

Agilent EasyValid Validation Kit

User Manual Organic, version 03A



Notices

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Manual Part Number

5994-7137EN

Edition

First edition, March 2024

Printed in USA

Agilent Technologies, Inc.
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Introduction

The EasyValid Validation Kit is designed for comprehensive and fast validation of GPC/SEC systems independent of instrument model and manufacturer. All concentration detectors, such as refractive index (RI) or ultraviolet (UV) can be validated at the same time with the universal EasyValid approach. Molar-mass-sensitive detectors (online viscometers and online MALS/LALS/RALS detectors) can be part of the validation, but should also be validated using light scattering/viscometer validation kits (e.g., part number PSS-PSKITV).

This validation procedure checks the interaction of all components (pump, injection system, detectors, software) needed for GPC/SEC data capture and analysis (system suitability test); testing vendor specifications for components is not part of this test. The validation will only pass if all instrument modules are suitable for GPC/SEC measurements. Therefore, EasyValid is also ideal for mixed configurations where components from different vendors are used. The validation should be performed after installation, maintenance, or major repairs. It is also useful for checking company-developed standard operation procedures (SOPs), for identifying systematic user errors, and for training new employees.

One of the major advantages of EasyValid is that it is performed under GPC/SEC conditions and that true GPC/SEC results (molar mass averages and molar mass distribution) are validated. EasyValid uses GPC/SEC-certified reference materials characterized with extensive round robin tests.

EasyValid Validation Kit is designed:

For validations in:	Tetrahydrofuran (THF)
Temperature range:	20 to 35 °C
For GPC/SEC systems with*:	Degassers
	Pumps
	Sample injection system
	Column oven
	Detector(s)
	Capillaries
	Data acquisition hardware
	Data acquisition and evaluation software

*Any vendor may be used and not all components are required. Absolutely necessary components are printed in bold.

1 About EasyValid

Introduction

The validation approach can be performed with all (GPC/SEC) data acquisition and evaluation software packages that allow independent baseline and integration limits and calibration curve polynomial fit functions (fifth order).

Recommended Data System: Agilent WinGPC and later versions

Recommended Software Options: Report Designer

Sample evaluation (see **Chapter 3**) is described in a general, software-independent way. Useful WinGPC software features and functions are marked as a WinGPC tip.

General Description of the Validation Steps

The validation procedure consists of three steps:

- 1 The overall performance of the system is checked using a monodisperse, low molecular weight substance. If the overall performance is acceptable, the main validation can begin.
- 2 A calibration curve is constructed using the 12 EasyValid molar mass calibration standards.
- 3 This calibration curve is used for evaluating two different certified reference materials. The obtained molar mass averages are compared with the expected values. If the obtained molar mass deviations are in the accepted range, the validation will pass. If one or more molar mass averages are outside the allowed range, the validation has failed. Potential reasons leading to a failed test are summarized in **Chapter 5** of this user manual.

1 About EasyValid
General Description of the Validation Steps

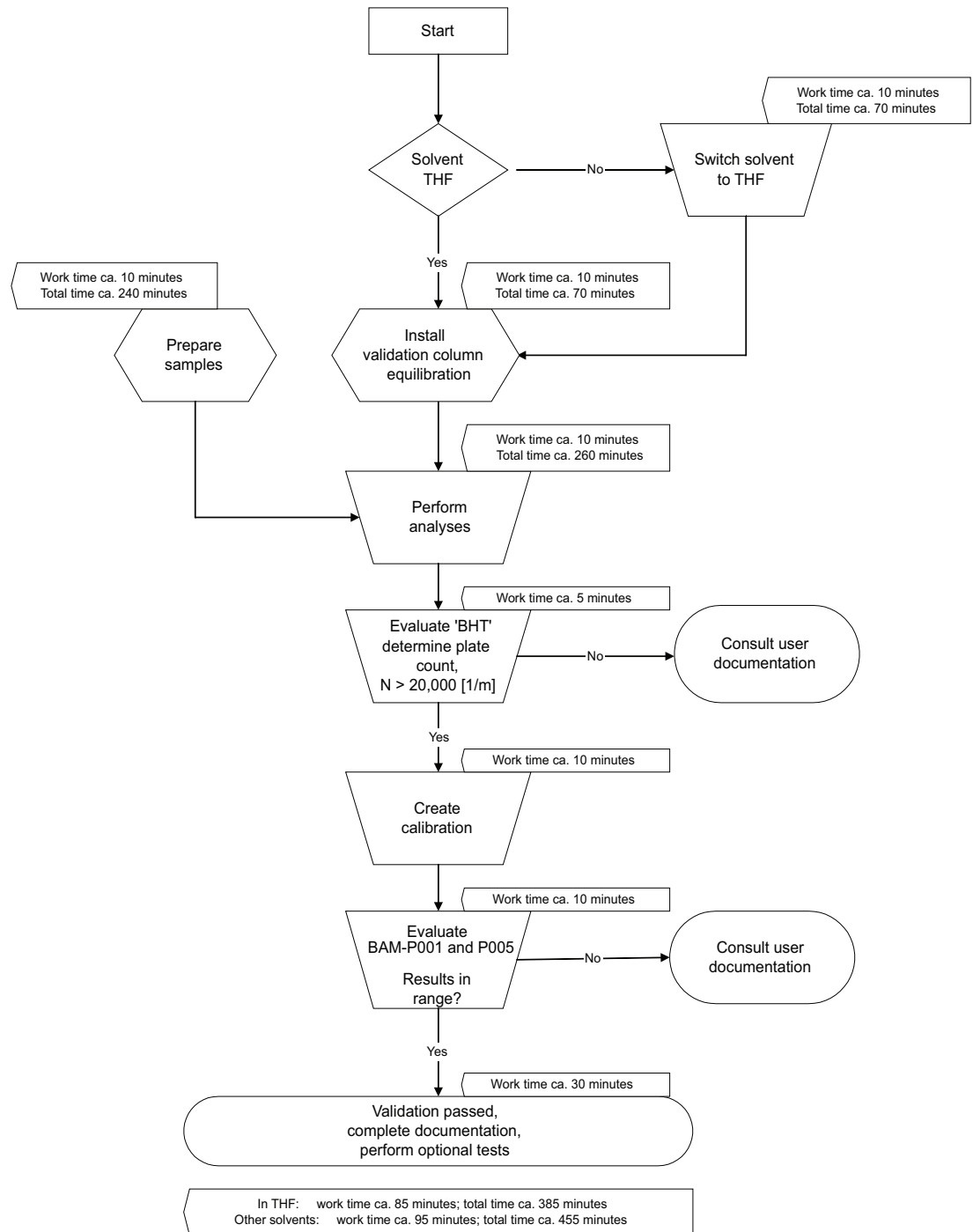


Figure 1. Validation scheme and time consumption.

*BAM-P001 is identical to ERM-FA001 and PS2088 and BAM-P005 is identical to ERM-FA005 and PS980422.

EasyValid in Certified Laboratories

Validation with the EasyValid Validation Kit can be part of an operational qualification/performance verification (OQ/PV). OQ/PV is an essential step of a complete validation that is performed during the whole life cycle of a chromatographic system. Design qualification (DQ) and installation qualification (IQ) should pass before starting OQ/PV. OQ/PV does not free the user from the requirement to validate their specific application and the separation columns (column combination) used for this application.

EasyValid validations can also be part of a performance qualification (PQ) when used after maintenance of the system or one of its components. A passed validation shows that the maintenance has been successful, and that the system is in working order again.

Tip: It is recommended to use EasyValid after installation of the system, after maintenance, after major repairs, or after exchange of one or more components. In between these validations, the system can be checked using a company-related reference sample.

Tip: A GPC/SEC ICH norm, ISO/EN norm 13885, and an ASTM norm, ASTM D5296-97, are available. EasyValid validation conforms with these norms.

EasyValid Validation Kit Contents

The EasyValid Validation Kit organic (part number PSS-PSTKITVAL) consists of:

- One GPC/SEC validation column with the following specifications:

Column Description: EasyValid organic (p/n EASYVALIDO-03)
 Column Dimensions: 8 × 300 mm
 Manufacturer: Agilent Technologies, Inc.

- A set of six different reference and test substances in color-coded glass vials (part number PSS-PSVAL). Five vials per substance are available, which can be used for five complete validation cycles. The primary standards and certified reference materials PS2088 (formerly ERM-FA001) and PS980422 (formerly ERM-FA005) were characterized through an extensive and comprehensive independent round robin test by the German Institute for Material Research (BAM).

Manufacturer: Agilent Technologies, Inc.

Table 1 Overview of EasyValid Validation Kit contents.

Substance	Type	Vial Color Code	Content
BHT	System suitability	Brown vial with blue label and black cap	2,6-di- <i>tert</i> -Butylhydroxytoluol p.a.
PS ReadyCal green* ReadyCal Kit Poly(styrene)	Calibration	Vial with green cap	Four poly(styrene) molar mass standards with narrow distribution Molar mass comp. 1 = 2,520,000 Da Molar mass comp. 2 = 277,000 Da Molar mass comp. 3 = 34,800 Da Molar mass comp. 4 = 3,470 Da
PS ReadyCal red* ReadyCal Kit Poly(styrene)	Calibration	Vial with red cap	Four poly(styrene) molar mass standards with narrow distribution Molar mass comp. 1 = 1,201,000 Da Molar mass comp. 2 = 130,000 Da Molar mass comp. 3 = 17,600 Da Molar mass comp. 4 = 1,250 Da
PS ReadyCal white* ReadyCal Kit Poly(styrene)	Calibration	Vial with white cap	Four poly(styrene) molar mass standards with narrow distribution Molar mass comp. 1 = 549,000 Da Molar mass comp. 2 = 66,000 Da Molar mass comp. 3 = 9,130 Da Molar mass comp. 4 = 474 Da
PS2088 (formerly ERM-FA001)	Validation	Brown vial with blue label and blue cap	Poly(styrene) with narrow distribution PS2088 M _n = 74,000 Da M _w = 79,600 Da M _p = 82,800 Da

1 About EasyValid

EasyValid Validation Kit Contents

Table 1 Overview of EasyValid Validation Kit contents.

Substance	Type	Vial Color Code	Content
PS980422 (formerly ERM-FA005)	Validation	Brown vial with blue label and yellow cap	Poly(styrene) with broad distribution PS980422 $M_n = 139,600$ Da $M_w = 311,800$ Da

* Lot # PSS-pskitr1-07

- A column test certificate, a calibration certificate, two certificates for PS2088 and PS980422, this user documentation, and two reference validation reports for PS2088 and PS980422 measured using the validation column.
- Two WinGPC report layouts for WinGPC. For report layouts for older WinGPC versions, please contact: pss.gpcsupport@agilent.com

Storage and Shelf Life

Storage

New, unopened reference samples should be stored in a cool, dark, and dry environment. The validation column, if not in use, should be plugged tightly and stored in a cool, dark, and dry environment. Use the plugs delivered with the column to plug the column. After one day of storage, the plugs should be checked and tightened again, if necessary. Do not freeze the validation column since this may destroy the column package.

Shelf life

- Sample solutions can be used for 2 to 3 days and should be discarded afterwards.
- The undissolved reference substances can be used for the stated shelf life (printed on the product label) when stored properly, as described earlier.
- Only reference substances purchased with the column and filtered poly(styrene) calibration standards should be measured on the validation column. Unknown high or low molecular weight samples or solvents other than THF can decrease column lifetime, lead to failed validations, or destroy the column.
- The validation column should be exchanged 5 years after purchase or if the plate count is below 20,000 [1/m].

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Sample Preparation

The samples are prepared by adding the proper amount of solvent (THF) to the vial. The best results (e.g., low baseline drift and fewer system peaks) are obtained when the solvent used for sample preparation is taken from the solvent bottle of the GPC/SEC system.

Tip: Add an internal flow marker (e.g., 20 ppm toluene) to the solvent that is used for preparing the samples.

The colored caps indicate the content of the vial and should not be mixed up.

The substance concentration in the vial is known. The optimum sample concentration is achieved by adding a defined amount of solvent. The validation is developed for measurements with 20 μL injection volume. For systems where 20 μL injection volume is not possible (e.g., injection systems with fixed loops larger or smaller than 20 μL), the concentration needs to be adjusted as described below.

Preparing the sample solution

- 1 Select a vial and unscrew the cap.
- 2 Add 1.5 mL THF (optional: with internal flow marker) using a syringe or pipette.
- 3 Close the vial with the corresponding cap and let it sit for 4 to 5 hours, then shake gently for homogenization.

Tip: Gentle shaking can decrease the time needed for dissolving the substance by a factor of two.

For injection systems that allow a 20 μL injection volume, the vials can be used without further action.

Deviating test conditions

For an injection volume of 100 μL (50 μL), remove 1.2 mL (0.75 mL) solution from the sample vial and discard it. Add 1.2 mL (0.75 mL) pure solvent to the vial, shake it gently, and use this vial as is.

Column Installation

Systems where THF is not the solvent used

GPC/SEC systems running with solvents other than THF need a solvent change to THF. Follow the instructions below for a quick and easy exchange:

- 1 If the GPC/SEC system is running with salt solutions, changing to the salt-free original solvent is necessary. The exchange is completed after 45 mL solvent per column has passed through the complete system. If an RI detector is installed, purge the RI reference cell for at least 15 minutes (3 × 5 minutes is best).
- 2 Stop the pump and remove the column (set). Plug the column ends tightly with the corresponding plug.
- 3 If the original solvent is not miscible with THF, use an intermediate solvent before changing to THF. A good intermediate solvent for most applications is acetone. If the original solvent is miscible with THF, users can change to THF directly.

NOTE

Some autosampler models require their own solvent bottles with solvent for cleaning the injection needle. Please do not forget to replace this solvent too.

NOTE

Viscometers require special conditions for changing the solvent. Please check the corresponding user documentation for details when using a viscometer.

Validation of column installation

- 1 For best practice and to avoid any damage to the column, use new connectors before connecting the EasyValid column. Check the validation column user documentation for further information.
- 2 Connect the column inlet (see marker for flow direction on the column) to the injection system and the column outlet directly to the waste. Please make sure that all parts of the GPC/SEC system, including capillaries, are filled with solvent before tightening the connections.
- 3 Start the pump with a flow rate of 0.2 mL/min and check for leaks. When solvent elutes from the column, the flow rate can be increased to 1 mL/min. If it takes more than 1 minute until the solvent leaves the column, stop the pump after the solvent elutes. Install the column in the reverse flow direction and flush the column for 1 hour at 0.2 mL/min before returning to the normal flow direction.
- 4 Flush the column with at least 45 mL solvent, then connect the column outlet to the detectors.
- 5 Flush the complete GPC/SEC system with at least 45 mL solvent. If an RI detector is installed during this time, the reference cell can be flushed/purged.

3

Measurement and Reference Substance Data Evaluation

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Measurement

After sample preparation as described in **“Sample Preparation”** on page 13, all samples must be injected at least three times. It is recommended to inject the samples in the same order as listed in **Table 1**.

The following conditions must be set for the measurement:

Solvent:	THF
Flow rate:	1.0 mL/min
Temperature:	20 to 35 °C
Injection volume:	20 µL
Injection interval:	16 minutes
Number of injections per sample:	3
Detector(s):	Any, but at least one UV detector (254 nm) or one RI detector

WinGPC Tip: The WinGPC sample import option can be used to load the sample and sequence information into the sample editor.

- File: PSS-PS(K)VAL03A.txt
- Location: WinGPC program directory

Start the data acquisition using the chromatography software. Do not forget to add the correct column information (e.g., dimensions, serial number, and specifications) before starting the measurement.

Data Evaluation

For all samples:

- 1 Activate the internal flow correction (optional).
- 2 Set the baseline limits to left limit 4 mL and right limit 14 mL.

WinGPC Tip:

With the raw data window active, from the **Options** menu, select **Analysis > quick analysis**. Set the values for the parameters as shown below:

For all injections:

Baseline left limit (mL): 4

Baseline right limit (mL): 14

Integration left limit (mL): 4

Integration right limit (mL): 14

Optional internal flow correction is also possible here (see WinGPC Application 25, #30025).

General system performance with reference substance BHT

Two performance tests can be done using the monodisperse, low molecular weight substance BHT (butylated hydroxytoluene).

Checking the inter-detector delay for GPC/SEC systems with UV and RI detector

- Evaluation: last injection of the BHT series for all concentration detectors
- If more than one detector is present in a GPC/SEC system, the inter-detector delay needs to be determined. If the software solution does not support this concept, calibration curves for every concentration detector have to be established.

To verify the correct inter-detector delay, set the integration limits closely around the peak. Compare the signals visually and check if the detector signal maxima match. Please note: if the signal for the last detector is slightly broader than that of the first detector, this might be due to band broadening in the capillaries and detector cells. This effect is not critical if the deviation is not too large.

WinGPC Tip:

The visual check should be done in the elugram window. If a calibration curve is loaded, WinGPC evaluates the data automatically and shows the elution volume of the peak maximum in the information window of the mass-distribution window. Compare these two values for UV and RI—they should match properly if the inter-detector delay is correct.

If the peak maxima match, the steps **Determination of the plate count** and Establishing a calibration curve need to be performed only for one concentration detector. Otherwise, the inter-detector delay needs to be adjusted or two plate count determinations and calibration curves are needed.

Determination of the plate count

Evaluation: last injection of the BHT series for one concentration detector.

- Set the integration limits and determine the plate count. This can be done automatically in the evaluation software or manually using the following formula:

$$N = 5.54 \times \left(\frac{V_e}{w_{1/2}} \right)^2 \times \frac{100}{L}$$

Where:

V_e : Elution volume peak maximum (mL)

$w_{1/2}$: Peak width (mL) at half peak height

L: Column length (cm), insert 30 for the validation column

WinGPC Tip:

- 1 Activate the elugram window. Right-click the x-axis and select **Set Peak Integration**.
- 2 From the **Options** menu, select **System Test**. Check column dimensions and diameter. If wrong, enter the correct values and select **Calculate**.
- 3 Use **Print** to print the results of the system test.

The system test is passed when the plate count is > 20,000 [1/m].

It is recommended to have a plate count of at least 35,000 [1/m] for the validation column.

A passed system test indicating that the general performance of the GPC/SEC system is sufficient as a precondition for completing the validation.

Calibration

Evaluation: for all injections, use ReadyCal Kit Poly(styrene) with green, red, and white caps.

- 1 Establish a calibration curve using all 12 molecular weight standards. Use M_p (the molar mass at the peak maximum) for the construction.
- 2 Select the fitting function for the calibration curve: Mandatory fitting function: Polynomial 5.
- 3 Save the calibration curve and print it for the validation documentation and further evaluation.

WinGPC Tip:

Automatic construction of the calibration curve (including optional flow correction) is also possible. Use the automation feature and the integration limits:

Left limit (mL): 5, right limit (mL): 11.4

Additional information can be found in the [Agilent WinGPC User Guide](#).

Evaluation for the certified reference materials PS2088 and PS980422

Evaluation: for all injections samples PS2088 and PS980422.

- 1 Use the newly constructed calibration curve to determine the molar mass averages and the molar mass distribution for PS2088 and PS980422.

WinGPC Tip: Activate the raw data window and select **Load** from the **Calibration Data** menu. Load the calibration curve for all injections.

- 2 Set the integration limits as described in **Table 2** with reference to the elution volumes measured for certain molar mass standards of the calibration curve (average of three injections).

Table 2 Integration limits reference substances PS2088 and PS980422.

Sample	Limit (from Calibration Curve)	Integration Limit
PS2088	Elution volume standard Mp = 130,000 Da	Left (mL): ____
	Elution volume standard Mp = 17,800 Da	Right (mL): ____
PS980422	Elution volume standard Mp = 2,400,000 Da	Left (mL): ____
	Elution volume standard Mp = 8,400 Da	Right (mL): ____

- WinGPC Tip:
- 1 Activate the elugram window.
 - 2 After a right mouse click on the X-axis underneath the peak maximum, select **Manual borders** from the context menu.
 - 3 Enter the values in **Table 3** in g/mol into the Minimum and Maximum cells.

Table 3 Integration limits WinGPC.

Sample	g/mol
PS2088	130,000 to 17,800
PS980422	2,400,000 to 8,400

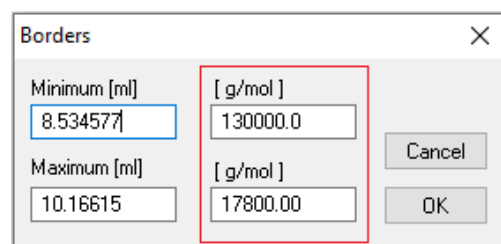


Figure 2. Limit of PS2088.

Then, print the results.

3 Measurement and Reference Substance Data Evaluation

Evaluation for the certified reference materials PS2088 and PS980422

The validation passes if the results for the samples are in the allowed range summarized in **Table 4**.

Table 4 Reference values for the reference substances PS2088 and PS980422.

Sample	Passed if: Mw (Da) in the range of	Passed if: Mn (Da) in the range of	Passed if: Mp (Da) in the range of
PS2088	83,580 to 75,620	77,700 to 70,300	86,940 to 78,660
PS980422	296,210 to 327,390	132,620 to 146,580	—

For samples with a broad molecular weight distribution (e.g., sample PS980422), the determination of M_p is difficult. This is one of the reasons why calibration standards, where M_p is needed, have a narrow molecular weight distribution.

WinGPC Tip:

WinGPC with Report Designer:

- 1 Activate the Raw Data window.
- 2 From the **Raw Data** menu, select **Print Report** and print the report "Validation PS2088" for sample PS2088 and "Validation PS980422" for sample PS980422.

These reports set automatic passed/failed flags for the reference substances.

Enhanced Validation (Optional)

To pass the validation, the raw data quality needs to be good to very good. This means that baseline drifts can be corrected and that the signal-to-noise (S/N) ratio is sufficient for GPC/SEC analysis.

Therefore, the following tests are optional for enhanced validation.

Determination of the detector drift

Evaluation: last injection of the BHT series for all concentration detectors.

- 1 Print the raw data and draw a line from the injection point to the elution volume of the peak maximum, as shown in **Figure 3**.

The drift, D , is defined as:

$$D = \text{Detector signal}_{\text{peak maximum}} - \text{Detector signal}_{\text{injection}}$$

- 2 Read the signal height at the peak maximum.

The signal height, H , is defined as:

$$H = \text{Signal height}_{\text{Maximum}} - \text{Signal height}_{\text{Baseline, 0 mL}}$$

- 3 Calculate the drift ratio, DR, according to:

$$DR = \frac{D}{H} \times 100\%$$

The detector drift test passes when the drift ratio $DR < 5\%$.

3 Measurement and Reference Substance Data Evaluation

Determination of the signal-to-noise ratio

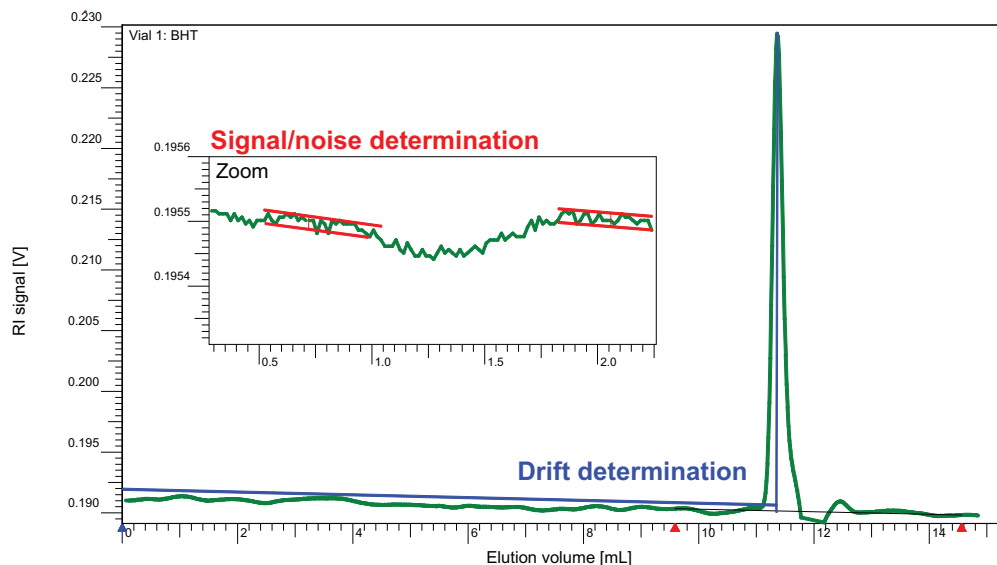


Figure 3. Determination of the drift ratio and the signal-to-noise ratio. Please note that the blue line has been shifted for better visibility.

Determination of the signal-to-noise ratio

Evaluation: last injection of the BHT series for all concentration detectors.

- 1 Zoom into the detector signal in the raw data window as shown in **Figure 3**, print it, and draw two parallel lines around the signal. For this test only, the short time noise is relevant; long-term signal instabilities should not be considered.
- 2 Measure the vertical distance between the two lines (the noise, R).

$$R = \text{Signal2} - \text{Signal1}$$

- 3 Calculate the signal-to-noise (S/N) ratio according to:

$$SN = \frac{R}{H} \times 100\%$$

The signal-to-noise test passes when the S/N ratio < 1%.

Determination of the repeatability

Evaluation: last injection of the BHT series, one concentration detector.

Note the elution volumes at the peak maximum for all repeat injections of the BHT series.

3 Measurement and Reference Substance Data Evaluation

Determination of the repeatability

The deviation, D, for the peak maxima is calculated according to:

$$D = \frac{E_{\text{Maximum_value}} - E_{\text{Minimum_value}}}{E_{\text{Maximum_value}}} \times 100\%$$

The deviation test passes when the deviation $D < 0.3\%$.

WinGPC Tip: Overlay all BHT injections and print the molar mass window overlay. The volumes for the peak maxima are summarized in the Vp line.

Test Documentation

For complete documentation of the validation, the following documents should be available:

- Printout of system test with plate count or printout of the graphic used for calculating the plate count.
- Printout of calibration curve.
- 3× printout results PS2088; a layout of a document with all values is also part of this documentation. Copy this and sign the document. Alternatively, sign the WinGPC Report Designer printouts.
- 3× printout results PS980422; a layout of a document with all values is also part of this documentation. Copy this and sign the document. Alternatively, sign the WinGPC Report Designer printouts.
- Document “**EasyValid Validation Kit Organic – Results Overview**” (also part of this user documentation). Copy this page and sign it.

Optional:

- Printout overlay of the repeated BHT injections to determine the repeatability.
- Printout of the measured raw data.
- Printout of the raw data used to determine the baseline drift and the S/N ratio.

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4

Example of a Successful Validation

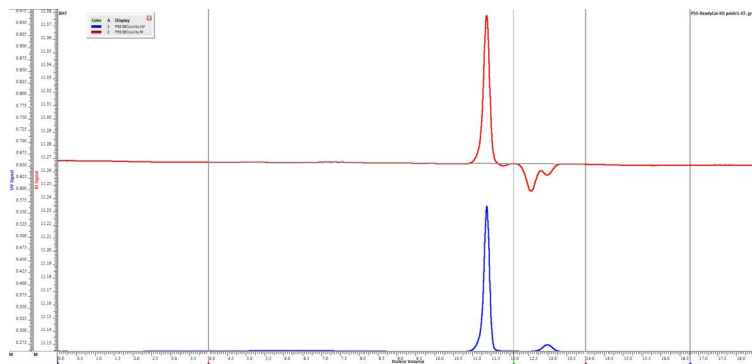
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Raw Data of the Reference Substances

The following pictures show the raw data of the reference substances for two detectors, one UV detector at 254 nm (blue) and one RI detector (red). The inter-detector delay for the RI detector was determined and corrected before the measurement. BHT was used as the (optional) internal flow marker for all samples.

For all raw data figures:

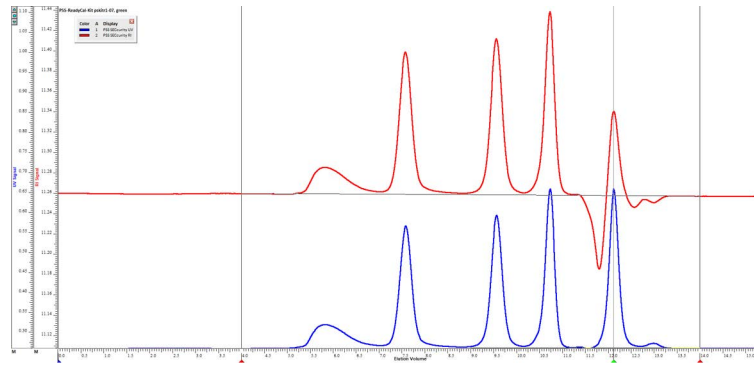
- Blue triangles: Injection marks
- Red triangles: Baseline limits
- Light green triangle: Internal flow marker



Sample: BHT

The BHT peak is followed by the system peaks. For RI detectors, system peaks are often negative.

4 Example of a Successful Validation Raw Data of the Reference Substances



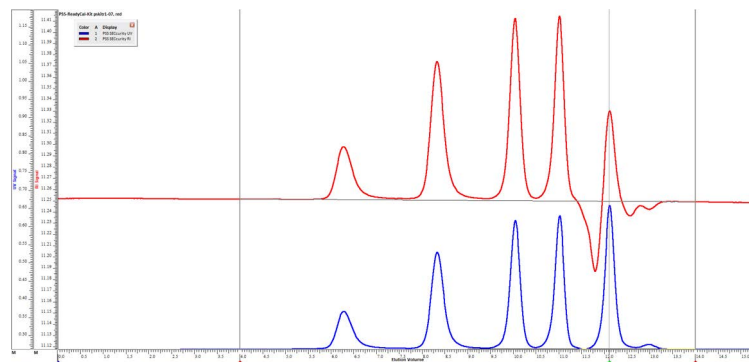
Sample: ReadyCal Kit Poly(styrene), green cap

Peak order:

Four molar mass standards:

- Molar mass comp. 1 = 2,520,000 Da
- Molar mass comp. 2 = 277,000 Da
- Molar mass comp. 3 = 34,800 Da
- Molar mass comp. 4 = 3,470 Da

System peaks and internal flow marker (toluene, light green triangle)



Sample: ReadyCal Kit Poly(styrene), red cap

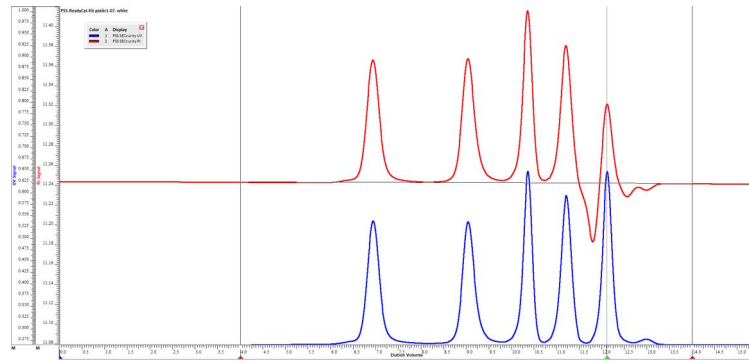
Peak order:

Four molar mass standards:

- Molar mass comp. 1 = 1,210,000 Da
- Molar mass comp. 2 = 130,000 Da
- Molar mass comp. 3 = 17,600 Da
- Molar mass comp. 4 = 1,250 Da

System peaks and internal flow marker (toluene, light green triangle)

4 Example of a Successful Validation Raw Data of the Reference Substances



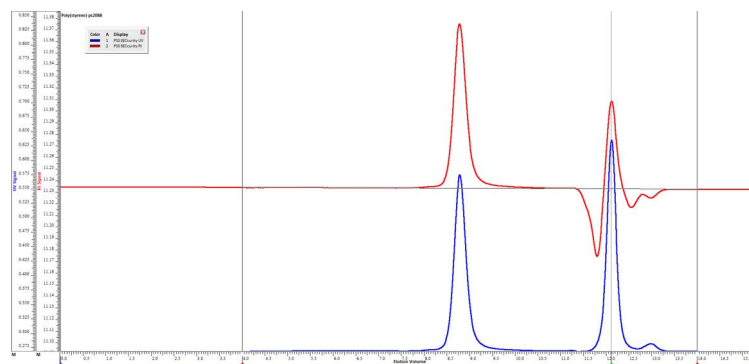
Sample: ReadyCal Kit Poly(styrene), white cap

Peak order:

Four molar mass standards:

- Molar mass comp. 1 = 549,000 Da
- Molar mass comp. 2 = 66,000 Da
- Molar mass comp. 3 = 9,130 Da
- Molar mass comp. 4 = 474 Da

System peaks and internal flow marker (toluene, light green triangle)

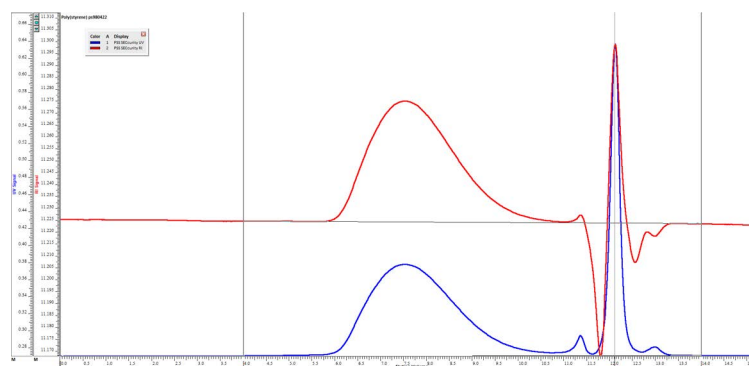


Sample: PS2088

Peak order:

One peak PS2088, reference substance with narrow molecular weight distribution

System peaks and internal flow marker (toluene, light green triangle)



Sample: PS980422

Peak order:

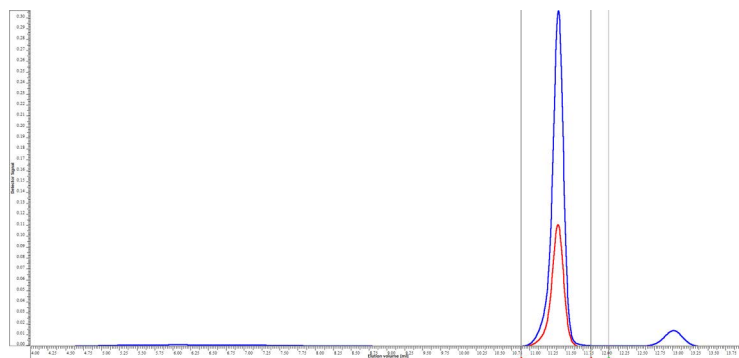
One peak PS980422, reference substance with broad molecular weight distribution

System peaks and internal flow marker (toluene, light green triangle)

Reference Substances with Integration Limits

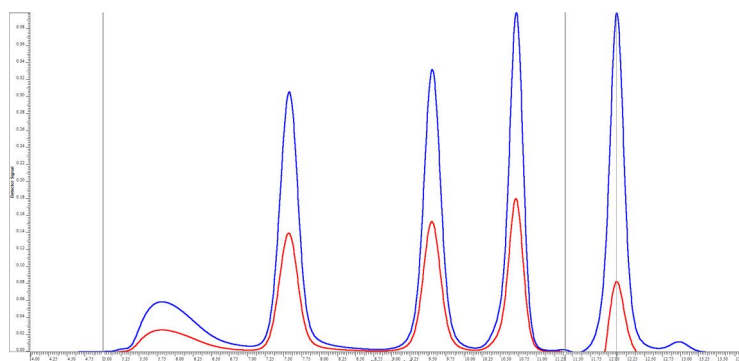
For all elugram figures:

Red triangles: Baseline limits
Light green triangle: Internal flow marker



Sample: BHT

Integration limits for plate count determination.



**Sample: ReadyCal Kit
Poly(styrene), green cap**

Peak order:

Molar mass comp. 1 =
2,520,000 Da

Molar mass comp. 2 =
277,000 Da

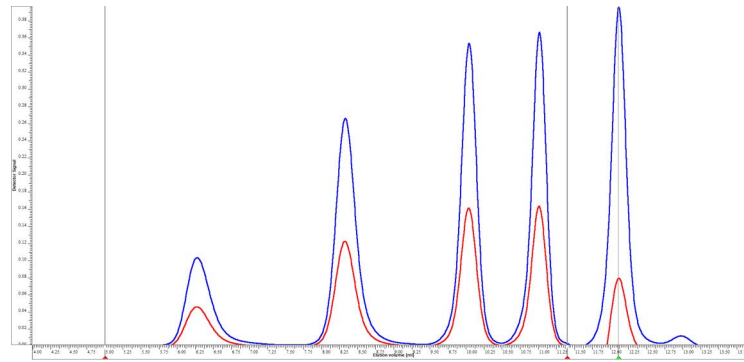
Molar mass comp. 3 =
34,800 Da

Molar mass comp. 4 =
3,470 Da

Toluene (light green triangle)

Integration limits are set by the WinGPC automation. The integration limits can be different for establishing the calibration curve.

4 Example of a Successful Validation Reference Substances with Integration Limits



Sample: ReadyCal Kit Poly(styrene), red cap

Peak order:

Molar mass comp. 1 =
1,210,000 Da

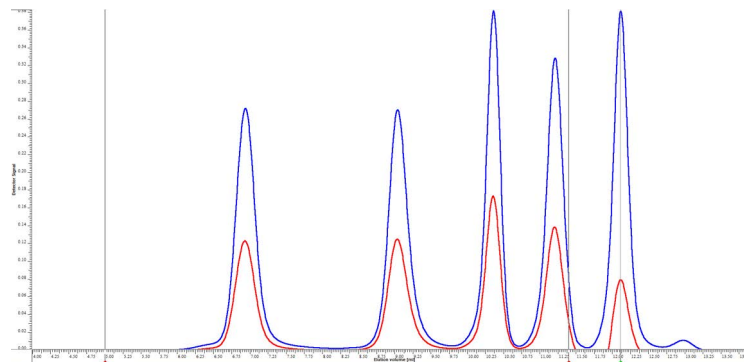
Molar mass comp. 2 =
130,000 Da

Molar mass comp. 3 =
17,600 Da

Molar mass comp. 4 =
1,250 Da

Toluene (light green triangle)

Integration limits are set by the WinGPC automation. The integration limits can be different for establishing the calibration curve.



Sample: ReadyCal-Kit Poly(styrene) white cap

Peak order:

Molar mass comp. 1 =
549,000 Da

Molar mass comp. 2 =
66,000 Da

Molar mass comp. 3 =
9,130 Da

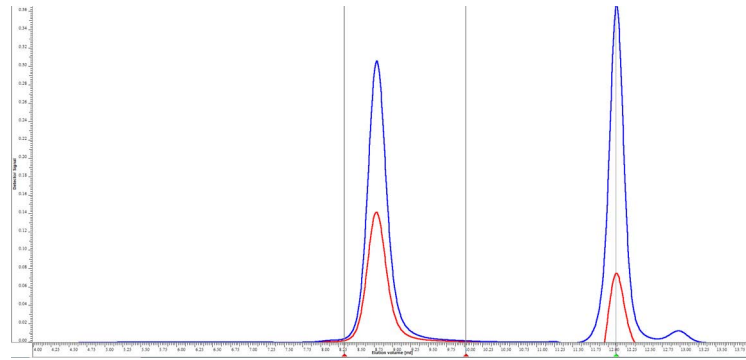
Molar mass comp. 4 =
474 Da

Toluene (light green triangle)

Integration limits are set by the WinGPC automation. The integration limits can be different for establishing the calibration curve.

4 Example of a Successful Validation

Reference Substances with Integration Limits

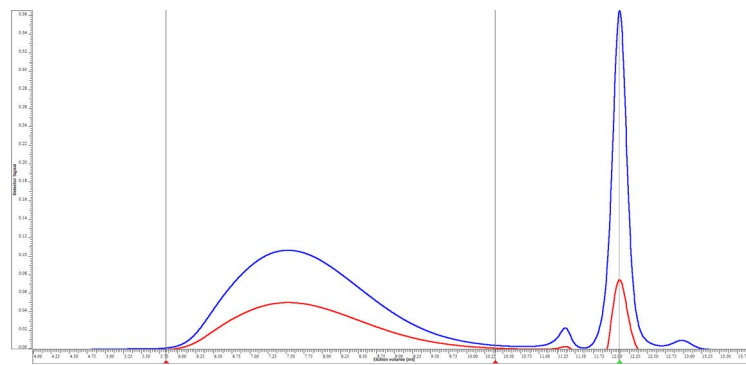


Sample: PS2088

Peak order:

- PS2088, reference substance with narrow molecular weight distribution
- Toluene (light green triangle)

The integration limits for PS2088 are fixed and need to be set as in the example.



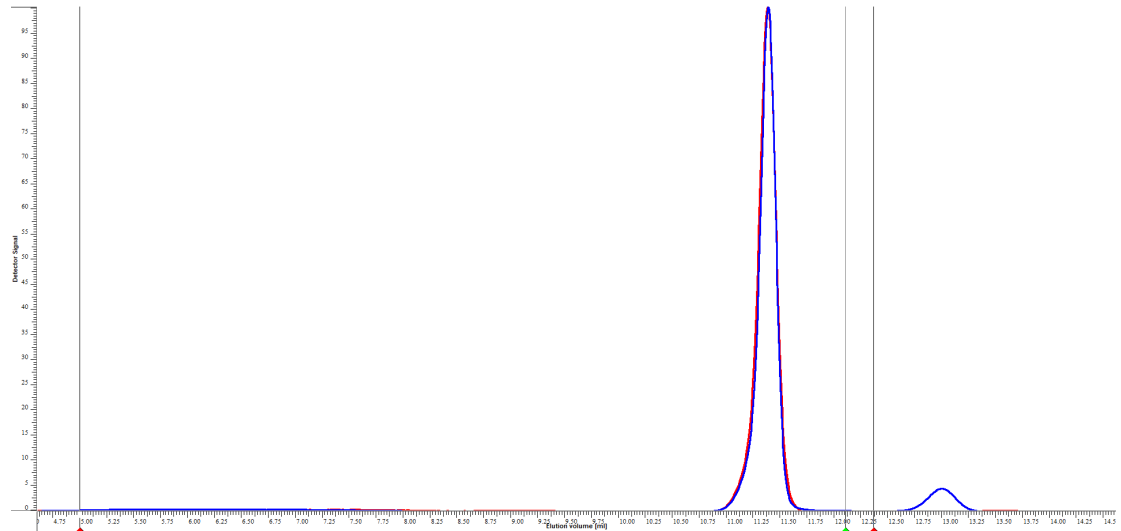
Sample: PS980422

Peak order:

- PS980422, reference substance with broad molecular weight distribution
- Toluene (light green triangle)

The integration limits for PS980422 are fixed and need to be set as in the example.

General System Performance with the Reference Substance BHT

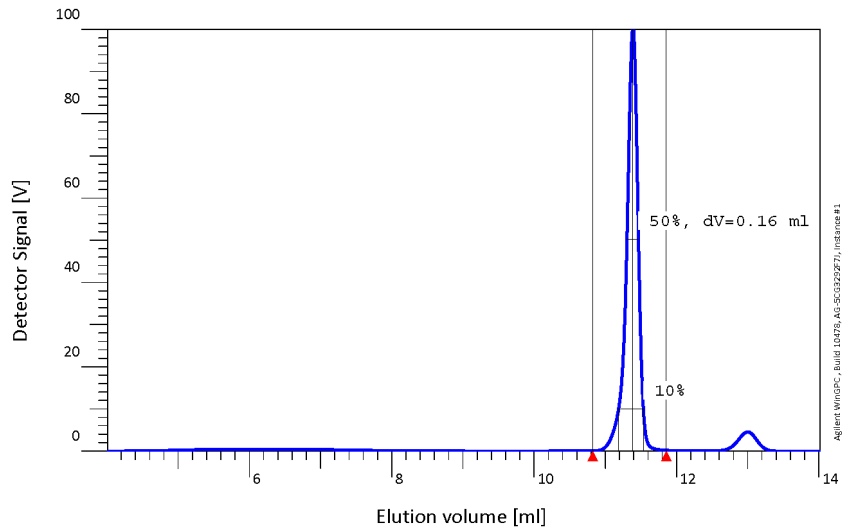


Checking the inter-detector delay using visual control in the WinGPC elugram window

The UV and RI signal for BHT match, which is expected when the inter-detector delay is correct.

4 Example of a Successful Validation
 General System Performance with the Reference Substance BHT

Determination of plate count and WinGPC result report



Sample :	BHT	Method :	RL3.MET
Baseline from :	4.000 ml	to :	14.000 ml
Integration from :	10.822 ml	to :	11.856 ml
Calibration File :	\\EasyValid orga...N230421H01_PG01.CAL	Eluent :	Tetrahydrofuran
MHK - A (Cal.):	0.000E+0	MHK - K (Cal.):	1.000E+0 ml/g
Int.stand.-cal.:	12.110 ml	Int.stand.-sam.:	12.095 ml
Pump :	Agilent 1200	Flowrate :	1.000 ml/min
Concentration :	1.000 g/l	Inject volume :	20.000 ul
Column 1 :	Agilent EasyValid organic	Temperature :	23.000 °C
Length :	30.000 cm	Diameter :	0.800 cm
Detector 1 :	UV 254nm	Delay volume :	0.000 ml
Detector 2 :	RID	Delay volume :	0.176 ml
Operator :	J.Preis	Acquisition interval :	1.000 sec

PSS Systemtest nach ISO 13885-1/DIN 55672-1 :

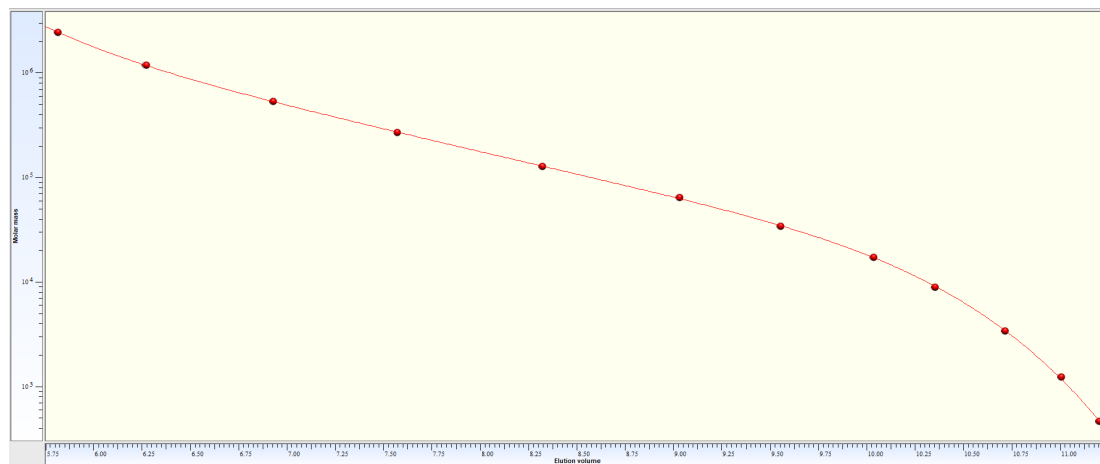
	Results	Requirements	Unit
Vol. Peakmax.:	11.386		ml
Plate Count :	91208	> 20000	1/m
Asymmetry :	1.351	> 0.85 < 1.15	-
Resolution :	1.648	> 2.5	-
Efficiency :	0.906	> 6	cm
signal drift :	-2.639E-3	< 5 %Imax	V/h
signal noise :	4.065E-5	-	V
signal wander :	1.344E-4	-	V
signal/noise :	14998.646	> 100	-
pump pressure fluct. :	0.000E+0	-	bar
column temp fluct. :	0.000E+0	-	°C

The measured plate count is above the minimum value of 20,000 1/m plates. The validation can be performed.

Calibration Curve

A calibration curve established from 12 different molar mass standards. The elution volume is averaged from three single injections.

Fit function: Polynomial fifth order.



The calibration curve shows the expected sigmoidal shape.

Evaluation of the Reference Materials PS2088 and PS980422

The molar mass averages for six injections (3 × PS2088, 3 × PS980422) need to be compared with the reference values and printed. Alternatively, six WinGPC report layouts should be printed. The following figures show examples with automatic passed/failed flags from the WinGPC Report Designer.

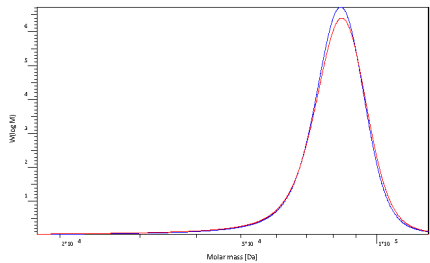
WinGPC Report ps2088

This OQ/PV validates the GPC/SEC system and the data flow within the data acquisition system.

OQ/PV was executed

for GPC-System: PG01
 on: AG-5CG3292F7J with Windows 10 22H2 Enterprise,
 with Agilent WinGPC, Build 10478 S/N: DE930055
 by: J.Preis
 at (Month, Day, Year): 01/18/2024 03:04:48 PM
 with Validation column: EasyValid organic

Molar mass distribution



Processing parameters

Sample name: Poly(styrene) ps2088
 Injection time: 18:09:4
 Project: W\GPC_DATEN\POLY\Projekt\EasyValid\EasyValid23.LDX
 Calibration: EasyValid organic vsn03a SN230421H01_PG01.CAL
 created: 4/25/2023
 last modified: 4/25/2023
 MH-alpha (Cal): 0,000
 MH-K (Cal): 1,0000
 Baseline from: 4,00 ml
 to: 14,00 ml
 Integration from: 8,33 ml
 to: 10,02 ml
 Solvent: THF
 Flow rate: 1,00 ml/min
 Injection volume: 20 µl
 Detector(s): UV 254 nm
 RI

Validation results

	Reference value	Allowed range	Value	Deviation [%]	Status
<i>Results for detector: UV 254 nm</i>					
Mn [Da]	74000	70300 - 77700	76843	3,841	passed
Mw [Da]	79600	83580 - 75620	80866	1,590	passed
Mp [Da]	82800	78660 - 86940	82979	0,216	passed
<i>Results for detector: RI</i>					
Mn [Da]	74000	70300 - 77700	76287	3,090	passed
Mw [Da]	79600	83580 - 75620	80873	1,599	passed
Mp [Da]	82800	78660 - 86940	83391	0,713	passed
End of Table					

This OQ/PV is passed when all results are within the allowed range.

Validation passed: Yes* No**

Signature: _____ Date (Month, Day, Year): _____

* Validation passed, no further action required.

** Validation not passed. Please perform another measurement following the instructions in the user documentation.
 If the validation still fails please contact:

Print date (Month, Day, Year): 01-18-2024

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Validation Report ps2088 Version 1.0

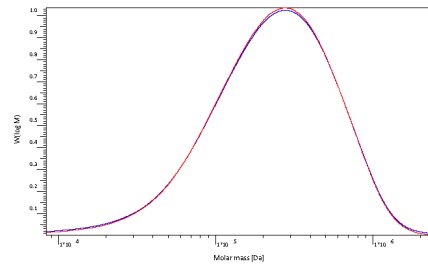
WinGPC Report ps980422

This OQ/PV validates the GPC/SEC system and the data flow within the data acquisition system.

OQ/PV was executed

for GPC-System: PG01
 on: AG-5CG3292F7J with Windows 10 22H2 Enterprise,
 with Agilent WinGPC, Build 10478 S/N: DE930055
 by: J.Preis
 at (Month, Day, Year): 01/18/2024 03:07:38 PM
 with Validation column: EasyValid organic

Molar mass distribution



Processing parameters

Sample name: Poly(styrene) ps980422
 Injection time: 19:00:0
 Project: W:\GPC_DATEN\POLY\Projekte\EasyValid\EasyValid23.LDX
 Calibration: EasyValid organic vsn03a SN230421H01_PG01.CAL
 created: 25.4.2023
 last modified: 25.4.2023
 MH-alpha (Cal): 0,000
 MH-K (Cal): 1,0000
 Baseline from: 4,00 ml
 to: 14,00 ml
 Integration from: 5,84 ml
 to: 10,39 ml
 Solvent: THF
 Flow rate: 1,00 ml/min
 Injection volume: 20 µl
 Detector(s): UV 254 nm
 RI

Validation results

	Reference value	Allowed range	Value	Deviation [%]	Status
<i>Results for detector: UV 254 nm</i>					
Mn [Da]	139600	132620 - 146580	139047	0,396	passed
Mw [Da]	311800	296210 - 327390	319767	2,555	passed
<i>Results for detector: RI</i>					
Mn [Da]	139600	132620 - 146580	142207	1,867	passed
Mw [Da]	311800	296210 - 327390	316949	1,651	passed
					End of table

This OQ/PV is passed when all results are within the allowed range.

Validation passed: Yes* No**

Signature: _____ Date (Month, Day, Year): _____

* Validation passed, no further action required.

** Validation not passed. Please perform another measurement following the instructions in the user documentation. If the validation still fails please contact:

Print date (Month, Day, Year): 01-18-2024

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Validation Report ps980422 Version 1.0

All three injections for PS2088 and PS980422 show the status "passed" for all concentration detectors. This result means that the overall validation has been passed.

Enhanced Validation (Optional)

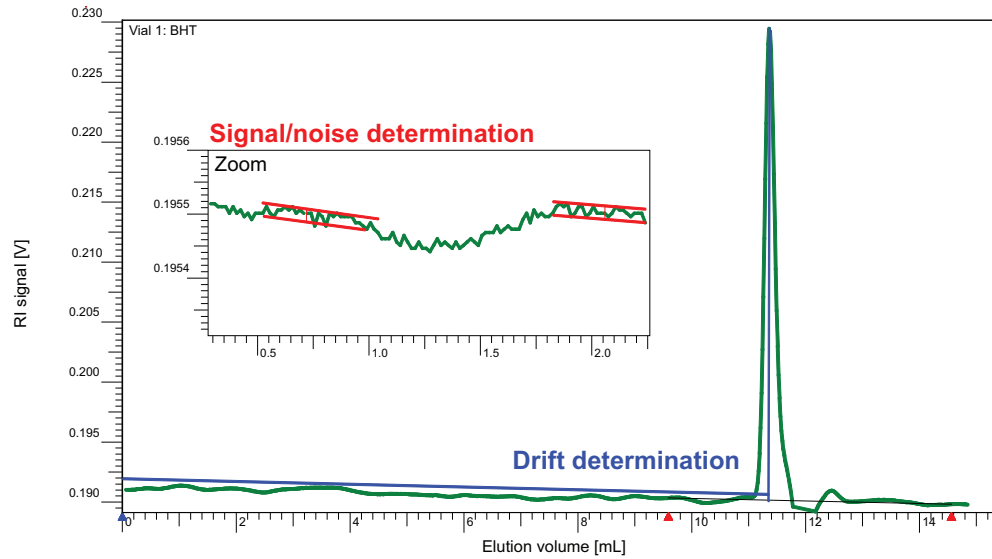


Figure 4. Note that the blue line has been shifted for better visibility.

Determination of detector drift for one concentration detector

Example: RI detector

$$D = \text{Detector signal peak maximum} - \text{detector signal injection} \\ = 0.1910 \text{ V} - 0.1920 \text{ V} = -0.001 \text{ V}$$

$$H = \text{Detector signal peak height} - \text{detector signal baseline at intersection} \\ = 0.2295 \text{ V} - 0.1905 \text{ V} = 0.039 \text{ V}$$

$$DR = D/H \times 100 \\ = -0.001 \text{ V} / 0.039 \text{ V} \times 100\% = -2.56\%$$

If the drift ratio is below the maximum drift ratio of $\pm 5\%$, the test passed.

Determination of the S/N ratio for one concentration detector

Example: RI detector

$$R = \text{Signal 2} - \text{Signal 1} = 0.00001 \text{ V}$$

$$S/N = R/H \times 100 = 0.00001 \text{ V} / 0.039 \text{ V} \times 100\% = 0.25\%$$

If the S/N ratio is below the maximum S/N ratio of 1%, the test passed.

Determination of the repeatability

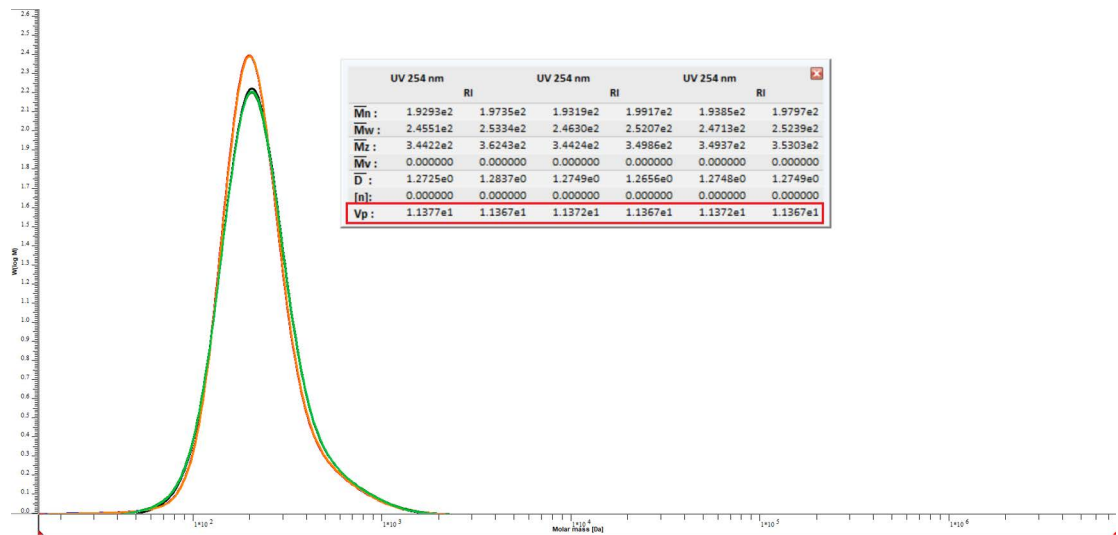


Figure 5. Overlay of three repeat injections of the reference substance BHT with UV and RI detection.

$$D = \text{Maximum volume at the peak maximum} - \text{minimal volume at the peak maximum}$$

Repeatability UV detector:

$$D(\text{UV}) = (11.377 \text{ mL} - 11.372 \text{ mL}) / 11.377 \text{ mL} \times 100\% = 0.04\%$$

Repeatability RI detector:

$$D(\text{RI}) = (11.367 \text{ mL} - 11.367 \text{ mL}) / 11.367 \text{ mL} \times 100\% = 0\%$$

If the deviation is below the maximum deviation of 0.3%, the test passed.

This test was performed without correction using the internal flow marker.

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Possible Reasons for a Failed System Validation

Possible Reasons for a Failed General System Performance Test 39

Possible Reasons for a Failed Validation 41

Possible Reasons for a Failed General System Performance Test

The following (partial) list provides possible explanations as to why a system fails the general system performance test and plate counts below 20,000 [1/m] plates are measured:

- The validation column is damaged due to incorrect storage or handling, injection of incorrect samples, or use of noncompliant solvents. The column user documentation gives hints for handling, storage, and use.
- The installed system capillaries are too long, or the inner diameter is too large.
- Capillary connections have excessive dead volume and reduce the overall performance of the system.

The following (partial) list shows possible explanations why the repeatability test fails and deviations > 0.3% are measured:

- The pump shows fluctuations despite regular pumping.
- There is a leakage or a microleakage in the system.
- The system and the validation column are exposed to strong temperature fluctuations (>10 °C difference).
- The solvent quality is poor.

Corrective actions after a failed plate count test

- 1 Check the capillaries and exchange them if fittings and ferrules do not sit properly.
- 2 If possible, check the validation column on another GPC/SEC system.

If these steps cannot be taken or if a second system also reports plate counts below 20,000 [1/m], contact Agilent with the following information:

- Serial number validation column.
- Plate count and asymmetry of the validation column compared to the values reported on the column certificate.
- Age of the validation column, the number of passed validations that have been performed, and when the validation column was last used.

Corrective actions after a failed repeatability test

- 1 Check all connections for leakages or microleakages with a clean tissue.
- 2 Check if a used column compartment is tightly closed.
- 3 Check when the pump was last maintained and if maintenance is due.
- 4 If the test fails directly after maintenance and pump seal exchange, give more time for equilibration (e.g., overnight), then repeat the reproducibility test.
- 5 Measure the flow rate using a graduated cylinder and a stopwatch. If the measured flow rate deviates strongly from the set flow rate, check if the pump offers test routines that can be executed by the user.
- 6 If a degasser is not used, install a degasser, or use other methods to clean the solvent.

If these tips do not help, contact Agilent or the pump manufacturer with the following information:

- Pump type, model, and serial number.
- Age of the system and what components are installed.
- When the pump was last maintained.
- The measured flow rate compared to the set flow rate.

Possible Reasons for a Failed Validation

If one or more molar mass averages are outside the allowed range, the validation failed. This section shows possible explanations (others may exist) why the validation could fail.

The parameters for the measurement were incorrect

Please check:

- Injection volume
- Whether the concentration was optimized for the injection volume.
- Flow rate
- Baseline limits
- Integration limits
- Calibration curve: have all molar mass standards been used and are the entered molar masses correct?
- Fit function of the calibration curve: has a polynomial function fifth order been used?
- Plate count of the validation system
- Whether the validation column was only used for the reference substances.

If only one detector fails in a multidetector system, is the inter-detector delay correct?

Does the second detector need its own calibration curve? (Not WinGPC)

The raw data quality is insufficient

The following figures show two examples of insufficient raw data quality leading to a failed validation.

Baseline drift

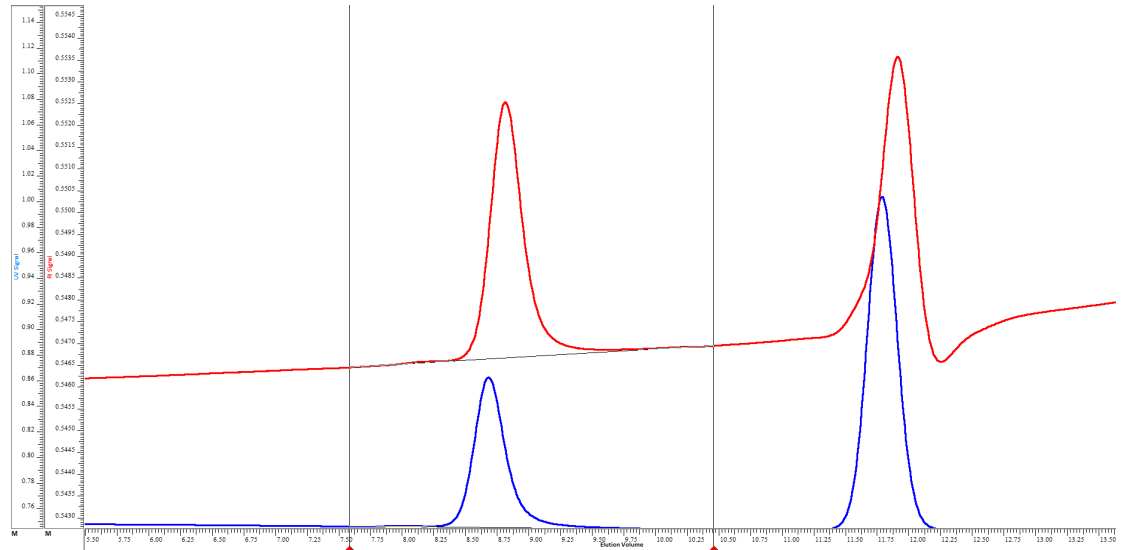
A baseline drift does not automatically mean that the validation is failed. If the drift is constant and can be corrected with the baseline, there will be no influence on the results. If the drift is not constant, parts of the sample will be integrated and the validation will not pass.

5 Possible Reasons for a Failed System Validation

The raw data quality is insufficient

Example:

An RI signal with baseline drift that can be corrected with the baseline setting. All parts of the sample are above the baseline and can be integrated. The validation will pass.

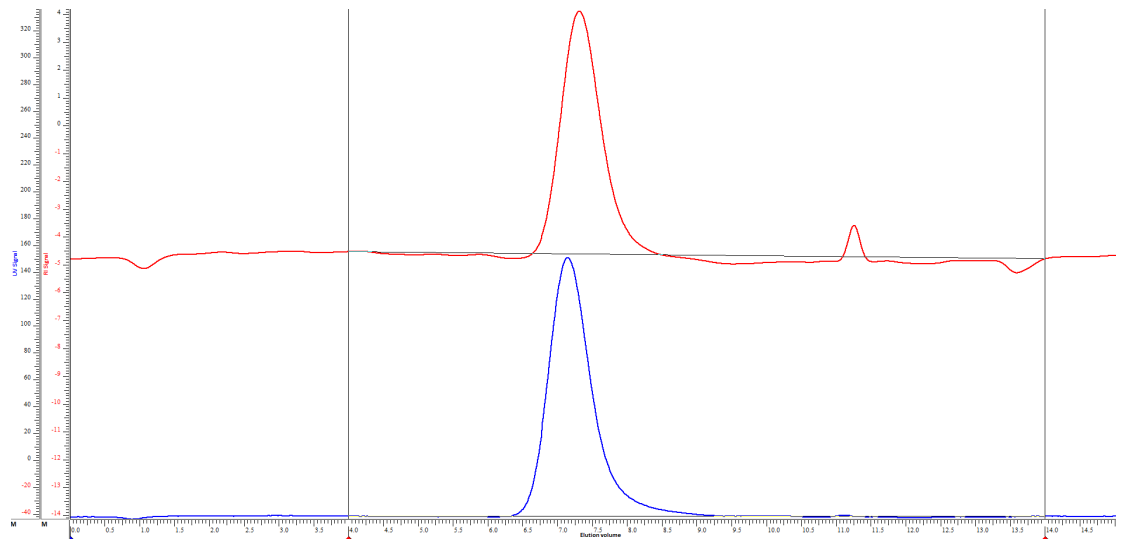


Detector wander

More problematic than baseline drift is detector wander. Wander leads (independently of the baseline setting) to a failed validation since parts of the sample cannot be integrated.

Example:

An RI signal with wander. A part of the sample is below the baseline and cannot be integrated. This leads to a failed validation. The UV detector shows only a light wander.



Baseline drift and detector wander can be combined. In these cases, the validation cannot be passed.

5 Possible Reasons for a Failed System Validation

Corrective actions after a failed validation

Possible reasons for insufficient raw data quality (not a comprehensive list):

- Bad solvent quality, the solvent is too old, has too many peroxides, or a too high water content.
- The system eluent has not been used for sample preparation.
- High temperature changes or drafts (e.g., due to air conditioning).
- The time for stabilizing the system was insufficient and the RI reference cell has not been purged long enough.
- Impurities in the pump head, the injection system, the capillaries, or the detectors (cells).
- A trapped air bubble in the system or the validation column.
- Not all parts of the system (e.g., pump, injection system, or detectors) are THF resistant.
- A broken or damaged RI detector purge valve.

Corrective actions after a failed validation

- 1 Change the solvent bottle. Use only clean, freshly opened HPLC-grade water.
- 2 Allow enough time for system equilibration; overnight is best. Reduce the flow rate to 0.2 mL/min for 15 minutes, then increase the flow rate stepwise (0.1 mL/min steps) until 1 mL/min is reached. This procedure might help to get rid of small, trapped air bubbles.
- 3 Purge the reference cells of RI detectors thoroughly.
- 4 Perform the system validation again.

If the validation fails again, contact Agilent with the following information:

- Measured results for the molar mass averages. If WinGPC has been used, send the raw data to Agilent.
- Age of the system and what components are installed.
- When the system was last maintained.
- Accuracy and stability of the pump flow.
- Age of the validation column, the number of passed validations performed, and when the validation column was last used.

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6

Appendix

This appendix contains a printable Results Overview form for the EasyValid Validation Kit Organic.

EasyValid Validation Kit Organic – Results Overview

Results for system: _____

Pump: _____	S/N: _____
Injection system: _____	S/N: _____
Detector 1: _____	S/N: _____
Additional components: _____	S/N: _____
_____	S/N: _____
_____	S/N: _____
_____	S/N: _____

Results validation step 3.2.1:

Inter-detector delay: _____ (only for systems with more than one concentration detector)
 Passed Failed Not measured

Plate count:
Expected: N > 20,000 [1/m] Measured: N = _____ 1/m
 Passed Failed

Results validation step 3.2.3:

Results sample PS2088:
Expected: _____ molar mass averages for all three injections within the allowed range.
 Passed Failed

Results sample PS980422:
Expected: _____ molar mass averages for all three injections within the allowed range.
 Passed Failed

Results validation step 3.3 (optional):

Detector drift: _____ Expected: DR < 5% Measured: DR = _____%
 Passed Failed Not measured

Signal-to-noise: _____ Expected: S/N < 1% Measured: S/N = _____%
 Passed Failed Not measured

Repeatability: _____ Expected: D < 0.3% Measured: D = _____%
 Passed Failed Not measured

The validation passes when all performed and non-optional tests have passed.

Passed Failed

Date: _____ Signature: _____

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First edition, March 2024

5994-7137EN

