiß - 1	-0.7654	10.8519
4	6.3505	277000.00	1.00	99.6957	Montag 22.02.16	19:24:37,54	Vial 4: Polystyrol ReadyCal grün - 1	-0.7129	7.9236
5	6.7997	130000.00	1.00	57.2160	Montag 22.02.16	19:41:47,55	Vial 5: Polystyrol ReadyCal rot - 1	-0.6714	12.5449
6	7.3123	66000.00	1.00	37.4077	Montag 22.02.16	19:58:57,79	Vial 6: Polystyrol ReadyCal weiß - 1	-0.6425	2.2714
7	7.7672	34800.00	1.00	21.3564	Montag 22.02.16	19:24:37,54	Vial 4: Polystyrol ReadyCal grün - 1	-0.6333	-0.4256
8	8.2997	17800.00	1.00	12.7851	Montag 22.02.16	19:41:47,55	Vial 5: Polystyrol ReadyCal rot - 1	-0.6422	-10.7416
9	8.7123	9130.00	1.00	12.3422	Montag 22.02.16	19:58:57,79	Vial 6: Polystyrol ReadyCal weiß - 1	-0.6636	-6.3239
10	9.3172	3470.00	1.00	0.0000	Montag 22.02.16	19:24:37,54	Vial 4: Polystyrol ReadyCal grün - 1	-0.7179	-5.5173
11	9.8830	1250.00	1.00	1.7482	Montag 22.02.16	19:41:47,55	Vial 5: Polystyrol ReadyCal rot - 1	-0.7934	-1.7456
12	10.3290	474.00	1.00	4.2705	Montag 22.02.16	19:58:57,79	Vial 6: Polystyrol ReadyCal weiß - 1	-0.8698	10.4434
13									
14									

Figure 51 Integrated calibration software with graphics and editor section for multiple calibration methods

Calibration Selector Functions

The File selector is a drop-down box which contains all loaded calibration files within in the **Calibration** window. The filename displayed in the box itself is the calibration file which is currently displayed in the calibration table and the calibration graphics section. However, if overlay mode is active (overlay button on the icon bar pressed), then all calibration curves will be shown in the graphics section, while only the data of the file shown in the selector box are displayed in the calibration table. Newly created calibration files (e.g., by **File > New** or by a calibration method in the **Calibration** menu will be appended automatically to the file list in the file selector.

The *Fit* selector is a similar drop-down box which lists all calibration functions which can be used for interpolating the calibration data. The list contains conventional polynomial regression models (linear to 7th degree) and three calibration functions (PSS...) designed by PSS to reflect the sigmoidal shape of calibration curves better than conventional polynomials. When a regression model is picked from the fit selector, the calibration data are automatically interpolated, and the fit result is shown in the graphics section and the calibration table.

The Compare selector allows to specify additional information to be displayed in the calibration graphics section. Either the **Slope** or the **Deviation** of the current calibration curve can be displayed on a separate Y-axis. This second Y-axis can be scaled and moved in the same way all other axes are changed (cf. chapter "Raw Data Window" on page 195 for details). The **Slope** selection will show the first derivative of the fitted calibration curve. It can be used to judge the quality of the regression model used, since too high polynomial degrees will force the function to "snake" through the calibration points. This will show up in the first derivative of the calibration curve as relative minima and maxima, which can cause erroneous bumps and shoulders in the molar mass distribution curve. The **Deviation** option shows the molar mass residuals; i.e. the relative deviation of the molar mass of the calibration standard from the calculated molar mass using the current regression model. Both comparison options follow requirements from the ISO 13885 and DIN 55672 GPC standards. The results of the comparison are always calculated independent of the option selected in the Compare selector and the current setup in the **Table > Columns...** menu. By default, the **Slope** and **Deviation** columns will be shown. They can be toggled on/off any time in the **Table > Columns...** menu of the Calibration window.

The Method selector allows to use the calibration window interface for different calibration methodologies. The default method is molar mass calibration, where the logarithm of molar mass at a certain chromatogram position is plotted and fitted versus elution volume. This method is used for conventional calibrations with

narrow or broad standards and for universal calibration using Mark-Houwink parameters. The **mass*[h]$_g$** option is used to display and fit the universal calibration curve log M*[h], which is *measured* by using an online viscometer. The WinGPC Software uses a single calibration file to store conventional (lg M *vs.* V) and universal (lg M*[h] *vs.* V) calibration data. To use viscosity detection for calculation of molecular weights, both calibration curves have to be fitted and then saved into the single calibration file. Please note that the term "universal calibration" is sometimes used for Mark-Houwink transformations and for calibrations using viscosity measurements with online viscometers. The **Chemical Heterogeneity** selector option is used to calibrate interaction chromatography analyzes (polymer HPLC) using copolymer standards of known chemical composition. The calibration data are presented on a linear Y-axis (composition in %) vs. elution volume. Please note that copolymer heterogeneity calculations require either the WinGPC Software copolymer module (if composition is derived from detector calibrations) or the chemical heterogeneity module (if composition is measured in interaction mode and derived from a composition calibration).

The **Radius [nm]** selector option is used to determine radius distributions as an alternative to DLS or for membrane characterization. For details see chapter "Determination of Radius Distributions" on page 323.

Calibration Window Layout

The calibration window below the selector bar is divided horizontally in two parts. The upper part displays the graphical information of the data entered in the data editor (lower section). The lower part shows the data table (editor) of the calibration window, where data can be entered and edited. The relative size of the parts can be easily adjusted by moving the section separator by dragging the mouse pointer.

Calibration curves can be overlaid for comparison using the overlay icon on the icon bar. In overlay mode the graphics section will show the overlay of all loaded calibrations, while in the calibration table only the data of the active calibration (this is the one which is shown in the *File* selector). The active calibration can be selected from the list of all loaded calibrations using the **File** drop-down box.

The Calibration Graphics Section

The graph window shows the calibration data of the currently opened calibration, the fitted calibration curve through the calibration points and the calibration slope or residuals, if selected in the *Compare* selector. The selection which calibration is currently displayed is done by the *File* selector drop-down list. The calibration table section will always show the information belonging to the active calibration as shown in the *File* selector. The selection of the type of displayed calibration curve (conventional, universal or chemical composition), is done in the Method selector drop-down list.

If the overlay button is activated, all loaded calibration curves will be displayed, as selected by **Options > Current Settings**. If an option is selected in the *Compare* list, the information for residuals or slope of the active calibration will be displayed as well. To enhance readability WinGPC Software a 0%-deviation line in the residual plot. This allows a faster understanding of the distribution of the residuals and how serious the deviation of a single calibration point might be.

The X- and Y-axis styles can be changed, by selecting **properties** from the context menu (click with right mouse button on the respective axis). You can edit the labels, the font and color of the labels and numbers as well as the color of the axis background itself. Manual scaling of the axis is also only a mouse klick away.

Alternatively, an interactive axis expansion/compression is available by clicking on the arrows which appear when moving the mouse pointer over the X-or Y-axis scaling region. They work identical as in all other windows of the WinGPC Software. The axis can be moved in the same way as for all other windows in the WinGPC Software. When moving the mouse pointer onto the axis, it changes its cursor to a pointing hand. Press the left mouse button and keep it pressed, while moving the axis. Then release it at the desired position.

You can zoom in on any part of the graphics by simply selecting the first corner of the box using the left mouse button. Keep it pressed and move the mouse pointer to the desired position. The zoom box will follow. Upon releasing the mouse button, the area within the zoom box will be expanded.

When using the right mouse button within the graphics window, a window opens with the following options:

Autoscale:	Automatically scales the window contents to get a maximized view of all calibration curves. Autoscale can also be invoked from the Options menu.
Unzoom:	Undoes previous zooms stepwise. Alternatively, use the **Options > Autoscale** command to undo all previous zooms. This option is also available from the **Options > Unzoom** menu.
Current Settings:	Opens a window which allows to change the color and type setting for the currently active calibration file. You also can define the selected settings to become the default settings for all calibration files opened later on using the **make default for ...** option. This option can also be called from the Options menu. This command will overwrite the definitions in the **File > Settings > Default...** menu.
Open this calibration run:	Calibration traceability: Each calibration data point is linked with it´s injection in the WinGPC Software project and saved in the CAL file. This menu item will open the original data. If the project has been moved to a different folder (e.g., for backup), the new path can be updated easily.

The Calibration Table

The calibration table shows a variety of columns and rows. Up to 1024 data points (rows) can be used to build a calibration curve. The column design can be adjusted, as well as the number of columns that are shown by clicking with the right mouse button on a column header. The columns can be sorted by any column parameter just by clicking on the column header with the left mouse button. All the columns will be sorted according to the selected column in increasing (↑) or decreasing (↓) order. Clicking more than once on the column header will toggle between increasing and decreasing sort order. The column header will display an arrow to indicate the sort order.

The columns for residuals and slope of calibration curve will be automatically calculated for the current calibration when a fitting function was selected.

A right click with the mouse on a cell of the calibration table will bring up a copy&paste context menu (when the cursor is inside this cell). The available copy&paste commands for not selected values will be **paste** and **select all**. For a highlighted entry **cut**, **copy**, **paste** and **delete** will be available.

Clicking with the right mouse button within a cell of the calibration table, a box is opened showing a variety of options (when the cursor is not inside this cell):

Columns: Allows to select which columns will be displayed in the calibration table. This command is also available from the column and row context menus by clicking on the column or row header with the right mouse button.

Column setup: Defines the header of the current column, adjust colors and text styles for column headers and column values. This command is also available from the column context menu by clicking on the column header with the right mouse button.

Clear column: Deletes all entries entered in the selected column (i.e. the column with the cursor). This command is also available from the column context menu in the calibration table by clicking on the column header with the right mouse button.

Reset rows: Clears empty rows from the calibration table or deletes and clears invalid row entries. This command is also available from the row context menu by clicking on the row header with the right mouse button.

Insert row: Inserts a new line at the cursor position (i.e.: before the cursor). This command is also available from the row context menu by clicking on the row header with the right mouse button.

Delete row: Deletes the line in with the cursor position. This command is also available from the row context menu by clicking on the row header with the right mouse button.

Clear row: Deletes all entries in the line with the cursor. This command is also available from the row context menu by clicking on the row header with the right mouse button.

File Menu

The **File** menu contains all input and output options, as well as the global view settings of the calibration software.

New: Creates an empty calibration table and automatically makes this file the active calibration file. The default temporary file name is always **noname.cal**. When the file is saved this default file name can be modified.

Open: Loads an existing calibration file and automatically makes the opened file the active calibration. Please note, that this file open command does not influence the calibration file which is used to process raw data.

Import ASCII: Imports a data table containing elution volume, molar mass, sample name. This command automatically opens a new calibration and makes this calibration the active calibration. The file name of the ASCII file will be the default file name of the calibration file.

Save: Saves the active calibration using its current name without prompting for a file name unless the default filename (Noname_n.cal) is in use.

Save as..: Saves the active calibration and prompts for a new/modified file name.

Export HTML: Creates an HTML file from the active calibration using export.htm as the default HTML file name. The HTML file is encoded according to the W3C HTML 4.0 specifications. The graphics part is saved (as a separate JPG file). The advantage of HTML file exports is their universality. They can be viewed on any computer with any Internet browser without the need to have a local copy of the WinGPC Software. This can be very useful when sharing results over the Internet or local network.

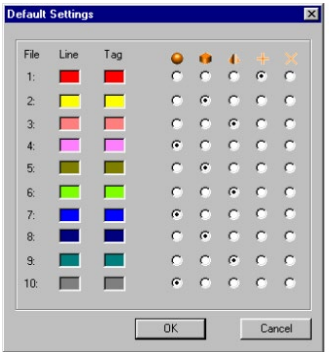
Export ASCII: Exports the active calibration data in ASCII format to a file with the same file name but a TXT file extension. The columns are TAB separated and the lines are CR-LF separated. The ASCII export allows for simple transfer of calibration data to other applications.

Close: Closes the actual calibration file. The software will prompt the user if the file has been modified or not saved at all before closing the file and the WinGPC Software. Please note, that this file close command does not influence the calibration file which is used to process raw data.

Print...: Prints the current display in the graphics and the calibration table according to the current language selection. The report can be directed to any installed printer. The layout of the calibration reports are be located in the program folder (general file mask: cal0n_m.LST (m: d/e German/English report, n: ½ individual/overlay calibration). Curve colors are automatically mapped to different line patterns if b/w printers are selected.

Page Preview: Shows a print preview in a separate print-preview window which allows zoom and direct printing from the preview window.

Settings: Allows to load, modify and save the calibration settings. The **Default...** command allows to set line and tag colors as well as the tag symbol for each loaded calibration curve. These properties will be saved with the calibration settings file.



The default calibration settings file located in the program folder (calibration.cfg) contains the information on the view and number of displayed columns, color scheme for data points as well as the color scheme and captions of the axis. Upon loading a setting file all parameters will be updated and overwritten if not saved previously.

Lock Data Points If the Data Point lock is deactivated, you can shift a data point of the active calibration to any location within the graph. To do so, just press the left mouse button on the desired data point, and move the data point to the desired location. Then release the mouse button. The coordinates (V_e, M) will be permanently updated in the editor. Using this feature, you can find out, where a special data point has to be located to match the calibration. Note, that the location of the moved data point also influences the calibration curve itself, if not weighted with zero.
As default, the data points will be locked, in order not to move data points accidentally.

Exit: Exits from calibration window.

NOTE

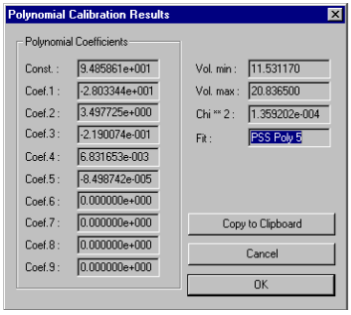
The software will assign the calibration file's display properties from the current number of the loaded file. The color scheme is not saved with the calibration file. When opening calibrations files, these settings will be used to auto-increment the file display properties.

Calibration Menu

The **Calibration** menu allows the selection of different calibration methods. Additionally, this menu contains the calibration parameters as well as the calibration results. The selection of the appropriate fit function is done using the *Fit* drop-down list on the Selector bar.

Parameters...: Allows to enter the operator name, the name and the elution volume of the internal standard, and the name of the column (set). The Mark-Houwink parameters for the polymer-eluent-temperature used for the calibration can be entered here too. The values entered here will be used by default for universal and other calibration procedures. The default Mark-Houwink values in the WinGPC Software are K=1.0 ml/g and a=0.0. This ensures that the M_v and [η] results will be zero, if no proper Mark-Houwink coefficients have been entered. Please modify them, if you want to report a proper viscosity average molar mass or and correct intrinsic viscosity number. Comments can be also stored with the calibration data file in a free text form; the text is entered directly in the edit line.

Polynomial Results Shows the coefficients of the calibration curve fit. This result box also shows the regression coefficient R and the selected calibration function. These parameters are not part of the standard calibration report but can be added to the report if the WinGPC Software ReportDesigner is used. The results of the calibration regression can be copied to the Windows clipboard (button **Copy to Clipboard**) and used in other applications by pasting the polynomial results.

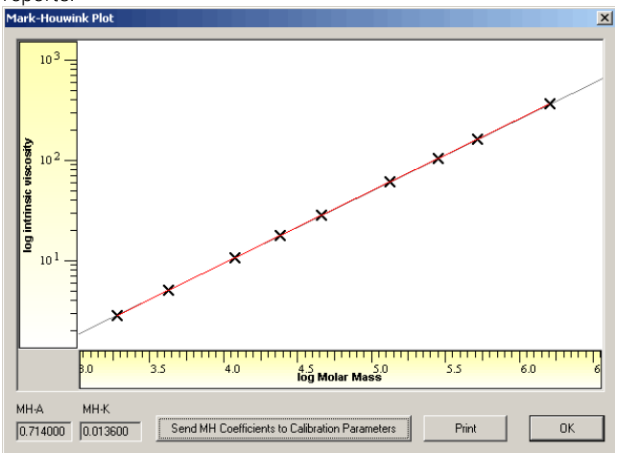


Guided Broad Calibration: Performs a broad standards calibration based on a narrow calibration (sometimes called broad-on-narrow calibration). The user is guided through the individual steps of this calibration procedure. See chapter "Calibration Curve Creation by Broad Standard Calibration" on page 314 for an in-depth explanation of all the necessary steps or follow the step-by-step instructions in the online help. Cf. chapter "Calibration" on page 21 for an explanation on the theory of that calibration.

Guided Integr. Calibration: Performs a calibration using broad samples with known integral (cumulative) molar mass distribution. This method does not require any base calibration at all. The user is guided through the individual steps of this calibration procedure. See chapter "Calibration Curve Creation by Integral Calibration" on page 318 for a in-depth explanation of all the necessary steps or follow the step-by-step instructions in the online help. Cf. chapter "Calibration" on page 21 for an explanation on the theory of that calibration.

Universal Calibration: Transforms an already existing narrow standards calibration according to the Mark-Houwink parameters for the narrow standard and the new polymer type entered in the dialog box. Only the molar mass values in the calibration table are recalculated in this method. See chapter "Calibration Curve Creation by Mark-Houwink Transformation" on page 313for a in-depth explanation of all the necessary steps or follow the step-by-step instructions in the online help. Cf. chapter "Calibration" on page 21 for an explanation on the theory of that calibration.

Mark-Houwink Plot: Displays the Mark-Houwink plot of the currently active calibration curve. This option is only available if the calibration file contains viscosity information; otherwise, the menu item is grayed out. The results of the linear regression of log[η] vs. log M are also calculated and can be copied to the **Calibration Parameters....** This graph can be printed directly or can be incorporated in ReportDesigner layouts for individualized reports.



Overlay: Toggles overlay mode on/off. In active state the menu item is ticked on and the overlay icon in the icon bar is depressed. In overlay mode the graphics section will show the overlay of all loaded calibration files, while in the calibration table only the data of the active calibration (this one is shown in the *File* selector). The active calibration can be selected from the list of all loaded calibrations using the *File* drop-down box. This menu has identical functionality as the overlay icon in the icon bar.

Table Menu

The **Table** menu allows to define which columns will be displayed, and their display options. The cursor has to be in the calibration table section of the window to have all menu items available.

Columns: Allows to select which columns will be displayed in the calibration table. This command is also available from the column, row and cell context menus in the calibration table by either clicking on the header or on a cell with the right mouse button.

Column setup: Defines the header of the current column, adjust colors and text styles for column headers and column values. This command is also available from the column and cell context menus in the calibration table by either clicking on the column header or on a cell with the right mouse button.

Clear column: Deletes all entries entered in the selected column (i.e. the column with the cursor). This command is also available from the column and cell context menus in the calibration table by either clicking on the column header or on a cell with the right mouse button.

Reset rows: Clears empty rows from the calibration table or deletes and clears invalid row entries. This command is also available from the column, row, and cell context menus in the calibration table by either clicking on the header or on a cell with the right mouse button.

Insert row: Inserts a new line at the cursor position (i.e.: before the cursor). This command is also available from the row and cell context menus in the calibration table by either clicking on the row header or on a cell with the right mouse button.

Delete row: Deletes the line in with the cursor position. This command is also available from the row and cell context menus in the calibration table by either clicking on the row header or on a cell with the right mouse button.

Clear row: Deletes all entries in the line with the cursor. This command is also available from the row and cell context menus in the calibration table by either clicking on the row header or on a cell with the right mouse button.

Options Menu

The **Options** menu defines how the data in the graphics section of the calibration window will be displayed. All **Options** commands are also available from the context menu in the graphics display.

Autoscale: Sets the logM and V_e axes automatically to display all data in single or overlay mode. Autoscale can also be used to undo many "Zoom" steps with a single command. Autoscale can also be invoked from the context menu when right-clicking in the graph section of the calibration window.

Unzoom: Undoes previous zooms stepwise. Alternatively, use the **Options > Autoscale** command to undo all previous zooms. This option is also available from the context menu when right-clicking in the graph section of the calibration window.

Current Settings: Opens a window which allows to change the color and type setting for the active calibration. You also can define the selected settings to become the default settings for all calibration files opened later on using the **make default for ...** option. This option can also be invoked from the context menu when right-clicking in the graph section of the calibration window. This command will overwrite the definitions in the **File > Settings > Default...** menu.

Interactive Creation of Conventional Calibration Curves

Before creating a narrow standards calibration, please make sure that you have run the narrow standards samples at proper conditions and that you have the molar mass (preferentially the M_p value) available.

In order to create a conventional calibration curve, the elution volumes, molar masses and statistical weights have to be entered in the calibration software. This can be done manually using the keyboard when entering them from an existing printout (select **File > New** first), or by importing a data table **File > Import ASCII**, if a file is available. In most cases the calibration standards will have been run with the WinGPC Software hardware and software. Then the **find minimum/maximum** dialog in the X-axis context menu of the **Elugram** window is the best approach to generate calibration data. Please note, that the **Add to calibration** button will be grayed out if no calibration file is open; use **File > New** to open an empty calibration table.

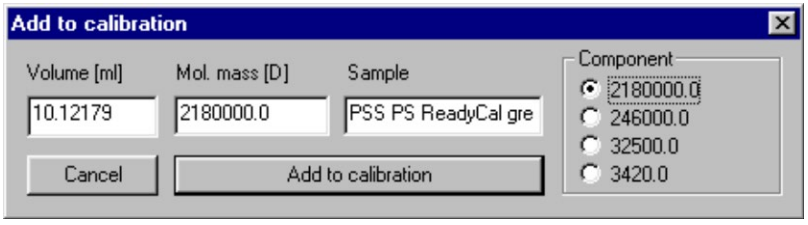


Figure 52 Peak search dialog to ease calibration table creation

Immediately after input of a calibration point it will be displayed in the graphics section of the calibration window. The calibration molar masses can only be selected from the list, if they have been entered previously in the sample editor.

Make sure that you have selected *Molar Mass* in the Method selector to show the conventional plot of log M *vs.* elution volume. The individual calibration points will be displayed when entered into the calibration table.

When all calibration points have been entered into the calibration table section, you can select a suitable calibration function from the *Fit* selector drop-down list. (WinGPC Software supports linear and polynomial regression models as well as three optimized proprietary calibration functions (PSS Poly3, PSS Poly5 and PSS Poly7)). The software performs a least squares regression calculation and shows the resulting curve fit in the graph section of the calibration window.

Please note that the statistical weight assigned to the data point defines whether the calibration point will be used for the creation of the conventional calibration curve. If the statistical weight is zero, the data point is shown, but not used in the regression calculation.

NOTE The statistical weight is the same for the molar mass and the intrinsic viscosity.

Hints for Finding the Best Calibration Fit

Unfortunately, no analytical function exists which describes the shape of a calibration curve for all cases. The user must rely on knowledge when selecting the calibration curve for the calibration data. The quality of the adjustment can be determined by 3 criteria:

1. The deviation between calibration points and calibration curve should be low.
2. The distribution of residuals along the volume axis should be random.
3. The slope of the calibration curve should be physically meaningful, i.e. should not have inflection points.

To rely only on first requirement can easily create errors. E.g., it is always possible to fit a 5^{th} order polynomial to a 6-point calibration without any deviation. However, the fitted curve will in general "snake" thought the calibration data. This will not reflect the error level of the individual data points.

The slope of the calibration curve should be highly negative for small and large elution volumes, while there should be a broad region with relatively constant value in between. To get a better understanding about the quality of the calibration curve, you can add the information for residuals or the slope of the calibration curve to the graph. This is done by selecting the appropriate entry in the **Compare** drop-down list. It is highly recommended to try different regression models to find the best calibration curve. Select **Calibration > Parameters** and add the values for internal standard etc. Save your calibration curve using the options in the **File** menu.

For a detailed work list for performing calibrations with narrow standards consult the WinGPC Software Quick Reference Guide. Step-by-step instructions are also available in the WinGPC Software online help menu (**Help > Step-by-Step**).

Calibration Curve Creation by Mark-Houwink Transformation

The universal calibration method requires the Mark-Houwink coefficients for the standards and the new polymer under identical conditions and the existence of a calibration file, which was created from the narrow polymer standards.

At first load the existing calibration curve, which you like to modify into a new one for another polymer type. Select **Calibration > Universal Calibration** from the calibration menu and activate the check box **calculate transformation**. Enter the Mark-Houwink coefficients for the new polymer (if the Mark-Houwink coefficients of the original calibration curve are not correct, you have to correct those in the original calibration curve using **Calibration > Parameters** before continuing. If you wish the intrinsic viscosities to be calculated from the molar masses, (e.g., to establish a universal calibration curve from a calculation) activate the **calculate transformation** option and select **molar mass** and **calculate** $[\eta]_g$. Agree to the **Create a new calibration table?** question to create a calibration table with the calculated intrinsic viscosity data.

The curve fitting and optimization is the same as in the conventional calibration case. The only difference in universal calibrations is the source of the molar mass data, which come from a calculation and not from a molar mass measurement by the polymer standards vendor. Additionally, the entered “new” Mark-Houwink coefficients for the substance under investigation will be copied to the **Calibration > Parameters** section of the universal calibration curve.

Calibration Curve Creation by Broad Standard Calibration

Before creating a broad standards calibration, please make sure that you have run the broad standards sample(s) at proper conditions and that you have the molar masses (normally M_w values have the best accuracy) and/or intrinsic viscosity available. A narrow standards calibration file established under similar conditions is also necessary.

The WinGPC Software's broad standard calibration will guide the user through the complete process to make it as convenient as possible.

The guided broad standard calibration method needs an existing narrow standards calibration. By default, the active calibration curve in the calibration window is used as base calibration. This calibration will be transformed into the new one matching the molecular parameters of the broad standards, by recalculating it from the chromatogram and the molar masses provided. For a more detailed description of background of broad standard calibration, please refer to the theory section of this user guide (cf. chapter "Calibration" on page 21) or to published literature.

Start a broad calibration by selecting **Calibration > Guided broad calibration** from the menu in the calibration window. Please make sure that you have already loaded the narrow standards calibration that shall be modified and a chromatogram (or an overlay) of the broad standard(s) loaded. Initially the software informs about the name of the currently active calibration curve, which will be used as base calibration. If this calibration curve is correct, go on to *Step 1* using the **OK** button, otherwise select **Cancel** to leave the guided broad calibration and select the correct calibration as active calibration before starting again.

In *Step 1* the broad standard chromatograms will be chosen either from the current elugram or from a saved overlay file. The overlay file can contain up to 8 elugrams, whereas the elugram can only be used for a single broad sample. The average molar masses of these chromatograms will be fitted simultaneously. The creation of overlay files is described in chapter "Overlay Menu" on page 230 in this guide; alternatively use the step-by-step instructions in the WinGPC Software online help.

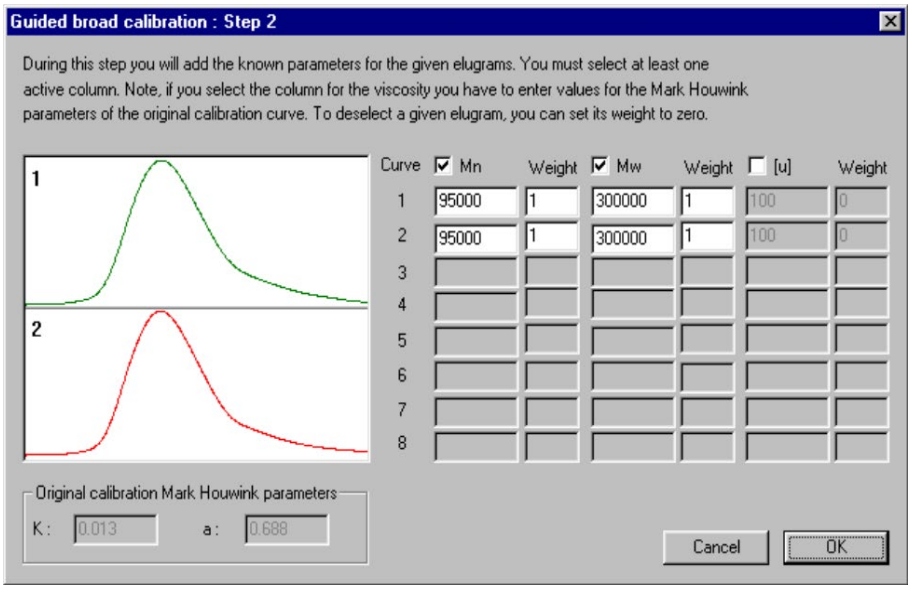


Figure 53 Guided broad standard calibration with interactive reference value dialog

In *Step 2* you have to select which average values shall be used (M_n, M_w, [h]). Enter the corresponding averages and use a statistical weight of 1, if you do not have any reason to give a higher or lower statistical weights to any of the entered averages.

NOTE

If you are using the intrinsic viscosities ([h]) the Mark-Houwink coefficients of the base calibration have to be correct. You can correct them in Step 3 of the broad calibration routine.

In the next *Step 3,* the fitting procedure will be performed. WinGPC Software first performs a raw search for a useful starting point of the fitting routine. If you select **skip** in the grid evaluation section of the window, the start parameters used are identical to the start parameters entered in the **start** section. Otherwise, the software performs a grid evaluation within the limits given in the **fit parameters** section, in order to find a good starting point for the actual fitting procedure. The calculations will be done by pressing the **Start** button.

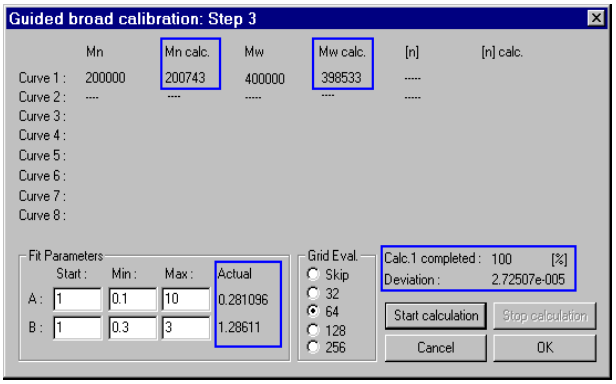
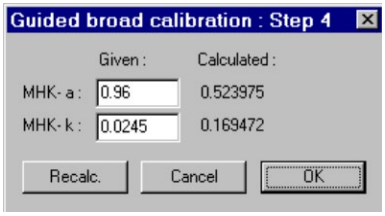


Figure 54 Finding the best fit for broad standard calibrations (result in blue)

The dialog box continuously displays the target values and actual values (shown in blue boxes in adjacent Figure) for the molar masses in the upper part of the window, while in the lower part the deviation (sum of the squared relative deviations of target and actual value) are shown. The fitting procedure can take some time depending on the complexity of the 3-dimensional data surface. The optimization can be stopped anytime using the **stop** button. If the fitting limits are reached by one of the parameters, the optimization will be interrupted. You can then change the fitting limits, and can start the evaluation again, or go on with the evaluation starting at the present set of parameters after entering them in the **start** boxes and using the skip option within the **grid evaluation** section. After the fitting procedure is completed use the **OK** button to continue to *Step 4*.



The parameters A and B calculated during the optimization process can be expressed as Mark-Houwink coefficients of the base calibration and the polymer for which the calibration curve has to be created (cf. the theoretical aspects of broad calibration). The dialog box allows to calculate the Mark-Houwink coefficients of the substance under investigation using the Mark-Houwink parameters of the base calibration and the parameters A and B. The Mark-Houwink coefficients entered for the base calibration are shown in the **Given** section. If these are not correct, enter the correct ones here. You can then recalculate the new Mark-Houwink parameters (see adjacent Figure). Press the **OK** button to create a new (broad standards) calibration table, where the molar masses of the base calibration are replaced by

the molar masses calculated from the base calibration and the fitting parameters A and B. Furthermore, the calculated Mark-Houwink coefficients for the substance under investigation will be copied to the **Calibration > Parameters** section of the new calibration curve.

The calibration points of the new calibration can now be fitted and optimized as described in "Interactive Creation of Conventional Calibration Curves".

For a detailed work list for performing calibrations with broad standards consult the WinGPC Software Quick Reference Guide. Step-by-step instructions are also available in the WinGPC Software online help menu (**Help > Step-by-Step**).

Calibration Curve Creation by Integral Calibration

This calibration method requires the chromatograms and the integral (cumulative match) molar mass distribution of one or more broad standards. A base calibration is not needed, however.

The other calibration procedures mentioned before were based on an existing narrow calibration which was recalculated. In this process a new calibration table was always automatically created. The integral calibration procedure starts from an empty calibration file. If the integral calibration method is selected while an existing calibration is open, the new data points created by integral calibration procedure will be added to this calibration. Thus, if the integral calibration should contain only its own data (and not add to existing calibration data), start from an empty calibration curve by selecting **File > New** from the menu in the calibration window.

In WinGPC Software, the integral calibration method guides the user step by step to perform integral calibrations as convenient as possible.

Start the guided integral calibration procedure by selecting **Calibration > Guided Integral Calibration** from the menu in the calibration window. Continue by either selecting the current elugram of the broad sample or chromatograms from multiple broad samples from a saved overlay file. The overlay file can contain up to 8 elugrams, whereas the elugram can only be used for a single broad sample. The creation of overlay files is described in chapter "Overlay Menu" on page 230 in this guide; alternatively use the step-by-step instructions in the WinGPC Software online help.

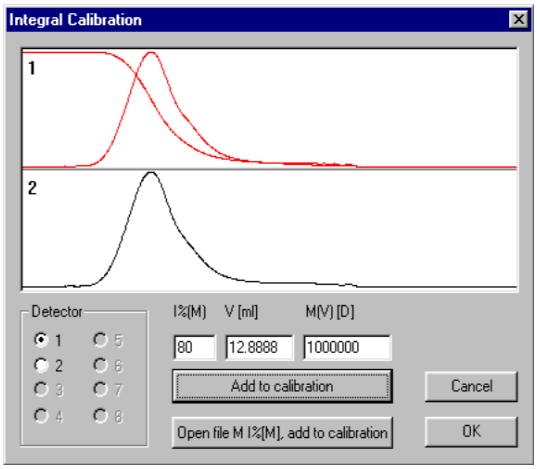


Figure 55 WinGPC Software Integral Calibration Wizard

Then select the signal that you want to use for the integral calibration. The software calculates the corresponding cumulative concentration distribution for the selected signal (see red trace for signal 1 in adjacent Figure).

Now enter the molar mass and integral distribution I(M) values for your broad standard. This can be done either manually by typing in the values from a list (as shown in the Figure) or by reading them in from an ASCII file (use the **Open file M, I%(M), add to calibration** option). When the value for the integral molar mass distribution is entered, the corresponding elution volume is displayed immediately. Use the **Add to Calibration** button, to manually add the desired data set to the calibration table. The calibration data can now be fitted and optimized as described in "Interactive Creation of Conventional Calibration Curves". Within one session, you can change the curves within an ADD-file, can load new ADD-files and the corresponding ASCII-files. When you have processed all your data, leave the window for integral calibration using the **OK** button.

For a detailed work list for performing calibrations with integral broad standards consult the WinGPC Software Quick Reference Guide. Step-by-step instructions are also available in the WinGPC Software online help menu (**Help > Step-by-Step**).

ASCII files for integral calibration can be easily created using the WinGPC Software data editor, or by entering the molar masses into the first, the value for the integral distribution into the second column of the calibration editor, and exporting these data as ASCII file.

The ASCII file should have the following structure with M(Da) as the first column and I(M)(%) as the second colum. The file needs to be provided without a header line:

1.8458E+06	100
1.5594E+06	99.98
1.3229E+06	99.93
1.1268E+06	99.87
....	....
2.8385E+03	0.001
2.6406E+03	0

Universal Calibration Curve Creation with Viscosity Detectors

In order to create universal calibration curves, viscosities for the standards have to be added to the calibration table. There are various methods to do this:

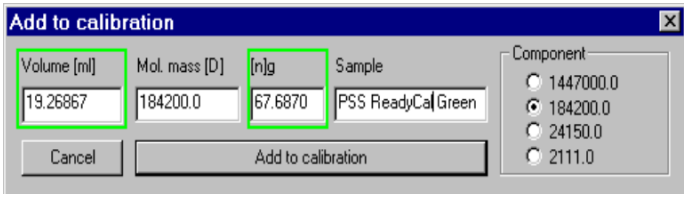


Figure 56 Adding peak position and viscosity to create a conventional and universal calibration table simultaneously

- Manually enter the intrinsic viscosity data, or
- Use the search peak maximum dialog in the **Elugram** Window to transfer peak elution volume, the experimentally determined viscosity and the sample description from the elugram into the calibration table. The software detects automatically if a viscometer signal is present. If missing, the intrinsic viscosity will not be calculated and transferred to the calibration table.
- Calculate the intrinsic viscosities using the Mark Houwink coefficients of the substance under investigation, using **Calibration > Universal Calibration**, and activate the **Calculate [h]?** option.

In most cases universal calibrations are done when an online viscometer is present in the instrument. Besides the viscosity detector, polymer standards with narrow molar mass distribution are needed to perform a universal calibration. They have to be analyzed with a concentration detector and the viscometer.

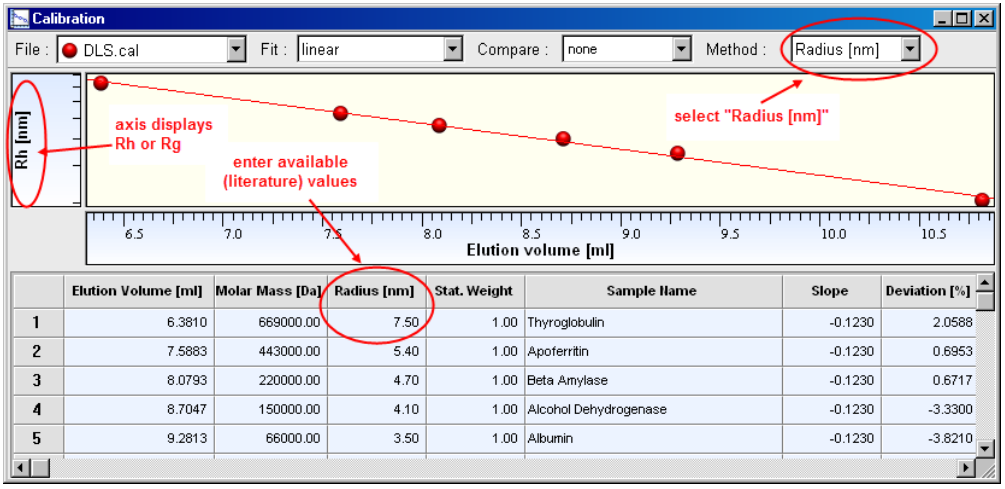
Conventional and universal calibration curves are stored in the same calibration file. Different views can be created from the same calibration table. To look at the universal calibration the *Method* selector in the calibration window should be set to **M*[h]**$_g$. Then log(M*[h]$_g$) is plotted *vs*. V_e and all curve fitting and optimization procedures can (and have to) be applied as for a conventional calibration. Save the calibration file after *both* logM(V) and log(M*[η]$_g$)(V) have been fitted with a selected regression model. If either calibration data are not fitted WinGPC Software will respond with the error message "An error occurred during analysis! Control

calibration data." when processing chromatograms. The evaluation of unknowns can be done with this calibration either by doing conventional calculation or calculations based on the universal calibration curve; refer to chapter "X-Axis Context Menu" on page 377 and "Viscosity Window" on page 372 for details of molar mass determination and viscosity data processing.

Please note that the statistical weight assigned to the data point defines whether the calibration point will be used for the creation of the conventional calibration curve. If the statistical weight is zero, the data point is shown, but not used in the regression calculation. The statistical weight is the same for the molar mass and the intrinsic viscosity. The columns for deviation and residuals will display the corresponding values for the universal calibration curve, if the Method selector is set to $\mathbf{M^*[h]_g}$. Please also note that the calibration parameters are identical to those defined for the conventional calibration curve.

Determination of Radius Distributions

WinGPC Software offers to determine radius distributions by activating the method **Radius** located on the right hand side of the calibration window.



	Elution Volume [ml]	Molar Mass [Da]	Radius [nm]	Stat. Weight	Sample Name	Slope	Deviation [%]
1	6.3810	669000.00	7.50	1.00	Thyroglobulin	-0.1230	2.0588
2	7.5883	443000.00	5.40	1.00	Apoferritin	-0.1230	0.6953
3	8.0793	220000.00	4.70	1.00	Beta Amylase	-0.1230	0.6717
4	8.7047	150000.00	4.10	1.00	Alcohol Dehydrogenase	-0.1230	-3.3300
5	9.2813	66000.00	3.50	1.00	Albumin	-0.1230	-3.8210

If the radius of the polymer standards is known (e.g., from literature), those values need to be entered in the respective column of the calibration table. Please adjust the axis description of the x-axis according to the values used (e.g., Rh or Rg).

If this calibration file is loaded to the data, the x-axis of the MWD window will automatically switch to **Radius**.

This function can be used as:

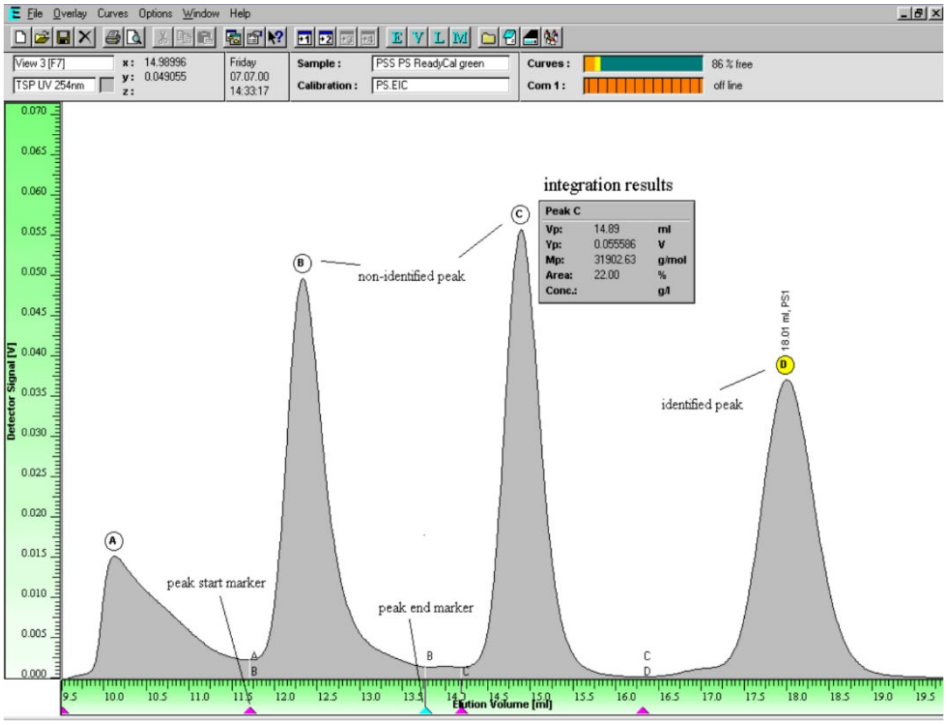
- high-resolution competition to DLS measurements (based on Rh calibration)
- membrane characterization with pore width (based on Rg calibration)

8 Application of the HPLC Mode

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter "WinGPC Compliance " on page 507 of this user guide.

The HPLC mode of the WinGPC Software offers an integrated route to perform HPLC evaluations using standard peak area or peak height-based calculations. Peaks can be searched and compared with a reference table, to identify a substance by retention volume. Furthermore, the amount of the identified peaks can be calculated using the external standard method. For the calculation of the peak areas of different peaks vertically dropped baselines or a valley-to-valley calculation can be selected (see **Options > Baseline** in chapter "Elugram Window" on page 224 for details).

The activated HPLC mode is identified by the tick mark on the Menu item **Options > HPLC Analysis** in the area or height sub menu and the pressed HPLC icon on the icon bar. If the HPLC mode is activated, the peaks in the elugram window are color-filled by default and the standard printout of the elugram window shows the HPLC results. The HPLC mode does not influence other windows. Thereby it is possible to perform HPLC calculations as well as molecular weight calculations at the same time without the need to change the method or to re-load and re-process data.



WinGPC Software HPLC Mode for quantification and identification of additives, monomers etc.

To activate the HPLC mode select **Options > HPLC-analysis** and select peak area (**Area**) or peak height (**Height**) based processing. Alternatively, the HPLC icon in the icon bar can be used to switch HPLC mode to peak area processing by default. After the baseline has been set and the internal standard has been marked in the raw data window, the HPLC evaluation can be performed in the elugram window. When selecting **Options > Peaklist sort for** the software performs a peak search in the active curve. The peaks found are marked with pink (peak start left) and turquoise colored (peak end right) triangles on the X-axis and the peak maximum is identified by peak identifier letters in yellow (white) circles (peaks with (without) match in reference table). Peaks which have not been detected automatically can be added with the X-axis function Insert Peak. The left (pink) and right (turquoise) peak limits can be moved by clicking onto them and holding the left resp. the right mouse button. A left mouse click on the peak identifier letter of a peak, you receive information regarding substance name, elution volume at peak maximum, peak height at maximum, molar mass, %area, concentration (if response factor is known). Identified peaks can be deleted by clicking the right mouse button on the

letter of the peak maximum. The list of found peaks can be transferred to the data editor (**Options > Editor peaklist**).

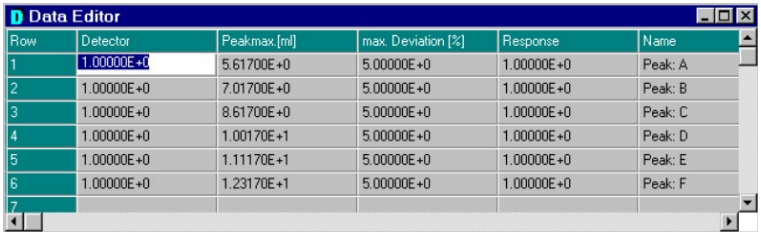
HPLC calculations:

- From the raw chromatogram peaks are identified with the create peaklist command.
- The peaklist contains peak position V_p, peak height Y_p, peaks area and relative amounts calculated from area ratioing. The reference area is the sum of all areas from the peaks found.
- Peak identification and absolute quantification needs input from a reference table. Only after a reference table with response factors and reference peak positions has been created and loaded, absolute amounts can be determined, and compounds identified.

HPLC Reference Table

A reference table can be created in the data editor or any other text editor. It contains: detector number, volume at peak maximum, allowed deviation in %, response factor, substance name, whereas the entries will be separated by tabulators. The simplest way to create a reference table is as follows:

1. Make sure HPLC mode is active, select **Options > Peaklist sort for** in the **Elugram** window.
2. Transfer the list of identified peaks with **Options > Reference Table... > Create**] into the data editor, where the parameters for reference tables can be edited.
3. Use **File > HPLC Export** in the **Data Editor** window to save the reference table.



Row	Detector	Peakmax.[ml]	max. Deviation [%]	Response	Name
1	1.00000E+0	5.61700E+0	5.00000E+0	1.00000E+0	Peak: A
2	1.00000E+0	7.01700E+0	5.00000E+0	1.00000E+0	Peak: B
3	1.00000E+0	8.61700E+0	5.00000E+0	1.00000E+0	Peak: C
4	1.00000E+0	1.00170E+1	5.00000E+0	1.00000E+0	Peak: D
5	1.00000E+0	1.11170E+1	5.00000E+0	1.00000E+0	Peak: E
6	1.00000E+0	1.23170E+1	5.00000E+0	1.00000E+0	Peak: F
7					

The detector number defines which detector signals will be used to compare them with the reference table. In a single reference table, the reference data of several detectors can be saved simultaneously. Identification and assignment of detector signals is done by the detector number in the reference file which must match the detector number in the WinGPC Software method (check the detector settings in the Method window if in doubt. A common reference table for all detectors is advantageous because only one reference table must be loaded and maintained. Which data will be used for HPLC evaluation can be chosen by simply selecting the active curve.

The response factor, F_c, reflects the correlation between injected sample amount, mc, and the peak area, A_c, and must be determined for every compound as:

$$F_c = \text{amount } m_c \,/\, \text{peak area } A_c$$

NOTE

The concentration is that of the injected sample as specified in the sample editor for the component, not the concentration measured at the detector.

The response factors can be determined from a single injection (concentration, level) or from multiple injections with different concentrations of the same sample or sample mixture (multi-level calibration) using linear regression analysis. The calculation of response factors is available from the **Options > Reference table... > Response factors** menu. The results for multiple peaks and multiple detectors can be transferred directly to a reference table file (*.REF) using the **to "Ref"- File** button in the response factor calculation dialog. Additional details can be found in the Options menu of the Elugram window (cf. chapter "Elugram Window" on page 224).

NOTE

Please note that the component concentrations in the sample editor will be assigned in ascending elution order to the peaks for HPLC response factor calculation; e.g., concentration "comp. 1:" will be assigned to "Peak A", etc.

In order to evaluate a sample or a sample series with the reference table, the HPLC reference file must be loaded in the elugram window (**Options > Reference table > Load**).

The comparison with the peaks found in the HPLC mode is done automatically when a reference file is loaded or when a peaklist is generated with **Options > Peaklist sort for...**. If peaks were inserted or deleted in the elugram, a new comparison with the reference table can be enforced using **Options > Compare with list**.

Additional information on HPLC related menus can be found in the Options menu of the Elugram window (cf. chapter "Elugram Window" on page 224).

Peaklist from File

If the automatic peach search function doesn't meet your peak search criteria (e.g., if no relative minimum between structural sample features is observed), you can preconfigure the peak settings according to your needs.

WinGPC Software will read the settings from a text file of the following structure ("i" is the peak increment, starting at 1):

PEAK_i=	peak description (name) as it shall be displayed
UNIT_i=	possible values for units are: ml (milliliters), min (minutes), sec (seconds), Da (Dalton); this unit will be used as unit for following parameters

and

MAX_i=	expected maximum of peak (in given units)
MAXDEV%_i=	optional: peak search window as in HPLC mode to identify a peak correctly; if missing or value=0 next peak (no matter which deviation) is found

or

START_i=	start value (in given units), START value must be smaller than END value (also with Da)
END_i=	stop value (in given units)

optional

RESPONSE_i=	response factor as in HPLC mode, i.e. defined as conc/area; if missing or value=0 no concentration calculation is performed

Example:

```
PEAK_1=Peak 1 - maximum
UNIT_1=ml
MAX_1=6.5
```

⇒ peak with maximum next to 6.5 ml (no max. deviation!)

```
PEAK_2=Peak 2 - range
UNIT_2=ml
START_2=7
END_2=8.5
```

⇒ peak within 7.0 and 8.5 ml

```
PEAK_3=Peak 3 - maximum with MAXDEV
UNIT_3=ml
MAX_3=9.8
MAXDEV%_3=5
```

⇒ peak with maximum within 5% of 9.8 ml

The peaklist can be created and edited in any standard text editor, inclusive notepad.

To apply the peak list to your data acquisition, procede as shown below:

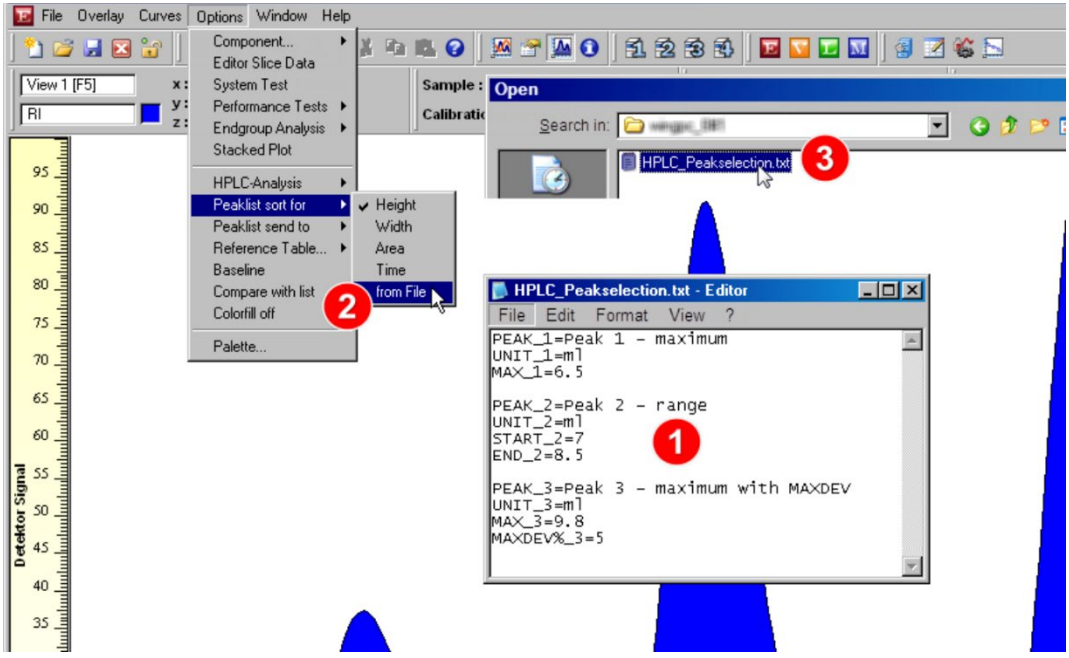
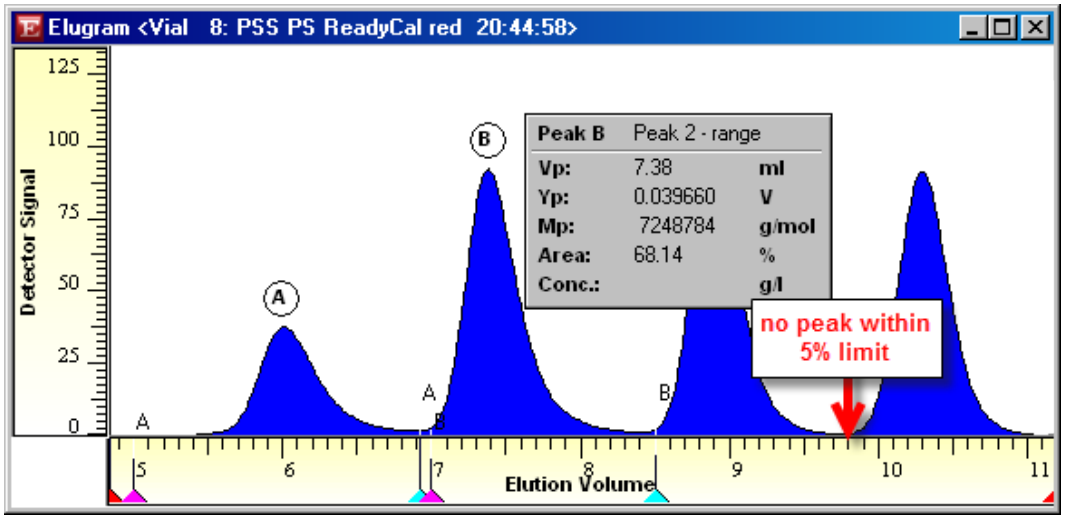


Figure 57 Create a peaklist and save it as *.txt file (1), activate the HPLC mode in the elugram window and select [Options][Peaklist sort for][from File] (2), select the preconfigured file (3)

Depending on your global settings (display of Inject Options), you may be asked for the injections the peaklist should be applied to.

The result is shown in the figure below:



Peak A and B are analysed as defined in the peaklist (Peak 1 and 2), the third peak was not assigned, because the deviation was greater than the limit set by MAXDEV%.

X-Axis Context Menu

These functions are accessible by clicking on the X-axis labeling with the right mouse button.

Table 43 X-axis context menu

Function	Description
Find maximum/minimum:	Searches to the next relative maximum/minimum in the active curve from the mouse cursor position where the function has been invoked. When the next relative minimum/maximum is found a dialog box opens, where peak position (V_p), molar mass and sample name for the creation of calibration tables are displayed. When using a viscosity detector, the intrinsic viscosity from the calculation column of the viscosity information box will be shown. These can be edited, and molar masses can be selected from the radio button list on the right. The calibration molar masses displayed there must be entered in **Editor > Samples**. The **Add to calibration** button appends the data set to the active calibration table. If no calibration table is loaded in the Calibration Window, this button is grayed out. Please remember to use the **File > New** command in the calibration window to create a new calibration curve; otherwise, data sets will be appended to the one currently displayed in the calibration window. **Cancel** will close this dialog without any changes.
Set peak integration:	Performs a peak search in all displayed curves. The integration limits are placed around the found peak, whereby the first local minimum to the left or right of the mouse pointer position will be used.
Insert Peak:	Inserts peak start and peak end markers at the current mouse cursor position. This option is useful if the automatic peak finder will not work, e.g., with very small peaks or shoulders which do not possess a relative minimum. The peak markers have to be dragged to the correct start and end positions manually before useful results can be obtained. Delete peaks: Peaks can be deleted by clicking on the peak identifier label with the right mouse button.
Set standard scale:	Allows the manual setting of the displayed X-axis range of the chromatogram and sets the default standard scale.
Standard scaling:	Restores the scale of the last manual scaling (e.g., after editing the scaling by the arrow keys or the scroll bar of the X-axis.). A tick mark in the context menu indicates if the standard scale is in use.
Properties:	This context menu is available on each axis in the WinGPC Software. It allows setting axes properties individually. For example, text attributes (font, size, color etc) and background properties of the axis can be defined. It is also possible to switch from elution volume to elution time representation for X-axis properties. The axis properties will be saved for each instrument individually.

9 WinGPC Software ReportDesigner

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter "WinGPC Compliance " on page 507 of this user guide.

The WinGPC Software ReportDesigner is an optional module which allows the creation of individual report layouts in desktop publishing quality. The layout is a template which can hold different objects like formatted text, graphics, tables. Report layouts can contain any piece of WinGPC Software information (parameter, result, graphics, file name and properties, etc.) independent of the window they are located in. This allows to create a single report layout which contains all items which are needed on a single or multiple page printout with just one mouse click or even fully automated.

The menu items **Edit Report** and **Print Report** are located in the Raw data window in the Raw data menu. Alternatively, printing from the ReportDesigner or editing a report layout can be done by clicking on the respective icons on the icon bar. A mouse click on the icon **Quick Report** will print directly with the previously selected report layout on the default printer defined in the layout. Please note that these commands are only available if the ReportDesigner module has been purchased; otherwise, they are grayed out.

ReportDesigner functionality can be used with the WinGPC Software automation to create reports which automatically check user defined pass/fail criteria and modify the report contents automatically. In **Automation properties**, report layouts can be chosen for automated sample processing in the Reporting section of the **Report** tab using the **…Browse** button. Similar to the Quick Report icon, printouts are automatically directed to the printer for which this layout was created.

The ReportDesigner offers several ways to direct the output, which can be easily selected from the dialog in **Raw data > Print Report**. The **Options...** button on the

right of the **Direct to...** provides more options. You can edit here printer properties and even page formats individually for each region defined in the layout. If required changes can be made permanent.

Table 44 Printer properties

Option	Description
Printer:	prints the previously selected report layout to the selected printer (by default the printer for which the layout was generated). A different printer can be chosen using the **Change...** button. The number of copies and printing selected pages only can be set in the **Options** section.
Preview:	creates a screen preview of the current report. If the preview looks ok, printing can be generated from the preview menu bar. Please note that the ReportDesigner Preview does not copy the printout to the Windows clipboard (as in the case of the standard WinGPC Software report). Use JPEG output instead or the menu **Raw Data > Report to clipboard** to import ReportDesigner reports into other applications.
Presentation:	Display of the current report in the presentation mode. The presentation mode will be stopped by pressing the **Esc** key.
Adobe PDF:	a *.PDF-document is created from the report and can be saved with a user-specified name. An additionally installed PDF-writer is not needed with ReportDesigner. **Options...:** Document options (Title,...), Properties (Compression Type, Font embedding,...), Security (Encrypt document,...)
Multi-Mime HTML Format, XHTML/CSS Format:	can be used to publish reports in the Internet, Intranet or Extranet as HTML or Multi-Mime HTML files. These files meet the W3C specification and generate HTML 3.2 code which can be opened by any modern browser. The default file name is the sample name (or the calibration file name). Graphics will be saved separately as JPEGs and linked to the HTML file or embedded into the Multi-Mime HTML file. **Options...:** Print resolution and JPEG-options (Color mode, Quality) *Note:* HTML specifications do not support overlapping objects. If the layout uses overlapping objects, an error message will appear which lists all objects which cannot be exported to HTML. In such cases we recommend using JPEG export instead, which can be viewed in every modern browser, too.
Microsoft Excel Format:	complete reports with all layout elements or table objects can be exported directly as *.XLS files. They can be edited with spreadsheet software for further evaluations or special plots. **Options...:** JPEG Generation (Resolution, Color mode, Quality) Data Export (tables only, all pages in one table)
Microsoft Word Format:	reports will be generated in MS Word format; the creation is executed inependently from the installation of the product; due to format restrictions some format features, e.g., mixed page orientations and tabulators are not supported.

Table 44 Printer properties

Option	Description
Rich Text Format (RTF):	exports the report with a user-defined name as formatted text. You can open RTF files with any word processing program and edit text and graphics separately. **Options...**: Color mode, Resolution, General Options
Microsoft XPS Format:	a *.XPS document is created from the report and can be saved with a user-specified name. An additionally installed XPS-writer is not needed with ReportDesigner.
Multi-TIFF, TIFF:	creates TIFF reports with a user-specified name. **Options...:** Picture-Export Options (Color mode, Resolution, Quality) *Note*: Multi page reports are converted into one single TIFF file (Multi-TIFF) or each page into automatically consecutively numbered TIFF-files. Therefore, its recommended to create separate folders for each report.
Bitmap, Metafile (EMF), PNG, JPEG:	exports the report as an uncompressed JPEG file which meets the JPEG specifications and can be viewed in every modern browser. The file name can be specified by the user. This file format can be used to import ReportDesigner images to any other Windows application. **Options...**: Picture-Export Options (Color mode, Resolution, Quality) Note: For multi page reports, every single page is converted into a graphic which is consecutively numbered automatically. Therefore, we recommend creating a separate folder for each printed report.
Fax Device (local):	sends a fax using the fax project parameters
File:	creates a *.prn file with user defined file name; output via copy on any printer
HTML, HTML jQuery Mobile:	Generates HTML formatted reports using jQuery mobile framework and Javascript, the files are optimized for the display on mobile devices.
Pinwriter (TTY):	like print option “File”, a *.PRN file is created that can be used to copy them any time to the printer. **Options...**: Printer Emulation (ESC/P 9Pin [Epson LX],..., ASCII, ANSI, UNICODE)
Text Format:	the content of table objects can be exported as *.TXT file with user-defined name to be opened with any editor or word processing program. **Options...**: Character set (ASCII, ANSI, UNICODE), export only data, data export options (separator,...)
XML Format:	creates *.XML files with user-specified names that can be imported into other software (e.g., database). **Options...**: Layout (resolution, all pages in one file, tables only), JPEGs (color mode, quality), JPEG export (as file, embedded, none) *Note*: As depending on the settings, one or more JPEGs will be created. So, it might be recommended to create a folder for each XML-report.

NOTE

Default report output devices can be selected in and saved with the report layout, e.g., PDF, preview, etc. by selecting **Project > Page setup** in the **Export Media** tab.

The ReportDesigner Quick Reference Guide (in "Documentation" folder of installation medium) or the ReportDesigner online help should be consulted on how to create report layouts. Many important tips and tricks can be found there on how to optimize the layouts and make the most outstanding presentation of WinGPC Software.

Please note that the WinGPC Software ReportDesigner will not show up as a module in the WinGPC Software Login window (as the viscometry or copolymer module would). However, it is always present in the background when needed and purchased. Menu items and icons related to ReportDesigner functionality are grayed out if no valid ReportDesigner license has been obtained.

A new report layout is created when the ReportDesigner editor is opened with a new (non- existing) file name. A software wizard starts automatically and assists you during the first steps. Agilent provides sample report layouts for various applications that illustrate the powerful desktop publishing features of this software module. These reports can easily be modified and, of course, you can copy useful elements into your own layouts.

Converting Existing Layouts into ReportDesigner Layouts

ReportDesigner uses a new layout format. If you open an existing layout for the first time with ReportDesigner, it will be converted automatically into the new format. A message will inform you that ReportDesigner cannot open converted layouts anymore. Therefore, it is recommended to save converted reports using another name or just leave the ReportDesigner without saving changes if you want to keep existing reports unmodified.

The ReportDesigner variable list is extended compared to the ReportDesigner with retained variable names (exclusions see below). Existing layouts can be converted into ReportDesigner format without difficulty. Only table objects have to be treated in a special way.

In the ReportDesigner, tables could be placed everywhere on the layout. The ReportDesigner introduces an element called report container in which tables are placed subsequently. So, only one table per layout can be processed during conversion. The table printed out first is selected by default. All other elements in the base layout (text boxes, diagrams,...) are converted without any problem. To be sure that the most important or complicated table will be converted, just delete all other tables (e.g., simple headerline tables that can be recreated very easily).

It's also possible to copy/paste each single table into a separate ReportDesigner layout. In this way you generate conventional layouts with only one table each. These reports you can convert subsequently into ReportDesigner format, name them appropriately and save. Now you can copy/paste one table after another into your base layouts report container. As copy/paste operations can only be done for layouts of the same version, one has to perform the conversion steps for each single table.

Please do always check converted layouts for correct functionality and consider the following changes in ReportDesigner variables and variable types!

NOTE

You can remove the message "This project contains a table that is not available now..." by the following procedure:

- open the *.LST-file of the ReportDesigner layout with any text editor
- search for "**Table:**"
- delete "**Table:**"and save the corrected layout

Modifications for a Successful Layout Conversion

Variables with the prefix *Calibration* refer to calibration curves stored with the measurement, the prefix *Calibration_file* refers to the calibration opened in the calibration window. Similarly, in ReportDesigner exist the variables *Diagrams.Calibration* and *Diagrams.Calibration_file* that has not been distinguished before.

Furthermore, in ReportDesigner, the date variable *Calibration.Creation_date* and the string variable *Calibration.Creation_time* have replaced the previous string variable *Calibration.Created*. The same is true for *Calibration.Update_date* and *Calibration.Update_time* and *Calibration_file.Creation_time*.

This means, you have to check converted layouts that contain calibration curves and related creation/modification dates and edit them manually.

If you want to round significant digits, you have to use self-made functions adjusted to ReportDesigner. Some suggestions can be found in the example layouts 2, 7, 10 and 11.

Please do also consider the document *Update Information on ReportDesigner* where all changes are summarized.

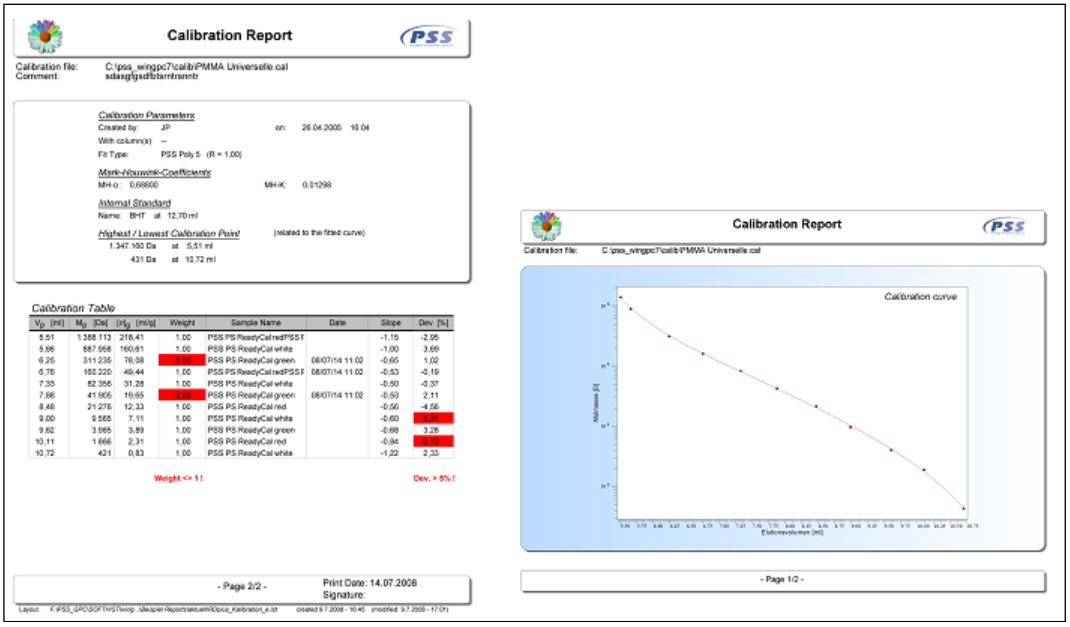
Properties and Features of the Example Layouts

This section gives an overview on the features of the example layouts delivered with your WinGPC Software installation and automatically installed in your program folder.

Example Reports

Calibration Report

(**enhanced_calibration_report.lst**):



multi page layout:

- extended tables continue on next page(s), page break is triggered automatically
- print date, signature and layout used are printed out on last page only
- different page format (portrait or landscape) for following page(s):
- here large diagram (landscape) on first page and tables/parameters (portrait) on following page(s)
- changing paper size from portrait to landscape without any problem; report container covers both paper sizes

parameter table:

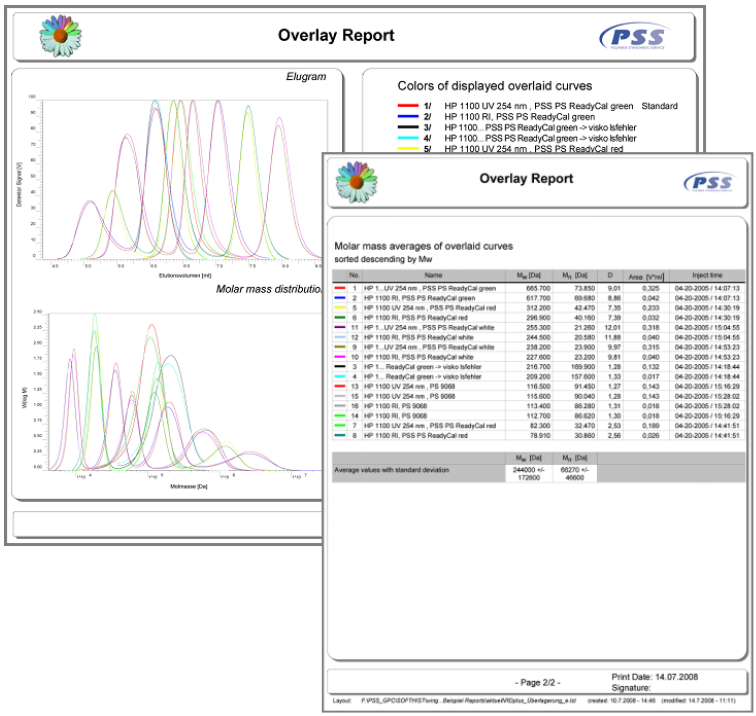
- MH-coefficients are displayed only if MH-a is not 0 (cf. appearance conditions)
- background height adapts dynamically to the table height (controlled by userdefined variable @MHparameter)

Calibration table:

- Zebra pattern (easily defined and activated as data line option)
- header and data line are adapted automatically depending on visco data being present or not
- if weight <> 0 or deviation > ± 5% (each can be user specified), the concerning field will be marked red, and a warning will be displayed (controlled by userdefined variables)

Overlay Report

(**overlay_statistics.lst**):



multi page capability (cf. Calibration Report) and selectable paper size for following page(s).

caption:

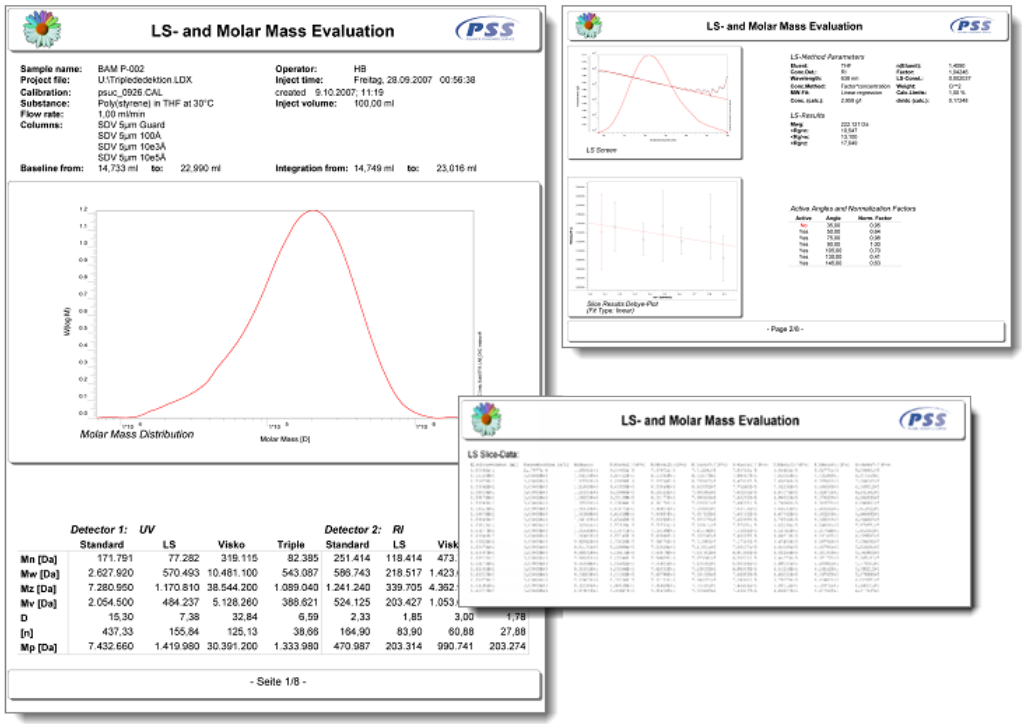
- up to 32 curves with related colors
- only curves displayed in the overlay are listed (data line appearance condition)
- curve names abbreviated (depending on overall length of the name string)

results:

- table sorted (here: decreasing by M_w)
- **intelligent round function**: M_n, M_w are rounded to 4 significant digits
- calculation of average with standard deviation (here: M_n, M_w; ReportDesigner statistic functions)

LS and Molar Mass Evaluation

(**comprehensive_LS_report.lst**):



Multi page capability (cf. Calibration Report):

- changing paper size from portrait (first page) to landscape (following page(s)):
- report container height must be defined differently for first and following page (condition controlled by page()-variable)
- user input with combobox while printing (new ReportDesigner function: AskString-Choice$)

Parameter:

- sample name and project file name abbreviated (depending on overall length of the name strings)
- column names are only displayed when present in the method (cf. appearance conditions) a line feed is produced for every column not present
- ⇒ table height is dynamically adapted, correct positioning of following tables

MWD results:

1 Molar mass averages only for concentration detectors (conditioned by detector signal type)

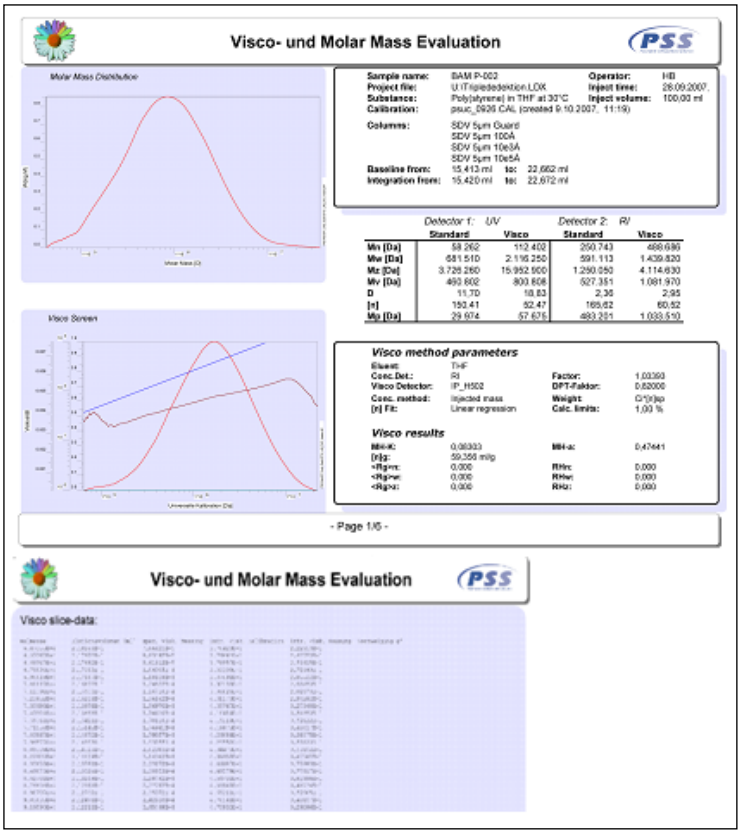
2 All available calibration types in one table

Lightscattering angles:

- marked red if angle is deactivated
- caption **Slice Results** is adapted automatically (by variable plottype)
- on demand (user input while printing): slice lists for all angles with molar mass, radii of gyration and branching (if activated in the LS screen)

Visco and Molar Mass Evaluation

(**comprehensive_viscometer_report.lst**):



user input with combobox while printing (ReportDesigner function: AskString Choice$)

Parameter:

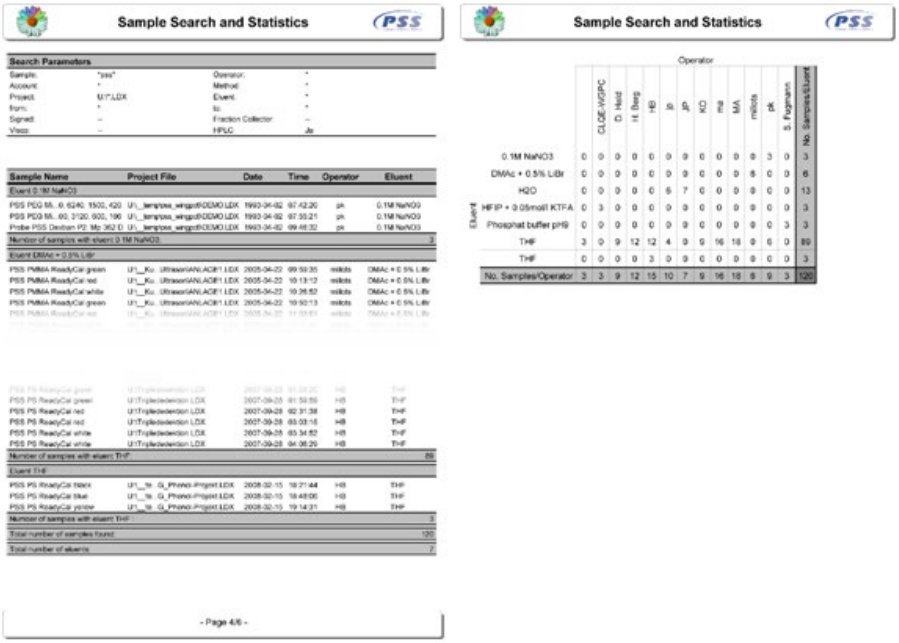
- only columns present in the method are shown; dynamic adaption of table height by linefeed (see appearance condition)

MWD results:

- only displayed for concentration detectors (appearance condition controlled by variable signal type)
- zebra pattern (easily defined and activated as data line option)
- on demand (user input while printing with AskStringChoice$) visco data slice list with all curves activated in the visco screen; automatic page break

Sample Search and Statistics

(**sample_search_statistics.lst**):



Sample Search and Statistics

Search Parameters

Sample:	"pss"	Operator:	*
Account:	*	Method:	*
Project:	U:*.LDX	Eluent:	*
from:	*	to:	*
Signed:	--	Fraction Collector:	--
Visco:	--	HPLC:	Je

Sample Name	Project File	Date	Time	Operator	Eluent
Eluent 0.1M NaNO3					
PSS PEG Mi...6 6240, 1500, 420	U:\...\temp\pss_wingpc6\DEMO.LDX	1993-04-02	07:42:20	pk	0.1M NaNO3
PSS PEG Mi...60, 3120, 600, 196	U:\...\temp\pss_wingpc6\DEMO.LDX	1993-04-02	07:55:21	pk	0.1M NaNO3
Probe PSS Destran P2: Mp 362 D	U:\...\temp\pss_wingpc6\DEMO.LDX	1993-04-02	09:46:32	pk	0.1M NaNO3
Number of samples with eluent: 0.1M NaNO3:					3
Eluent DMAc + 0.5% LiBr					
PSS PMMA ReadyCal green	U:\...Ku...Ultrasan\ANLACE1.LDX	2005-04-22	09:59:35	mikids	DMAc + 0.5% LiBr
PSS PMMA ReadyCal red	U:\...Ku...Ultrasan\ANLACE1.LDX	2005-04-22	10:13:12	mikids	DMAc + 0.5% LiBr
PSS PMMA ReadyCal white	U:\...Ku...Ultrasan\ANLACE1.LDX	2005-04-22	10:26:52	mikids	DMAc + 0.5% LiBr
PSS PMMA ReadyCal green	U:\...Ku...Ultrasan\ANLACE1.LDX	2005-04-22	10:50:13	mikids	DMAc + 0.5% LiBr
PSS PS ReadyCal green	U:\Tripledetection.LDX	2007-09-28	01:59:35	HB	THF
PSS PS ReadyCal green	U:\Tripledetection.LDX	2007-09-28	01:59:59	HB	THF
PSS PS ReadyCal red	U:\Tripledetection.LDX	2007-09-28	02:31:38	HB	THF
PSS PS ReadyCal red	U:\Tripledetection.LDX	2007-09-28	03:03:16	HB	THF
PSS PS ReadyCal white	U:\Tripledetection.LDX	2007-09-28	03:34:52	HB	THF
PSS PS ReadyCal white	U:\Tripledetection.LDX	2007-09-28	04:36:29	HB	THF
Number of samples with eluent: THF:					89
Eluent THF					
PSS PS ReadyCal black	U:\...\9...G_Phenol-Propiat.LDX	2008-02-15	18:21:44	HB	THF
PSS PS ReadyCal blue	U:\...\9...G_Phenol-Propiat.LDX	2008-02-15	18:48:06	HB	THF
PSS PS ReadyCal yellow	U:\...\9...G_Phenol-Propiat.LDX	2008-02-15	19:14:21	HB	THF
Number of samples with eluent: THF:					3
Total number of samples found:					120
Total number of eluents:					7

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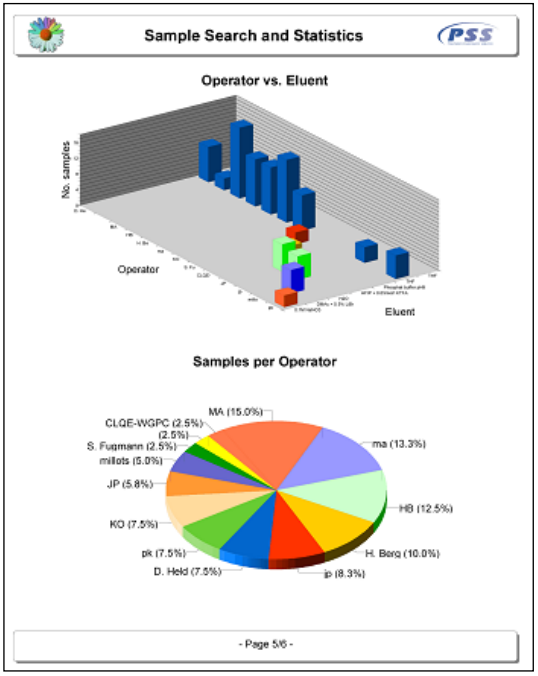
Sample Search and Statistics

Operator

Eluent	CLOE-WGPC	D. Held	H. Berg	HB	js	JP	KO	ma	MA	mikids	pk	S. Pugmann	No. Samples/Eluent
0.1M NaNO3	0	0	0	0	0	0	0	0	0	0	3	0	3
DMAc + 0.5% LiBr	0	0	0	0	0	0	0	0	0	6	0	0	6
H2O	0	0	0	0	0	6	7	0	0	0	0	0	13
HFIP + 0.05molI KTFA	0	3	0	0	0	0	0	0	0	0	0	0	3
Phosphat buffer pH9	0	0	0	0	0	0	0	0	0	0	0	3	3
THF	3	0	9	12	12	4	0	9	16	18	0	6	89
THF	0	0	0	0	3	0	0	0	0	0	0	0	3
No. Samples/Operator	3	3	9	12	15	10	7	9	16	18	6	9	120

results:

- sorted: ascending by eluent (table property; easily to be changed)
- ✓ grouped by eluent
- ⇒ number of samples per eluent
- number of eluents (userdefined variable)
- overall number of samples (userdefined variable)



3D bar chart:

- operator, eluent and number of samples plotted
- declination and rotation can easily be adapted
- colors can be defined by user

2D pie chart:

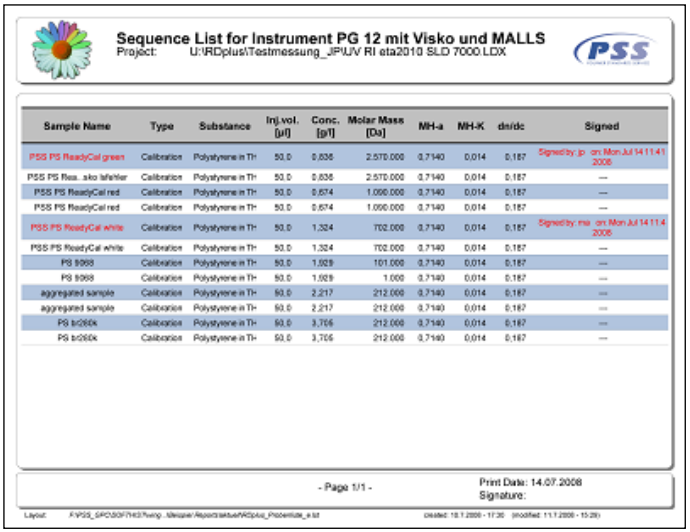
- operator vs. number of samples
- declination and colors user defined

Cross table:

- eluent vs. operator
- each number of samples/operator and samples/eluent and total sum

Sequence List

(**enhanced_sequence_list.lst**):



Sequence List for Instrument PG 12 mit Visko und MALLS
Project: U:\RDplus\Testmessung_JP\UV RI eta2010 SLD 7000.LDX

Sample Name	Type	Substance	Inj.vol. [µl]	Conc. [g/l]	Molar Mass [Da]	MH-a	MH-K	dn/dc	Signed
PSS PS ReadyCal green	Calibration	Polystyrene in TH	50.0	0.836	2.570.000	0,7140	0,014	0,187	Signed by: jp on: Mon Jul 14 11:41 2008
PSS PS Rea...sko lafehler	Calibration	Polystyrene in TH	50.0	0.836	2.570.000	0,7140	0,014	0,187	---
PSS PS ReadyCal red	Calibration	Polystyrene in TH	50.0	0.674	1.090.000	0,7140	0,014	0,187	---
PSS PS ReadyCal red	Calibration	Polystyrene in TH	50.0	0.674	1.090.000	0,7140	0,014	0,187	---
PSS PS ReadyCal white	Calibration	Polystyrene in TH	50.0	1,324	702.000	0,7140	0,014	0,187	Signed by: ma on: Mon Jul 14 11:4 2008
PSS PS ReadyCal white	Calibration	Polystyrene in TH	50.0	1,324	702.000	0,7140	0,014	0,187	---
PS 9068	Calibration	Polystyrene in TH	50.0	1,929	101.000	0,7140	0,014	0,187	---
PS 9068	Calibration	Polystyrene in TH	50.0	1,929	1.000	0,7140	0,014	0,187	---
aggregated sample	Calibration	Polystyrene in TH	50.0	2,217	212.000	0,7140	0,014	0,187	---
aggregated sample	Calibration	Polystyrene in TH	50.0	2,217	212.000	0,7140	0,014	0,187	---
PS br266k	Calibration	Polystyrene in TH	50.0	3,705	212.000	0,7140	0,014	0,187	---
PS br266k	Calibration	Polystyrene in TH	50.0	3,705	212.000	0,7140	0,014	0,187	---

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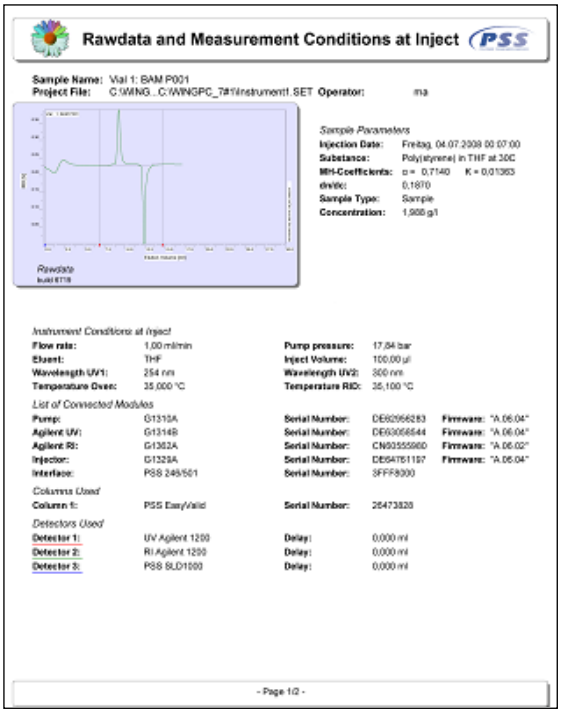
Print Date: 14.07.2008
Signature:

- extended sample names are abbreviated
- ✓ either substance or **n.a.** will be displayed (conditioned display)
- signed samples are written in red (see appearance conditions)

Raw Data and Measurement Conditions at Inject

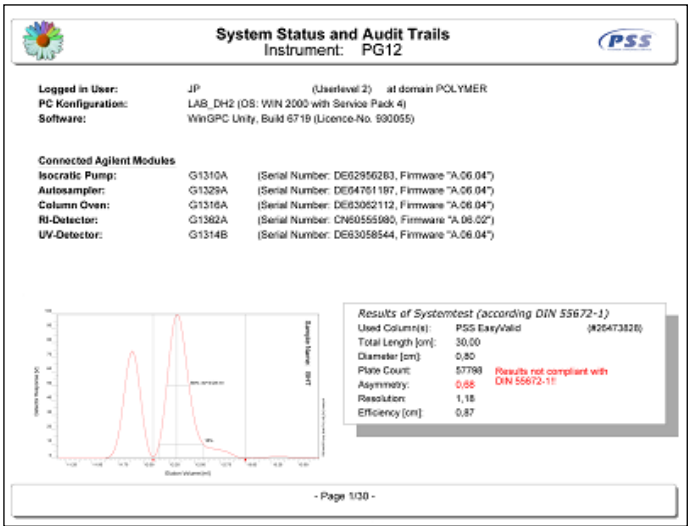
(**system_status_details.lst**):



- sampletype **calibration**: red color if concentration = 0 g/l (toggled by appearance condition)
- name, serial number and firmware of Agilent modules only displayed if connected (cf. appearance conditions)
- **Intelligent round function**: M_n, M_w are rounded 3 significant digits

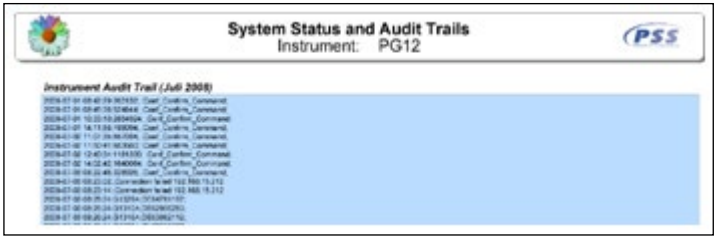
System Status with Audit Trails

(**system_status_AuditTrails.lst**):

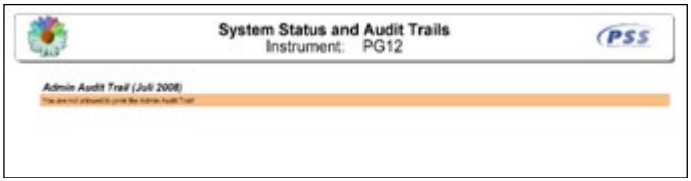


- user input with combobox while printing (new ReportDesigner function AskString-Choice$)
- Agilent modules with serial number and firmware displayed if present (see appearance conditions)
- user input sample name of last system test
- system test compliance with DIN 556721-1 is checked automatically

  ⇒ warning if not compliant



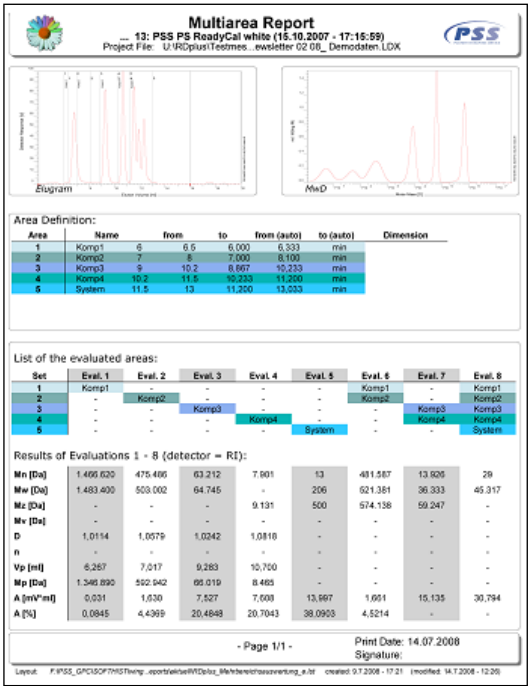
- on demand (user input AskStringChoice$ with combobox **Yes / No**) printing of audit trails: instrument, session, sample and administrator



- user level is checked
- ⇒ administrator audit trail can only be printed out if administrator is logged in!

Multiarea Report

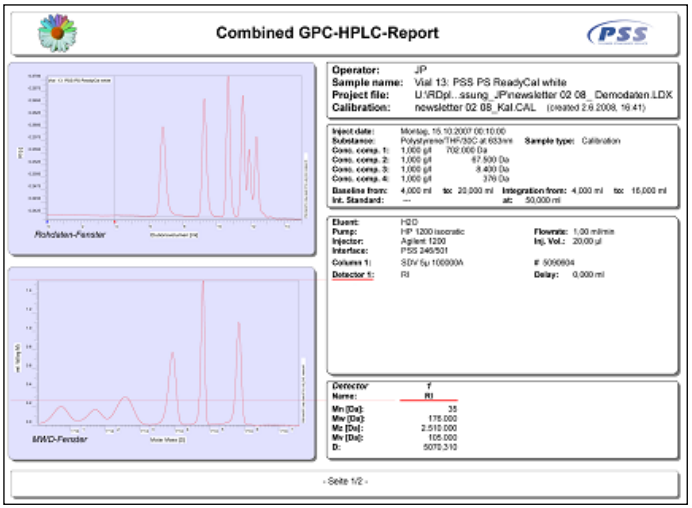
(**mutiarea_report.lst**):



- only defined areas are displayed (see appearance conditions)
- dynamic adaption of table height by conditioned linefeeds
- display of results or "-" (conditioned display)

Combined GPC-HPLC-Report

(**GPC-HPLC_report.lst**):



extended project path abbreviated

sample parameters:

- ✓ sample type calibration: red color if concentration = 0 g/l (cf. appearance condition)
- ✓ dynamic adaption of table height by linefeeds (appearance condition: if substance is not defined or sample type <> calibration)

instrument parameters:

- only columns and detectors present in method window are displayed
- table height adapted by conditioned linefeed

MWD results:

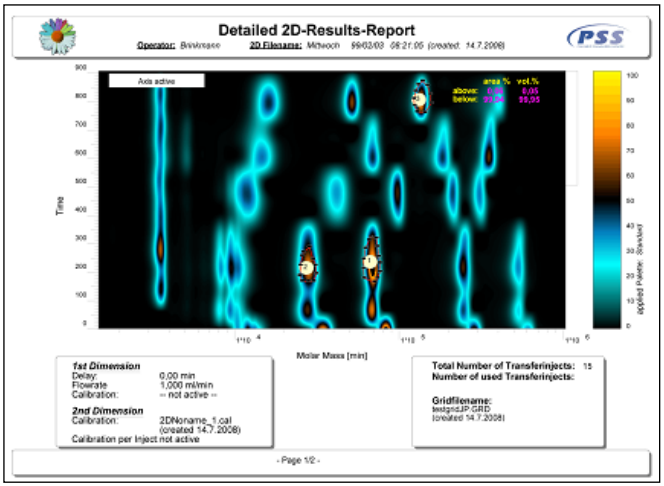
- only concentration detectors are shown (conditioned by variable signal type)
- **Intelligent round function**: M_n, M_w are rounded to 3 significant digits

HPLC evaluation:

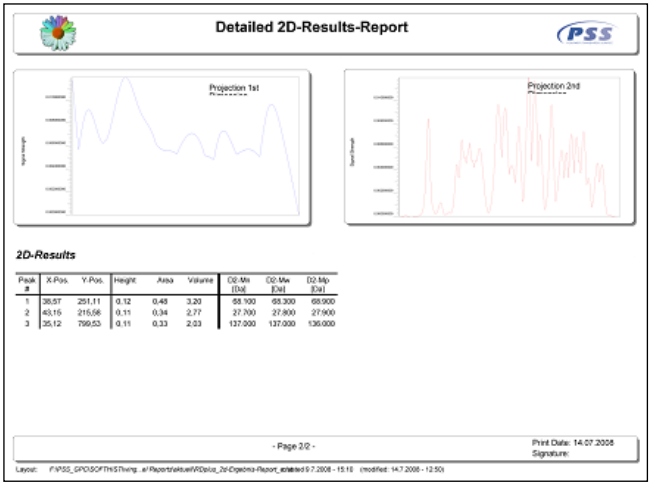
- peaklist is sorted by area or height corresponding to WinGPC Software settings (see appearance conditions and table properties sorting)

Detailed 2D-Results-Report

(**2D_results_report.lst**):



- selective display of declination and rotation or **Axis active** (see appearance conditions)
- display of active grid and grid name or **not active** (appearance conditions)
- calibrations of 1st and 2nd dimension are shown if active



2D-Results:

- only shown if calibration(s) are active
- **intelligent round function**: M_n, M_w are rounded to 3 significant digits

The ReportDesigner Variable List

The *List of Variables* contains an explorer-like table with all WinGPC Software parameters, results, graphics, file and date information, etc. It consists of two major elements: *variables* and *fields*. Variables represent a fixed WinGPC Software parameter which is defined by its absolute properties (e.g., a results value of detector 1). In contrast, fields are used to create tables (with headers and data rows). WinGPC Software will calculate the number of samples in overlays or detectors in multi-detector setups during printing. This means that a single report layout can be used for analyses with very different instrument configurations and the user need not think or take care about this.

Example:

You are using a data acquisition method with UV and RI detection. Depending on the type of sample, the report should sometimes contain the results of UV and RI detectors, sometimes only RI data should be reported.

This can be accomplished easily with a report layout that contains a table filled with fields from the *List of Variables* in **Fields > Detector**. The ReportDesigner layout preview fills all cells with 0. The table cells and number of table rows will be calculated during printing depending on the number of displayed detectors: in case only the RI detector is displayed in WinGPC Software, only a single data (result) row will be printed, otherwise 2 data (result) rows will be printed if both detectors are displayed.

NOTE

In contrast to tables filled with fields, the fixed variable of a detector result is also available in the *List of Variables* **Variables > Results > MWD** for each detector. They should be used for layouts in which the instrument configuration does not change. The correct contents of the variables can already be checked in the layout editor Preview window, which refers to the data currently evaluated in WinGPC Software. If no sample is under evaluation in WinGPC Software, the variables are empty or 0. In contrast to fields, the detectors which are not displayed in WinGPC Software will be reported nonetheless, if they have been entered in the layout as variables.

Contents of the Variable List

Variables

Table 45 Variables

Variable	Definition
ReportVar	Information related to the layout used (e.g., **Report_name**, **Creation_date**)
PC_configuration	Information related to the workstation and the WinGPC Software installation (e.g., operating system: **Operating_system**, WinGPC Software license number: **WinGPC_serial_number**)
UDC	Information related to the PSS UDC810 Universal Data Center (e.g., **UDC_Name**, **UDC_Connection**)
CFR	Information related to the logged in user (e.g., **UserName**, **Userlevel**) and audit trails (e.g., **Session_Audittrail**)
Acquisition	Method and instrument information (e.g., **Project**, **Operator**, **Instrument**...), columns and detectors (also detector color); information on instrument options for viscometer and light scattering detectors from the **method** window (**DPT-sensitivity**, **Wavelength**, **Scattering_angle**, **LS_constant**, etc.)
Processing	Data processing information of the current sample, e.g., inject time (**Injection_time**), baseline and integration limits (**Baseline_left**, ...), used calibration file (**Calibration_file_1**) and calibration type (**Calibration_type**; cf. variable contents below), etc. Settings in the viscometry, light scattering window, in the HPLC mode (e.g., **Reference_file**) and automation settings (e.g., **Calibration_file**, **Internal_standard_correction**,...)
Sample	Sample information from the sample editor, e.g., name of current sample (**Sample_name**)
Calibration	Information on the calibration used in the current project: calibration file (**File**), fit function (**Fit**), file create date/time (**Created**) and file modify date/time stamp (**Updated**), regression results of the calibration fit (**Coefficients**)
Calibration_File	Information on the active calibration file in the calibration window: e.g., calibration file (**File**), fit function (**Fit**), file create date/time (**Created**) and file modify date/time (**Updated**), regression results of the calibration fit (**Coefficients**) and regression coefficient *R*
Overlay_calibration	Information on each of up to 16 calibration curves overlaid in the calibration window, e.g., name (**File**), curve color (**color**), internal standard used (**Name_internal_standard**), columns used (**Columns**), etc.

Table 45 Variables

Variable	Definition
System_test	System suitability test results for the selected standard: all parameters and results of the system test incl. which guideline is used for testing (**reference_standard**); the subfolder lists all limits (requirements) which have been set. *Note*: • system test results are kept until a new system test has been calculated or WinGPC Software is closed • use the **Fields > System_test_table** for simultaneous output of different reference standards in comparison
Performance_tests	Performance test results for the selected signal including the type of test (type) for all detectors (sub folder **detectorN**); the test result parameters of each performance test are listed in the sub folders for each detector *Note*: There is no corresponding *Field* variable for tables
Sieve_settings	Set of parameters which are entered in the **Retention %** dialog box and the current sieve curve equation
Search_Settings	Parameters of the sample search mask (e.g., **Sample_Name**, **Inject_Date**, **Eluent**)
Results	MWD results for each individual detector (*Mn*, *Mw*,..), results from different types of molar mass determination (conventional, viscometry, light scattering, triple), results from light scattering and viscosity window (Mark-Houwink coefficients: **Mark_Houwink_K**, **Mark_Houwink_A**, bulk intrinsic viscosity: *ng*, bulk *Mw,g* from light scattering...), chemical heterogeneity results for each detector (e.g., **average_comp**)
Overlay	Results on each of up to 32 overlaid signals (*Mn*, *Mw*...) of the active overlay, including curve details like inject date/time (**Inject_time**) and curve properties (**Smoothing**, **Color**, **Calibration_file**), etc.
MultiArea	multi-area settings (e.g., area start **Settings > Area_1-Start** and end **Settings > Area_1-End**, used *.MAS parameter file (**File**) and results for each range and detector)
Two_D	File information and settings of the 2D window (e.g., **2D_file_name**, **grid_file_name**, **2D_area_above**)
Diagrams	WYSIWYG graphics of all WinGPC Software windows: raw data, elugram (shows overlay, HPLC mode or system test, if active), MWD, viscosity, light scattering, calibration, 2D
LL	internal ReportDesigner variables (e.g., **OutputDevice**, page size and printable area of the defined printer)

Project Variables

Table 46 Project variables

Variable	Definition
@LLFAX	Recipient/sender information for FAX and e-mail transmissions

Fields

Table 47 Fields

Variable	Definition
Sample_Search	Parameters of the sample search mask (e.g., **Sample_Name**, **Inject_Date**, **Eluent**)
Overlay_Calibration	Parameters and coefficients of the curves overlaid in the calibration window (e.g., **No**, **Color**, **Mark_Houwink_A**, **Creation_Date**)
Detector	Molar mass results (**Mn**, **Mw**,...) and detector information (**Name**, etc.) for data table The detector assignment is not static (fixed) as in the case of **Variables > detector**; the number of used detectors is automatically updated by WinGPC Software
Calibration	Contents of the calibration table of the active calibration file in the calibration window selectable by columns (**Vp**, **Mp**, ...)
Data_Editor	Provides content of all data editor columns, flexible use
System_test_table	Simultaneous system suitability test results for all standards: all parameters and results of the system test incl. which limits have been set for SST testing
HPLC_Parameters	Results of the peaklist generated from a HPLC mode analysis (**Peak_name**, **Peak_start**, **Peak_end**, etc.); Information on used HPLC reference file can be found in **Variables > Processing > HPLC**
HPLC_response_factors	Results from linear regression analysis of response calibration (in subfolder fordetector*N*) for multiple compounds (peaks); information on reference file can be found in **Variables > Processing > HPLC**
Light_scattering_angles	Information for multi angle light scattering like scattering angles (**result_angle**), normalization factor (**normalization_factor**) or angle used or not (**active**)

Table 47 Fields

Variable	Definition
Overlay	Information on all curves in the overlay mode (**Curve_name**, **Smoothing**, etc.) and their results (**Mn**, **Mw**,...) The curve assignment is not static (fixed) as in the case of **Variables > Overlay**; contains special sections on • **Viscosity_results** • **Light_scattering_results** • **Sieve_results**
Two_D_Parameters	Sample information of all samples transferred or loaded in the 2D window (e.g., sample name: **transfer_injection_available**, transfer injection shown in overlay: **transfer_injection_used**, calibration used: **calibration**)
Two_D_Isolines	Position (**position_value**) and status (displayed or not) of the isolines in the 2D window
Two_D_Results	Results and positions for the peaks analyzed in the 2D window (e.g., **peak_name**, **peak_x_position**, **Mn** and many more)
Sample_List	Parameters of the samples listed in the sample editor (e.g., **Sample_name**, Substance, **Inject_volume**, **Rawdata_Comment**)
LL	Internal ReportDesigner fields (e.g., number of printed data sets **LL.CountPrintedData**, name of the actual table **LL.CurrentTable**, etc.)

Variables with Preset Values

In most cases variables and fields are filled with parameters and results which are determined by the user of the analysis (sample names, file names, concentrations, date/time, etc.). A limited number of variables, however, can only contain parameters from a preset list (e.g., baseline type, calibration type, fit function), which can be renamed on the report, if the preset name does not seem appropriate for the end user.

Example:

Instead of using the internal variable name “RALLS, vert. pol.” the signal description “LS 90°” should be printed on the report.

This can be done in the ReportDesigner layout by entering variable conditions:

```
cond(Acquisition.Light_scattering.LS_method = "RALLS, vert. pol.", "LS
90°","")
```

Every time the LS method uses a RALLS signal with vertically polarized light, “LS 90°” will be printed as the LS method.

Additional information on fine-tuning reports can be found in the WinGPC Software ReportDesigner User Manual or in the online help.

Preset Variables

Method, processing, and sample parameters:

Table 48 Method, processing, and sample parameters

Parameter	Variable structure/name	Options
Processing	Variables > Processing > Calibration_type	• Conventional (calibration with standards) • viscometry (universal calibration lg(M[h]) vs. V_e) • LS (calibration from light scattering) • Triple (M from triple detection analysis)
HPLC	Variables > Processing> HPLC > mode	• off • height • area
Baseline type	Variables > Processing > Baseline_type	• Standard • 2 Points linear • 3 Points linear • 3 Points splined • 5 Points linear • 5 Points splined
Analysis Options positive/negative peaks	Variables > Processing > Peak_type	• positive peaks • positive + negative peaks • negative peaks
Viscometer model	Variables > Acquisition > Method > Viscometer	cf. resource tree in method window
Light scattering type	Variables > Acquisition > Light_Scattering > LS_method	• LALLS, KMX 6 • RALLS, no pol. • RALLS, vert. pol.
Sample type	Variables > Sample > Sample_type	• Sample • Calibration • Recalibration replace • Recalibration average • stock solution • filtrate • retentate
Curve smoothing	Variables > Overlay > Kurve_xx > Smoothing or Fields > Overlay > Smoothing	contains the number of points for smoothing (moving average) in **Curves** in the elugram or overlay; no smoothing returns "0"

Table 48 Method, processing, and sample parameters

Parameter	Variable structure/name	Options
Automation - do internal standard correction	Variables > Processing > Automation > internal_standard_ correction	yes, no *Note*: peak position of the internal standard can be found in **Variables > Calibration > Reference_internal_standard** for the loaded calibration curve, and in **Variables > Processing > internal_standard_acquisition** for the active sample
Automation - internal standard negative	Variables > Processing > Automation > internal_standard_negative	yes, no
sieve_results	Fields > Overlay > sieve_results>retention_n_unique	* , if result is not unique (space), if result is unique

Viscosity window parameters:

Table 49 Viscosity window parameters

Parameter	Variable structure/name	Options
method for IV calculation	Variables > Processing > Viscosity > Visco_method	normal or Solomon-Gatesman
Weight method used	Variables > Processing > Viscosity > Visco_weight	off, Ci, [n]sp, Ci*[n]sp
molar mass calculation method	Variables > Processing > Viscosity > Visco_calibration_type	Elution volume (x-axis: volume; conventional calibration) Standard (x-axis: molar mass, conventional calibration) Universal (x-axis: molar mass, universal calibration curve)

Light Scattering window parameters:

Table 50 Viscosity window parameters

Parameter	Variable structure/name	Options
Fit function	Variables > Processing > Light_Scattering > LS_fit	• linear regression • 3rd order polynomial • 5th order polynomial • 7th order polynomial
Weight method used	Variables > Processing > Light_Scattering > LS_weight	off, sqrt(Ci), Ci, Ci**2
use original (non-interpolated) data	Variables > Processing > Light_Scattering > LS_original_data	yes, no
angle used for MALLS evaluation	Fields > Light_scattering_angles > Active	yes, no

10 The Viscosity Module

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter "WinGPC Compliance " on page 507 of this user guide.

WinGPC Software supports the use of all types of on-line viscometers providing one or two pressure signals. The use of a viscometer in a GPC instrument allows the determination of molecular weights and molecular weight distributions for polymers where matching calibration standards are not available. Besides the viscometer a concentration detector is needed to determine on-line the concentration in every GPC slice. Common concentration detectors are refractive index (RI) and UV detectors, that can be used at a wavelength where the monomeric unit shows adsorption. Furthermore, calibration standards are needed to establish a universal calibration curve.

Supported Viscometers

The following viscometers are supported and listed in the WinGPC Software resource tree of the **method** window. They can be edited after a right mouse click on the detector name in the tree and by selecting **Edit**.

Viscometers can not be added (like other items) but have to be selected from the entries offered by the tree. Please contact your Agilent representative if problems identifying the viscometer device occur.

Viscotek Models

Model No.:	**100,110,150R/210R/220R,H502, 200/250, DDA270, TDA**
Viscometer type:	four capillary, symmetric bridge, inlet pressure and delta pressure signal
Data acquisition:	analog via 1 A/D converter per signal (either Agilent UIB or legacy PSS Interface)
Login option:	activate option *Viscometry*

Model No.:	**T50/T60**
Viscometer type:	four capillary, symmetric bridge, delta pressure signal
Data acquisition:	analog via 1 A/D converter per signal (either Agilent UIB or legacy PSS Interface)
Login option:	activate option *Viscometry*

Knauer Models

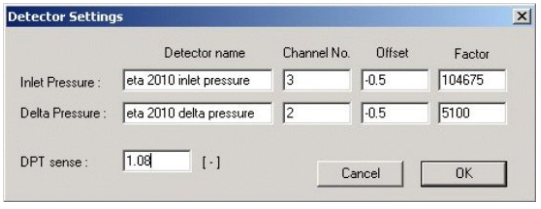
Model No.:	**200**
Viscometer type:	four capillary, symmetric bridge, inlet pressure and delta pressure signal
Data acquisition:	analog via 1 A/D converter per signal (either Agilent UIB or legacy PSS Interface)
Login option:	activate option *Viscometry*

Waters Models

Model No.:	**150CV, GPC-2000**
Viscometer type:	single capillary, delta pressure signal
Data acquisition:	analog via 1 A/D converter per signal (either Agilent UIB or legacy PSS Interface)
Login option:	activate option *Viscometry*

PSS Models

Model No.:	**DVD1260/ETA-100x, ETA-20x0**
Viscometer type:	four capillary, asymmetric bridge, inlet pressure and delta pressure signal
Data acquisition:	digital (COM, LAN, or USB), or analog via 1 A/D converter per signal (either Agilent UIB or legacy PSS Interface)
Login option:	activate option *Viscometry*



Agilent/PL Models

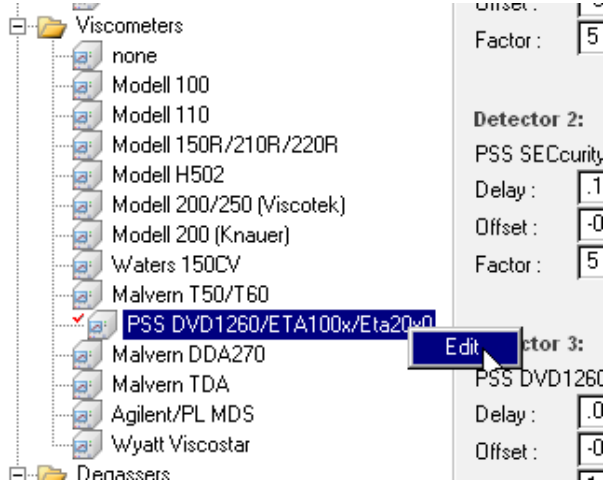
Model No.:	**MDS**
Viscometer type:	four capillary, symmetric bridge, inlet pressure and delta pressure signal
Data acquisition:	digital (USB), or analog via 1 A/D converter per signal (either Agilent UIB or legacy PSS Interface)
Login option:	activate option *Viscometry*

Wyatt Models

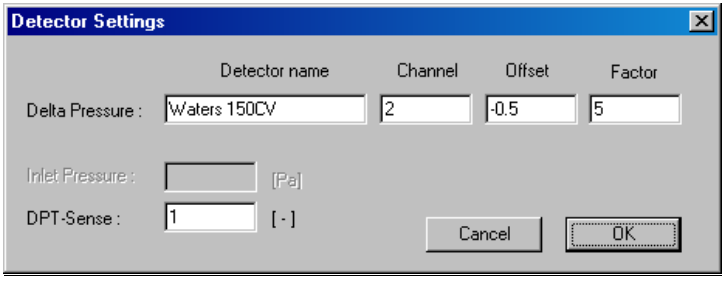
Model No.:	**Viscostar**
Viscometer type:	four capillary, symmetric bridge, inlet pressure and delta pressure signal
Data acquisition:	analog via 1 A/D converter per signal (either Agilent UIB or legacy PSS Interface)
Login option:	activate option *Viscometry*

Parameters Settings

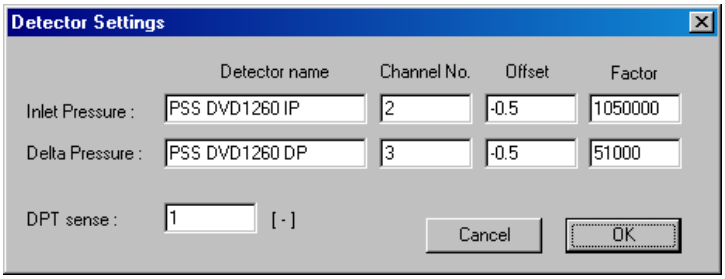
Parameters for all models will be set via right mouse click onto the detector name of the ressource tree (choose edit).



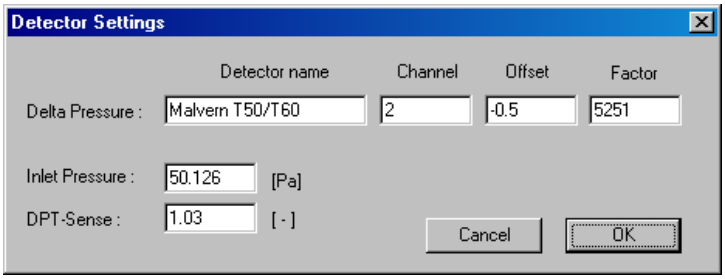
One capillary viscometers with just one pressure signal (differential pressure) will show following dialog:



Four capillary viscometer with two pressure signals (inlet and differential pressure) will show following dialog:



Four capillary viscometer with just one pressure signal (differential pressure) will show following dialog:



Detector name: Assigns a name to the detector signal. This name is displayed in the instrument layout view and the information window of the raw data window.

Channel No.: Enter the channel numbers for inlet pressure and delta pressure as retrieved for the digital signals or (in case of a legacy system) as connected to the interface.

Offset/Factor: Offset and factor will be 0 and 1 for digital data acquisition. In case of legacy hardware with analog data acquisition, the values can be determined according to the procedure described in the appropriate Method Window section (cf. chapter "Resource Tree" on page 166). For single capillary viscometer, where only voltage is needed use the default values for the PSS A/D converters.

Inlet Pressure: Optional: if the inlet pressure signal is not measured online a fixed value can be entered here

DPT sense: Default value: 1. Due to e.g., aging processes of the pressure transducer membranes it might be necessary to work with the DPT sense. The measured intrinsic viscosity is multiplied with this factor to correct deviations from the expected value. The DPT sense can be determined measuring a viscosity polymer standard with known intrinsic viscosity. WinGPC Software will automatically calculate and display the DPT sense in the information window of the viscosity window.

NOTE

If the calibration standards are measured under the same conditions the DPT sense does not influence the molar mass distribution and averages. The Mark-Houwink coefficient α, that provides structure information, is also not affected. However the global (overall) intrinsic viscosity and the Mark-Houwink coefficient K will be influenced if the DPT sense is changed.

NOTE

The viscometer properties dialog for finished measurements allows only to change the DPT sense and the inlet pressure default value (if not measured). The factor and offset values can be changed in the instrument layout view directly. Channel numbers and detector name can not be changed at all.

NOTE

For better usability the parameters are saved in the viscometer.ini file located in the c:\wingpc_8#1 folder. If the data acquisition PC is changed this folder can be directly copied to the new PC to keep all parameters and user specific settings.

Basics of Viscometry Theory

According to the concept of the universal calibration (see calibration in chapter "Calibration" on page 21) true molecular weights and molecular weight distributions can be measured if an on line viscometer is used.

Primary information obtained from a viscometer is the specific viscosity, η_{sp}. Please refer to the viscometer manual for detailed information on the calculation of the specific viscosity from the pressure signal(s), since this is detector dependent.

The ratio of the specific viscosity, h_{sp}, and the concentration, *c*, is the reduced viscosity:

$\eta_{red} = \eta_{sp}/c$. The limiting value at vanishing concentrations is the intrinsic viscosity $[\eta]$. In the case of GPC it is assumed, that the concentrations are small enough to ignore the concentration dependence, i.e. $[\eta] \cong \eta_{sp}/c$.

The intrinsic viscosity is not only measured globally, but also for every elution volume slice. This provides a Mark-Houwink plot and the chance to measure Mark-Houwink coefficients for samples with a broad molecular weight distribution.

Slice molar masses for unknown samples are obtained according to

$$M_i = \frac{([\eta]*M)_{i,calib}}{[\eta]_i}$$

where the numerator contains information from the universal calibration curve. The molecular weight distribution and the molecular weight averages are obtained with the slice molar masses in the usual way (cf. chapter "Molecular Weight Averages and Molecular Weight Distributions in GPC/SEC" on page 18).

A universal calibration curve is created with molar mass calibration standards. The result of log(M*[η]), where [η] is measured on line, is plotted against the elution volume and fitted with the appropriate fit function.

The concentrations needed to obtain the intrinsic viscosity can be obtained from a concentration detector e.g., a differential refractive index detector or a UV detector. Details on this are given in chapter "Determination of Slice Concentration" on page 48.

Performing Viscometry Measurements

This section describes the necessary steps for viscometry data acquisition and processing. Only new features and parameters will be discussed to keep the Agilent WinGPC Software User Guide compact and to avoid repetitions. The basic features of the WinGPC Software are described in their respective chapters. Please refer to them, if some notions and features are not explained here.

Method Window

Edit all items in the resource tree. Most important are the concentration detector(s) and the viscometer. Enter as many values as known. If the slice concentration should be determined using the concentration determination methods **Fact*dn/dc** and **Fact.*Conc.**, the factor for the concentration detector needs to be entered. If the factor is not known, it can be determined measuring a sample with known concentration and dn/dc.

Add the resources in the instrument layout view. Drag&drop the concentration detectors or define the number of concentration detectors in the **Method** Window using **Definition > Number of detectors** and select them manually from the pop-up list with a left mouse click on the corresponding item. Viscometers need to be added with drag&drop.

Please note that it is better to remove the viscometer (drag&drop of **none** from the resource tree on the icon) before changing the number of concentration detectors.

Raw Data Window

Enter the necessary sample dependent parameters:

- concentration
- injection volume

in the **Editor > Samples** dialog.

Injection volume and concentration are needed for the calculation of the slice concentration if the methods **injected mass**, **Fact.*Conc.** and **Conc.*dn/dc** should be used. Details describing the calculation of concentrations can be found in chapter "Determination of Slice Concentration" on page 48.

The refractive index increment dn/dc will be used for the calculation of the slice concentration, if the concentration determination method **fact.*dn/dc** is selected. If this method should be used, please enter the dn/dc value also.

NOTE

The dn/dc value for the concentration determination is not needed if chemically equal substances will be investigated. If the detector factor is determined with a certain material, it can be used for the same material without any restrictions. Only if different substances should be investigated the dn/dc is needed.

The first measurement should be performed with calibration standards with known concentration to establish the universal calibration curve. A description how the universal calibration curve is established is available in the "Calibration" chapter of this user guide, in the step-by-step instructions of the Online help (**Help** > **Step by step**) and in the WinGPC Quick Reference Guide (in the **Documentation** folder of installation medium).

NOTE

Universal curves have no special file extension. They are saved together with the conventional calibration curve under the same file name. WinGPC Software detects automatically if a calibration curve provides both, conventional and universal processing. The setting in the mass distribution window defines the kind of analysis that is executed.

Evaluation of Viscometry Measurements

The following data processing steps are necessary to obtain results from runs with a viscometer.

Raw Data Window

In the Raw Data Window you must set the baseline limits and perform the optional correction with the internal standard. If you are using the concentration determination methods **injected mass**, **Fact.*Conc.** and/or **Conc.*dn/dc** you must set the baseline in a way that the area under the RI Signal inside the baseline limits reflects the polymer quantity. I.e. the complete polymer peak must lie inside the boundaries, but signals like system, salt or solvent peaks are excluded. If not done yet load the universal calibration curve using **Calibration Data > Load** from the menu.

Elugram Window

Here the integration limits need to be defined just as described for conventional data processing (see chapter “Elugram Window” on page 224 for more details).

Viscosity Window

The viscosity window is the central window for selecting specific calculation options and to verify primary information. If the processing parameters are fixed by the method this window is only needed for trouble shooting.

Mass Distribution Window

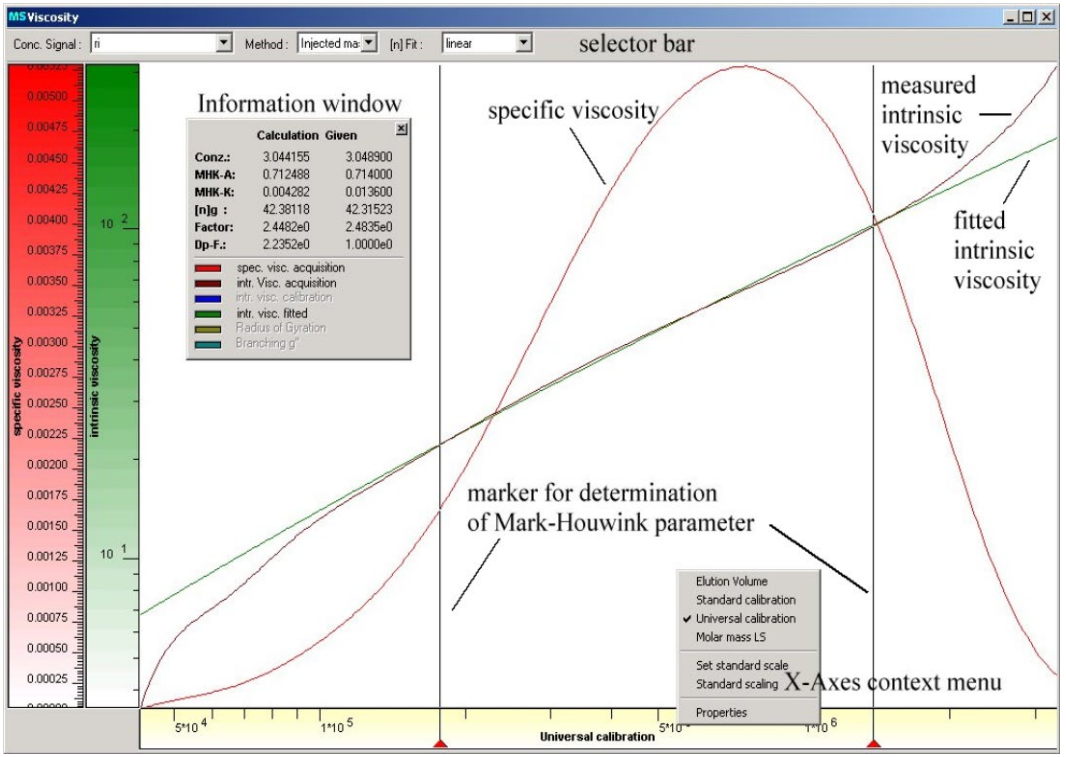
The molar mass distribution and the molar mass averages measured with viscometry are immediately displayed when the X-axis context menu item **Calib. Viscometry** is chosen. This setting can be changed anytime and be saved with the method.

NOTE

Besides the currently processed sample the status bar shows also the currently used calibration method: If **Viscometry** is shown **Calib. Viscometry** is active and the universal calibration curve is used. If the name of the calibration curve is shown **Calib. Standard** is used and the conventional calibration curve is used.

Viscosity Window

The viscosity window is the central window for selecting specific calculation options and to verify primary information.



The window shows by default the curves for the measured specific and intrinsic viscosity. Furthermore, the intrinsic viscosity fit as well as the calculated radius of gyration and the branching g' curve can be switched on/off by clicking on the curve name in the information window.

Besides the curves the information window shows the two columns **Calculation** and **Given**.

Table 51 Viscosity window

Curves	Calculation	Given
Conc.	Calculates overall concentration within the integration limits. The settings for concentration signal and method are used	Concentration entered in the sample editor. If the concentration for more than one component is entered, **Options > Component** from the light scattering window menu defines what concentration is displayed.
MHK-A MHK-K	Mark-Houwink coefficients K and α, obtained from a linear fit of log [η] vs. log M. The red markers define the fitting region. log K = intercept α = slope	Mark-Houwink coefficients saved and retrieved from the active calibration curve. The Mark-Houwink coefficients for the calibration standards can be entered using the calibration window menu item **Calibration > Parameters...**.
[η]g	Global intrinsic viscosity measured for this sample using the measured specific viscosity and the measured concentration. $[\eta]_g = \frac{\int \eta_{sp} dV}{\int c dV}$	Global intrinsic viscosity calculated using the measured specific viscosity and the concentration and injection volume given in the sample editor. $[\eta]_g = \frac{\int \eta_{sp} dV}{c_{inj} V_{inj}}$
Factor	Calculated factor for the concen-tration detector defined in the selector bar. The factor is needed if the method **fact.*dn/dc** or **fact.*conc.** should be used.	Factor of the selected concentration detector. Entered in the method window instrument layout view.
Dp-F.	Necessary DPT sense, to calculate the intrinsic viscosity [η] in the column **Given**. The DPT sense is calculated using the concentration of the column **Given** and the experimental specific viscosity.	DPT sense entered in the viscometer properties dialog of the method window.

Evaluation parameters can be set in the selector bar. The selector **Conc. signal** allows to define which detector should be used for the determination of the slice concentration. The **Method** selector defines the concentration determination method. The third selector in the bar allows to define the fit function used to fit the measured intrinsic viscosities vs. the elution volume. This is needed since at the peak onsets the uncertainty for data points increases. The fit (as well as the chosen weight function) allows to rely on points measured with a higher precision.

Table 52 Evaluation parameters

Parameter	Description
Conc. Signal:	Here the concentration detector can be chosen. Available are all concentration detectors defined in the method.
Method:	4 different methods are available: Injected mass, Fact.*dn/dc, Fact.*Conc. and Conc.*dn/dc see section "Determination of Slice Concentration" on page 48 for details.
Fit:	Here the fit function for fitting the measured intrinsic viscosity is chosen. Please try first the linear function. If the data are fitted badly change first the settings in the menu **Options > Viscometry > Weight** to get a better fit. Recommended setting is ci*[n]sp, this is the best choice for most samples.

NOTE

The mass recovery of samples can be easily determined if a calibrated concentration detector is used. The overall sample concentration can be measured using the method **Fact.*dn/dc**. The concentration result shown in the information window should be comparable to the concentration entered in the sample editor. Therefore, the mass recovery [%] is the ratio of the calculated and the given concentration multiplied with 100. If the recovery is less than 100% this might be a hint for e.g.,

- a wrong concentration determined with the balance
- sample interaction with column material
- micro gel content of the sample and/or therefore sample loss by filtering.

NOTE

The WinGPC Software ReportDesigner allows the automatic calculation of the mass recovery including an automatic warning on the report if it is too low.

X-Axis Context Menu

These functions are accessible by a right mouse click on the X-axis scale.

Table 53 X-axis context menu

Function	Description
Elution Volume:	The elution volume will be displayed on the X-axis. This setting is useful for the selection of the fit function. The accessible elution volume range depends on the setting of the integration limits in the elugram subtracted by the part excluded through the setting of the calculation limits (**Options > Viscometry**, default: 1%).
Standard Calibration:	The molecular weight of the standard calibration is used as X-axis.
Universal Calibration:	The molecular weights received by universal calibration are used as X-axis. The molecular weights in the slices are calculated as: $M = \left(\frac{[\eta]_{calib} * M}{k_{mess}} \right)^{\frac{1}{\alpha_{mess}+1}}$
Molar Mass LS:	This is only available for measurements with viscometry and light scattering data. The molecular weights calculated by light scattering will be displayed on the X-axis. The fit and weight options (**Options > Light scattering**) are considered, so it is important to specify the light scattering parameters first. This feature is useful to measure Mark-Houwink coefficients without any assumptions while relying on primary detector information.
Set standard scale:	Allows the manual setting of the displayed X-axis range of the viscosity window and sets the default standard scale.
Standard scaling:	Restores the scale of the last manual scaling (e.g., after editing the scaling by the arrow keys or the scroll bar of the X-axis.). A tick mark in the context menu indicates if the standard scale is in use.
Properties:	This context menu is available on each axis in the WinGPC Software. It allows setting axes properties individually. For example, text attributes (font, size, color etc) and background properties of the axis can be defined. It is also possible to switch from elution volume to elution time representation for X-axis properties. The axis properties will be saved for each instrument individually.

Y-Axis Context Menu

These functions are available from the Y-axis context menu on all axes, if you click on the Y-axis scale with the right mouse button. A popup menu appears in which the following functions can be selected:

Table 54 Y-axis context menu

Function	Description
Norm.:	Toggles between normalized and manually scaled view.
Set standard scale:	Allows the manual setting of the displayed Y-range of the chromatogram and sets the default standard scale.
Standard scaling:	Use preset Y-axis scale as defined in **Set standard scale**. If the standard scale is in use, this command shows a tick mark.
Tip: The method file also saves the scaling settings of the various axes. These will be loaded when the method file is retrieved from the file system.	
Properties:	This context menu is available on each axis in the WinGPC Software. It allows setting axes properties individually. For example, axis labels, text attributes (font, size, color etc) and background properties of the axis can be defined.
Tip: It can be very useful to assign the Y-axis caption in the same color as the detector signal and give the axis a descriptive name (e.g., measured LS intensity).	

File Menu

Table 55 File menu

Function	Description
Printer Setup:	Allows definition of parameters for the default printer defined in the Windows Control Panel. Landscape format prints the graphics on a full page. Portrait format prints the graphics, full method documentation and results by default. The printed information in the portrait layout can be selected for automated runs in the **Definition > Automation Settings** dialog box. The exact information of the portrait format printout depends on which window will be printed. For color printers you can select color or monochrome printing depending on the printer driver options. The color of the curves in monochrome printouts are mapped automatically to a line style to avoid unreadable black and white prints. The correlation between curve color and line style in monochrome-print is listed in "Curve Colors and Line Styles in Monochrome Printing" on page 534.
Print:	Prints the current contents of the viscosity window to the default printer. The graphics are always printed in WYSIWYG mode, i.e. the current window display will be printed identically as shown.
Page Preview:	Shows a print preview in a separate print-preview window, which can be sized and moved. At the same time this command copies the preview contents to the Windows clipboard. When printing in portrait format, the graphics and the text for the measurements will shown, while landscape format will print only the graphics.
ASCII Save as:	Saves the currently in the viscosity window displayed data as ASCII file for use in other applications or for the transfer data to other computer platforms.
Edit Comment:	Allows to enter a viscosity data related text (comments, hints for data treatment, data processing details, observations, etc.). Up to 1024 characters can be entered for each injection (sample) separately. Alternatively, the  icon in the status bar (see "Comment and Options" on page 159) can be used to open the comment dialog box. This icon is gray if no comment has been entered for this sample, if text has been entered it is highlighted in green.

Options Menu

Table 56 Options menu

Function	Description
Editor slice data:	Transfers the displayed data to the WinGPC Software data editor.
Component:	Assigns the sample editor's component concentration to the peak (see also **Raw Data** Window Menu **Editor > Samples**). The concentration of the selected component appears in the column **given** of the information window.
Viscometry	
• Calculation limits:	Filters less significant evaluation points so that only areas in which concentration and viscometer detector show significant data are used. A defined percentage of the concentration detector peak maximum (default: 1 %) is then discarded.
• Fit:	The measured intrinsic viscosity should be fitted linear or by a polynomial function of 3rd, 5th or 7th order. This fitted intrinsic viscosity curve will then be used to obtain the molar masses from the universal calibration curve. The original viscosity data are used if **Original Data** is chosen. For most samples the fit function **linear** is the best choice. The fit should be selected so that the curve **intr. visc. fitted** matches most of the **intr. Visc. acquisition** curve. The quality of the fit can be improved selecting the proper weight function (see below). The fit can also be chosen using the selector bar in the light scattering window.
• Weight....	Allows to assign different weights to the elution volume - intrinsic viscosity pairs. The chosen weight function influences the fit for the measured intrinsic viscosity shown in the viscometry window as **intr. visc. fitted** curve. The weight has no influence if **Original Data** is selected in the MW fit selector. If necessary, the weight function can be selected for each sample separately. In general, the weight option ci*[n]sp is the recommended one, since this leads to the best fit for the majority of samples. • **Off**: No special weight will be used, all pairs will be treated equal. • **ci**: Each point will be weighted with the height of the concentration signal. Because the viscosity signal on the lower molecular end loses signal intensity faster than the concentration signal, such points will be given high weight even if only a noisy viscosity signal is provided. **[n]sp**: Each point will be weighted with the height of the specific viscosity signal. Because the concentration signal on the high molecular end loses signal intensity faster than the viscosity signal, such points will be given high weight even if only a noisy concentration signal is provided. • **Ci*[n]sp**: Each point will be weighted with the product of concentration and viscosity signal, to fulfill the different molecular weight dependencies of both signals (recommended method).
Branching:	**MH-coef. branching** opens a window where the Mark-Houwink coefficients K and α can be entered. According to [η] = K * Mα the intrinsic viscosity for the linear counterpart can be calculated. The resulting values can then be used to calculate and show the “branching g’” curve that can be turned on/off using the menu item **branching g’** or the curve in the information window.

Table 56 Options menu

Function	Description
Radius of Gyration:	Calculation of the radius of gyration according to the Flory-Fox equation. The curve will be displayed in the viscosity window (alternately activate/ deactivate of the curve in the information box by clicking the *radius of gyration*).
SolomanGatesman	Calculation of the intrinsic viscosity according to Soloman-Gatesman to limit the concentration influence on the intrinsic viscosity calculation from GPC data. The calculation is done by: $[\eta] = \frac{\sqrt{2}}{c}(\eta_{sp} - \ln \eta_{rel})^{1/2}$

11 Light Scattering Module

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter “WinGPC Compliance ” on page 507 of this user guide.

WinGPC Software supports the use of all types of on-line light scattering detectors:

- right angle (RALLS)
- low angle (LALLS)
- and multi angle (MALLS, TALLS)
- DLS (see “Agilent/PL MDS/GPC 220” on page 393)

The use of a light scattering detector in a GPC instrument allows the determination of absolute molecular weights and absolute molecular weight distributions. Besides the light scattering device, a concentration detector is needed to determine on-line the concentration in every GPC slice. Common concentration detectors are refractive index (RI) and UV detectors, that can be used at a wavelength where the monomeric unit shows adsorption.

Supported Light Scattering Detectors

The following light scattering instruments are directly supported and listed in the WinGPC Software resource tree of the **method** window. They can be edited after a right mouse click on the detector name in the tree and by selecting **Edit**.

Please note that detectors supported via digital data acquisition can be edited only

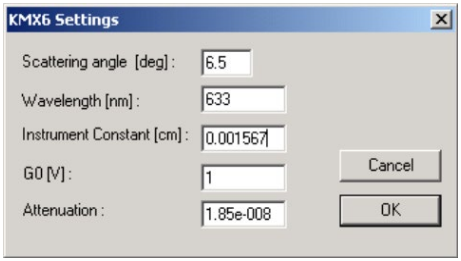
- if they are used in a finished run
- if they are used in a method and if the detector is on line

Light scattering devices can not be added (like other items) but have to be selected from the entries offered by the tree. Please contact your Agilent representative if problems identifying the light scattering device occur.

KMX6

LS type:	**LALLS**
Data acquisition:	analog via 1 A/D converter per signal (either Agilent UIB or legacy PSS Interface)
Login options:	activate option **Viscosity/Light Scattering**

Parameters KMX6:



Scattering angle [grd]:	see LS instrument user manual (typically between 6° and 7°)
Wavelength [nm]:	laser wavelength in nm, see LS instrument user manual
Instrument constant [cm]:	determine using the WinGPC Software Detector Setup, enter 1 as initial value
G0 [V]:	see LS instrument user manual, if not available use the default value 1. The corrected G0 may then be calculated using following equation: G0 = Mw(ref)/Mw(app)
Attenuation:	see LS instrument user manual, if not available use the default value 1

Wyatt DAWN DSP, DAWN EOS, miniDAWN, miniDAWN TREOS, HELEOS

LS type:	**MALLS**
Data acquisition:	*all but TREOS/HELEOS*: digital, adds 2 analog channels (Aux 01 and 02) which can be selected with a right mouse click in the method CH No. field of a concentration detector *TREOS/HELEOS*: analog via 3 A/D converter modules for the TREOS and 8 A/D converter modules for the HELEOS (8 signals out of 18 can be selected and recorded). Channels can be set in the miniDAWN TREOS and DAWN HELEOS properties respectively.
Login options:	activate option **Viscosity/Light Scattering** and **Wyatt**, select COM port connected to the light scattering device; for TREOS/ HELEOS: select TREOS or HELEOS as port to have access to the light scattering device

Parameters Wyatt DAWN, miniDAWN and TREOS:

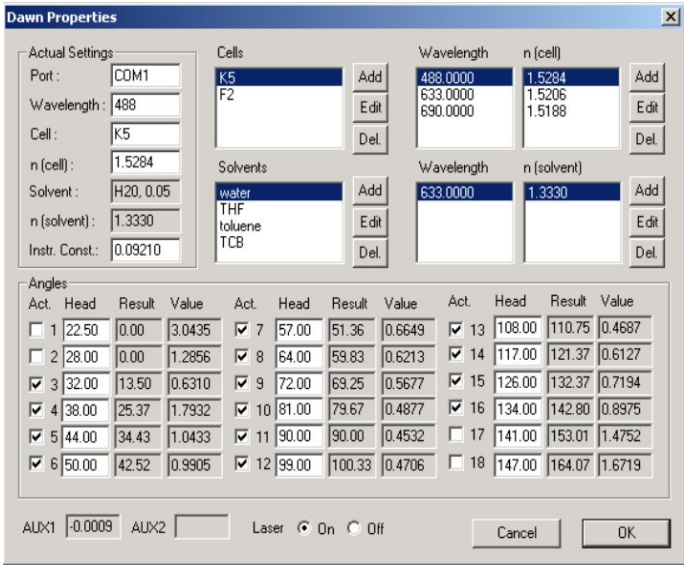


Figure 58 Wyatt DAWN settings

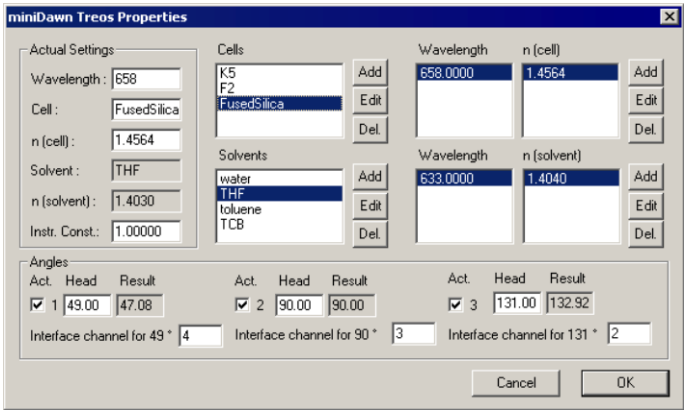


Figure 59 Wyatt miniDAWN TREOS settings

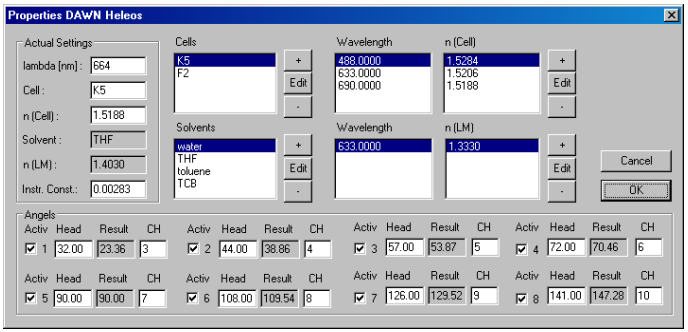
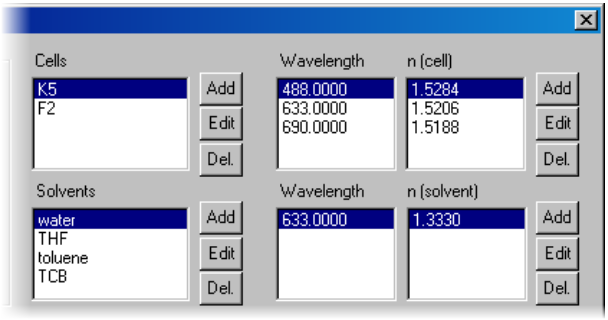


Figure 60 Wyatt DAWN HELEOS settings

Port:	COM port (chosen during Login) where the light scattering device is connected to; for TREOS: always select TREOS (chosen during **Login**) for this type of detector
Wavelength:	laser wavelength in nm, see LS instrument user manual
Cell:	cell type, see LS instrument user manual (most common K5 and F2)
n (cell):	refractive index of glass cell, depends on cell type and laser wavelength, see LS instrument user manual or enter the value displayed in the n(cell) section
Solvent:	solvent defined in the currently used WinGPC Software method, can only be changed in the instrument layout view
n (solvent):	refractive index of the solvent in the currently used method, can only be changed in the instrument layout view
Instr. const.:	determine using the WinGPC Software Detector Setup

Due to the cell design, the scattering angles of the DAWN instruments are not equal to the detector angles but depend on the glass type of the cell and the solvent used. However, the scattering angles can be calculated taken into account the refractive indices of the cell glass and the solvent. WinGPC Software calculates and uses the correct scattering angles as described in the light scattering detector manual.

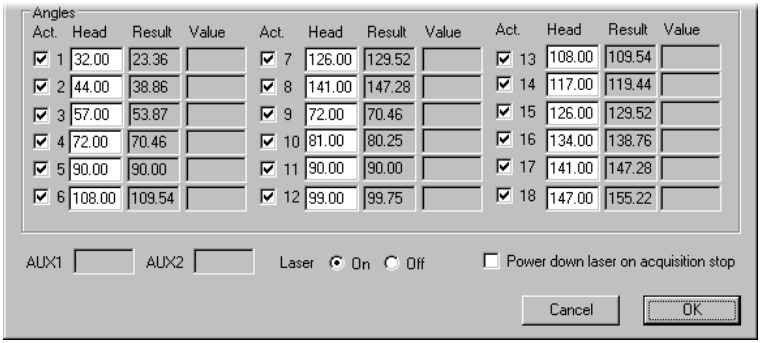
Cells and Solvents section:



Cells: This section shows the refractive index (depending on the laser wavelength) of the most common DAWN cell types K2 and F5. If the cell is marked, the refractive index can be read for the laser wavelengths 488, 633, and 690 nm. This value has to be entered manually in the n (cell) field. Cell types, wavelengths and the corresponding cell refractive indices can be added using the button **Add. Edit** allows to edit the selected entry while Del. deletes the selected entry.

Solvents: The solvent section shows the refractive indices of the most common solvents. This is for documentation and to check inside the DAWN Properties dialog if the parameters are set correctly. The solvent and the refractive index of the solvent can not be changed inside the dialog but have to be modified in the instrument layout view of the method window. Solvents and the corresponding refractive indices can be added using the button **Add**. **Edit** allows to edit the selected entry while Del. deletes the selected entry.

Angles section:



Act.: Defines if an angle is by default used for fitting the values in the Zimm/Debye/Berry plot. If the tick mark is set the value of this angle is used for the extrapolation, if not the value is displayed in red in Zimm/Debye/Berry plot and not used.
Please note that the use of an angle can be changed any time in the Zimm/Debye/Berry plot or in the light scattering detector properties dialog. The raw data signals of all angles will be recorded any time independent of the selection in the dialog.

Head: In the head section the angles of the detector are displayed. They can be edited if necessary and filled with the values given in the light scattering instrument user manual.

Result: Depending on the refractive index of the cell and solvent used the scattering angle differs from the head angle, where the detector is placed. The result fields shows the scattering angle calculated from the head angle.

Value: Actual value in Volt measured for this detector. These fields are empty if the light scattering device is not on line.

AUX1/AUX2 Shows the voltage values for the connected detectors (e.g., RI and/or UV). If PSS data acquisition hardware is used this fields can be empty.

Laser on/off: The laser of the light scattering device is automatically turned on (off) when WinGPC Software is launched (closed). This radio button can be used to turn the laser on/off during runtime, if the light scattering device does not have an external laser control button.

NOTE

Data acquisition via HELEOS requires additional configurations, for more information see the respective folder in your WinGPC Software Utilities directory of your WinGPC Software installation (only available if you chose “HELEOS” support during WinGPC Software installation).

NOTE For better usability the user defined settings of this dialog are saved in the dawn.ini file located in the wingpc_8#1 folder. If this ini-file is not available data transmission from this device is not possible and WinGPC Software will not go on line. A template file (treos.ini) which has to renamed to “dawn.ini” prior to WinGPC Software launch is available.

PDI-1 and PDI -2

LS type:	**PDI-1: 1 angle - LALLS (15°) or RALLS (90°)**
PDI-2: 2 angles - TALLS	analog via 1 (1 angle) or 2 (2 angle) A/D converter per signal (either Agilent UIB or legacy PSS Interface)
Data acquisition:	activate option **Viscosity/Light Scattering**

Parameters PDI:

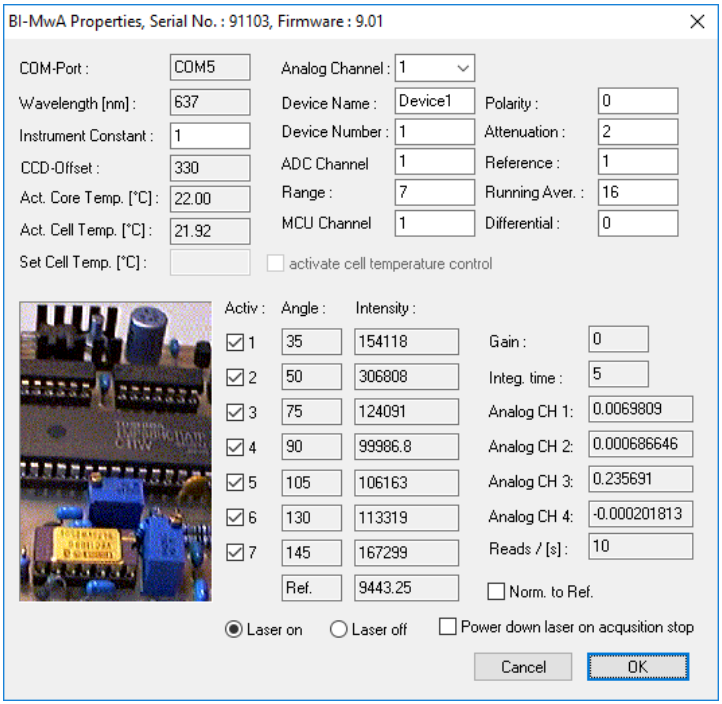
PDI-1 only:	Scattering angle:	see LS instrument user manual (typical 15° or 90°), for 15° select LALLS, for 90° select RALLS data analysis option
PDI-2 only:	Interface channel:	enter the PSS Interface channel numbers 15°/90° where the A/D converters of the 15° and 90° angle are connected to
	Wavelength:	laser wavelength in nm, see LS instrument user manual
	Instrument constant:	determine using the WinGPC Software guided detector setup
	G0:	see LS instrument user manual, if not available use the default value 1

NOTE This detector type can also be used to incorporate LALLS or RALLS instruments that are not listed in the resource tree.

PSS SLD 7x00/BIC BI-MwA

LS type:	**MALLS**
Data acquisition:	digital, adds 4 analog channels (Bi-MwA Analog 01 to 04) which can be selected with a right mouse click in the method CH No. field of a concentration detector
Login options:	activate option **Viscosity/Light Scattering** and **PSS SLD7x00**, COM port is automatically assigned if the light scattering device drivers are installed correctly

Parameters PSS SLD7x00 / BIC BI-MwA:



COM-Port:	COM port automatically assigned during driver installation and automatically chosen during login
Wavelength:	laser wavelength in nm, automatically read from the instrument configuration
Instrument constant:	determine using the WinGPC Software guided detector setup
CCD-Offset:	CCD and instruments dependent parameter automatically read from te instrument configuration
Act. Core Temp.:	Light scattering device processor temperature in ℃
Act. Cell Temp.:	Cell temperature in ℃, only available if the SLD7x00/BI-MwA has the optional temperature control
Set Cell Temp.:	After the check box activate cell temperature control is activated the desired cell temperature can be entered here in ℃. The check box can only be activated if the SLD7x00/BI-MwA has the optional temperature control and if data acquisition is not active.
activate cell temp control	This option can only be activated if the BI-MwA has the optional temperature control functionality. This is not possible while data acquisition is active.
Analog Channel:	The 4 (or optional 19) analog channels of the SLD 7000/BI-MwA can be programmed according to the description in the LS instrument user manual. This is not necessary if PSS data acquisition hardware is used for the acquisition of the concentration detector/viscometer signals. For most GPC applications the default settings are recommended.
Active:	Defines if an angle is by default used for fitting the values in the Zimm/Debye/Berry plot. If the tick mark is set the value of this angle is used for the extrapolation, if not the value is displayed in red in Zimm/Debye/Berry plot and not used. Please note that the use of an angle can be changed any time in the Zimm/Debye/Berry plot or in the light scattering detector properties dialog. The raw data signals of all angles will be recorded any time independent of the selection in the dialog.
Angle:	Detector and scattering angles. **Ref.** is for the intensity of the laser reference beam that is also measured.
Intensity:	Actual value measured for this angle. These fields are empty if the light scattering device is not on line. Dependent on the setting of the **Norm.to Ref.** check box, the values divided by the reference beam intensity (box checked) will be displayed or the original CCD units (box unchecked). This setting does not influence the recorded data. All raw data and the reference beam intensity are always recorded as needed independent from the settings in the properties dialog.
Norm. to Ref.:	If the check box is activated then the intensities divided by the reference beam intensity are shown in the **Intensity** fields. Otherwise the raw CCD signals are displayed. This setting does not influence the recorded data. All raw data and the reference beam intensity are always recorded as needed independent from the settings in the properties dialog.

Analog CH 1 - 4:	Shows the voltage values for the attached connected detectors (e.g., RI, UV and/or viscometers). If PSS data acquisition hardware is used this fields can be empty.
Gain/Integ. time:	Automatic WinGPC Software settings for optimum detection range.
Reads:	Number of data packages received per second. Automatic controlled by WinGPC Software. Optimum value is 7 reads per second.
Laser On / Off:	manual laser source control; by default laser on/off by WinGPC Software launch
Power down laser on acquisition stop	option to switch off laser beam after data capture has been closed automatically by WinGPC Software

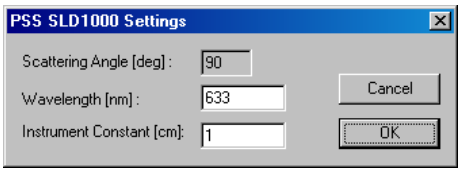
NOTE For better usability the user defined settings of this dialog are saved in the gpcmwa.ini file located in the wingpc_8#1 folder. If this ini-file is not available data transmission is not possible and WinGPC Software will not go on line.

NOTE The **Act. Cell temp.** field will show 1 if the cell temperature control option is not available and/or if the **TempTable** section of the gpcmwa.ini file is missing.

PSS SLD1000

LS type:	**RALLS (90°)**
Data acquisition:	analog via 1 A/D converter per signal (either Agilent UIB or legacy PSS Interface), or digital via ChromPilot
Login options:	activate option **Viscosity/Light Scattering**

Parameters PSS SLD1000

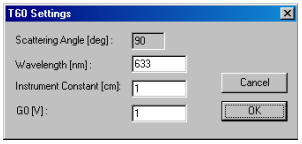


Scattering angle:	90°
Wavelength [nm]:	Laser wavelenth in nm, will be transferred automatically if controlled via ChromPilot
Instrument constant:	determine using the WinGPC Software Detector Setup

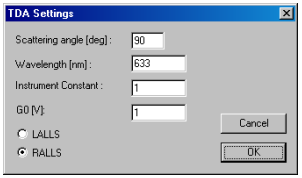
Malvern/Viscotek T60, TDA and TDA-2

LS type:	**T60: 1 angle RALLS (90°)** **TDA: 2 angles, 7° (LALLS) and/or 90° (RALLS)**
Data acquisition:	analog via 1 (1 angle) or 2 (2 angle) A/D converter per signal (either Agilent UIB or legacy PSS Interface)
Login options:	activate option **Viscosity/Light Scattering**

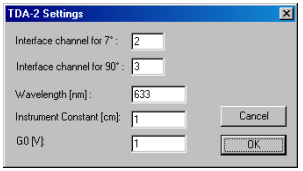
Parameters T60/TDA



T60



TDA



TDA-2

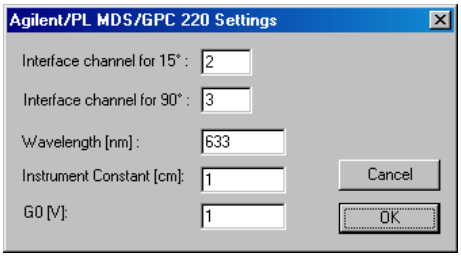
Scattering angle:	see LS instrument user manual (typical 7° or 90° for TDA and 90° for T60); TDA only: for 7° select LALLS, for 90° select RALLS
Wavelength [nm]:	laser wavelength in nm, see LS instrument user manual
Instrument constant:	determine using the WinGPC Software Detector Setup
G0 [V]:	see LS instrument user manual, if not available use the default value 1

Agilent/PL MDS/GPC 220

LS type:	**2 angle TALLS**
Data acquisition:	analog via 2 A/D converter per signal (either Agilent UIB or legacy PSS Interface), or digital
Login options:	activate option **Viscosity/Light Scattering**
LS type:	**DLS**
Data acquisition:	digital
Login options:	activate option **Viscosity/Light Scattering**

⇒ will record R_h directly, signal will be displayed with R_h axes.

Parameters Agilent/PL MDS/GPC 220

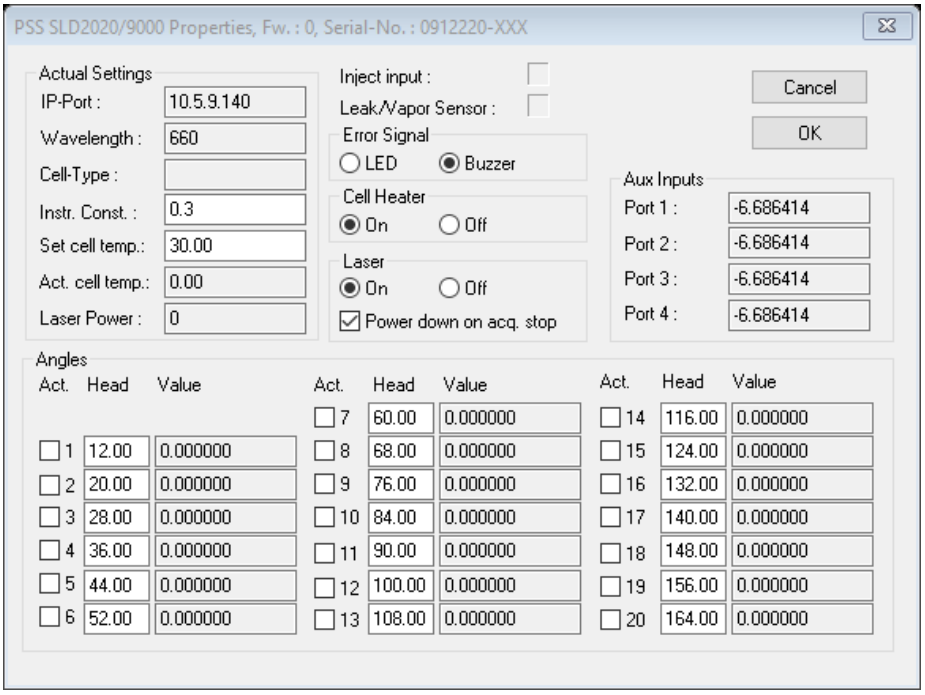


Interface channel	enter the PSS Interface channel numbers where the A/D converters of the 15° and 90° angle are connected to
Wavelength [nm]:	laser wavelength in nm, see LS instrument user manual
Instrument constant:	determine using the WinGPC Software Detector Setup
G0 [V]:	see LS instrument user manual, if not available use the default value 1

Agilent 1260 Infinity II MALS Detector

LS type:	**MALLS**
Data acquisition:	digital via LAN default IP: 192.168.254.140
Login options:	activate option **Viscosity/Light Scattering** and **Agilent 1260 MALS**

Parameters Agilent 1260 MALS



IP port	Needs to be entered in the login screen.
Wavelength	laser wavelength in nm, automatically read from the instrument configuration
Cell-Type:	automatically read from the instrument configuration
Instrument Constant	Determined during the WinGPC Software Detector Setup
Set cell temp:	Cell temperature can be set between room temperature + 10°C and 60°C, no active cooling.
Act. cell temp	Shows acutal cell temperature

Light Scattering Module

Laser Power	Between 10-100mW, can be adjusted in gpcsld2020.ini file
Inject input	Inject trigger
Leak/Vapor Sensor	Leak/Vapor Sensor ready
Error Signal	Set Error Signal type
Cell Heater	Turn on/off cell heater
Laser	The laser of the light scattering device is automatically turned on (off) when WinGPC Software is launched (closed). This radio button can be used to turn the laser on/off during runtime, if the light scattering device does not have an external laser control button
Power down on act. stop	Laser will turn off after measurements are finished.
Angles	Detection and scattering angles
Act.	Scattering angles to be shown in evaluation can be selected
Head	Scattering angle
Value	Actual value in Volt measured for this detector. These fields show 0.00000 if the light scattering device is not on line

NOTE

For better usability the user defined settings of this dialog are saved in the mals.ini file located in the wingpc_8#1 folder. If this ini-file is not available data transmission is not possible and WinGPC Software will not go on line.

Basics of Light Scattering Theory

Specific hints for the conversion of voltages into absolute scattering intensities can be found in the user manual of the light scattering instrument, as the calculations depend on the instrument type. Besides the light scattering intensity, which can be obtained from the connected light scattering instrument, the concentration in each slice of the chromatogram is required. This usually is measured by a differential refractive index detector. Details describing the calculation of concentrations can be found in chapter "Determination of Slice Concentration" on page 48.

WinGPC Software uses to the following equations for the different LS types to obtain molar masses:

Low Angle Light Scattering (LALLS):

$$M = \frac{R_\theta}{c(K - 2 \cdot A_2 \cdot R_\theta)} \qquad R_\theta = \frac{G_\theta}{G_0} \cdot \frac{D}{\sigma'} \qquad K = \frac{2 \cdot \pi^2 \cdot n^2}{\lambda^4 \cdot N_L} \left(\frac{dn}{dc}\right)^2 (1 + \cos^2($$

90° Light Scattering (RALLS):

$$M = \frac{R_\theta}{c(K - 2 \cdot A_2 \cdot R_\theta)} \qquad R_\theta = \frac{G_\theta}{G_0} \cdot \frac{D}{G_G} \cdot \sin(\theta)$$

$$K_{unpolarisiert} = \frac{2 \cdot \pi^2 \cdot n^2}{\lambda^4 \cdot N_L} \left(\frac{dn}{dc}\right)^2$$

$$K_{polarisiert} = \frac{4 \cdot \pi^2 \cdot n^2}{\lambda^4 \cdot N_L} \left(\frac{dn}{dc}\right)^2$$

Multi Angle Light Scattering (MALLS)

$$M = \frac{R_\theta}{c(K - 2 \cdot A_2 \cdot R_\theta)} \qquad R_\theta = G_\theta \cdot G_0 \cdot D$$

$$K_{polarisiert} = \frac{4 \cdot \pi^2 \cdot n^2}{\lambda^4 \cdot N_L} \left(\frac{dn}{dc} \right)^2$$

where	R(θ)	while θ ->0 leads to R(0)
	N(θ)	normalization coefficient
	D	instrument constant (related to Rayleigh ratio toluene)
	M	molar mass
	c	concentration
	K	optical constant
	A_2	second virial coefficient
	G(θ)	scattering intensity
	θ	scattering angle
	n	refractive index solvent
	λ	laser wavelength
	N_L	Avogadro's number
	dn/dc	refractive index increment

Performing Light Scattering Measurements

The next sections describe the necessary steps for light scattering data acquisition and processing. Only new features and parameters will be discussed to keep the Agilent WinGPC Software User Guide compact and to avoid repetitions. The basic features of the WinGPC Software are described in their respective chapters. Please refer to them if some notions and features are not explained here.

Method Window

Edit all items in the resource tree. Most important are solvent (refractive index), the concentration detector(s) and the light scattering detector. Enter as many values as known. If the slice concentration should be determined using the concentration determination methods **Fact*dn/dc** and **Fact.*Conc.**, the factor for the concentration detector needs to be entered.

If the factor is not known, it can be determined any time using the Detector Setup (see chapter "Detector Setup" on page 183). The instrument constant for the light scattering detector can also be determined using this setup.

Add the resources in the instrument layout view. Drag&drop the concentration detectors or define the number of concentration detectors in the Method Window using **Definition > Number of detectors** and select them from the pop-up list after a left mouse click on the corresponding item. Light scattering devices need to be added with drag&drop as the last detector in the instrument layout view.

NOTE

- Light scattering detectors need to be removed (drag&drop of **none** from the resource tree on the icon) before changing the number of concentration detectors or before adding/removing a viscometer.
- If no PSS data acquisition hardware is used: the analog channels of the light scattering device are only accessible if the light scattering device is part of the instrument layout view.

NOTE

If the LS instrument does not have a way to measure absolute calibration constants (as in the KMX6), then use a well known standard and calculate the instrument constant by rationing the known molar mass of the sample with the calculated apparent molar mass. Please make sure that the instrument constant is set to 1 (the default value) when doing this calculation. Alternatively use the **Method > Detector Setup** that is available for finished runs only.

Raw Data Window

Enter the necessary sample dependent light scattering parameters:

- concentration
- injection volume
- and dn/dc

in the **Editor > Samples** dialog.

The refractive index increment dn/dc will also be used for the calculation of the slice concentration, if the concentration determination method **fact.*dn/dc** is selected. Injection volume and concentration are needed for the calculation of the slice concentration if the methods **injected mass**, **Fact.*Conc.** and **Conc.*dn/dc** should be used. Details describing the calculation of concentrations can be found in chapter "Determination of Slice Concentration" on page 48.

If a UV detector is used as concentration detector, the extinction coefficient dA/dc can be entered as well. In order to activate the calculation with the dA/dc value the signal type needs to be set to **UV** as well (see chapter "Instrument Layout View" on page 170).

Evaluation of Light Scattering Measurements

The following data processing steps are necessary to calculate results from runs with a light scattering detector.

Raw Data Window

In the **Raw Data** Window you must set the baseline limits and perform the optional correction with the internal standard. If you are using the concentration determination methods **injected mass**, **Fact.*Conc.** and/or **Conc.*dn/dc** you must set the baseline in a way that the area under the RI Signal inside the baseline limits reflects the polymer quantity. I.e. the complete polymer peak must lie inside the boundaries, but signals like system, salt or solvent peaks are excluded.

Elugram Window

Here the integration limits need to be defined just as described for conventional data processing (see chapter "Elugram Window" on page 224 for more details).

Light Scattering Window

The light scattering window is the central window for selecting specific calculation options and to verify primary information. If the processing parameters are fixed by the method this window is only needed for trouble shooting.

Mass Distribution Window

The molar mass distribution and the molar mass averages measured with light scattering are immediately displayed when the X-axis context menu item **Calib. Light scattering** is chosen. This setting can be changed anytime and also saved with the method.

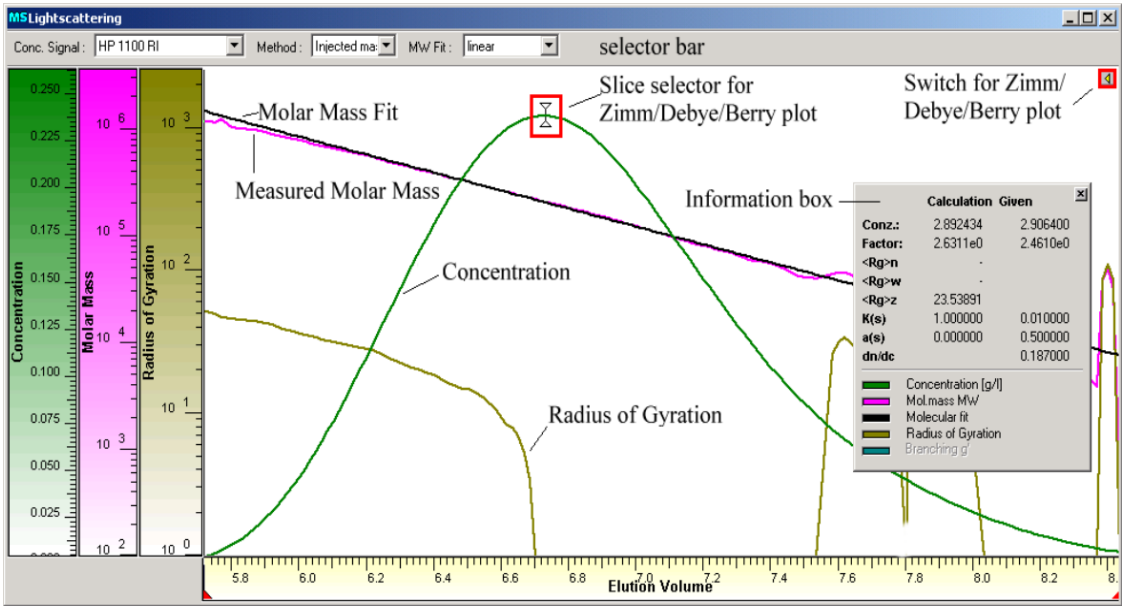
NOTE

Besides the currently processed sample the status bar shows also the currently used calibration method: If “Lightscattering” is shown “Calib. Lightscattering” is active and the light scattering data evaluation is used. If the name of the calibration curve is shown “Calib. Standard” is used and a conventional GPC data analysis is performed.

Light Scattering Window

The light scattering window is the central window for selecting specific calculation options and to verify primary information.

Window Description



The window shows by default the curves of the measured concentration and the measured molar mass. Furthermore, the fitted molar mass curve as well as the radius of gyration and the branching g curve (MALLS only) can be switched on/off by clicking on the curve name in the information window.

Besides the curves the information window shows the two columns **Calculation** and **Given**.

Table 57 Window

Curves	Calculation	Given
Conc.	Calculated overall concentration within the integration limits. The settings for concentration signal and method are used.	Concentration entered in the sample editor. If the concentration for more than one component is entered, **Options > Component** from the light scattering window menu defines what concentration is displayed.
Factor	Calculated factor for the concentration detector defined in the selector bar. The factor is needed if the method **fact.*dn/dc** or **fact.*conc.** should be used.	Factor of the selected concentration detector. Entered in the method window instrument layout view.
<Rg>n, <Rg>w, <Rg>z (MALLS only)	Radius of gyration averages. These results are only available with MALLS detectors. Reliable results are reported, otherwise "-" is shown.	
K(s), a(s)	Calculated parameters for the log M vs. log Rg relation.	Parameters for linear counterpart. Entered in the **Options > Light scattering > Branching > Coef. branching** dialog of the light scattering window menu
dn/dc	Calculated dn/dc. This is only available if **fact.* conc.** is used as concentration determination method. If a calculated dn/dc is displayed this value is used for light scattering data evaluation.	Sample dn/dc entered in the sample editor (Raw data window menu **Editor > Samples**). This dn/dc is used for light scattering data evaluation unless there is no calculated dn/dc.

Light scattering relevant evaluation parameters can be set in the selector bar. The selector **Conc. signal** allows to define which detector should be used for the determination of the slice concentration. The **Method** selector defines the concentration determination method. The third selector in the bar allows to define the fit function used to fit the measured molar masses. This is needed since at the peak onsets the uncertainty for data points increases. The fit (as well as the chosen weight function) allows to rely on points measured with a higher precision.

Conc. Signal: Here the concentration detector can be chosen. Available are all concentration detectors defined in the method.

Method: 4 different methods are available:
Injected mass, Fact.*dn/dc, Fact.*Conc. and Conc.*dn/dc (and Fact.*dA/dc and Conc*dA/dc for UV respectively).
See chapter "Determination of Slice Concentration" on page 48 for details.

Fit: Here the fit function for fitting the measured molar mass is chosen. Please try first the linear function. If the data are fitted badly change first the settings in the menu **Options > Light scattering > Weight** to get a better fit. Recommended setting is ci*ci, this is the best choice for most samples.

NOTE

The mass recovery of samples can be easily determined if a calibrated concentration detector is used. The overall sample concentration can be measured using the method **Fact.*dn/dc**. The concentration result shown in the information window should be comparable to the concentration entered in the sample editor. Therefore, the mass recovery [%] is the ratio of the calculated and the given concentration multiplied with 100. If the recovery is less than 100% this might be a hint for e.g.,

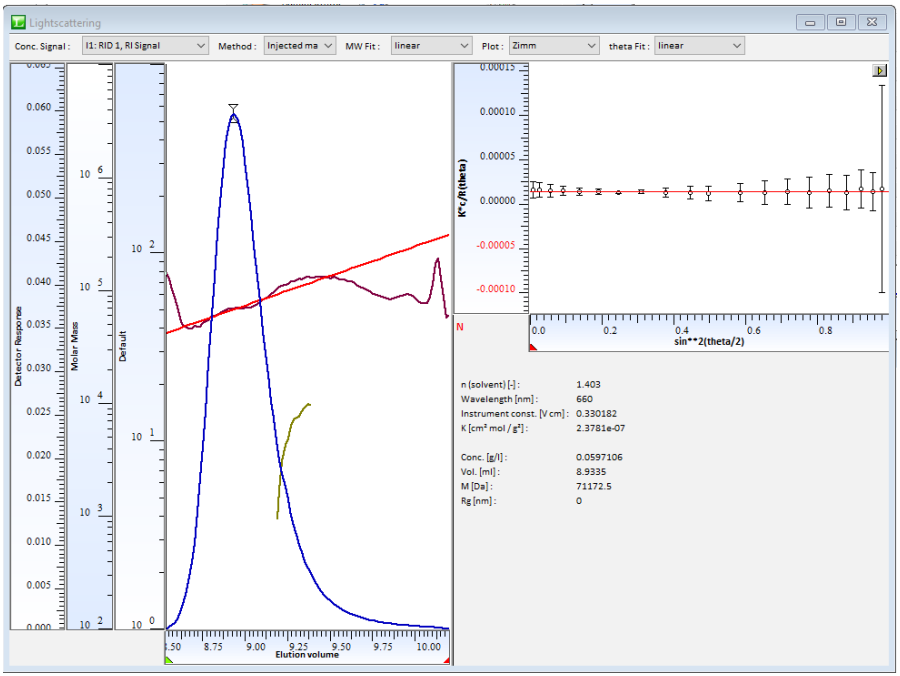
- a wrong concentration determined with the balance
- sample interaction with column material
- micro gel content of the sample and/or therefore sample loss by filter.

NOTE

The WinGPC Software ReportDesigner allows the automatic calculation of the mass recovery including an automatic warning on the report if it is too low.

For multi angle light scattering instruments the light scattering window can be expanded to display the slice results of the Zimm, Debye or Berry plot. The switch in the top right corner divides the light scattering window in two sections:

- the left part shows the part described above,
- the right part shows the angular dependence plot for the elution volume selected with the slice selector. Additionally, status information, slice results and an expanded selector bar is shown.



The expanded selector bar allows to define the plot type to be used for evaluation as well as the fit function to be used to fit the angular dependence.

Plot: Select between Zimm, Debye and Berry plot to evaluate your data.

Fit: This fit function is used to fit the data in the Zimm, Debye or Berry plot. Please choose the fit function carefully. For most samples linear is the best option.

NOTE

All these settings can be chosen inject dependent meaning that the option will be performed according to the chosen option displayed in the status bar (Actual injects, Actual inject + following, Actual inject and selectable, All injects).

Single angles can be disabled for the Zimm/Debye/Berry plot data evaluation. This is necessary for Wyatt DAWN EOS instruments when the first angles are not accessible, e.g., due to the solvent used. Also, for signals with strong baseline fluctuations it might be better to remove the angle from the analysis.

NOTE

Angles are considered even if the curve for this angle is not shown in the Raw data window. To disregard the angle in the analysis a left mouse click on the angle in the Zimm/Debye/Berry plot is necessary.

An angle is disabled if it is shown in red. It can be added to the analysis again by another left mouse click on its data point.

NOTE

Angles can also be enabled/disabled in the settings dialog that is opened by a right mouse click on the light scattering resource in the method window's resource tree. This setting can be saved with the method. Disabling angels is only important for the data analysis. The raw data will always be recorded, even if the angle is disabled.

X-Axis Context Menu

These functions are accessible by a right mouse click on the X-axis scale.

Table 58 X-axis context menu

Function	Description
Elution Volume:	The elution volume will be displayed on the X-axis. This setting is useful for the selection of the fit function. The accessible elution volume range depends on the setting of the integration limits in the elugram subtracted by the part excluded through the setting of the calculation limits (**Options > Light scattering**, default: 1%).
Standard Calibration:	The molar masses of the currently active standard calibration will be displayed on the X-axis. This setting can be useful if conventional results and light scattering results should be compared, e.g., in the overlay mode.
Molar Mass LS:	The molecular weights calculated by light scattering will be displayed on the X-axis. The fit and weight options (**Options > Light scattering**) are taken into account. This setting is useful e.g., for multi angle light scattering measurements where the radius of gyration or the branching coefficient g should be plotted against the molecular weight. Also, the coefficients K(s) and a(s) can be determined in this mode.
Triple Detection:	Triple detection is only possible by simultaneous use of a viscometer and a light scattering instrument, so this selection is only accessible for runs containing viscometer and light scattering data. The molecular weight displayed on the X-axis will be calculated by triple detection.
Set standard scale:	Allows the manual setting of the displayed X-axis range of the light scattering window and sets the default standard scale.
Standard scaling:	Restores the scale of the last manual scaling (e.g., after editing the scaling by the arrow keys or the scroll bar of the X-axis.). A tick mark in the context menu indicates if the standard scale is in use.
Properties:	This context menu is available on each axis in the WinGPC Software. It allows setting axes properties individually. For example, text attributes (font, size, color etc) and background properties of the axis can be defined. It is also possible to switch from elution volume to elution time representation for X-axis properties. The axis properties will be saved for each instrument individually.

Y-Axis Context Menu

These functions are available from the Y-axis context menu on all axes, if you click on the Y-axis scale with the right mouse button. A popup menu appears in which the following functions can be selected:

Table 59 Y-axis context menu

Function	Description
Norm.:	Toggles between normalized and manually scaled view.
Set standard scale:	Allows the manual setting of the displayed Y-range of the chromatogram and sets the default standard scale.
Standard scaling:	Use preset Y-axis scale as defined in **Set standard scale**. If the standard scale is in use, this command shows a tick mark.
Tip: The method file also saves the scaling settings of the various axes. These will be loaded when the method file is retrieved from the file system.	
Properties:	This context menu is available on each axis in the WinGPC Software. It allows setting axes properties individually. For example, axis labels, text attributes (font, size, color etc) and background properties of the axis can be defined.
Tip: It can be very useful to assign the Y-axis caption in the same color as the detector signal and give the axis a descriptive name (e.g., measured LS intensity).	

File Menu

Table 60 File menu

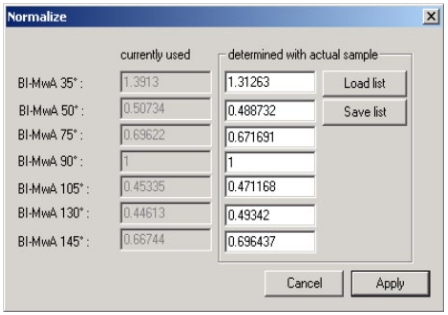
Function	Description
Printer Setup:	Allows definition of parameters for the default printer defined in the Windows Control Panel. Landscape format prints the graphics on a full page. Portrait format prints the graphics, full method documentation and results by default. The printed information in the portrait layout can be selected for automated runs in the **Definition > Automation Settings** dialog box. The exact information of the portrait format printout depends on which window will be printed. For color printers you can select color or monochrome printing depending on the printer driver options. The color of the curves in monochrome printouts are mapped automatically to a line style to avoid unreadable black and white prints. The correlation between curve color and line style in monochrome-print is listed in "Curve Colors and Line Styles in Monochrome Printing" on page 534.
Print:	Prints the current contents of the light scattering window to the default printer. The graphics are always printed in WYSIWYG mode, i.e. the current window display will be printed identically as shown.
Page Preview:	Shows a print preview in a separate print-preview window, which can be sized and moved. At the same time this command copies the preview contents to the Windows clipboard. When printing in portrait format, the graphics and the text for the measurements will shown, while landscape format will print only the graphics.
ASCII Save as:	Saves the currently in the Light scattering window displayed data as ASCII file for use in other applications or for the transfer data to other computer platforms.
Edit Comment:	Allows to enter a light scattering data related text (comments, hints for data treatment, data processing details, observations, etc.). Up to 1024 characters can be entered for each injection (sample) separately. Alternatively, the  icon in the status bar can be used to open the comment dialog box. This icon is gray if no comment has been entered for this sample, if text has been entered it is highlighted in green.

Options Menu

Table 61 Options menu

Function	Description
Editor slice data:	Currently not used
Component:	Assigns the sample editor's component concentration to the peak (see also **Raw Data Window** Menu **Editor > Samples**). The concentration of the selected component appears in the column **given** of the information window.
Sub menu Light scattering:	
Calculation limits:	Filters less significant evaluation points so that only areas in which concentration and light scattering detector show significant data are used. A defined percentage of the concentration detector peak maximum (default: 1 %) is then discarded.
Fit:	The measured molar mass should be fitted linear or by a polynomial function of 3rd, 5th or 7th order. This fitted light scattering molar mass curve will then be used for further calculations. The original light scattering data are used if **Original Data** is chosen. For most samples the fit function **linear** is the best choice. The fit should be selected so that the curve **Molecular fit** matches most of the **Mol. mass MW** curve. The quality of the fit can be improved selecting the proper weight function (see below). The fit can also be chosen using the selector bar in the light scattering window.
Weight...	Allows to assign different weights to the elution volume - molar mass pairs. The chosen weight function influences the fit for the measured molar masses shown in the light scattering window as Mol. mass MW curve. The weight has no influence if **Original Data** is selected in the MW fit selector. The weight function can be selected for each sample separately, if necessary. In general, the weight option ci*ci is the recommended one since this leads to the best fit for most samples.
Off:	No special weight will be used, all pairs will be treated equal.
sqrt(ci)	Each point will be assigned a weight equal to the square root of the concentration signal. Because the light scattering signal loses signal intensity faster than the concentration signal on the low molecular weight end, points on the low molecular weight end might have a high weight even if only a noisy light scattering signal is present.
ci:	Each point will be assigned a weight equal to its concentration. Because the light scattering signal at the lower molecular end loses signal power faster than the concentration signal, points on the high molecular weight side might have a high weight even if only a noisy concentration signal is present. In comparison to the weighting method sqrt(ci) however, the center of the distribution will be weighted more heavily;
ci*ci:	Each point will be assigned a weight equal to the squared concentration. The squared weight gives a high weight to the center of the distribution.

Table 61 Options menu

Function	Description
Coeff. branching:	Multi angle laser light scattering allows to measure the radius of gyration. For branched samples this enables the investigation of the amount of branching. The menu item **Coef. branching** opens a window where the coefficients K(s) and a(s) can be entered. According to Rg = K(s) * M a(s) the radius of gyration for the linear counterpart can be calculated. The resulting values can then be used to calculate and show the "branching g" curve that can be turned on/off using the menu item **branching g** or the curve in the information window.
Normalize:	Only applicable for multi angle light scattering. Normalizing is necessary to ensure that at all angles the same scattering intensity produces the same measured voltage value. Deviations can occur e.g., according to different electronic parts used to build the detectors at the angles. More details to this subject can be found in the light scattering device manual. Please verify the normalization factors regularly. Agilent recommends normalizing using an isotropic scatterer, e.g a narrow distributed molecular weight standard with molecular weight about 100 000 Da (most organic solvents: polystyrene, aqueous solvents: pullulan). The normalization strongly effects the determination of the radius of gyration, the molar masses are also effected but less significant.  The look of the **Normalize** Window depends on the light scattering detector used. For every angle a separate factor is needed, so the number of entries in the window is related to the number of angles acquired. The window shows the currently used normalization coefficients as well as the values determined with the actual sample. The newly determined normalization coefficients can be saved in a file using **Save list**. Existing lists can be loaded in this dialog with **Load list**. They can be loaded either to finished runs or to an instrument (to be saved with the method). **Apply** forces WinGPC Software to use the coefficients displayed in the **determined with actual sample** section for all samples in the currently open login/sample sequence. With **Cancel** the dialog can be left, in this case the currently used coefficients will be kept. The normalize procedure is also part of the detector setup located in the method window.

NOTE

Save the normalization coefficients as well as other light scattering parameters with the method. This allows to skip the light scattering window during evaluation and saves mouse clicks.

12 Triple Detection

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter "WinGPC Compliance " on page 507 of this user guide.

While the conventional GPC with viscosity detection is based on the validity of universal calibration, light scattering detection with only one angle has the disadvantage, that for molecules with high molecular weight, especially at a high scattering angles, the angular dependence of the scattering function, P(Θ) is not neglectable. Thus, the molecular weights by light scattering will underestimate the true molecular weights. Triple detection offers a possibility to work with an iterative algorithm using light scattering and viscosity signals to calculate molecular weights irrespective of elution mode but under consideration of the angular dependence of the scattering function.

To perform a triple detection the algorithm proceeds as follows:

1. In a first step a molecular weight (M_1) will be calculated from the light scattering signals without consideration of the angular dependence using the equations of the light scattering modules (see chapter "Light Scattering Window" on page 401). Generally, this will underestimate the molecular weight.
2. From the calculated molecular weight and the experimental intrinsic viscosity [η] the radius of gyration of the molecule can be deduced by the Flory-Fox equation.
3. Using the estimated radius of gyration the scattering function at the given scattering angle can be calculated based on the assumption of a Gaussian coil.
4. Now a new molecular weight can be calculated from the intensity of the light scattering instrument and the estimated scattering function.

The steps 2-4 will be iterated until you receive stable values for radius of gyration and molecular weight.

Performing Triple Detection Analyzes

Data acquisition for triple detection does not differ from data recording with light scattering and. viscometer detector. The options and parameters are explained in chapters “The Viscosity Module” on page 362, “Viscosity Window” on page 372, “Light Scattering Module” on page 382 and “Light Scattering Module” on page 382 respectively.

Data Evaluation with Triple Detection

As the measurement of the triple detection does not differ from ordinary light scattering and viscometry runs, the evaluation does not differ substantially either. Thus, select baselines and internal standard in the **Raw Data** Window and set the integration limits in the *elugram*.

Mass Distribution Window

To obtain the molecular weights from triple detection in the Mass Distribution Window, select **Calib. Triple Detection** from the X-axis context menu. The results in the information boxes of the mass distribution window relate now to the molecular weights received by the triple detection.

NOTE Triple detections helps to overcome the limitations of 90° light scattering.

WinGPC Software allows not only to combine one angle light scattering devices with viscometers, but also to use MALLS instruments with viscometers. If the Triple evaluation is selected for MALLS/viscometry data WinGPC Software picks the 90° signal and evaluates this signal according to the triple procedure. This allows to compare the results obtained with two different evaluation approaches.

13 2-Dimensional Chromatography

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter "WinGPC Compliance " on page 507 of this user guide.

2-dimensional chromatography allows the separation of complex samples with highest peak capacity and efficiency by combining two chromatographic separation techniques. Details on theory and application can be found in chapter "2-Dimensional Combination of Separation Techniques" on page 59. The 2D software is an integrated part of the WinGPC Software main program. The look and feel as well as the user interface are identical. So, users already familiar with WinGPC Software will be able to use the 2D software without additional training for this module.

General Setup of the 2D Window

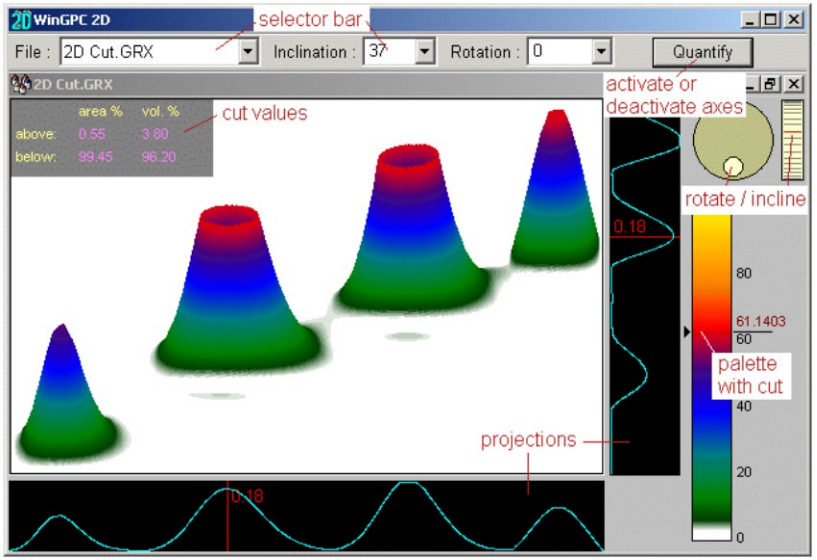
The **WinGPC 2D** window is very similar to other WinGPC Software windows. At the top and the 2D workspace it consists of the selector bar, the 2D workspace holds the 2D sub windows. The WinGPC Software icon bar contains a few icons at the right which are 2D window specific:


brings the 2D window to front/background (toggle)


toggles 2D view between full and partial (black margin around image) window mode


invokes the generation of the first derivative of the displayed 2D image


displays concentration iso-lines and toggles between 2D area and line display



The selector bar is static and will act on the currently active sub window. The file list box will show and allow the selection of all open 2D sub windows. The inclination list box allows to select pre-defined vertical view angles (0° to 90°) of the 3D data array. The rotation list box allows to select pre-defined horizontal view angles (0° to 360°) of the 3D data array. Inclination and rotation of the 2D graphics can also be done interactively by dragging the mouse over the controls on the top right of the 2D sub window.

Each sub window contains the contour plot with the projections and cuts respectively for both dimensions. The properties will determine if a cut or the 2D projection (default) is displayed. When the projection view is deactivated, the mouse position in the 2D graphics section will determine the cut shown (as identified by the red cross hairs). A palette visualizes the signal intensities of the contour plot. A mark can be dragged from 100 % downwards to cut the 2D plot at a certain value. The calculated cut values will be given in the 2D plot as well. For a quantitative analysis of the 2D data axes can be added to the contour plot (Inclination and Rotation = 0) by pressing the **Quantify** button.

Performing 2D Analyses

The next sections describe how to prepare, perform, process and report 2Danalyses using WinGPC Software.

Preparing for a 2D Run

Before you start a 2D measurement you need to adapt the chromatographic conditions of your single methods. As a rule of thumb keep the elution volume of the first dimension as small as possible and optimize (i.e. shorten) the time between two injections for the second dimension.

The quotient of the loop volume of the transfer valve and the time until the next injection into the second dimension defines the flow rate of the first dimension for complete sample transfer to the second dimension:

loop volume / fraction time = flow rate 1st dimension

The quotient of the 1st dimension elution volume and the loop volume corresponds to the total number of transfer injections:

volume 1st dimension / loop volume = total fractions

Once you do have that information, you just have to create one method for each dimension, save it and then start the data acquisition.

This can be done either with the comfortable guided 2D valve setup according to section "Guided 2D Valve Setup" or by a manual programming of the Preparing for a 2D run. Make sure you activated a minimum of 2 instruments as well as 2D GPC in the login screen.

Data Processing

The acquired data from 2D runs can be processed at any time:

1. Set baselines and integration limits as required
2. Activate the Elugram window and select **File > ... to 2D-graphic**; all injections will be sent to the 2D window**.**
3. Press the  icon to bring the 2D window into the foreground.
4. Press **Quantify** to see the axes of your contour plot.
5. You can set the calibration information with the **Properties** function (either in the context menu or under **Options > Properties**).

NOTE

if you use the calibrate function of the axes context menu make sure you already chose a valid calibration curve for this sample.

6. Set a grid (**Options > Grid > Design mode**) or load an already created grid (**Options > Grid > Open...**, see also the chapter "Performing 2D Quantifications" on page 423).

NOTE

You can create/change a grid only in the non calibrated view! (there will be the information **calibrated** in the status bar if a calibration is active).

7. Analyze your 2D Plot by selecting **Analysis > Results**, you will get the position and amount of the selected contour areas; when a calibration is activated you will get the molar mass averages as well.
8. The preview button will create a pre-configured 2D report, you can change this report according to your needs if you have a WinGPC Software ReportDesigner license.
9. Save the 2D results (2D data, calibration and grid information) with  (or **File > Save as...**) as a *.grx file.

Generating and Reporting Results

Quantitative results on the 2D data are generated from the **Analysis > Results** menu. A grid has to be designed or selected from the menu in order to have any results being displayed. A table will show the results for each grid area. In its simplest form the table will show results for peak positions, peak areas and peak volumes (the latter correspond to the relative masses of the different species). If a calibration is selected and activated, MWD results are calculated for each area. The quantitative results of the 2D experiment can be copied to the Windows clipboard by pressing the copy to clipboard button within the result box. The most comfortable export function is the 2D report, which can be chosen with the print preview button (). Users who have a WinGPC Software ReportDesigner license may create a user defined report, otherwise a pre-configured report will be loaded.

Sometimes it might be useful to compare the projections from a 2D experiment with one dimensional measurements. Therefor the function send trace to overlay was created. This function can be activated either over the axes context menu or over **File > send ... trace to overlay**. There are three different options. The first or the second dimension trace or both can be sent at one time. To transfer the cuts position the mouse cursor, use the keyboard command **Alt + F** and choose the wanted menu item.

NOTE

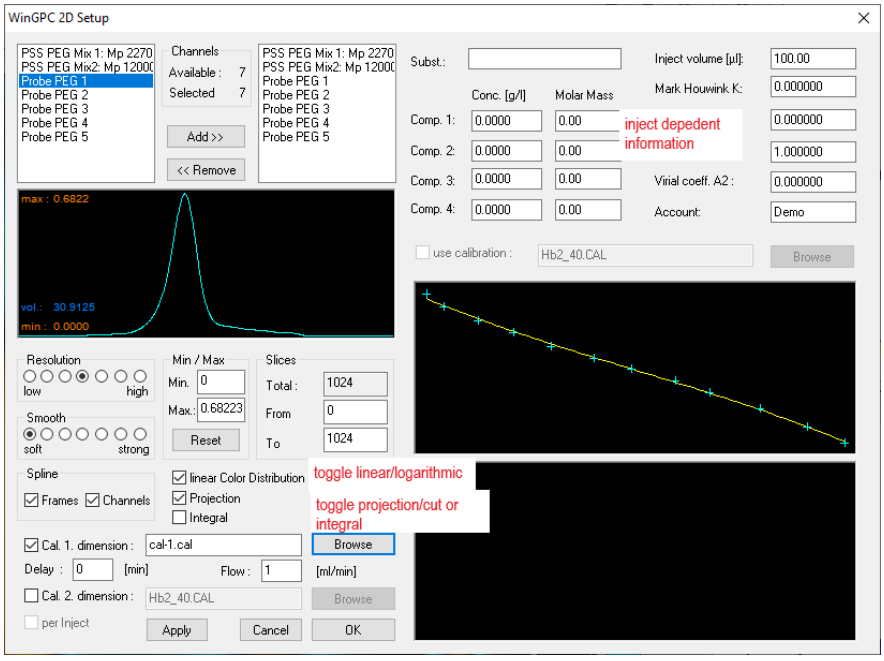
The traces will be sent to the elugram window, the axis context menu item will not be shown when the axis is calibrated. You can recalibrate the elugram later again. If you need the molar mass distribution out of the 2D window, it is possible to send calibrated data via **File > send 2D trace to overlay**. Those data will be transferred with logarithmic x-values (i.e. 5.5 instead of 1·105.5).

Graphical images can also be sent to the clipboard using the **File > Preview** command and selecting the proper item (2D plot, horizontal/vertical cut/projection). If the image of a cut should be copied, please position the mouse cursor first and then make sure that the command is issued from the keyboard (otherwise the mouse position is changed inadvertently) by selecting **Alt + F + P** and pressing **D** (**H**, **V**) for the 2D graphics (horizontal cut, vertical cut).

Another option is the export of ASCII data directly from the 2D window. This option is accessible through **File > ASCII Export**. This will export a txt file of the displayed 2D plot. The decimal separator settings can be changed in the data editor window **Column > Decimal sign**.

Setting 2D Properties

The 2D module contains a variety of different functions for a comprehensive data analysis. The first step is to activate the x- and y-axes by pressing the **Quantify** button in the selector bar.



It is possible to get the **Properties** dialog (see figure) via **Options > Properties** or with the context menu of the 2D plot. The upper part of the properties dialog lists all available transfer injects, their individual sample information and their elugrams. The transfer injection section lists the loaded (left) and currently used (right) samples for 2D evaluation. The lower part of the properties window includes display (projection or integral) as well as calibration options. The resolution, smooth factor, spline, z-range and number of slices are preset with optimal values – there is no need to change these settings during a normal 2D data evaluation (it might be necessary for imported data).

You can toggle between a linear and a logarithmic display of the 2D data. Linear is the default selection. A logarithmic z-scale can become handy when small amounts of byproducts or the influence of baseline noise shall be monitored beside other signals.

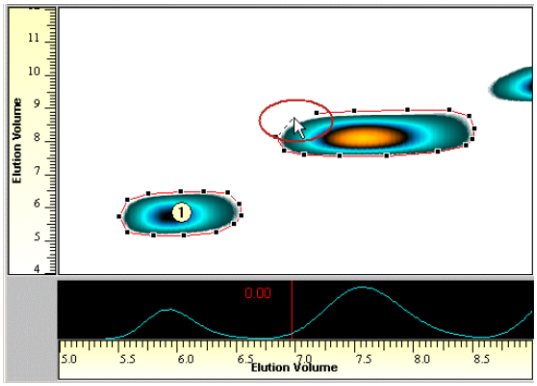
As mentioned above the 2-dimensional plot can be projected onto the x- and y-axes. The **Projection** field determines whether the projection (sum of all slices in the x- or y-direction) or a cut at a specified point of the diagram will be shown. The first as well as the second dimension can be calibrated with any calibration file. Because the first dimension data can have different origins which are not necessarily monitored with the WinGPC Software, the scale of the y-axes should be corrected first. If the fraction collection was started with a delay, this value should be entered into the properties window. The flow rate of the first dimension has to be entered into the field next to the delay. The y-axes will be transformed from a time scale to a volume scale by this procedure. Usually, the second dimension will be a GPC and if a calibration curve was already loaded to the raw data this curve will be shown as the default calibration in the lower right side of the properties window. A different calibration file can be chosen with the **Browse** function.

The 2D chromatography is often used to separate complex samples first according to their chemical heterogeneity and then according to their molecular size. Because the calibration curves for different components will differ from each other, the new *WinGPC 2D* add-on provides an opportunity to specify different calibration curves for the same 2D plot. Therefore, the *per Inject* item should be activated (only possible, when *Cal. 2. dimension* is activated as well). This deactivates the overall calibration and will activate the calibration field on the upper right side of the properties window. Now single calibration curves for the transfer injects with known composition and existent calibration curve can be chosen and activated. For those injects, where no calibration curve is chosen, the calibration will be interpolated between given calibration curves (e.g., a calibration A is chosen and activated for sample 3 and a calibration B is chosen for sample 27 - then the samples 4 to 26 will be calibrated with a curve which is a linear interpolation between A and B; sample 1 and 2 will get curve A and sample 28 to 30 will get curve B if not chosen otherwise). This procedure gives an approximated calibration for copolymers which elute between their homo polymers. The more sample- and calibration information are available the better this approximation will be. The calibration can be activated and deactivated in the properties window and (once the calibration files are selected) in the context menu of the axes. The status bar of the sub window will indicate when a calibration is active.

Performing 2D Quantifications

The calibration information can be entered and changed in the properties window, to get quantitative results for the sample it is necessary to specify the components that should be analyzed.

This can be done by drawing a grid around the single components. To use the grid function, activate **Options > Grid > Design mode** and load an existing grid (**Options > Grid > Open...**) or draw a new grid.



NOTE

Make sure that the 2D plot is not calibrated and you cannot zoom when the Design Mode is active. Use the scaling function of the axes or deactivate the Design Mode, zoom to the desired section and activate the Design Mode again.

To create a grid, just:

- click with left mouse button to set a grid point
- drag the cursor with your mouse to draw a line to the next grid, do not drag mouse with button pressed
- set next grid point: click left with mouse, release mouse button and continue
- to finish the polygon, click onto the first grid point again and then hit **Esc** key, the polygon will be numbered automatically
- to add additional grid points (or attach a new polygon to an already existing polygon) press **Ins** key
- to delete a grid point: bring the cursor over the grid point and press **Del** key
- to delete a grid line: bring the cursor over the line and press **Del** key
- to move a grid point: move the mouse cursor over an existing grid point until the cursor changes its shape – then move the point with the mouse.

To mark all polygons in order to move the grid or to vary its size press **Home** key. The grid can be saved with **Options > Grid > Save as...**.

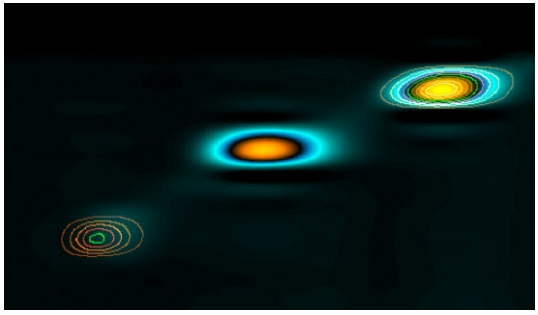
Using 2D Color Palettes

The color palettes are used to map the concentration (Z-axis) in the 3D data array. 4 system palettes are available or individual palettes can be created, saved, and opened selecting **Options > Palette** from the menu. New palettes are created by selecting an existing one as a template and modifying it. The new palettes have to be saved separately to keep them permanently; otherwise, they will be lost when closing the WinGPC Software session. A new palette is created easily by selecting an existing one, clicking on one of the color set points and modifying its color by either selecting it from the pre-defined colors in the matrix or using the color control panel on the right. The color distribution preview bar shows how the selected color set point will be interpolated to create a continuous color distribution.

Additionally the background colors for the screen and for the printout can be chosen separately. The default colors are black for the screen and white for the print version. The colors are changed in the same way like those for the palette.

Comparing Samples with the 2D Overlay

Sometimes it is reasonable to compare the results for two samples. One way is to compare the numerical results (see "Generating and Reporting Results"), but often an overlay will help to identify at the first glance, if two samples are identical or different. The WinGPC Software 2D module allows to overlay two 2D plots into one graphics box. Load the first contour plot and then add a second one with into the same sub window. The first one will be displayed as created before and the second plot will be shown in transparent isolines. Please make sure that both files have the same background color and are both calibrated or not calibrated. As soon as the second diagram is overlaid, the context menu for the graphics box will be extended by two menu items (properties overlay and palette overlay), so all information of both measurements are still available.



Transfer Valve Setup for 2D Runs without Guided 2D Valve Setup

If you do not want to use the automated valve setup you have the possibility to program the relay on your own. Use the following steps as a guide:

- Get the method parameters for the 1st and 2nd dimension: run times for both dimensions, number of samples, etc...
- Launch the WinGPC Software with a 2-instrument setup
- Choose "**Instrument No. 2**" and load or edit the method in the **Method** Window
- Select **Definition > Start Condition > Inject** and set it to **Inject No.**: "1"
- Do the same for **Definition > Stop Condition > Inject** and set **Inject No.** to the max. number of intended transfers + time for last transfer injection
- optional step if more than a single sample will be injected in the first dimension: select **Definition > Start Condition > Repeats** and enter the number of samples injected into the first dimension*
- In the **Method Information** Window click on **Pause** to set data acquisition for Instrument No. 2 to standby
- Choose Instrument No. 1 and load or edit the method in the **Method** Window
- Select **Definition > Start Condition > Inject** and set it to **Inject No**: "1"
- Do the same for **Definition > Stop Condition > Inject** and set **Inject No.** to the number of 2D samples injected into the first dimension
- Set the 2D transfer valve control parameters (**timed events > relay**) according to the before determined parameters, i.e. set the signal dependence to **inject and periodical** and for the signal type:
- Timing delay: for relay 2 the delay until the fractionation shall begin, respectively the delay and (for relay 1) additional the time between 2 injections of the 2nd dimension.
- Timing interval: the interval has to be twice the time between two injections of the 2nd dimension.
- No. of switches: have to be half of the total transfer injections.
- Switch time: should be set to 1
- Hint: do not open the timed events dialog after a run has been started

- In the Method Information Window click on **Pause** to set data acquisition for **Instrument No. 1** to standby
- OK the message that timed events are activated when setting instrument 1 on standby
- Inject the first sample in the first dimension; this will trigger data acquisition of both dimensions
- Enter sample names, concentrations etc. into the sample editor of **Instrument No. 1**

*) the "repeat" feature will automatically generate a new 2nd dimension sequence for each injection in the first dimension. It is only necessary if more than a single sample is injected into the first dimension; please note that the number of repeats is the number of samples minus one.

14 Copolymer Analysis Software Module

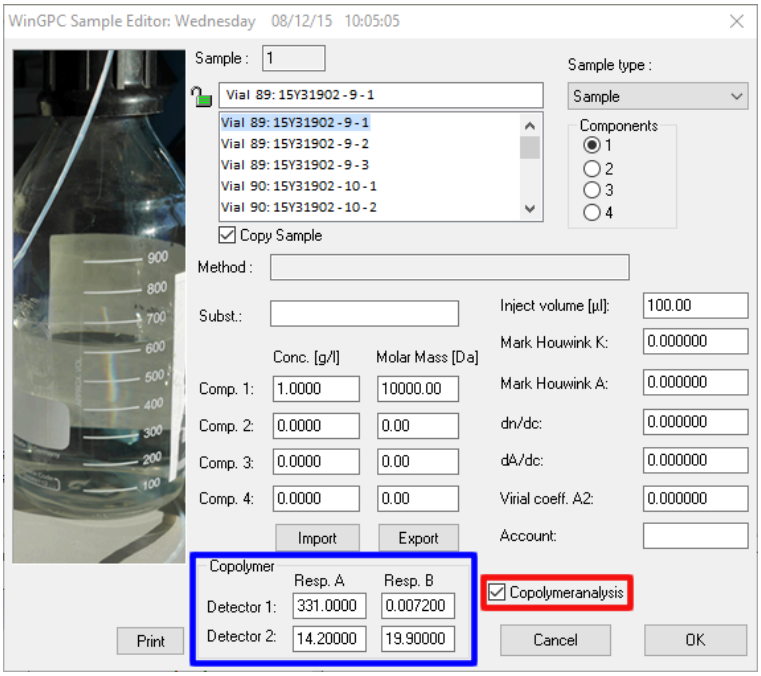
Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter "WinGPC Compliance " on page 507 of this user guide.

This WinGPC Copolymer add-on allows the analysis of chemical composition of copolymers and blends (e.g., block copolymers, graft copolymers, polymer mixtures) in the GPC mode of chromatography. WinGPC Software will report chemical composition, the chemical composition distribution (variation of composition with molar mass, CCD), homopolymer content, and average copolymer molar masses if applicable. No special sample preparation or data processing is necessary to obtain this type of information. This method requires two independent detector signals (e.g., RI and UV) and two different calibrations (molar mass calibration for homopolymer A and B as well as a detector response calibration). Further details on this method, its applicability and background on calculations can be found in chapter "Copolymer GPC Analysis by Multiple Detection" on page 53.

This WinGPC add-on is an alternative for the WinGPC Software Chemical Heterogeneity Module. Please note that these software modules are only available with the scientific version of the product.

Procedure:

To process chemical composition data the WinGPC Software **Copolymer** software option has to be marked in the **Login** Window when WinGPC Software is launched. To use the copolymer composition data processing this option has to be activated in the sample editor (see Figure below). This will toggle between conventional data processing and compositional analysis for each sample individually.

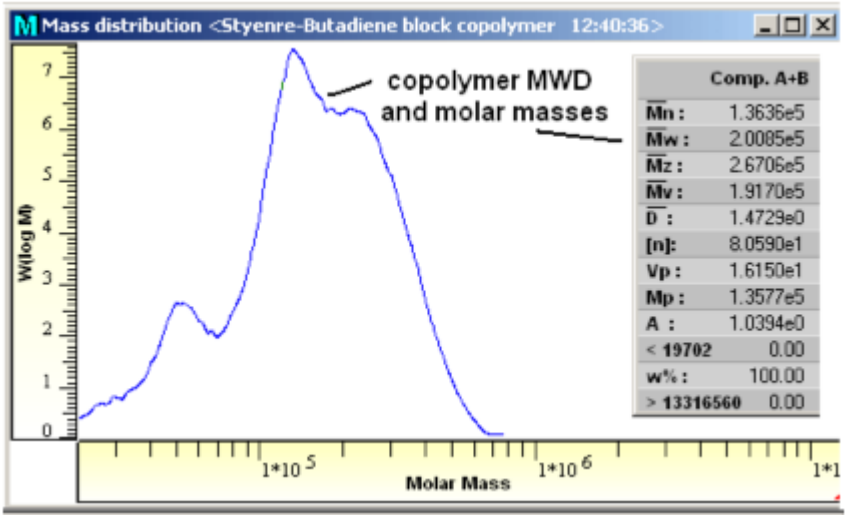


The only difference to conventional GPC data processing is the special mathematical treatment of raw data to calculate the composition of component A and B in each analytical fraction based of the detector response factors entered for the sample in the sample editor. The copolymer molar mass calculation requires that two molar mass calibration files have to be loaded using the **Calibration Data** menu in the **Raw Data** Window.

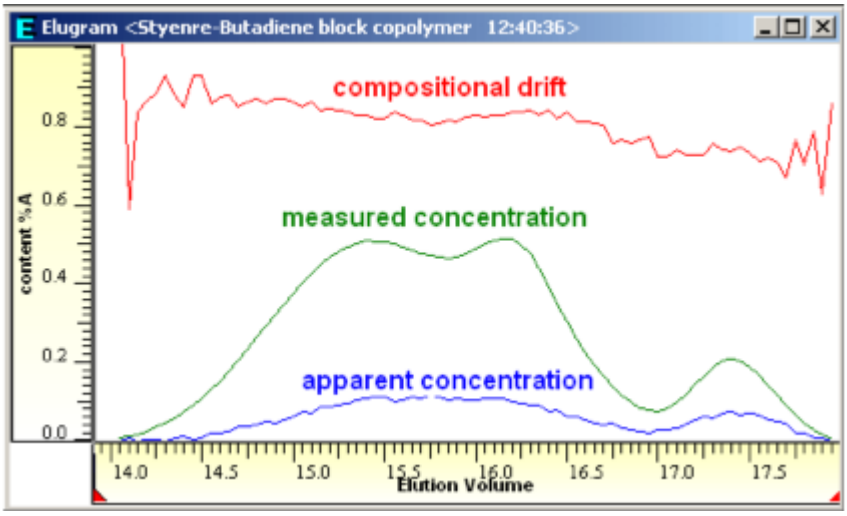
The data acquisition is done in the usual way and there are no special requirements for the sample preparation and no additional sample parameters necessary.

The following data processing steps are performed when a chemical composition analysis is done:

1 The true concentration of components A and B and their %composition are calculated from the detector signals according to the detector response factors in the sample editor. In the **Elugram** Window the true concentration traces for **Comp.A** and **Comp.B** and the true compositional drift of the sample (signal shown as fraction of A denoted by Comp.A/(A+B)) are displayed by default. The apparent concentration signal in the figure below is shown as a blue trace, the true concentration of the copolymer sample is shown in green.



2 The additional signal can be deselected in the **Curve** menu by clicking on a curve identifier and ticking off the trace "visible" option. This is useful if the individual concentrations shall not be shown in the **Elugram** and **Mass Distribution** Windows (e.g., in the Figure below the individual traces for **Conc.A** and **Conc.B** are not shown for simplicity and better readability).



3 The calculation and the display of the results of the copolymer molar mass averages and distribution identified by **Comp.A/(A+B)** are done in the **Mass Distribution** Window, which has otherwise the same functionality as in conventional GPC. This requires that the correct homo polymer molar mass calibration files have been assigned to the sample by selecting **Load** for the calibration file for the first comonomer and **Load Copo sec.** for the second comonomer in the **Calibration Data** menu in the **Raw Data** Window.

4 The standard report will be printed using the internal WinGPC Software printing functionality. However, more print design options are available in the WinGPC Software ReportDesigner which is available as a software option. For further graph annotation the ChromEdit software add-on for WinGPC Software might be useful for tweaking and fine-tuning of result presentation.

15 Chemical Heterogeneity Module

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter “WinGPC Compliance ” on page 507 of this user guide.

This WinGPC add-on allows the analysis of chemically heterogeneous polymers (e.g., copolymers, endgroup functionalized polymers) in the interaction mode of chromatography (HPLC). This method requires a calibration of (HPLC) retention with standards of known chemical composition. This WinGPC add-on is an alternative for the WinGPC Copolymer add-on which calculates composition based on the detector response. Please note that these software modules are only available with the scientific version of the product.

The module calculates the average composition, G, the width of the distribution, dG, and the skew, S, of the chemical composition distribution from the composition calibration. Details on the method, its application and background on calculations can be found in chapter “Copolymer HPLC Analysis” on page 57.

The slice concentration relates to the “true” concentration of the species in the LC fraction, not the apparent concentration measured by the detector without applying corrections. The true concentration can be measured by entering the detector response factors of the components in the WinGPC Software sample editor. These values are obtained from a detector response calibration.

NOTE

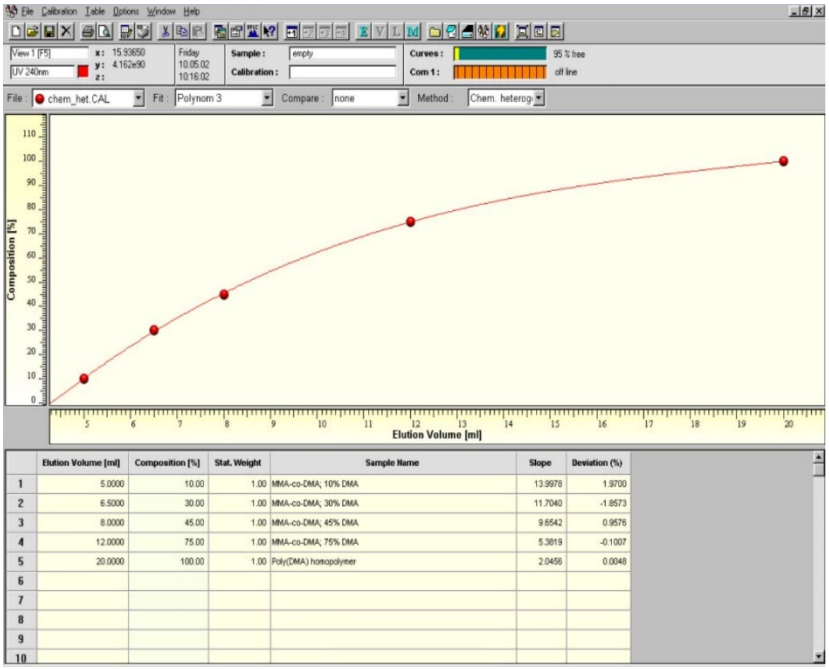
This correction can be neglected if the second component makes up <5% of the sample, e.g., with endgroups. In such cases the apparent detector concentration is very close to the “true” concentration of the species.

Procedure:

To process chemical heterogeneity data the WinGPC Software **Chemical Heterogeneity** option has to be marked in the **Login** window when WinGPC Software is launched. To use the chemical heterogeneity data processing option the menu item **Definition > Chem.Heterogeneity** in the **Method** Window has to be selected. This will toggle between conventional data processing and compositional analysis during a WinGPC Software session.

The only difference to conventional GPC data processing is the special mathematical treatment of raw data and the composition retention calibration option in the Calibration Window. Please note that this software option will not be available if it is not the scientific version of the product.

The data acquisition is done as usual in WinGPC Software. The retention calibration is performed with standards of known composition and relates %composition to the elution volume of the standard. The calibration curve is created in the **Calibration** Window, but the **Chem. Heterogeneity** mode has to be selected in the **Method** field. All calibration points are entered into the calibration table in the same manner as in a conventional molar mass calibration. The regression of the chemical composition calibration and its optimization are also done similar to a molar mass calibration. Please note that the method flag will be saved with the calibration file and WinGPC Software will recognize chemical heterogeneity calibrations automatically when opened. There is no special file extension for this type of calibration.



	Elution Volume [ml]	Composition [%]	Stat. Weight	Sample Name	Slope	Deviation (%)
1	5.0000	10.00	1.00	MMA-co-DMA; 10% DMA	13.9978	1.9700
2	6.5000	30.00	1.00	MMA-co-DMA; 30% DMA	11.7040	-1.8573
3	8.0000	45.00	1.00	MMA-co-DMA; 45% DMA	9.6542	0.9576
4	12.0000	75.00	1.00	MMA-co-DMA; 75% DMA	5.3819	-0.1007
5	20.0000	100.00	1.00	Poly(DMA) homopolymer	2.0456	0.0048
6						
7						
8						
9						
10						

The following data processing steps are performed when a chemical heterogeneity analysis is done:

1. The true concentration of components A and B is calculated from the detector signals according to the detector response factors in the sample editor (if present; otherwise the apparent concentrations are used). In the **Elugram** Window the true concentration traces for **Conc.A** and **Conc.B** and the true overall concentration of the sample (Conc.A+B) are displayed by default. The apparent concentration signal in the figure below is shown as a green trace, the true concentration of the copolymer sample is shown in red.
2. The additional signal can be deselected in the **Curve** menu by clicking on a curve identifier and ticking off the trace “visible” option. This is useful if the individual concentrations shall not be shown in the **Elugram** and **Mass Distribution** Windows. (E.g., in the Figure below the individual traces for **Conc.A** and **Conc.B** are not shown for simplicity and better readability.)
3. The calculation and the display of the results of the composition distribution are done in the **Mass Distribution** Window, which has the same functionality as in conventional GPC. Please note that the original detector signal is no longer displayed in this window, because it no longer has any physical meaning. All information on the chemical heterogeneity distribution of the sample is summarized in curve **Conc.A+B**, which replace the original signal.

4 The Y-axis of the **Mass Distribution** Window now corresponds to the weight fractions of the composition distribution, *w*. This axis will automatically be used if the **Chem. Heterogeneity** option is selected in the **Definition** menu of the **Method** Window. Please note that the user has to take care about the proper X-axis label.

5 A chemical heterogeneity report will be created and can be printed using the internal WinGPC Software printouts. However, more print design options are available in the WinGPC Software ReportDesigner which is available as a software option.

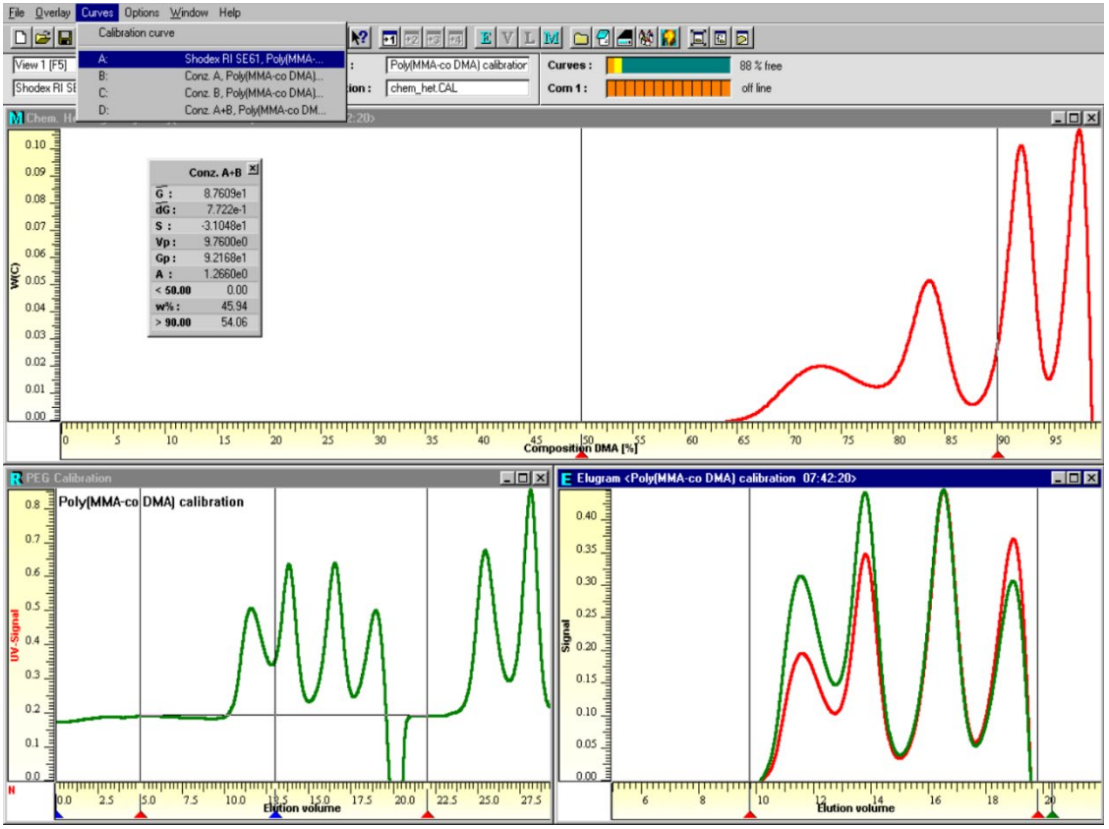


Figure 61 WinGPC Software displaying curves and results in Chem.Heterogeneity mode

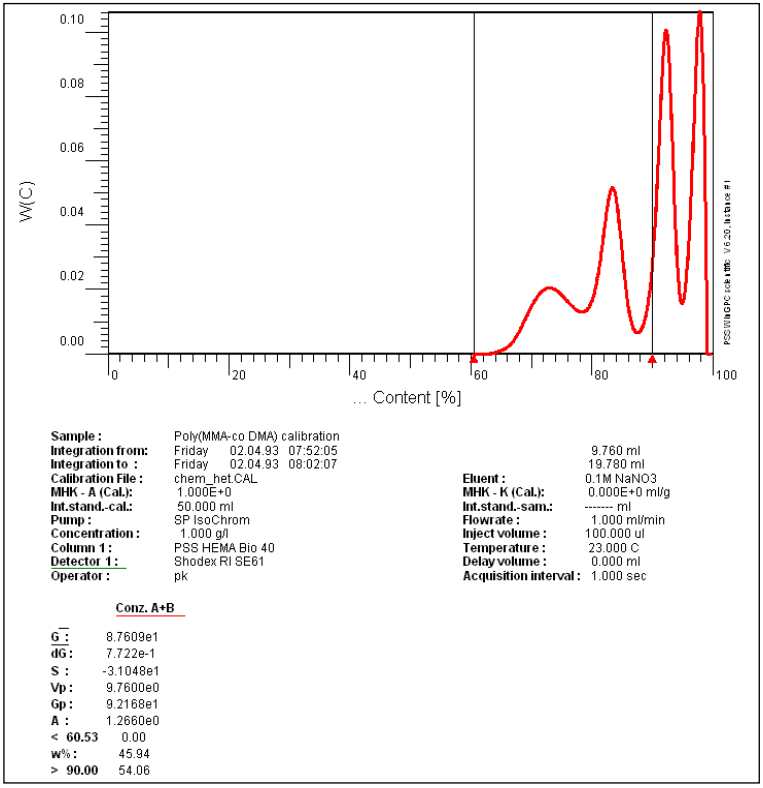


Figure 62 Example of a Standard WinGPC Software Report for a Chem. Heterogeneity Analysis

16 Endgroup / Heparin Analysis

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter "WinGPC Compliance " on page 507 of this user guide.

The WinGPC Software Endgroup Analysis allows the creation of a calibration curve for substances, which carry a detectable endgroup. It can also be used in more general terms to determine the degree of functionalization e.g., in branched or graft polymers, if the endgroup detector factor, *f*, is known. Please note that these software modules are only available with the scientific version of the product.

Specifically, it is useful for low molecular weight heparin. The creation of the heparin calibration fully conforms to the requirements of different Pharmacopeia (EP, USP, DAB).

This software module permits the creation of calibration curves under the following requirements:

1. the sample possesses an endgroup signal (for heparin UV 234nm)
2. a mass proportional signal is measurable (for heparin RI)
3. a sample with known number average molecular weight is available.

Because the UV signal is proportional to the number of eluting molecules while the RI proportional to the mass eluting, the following equations hold true:

$$M_n = f \cdot \frac{\int RI \, \mathrm{d}V}{\int UV \, \mathrm{d}V}$$

This relationship can be used for the determination of the response factor f. If the number average molecular weight of the calibration substance is given, the factor f can be calculated for the whole sample. Consequently, the molar masses in each slice can be calculated from the UV and RI chromatograms according to:

$$M = f \cdot \frac{RI}{UV}$$

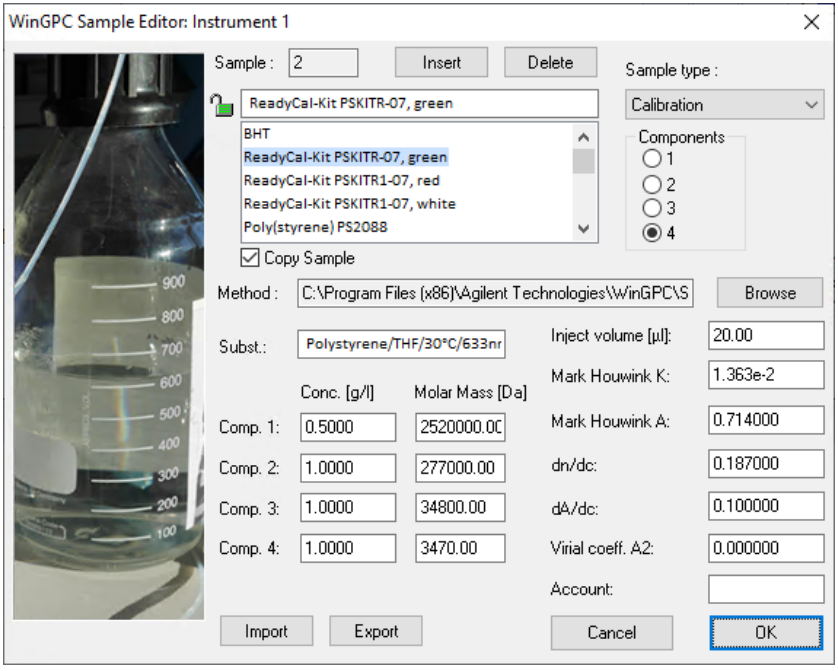
The next sections describe the steps which are necessary to do data acquisition and data processing for endgroup analyzes. Only the important new features and parameters will be discussed in order to keep the Agilent WinGPC Software User Guide compact and avoid repetitions. The basic features of the WinGPC Software are described in their respective chapters. Please refer to those if some notions and features are not explained here.

Method Window

Define both detectors in the Instrument Layout View and assign the signal type **UV** to the number-proportional signal, and the signal type **RI** to the mass-proportional signal (see chapter "The Method Window" on page 165). All other parameters can be used in the ususal way without any limitations.

Raw Data Window

Unknown samples are processed as any unknown sample would be analyzed using the calibration created with a heparin reference material.



This type on calibration can be created easily with WinGPC Software by running the reference standard and entering its reference molar mass (M_n) in the sample editor (**Editor > Samples**) for component 1. The internal standard and the baselines are set in the same way done in conventional GPC data treatment. All other features, options and parameters can be used in the ususal way without any limitations.

Elugram Window

Unknown samples are processed as any unknown sample would be analyzed using the calibration created with a heparin reference material. In the elugram the integration limits are set in a way that they cover the peak of the unknown sample completely.

The calibration created from a heparin reference sample is invoked from the **Options > Endgroup analysis** menu by selecting the **Endgroup analysis** option. Now the factor, f, will be calculated, and the slice molecular weights will be obtained. In the elugram the calibration curve calculated from the endgroup signal is displayed besides both detector signals. At the same time value pairs V_e/M are calculated. Please make sure that no other peaks should lie within the integration limits to avoid misinterpretation of reference molar masses.

Use the **Options > Endgroup analysis > Create Calibration** to transfer the calibration data table to the **Calibration** window. The creation of the calibration curve is similar to conventional calibrations described in chapter "Interactive Creation of Conventional Calibration Curves" on page 311. This calibration file should be saved for later use to process unknown heparin samples regarding their correct molecular weight distribution.

NOTE

It might be useful to remove some outliers in the calibration table which might occur at the onsets of the reference sample peak due to limited signal-to-noise.

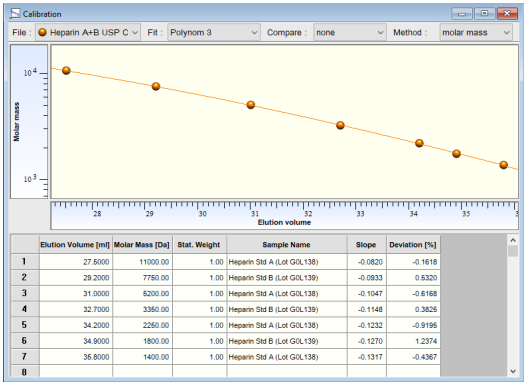
Performing Heparin Analysis based on USP / EP

Performing Heparin Data Analysis based on USP Methods

1 Load the heparin samples (Standard solutions and Test solutions) acquired by WinGPC Software or import heparin data acquired by Chromeleon as described in section "Importing Chromeleon Data" on page 273.

2 Set baselines and integration limits for all samples / injections. Guidelines are available in the **Help > Step-by-step** menu and in chapter "Performing a Simple GPC Measurement" on page 119. Alternatively, the the wizards can be used (**Method** Window, menu item **Method > WinGPC Wizard**).

3 Create a molecular weight calibration curve (see chapter "Creation of a Conventional Calibration Curve" on page 124 for details) from the Heparin **Standard solution** runs (USP Molecular Weight Calibrants). Save the calibration as a file, e.g., "Heparin calibration USP Standard solution A+B.cal".



	Elution Volume [ml]	Molar Mass [Da]	Stat. Weight	Sample Name	Slope	Deviation [%]
1	27.5000	11000.00	1.00	Heparin Std A (Lot G0L138)	-0.0820	-0.1618
2	29.2000	7750.00	1.00	Heparin Std B (Lot G0L139)	-0.0933	0.5320
3	31.0000	5200.00	1.00	Heparin Std A (Lot G0L138)	-0.1047	-0.6168
4	32.7000	3360.00	1.00	Heparin Std B (Lot G0L139)	-0.1148	0.3825
5	34.2000	2260.00	1.00	Heparin Std A (Lot G0L138)	-0.1232	-0.9196
6	34.9000	1800.00	1.00	Heparin Std B (Lot G0L139)	-0.1270	1.2374
7	35.8000	1400.00	1.00	Heparin Std A (Lot G0L138)	-0.1317	-0.4367
8						

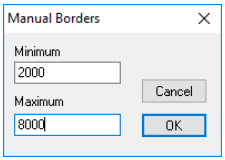
Click on **Help > Step-by-step** and select **Creating Calibration Curves** for instructions. A related calibration curve is shown in the Figure above.

NOTE

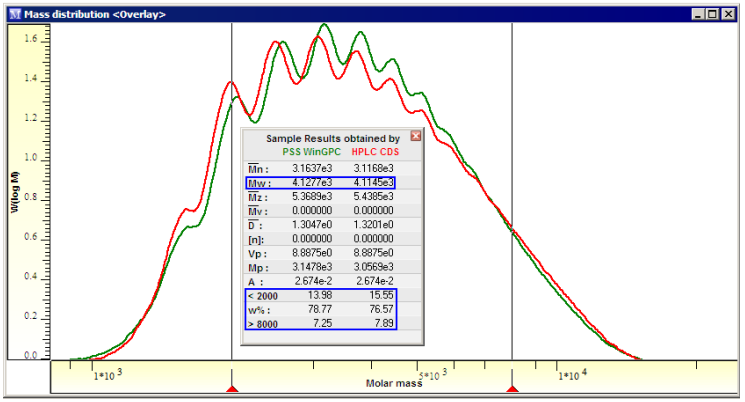
If a broad heparin standard has been used as calibrant create a broad calibration as described in **Help > Step-by-step** and select **Performing an Integral (Cumulative Match) Calibration**.

4 Assign this Heparin calibration to the related data set(s) imported in step no. 1 above: Highlight the WinGPC Software **Raw Data** Window with a mouse click, select **Calibration Data > Load…** and choose the related calibration file from the file list, e.g., "Heparin calibration USP Standard solution A+B.cal".

5 Right mouse click on the x-axis in the **Mass Distribution** Window and select "**Manual Borders**" from the x-axis context menu. Enter the respective molar mass specification (refer to the relevant USP) into this dialog as shown in the next Figure (here: 2000 Da and 8000 Da, respectively).



6 The numerical results and the molar mass distribution of a single sample or an overlay of multiple samples will be shown on screen. An example is shown in the Figure below.



7 Report results either by using the default WinGPC Software reports or a custom report (this requires the optional ReportDesigner software module). Click on **Help > Step-by-step** and select **Printing Results** for instructions.

8 Comprehensive batch data analysis is also available for multiple samples. Click on **Help > Step-by-step** and select **Automated Data (Re-) Processing** for instructions.

NOTE

Fully automated data processing during data capture is not possible with imported data. However, this functionality is available when using WinGPC Software for data acquisition.

Performing Heparin Data Analysis based on EP Method

Background information on this data processing method is available at the beginning of chapter “Endgroup / Heparin Analysis” on page 438.

If the Heparin option is enabled data processing according to the EP low molecular weight heparin method can be done.

NOTE

The Heparin/Endgroup analysis was not included in older WinGPC versions and had to be purchased separately. All Agilent WinGPC licenses are delivered with this option automatically.

Please follow the instructions below:

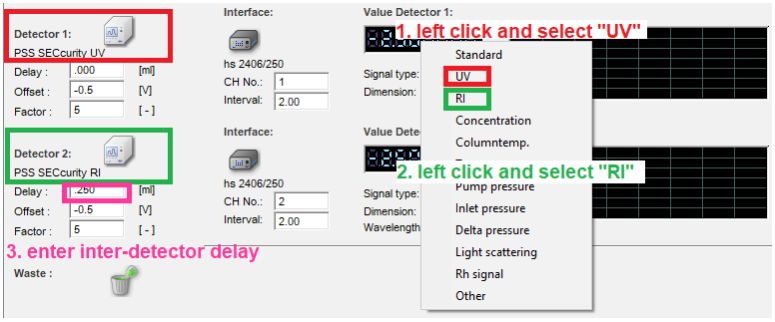
1 Load the heparin samples (EP Heparin CRS standard and (optionally) unknowns) acquired by WinGPC Software or import heparin data acquired by Chromeleon as described in section “Importing Chromeleon Data” on page 273.

NOTE

This method requires UV and RI channels in the Chromeleon data set(s).

2 Adjust the WinGPC Software method by clicking into the WinGPC Software Method Window and reviewing the **Signal type** for both the UV and RI detector as they determine the way data processing and EP heparin calibration is performed.

Modify the **Signal type** for the UV and RI detector signal by a left mouse click into the (black) **Value Detector** number field and select the appropriate signal description as illustrated in the next Figure.

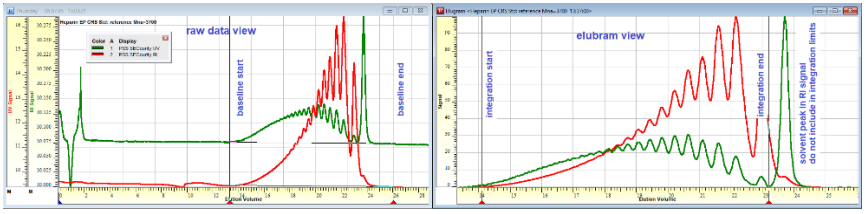


3 Enter the detector delay volume between the UV and RI signals in the detector **Delay** field for **Detector 2** as highlighted in pink in the Figure above. The simplest procedure is to use the **Guided Detector Setup** accessible from the

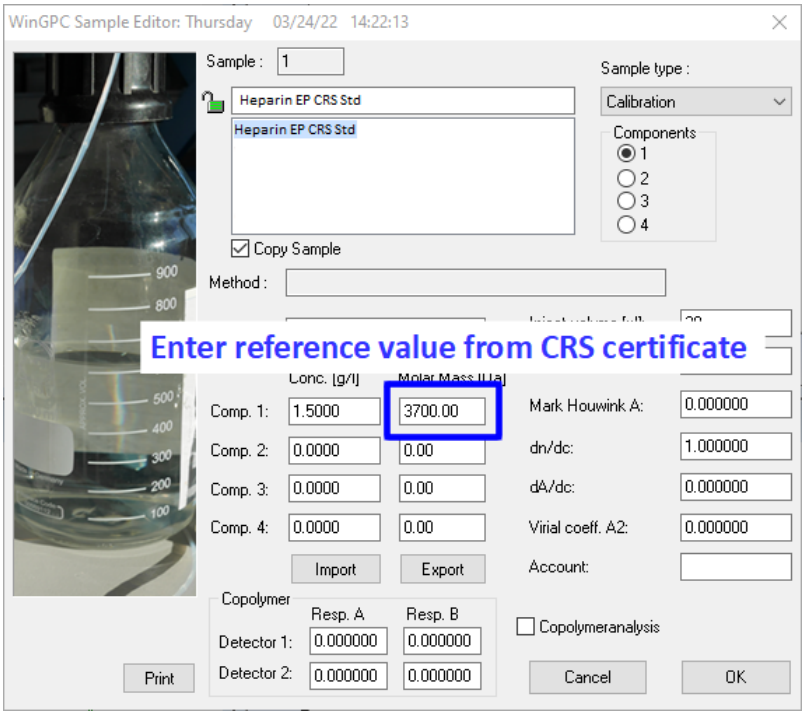
menu item **Method**. Optionally save the method as a separate file for future use.

4 Change the view to the **Raw Data** Window by clicking into it. Set baselines and integration limits for all samples / injections. Guidelines are available in the **Help > Step by step** menu and in chapter "Performing a Simple GPC Measurement" on page 119. Alternatively the the wizards can be used (**Method** Window, menu item **Method > WinGPC Wizard**).

5 Ensure that no solvent or trash peaks are included in the in integration limits; this is especially important as RI responds to many compounds.



6 Select the Heparin EP CRS standard from the injection list and edit the sample information from the **Editor > Samples** menu of the **Raw Data** Window.

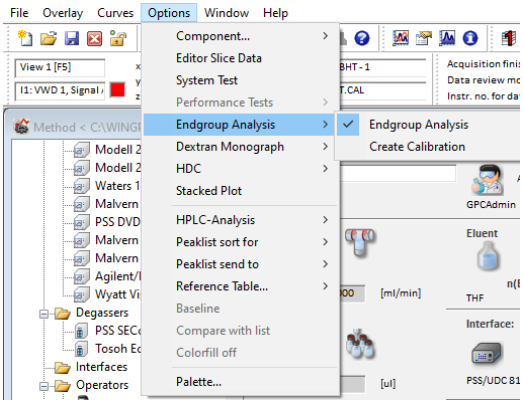


Enter the molar mass reference value from the EP CRS standard certificate in the molar mass field in the sample editor (see Figure above). Accept changes with **OK**, otherwise cancel out.

NOTE

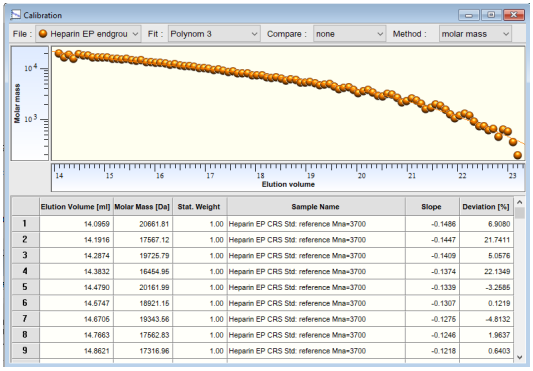
Entering the standard concentration is recommended, but not required for proper data processing

7 Click into the WinGPC Software **Elugram** Window and select **Options > Endgroup Analysis > Endgroup Analysis**. When **Endgroup Analysis** shows a tick mark the contents of the **Elugram** Window adds the absolute molar mass determined by endgroup calibration as specified in the EP low molecular weight heparin standard.



8 Create a molecular weight calibration curve from the Heparin EP CRS standard by selecting **Options > Endgroup Analysis > Create Calibration**.

9 Change to the **Calibration** view by clicking on the **Calibration** icon in the Icon Bar. Review the data and select an appropriate calibration “Fit” (3rd order polynomial is often used). A related calibration curve is shown in the next Figure.

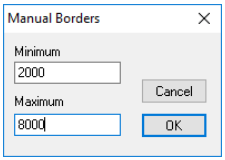


	Elution Volume [ml]	Molar Mass [Da]	Stat. Weight	Sample Name	Slope	Deviation [%]
1	14.0969	20661.81	1.00	Heparin EP CRS Std: reference Mna=3700	-0.1486	6.9080
2	14.1916	17567.12	1.00	Heparin EP CRS Std: reference Mna=3700	-0.1447	21.7411
3	14.2874	19725.79	1.00	Heparin EP CRS Std: reference Mna=3700	-0.1409	5.0576
4	14.3832	16454.95	1.00	Heparin EP CRS Std: reference Mna=3700	-0.1374	22.1349
5	14.4790	20161.99	1.00	Heparin EP CRS Std: reference Mna=3700	-0.1339	-3.2686
6	14.5747	18921.15	1.00	Heparin EP CRS Std: reference Mna=3700	-0.1307	0.1219
7	14.6705	19343.66	1.00	Heparin EP CRS Std: reference Mna=3700	-0.1275	-4.8132
8	14.7663	17562.83	1.00	Heparin EP CRS Std: reference Mna=3700	-0.1246	1.9637
9	14.8621	17316.96	1.00	Heparin EP CRS Std: reference Mna=3700	-0.1218	0.6403

Save the created calibration curve as a file, e.g., as “Heparin EP endgroup calibration.cal”.

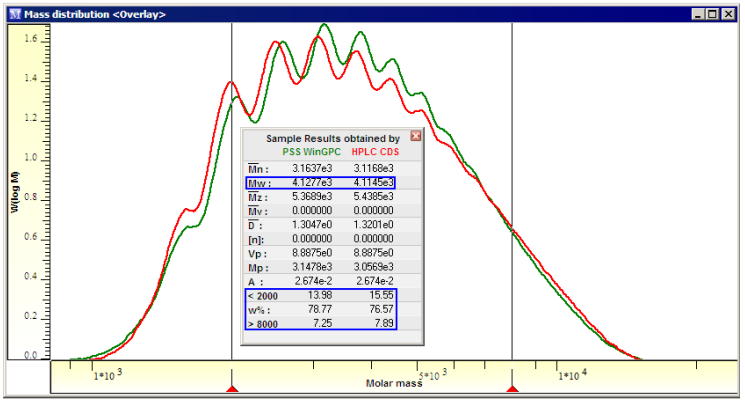
10 Assign this Heparin calibration to the related unknown heparin sample imported in step no. 1 above: Highlight the WinGPC Software Raw Data Window with a mouse click, select **Calibration Data > Load…** and choose the related calibration file from the file list, e.g., “Heparin EP endgroup calibration.cal”.

11 Right mouse click on the x-axis in the **Mass Distribution** Window and select **Manual Borders** from the x-axis context menu.



Enter the respective molar mass specification (refer to the EP standard) into this dialog as shown in the Figure above (here: 2000 Da and 8000 Da, respectively).

12 The numerical results and the molar mass distribution of a single sample or an overlay of multiple samples will be shown on screen. An example is shown in the Figure below.



13 Report results either by using the default WinGPC Software reports or a custom report (this requires the optional ReportDesigner software module). Click on **Help > Step-by-step** and select **Printing Results** for instructions.

14 Comprehensive batch data analysis is also available for multiple samples. Click on **Help > Step-by-step** and select **Automated Data (Re-)Processing** for instructions.

NOTE

Fully automated data processing during data capture is not possible with imported Chromeleon data. However, this functionality is available when using WinGPC Software for data acquisition.

17 3D Spectra Module

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter “WinGPC Compliance ” on page 507 of this user guide.

GPC Data are usually twodimensional, i.e., the intensity of one ore more detector signals is plotted against the time and then further processed. Special applications might require recording and analyze complete spectra dependent on time (e.g., using a DAD or a FLD). WinGPC Software offers a 3D spectra add-on which allows to record those signals in combination with ChromPilot and to create a threedimensional display of the data.

Acquisition of 3D Spectra

ChromPilot Spectra acquisition needs to be activated to record spectra in addition to the signals configured in the WinGPC Software **Method** window. You need to activate the spectra acquisition in the detailed view and will always see the status in the system view as well (see figures below).

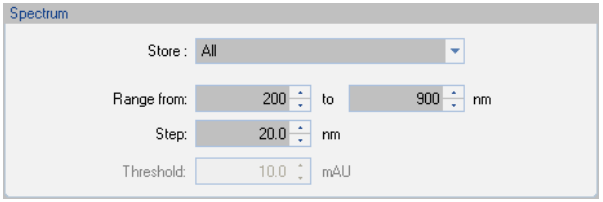


Figure 63 DAD spectra configuration/activation in ChromPilot Instrument Manager detailed view

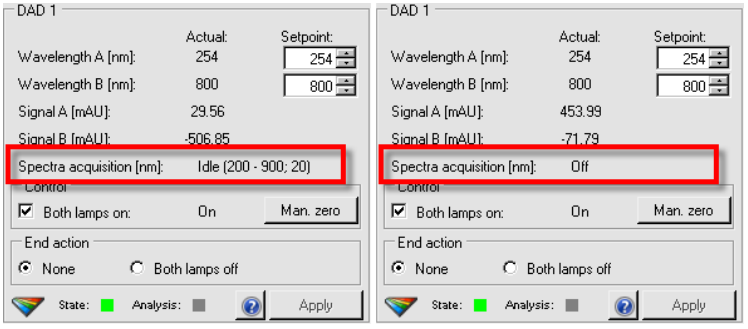


Figure 64 DAD Configuration (system view) with and without spectra acquisition

Spectra will be recorded in addition to and not instead of the conventional concentration signals. Thus, the WinGPC Software Method layout requires the definition of minimum one concentration signal (e.g., one or two selected UV/Vis signals of the DAD (usually defined as signal A, B, C etc.) and/or a RI signal). Since spectra files are larger than conventional GPC data, we recommend to deactivate spectra acquisition for those samples which don´t need 3D information (e.g., system tests, calibration samples,...). You can activate/deactivate spectra acquisition within a sequence by loading corresponding instrument settings. WinGPC Software creates an additional project file (*.SPX) for those projects that contain spectra. Depending on the settings the spectra of one sample might require several MB of storage space.

Import and Display of 3D Spectra

If you want to process data with 3D spectra, you can load the respective sequence as other data using the **Project Management** window.

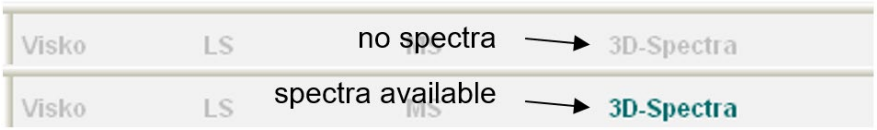


Figure 65 Preview with display of 3D spectra availability

The sample information preview for each sample shows if spectra are available or not (**3D-Spectra** black or greyed out, see figure above).

Sequences with 3D spectra can be evaluated as usual. To create a threedimensional display of a certain sample, baseline and integration limits need to be set first. Afterwards the spectra are transferred to the 3D window via the **elugram** menu **File > … to 3D Spectra**. This menu item is only available, if the 3D spectra module is licensed and the sample contains 3D information (this may vary from sample to sample).

The 2D/3D window ( ) will display the 3D spectra as soon as they are transferred via **File > … to 3D Spectra**.

General setup of the 3D window

The WinGPC Software 2D-, 3D spectra and MS modules use the same window structure. The workspace at the top consists of the selector bar, below you will find one or more subwindows containing the 3D spectra plot(s).

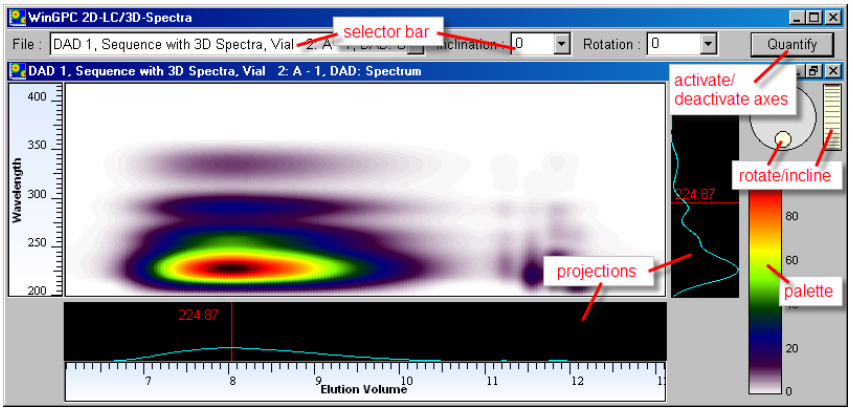
The WinGPC Software icon bar contains additional icons at the right which are 3D window specific:


brings the 2D/3D window to front/background (toggle)


toggles 3D view between full and partial (black margin around image) window mode


invokes the generation of the first derivative of the displayed 3D spectra


displays concentration iso-lines and toggles between area and line display



The selector bar is static and will act on the currently active sub window. The file list box will show and allow the selection of all open sub windows. The inclination list box allows to select pre-defined vertical view angles (0° to 90°) of the 3D data array. The rotation list box allows to select pre-defined horizontal view angles (0° to 360°) of the 3D data array. Inclination and rotation of the graphics can also be done interactively by dragging the mouse over the controls on the top right of the sub window.

Each sub window contains the contour plot with the projections and cuts respectively for both dimensions. The properties will determine if a cut or the projection (default) is displayed. When the projection view is deactivated, the mouse position in the 2D/3D graphics section will determine the cut shown (as identified by the red cross hairs). A palette visualizes the signal intensities of the contour plot. A mark can be dragged from 100 % downwards to cut the 3D plot at a certain value. The calculated cut values will be given in the 3D plot as well. For a quantitative analysis of the 3D data axes can be added to the contour plot (*Inclination* and *Rotation* = 0) by pressing the **Quantify** button.

3D Menu

The **File** menu contains all input and output options as well as the display options of the 3D window.

Table 62 File menu

Option	Description
New	Creates a new subwindow that can be used to transfer new 3D data or to load already processed 3D spectra
Open	Loads 3D spectra (*.GRX) into the active subwindow
Save as...	Saves active spectra as *.GRX file
Close	Closes active subwindow
Send 1D trace to overlay	Corresponds to **Send spectra to overlay**"
Send 2 D trace to overlay	Corresponds to **Send elugram to overlay**
Send 1D+2D traces to overlay	Sends spectra and elugram to overlay in elugram window (only recommended if information is to be separated again afterwards, e.g., with an external application).
Preview	Displays following figures as a preview (will be copied to the clipboard at the same time and may be pasted via **Ctrl**+**V** into other applications): • 2D Plot: 3D spectra • Horizontal Cut: elugram • Vertical Cut: single spectrum
ASCII Export	Exports the displayed 3D window into ASCII format

The **Analysis** menu can be used to view and print the results.

Table 63 Analysis menu

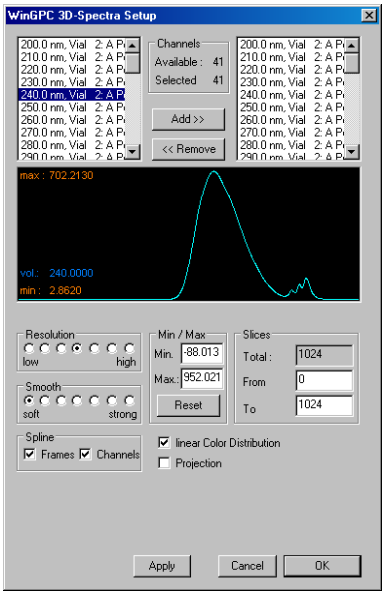
Option	Description
New Analysis	*The menu item is not available unter these conditions*
Results	Opens Evaluation Results dialog with all peaks / spectral areas that were previously defined using a grid

The **Options** menu contains different edit functions.

Table 64 Analysis menu

Option	Description
Properties	Opens properties dialog (see chapter „3D Properties Dialog“ on page 456)
Palette	Opens dialog for color palette management (see chapter „Using 2D Color Palettes“ on page 425)
Unzoom	Unzooms to initial values
Isolines	Offers the creation/modification of (additional) isolines (default: 9 isolines between 10 and 100%)
Grid	Grid to define certain peaks / spectral areas
• Design mode	Toggle; needs to be activated (tick mark in front of menu item) to create a grid
• Delete	Deletes current grid
• Save as ...	Saves current grid as *.GRD file
• Open ...	Loads a grid
Overlay	Overlay function; requires a previously saved contourplot that can be overlayed with the current plot

3D Properties Dialog



The 3D spectra module contains a variety of different functions for a comprehensive data analysis. The first step is to activate the x- and y-axes by pressing the **Quantify** button in the selector bar.

It is possible to get the **Properties** dialog (see figure) via **Options > Properties** or with the context menu of the contour plot. The upper part of the properties dialog lists the elugrams for each wavelength which was recorded for the given sample. The currently selected elugram will always be displayed below the list in a preview. It is possible, to activate/deactivate single wavelengths for the 3D display.

The lower part of the properties window includes display as well as calibration options. The resolution, smooth factor, spline, z-range and number of slices are preset with optimal values - there is no need to change these settings during a normal evaluation.

If negative data shall not be displayed, the **Min.** value may be set to “0”. This will also result in a recalculation of the color palette where 0% and 100% correspond to the minimum and maximum values respectively.

You can toggle between a linear and a logarithmic display of the 3D data. Linear is the default selection. A logarithmic z-scale can become handy when small amounts of byproducts or the influence of baseline noise shall be monitored beside other signals.

As mentioned above the 2-dimensional plot can be projected onto the x- and y-axes. The Projection field determines whether the projection (sum of all slices in the x- or y-direction) or a cut at a specified point of the diagram will be shown.

3D Spectra Data Evaluation

Data Processing

- The 3D spectra can be processed at any time:
- Set baselines and integration limits as required
- Activate the **Elugram** window and select **File > ... to 3D Spectra**; all data will be transferred to the 3D spectra window
- Press the  icon to bring the 3D window into the foreground
- Press **Quantify** to see the axes of your contour plot
- Single spectra may be sent to the overlay (**Elugram** Window) by selecting a certain position and choosing **Send spectra to overlay** (context menu, right mouse click)
- You can set the calibration information with the **Properties** function (either in the context menu or under **Options > Properties**)
- Set a grid (**Options > Grid > Design mode**) or load an already created grid (**Options > Grid > Open...**) to quantify single components of the contourplot
- Analyze your contourplot by selecting **Analysis > Results**, you will get the position and amount of the selected contour areas; when a calibration is activated you will get the molar mass averages as well
- The preview button will create a pre-configured 3D spectra report, you can change this report according to your needs if you have a WinGPC Software ReportDesigner license
- Save the 3D results (3D data, palette, calibration and grid information) with  (or **File > Save as...**) as a *.grx file.

Generating and Reporting Results

Quantitative results on the 3D data are generated from the **Analysis > Results** menu. A grid has to be designed or selected from the menu in order to have any results being displayed. A table will show the results for each grid area. In its simplest form the table will show results for peak positions, peak areas and peak volumes (the latter correspond to the relative masses of the different species). If a calibration is selected and activated, MWD results are calculated for each area. The quantitative results can be copied to the Windows clipboard by pressing the copy to clipboard button within the result box. The most comfortable export function is the 3D spectra report, which can be chosen with the print preview button (). Users who have a WinGPC Software ReportDesigner license may create a user defined report, otherwise a pre-configured report will be loaded.

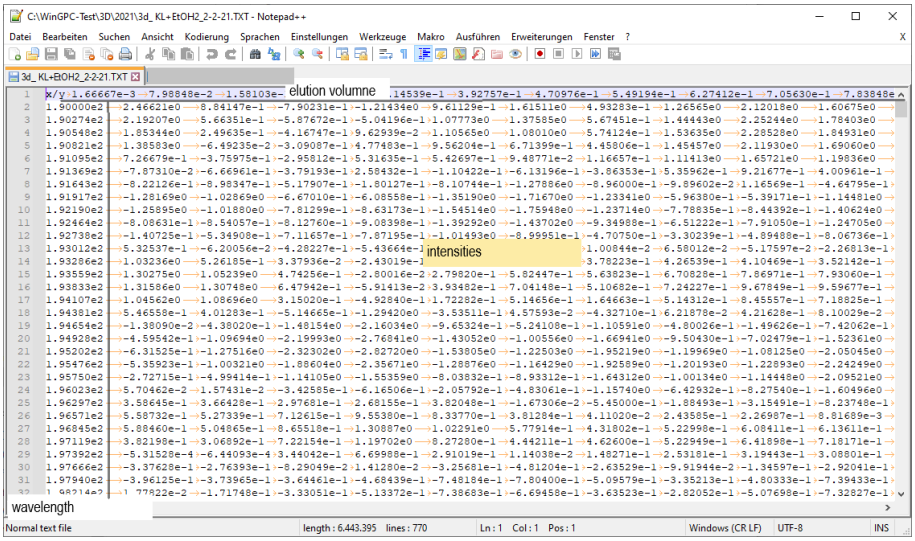
Sometimes it might be useful to display single spectra or to compare elugrams of a certain wavelength with the elugram of the concentration detector. Therefor the function send trace to overlay was created. This function can be activated either over the axes context menu or over **File > send ... trace to overlay**. There are three different options. The first or the second dimension trace or both can be sent at one time. To transfer the cuts position the mouse cursor, use the keyboard command **Alt+ F** and choose the wanted menu item.

NOTE

The traces will be sent to the elugram window, the axis context menu item will not be shown when the axis is calibrated. You can recalibrate the elugram later again. If you need the molar mass distribution out of the 3D window, it is possible to send calibrated data via **File > send 2D trace to overlay**. Those data will be transferred with logarithmic x-values (i.e., 5.5 instead of 1·105.5).

Graphical images can also be sent to the clipboard using the **File > Preview** command and selecting the proper item (2D plot, horizontal/vertical cut/projection). If the image of a cut should be copied, please position the mouse cursor first and then make sure that the command is issued from the keyboard (otherwise the mouse position is changed inadvertently) by selecting **Alt + F + P** and pressing **D** (**H**, **V**) for the 2D graphics (horizontal cut, vertical cut).

Another option is the export of ASCII data directly from the 3D window. This option is accessible through **File > ASCII Export**. This will export a txt file of the displayed 2D plot. The decimal separator can be changed in the **Data Editor** window, **Column > Decimal sign** (Chapter "File Menu"). The x-axis (elution volume) is exported to the first line, the y-axis (wavelength) to the first column.



Advanced 3D Data Processing

Since the setup of the 3D spectra window and the 2D window is basically the same, refer to the respective sections of the 2D window description for following topics:

- For details on quantifications (how to set a grid) refer to chapter "Performing 2D Quantifications" on page 423.
- Creating and managing color palettes is described in chapter "Using 2D Color Palettes" on page 425.
- The overlay function (comparing 3D spectra) is described in chapter "Comparing Samples with the 2D Overlay" on page 426.

18 Mass Spectrometry Module

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter “WinGPC Compliance ” on page 507 of this user guide.

The WinGPC Software Mass Spectrometry module offers a quick and comprehensive solution for GPC-MS data analyis. The concentration detector signals will be aquired by WinGPC Software directly, while the MS is controlled and recorded by the MS instrument software.

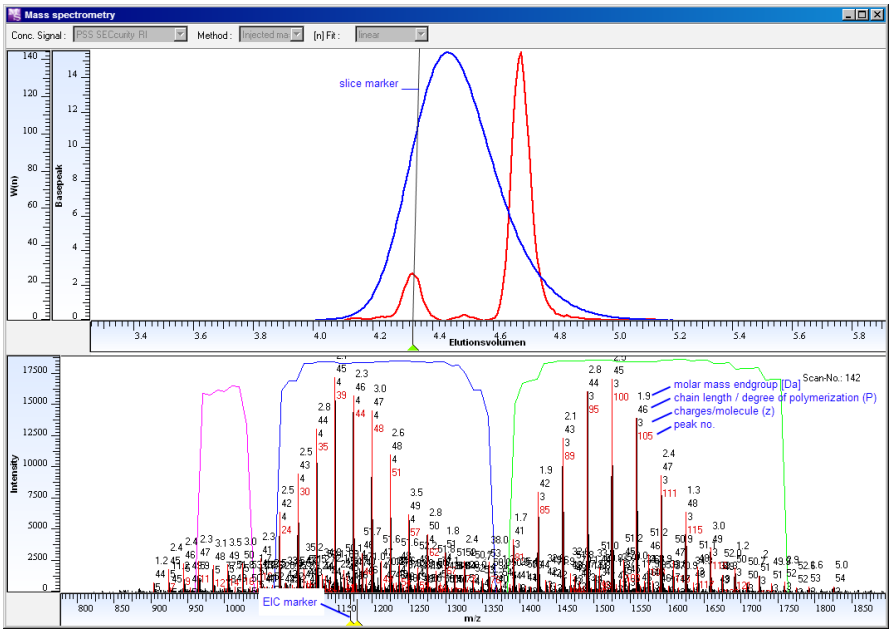
After the MS-GPC experiment finished, the MS data can be imported once into the WinGPC Software data base and may be reprocessed afterwards at any time without a new import.

We recommend importing and analyzing MS data in WinGPC Software second instance and importing MS data to finished GPC runs only.

Mass Spectrometry Window

The Mass Spectrometry Window is set up similar to the LS or viscometry window. The upper poart displays the concentration signal (here RI, blue) and the MS signal (here red). The selector **Conc. Signal** allows to define which detector should be used for the concentration signal.

Using the green fraction marker (x axis) a spectrum corresponding to a certain elution volume can be selected and displayed in the lower part of the window.



Information given in MS window:

- Above each peak (upper to lower information): endgroup molar mass [Da], chain length degree of polymerization (P), charges/molecule (z), peak number
- Scan-No.: number of spectrum (display defined by green fraction marker)
- EIC-Marker: yellow marker on x axis, define molar mass (region) (= base peak or structure) of EIC (extracted ion chromatogram)
- Charge state: different charge states will be visualized by colors (here: green: z=3, blue: z=4, pink: z=5)

MS Menu

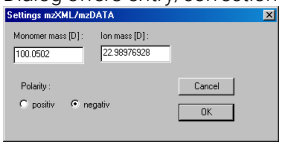
The **File** menu contains all input and output options as well as display options.

Table 65 File menu

Option	Description
Load MS spectra:	Opens a dialog to load/import new MS data
Printer Setup:	Allows definition of parameters for the default printer defined in the Windows Control Panel.
Print:	Prints the current contents of the viscosity window to the default printer. The graphics are always printed in WYSIWYG mode, i.e. the current window display will be printed identically as shown.
Page Preview:	Shows a print preview in a separate print-preview window, which can be sized and moved. At the same time this command copies the preview contents to the Windows clipboard. When printing in portrait format, the graphics and the text for the measurements will shown, while landscape format will print only the graphics.
ASCII Save as:	Saves the currently in the MS window displayed data as ASCII file for use in other applications or for the transfer data to other computer platforms.
Edit Comment:	Allows to enter a MS data related text (comments, hints for data treatment, data processing details, observations, etc.). Up to 1024 characters can be entered for each injection (sample) separately.

The **Options** menu contains functions for MS data evaluation:

Table 66 Analysis menu

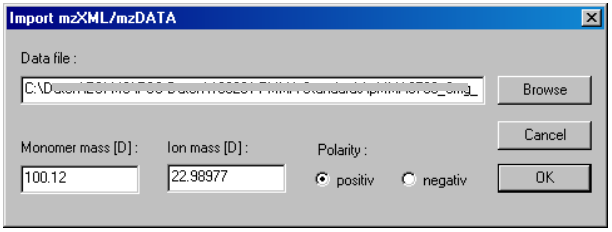
Option	Description
Editor Slice Data:	Transfers the displayed data to the WinGPC Software data editor.
Component:	Number of components (currently supported: 1)
Light scattering	*Menu item not available*
Viscometry	*Menu item not available*
Mass spectrometry	
Settings	Dialog offers entry/correction of molar masses for repeating unit and ion. 
Data to Calibration	Creates a calibration curve based on current EIC.
Calculation limits	Sets MS-Display parameters in x and y – axes

Import of MS Data

MS data need to be imported just once and will be combined with the concentration signals aquired by WinGPC Software:

1 Load WinGPC data and set baseline and integration limits.

2 Open MS window ( ) and choose **File > Load MS spectra**. Select corresponding data in the following dialog (file format dependent on MS manufacturer, e.g., *.RAW) and enter molar masses of repeating unit and ion.



3 Check the detector delay in the **Method** Window and adjust if necessary.

MS data formats of following software packages can be imported:

- mzXML
- Agilent Mass Hunter (.d/.D)
- Agilent ChemStation (.D)
- Thermo xCalibur (.raw)
- Waters MassLynx (.raw)

For ChemStation Import, the scan quality can also be selected.

WinGPC Software data (concentration signal) has be imported/opened first.

Performing MS Analysis

The evaluation and assignment of charge states, degree of polymerization and molar mass of endgroups will be calculated automatically (prerequisite: correct detector delay, information about molar masses of repeating unit and ion).

The green fraction marker can be used to display a mass spectrum for each elution volume. Base peaks or certain structures may be selected using the yellow EIC markers and displayed as chromatogram.

19 ChromPilot System Control

The WinGPC Software ChromPilot is a powerful tool to actively control and manage GPC systems. The control functionality is available for Agilent GPC/SEC instruments/systems. Licenses of predecessors of Agilent WinGPC may contain instrument control for systems of other vendors as well (such as Aurora, ERC, Sedere, Shimadzu, Shodex, SofTA, Thermo/Dionex, Tosoh, Varian/PL, VICI/Valco and Waters). All references to systems of other manufacturers within this manual apply to older WinGPC licenses only.

This option is integrated in WinGPC Software and any existing WinGPC Software license can be upgraded easily. The ChromPilot option can control up to 4 complete systems per software license via the existing communication ports.

WinGPC Software ChromPilot can only be used with 3rd party systems if the ChromPilot and at least one driver package for the above-mentioned GPC/LC systems was purchased and activated in the WinGPC Software license key.

Additional driver packages may be licensed at any time without the need to re-qualify the software installation. Your local Agilent representative will be able to assist you with this.

For details regarding the supported systems and modules as well as their configuration please confer chapter "Overview of Controlled Systems" on page 541.

The ChromPilot consists of 3 major dialogs:

Table 67 ChromPilot dialogs

Dialog	Description
Configuration Manager	select and configure systems/modules for control
Instrument Manager	shows system/module actuals, setpoints and actions
Sequence Manager	allows to run samples but also offers workflow control

NOTE

If Dionex systems shall be controlled, according Dionex software modules need to be installed separately from the installation medium. Please refer to the WinGPC Software System Control documentation for Dionex systems for detailed information.

Compliant with ChromPilot

Please note that some WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. For further information consult the chapter "WinGPC Compliance " on page 507 of this user guide.

User Level Settings for ChromPilot

Configuration Manager

Access is regulated by the user admin right **Add WinGPC Software User**. In the default Administration Database configuration this right is set for **Administrators** and **Advanced Users**, but may be altered for each user individually (i.e. deny the right or even allow it for a **User**). The misuse of the **Add WinGPC User** right is prohibited if the access to the Administration Database is disabled (default for all but **Administrators**). See also chapter „WinGPC Users Screen“ on page 524.

Instrument Manager

Administrator:	full access
Advanced User:	full access
User:	load settings, direct control (switch modules on/off, perform purge and zero), view logbook
Guests:	no access

Sequence Manager

Administrator:	full access
Advanced User:	full access
User:	load sequences, enter samples; not allowed: enter/alter change conditions, end actions
Guests:	no access

ChromPilot Configuration Manager

Configuration changes/modifications may be done either on WinGPC Software startup or later on during the session if the system to be configured is not in run state. The Method Window menu item **Method > ChromPilot Configuration** will open the **Configuration Manager**. If the **Configuration Manager** shall not be displayed (skipped) during a regular WinGPC Software startup, it may be disabled (and enabled again) by removing (adding) the tick mark in the Method Window menu **Method > Show ChromPilot on startup**.

Instrument Configuration

Make sure that the hardware of all GPC/LC systems which shall be configured in WinGPC Software, ChromPilot is correctly installed and connected to the PC or network according to the corresponding WinGPC Software system control documentation.

NOTE

A separate WinGPC System Control documentation is availabe in the **Documentation** folder of the installation medium. Please prepare your system according to this documentation.

On first startup of WinGPC Software with ChromPilot option all controlled instruments need to be configured correctly. After clicking on the WinGPC Software shortcut, the authentication dialog will open. The next three dialogs (WinGPC Software Wizard, login screen and UDC Device Connection dialog) are optional. If activated in the **Method** Window, the WinGPC Software ChromPilot **Configuration Manager** (see second Figure below) will show after the UDC device connection dialog. The initialization process may take some time, the progress is indicated by a status dialog as shown in the adjacent Figure.

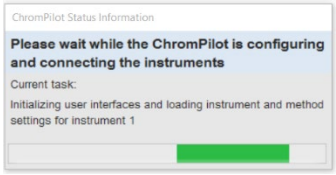
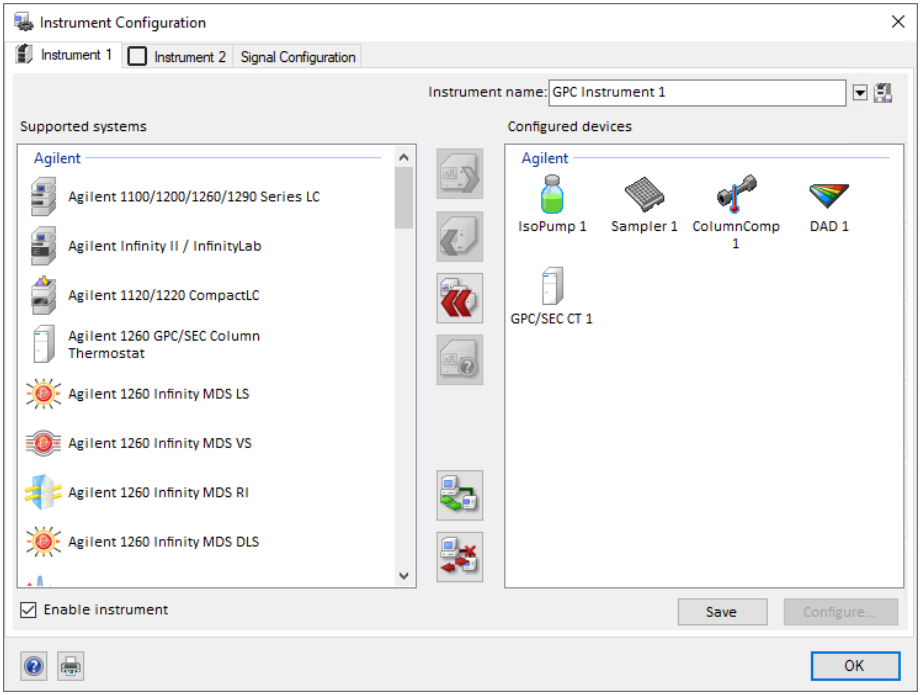


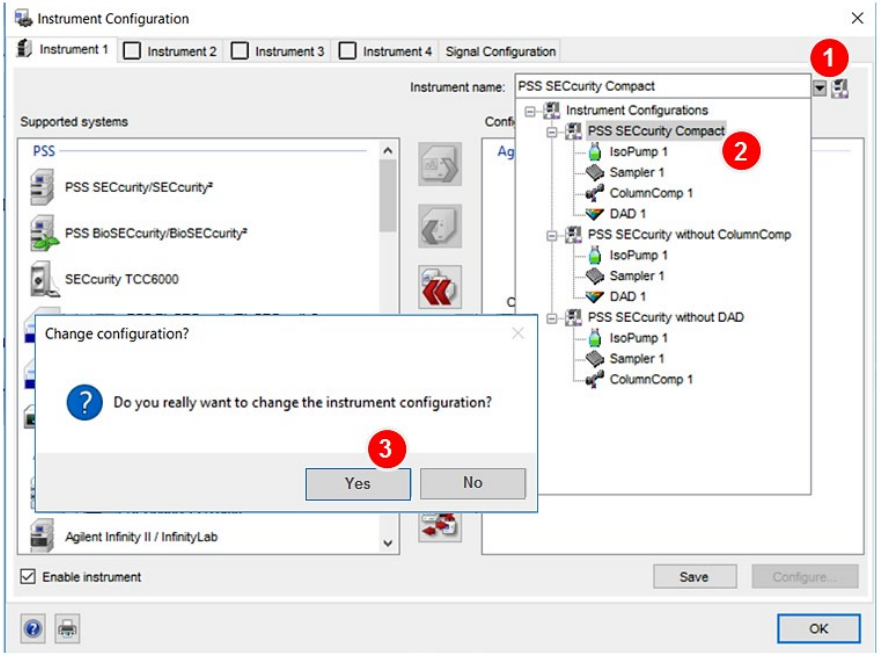
Figure 66 Status dialog – configuration initialization

The WinGPC Software ChromPilot **Configuration Manager** screen shown in the following Figure is divided into two main sections with all supported systems (manufacturers) on the left hand side and the configured modules of a given instrument on the right hand side.



The maximum number of controlled instruments will be indicated by the number of tabs (Instrument #) which are available. It depends on the number of instruments which were activated in the WinGPC Software Login screen (limited by liscence key). By clicking on one of the tabs, the corresponding instrument layout will be shown on the right side of the **Configuration Manager**. The configuration status of each instrument is indicated by the symbol on the specific tab (configured and enabled , configured but disabled , not configured ☐). The last tab, **signal configuration** is needed for digital data acquisition. This tab can be used to define and preconfigure the signals which shall be recorded during data acquisition.

Two buttons at the left bottom of the window are used to open the online help function ( ) and to print ( ) the actual configuration of all instruments with description, serial numbers, firmware and address (connection type) of all modules. A new function is the drop-down menu button of the instrument name box (top right). Here you can load/ change already configured devices (see next Figure). But before one can load/ change an already existing instrument configuration, you have to at least once save a configured device. Therefore, provide a name for the devices and confirm the configuration by pressing the **Save** button (bottom right).
Note that once saved, the different instrument configurations will be shown if you left-click on the drop-down menu button (1).



To load/ change to an already configured devices left mouse-click on the **+** Symbol in front of *Instrument Configurations* (will show all saved devices), select the desired instrument configuration by a left-double click on the instrument name (2) and confirm the following dialog (3) with **Yes**. The **+** or **-** Symbol in front of the saved instrument will show or hide the complete device configuration, respectively. With a right mouse-click on the saved instrument name you are able to delete the configured Instrument.

Instrument Activation / Deactivation

An instrument will be treated as "controlled", if the tick mark **Enable Instrument** on the lower left side of the **Configuration Manager** is activated and at least one configured module is visible for this instrument tab on the right hand side of the **Configuration Manager**. If you uncheck the **Enable Instrument** checkbox, the respective configured devices will be greyed out and the system will be treated as an uncontrolled instrument (no *ChromPilot* and *Sequence* – button available).

If the **Configuration Manager** is opened during a running WinGPC Software session, the additional buttons **reconnect** () and **disconnect** () are available. If a system is deactivated using the disconnect option, the instrument will still be treated as a controlled instrument, but none of the modules will be displayed as available in the **Instrument Manager** (red status diode, documented as "Connection state: Disconnected, Ready state: Error, Reason: Communication error"). The reconnection will be achieved using the reconnect button. If a system is disconnected due to slow response of the communication ports, ChromPilot will attempt to reconnect automatically without any user input.

Adding or Changing System Configurations

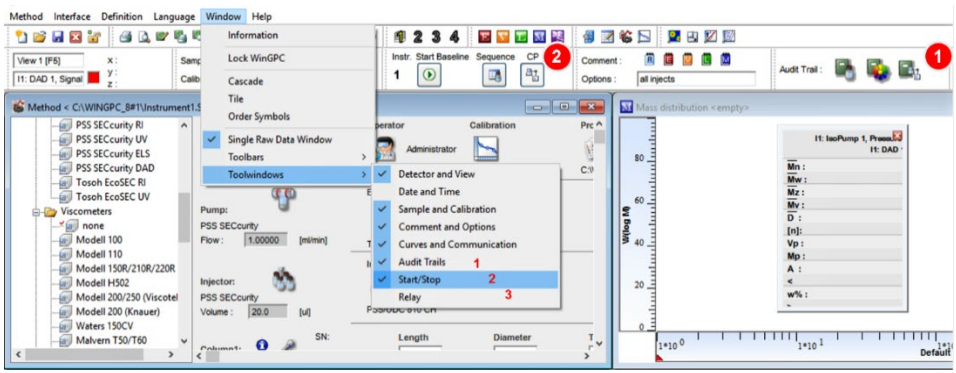
Each instrument which is licensed by WinGPC Software may be controlled if ChromPilot with at least one driver package was purchased. In order to define the instrument which shall be configured, the respective tab (e.g., "Instrument 1" or "Instrument 2") needs to be chosen and enabled first (check tick mark **Enable instrument**). A new module/system can be added by choosing the respective item on the left hand side of the **Configuration Manager** (see **Supported Systems** box), then the **add module(s)** button () will get available if the driver package is licensed and the module(s) may be added. Depending on the module type (manufacturer) the following configuration will be processed automatically after the definition of the communication ports or requires additional user input. Please refer to the respective WinGPC Software System Control documentation in the **Documentation** folder of the installation medium for further details of the specific system. This document can be viewed using the **help** button (), a PDF version is located in the WinGPC Software program folder in the subfolder **Documentation\en**.

As soon as the initial configuration has finished, the configured modules are visible as **Configured Devices** on the right hand side of the **Configuration Manager** and furthermore the empty box in front of the respective instrument tab shows now a device symbol. The configuration can be modified by selecting the respective module and pressing **Configure…**. If a module shall be removed, the button **remove module** () needs to be pressed. The button **remove system** () will remove all configured modules of the actual instrument. ChromPilot also allows to configure mixed systems which are connected via different communication ports, if they belong to the same driver package (e.g., SECcurity GPC1260 with SECcurtiy TCC6000 and SECcurity ELS1400) or if they consist of modules from different manufacturers (e.g., Agilent GPC system with SECcurity TCC6000 and Shodex RI detector) as long as all driver packages are activated.

To minimize time and effort for the configuration of instruments you can save your configured devices and load / change them by using the new drop-down menu function of the Instrument Name box (see chapter “Instrument Configuration” for details).

WinGPC Software Method Window with ChromPilot

Controlled systems will start with a specifically designed Method Window. For each instrument which was defined as a controlled instrument, the Toolwindow (**Start/Stop**, **Audit Trails** and **Relay**) are modified. With default settings the Toolwindow **Start/Stop** (2) and **Audit Trails** (1) are shown in the status bar (available by **Window > Toolwindows**), as indicated in the following Figure.



Start Baseline Record starts data acquisition, but not the autosampler sequence. The **Sequence** button opens the **Sequence Manager** dialog which is used to define, start and stop sequences. A button for the ChromPilot you can find either on the upper left side of the **Method** Window or in the Toolwindow **Start/Stop** (see **CP** button). Both buttons open the ChromPilot **Instrument Manager**.

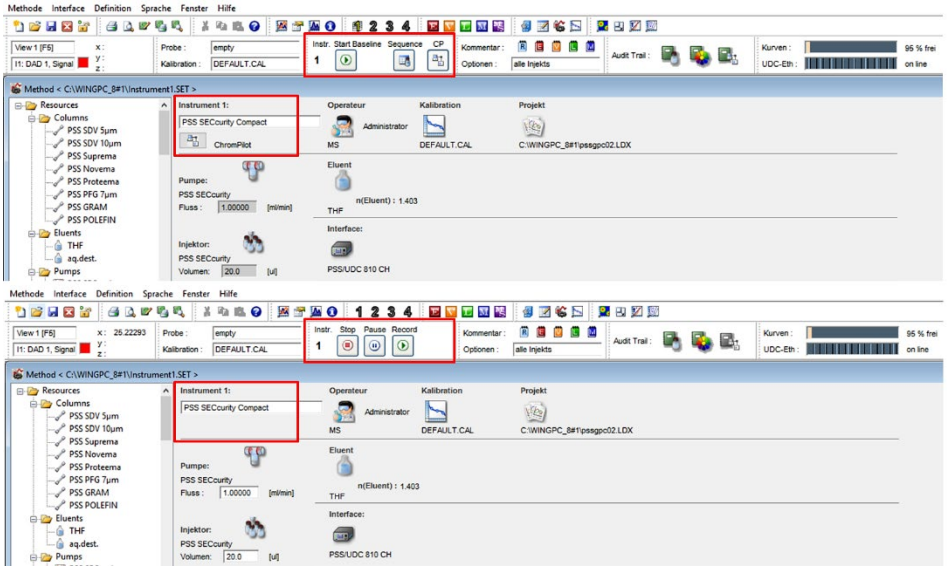


Figure 67 Method Window for controlled (top) and uncontrolled instruments (bottom). Compare the indicated red areas for details

Some fields of the instrument layout (**Method** Window) are filled automatically using the the settings which are defined in the ChromPilot *Instrument Manager*. These settings are flow rate and column temperature as well as the injection volume which is taken from the **Sequence Manager**. Latter will be updated for each injection.

NOTE

Controlled and not controlled instruments can be used in parallel. For not controlled instruments, the layout is the same as in WinGPC Software versions without ChromPilot.

ChromPilot related menu items of the **Method** Window (subentries of the **Method** menu) are:

Show ChromPilot on startup	Activates/deactivates display of **Configuration Manager** on WinGPC Software startup (active: indicated by a tick mark)
ChromPilot Configuration	Opens ChromPilot Configuration Manager

ChromPilot Instrument Manager

The button **ChromPilot** in the **Method** Window (or use additional button **CP** integrated in status bar) opens the ChromPilot **Instrument Manager**. This dialog will always be shown for the current instrument. The Instrument number and Name (as entered in the **Configuration Manager**) is displayed in the title bar. In addition, the file name of the actual instrument settings (*.spm file) is shown. If the settings were not saved as a specific file, it will be displayed as **DEFAULT**, saved and modified settings will be marked as **modified**.

The dialog consists of a **System View** (compare **System** tab) which displays all configured modules with the most important parameters at one glance, and - depending on the driver package - of a **Module View** with in depth details for modules which are supported by special rc.NET drivers (information on the availability may be taken from the respective WinGPC Software system control documentation). Switching between different views is realized by different tabs (**System** for the **System View** and the name of the respective module for its detailed **Module View**). A menu above the tabs (**File** or **View**) allows to manage (load/save) different settings or to access a detailed logbook.

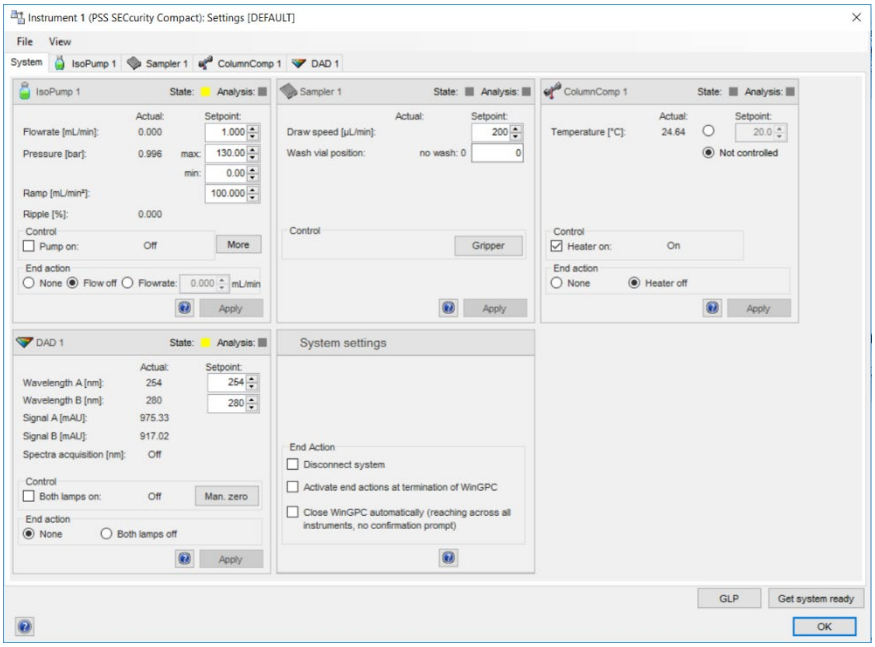


Figure 68 ChromPilot Instrument Manager System View

The standard view is the **System View**. Status information may be viewed and parameters changed in the same dialog. End actions may be activated/deactivated for each module separately. As soon as all modules are "ready" (TIP: use **Get System Ready** button for a global get ready of all modules), you may close the dialog with **OK** in order to start the sequence in the **Sequence Manager**. The flow rate of the pump, the column oven temperature, and the injection volume (taken from the **Sequence Manager**) are transferred to the **Method** Window automatically.

Each panel of the **System** tab shows an online help-button, where you can find additional information for the respective module.

NOTE

The **System-** user interface (**System** tab) shows now a new panel for *System Settings* (see Figure above), which allows the definition of additional end actions, e.g., "Disconnect system", "Activate end actions at termination of WinGPC Software" and "Close WinGPC Software automatically". Only the first one requires additional activation in the Sequence Manager, the last one even reaches across all instruments and will shut down WinGPC Software without further confirmation prompt as soon as the sequences on all instruments are finished.

Instrument Manager Menu

The **File** menu allows to organize and print instrument settings:

Table 68 File menu

Setting	Descriptions
Load settings...	Loads a complete parameter set of all configured modules (warning "Instrument settings for at least one device could not be found in file [filename].spm" if one or more modules doesn´t match). Settings are loaded, but not executed directly (requires **Get system ready** or **OK**)
Load settings with comparison...	Loads a complete parameter set and displays a dialog with deviating parameters for each module. New parameters can be acknowledged (**Send from ChromPilot to instrument**) or discarded, if current values shall be kept (**Send from instrument to ChromPilot**). If **Send from ChromPilot to instrument** is selected, those values will be loaded and sent directly (no additional **OK** necessary).
Save settings...	Saves the complete parameter set of all configured modules (instrument settings) as a *.spm file including end action definitions and timetables, but not the direct control status (e.g., pump on)
Load previous logbook...	Loads WinGPC Software *.log files as a logbook history file; only active if **View** is set to **Logbook**
Print	**Method...**: Prints the actual instrument settings (module parameters), including timetables. **Device config...**: Prints the actual module configuration (same as print button in Configuration Manager), including serial numbers, firmware etc. **Logbook**: Prints the actual logbook; only active if **View** is set to **Logbook**.
Close	Closes Instrument Manager dialog

The **View** menu is used to switch between instrument settings and logbook information:

Table 69 View menu

Setting	Descriptions
Settings	Shows the (default) **System View** or (if available and selected) the **Module View** of one of the configured modules
Logbook	Shows the actual logbook on the right hand side and available EMF information about the system on the left hand side (see chapter "Logbook and GLP/EMF Information" on page 484); menu items **File > Load previous logbook...** and **File > Print > Logbook** will be active in this view

System View – Entering Module Parameters

The ChromPilot **Instrument Manager** will always start with the **System View** and the setpoints used for the last measurements (on first initialization after installation default setpoints as integrated in the driver package will be used – for details review according WinGPC Software System Control User Manual.

The **System View** user interface is subdivided in several panels with dynamic arrangement (exceptions: Dionex systems have a different user interface, in addition some special modules will only be supported in a module view. Please refer to the separate WinGPC System Control documentation in the **Documentation** folder of the installation medium for details).

Each configured module will be displayed with its actual and set values, a direct control box and (if applicable) end action definitions (see chapter "System Settings Defined by End Actions" on page 481). Two status diodes on the upper right side of each module panel inform about the actual status of the module and of the sequence.

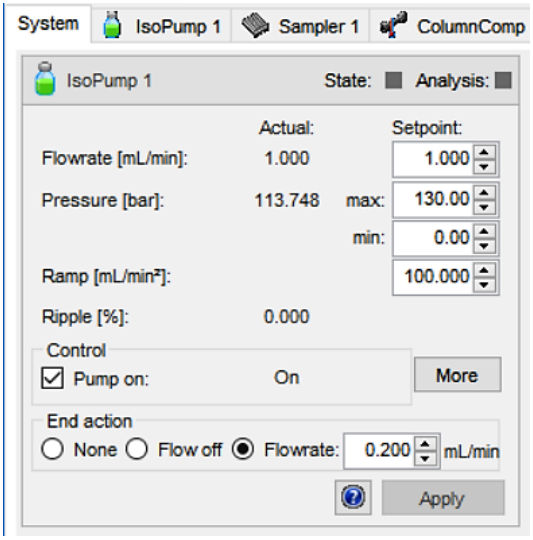


Figure 69 ChromPilot Intrument Manager System View (Panel: Iso Pump)

The adjacent Figure shows an example of the user interface for one module which is displayed in the **System View**. The upper left side will always display the type and number of the module (here: "IsoPump 1"), it is possible to configure more than one module of the same type (e.g., pumps, column compartments…) which will be numbered automatically. Setpoints and actual values of module specific parameters are displayed in the upper part of user interface. Setpoints may be entered/edited by clicking into the value field and typing in a number or using the up/down controls. To apply the changes and to send them to the system the **Apply** button has to be pressed.

NOTE

The **Apply** button will be enabled only if a parameter field has been edited and left again (e.g., by using the tab key or by moving the cursor to a different field). Only **Apply** will send the modified setpoints to the system.

Two group boxes provide special functions:

Control box:

The direct control group box contains commands which are sent directly to the system, e.g., setting the tick mark for **Pump on** will switch the pump on or pressing the **Zero** button of a detector will execute a zero directly without pressing the **Apply** button. The functions/status of the direct control group box are not part of the instrument settings which may be saved as *.spm files.

End action box:

Several modules provide optional end actions. Those will be defined in the end action group box and need to be acknowledged by pressing the **Apply** button. The end action definitions are part of the instrument settings which may be saved as *.spm files. End actions will either be executed at sequence end or on WinGPC Software shutdown (see also chapter "Definition of End Actions" on page 481).

NOTE

If the end actions which are defined in the **Instrument Manager** shall be executed after a sequence is finished, it is necessary to activate them in the **Sequence Manager** as well. Alternatively, they may be executed on WinGPC Software shutdown, then the tick mark on the **Activate end actions at termination of WinGPC Software** checkbox in the panel **System Settings** part of the **System** user interface needs to be checked.

Definition of End Actions

Instrument specific end actions (e.g., reducing the flow rate or switching off the UV lamp after the sequence) can be defined in the **Instrument Manager**. If such an end action shall be performed, ensure that it is activated in the **Instrument Manager** (radio button) and in the **Sequence Manager** as well (see also chapter "System View – Entering Module Parameters" on page 479). The checkbox for activation of selected end actions in the **Sequence Manager** is located on the lower left side below the sequence table.

Possible end actions:

- Switching off the pump
- Reducing the flow rate
- Switching off the thermostat of thermostated autosamplers
- Switching off the UV lamp
- Recycle the eluent (only SECcurity/Agilent/HP RI detector)
- Switching off the heater of the column compartment (for EcoSEC: pump compartment as well)
- Switching a valve to a user-defined "standby" position
- Disconnect system (selectable in the **Instrument Manager**)

System Settings Defined by End Actions

Following end actions will influence the WinGPC Software session after sequences are finished:

- Disconnect system

  The instrument will be disconnected after execution of all other end actions, thus the instrument will be available for other users again. This end action has the same function as the respective button in the **Instrument Manager**, the instrument can be reconnected using the **reconnect** button:

   (disconnect)  (reconnect)

  ⇒ Additional requirement: Activation of end actions in the Sequence Manager

- Activate end actions at termination of WinGPC Software

  If end actions are defined for the current instrument (e.g., reduce flow rate), these will be executed independent of an activation in the Sequence Manager at the termination (shut down) of WinGPC Software.

  ⇒ Requires no additional activation in the **Sequence Manager**.
- Close WinGPC Software automatically (reaching across all instruments, no confirmation prompt)

  After all sequences are executed, WinGPC Software will be closed. This end action reaches across all instruments, i.e. it will automatically be activated or deactivated for all controlled instruments. All measurements / calibrations currently loaded for evaluation will be saved and closed without request for confirmation.

  ⇒ Requires no additional activation in the **Sequence Manager**.

Status Information

The **SystemView** offers to check the status of all configured modules at one glance (except for light scattering detectors). The user interface component of each module shows two status diodes on the upper right side. The first one indicates the state of the module itself, the second displays the state of the sequence (status of analysis). Details on the state (e.g., reason for a not ready state (yellow diode) or an error (red diode)) will be shown as a tooltip (TIP: place the mouse cursor over status diode). An overview on different states is shown in the Table below.

Table 70 Module states

Icon	Description	Meaning
Module Status		
■	module ready	module fully operational
■	module ready	module operational and in run status
■	module not ready	module setpoints not reached
■	module error	communication error (disconnected), leak, etc.
Analysis Status		
■	no analysis is running	no sequence started
■	analysis is running	sequence started and running
■	analysis is running and paused	sequence is paused (data acquisition still active); sequence will continue when start button is pressed again

The sequence will not start unless all configured modules are in ready state. To initialize the modules, the individual direct controls of the single modules or the global **Get system ready** button might be used. However, the sequence start command () can be issued even if the system is in **not ready** (■) state. In that case, the system tries to get all modules ready (e.g., bringing the temperatures into the accepted range) and then it will automatically process the first line of the sequence table without further user interaction.

During a sequence the next injection will not start if one of the modules has a state change to **not ready** or **error**. This will be reflected in the WinGPC Software *Sequence Manager* **System** status LED being *yellow* (■) or *red* (■). Only if the system cannot get itself back to normal (**ready** ■) it will ultimately time out. However, if an error has been recorded (**Alarm** ■) in any of the modules the sequence will terminate. In such a case open the WinGPC Software **Instrument Manager** and get the system ready.

Logbook and GLP/EMF Information

In order to get comprehensive information about the status of the GPC system at the moment of a measurement, the ChromPilot monitors EMF (Early Maintenance Feedback) data as well as the complete communication between WinGPC Software and the modules of each instrument. These data can be viewed by the menu item **View > Logbook** of the **Instrument Manager**. The logbook described in this section is an Instrument Audit Trail. Additional logbooks/audit trails are described in chapter "Audit Trails and Revision History" on page 518.

Helpful information regarding status changes and error messages may be viewed using the comfortable logbook filter functions.

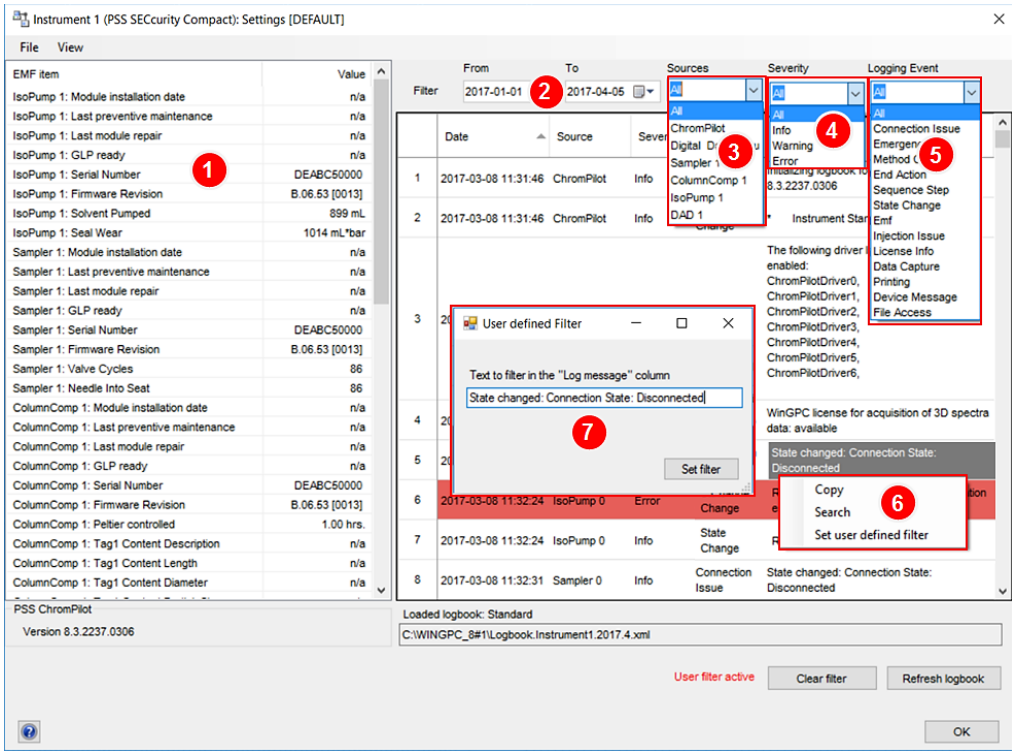


Figure 70 Logbook view of the ChromPilot Intrument Manager

The left-hand side (Figure 70, 1) of the logbook dialog gives instrument specific information about the GPC system (e.g., lamp burn time, total number of injections, serial numbers and firmware version). The kind of information available and displayed depends on the system. For details refer to the according WinGPC Software System Control User Manual.

A detailed logbook is shown on the right-hand side of the logbook dialog. It contains the communication between ChromPilot and the respective system, including sample injection times and names. Status information / changes and error messages will be logged as well.

The logbook is displayed as a table with comfortable filter functions. The available columns and filters are listed below:

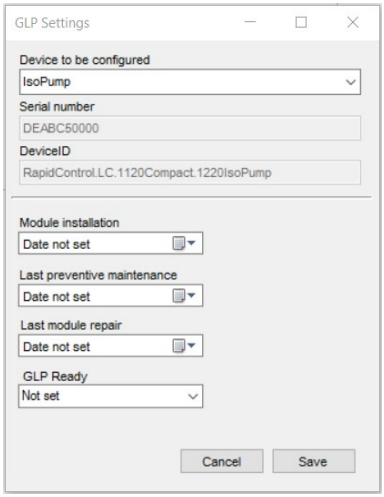
Table 71 Columns and filters of the ChromPilot Intrument Manager (Figure 70)

Item	Name	Filter Name and Meaning
(2)	Date	period from/ to
(3)	Source	Source of entry (e.g., ChromPilot, instrument or module name)
(4)	Severity	Meaning (Info, Warning or Error)
(5)	Logging Event	Kind of event (e.g., Connection Issure, Emergency, Sequence Step)
(6)	Log Message	User defined filter: mark entry and click with right mouse button. Context menu (6) offers **Set user defined filter**. Enter in following dialog (7) your own search criteria. The filter can be reseted by pressing the **Clear Filter** button (below table).
(7)	Date	period from/ to

The logbook has the function of an Instrument Audit Trail and is saved monthly as an externally encrypted file for each instrument. Each instrument has an own logbook, so up to four parallel instrument logbooks might exist. The actual logbook can be viewed by pressing the button **Refresh logbook**, older files can be loaded via the menu item **File > Load previous logbook…**. The files are always automatically encrypted and saved in the wingpc_8#1 folder and are named "Logbook.Instrument[Instrument#].[year]. [month].xml", thus e.g., the December 2013 logbook of Instrument 1 would be "Logbook.Instrument1.2013.12.xml".

GLP Information

In addition to the automatically logged information about the system you may also enter GLP information. This information is entered via the **View > Settings** dialog of the **Instrument Manager**. Press the **GLP** button on the lower right side of the window and enter all necessary/available information about installation date, preventive maintenance or repair for each configured module in the upcoming dialog (see next Figure).



ChromPilot Sequence Manager

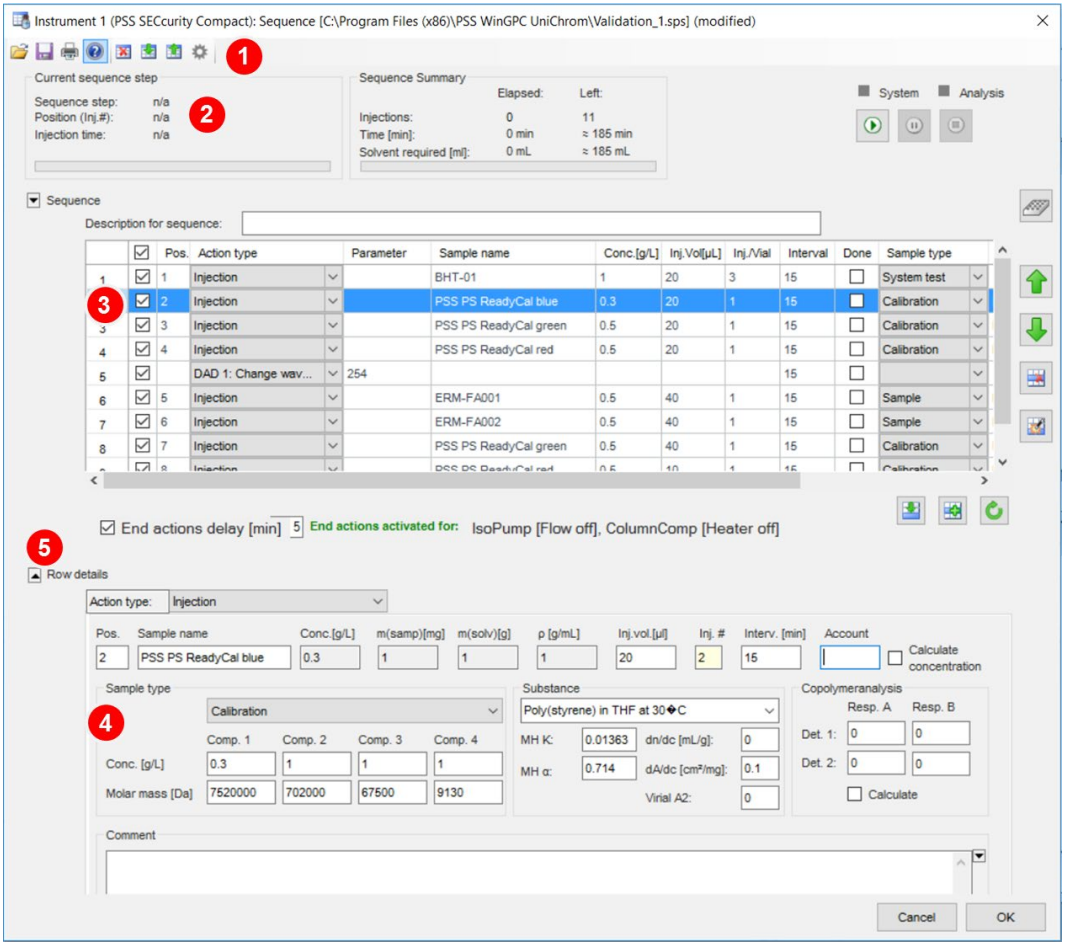


Figure 71 ChromPilot Sequence Manager

The **Sequence Manager** can be used to create, manage, start and stop sequences. For this reason, the **Sequence Manager** is the most prominent window during data acquisition. The Sequence Manager is divided into different sections:

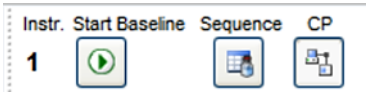
- The title bar displays (in accordance with the Instrument Manager) the instrument number and name as well as the file name which was used to save the sequence. Sequences which are not saved yet are marked as **DEFAULT**, saved/loaded and modified Sequences will be shown file name and the appendix **modified**.
- The Icon bar (Figure 71, 1) offers different import, export and print functions as well as a Settings-button to set default parameters for intervall and injection volume (see for details chapter "Sequence Manager Icon Bar" on page 490)
- A status area (Figure 71, 2) gives an overview on the current sequence step and sequence specific informations, e.g., summary about estimated/ elapsed time and solvent amount (see chapter "Sequence Manager Status Bar" on page 491).
- The Sequence table (Figure 71, 3) contains all sample information and (if applicable) programmed change conditions and sequence commands within the sequence (see chapter "Sequence Table" on page 492). Directly under the sequence table you will find the checkbox for activation of end actions, the delay time, and all selected end actions.
- An entry mask (Figure 71, 4) offers a detailed view on the currently selected sequence table row in order to ease the user input (see chapter "Creating a Sequence – Entering Sample Information" on page 495 and "Creating a Sequence – Using Change Conditions" on page 499).

NOTE

If the Entry mask is not visible you have to click on the button for Row details (Figure 71, 5), which is located on the lower left side of the **Sequence Manager** window.

Starting the Sequence Manager

The Toolwindow **Start/Stop** (available by **Window > Toolwindows > Start/Stop**) changes when a mixed combination of controlled and non-controlled GPC systems is used. WinGPC Software shows the standard Toolwindow **Start/Stop** for each non-controlled instrument. The **Start/Stop** Toolwindow for controlled instruments shows three buttons (see next Figure) - one for **Start Baseline** record, one called **Sequence** and on for the ChromPilot called **CP**.



The **Start Baseline** record button starts the data acquisition to record the baseline without injecting any samples. The baseline record may be stopped with **Stop Baseline** (pressing the same button again) or continued by a regular sequence start. If a sample sequence shall be entered and/or started, press the **Sequence** button. This button opens the **Sequence Manager**. In this case, the Sequence Manager substitutes the WinGPC Software Sample Editor. The **Raw Data** Window menu item **Editor > Samples** will also open the **Sequence Manager** for the actual sequence. Finished sequences will automatically be edited in the WinGPC Software Sample Editor. It is not necessary to start (or stop) a baseline record prior to the sequence. If the data acquisition is already started, it will be continued. If data acquisition was started with the **Start** button of the **Sequence Manager**, the **Start Baseline** button is not available if a sequence is running.

NOTE

If data acquisition is started with the **Start Baseline** button and afterwards the sequence is started, this button will not be shown during data acquisition and the baseline record is automatically stopped by end of the sequence. In contrast to the older version *WinGPC Software 8.2*, where the **Start Baseline** button will be available throughout the sequence and data acquisition needs to be stopped manually by this button as well (otherwise the baseline will still be recorded after end of sequence, even if end actions were executed)!

Hint for *WinGPC Software version 8.3 or higher*: If you need to extend baseline records after specific injects, it is useful to insert a Sequence Command called **WinGPC Software: Pause sequence**, which allows a baseline record for a certain time with the opportunity to activate the user acknowledge option.

Sequence Manager Icon Bar

Sequences or partial sequences may be saved and loaded / combined again. The icon bar offers administration and print functions (see also chapter "Import and Export Options for Sequences" on page 504).


Opens a ChromPilot sequence (*.sps file), overwrites current sequence


Saves the current sequence as *.sps file


Prints the current sequence


Opens the ChromPilot help

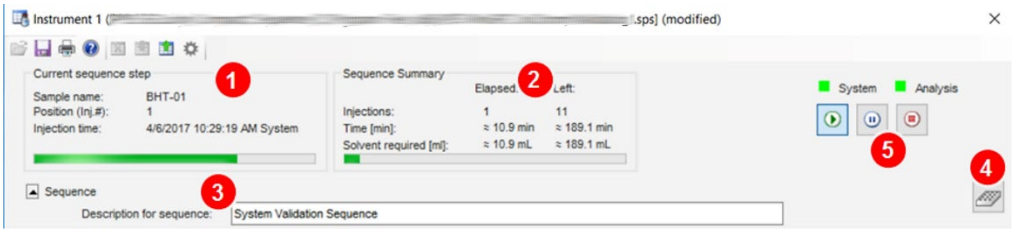

Clear table – deletes the current sequence


Import – can be used to import *.sps files afters a selected sequence row or to import WinGPC Software *.txt sequences which were created without ChromPilot


Exports the current sequence as *.sps or WinGPC Software *.txt file


Default Settings – set the interval time and injection volume

Sequence Manager Status Bar



The status bar (see Figure above) displays the status of system and sequence as well as general information about the sequence.

The section **Current sequence step** (1) contains information about the current sequence step (sample name, position with injection number and the injection times which were recorded from the autosampler (system) and from the interface).

If system and interface time differ more than 20 seconds, an error will be logged in the logbook and a wrong injection will be marked in the sample name as **wrong injection trigger**.

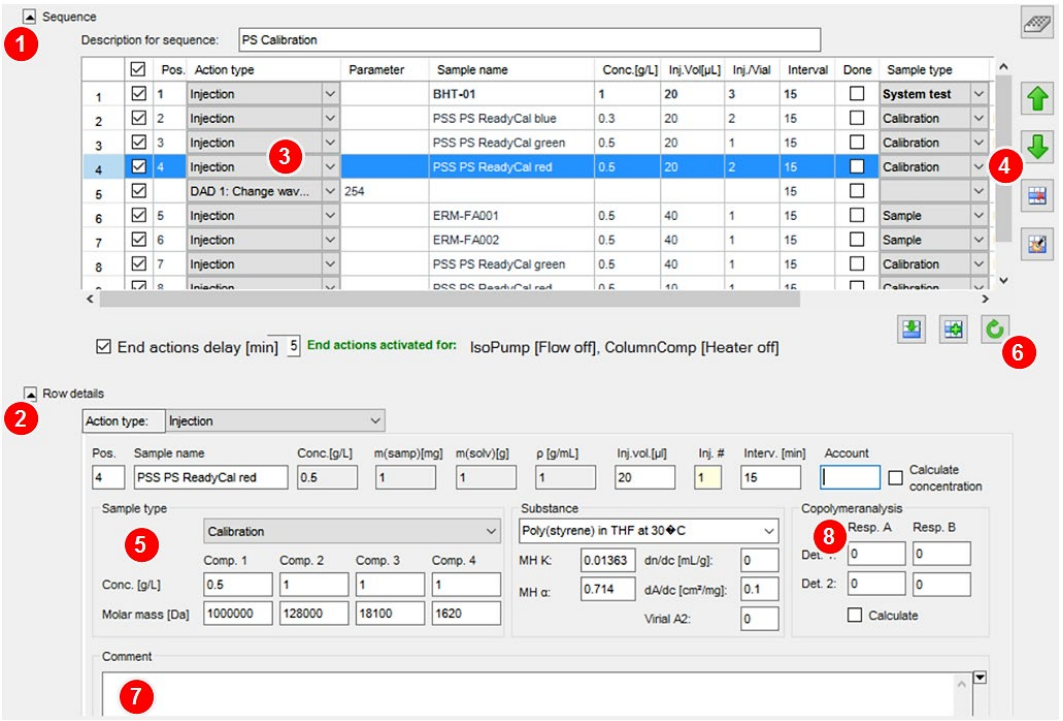
The section **Sequence Summary** (2) gives an overview on the total number of injections, the estimated total run time and solvent consumption before initialization of the sequence. Once the sequence is started, the **Sequence Summary** box shows the already elapsed/ remaining time, solvent amount and number of injects. The effective run time and solvent consumption might differ, because the get ready times for autosamplers and other modules cannot be calculated in advance. All wait times which are defined within the sequence (e.g., after the execution of a sequence command) are included in the calculations.

Below the status bar a description for the actual sequence may be entered (3). This description will appear in the Project Manager as the description of the login (same function as **Raw Data > Name Raw Data** of the **Raw Data** Window). The sequence description cannot be modified during a running sequence.

The correlation of a vial position to a vial number may be visualized using the tray view button (4). For details refer to the chapter "Assignment of Autosampler Positions" on page 496.

Start and stop functions as well as color coded status information are located on the the right hand side of the status bar (5).

Sequence Table



The sequence table (see Figure above) contains all sample information as well as all change conditions (e.g., activation of different methods or execution of sequence commands). In addition, the complete sequence table can be shown or hidden by pressing the Show details button (1). To expand the information for a specific row of the sequence table you have select the row by a left-mouse click and if the row details are not visible below the table click on the Show details button (2).

The table itself (3) may be edited with adjacent buttons (4). Entries/changes in the entry mask (5) need to be acknowleged with the corresponding buttons (6) to transfer the changes to the table. These functions are described in the chapter "Edit Functions ChromPilot Sequence Table" on page 494.

Entries made in the comment field (7) will be used as a raw data comment for the respective sample. The section **copolymeranalyis** (8) is only visible if the copolymer module is licensed and activated.

Sequence Manager Columns

The display of the columns may be user defined by a right mouse click onto the column headers. Setting/removing the tick mark will activate/deactivate the columns. You may also user define the column width. The settings for column width and display will automatically be saved and adjusted for all instruments.

Following columns are available:

☑	Run check box: Always visible – marked entries will be executed
Pos.	Vial position (only action type **injection**) (see chapter "Assignment of Autosampler Positions" on page 496)
Action type	Action type (using drop-down menu different options selectable (e.g., injection, sequence command or load WinGPC Software method/ instrument setup)
Parameter	If action type ≠ injection: value of new parameter (e.g., wavelength)
Sample name	Sample name (only action type **injection**)
Conc. [g/L]	Concentration in g/l (only action type **injection**)
Inj.Vol.[µl]	Injection volume in µl (only action type **injection**)
Inj./Vial	Number of injections per vial (only action type **injection**)
Interval	Action type = injection: sample run time, other action types: wait time
Done	Will be set by system: selected rows are completed
Sample type	Sample type (drop-down menu only available with action type **injection**)
Substance	Substance (only active/ editable with action type **injection**)
Account	Account

Some sample information (position, sample name, concentration, injection volume, interval and account) may be entered and edited in the table itself, all other entries/changes will be performed in the entry mask (Row details) below the table. The mask (hidden by default) will always show the content of currently selected sequence row. Furthermore, some additional information may be entered in the mask which are saved in the table, but not displayed as a separate table column. This additional information may be displayed and modified exclusively in this entry mask. For details refer to chapter "Creating a Sequence – Entering Sample Information" on page 495 and chapter "Creating a Sequence – Using Change Conditions" on page 499.

Edit Functions ChromPilot Sequence Table

In addition to the save/load and import/export functions (see chapter "Import and Export Options for Sequences" on page 504) several edit functions for the sequence table are available. The corresponding buttons are located beside the table and on the right side above the entry mask (Sequence table).

To select/edit an existing sequence row click on it. The row will be highlighted, and the content displayed in the entry mask.

The available edit functions are described below.

Table:

Table 72 Edit function

Icon	Description
	*Move up*: move selected sequence row one position up
	*Move down*: move selected sequence row one position down
	*Delete row*: deletes selected sequence row
	*Sample Sequence Wizard*: opens Sample Wizard dialog for comfortable sequence generation (especially helpful if samples have similar parameters, position and sample name will be incremented automatically), action type injection, entries will be added to the end of the table

Entry mask:

Table 73 Edit function

Icon	Description
	Enters (appends) the selected entry (with all information currently entered in the entry mask) to the end of the sequence table
	Inserts a new row at the selected sequence table position (duplicates selected entry), can be used to enter priority samples during a sequence
	Overwrites selected entry with the information currently entered in the entry mask – Save current row

NOTE

As soon as a sequence is started, all sequence rows which have already been processed (indicated by a "done" tick mark in the table) or are currently being processed may not be edited anymore. If a sequence is stopped and restarted again before the regular sequence end, it will continue with the last row without "done" tick mark.

Creating a Sequence – Entering Sample Information

Sequence creation with WinGPC Software ChromPilot may be done according to the individual needs. You can enter each sample separately, (re)use and/or combine existing sequences (see respective functions in chapter "Edit Functions ChromPilot Sequence Table" on page 494) or create a new sequence using the Sample Sequence Wizard.

Most information could be entered directly into sequence table, but it is more comfortable, to use the entry mask. The information, which need to (or may) be entered are following:

Pos. *)	Vial position of the autosampler, (default: 1, automatically increased with each row); the mapping of the individual tray positions can be visualized using the button  (see chapter "Assignment of Autosampler Positions" on page 496)
Sample name *)	Sample name (max. 54 characters)
Conc. [g/L] *)	Concentration in g/l (default: 1); deactivated and filled automatically if **calculate concentration** is active
m (samp)[mg]	Enabled if **calc. conc.** is activated: sample weight in mg
m (solv)[g]	Enabled if **calc. conc.** is activated: solvent weight in g
ρ [g/mL]	Enabled if **calc. conc.** is activated: solvent density in g/ml
Inj.vol.[µl] *)	Injection volume in µl (default as last entry or for new tables as defined in the **Sequence Manager** Status Bar **Settings** section)
Inj. # *)	Number of injections for this vial (default: 1)
Interv. [min] *)	Injection interval in minutes (if less than sample analysis time: overlaid injection) (default as defined in the Instrument Manager)
Account *)	User defined account for this sample, e.g., an internal ID – useful for search operations (default: empty)
Calculate concentration	Tick mark may be set if concentration shall be calculated automatically using sample and solvent mass as well as solvent density; not available for sample types calibration and recalibration
Sample type	Useful for automation procedures and to differentiate e.g., between normal samples (just one component) and calibration samples with up to 4 components (list box, default: Sample); up to 4 components with molar masses and concentrations may be entered
Substance	Substance specific parameters for standard polymers (list box, default: empty), may also be edited manually; contains Mark Houwink coefficients, refractive index increment dn/dc, extinction coefficient dA/dc and second Virial coefficient A2; since WinGPC Software version 8.3, the substance list contains polymer classes as well to support e.g., more comprehensive statistics
Comment	Sample specific comment, will be transferred to the raw data comment

Copolymer-analysis	Only available if copolymer module is licensed and activated (for details see chapter "Copolymer Analysis Software Module" on page 429)

*) These parameters may also be edited in sequence table itself

Once all sample information is entered for a sample, this has to be confirmed by pressing and the sample will automatically be added at the last position of the sequence table. The vial position of the next sample will be incremented by one.

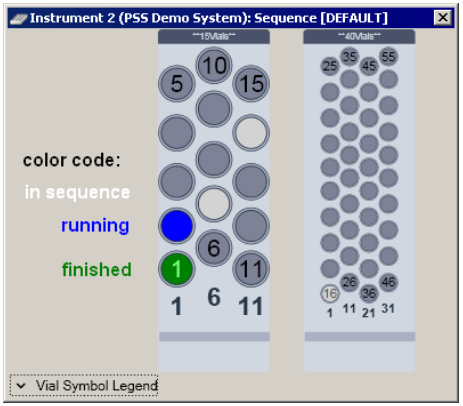
Priority Samples, Modifying Entries

Priority samples may be inserted, and entries may be entered even if a sequence is already started, provided that the position (sequence row) in question is not processed yet.

Using the *insert row* ( ) button priority samples may be inserted. A Sequence row that was already started cannot be modified anymore (buttons overwrite, insert, and delete are deactivated), but a copy may be added to the end of the table by pressing the *Append row* button ( ).

Assignment of Autosampler Positions

The WinGPC Software **Sequence Manager** is designed for vendor independent operation and use. Therefore, vial positions are always entered as numbers. The ChromPilot *Tray View* ( ) depicts the currently installed autosampler trays and shows the current status of the sequence and the vial position numbers which have to be entered as **Pos.** in the sequence table.



The title bar shows the instrument number, instrument name and sequence name. The sequence name is **DEFAULT** if the sequence table has not been saved. The designation **(modified)** in the title bar means that a saved sequence last been modified by the user.

The number in the vial is the WinGPC Software vial number, while the numbers at the tray bottom are the vendor specific vial numbers (e.g., the first vial in the right hand tray is vial position “1” while the number (vial position) to be entered in the ChromPilot is “16”).

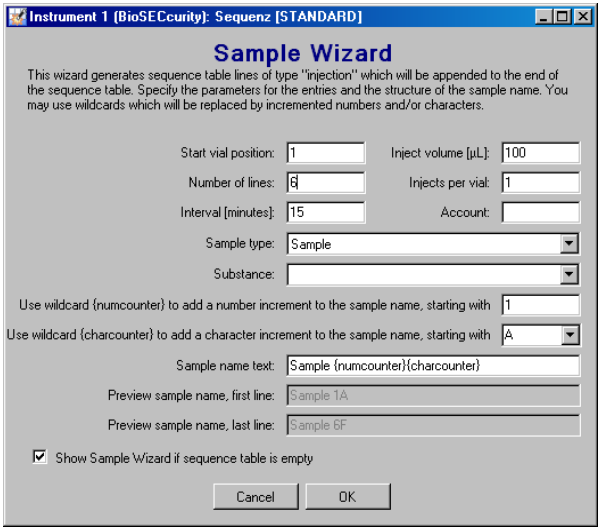
Vial position color codes:

Green	vial position already run
Blue	currently running sample
Gray	vial position entered in sequence but not started yet

For details on the supported autosampler trays refer to the specific WinGPC Software system control documentation.

Sample Wizard

If several samples with consecutively numbered names shall be measured using same conditions, the sequence may be created using the Sample Wizard. The dialog will be opened by pressing the *Sample Wizard* button () besides the sequence table. As an option the dialog will be shown automatically if the sequence table is empty.

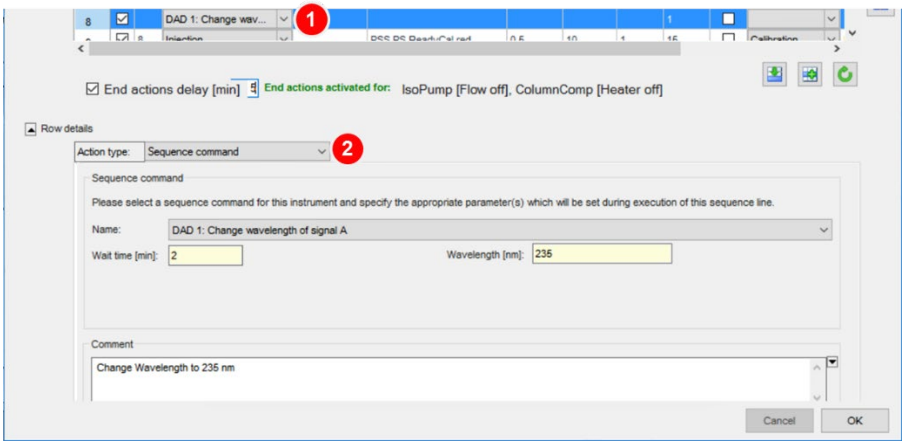


Numbers and/or characters can be used to increment the sample name. The entries will be added to the end of the sequence table.

Available entries are as follows:

Start vial position:	Autosampler position (default 1, will be incremented by 1)
Inject volume [µl]:	Incect volume in µl
Number of lines:	Sequence length (number of samples)
Injects per vial:	Multiple injection (default: 1)
Interval [minutes]:	Injection interval in minutes
Account:	Text field for account (default: empty)
Sample type:	List box for sample type, default: sample (for calibration samples we recommend to import preconfigured calibration sequences, since the Sample Wizard does not manage molar mass information)
Substance:	Substance specific parameters, list box (default: empty)
{numcounter} increment, start with:	Start value (number) for the counter (default: 1)
{charcounter} increment, start with:	Start value (character) for the counter (default: A)
Sample name:	Sample name (max. 54 characters less the counter characters), e.g., Samplename{numcounter}{charcounter}
Preview sample name, first line:	Preview displayed, but not editable (thus always greyed out), e.g.: Samplename1A
Preview sample name, last line:	Preview displayed, but not editable (thus always greyed out), e.g.: Samplename6F
Show Sample Wizard if sequence table is empty	If tick mark is set, Sample Wizard will be started if Sequence Manager is opened with empty table (e.g., after deleting all entries)

Creating a Sequence – Using Change Conditions



The list box **Action type** which is located between the sequence table and the entry mask can be used to program sample injections or adding different change conditions (*Sequence Commands*), which can be performed in between two injections. Such Change conditions are available by using the drop-down menu either of the column **action type** within the sequence table (1) or the **action type** box below the table (2). With a left mouse click on the drop-down function different commands are selectable (default **Injection**), as shown in the following Figure.

The dialog changes according to the selected action type. Either a browse button to load a certain file or a list box to choose the target parameter will appear.

All action types apart from **Injection** include a **wait time** field which can be used to delay the execution of the next row to get the system equilibrated after the change condition was performed.

Available Action Types

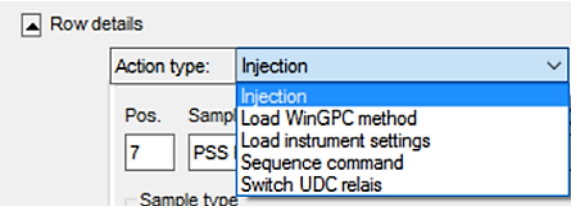
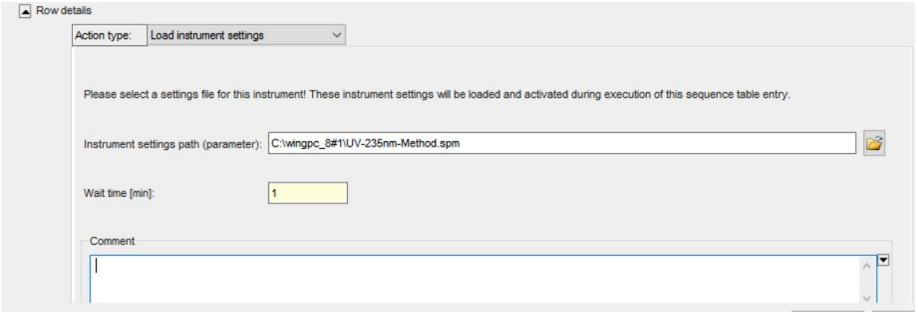


Table 74 Action types

Action type	Action type
Injection	Used to program a regular injection (see previous chapter)
Load WinGPC method	Loads a WinGPC Software Method (*.MET file), can be used to automatically start a new login and is recommended if flow rate is changed within a sequence
Load instrument settings	Loads ChromPilot instrument settings (*.spm file), can be used to continue next samples with a completely different instrument parameter set
Sequence command	Changes a single parameter of a module (e.g., wavelength of UV detector), available commands depend on module type and will be shown as a list box (alternatively use **Load instrument settings**) *Note*: The sequence command **Change Operator** is availabe for all configurations (see example below), the sequence command **Pause Sequence** allows to extend the baseline record between two injects or at the end of the sequence (with option of user acknowledge), the sequence command **WinGPC: Load automation file** loads automation settings/ parameters (*.AUT file), which will be applied to not yet processed and following injections of the sequence. The automation properties will be activated automatically.
Switch UDC relais	Switches one of the available UDC relais (o1 to o8), can be used to control external devices which are not supported by ChromPilot

Examples on Action Types

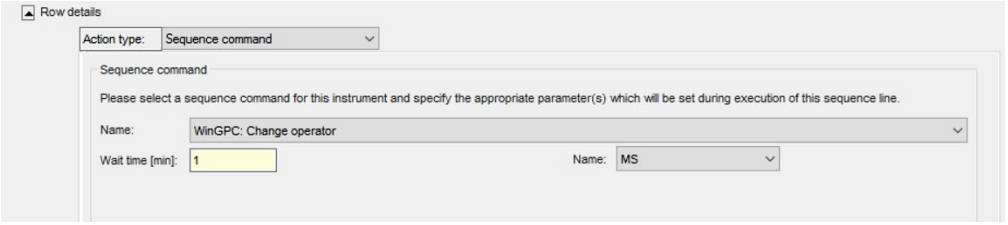
Load Instrument Settings



This action type will replace the settings of all modules with those defined in the *.spm file to be loaded. This is especially helpful if complex settings (e.g., gradients in form of timetables) shall be loaded.

Alternatively, a sequence command can be used if just a single parameter is to be modified (e.g., change UV wavelength).

Sequence Command Change Operator

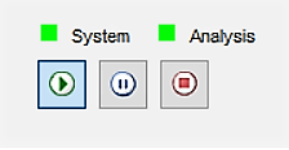


Use the sequence command **Change Operator** to assign certain sections of a sequence to different operators. The list of available operators is taken from the ressource tree (TIP: Operators can be added to the ressource tree by right-mouse click on operators selecting **Add**). On execution of this command WinGPC Software will stop the current sequence and start a new login (sequence) with the new operator's name within the same project. Thus, it is recommended to choose the run time of the preceding sample long enough to cover the complete elution volume (*important*: no overlapped injection during change operator). The command will only be executed after a regular inject. If the WinGPC Software method shall be changed as well (e.g., because a different project shall be used or method settings like evaluation parameters are different) it is recommended to preconfigure the *.MET file with operator information and load only the *.MET file (Action type **Load WinGPC Software Method**).

NOTE

Please note that sequence rows with action types described above will be executed prior or after a sample run according to their position in the sequence table. If certain system parameters are to be changed during a sample run (e.g., a solvent gradient needed for HPLC runs), this needs to be defined using a time table. For information about the availability of timetables please refer to the according WinGPC Software system control documentation. Once defined, a timetable can be saved and managed in the instrument settings (*.spm). Timetables will be started with the next injection.

Sequence Start and Stop Functions



If a sample sequence is entered (or imported) into the Sequence Manager and all available modules are in the **Ready** status, the sequence can be started by pressing the green **Start** button (see Figure above).

All rows which are selected (tick mark in the column ☑, 2nd column header in the sequence table, default: activated) will be executed.

In some cases, it is reasonable to run a partial sequence. Activate/ deactivate the entries by manually setting/ removing the tick mark for ☑. Entries without tick mark will be skipped during sequence execution. All entries will be activated again by setting the tick mark in the checkbox of the 2nd column header.

As soon as a row of the sequence table is executed (including interval/wait time) and the autosampler starts to inject the sample of the next row, this will be indicated by a check mark in the column **done** of the finished row.

During the **Run** status the **Instrument Manager** can be viewed, but not edited. Pressing the **Stop** button will stop the autosampler and the data acquisition immediately. If the same sequence shall be continued, the ChromPilot will start again with the first injection of the last row that was not finished yet. Because the data acquisition was stopped as well, the WinGPC Software will start a new login.

If a sequence is completed, all tick marks in the column **Done** will be removed.

If a sequence shall be interrupted but not stopped, the **Pause** button may be used. **Pause** will delay the next injection, but it will not stop the data acquisition. It might be useful when the injection interval is chosen too short or when some parameters of the GPC system shall be changed in between two injections (e.g., a “man. zero” for the detector). A paused sequence will alter the **Instrument Manager**: The direct controls will be available in order to reactivate a module which was lost or out of range (e.g., the RI needs to be purged because the diodes are unbalanced). All other changes would influence the run in a way that requires a new start of a sequence. Pause is only recommended for small adjustments or the delay of the next injection. The data acquisition will be active during the whole time, so no data are lost – even if the actual sample was not finished yet. As soon as the pause state is left, the autosampler will continue with the next scheduled injection.

NOTE

Once the last sample of a sample sequence was injected, the **Pause** button will not extend the data acquisition time. Pause delays the next injection, but not the end actions. If the last sample of a sequence is finished, no samples can be attached any more - even if the data acquisition is still running due to the execution of the end action wait time.

If data acquisition or baseline records between two injects or after the last inject needs to be extended, insert the Sequence Command **WinGPC Software: Pause sequence** (see chapter “Creating a Sequence – Using Change Conditions” for more details).

Sample Names within the Project

As soon as a sample is injected, the sample name with the vial position as a prefix will be displayed in the **Raw Data** Window next to the injection marker. At the same time the vial position becomes part of the sample name. To get distinct sample descriptions if multiple injections are programmed (Inj. # > 1), the number of the injection will be added to the sample name as well. Thus, it is possible to always reproduce the vial position even for finished runs.

Example: You inject sample „PS0815“ once using vial 1, then the resulting sample name will be „Vial 1: PS0815 –1“. If you inject it twice by entering „Inj. #“ „2“, then the resulting names will be „Vial 1: PS0815 –1“ and „Vial 1: PS0815 –2“.

Import and Export Options for Sequences

The **Sequence Manager** offers several functions to load/import sequences and to create outputs. All corresponding functions are available using the icon bar (see chapter "Sequence Manager Icon Bar" on page 490).

Load/Save and Import/Export

Open () and **Save** may be used for ChromPilot *.sps sequence files which include all information on the sequence table. Please note that you will overwrite all existing entries if you load a sequence table that way. If you need to combine different sequences or to add a partial sequence to existing entries, you should use the **Import** () option.

Import may also be used to load sample sequences which were created with the regular WinGPC Software sample editor and are available as *.txt files (e.g., ReadyCal standards). The sequence **Export** () enables an *.txt output to provide the sequence information for other applications. In addition, you may highlight parts of the current sequence table and export it as a *.sps (or *.txt) file.

NOTE

Please check all information regarding vial position, interval and inject volume if you load/import existing (partial) sequences, since these fields may contain different values than needed.

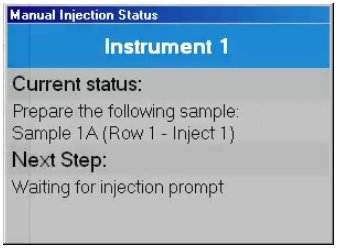
Sequences with Manual Injection

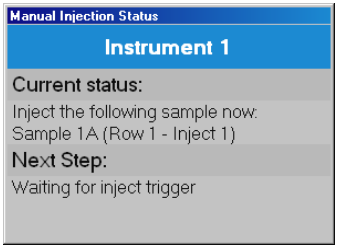
If a partially controlled system has no autosampler, but a manual injector or an autosampler which is not supported by the ChromPilot, the following steps will apply:

The current sequence status will be indicated by status dialogs in the **Sequence Manager**. Thus, you will always know when to prepare and inject a sample respectively.

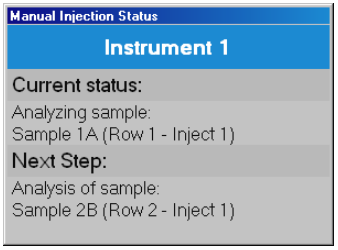
Create a sequence as described in chapter "Creating a Sequence – Entering Sample Information" on page 495. Note that the entered interval is a minimum interval between two injections. Injections which are executed within this interval will not be recorded by WinGPC Software (no Injection marker will be set before the instrument returns to idle state)! However, you may exceed the interval at will, e.g., you may inject the next sample after 30 or 60 minutes, even if an interval of only 10 minutes is entered. Check the WinGPC Software dialogs prior to each injection to prevent any problems:

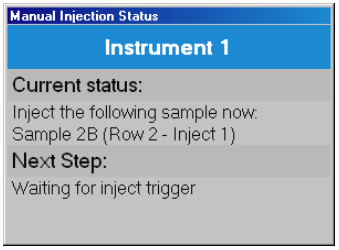
1 Initialization of the first sample: If the status prompts for injection, you may inject the sample.



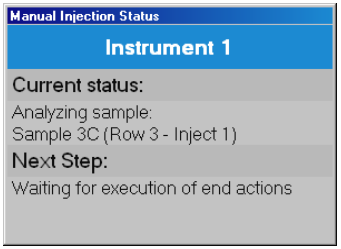


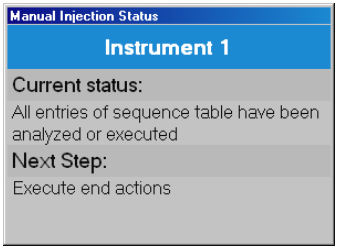
2 The dialog will always specify the sample (sample name) which shall be injected, thus mistakes will be prevented if multiple samples are to be injected within the same sequence.



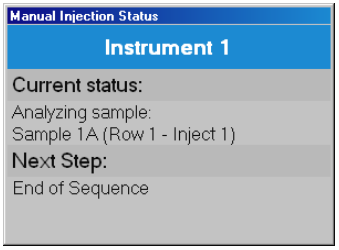


3 Optional end actions will be executed after the interval of the last sample and the additional wait time which is defined in the end actions settings.





4 If the end actions are not activated, the data acquisition will stop after the interval of the last sample line.



20 WinGPC Compliance Edition

WinGPC Software functions might be deactivated due to certain user level restrictions. These menu items are either erased (in context menus) or greyed out with a padlock symbol in front of it. Some buttons are not functional. Details are available in this chapter.

WinGPC Software supports all aspects of macromolecular characterization (see previous chapters in this user guide) and complies to all international and national standards, regulations, directives and guidelines for data capture and analysis in regulated environments. Among those are:

ASTM standards	EP regulations	IP regulations
BSI standards	EU directives	ISO standards
CFR guidelines	FDA requirements	JIS standards
ChP regulations	GAMP requirements	JP regulations
DAB regulations	GB(/T) standards	OECD recommendations
DIN standards	GxP requirements	USP regulations
EN standards	ICH recommendations	

Conformity declarations, software verification and validation reports are shipped with each WinGPC Software Scientific software license; examples are shown in chapter “Reference Printout of System Verification” on page 531. Additional services (qualification services, relocation services, instrument qualification, etc. are available).

WinGPC Software is an ISO 9001 certified Quality Management System. The established product life cycle management system complies with the requirements of supervising agencies. According to the GAMP directives the WinGPC Software MCDS is a “commercial of the shelf” standard software product and a “Closed System”, which is a controlled software environment with user access control and with full traceability of data, meta data and result generation.

Compliance Edition Overview

The WinGPC Software Compliance Edition is a seamlessly integrated software module (option) which adds all functions and features which are necessary to work in regulated laboratories and enhances traceability of results and processes. Basic features comprise:

- enhanced raw data and meta data integrity and traceability via data storage in database
- prohibition of accidental data interference by record versioning
- automated version history of meta data
- each data point tagged by universal date/time stamp
- prevention of modification of data which have been recorded/modified using the Compliance Edition by WinGPC Software installations without Compliance Edition functionality
- proof of proper local software installation and identity with master files via installation verification
- proof of correct data processing procedures and numerical calculations via AutoValid procedures
- detailed logbooks and audit trails for complete traceability of systems, sessions, user access and samples
- audit trail and logbook encryption for ultimate integrity
- enhanced user access control to WinGPC Software with automatic adaptation of corporate password policies (requires Windows domain controller)
- automatic local re-enforcement of password policies in case of offline work or network failure
- easy user rights and functionality assignment via WinGPC User Administration Console
- pre-defined user levels for all laboratories
- no need to re-qualify existing WinGPC Software data system installations when enabling additional Add-ons (no software installation is necessary)
- comprehensive support of electronic signatures with separate approval functionality and specific meanings and/or comments
- batch processing of electronic signatures

- all WinGPC Software standard reports show e-signature tag with information on who/when the electronic signature was issues on which device
- extensive ReportDesigner ComplianceEdition support

Secure WinGPC Software Login

The secure WinGPC authentication verifies the WinGPC user against the WinGPC user database (global or local) created by the WinGPC User Administration Console (see chapter “WinGPC Administration Console and User Database” on page 522) and assigns its user levels and software functionality. The passwords and the password policies are automatically read by WinGPC Software from the Windows primary domain controller. This eliminates the need to define, specify and maintain WinGPC Software specific rules and additional user passwords and helps the IT departments to enforce global security measures throughout the organization across all application levels.

Hacking of passwords can effectively be prevented by applying the respective Windows Active Directory group policies.

If no domain controller is available at login due to network problems or offline work, WinGPC Software will use a local copy of the network profile for authentication to prevent unauthorized access or hampering with user profiles.

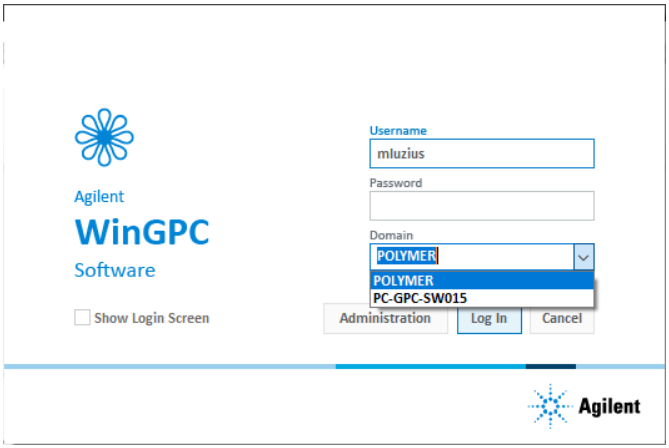
All logins (successful and unsuccessful) are logged in the WinGPC Software administration audit trail and can be reviewed by authorized WinGPC administrators.

The WinGPC Software Authentication dialog allows to select the correct Microsoft server (domain controller, PDC) which holds the user identity information or the local computer security database (select the “computer name” as specified in the system properties). The Figure below shows the domain controller “POLYMER” and the local computer “NB-MARK002" as authentication pools. Please make sure that the username and password are correctly entered.

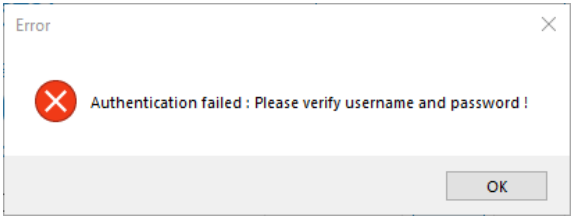
Notes:

- passwords can be case sensitive; take care to enter them in the correct form
- passwords may change when the expiration date (group policy) has been reached
- a network domain is only listed if the Windows logon is at the domain; local Windows users will only have access to the local domain

By default, the WinGPC Software login screen which shows the active modules will not be shown. The extended WinGPC login dialog is activated by setting the tick mark at **Show Login Screen**. This option becomes accessible when the cursor is in the password field and the user is authorized to access this dialog in the WinGPC administration database.



Additionally, the WinGPC User Administration Console can be accessed from this point which allows to add WinGPC users from the domain(s) to the WinGPC user database, specify user levels and set user privileges and access to WinGPC Software features. This administration tool is described in detail in chapter “WinGPC Administration Console and User Database” on page 522.



If a user fails to authenticate at the WinGPC user database repeatedly (see Figure above), please contact the WinGPC administrator and or the IT department for support. Have them check the following:

- User has not entered the user name and/or password incorrectly and/or exceeded the authentication policy as specified in the group policies for the local or network domain controller
- Access to WinGPC user database (network or local) is available (no error message at login in)
- User is listed in the WinGPC user database (visible on the right-hand side listing in the Administration tool, see chapter „WinGPC Users Screen“ on page 524)
- User is listed as a domain member (visible on the left-hand side listing in the Administration tool, see chapter „WinGPC Users Screen“ on page 524)
- User is not (temporarily) disabled in the network/domain

WinGPC User Levels

The Compliance Edition offers WinGPC User Levels, which allow to allow or restrict user access to WinGPC Software menus, functionality and features.

The WinGPC Software Compliance Edition comes with 4 pre-configured user levels:

WinGPC Administrators:	add/delete users in the WinGPC user database
WinGPC Advanced Users:	modify user privileges, special rights and user levels
WinGPC Users:	can access all WinGPC functionalities but admin tasks;
WinGPC Guests:	create methods, calibrations, and report layouts and define data processing standards

Details of the user level assignment and configuration of WinGPC Software functions are discussed in the WinGPC User Administration Console (see chapter "WinGPC Administration Console and User Database" on page 522).

Features, menus, etc. which are restricted to certain user levels by the Compliance Edition are highlighted in this Agilent WinGPC Software User Guide by the symbol shown on the left. WinGPC Software menu items show a padlock icon if they are grayed-out due to restrictions. In context menus and other special cases this is not possible (Windows interface limitations). Then these items will be missing.

Electronic Signatures

WinGPC Software with Compliance Edition options allows to lock and authorize meta data and results by so-called electronic signatures in complete compliance to ICH, GxP, CFR and FDA rules. Electronic signatures can be set by authorized WinGPC users as specified in the WinGPC User Level configuration. They are invoked by selecting the *sign sample* command ( ) from the WinGPC Software icon bar.

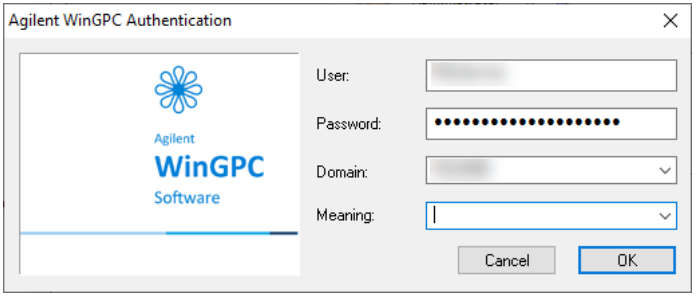


Figure 72 Initial e-signature assignment dialog

The WinGPC Software authentication dialog pops up to ask for a username and password combination to verify the right to set and/or remove electronic signatures. A reason (Meaning) has to be entered in the respective field (see red marked area Figure above). A list of existing reasons (identical to the **reason for change** dialog) pops up and new meaning will be saved automatically for future use. If either entry is incorrect or the privilege to assign electronic signatures is not granted to the selected user, then an authentication failure dialog will appear to inform the user. Please ask your WinGPC administrator for permissions to set and/or remove electronic signatures. Such privileges can be assigned to each user independently from the WinGPC User Level. Please refer to chapter "WinGPC Administration Console and User Database" on page 522 if the authentication repeatedly fails despite the fact that e-signature privileges are granted.

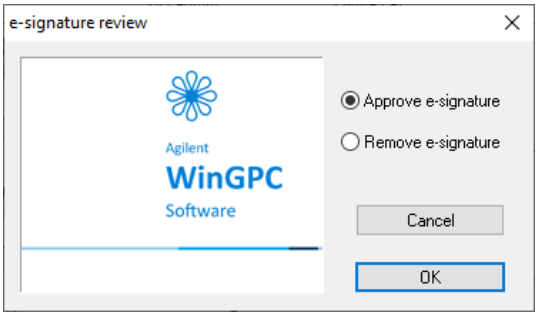
Setting, approving, and removing of electronic signatures is permanently saved in the sample audit trail in the following form:

electronic signature assigned/removed at: date/time stamp, WinGPC Software category ID, unique sample ID, user login name, WinGPC User Level, user PC name, event description.

Example:

```
Friday, August 11, 2006, 13:41:21, CAT=0,
SAMPLEID=797654028, USER=xx, USERLEVEL=1, PC=LAB-SW1,
EVENT=Electronic signature assigned by xx,
Meaning: initial e-signature
```

Electronic signatures prevent permanent changes in the raw data, processing parameters and settings and all related meta data and results. Temporary modifications are still possible for short-term review. However, the electronically signed data set will be used and the temporary modifications will be discarded without further notice, as soon as data shall be printed, exported overlaid or another sample is reviewed or processed. This ensures ease of use and still maintains highest security levels that prevent improper data handling.



Electronic signatures are assigned and removed in a single-step or with an additional approval level by a user which as e-signature approval privileges. Electronic signature approval is the final step in result verification. All users can process data according to their user levels in preparation for the review and assignment of the electronic signature by privileged users. The WinGPC Software icon bar shows the e-signature status of a sample with a padlock in the following way:


(Green dot and open padlock) – Sample not signed


(Yellow dot and closed padlock) – Electronic signature of sample assigned


(Red dot and closed padlock) – Electronic signature of sample approved

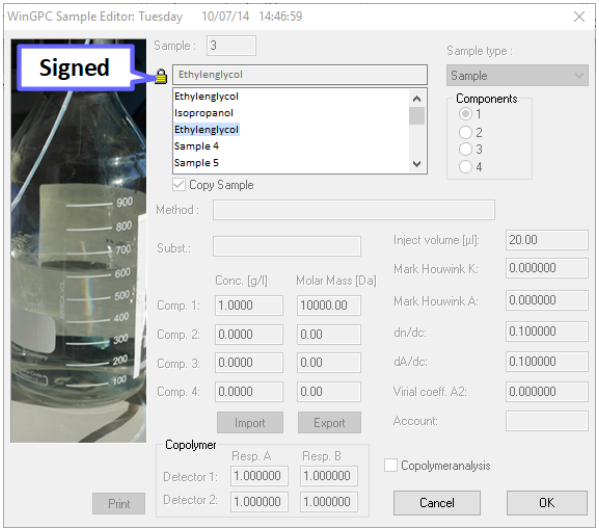
Please note that separate privileges exist to set, approve, and remove electronic signatures. This method allows two level assignments if they seem necessary for the organization.

Agilent recommends in that case that the initial data processing and result evaluation is done by regular lab staff (e.g., WinGPC user level: USER). Review is done by a user with WinGPC user level **ADVANCED USER** who has the right to set electronic signatures. Final approval will then be done by the supervisor with WinGPC user level **ADMINISTRATOR** or **ADVANCED USER** who has the sole right to approve electronic signatures.

Electronic signatures can be assigned / removed / approved for single samples or multiple samples (batch). Batch assignment or removal requires a special user privilege to be operational. If batch processing is used for e-signature management, then the WinGPC Software inject options can be used to direct the batch process (for details see chapter "Description of Menus and Options" on page 154).

NOTE

Preset **reason for change** comments or **meaning** phrases can be selected from the drop-down box for ease of use and keep comments in similar style.



Electronically signed (approved) samples can be easily identified in a sequence:

- in the **Inject** menu electronically signed (approved) samples are listed with asterisk* (**) after the injection number and before the injection time of the sample name
- in the raw data window the sample name is shown in yellow (red) instead of black for unsigned samples
- electronically signed samples are shown in the sample editor with a yellow (red) locked padlock in front of the sample name (cf. Figure); otherwise the padlock (green) will be open. No changes in the sample details are possible for signed samples; those details are for review only and grayed out.

Batch processing and automation features will skip samples which have been electronically signed. A message box will appear to inform the user about a possible conflict and the number of samples affected (see Figure above).

When electronically signed samples are printed a watermark is automatically added to each printout generated by WinGPC Software showing who and when the electronic signature was issued. This watermark is always located on the top of the page above the graphics. The watermark cannot be edited and has always the following format:

```
Sample signed (approved), {user name}, {computer name},
{at date/time}, Meaning:: {text}
```

User-specific electronic signature designations can be created by the WinGPC Software ReportDesigner (option). When user-created reports are printed, the users are responsible for the design and contents of the report layouts. Agilent recommends that all layouts should contain the e-signature variables which are located in the **Sample** folder in the ReportDesigner **Variables** list. Additional variables are available in the **CFR** and **PC Configuration** sections. Sample related instrument data like column bacak pressure, temperatures, etc. can also be added for further sample details.

Audit Trails and Revision History

Extensive audit trails are available to document changes and user interactions in WinGPC Software configuration, data treatment and administration. For increased security all audit trails are encrypted and cannot be edited inside or outside WinGPC Software. Printing of audit trails is only possible with the WinGPC Software ReportDesigner or for temporary use by copying and pasting via the Windows clipboard.

The audit trails are split into 4 separate files for ease of use which are cross-referenced for additional security.

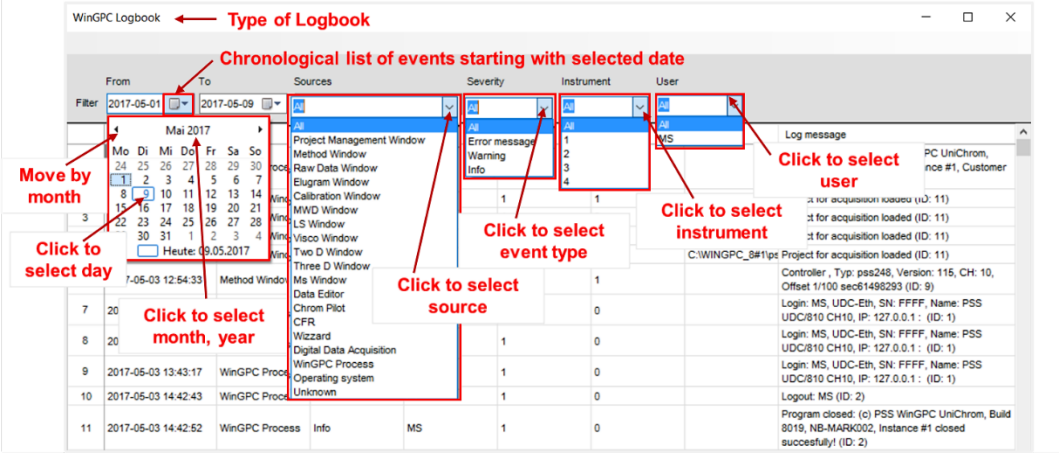
- administration audit trail: contains all events and modifications in setting up WinGPC users and WinGPC user rights; unsuccessful attempts to access to the administration tool are also logged
- instrument audit trail: logs all communication to/from the GPC systems actively controlled by WinGPC Software; this audit trail also lists instrument status, GLP and maintenance relevant data if available from the instrument/module; each system has its own instrument audit trail
- session audit trail: lists all actions performed at the PC during a WinGPC Software session including WinGPC Software login parameters and options, automation and batch processing messages, file access errors, missing user privileges, etc., the session audit trail is equal to the WinGPC Session logbook
- sample audit trail: logs all user actions, events, commands and modifications for each sample separately and automatically relates the audit trail to the respective sample; all meta-data activities including electronic signature assignment and removal are listed chronologically

Sample audit trails are integral part of the WinGPC Software project structure.

The administration audit trail (WinGPC_CFR.log) is located in the WinGPC Software program folder from which the administration tool is launched.

Instrument and session audit trails are automatically created and archived monthly in the WinGPC7_#1 folder with the following file name convention:

```
instrument audit trail: logbookN_YYYY-MM.LOG
session audit trail: WinGPC_8_YYYY_MM.LOG
(YYYY: year; MM: month; N: WinGPC instrument number)
```

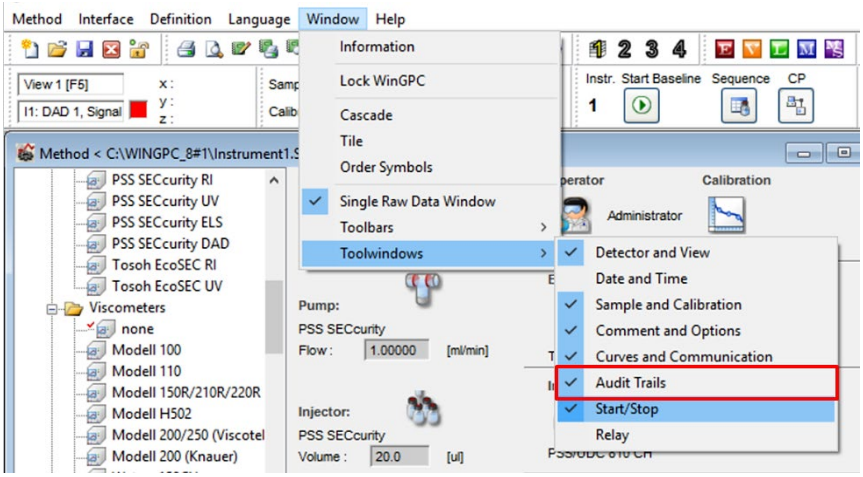


All audit trails are organized in chronological order and have the same user interface (see Figure above, e.g., WinGPC Session Logbook). For quick and easy access to the required information a calendar will be shown at the upper left corner of the audit trail window by clicking the respective calender button, as indicated in the Figure above. Here, one can select year, month and/ or a specific date to display the logged information, which is listed below in ascending order. If no event has been logged for the selected date, the next earlier information available is shown in the listing.

Which type of audit trail is displayed will be shown in the title bar of dialog window at the top left of the audit trail window, as illustrated in the Figure above.

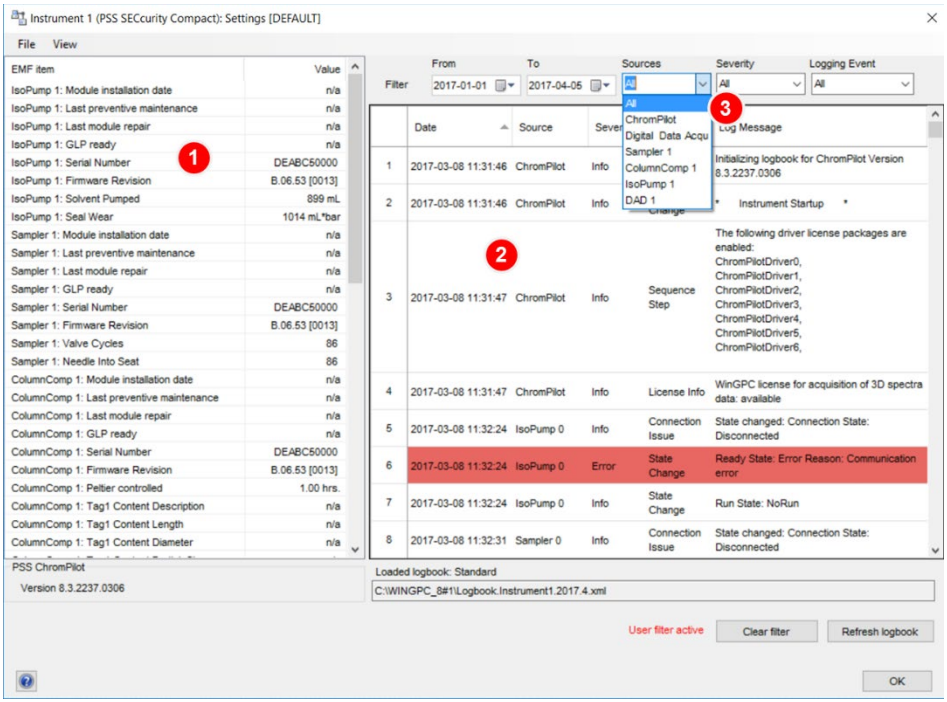
The audit trails are accessed from their workflow context which means that they are readily available by a mouse click for inspection.

Sample, Session and Instrument Audit Trails are always visible in the status bar of the main WinGPC Software screen (see Figure above, red marked area). If the Toolwindow **Audit Trail** will not be visible (deactivated), it can be activated by selecting **Window > Toolwindows > Audit Trail** (see Figure below).



Only WinGPC Software sessions which are operated with the Compliance Edition license (optional software module) will show an active sample audit trail button, in all other cases the button will be greyed out. If the connected instrument is not controlled by the WinGPC Software ChromPilot, the button for the instrument audit trail will be greyed out as well.

All audit trails from the Toolwindow **Audit Trail** except for the instrument audit trail share the same user interface and logbook fields. The instrument audit trail is dependent on the connected system and its communication history. The instrument audit trail can be accessed in the WinGPC Software ChromPilot dialog by selecting **View > Logbook**. The left panel (1) lists all modules in the system or instrument including serial numbers, firmware versions and maintenance information. The right panel (2) contains the history of instrument communication, error messages, injection requests and triggers (see next Figure). Helpful information concerning status changes and error messages may be viewed using the comfortable logbook filter functions. For example: module specific informations can be easily filtered by using the filter box **Sources** (3) to selecte the desired module for detailed inspection (refer to chapter "Logbook and GLP/EMF Information" on page 484 for details). Furthermore, the listed information are instrument specific and they depend on the manufacturer and model.

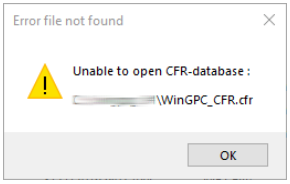


All audit trails, which are selectable from the Toolwindow **Audit Trail**, (except for the admin audit trail) share the same logbook event structure. These logbooks are organized as a table with columns, which have comfortable filter functions. In contrast the Admin Audit Trail (available by the *Administration Console*, see detailed information in chapter “WinGPC Administration Console and User Database” on page 522) contains events in the following form:

The admin audit trails is organized in chronological order (see Figure above). For quick and easy access to the required information a calendar is shown at the right-hand side of the window. Year, month and date can be selected for the display of the logged information, which is listed below in ascending order.

WinGPC Administration Console and User Database

The secure WinGPC authentication verifies a potential WinGPC user against the WinGPC Software user database. The WinGPC user database can be global or local depending on the location of the database. If it is located on a shared network drive (drive letter assignment necessary), then all users will share the same WinGPC user database. This is the recommended setup. However, in single system and/or single user installations the administrator can also choose to install the WinGPC user database on a single PC with a non-shared drive. In this case the WinGPC user rights are only available on this PC. Consequently, if a second PC is installed the WinGPC user administration has to be setup again, which makes it more difficult to keep the authentication policies consistent.



Agilent provides a master copy of the WinGPC user database located in the WinGPC Software support folder of the installation. The administrator has to copy this master database to the respective drive before user rights are assigned. If the WinGPC user database is not available at all or cannot be located in the expected drive, a message box will appear showing the expected location of the WinGPC user database. Please ask your WinGPC administrator and/or your IT department and let them check the following if the error persists:

- Database file is existing in the path shown in the message box.
- All WinGPC users have read/write access to this path.
- In case of a network drive, make sure the network path is accessible from the user PC.
- Please make sure that a drive letter is specified (the UNC convention is not sufficient).
- The database file has the correct size, date, and contents.

- Save and temporarily replace the database file with a backup copy or the master WinGPC user database provided in the support folder of the WinGPC installation.
- Contact your Agilent representative if the problem persists for assistance.

The administration console (identified as **WinGPC Software User Accounts**) is launched from the WinGPC Software authentication screen by pressing the **Administration** button.

The initial login must be done with a local administrator account

Username: administrator

Password: {of the local administrator account on this PC}

Every authenticated WinGPC user can enter the administration console, however, only those functions are accessible which are granted to his/her user level; other menus and commands will be grayed out. Each access and action will be logged in the admin audit trail.

NOTE

Opening the WinGPC User Administration Console can take a while if the Microsoft domain contains many users. Please wait a few minutes in the case of large networks or complex organizations.

The WinGPC User Administration Console contains three parts which will be discussed separately and can be accessed from their corresponding tab:

- WinGPC Users: lists existing WinGPC Software users and allows to add new users from the domain and remove WinGPC users from the list
- Group Properties: lists details of WinGPC user privileges based on the membership to a WinGPC user group (user level)
- Logfiles: shows the administration audit trail (cf. chapter "Audit Trails and Revision History" on page 518 and chapter "Administration Audit Trail" on page 529)

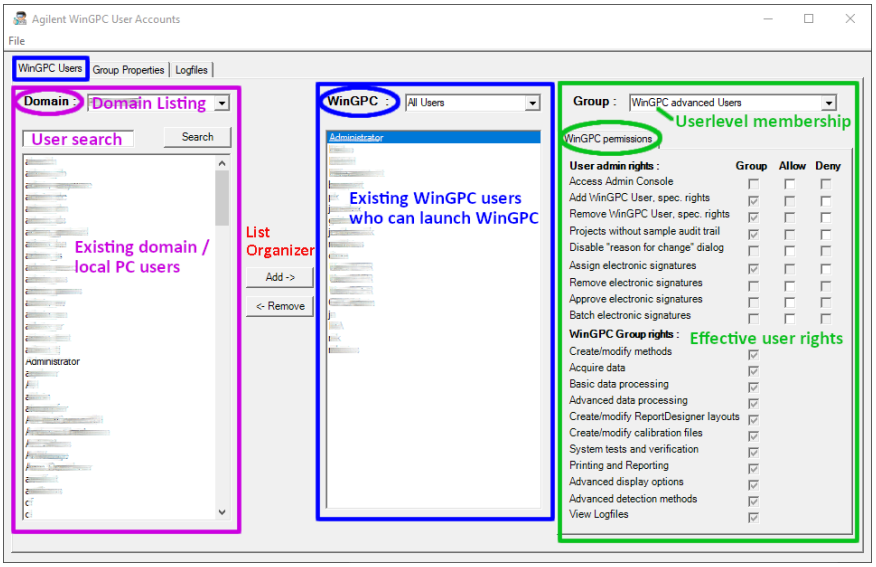
Changes in the WinGPC user database can be done by selecting [File] [Save] from the menu. Alternatively, the application will prompt for saving/discarding all modifications during the current session on closing the WinGPC user database.

WinGPC Users Screen

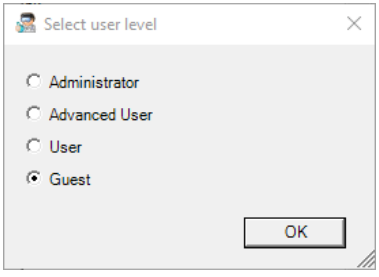
The main screen of the administration console (a.k.a. **WinGPC User Accounts**) is located in the **WinGPC Users** tab. This screen is used to add, remove, modify WinGPC users and their privileges. The **WinGPC Users** screen is divided in three major sections (see Figure below):

- Domain viewer: shows the user list of the selected domain (network or local PC) sorted by user name
- WinGPC user list: shows all WinGPC users according to the selected WinGPC group as specified in the WinGPC user database
- WinGPC user permissions: list all permissions and the user level of the selected WinGPC user as specified by the WinGPC administrator

If the selected domain is large or complex, it may take a few moments to list all members in the left panel. During that time the application may appear as not responding, but it is reading the users from the Microsoft domain controller (server), which might take a while to complete. Fast access to a certain username is possible by entering a (partial) name in the **user search field** and pressing the **Search** button. This search is performed on the existing user list. The complete user list can be displayed again by entering nothing in the **user search field** and pressing the **Search** button.



The selected user can then be assigned to a WinGPC user group by clicking on the **Add** button and selecting the appropriate user level from the dialog. In this dialog the user level with the least privileges (Guest account) is selected by default to prevent unintended access to WinGPC Software.



Default user group properties are automatically assigned depending on the selected user level (see following section). Special user admin rights can be granted by WinGPC administrators to users with special needs (depending on the local workflow requirements) from a list on the right-hand side of WinGPC Users dialog. They comprise the addition/removal of GPC users, the right to set/remove electronic signatures and the right to enable/disable sample audit trails and reason-for-change dialogs when a new WinGPC Software project is created by this user.

NOTE

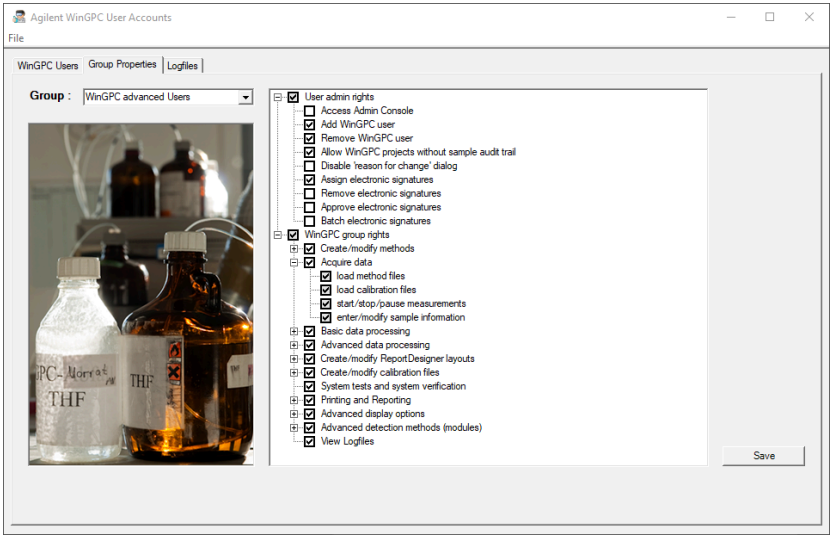
With WinGPC Software version 8.3 SR1 new options to be set in the user admin rights are implemented:

- Approvement of electronic signatures with four eyes principle: The user who signed a sample is not allowed to approve his own signature
- Access of **Config** dialog in column database linked with the individual user administration right **Remove WinGPC User**.
- Access of ChromPilot Configuration linked with the individual user administration right **Add WinGPC User**.

The lower part on the right-hand side of this dialog shows the major user rights which are automatically granted by their group membership assignment.

WinGPC Group Properties Screen

Details of the WinGPC user group definitions can be reviewed in the **Group Properties** tag of the administration console (a.k.a. **WinGPC User Accounts**). This screen lists in an explorer-like style all WinGPC Software tasks and their accessibility for each WinGPC user group.

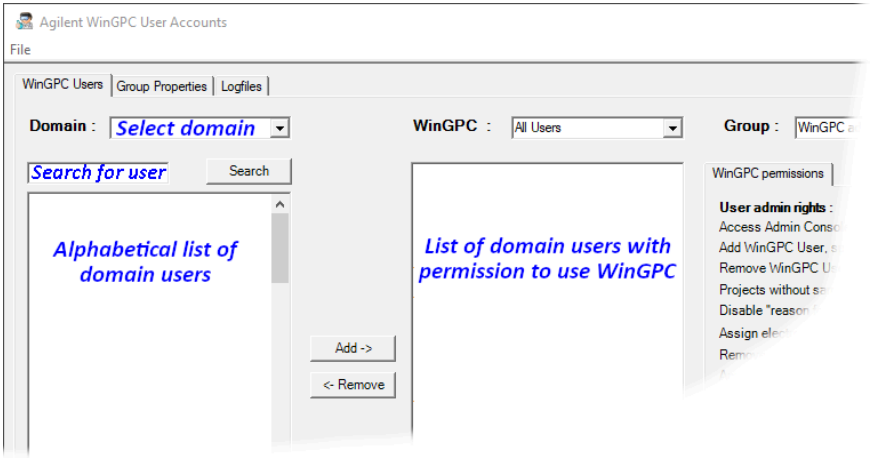


Assign User Privileges – Step by Step

User specific WinGPC Software feature acess can only be defined with the WinGPC Software Compliance Edition module.

This option is installed if the WinGPC Software authentication dialog shows the **Administration** button not grayed out (cf. Figure below). Only authorized WinGPC users have the permission to set or modify WinGPC user access and specify user rights as specified in the WinGPC CFR database (configured in the WinGPC Administration Console).

Current user properties (either general **admin rights** or **group rights**) are shown in the **WinGPC permissions** section in the WinGPC Administration Console and will in general depend on the WinGPC User Level (which is predefined) of a specific user as shown below.



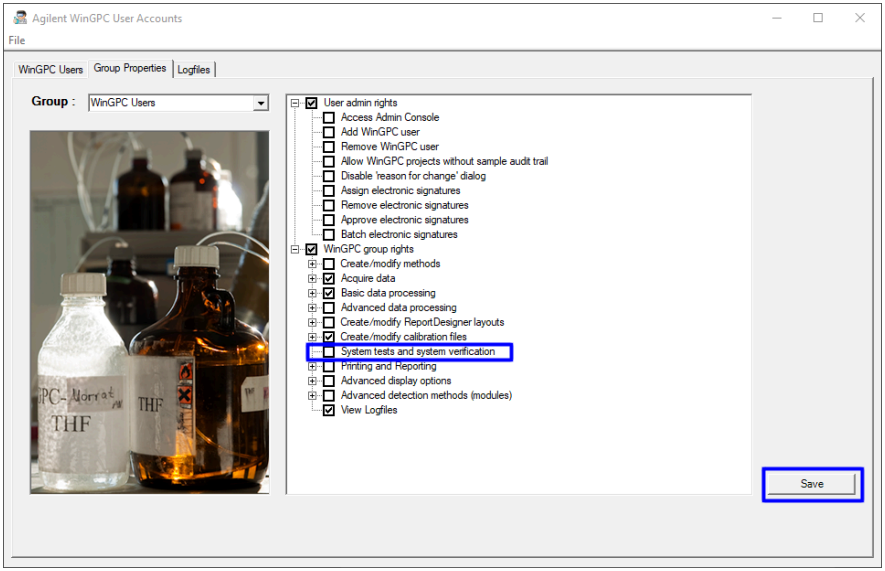
Step-by-step instructions on how to modify the WinGPC Software feature access in the WinGPC User Administration Console:

1 Launch WinGPC Software from a WinGPC Software computer with access to the WinGPC CFR database.

2 Enter user credentials which allow to access the WinGPC User Administration Console and modify WinGPC user rights. An error message will be shown if credentials are insufficient. Such users (normally assigned **WinGPC Administrator** status) must have the **Access Admin Console** permission (right).

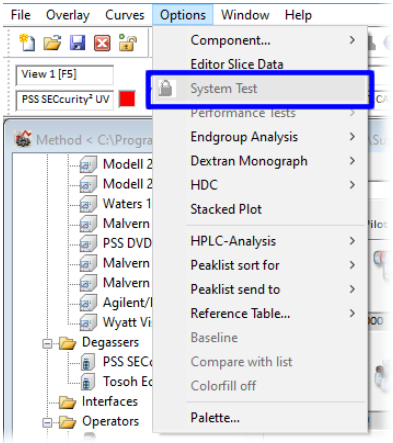
3 In the list of WinGPC users (list in the middle below **WinGPC:**) select the user whose WinGPC Software feature access permissions (right) are to be changed. The current user settings will be shown in the **WinGPC Software permissions** section.

4 Click on the **Group Properties** tab to access the group properties definition screen. Select the proper user level (**Group**) and edit any changes to be active for future users of this user level (see below).



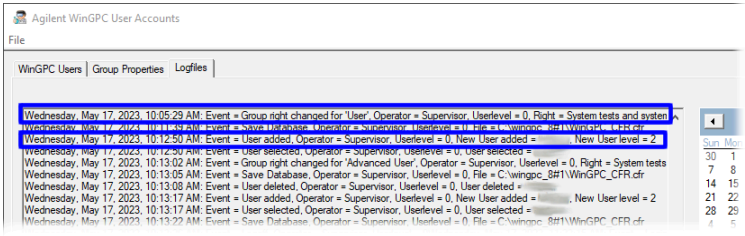
5 Return to the **WinGPC Users** view (tab), temporarily change the user level of this user, save it with **File > Save** and immediately set it again to its original value. This will ensure that this user will get the modified user rights as defined in step 4 above.

6 Have this user to log on to WinGPC Software and verify that the modified right is correctly set (you may also check the history of all permission settings/changes in the logbook, see chapter “Administration Audit Trail” on page 529).



Administration Audit Trail

All changes to the Administration Database are logged in the encrypted logbook (audit trail) which can be reviewed in the **Logfiles** tab.



You can easily check which user rights are applied to a specific user. All entries are displayed in chronological order. Please note that changes to group rights will not automatically be updated for existing users of the group. If existing users shall have the rights of a modified group policy, you need to set them intentionally (see chapter “Assign User Privileges – Step by Step” on page 527).

21 Appendix

The Appendix shows reference documents, lists background information and discussed hardware related issues.

Reference Printouts

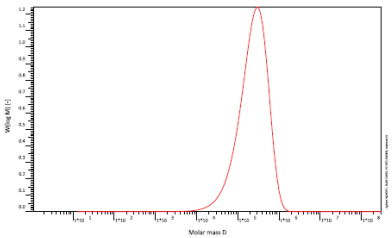
Reference Printout of System Verification

WinGPC Validation Report

This test validates all software algorithms and the complete data flow path within the data management.
The in-house software setup should be checked independently from this test by performing the install verification.

This OQ/PQ was performed on [redacted] with OS Windows 10 22H2 Professional,
for Agilent WinGPC, Build [redacted] with S/N: [redacted]
by: Validator
on (month/day/year): 06/30/2023 04:32:20 PM

Molar Mass Distribution Result



Conditions and parameters

Sample:	C:\Program Files (x86)\Agilent Technologies\WinGPC\Ps_valid.TXT
Calibration:	Ps_valid.CAL
MH-alpha (cal):	0,714
MH-K (cal.):	0,0136
integration from:	6,00 ml
to:	26,00 ml
Eluent:	THF
Flow rate:	1 ml/min
Injection volume:	20 µl

Validation Results

	expected value	*allowed range*	*calculated value*	*deviation (%)*	*status*
Mn [Da]	150 000	149 250 - 150 750	150007	0,005	passed
Mw [Da]	300 000	298 500 - 301 500	300014	0,005	passed
Mz [Da]	450 000	447 750 - 452 250	449985	0,003	passed
Mw/Mn	2	1.99 - 2.01	2,000	0,000	passed
Mv [Da]	280 869	279 465 - 282 273	280163	0,251	passed
[n] [ml/g]	105.68	105.15 - 106.21	105,669	0,010	passed
c [g/l]	4.343	4.321 - 4.365	4,3429	0,003	passed
					end of table

Status Statement Information: passed: result within error of expected value for theoretical data
failed: result deviation exceeds allowed deviation 0.5%

This OQ/PQ is passed, when all results show status 'passed'.

Validation passed: ☐ Yes* ☐ No** Signature: _____

* Validation has been successful, no further action required.
** Please read the technical note 'Agilent WinGPC Software Validation' or contact your IT department and if necessary your Agilent representative.

print date (month/day/year): 06/30/2023 04:32:20 PM
WinGPC Validation Report Version 1.0

page 1 of 1

Reference Printout of UDC Verification

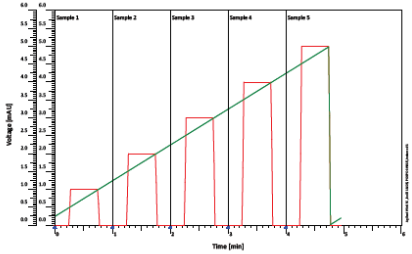
PSS UDC 810 Verification Report

A part of Agilent

This OQ/PQ checks the Universal Data center UDC, the correctness of data timings, data processing and communication of the PSS UDC data interface.

This OQ/PQ was performed on PC-GPC-SW015 with Windows 10 Enterprise (Ver. 2009, Build: 19045), for Agilent WinGPC, Build 10425 with S/N: DE432001
by: Validator
on (month/day/year): 05/22/2023 10:09:09 AM

Results UDC Verification



UDC Properties

UDC-Model:	PSS UDC/810 CH1
UDC Ser.No.:	00FFFF
UDC Firmware:	1.15

Test conditions UDC

UDC connection: Eth

UDC Selftest: passed

UDC Communication: stable

Result Details

Test	Status	Test relais	Status	Test relais	Status	AD conversion
Timing	passed	I1	N/A	O1	N/A	low test
Step 1: 0 V	passed	I2	N/A	O2	N/A	CH1 N/A
Step 2: 1 V	passed	I3	N/A	O3	N/A	CH2 N/A
Step 3: 2 V	passed	I4	N/A	O4	N/A	
Step 4: 3 V	passed	I5	N/A	O5	N/A	high test
Step 5: 4 V	passed	I6	N/A	O6	N/A	CH1 N/A
Step 6: 5 V	passed	I7	N/A	O7	N/A	CH2 N/A
Ramp test	passed	I8	N/A	O8	N/A	

Result summary :
test cycle 1 : passed
test cycle 2 : N/A

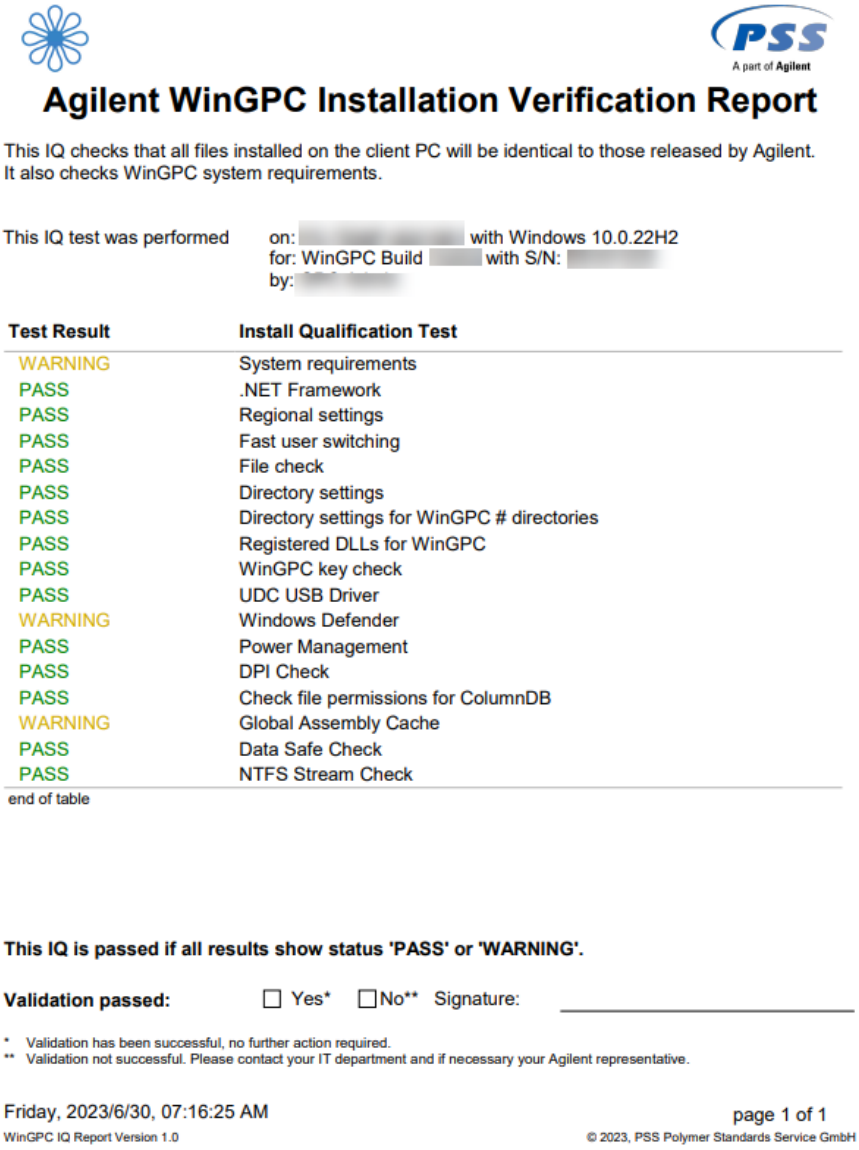
This OQ/PQ is passed when all results show status 'passed'.

Validation passed: ☐ Yes* ☐ No** Signature: _____

* Validation has been successful, no further action required.
** Validation not successful. Please contact:
PSS Polymer Standards Service e-mail: info@pss-polymer.com web: www.pss-polymer.com

print date (year-month-day): 2023-05-22 10:09:09 AM
page 1 von 1
WinGPC Validation Report Version 1.0

Reference Printout for Install Verification



PSS
A part of Agilent

Agilent WinGPC Installation Verification Report

This IQ checks that all files installed on the client PC will be identical to those released by Agilent.
It also checks WinGPC system requirements.

This IQ test was performed on: [redacted] with Windows 10.0.22H2
for: WinGPC Build [redacted] with S/N: [redacted]
by: [redacted]

Test Result	Install Qualification Test
WARNING	System requirements
PASS	.NET Framework
PASS	Regional settings
PASS	Fast user switching
PASS	File check
PASS	Directory settings
PASS	Directory settings for WinGPC # directories
PASS	Registered DLLs for WinGPC
PASS	WinGPC key check
PASS	UDC USB Driver
WARNING	Windows Defender
PASS	Power Management
PASS	DPI Check
PASS	Check file permissions for ColumnDB
WARNING	Global Assembly Cache
PASS	Data Safe Check
PASS	NTFS Stream Check

end of table

This IQ is passed if all results show status 'PASS' or 'WARNING'.

Validation passed: ☐ Yes* ☐ No** Signature: _____

* Validation has been successful, no further action required.
** Validation not successful. Please contact your IT department and if necessary your Agilent representative.

Friday, 2023/6/30, 07:16:25 AM page 1 of 1
WinGPC IQ Report Version 1.0

Curve Colors and Line Styles in Monochrome Printing

WinGPC Software automatically re-maps the curve colors to different line styles to make data reporting and data transfer as easy as possible. This is very useful if WinGPC Software reports are normally send to a color ink-jet printer and the printout must be send by fax (normally a black&white device). As soon as the user used the fax modem driver the colors of the chromatogram curves will be re-mapped to different line types to allow to read the information on the received fax with maximum clarity. The next printout to the local color ink-jet printer will be in the original colors again without user interaction. This can also be a handy feature if different network printers (e.g., color laser, slide processor) are used for printouts.

NOTE

The software investigates the printer driver information to find out if a color printer or a black&white device is used for output. Specifically, the printer driver will report the number of bit-planes to the WinGPC Software. However, Postscript printer drivers report only 1 bit-plane, even though they could be used for color output. For such cases, the software has an option to force the output to color (or black&white) mode. This option is available from the **Print Width Options > Prim.Color** or **Prim.Black_White** menu (see chapter "Raw Data Window" on page 195 for details).

The numbers in the following table are in the order of the color selection panel, from top left to bottom right:

Color selection panel

No.	Color	Line Style
1	white	middle-short-middle
2	black	short-short-short
3	grey	middle-middle-middle
4	dark grey	middle-short-middle
5	red	full
6	dark red	middle-middle-middle (no distance)
7	yellow	long-long-long
8	ochre	Dotted
9	green	middle -dot- middle
10	dark green	long-long-long
11	turquoise	long-short-long
12	dark turquoise	middle-middle-middle
13	blue	long-short-long
14	dark blue	long-short-long
15	purple	short-short-short
16	dark purple	Full
17	light green	Dotted
18	light blue	middle-middle-middle (no distance)
19	old white	middle-dot-middle
20	grey	Full

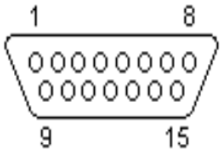
Digital I/Os Pin Assignment

All digital I/O on the PSS UDC810 or WinCHROM interface box are relays. Characteristics of the PSS UDC810 Universal Data Center are described in its User Documentation. The input relays are used to trigger the injection. The resistance of the input relays is approx. 500 Ohm. Thus, at a voltage of 5V approx. 10 mA is necessary to activate the relay. Contact closure should be 100 msec. or longer. The voltage should be taken preferentially from the external instrument (autosampler) to assure galvanic separation of the equipment. If no such voltage is supplied externally, the internal 5V power supply of the interface (digital I/O pins 1 and 9) can be used.

The output relays yield a contact closure if activated. Various external instruments (e.g., pumps, fraction collectors) can be activated and deactivated by these relays.

Pin assignment of legacy PSS WinCHROM Interface:

Wire to	Description
Pin 1/ 9	internal 5V voltage supply
Pin 2/10	input relay 1 (i1)
Pin 3/11	input relay 2 (i2)
Pin 4/12	input relay 3 (i3)
Pin 1/ 8	input relay 4 (i4)
Pin 5/13	output relay 3 (o3)
Pin 6/14	output relay 2 (o2)
Pin 7/15	output relay 1 (o1)



Relay Control

The Output relays can be controlled by the user. Activation/deactivation can be done manually by the user or in an automated fashion. For automatically activate/deactivate the relays see Method **Definition > Timed events** (see chapter “Definition Menu” on page 181).

Manual Relay Control

The relays are manually activated and deactivated by a mouse click on the relay buttons (o1, o2, o3) in the Toolwindow Relay (available by **Window > Toolwindows > Relay**). Light blue means activated output relay (see next Figure: o1 and o2) and dark blue means deactivated relay (compare o3 to o8).

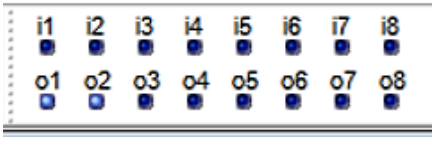


Figure 73 Toolwindow Relay

Inject Dependent Relay Control

The output relay will be inverted after a delay time for a given switch time. The standard relay state can be defined in the "**Standard**" box. The number of activations is equal to the number of injects. Overlaid injections are allowed, i.e. the time between two injects is allowed to be shorter than the delay time.

Periodic Relay Control

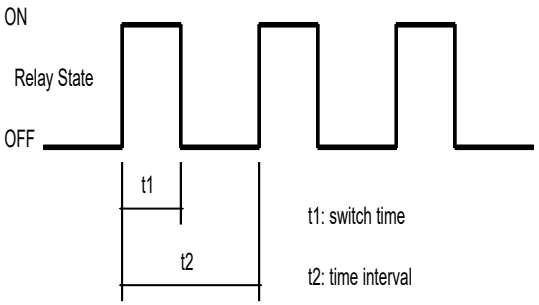


Figure 74 Schematic periodic relay control (standard relay state “OFF”)

The output relays state is inverted dependent on a given timing interval for a given switching time. The standard state can be selected.

Inject Dependent Periodic Relay Control

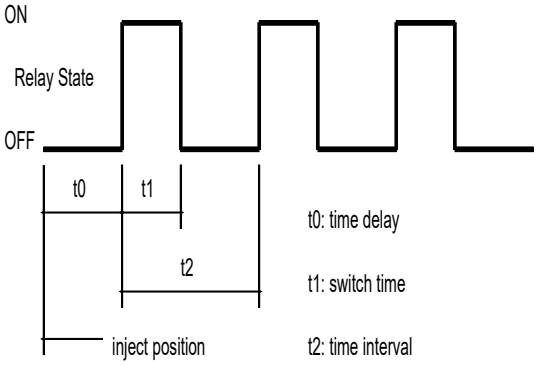


Figure 75 Inject dependent relay control (standard relay state “OFF”)

The output relays will be inverted dependent on a given delay time for a given switching time and time interval relative to the first inject. The standard state can be defined.

Detector Dependent Relay Control with Switch Borders

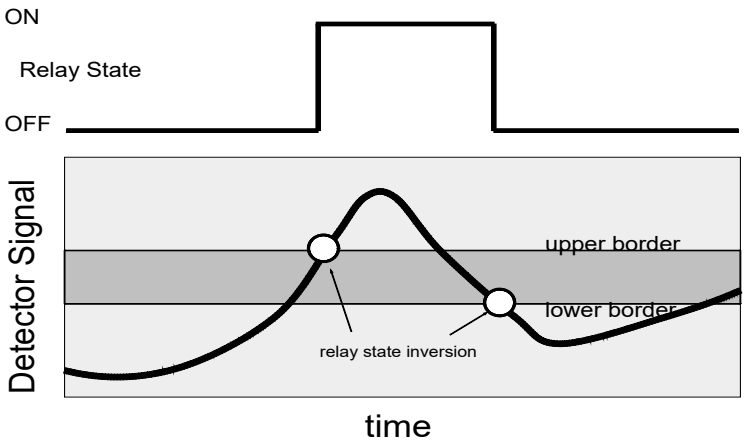


Figure 76 Detector dependent relay control with border switching

The relays will be inverted dependent on the detector signal. The standard state can be defined. At increasing detector signal the relays will be inverted at the upper switching border, at decreasing signal at the lower border. Upper and lower border are allowed to have the same value. In that case the relays will be in it's standard state below and in the inverted state above the switching border.

Detector Dependent Relay Control with Switching Region

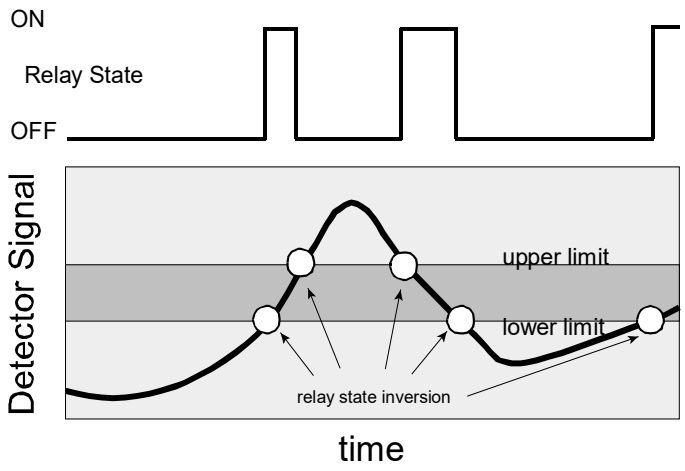


Figure 77 Detector dependent relay control with region switching

The relay will be inverted dependent on the detector signal. The standards state can be selected. Within the switching region the relays state is inverted, while outside the relays is in it's standard state.

ChromPilot: Supported Instruments and Modules

Overview of Controlled Systems

WinGPC Software acts as a universal macromolecular data system (MCDS) to organize the lab workflow efficiently and reliably with full tracability.

WinGPC Software allows to

- control of complete and mixed systems from a single or different vendors
- integrate old or not controlled components via analog or relay outputs

Comprehensive System Control and Sequence Creation

Comprehensive and fully traceable instrument control requires WinGPC Software with the ChromPilot option. Systems or modules purchased from third parties require a vendor specific driver which will communicate with the ChromPilot.

Initial Configuration

To actively control Agilent GPC/SEC systems with WinGPC Software, one module per system has to be connected to the data acquisition PC via LAN.

LAN (TCP/IP)

The LAN connection can be realized using either an already existing LAN Interface in the GPC system or by upgrading the system with a TCP/IP LAN card. Each system requires one card for remote control.

The allocation of the IP address and the (optional) integration into the local network should be done by a local administrator. If the GPC system is already integrated into the network, this step is not necessary.

If a PSS UDC810 is integrated in the network as well, the complete control and data acquisition can be performed using any PC in the network. Even more flexibility is possible with the WinGPC Software Client/Server system.

PC Requirements:

A free LAN port. The LAN connection can be realized with a cross-over patch cable (for multiple systems a hub and twisted-pair patch cables) to an extra ethernet card in the PC (recommended for more stability) or over the company network. The IP addresses of SECcurity system and PC need to belong to the same subnet.

Additional Requirements: each system that shall be controlled by ChromPilot requires a LAN port in at least one of it´s modules. The IP address needs to be assigned as a static address. This is described in detail in section “LAN Configuration” on page 543.

Settings in WinGPC Software ChromPilot Connection Dialog:

The dedicated IP address of the SECcurity system has to be entered as the “Port Address” in the Connection Dialog.

LAN Configuration

If a LAN connection is used, a unique IP address must be assigned to each GPC system. Please note, that WinGPC Software uses this connection to control the modules, but not for data acquisition.

The configuration of the SECcurity TCP/IP LAN interface depends on the available hardware. The IP address should be static since there is not BOOTP server available for WinGPC Software. The respective settings are described below.

LAN Interfaces

LAN Interface G1369A (part no. 404-0025), Firmware Minimum A.01.10

If a LAN Interface G1369A was installed, it might be necessary to configure its DIP switches. Therefor the card needs to be removed from the module (please note the Agilent hints on using electrostatic discharge wrist straps when handling electronics). The default settings will be “BOOTP” (DIP switches 1 to 8 “OFF”). In order to change to “Using Stored” (necessary for WinGPC Software ChromPilot), DIP switch no. 5 has to be set to “ON”.

The IP configuration can be done via the control panel as soon as the LAN Interface is re-installed in the SECcurity module.

Please note that this LAN Interface type does not allow the IP configuration via web browser.

Integrated LAN Interface

Some modules of the Agilent 1200 series (e.g., the DAD G1315C) have an integrated LAN Interface. The configuration is similar to the one described for G1369A, the DIP switches are on the rear of the module. In order to activate “Using Stored”, DIP switch no. 7 needs to be set to “ON”.

MIO LAN Interface card G1846A, Firmware Minimum K.08.20

If the configuration of the MIO card is unknown, the initial settings should be done via a control panel. Further configurations can be done using any web browser (see chapter “IP Configuration Using a Web Browser (Only Applicable for G1846A)” on page 545).

Programming IP Addresses Using a Control Panel

Entering the IP configuration section differs according to the kind of control panel which is used. Systems of the 1100 series will probably work with a control panel G1323B (part no. 404-0012, not available any more), systems of the 1200 series can be configured either with the old G1323 (if the firmware is compatible) or with the modern Instant Pilot G4208A (part no. 404-0036).

Control Panel G1323B:

```
[F5] = [Views]      => [System]  =>[Enter]
[F2] = [Configure]  => choose module with LAN MIO card      =>[Enter]
[F1] = [Interfaces] => [MIO]     =>[Enter]
```

You will get the message “Warning! If you continue, you will abort all LAN communications and the module will automatically restart after you have left the setup dialogue.” - press **continue**. You will get an overview of all the parameters that are currently set in the card. Choose **F1**=**Service** in order to modify the settings.

Instant Pilot G4208A:

On startup of a system with a new configuration you will automatically be guided to the LAN settings, but you can change them later as well using:

```
[More]      => [Configure]  => choose module with MIO card
            => LAN Settings => [Edit]     => [Modify]
```

Further steps for both control panels (G1323B and G4208A):

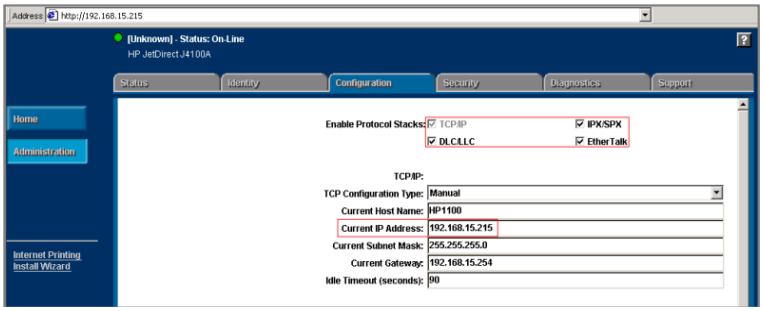
```
CFG Network NO  => [Enter] => choose “YES”
CFG TCP/IP  NO  => [Enter] => choose “YES”
BOOTP       YES => [Enter] => choose “NO”
```

As soon as the “BOOTP” value is changed to “NO”, a series of address bytes is displayed. Those addresses (IP, SM (subnet mask) and GW (gateway)) are usually written as XXX.XXX.XXX.XXX. The IP address must be of the same subnet as the IP address of the PC which controls the system. To enter the addresses, each byte (each block of three) has to be entered separately. Move to the parameter you want to change, enter the new value and press **Enter**. If you completed your changes, press **Done** to leave the service section.

The control panel G1323B requires a restart of the module by pressing **OK**. The Instant Pilot (G4208A) accepts all changes as soon as the LAN settings dialog is left.

IP Configuration Using a Web Browser (Only Applicable for G1846A)

As soon as the LAN MIO card has a dedicated IP address, further settings can be performed with any Internet browser. Enter the IP address into the address field of the browser to connect with the LAN MIO card. Press the **Administration** button in the following screen and choose the **Configuration** flag. Make sure that all protocol stacks are enabled (TCP/IP, DLC/LLC, IPX/SPX and EtherTalk). The TCP/IP settings can be verified or altered in this screen as well.



IP configuration using a Telnet session (1120 Compact)

Open the system (DOS) prompt window by clicking on Windows START button and select **Run...**. Type “cmd” and press **OK**. The connection to the 1120 Compact can be established by entering the actual IP address (default 192.168.254.11 or given at the instrument):

```
c:\> telnet <IP address>   (e.g.: c:\>telnet 192.168.254.11)
```

The system will answer with “Agilent Technologies G42...”

List all available commands (see figure):

```
> ?
```

Assign the new IP address:

```
> IP <new IP address>
```

Check the new settings:

```
> \
```

(shows actual status, see figure)

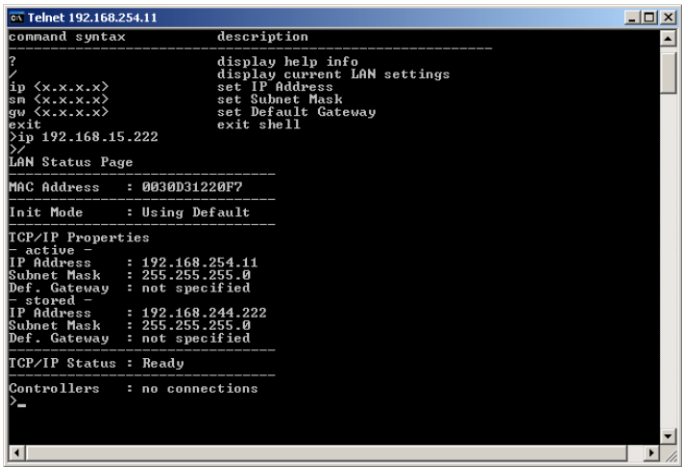
And leave the Telnet session:

```
> EXIT
```

In order to acknowledge these changes, the system has to be switched off and on again after the Telnet session was left.

NOTE

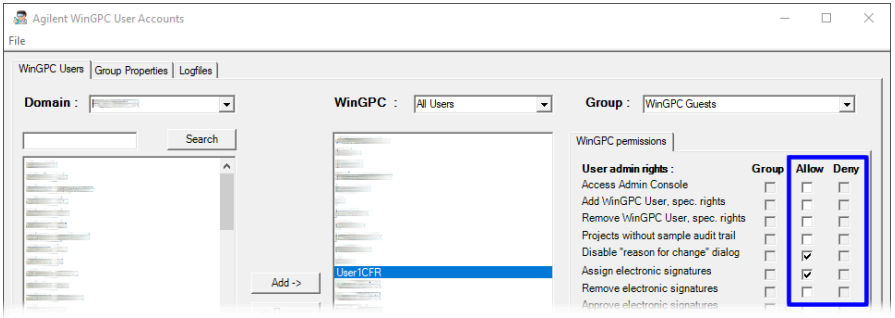
A similar Telnet session is possible for G1369A as well, but the changes have to be acknowledged with QUIT instead of just EXIT.



Compliance Edition: Installing and Configuring the Administration Database

This chapter describes the installation of the Agilent WinGPC Software Compliance Edition which adds full-featured software compliance as required by regulation issued by ICH, CFR, GxP, GAMP, etc.

Please note that regulations have to be provided by the WinGPC Software client organization to prevent unauthorized physical access to the WinGPC Software client PC.



1. Please make sure that you have administrator privileges before starting WinGPC Software installation.
2. Run WinGPC Software Setup as exemplified in the Agilent WinGPC Software Installation Instructions and the corresponding sections in this guide.
3. Make sure that all WinGPC Software folders have proper user rights. The WinGPC Session logbook (session audit trail) will show missing rights in case proper settings have not been applied.

NOTE Step 3 has to be performed only once for all client installations.

4 Copy the file WinGPC_CFR.cfr located in the WinGPC Software program folder to a directory with read/write privileges for all WinGPC users in the organization. This should be a shared location which can be accessed by all WinGPC Software users independent of time, place, and client PC. The user rights should be as specified in the Agilent WinGPC Software Site Preparation Checklist. If only a single PC will be used to access WinGPC Software, we recommend keeping this file in the WinGPC Software program folder as the location of the authentication database.

5 Launch WinGPC Software by clicking on the WinGPC Software icon or WinGPC Software.exe.

6 Select the location of the WinGPC Software authentication database as defined in step 3.

NOTE

Step 6 has to be performed only once for all client installations if a shared authentication database is used.

7 When the WinGPC Software Authentication dialog opens, enter **administrator** as user name, the corresponding local **administrator** password, select the local PC as **domain** and click on the **Administration** button to add all users to the WinGPC Software access database. Ensure the correct Windows domain for administrator authentication is selected.

If a local administrator password is not available, temporarily activate the **Guest** account and assign a password. Use this account for initial WinGPC Software database adminstration.

Please note that no user passwords have to be entered as they are read by WinGPC Software from the Windows domains. This allows to use the authentication policies of the customer organization (password strength, password validity, etc.).

8 Add users in the WinGPC Software User Accounts dialog from the list on the left and click on the **Add** button in the middle of the dialog screen. Select the WinGPC Software user group from the Select user level dialog. You may optionally grant or remove special **User admin rights** to non-WinGPC Administrators as specified in the corresponding section.

NOTE

- The displayed user list will depend on the selected **domain**. Please make sure you are using the correct domain as specified by the IT department.
- If the domain contains many objects, please be patient as reading the complete object list from the Microsoft server can take a while.

Agilent recommends adding all users at once, and never remove the **Administrator** account.

Do not modify the group properties.

Select **File > Save** to save changes or click the Close icon to save the WinGPC user account modifications.

WinGPC Software Launch

Now every user can launch WinGPC Software by clicking on the WinGPC Software icon on the desktop or opening WinGPC Software.EXE in the WinGPC Software program folder. Enter the Windows username and password to access WinGPC Software. Only **WinGPC Administrators** and **WinGPC Advanced Users** have the right to show the login window and modify details shown in the WinGPC Software login screen.

Only **WinGPC Administrators** have the right to make changes in the ChromPilot Connection Manager (other users must use a pre-defined instrument as setup by a WinGPC administrator).

WinGPC Software File Names and their Meaning

WinGPC Software creates and uses files of the following file types:

File/File type	Function
WinGPC_8.BTL [1)]	contains the item list for columns, detectors, operators etc.
WinGPC8.KEY	software license; defines the type and number of purchased modules
WinGPC_8.INI [1)]	general WinGPC Software user settings, e.g., default directories
WinGPC_8.PRB [1)]	contains the last sample search information
WinGPC_8.EDT [1)]	contains the last data editor contents
WinGPC_8.PAL [1)]	contains the defined color palettes for the background color
WinGPC_8.PRO [1)]	NetConnect settings; legacy WinGPC Software profile information
WinGPC_8_yyyy_mm.LOG [1)]	session audit trail (log file) created monthly (year_month); encrypted
WinGPC_CFR.cfr	WinGPC Software authentication database; only present with WinGPC Software Compliance Edition should be located on a network volume accessible by all WinGPC Software users
RFCDefaults.txt [1)]	user specific **reason for change** input list; for WinGPC Software Compliance Edition only
WinGPC_CFR.log [1)]	WinGPC Software administration and authentication audit trail (log file); encrypted;
Lang.DLL	only present with WinGPC Software Compliance Edition
Substanz.ACC	language resource file
*.LDX	user editable ASCII file; contains Mark Houwink Parameters, refractive index increments etc.; format description see chapter "WinGPC Software Sample Editor" on page 211
*.MDX	WinGPC Software Project file; contains login entries, start/stop time of runs
*.INX	WinGPC Software Project file; raw data file
*.FSX	WinGPC Software Project file; inject positions, sample names and baseline and integration limits
*.SAX	WinGPC Software Project file; processing parameters and method settings

*.ATX	WinGPC Software Project file; processing parameters and inject dependent settings
*.SPX	WinGPC Software Project file; sample audit trail; only present with WinGPC Software Compliance Pack
*.MSX	WinGPC Software Project file; 3D spectra; only present with WinGPC Software 3D spectra module
Instrumentn.* [1)]	default method files for instrument n
*.MET	WinGPC Software method file for data capture and default processing settings
*.nrm	normalization constants for MALLS detectors
*.REL	relay control table (ASCII file)
*.REF	HPLC reference table (ASCII file)
*.RBK	backup copy of HPLC reference table (ASCII file)
*.ADD	overlay file
*.CAL	calibration file
*.EMF [1)]	page preview contents in temporary file
*.LST	ReportDesigner layout
*.LSP	ReportDesigner printer definition for layout
*.spm	ChromPilot instrument method settings saved in ChromPilot Instrument Manager
*.sps	ChromPilot sequence file saved in ChromPilot Sequence Manager
System/ChromPilot.INI [1)]	stores if System/ChromPilot connection dialog is shown on startup
PSS-*.TXT	PSS ReadyCal calibration reference data for sample editor sequence import
cm*.*	ReportDesigner resource files
calibration.CFG	calibration settings profile
Sieve_settings.CFG	sieve curve dialog settings
CH.*, K.* or V.* [1)]	temporary curve cache file; automatically deleted on regular WinGPC Software termination can be deleted if WinGPC Software is not active
Logbook.Instrumentn_yyyy_mm.log [1)]	system audit trail (log file) created monthly (year_month); encrypted;
PSS.ChromPilot.DeviceConfiguration.Instrument.n.xml [1)]	only present with ChromPilot module; n+1 designates instrument configuration file in WinGPC Software ChromPilot Configuration Manager for instrument n

PSS.ChromPilot.Method.Instrument.n.xml [1)]	temporary method in WinGPC Software ChromPilot Instrument Manager for instrument n
PSS.ChromPilot.Sequence.Instrument.n.xml [1)]	temporary sequence in WinGPC Software ChromPilot Instrument Manager for instrument n
to_configuratorn.dat [1)]	temporary configuration file used with ChromPilot module; n+1 designates instrument
from_configuratorn.dat [1)]	temporary configuration file used with ChromPilot module; n+1 designates instrument
KonfPara.txt [1)]	module settings used with SystemPilot only
PSS.ChromPilot.Method.Instrument.n.xml	default ChromPilot instrument method for system no. n
PSS.ChromPilot.DeviceConfiguration.Instrument.n.xml	default ChromPilot instrument device/module configuration for system no. n
PSS.ChromPilot.Sequence.Instrument.1.xml	current ChromPilot sequence table for instrument no. n
S001_pss.drd [1)]	Calibration search index for drive d:
INJ.SDB and INJ.SDC [1)]	sample search database

[1)] These files are located in the WinGPC Software cache folder (C:\wingpc_8#n)

NOTE

The #n indicates the program instance number if WinGPC Software is launched several times (e.g., for multiple interfaces attached to the same PC).

The WinGPC Software help files are supplied for all supported languages.

Identifying Software Problems

If you observe a problem in the software we would be grateful to receive this information and assist you in overcoming the problem or defect. To do this efficiently, we require the following information:

- contact information: name, affiliation, phone number, e-mail address, etc.
- product information: WinGPC Software version and build number, cf. **Help > About**
- computer information: operating system, RAM size, swap size, user rights (on the keyboard, press the Windows key and Pause)
- problem description:
  - problem context: installation, data capture, data processing, calibration, viscometry, light scattering, copolymer, import/export, 2D, printing/reporting, utilities, help/documentation, etc
  - type of request: data loss, incorrect functionality, network, computer
  - severity of request: crash, cosmetic, feature request
  - step by step description: describe your observations in a reproducible way; send screen copies or data by e-mail if this seems useful

This information will allow the WinGPC Software support engineers to locate and identify the problem and resolve the problem fast and efficiently.

If the software crashes, you might want to copy the details of the Windows error message box. This can be done as follows:

1. Click on the **Details** button to show details from Windows message box.
2. Select the first part of the text displayed up to the section labeled **Registers**.
3. Press **CTRL** + **C** to copy this text to the Windows clipboard.
4. Paste (**CTRL** + **V**) this information into your report to the Agilent support.

If a WinGPC Software installation or configuration problem is suspected, then run the WinGPC Software Verification (WinGPC IQ) (see Agilent WinGPC Software Installation Instructions) and submit the test results to Agilent (**File > Send as E-Mail**) for further inspection.

If the problem is suspected to be PC or Windows related, then generating a MSINFO32 report run on the WinGPC Software data capture PC is recommended. MSINFO32 reports detail the hardware and software configuration of machines running Windows. These reports allow our technical support department to review your machine for hardware and software conflicts to recommend solutions to crashes and other issues.

How to run and save an MSINFO32 report:

1. Go to the Start button.
2. Select **Run...** (or press Windows Key + **R**).
3. Type msinfo32 and click **OK**.
4. While viewing the System Summary node go to **File** then **Save**.
5. Save your report as msinfo32.nfo and e-mail it to your Agilent support team.

Reference: http://support.microsoft.com/kb/308549/EN-US

In This Book

This guide describes the requirements, configuration and operation of the Agilent WinGPC Software version 1.0.

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Edition 07/2023

D0029603 Rev. A

Agilent