Agilent

Agilent EasyValid Validation Kit

User Manual
Aqueous, version 02A

Notices

Manual Part Number

5994-7069EN

Edition

First edition, February 2024

Printed in USA

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Foreword

The Agilent EasyValid column and the Agilent EasyValid Standards Kit are carefully matched products.

Please ensure that the column version number and the standard kit version number match one another. This is essential to ensure successful verification and validation of the GPC/SEC system.

It is possible to reorder the EasyValid column (p/n EASYVALIDA-02) as well as the EasyValid Standard Kit (p/n PSS-DXTVAL) for a specific version number independently.

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Contents

1 About EasyValid

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), infrared (IR), and/or evaporating light scattering (ELS) 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-DXTKITV).

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 SOPs (standard operation procedures), 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 aqueous is designed:

For validations in:	Water, sodium azide, 0.5 g/L
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

*Required components are printed in bold.

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 (Manufacturer: Agilent Technologies, Inc.)

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 eight 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.

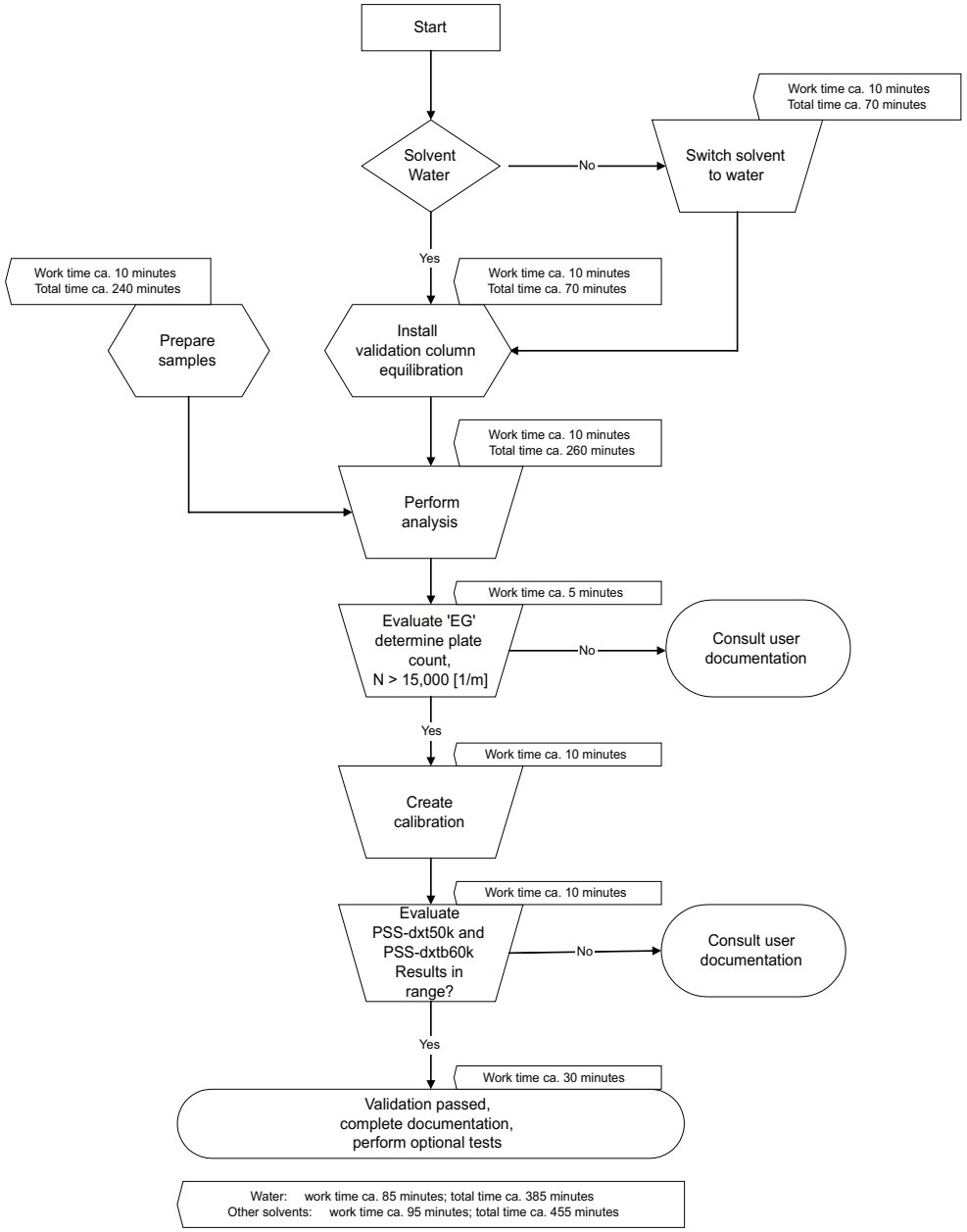


Figure 1. Validation scheme and time consumption.

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 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. 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 aqueous (p/n PSS-DXTKITVAL) consists of:

- One GPC/SEC validation column with the following specifications (part number EASYVALIDA-02):

Column Description:	EasyValid Aqueous version 02
Column Dimensions:	8 × 300 mm
Plate Number:	N ≥ 15,000 [1/m]
Asymmetry:	0.7 to 1.5
Supplier:	Agilent Technologies, Inc.

- A set of six different reference and test substances in color-coded glass vials (part number PSS-DXTVAL; batch number: DXTVAL02A). Five vials per substance are available, which can be used for five complete validation cycles. The primary standards PSS-DXT50K and PSS-DXTB60K were characterized by GPC/SEC and online light scattering. Manufacturer: Agilent Technologies, Inc.

Table 1 Overview of Validation Kit contents.

Substance	Type	Vial Color Code	Content
DXTP1	System suitability	Transparent vial with black cap	Dextran degree of polymerization 1
Pul ReadyCal green* ReadyCal Kit Pullulan, high	Calibration	Transparent vial with green cap	Four Pullulan molar mass standards with narrow distribution Molar mass comp.1 = 334,000 Da Molar mass comp. 2 = 47,100 Da Molar mass comp. 3 = 6,300 Da Molar mass comp. 4 = 504 Da
Pul ReadyCal red* ReadyCal Kit Pullulan, medium	Calibration	Transparent vial with red cap	Two Pullulan molar mass standards with narrow distribution Molar mass comp. 1 = 201,000 Da Molar mass comp. 2 = 22,000 Da
Pul ReadyCal white* ReadyCal Kit Pullulan, low	Calibration	Transparent vial with white cap	Two Pullulan molar mass standards with narrow distribution Molar mass comp. 1 = 106,000 Da Molar mass comp. 2 = 9,800 Da
Dextran DXT50-EV2	Validation	Transparent vial with blue cap	Reference material PSS-DXT50K with narrow distribution dextran M_n = 33,800 Da M_w = 45,500 Da
Dextran DXTB60-EV2	Validation	Transparent vial with yellow cap	Reference material PSS-DXT60K with broad distribution dextran M_n = 38,800 Da M_w = 59,200 Da

* Lot # PSS-PULMIX

- A column test certificate, a calibration certificate, two certificates of reference materials, this user documentation, and two reference printouts for PSS-DXT50K and PSS-DXTB60K measured using the validation column.
- Two WinGPC report layouts. These layouts are installed automatically with the software. 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. Please 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. Please 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 a minimum of 1 year after delivery when stored properly as described earlier.
- Only reference substances purchased with the column should be measured on the validation column. Unknown high or low molecular weight samples or solvents other than the aqueous solution can decrease column lifetime, lead to failed validations, or destroy the column.
- The validation column should be exchanged 3 years after purchase or if the plate count is below 15,000 [1/m].

2 System Preparation

Sample Preparation

The samples are prepared by adding the proper amount of solvent (water, sodium azide 0.5 g/L) 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: If an internal flow marker is used, be sure that the flow marker does not influence or overlap with the elution profile of the sample. The flow marker should elute after the integration limit.

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/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 aqueous solution (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 directly.

Column Installation

Systems where water is not the actual solvent

GPC/SEC systems running with solvents other than water need a solvent change to water. Please 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 water, use an intermediate solvent before changing to water. A good intermediate solvent for most applications is acetone.
4. If the original solvent is miscible with water, users can change to water 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 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

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:	Water, sodium azide 0.5 g/L
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:	RI detector required

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

- File: (PSS-)DXT(K)VAL02A.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 16 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): 16
Integration left limit (mL): 4
Integration right limit (mL): 16
Optional internal flow correction is also possible here (see **Agilent Technical Overview 5994-5940EN**).

General system performance with reference substance PSS-DXTP1

Two performance tests can be done using the monodisperse, low molecular weight substance Dextran DXTP1.

- Determination of the plate count
- Evaluation: last injection of the DXTP1 series

Set the integration limits and determine the plate count. This can be done in the data 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 passes when the plate count is N ≥ 15,000 [1/m].

It is recommended to have a plate count of at least 25,000 [1/m] for the validation column.

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

Calibration

Evaluation: for all injections, use ReadyCal-Kit Pullulan with green, red, and white caps.

1 Establish a calibration curve using all eight 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): 4, right limit (mL): 16

Additional information can be found in the **Agilent WinGPC User Guide**.

Evaluation for the reference materials PSS-DXT50K and PSS-DXTB60K

Evaluation: for all injections samples Dextran DXT50-EV2 and Dextran DXTB60-EV2.

1 Use the newly constructed calibration curve to determine the molar mass averages and the molar mass distribution for Dextran DXT50-EV2 and Dextran DXTB60-EV2.

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 the following table 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 PSS-DXT50K and PSS-DSTB60K.

Sample	Limit (from Calibration Curve)	Integration Limit
Dextran DXT50-EV2	Elution volume standard Mp = 337,000 Da	Left (mL): ____
	Elution volume standard Mp = 1,400 Da	Right (mL): ____
Dextran DXTB60-EV2	Elution volume standard Mp = 337,000 Da	Left (mL): ____
	Elution volume standard Mp = 1,400 Da	Right (mL): ____

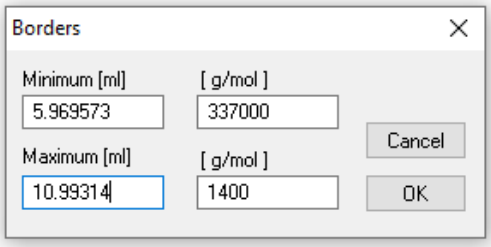
WinGPC Tip: a) Activate the elugram window.

b) After a right mouse click on the X-axis underneath the peak maximum, select **Manual borders** from the context menu.

c) Enter the values in **Table 3** in g/mol at minimum/maximum.

Table 3 Integration limits WinGPC.

Sample	g/mol
Dextran DXT50-EV2	337,000 to 1,400
Dextran DXTB60-EV2	337,000 to 1,400



3 Print the results.

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 PSS-DXT50K and PSS-DXTB60K.

Sample	Reference Value (Da)	Passed if: Mn (Da) in the Range of ±8%	Passed if: Mw (Da) in the Range of ±8%
Dextran DXT50-EV2	Mn = 33,800 Mw = 45,500	31,100 to 36,510	41,860 to 49,140
Dextran DXTB60-EV2	Mn = 38,800 Mw = 59,200	35,700 to 41,900	54,460 to 63,940

For samples with a broad molecular weight distribution, 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:

a) Activate the Raw Data window.

b) From the **Raw Data** menu, select **Print Report** and print the report "Validation DXT50-EV2" for sample DXT50-EV2 and "Validation DXTB60-EV2" for sample DXTB60-EV2.

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 eventual baseline drifts can be corrected and that the signal/noise 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 dxtp1-series, 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 2**.

The drift, D, is defined as:

$$D = S_{\text{Peak\_maximum}} - S_{\text{Inject\_time}}$$

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:

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

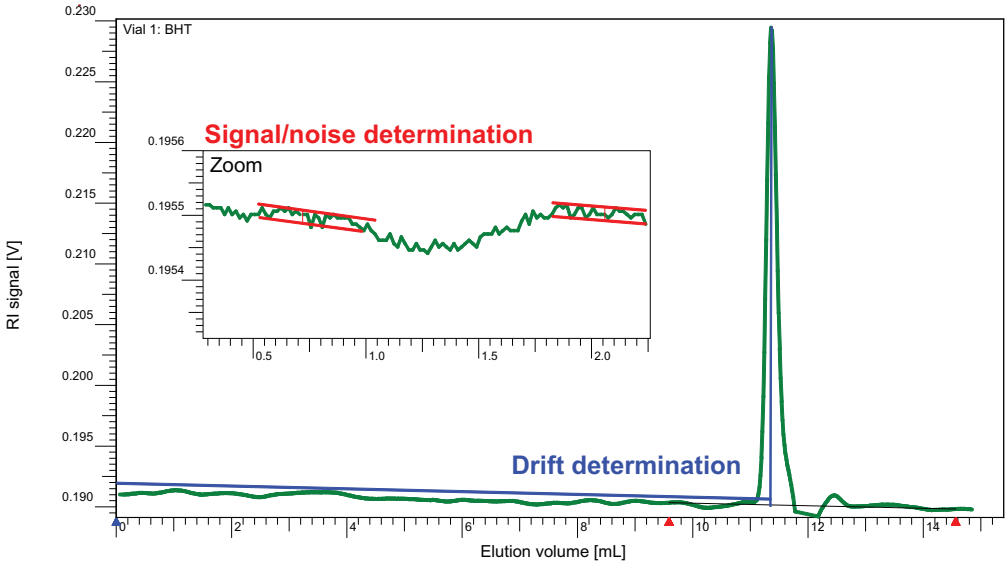


Figure 2. Determination of the drift ratio and the signal/noise ratio. Please note that the blue line has been shifted for better visibility.

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

Determination of the signal-to-noise ratio

Evaluation: last injection of the dxtp1-series.

1 Zoom into the detector signal in the raw data window as shown in Figure 2, 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: all injections of the dxtp1-series.

Note the elution volumes at the peak maximum for all repeat injections of the reference substance dxtp1 at the peak maximum.

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 dxtp1 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 DXT50-EV2; 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 DXTB60-EV2; 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 “Overview results” (also part of this user documentation). Copy this page and sign it.

Optional:

- Printout overlay of the repeat dxtp1 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.

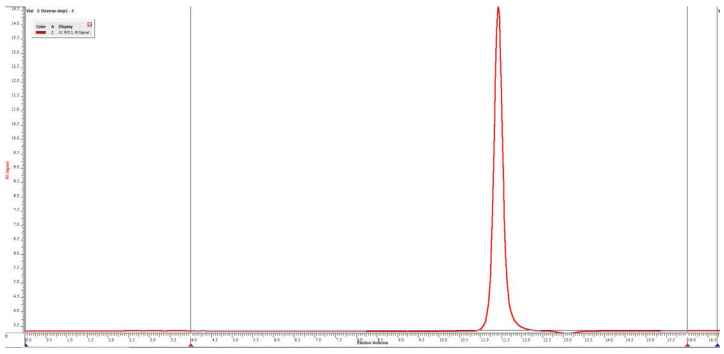
4 Example of a Successful Validation

Raw Data of the Reference Substances

The following pictures show the raw data of the reference substances for a RI detector.

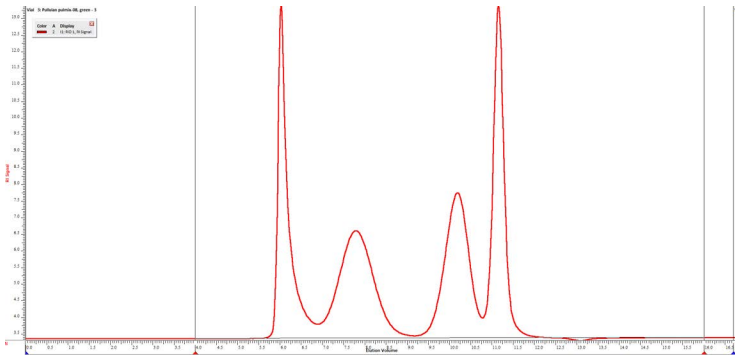
For all raw data figures:

Blue triangles: Injection marks
Red triangles: Baseline limits



Sample: DXTP1

The DXTP1 peak is followed by the system peaks. For RI detectors, system peaks are often negative.

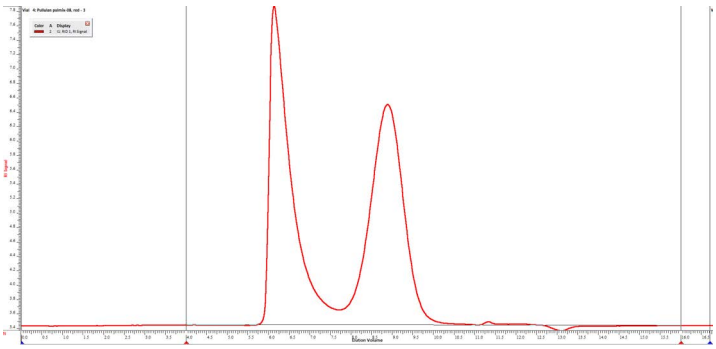


Sample: ReadyCal-Kit Pullulan, green cap

Peak order:

Four molar mass standards:

- Molar mass comp. 1 = 334,000 Da
- Molar mass comp. 2 = 47,100 Da
- Molar mass comp. 3 = 6,130 Da
- Molar mass comp. 4 = 504 Da



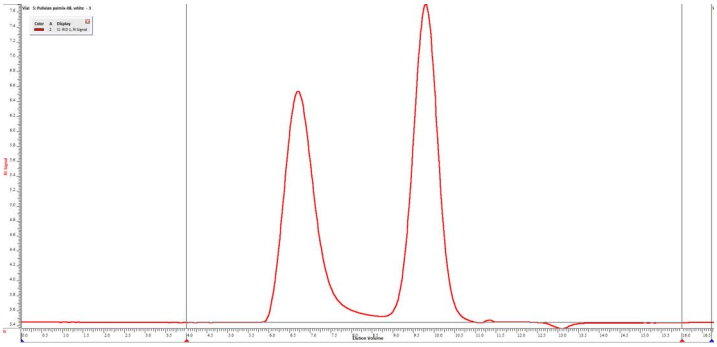
Sample: ReadyCal-Kit Pullulan, red cap

Peak order:

Two molar mass standards:

Molar mass comp. 1 = 201,000 Da

Molar mass comp. 2 = 22,000 Da



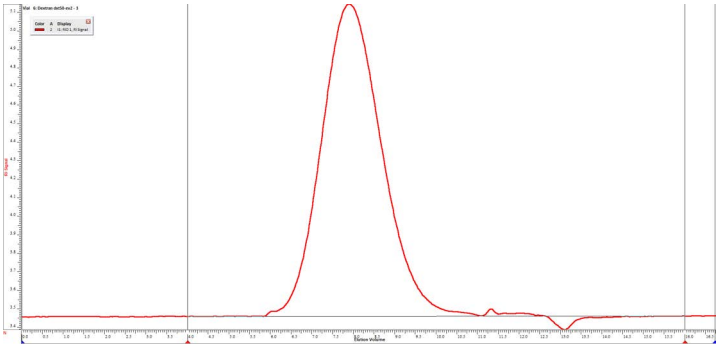
Sample: ReadyCal-Kit Pullulan, white cap

Peak order:

Two molar mass standards:

Molar mass comp. 1 = 106,000 Da

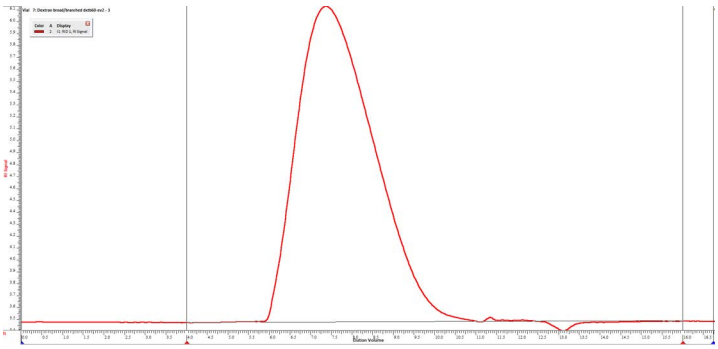
Molar mass comp. 2 = 9,800 Da



Sample: Dextran DXT50-EV2

Peak order:

Reference substance with narrow molecular weight distribution.



Sample: Dextran DXTB60-EV2

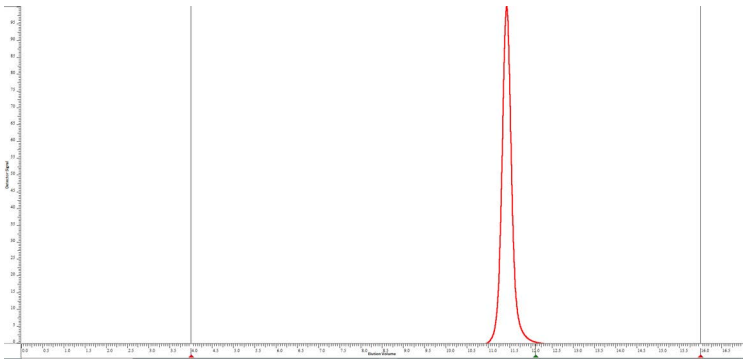
Peak order:

Reference substance with broad molecular weight distribution.

Reference Substances with Integration Limits

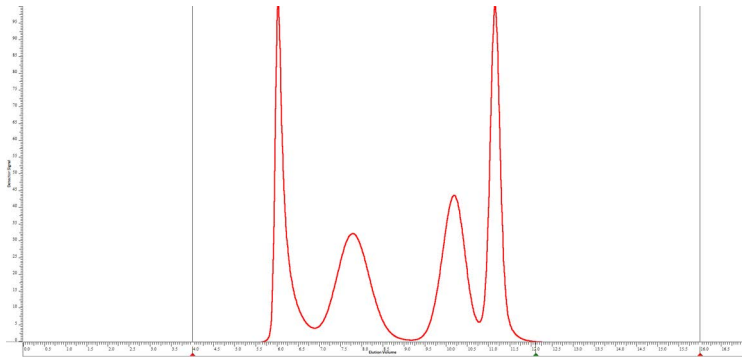
For all elugram figures:

Red triangles: Integration limits



Sample: DXTP1

Integration limits for plate count determination.



Sample: ReadyCal-Kit Pullulan, green cap

Peak order:

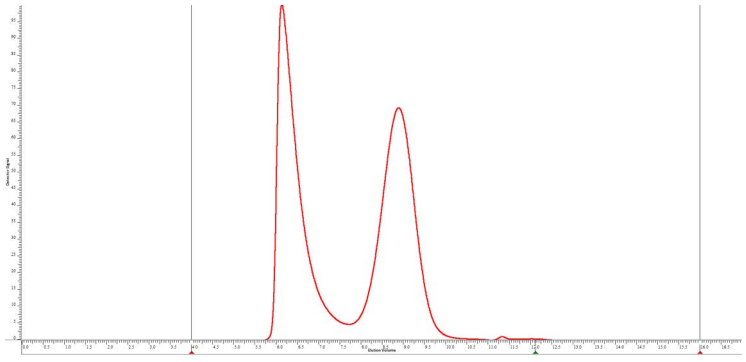
Molar mass comp. 1 = 334,000 Da

Molar mass comp. 2 = 47,100 Da

Molar mass comp. 3 = 6,300 Da

Molar mass comp. 4 = 504 Da

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



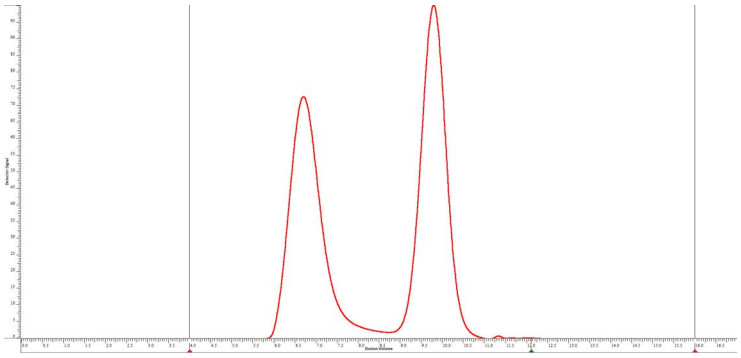
Sample: ReadyCal-Kit Pullulan, red cap

Peak order:

Molar mass comp. 1 = 201,000 Da

Molar mass comp. 2 = 9,800 Da

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



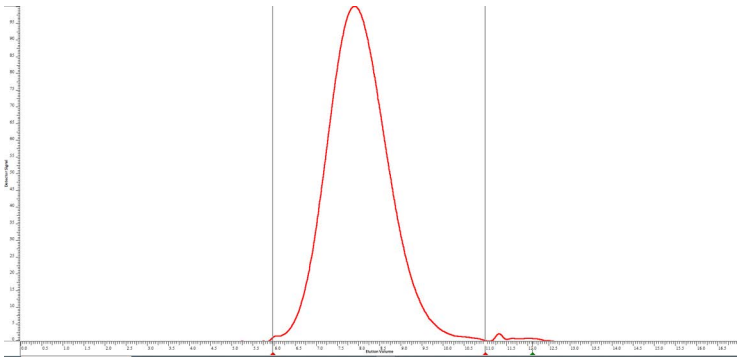
Sample: ReadyCal-Kit Pullulan, white cap

Peak order:

Molar mass comp.1 = 107,000 Da

Molar mass comp. 2 = 9,600 Da

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

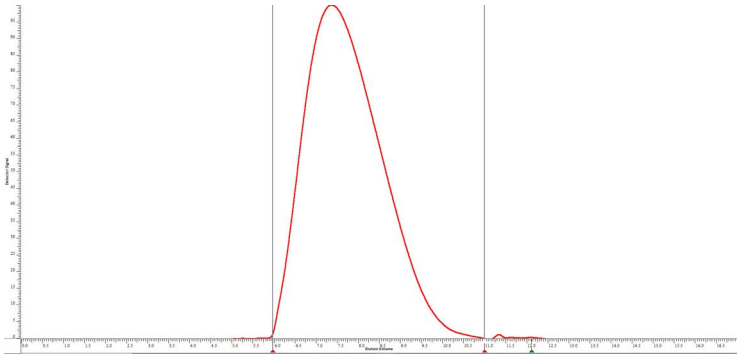


Sample: Dextran DXT50-EV2

Peak order:

Reference substance with narrow molecular weight distribution.

The integration limits for DXT50-EV2 are fixed and need to be set as described in the example.



Sample: Dextran DXTB60-EV2

Peak order:

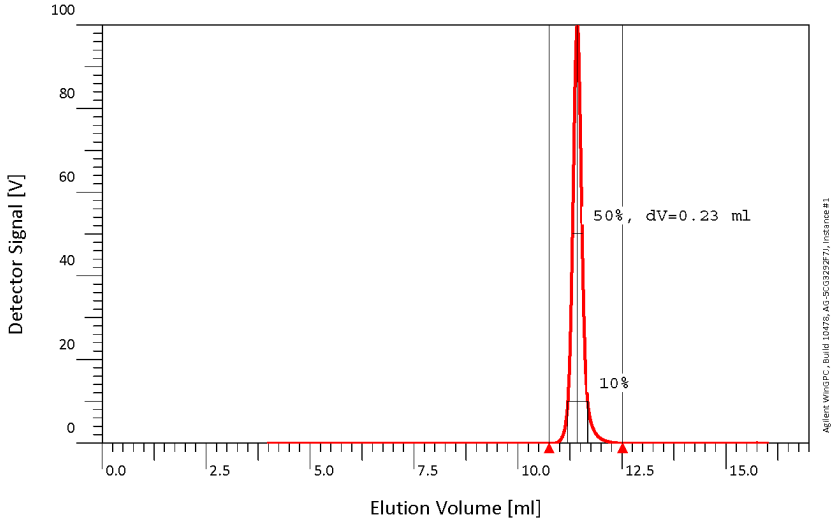
Reference substance with broad molecular weight distribution.

The integration limits for DXTB60-EV2 are fixed and need to be set as described in the example.

General System Performance with the Reference Substance PSS-DXTP1

Determination of plate count and WinGPC result report

If the measured plate count is above the minimum value of 15,000 [1/m] plates, the validation can be done.



Sample :	Vial 2: Dextran dxtp1 - 3	**Method :**	wr 13_10_22.MET
Baseline from :	4.000 ml	**to :**	16.000 ml
Integration from :	10.747 ml	**to :**	12.513 ml
Calibration File :	\EasyValid aque...PG30_dxtkit old.CAL	**Eluent :**	Water, Sodium azide 0.5g/L
MHK - A (Cal.):	0.000E+0	**MHK - K (Cal.):**	1.000E+0 ml/g
Int.stand.-cal.:	50.000 ml	**Int.stand.-sam.:**	------- ml
Pump :	Agilent	**Flowrate :**	1.000 ml/min
Concentration :	1.000 g/l	**Inject volume :**	20.000 ul
Column 1 :	PSS EasyValid aqueous Version 02	**Temperature :**	23.000 °C
Length :	30.000 cm	**Diameter :**	0.800 cm
Detector 1 :	I1: RID 1, RI Signal	**Delay volume :**	0.000 ml
Detector 2 :	I1: IsoPump 1, Pressure	**Delay volume :**	0.000 ml
Operator :	F. Ludwig	**Acquisition interval :**	1.000 sec

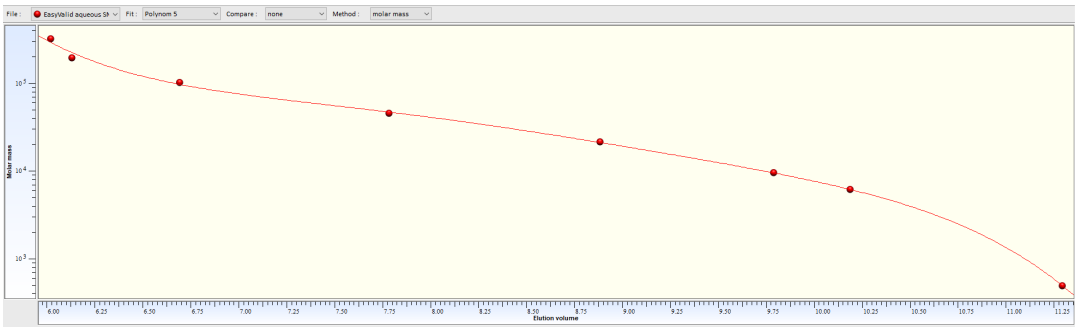
PSS Systemtest nach ISO 13885-1/DIN 55672-1 :

	Results	Requirements	Unit
Vol. Peakmax.:	11.422		ml
Plate Count :	44263	> 20000	1/m
Asymmetry :	0.884	> 0.85 < 1.15	-
Resolution :	1.436	> 2.5	-
Efficiency :	1.160	> 6	cm
signal drift :	2.369E-2	< 5 % Imax	V/h
signal noise :	2.862E-3	-	V
signal wander :	2.611E-3	-	V
signal/noise :	7847.003	> 100	-
pump pressure fluct. :	3.461E-2	-	bar
column temp fluct. :	0.000E+0	-	°C

Calibration Curve

A calibration curve established from eight Pullulan 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 PSS-DXT50-EV2 and PSS-DXTB60-EV2

The following figures show examples with automatic passed/failed flags from the WinGPC Report Designer.

WinGPC Report dxt50-ev2

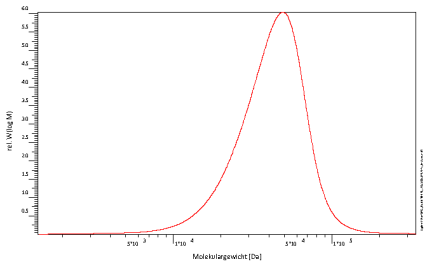
Version02

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

OQ/PV was executed

for GPC-System: PG-30
on: AG-5CG3292F7J with Windows 10 22H2 Enterprise,
with Agilent WinGPC, Build 10478 S/N: DE930055
by: F. Ludwig
at (Month, Day, Year): 01/25/2024 09:21:39 AM
with Validation column: EasyValid aqueous Version 02, SN: 91090443

Molar mass distribution



Processing parameters

Sample name:	Vial 6: Dextran dxt50-ev2 - 3
Injection time:	16:07:0
Project:	W:\GPC_DATEN\POLY\Projekte\EasyValid23.LDX
Calibration:	EasyValid aqueous SN9109443_PG30.CAL
created:	3/30/2023
last modified:	3/30/2023
MH-alpha (Cal):	0,000
MH-K (Cal.):	1,0000
Baseline from:	4,00 ml
to:	16,00 ml
Integration from:	5,97 ml
to:	10,99 ml
Solvent:	Water, Sodium azide 0.5g/L
Flow rate:	1,00 ml/min
Injection volume:	20 µl
Detector(s):	I1: RID 1, RI Signal I1: IsoPump 1, Pressure

Validation results

	Reference value	Allowed range	Value	Deviation [%]	Status
Results for detector: I1: RID 1, RI Signal					
Mn [Da]	33800	31100 - 36510	34143	1,015	passed
Mw [Da]	45500	41860 - 49140	45925	0,933	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 follwing the instructions in the user documentation. If the validation still fails please contact:

Print date (Month, Day, Year): 01-25-2024 Page 1 of 1

Validation Report dxt50-ev2 Version2.1

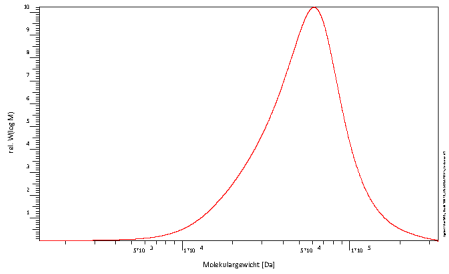
WinGPC Report dxtb60-ev2

Version02

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

OQ/PV was executed
for GPC-System: PG-30
on: AG-5CG3292F7J with Windows 10 22H2 Enterprise,
with Agilent WinGPC, Build 10478 S/N: DE930055
by: F. Ludwig
at (Month, Day, Year): 01/25/2024 09:38:19 AM
with Validation column: EasyValid aqueous Version 02, SN: 91090443

Molar mass distribution



Processing parameters

Sample name:	Vial 7: Dextran broad/branched dxtb60-ev2 - 3
Injection time:	16:57:0
Project:	W:\GPC_DATEN\POLY\Projekte\EasyValid\EasyValid23.LDX
Calibration:	EasyValid aqueous SN9109443_PG30.CAL
created:	3/30/2023
last modified:	3/30/2023
MH-alpha (Cal):	0,000
MH-K (Cal.):	1,0000
Baseline from:	4,00 ml
to:	16,00 ml
Integration from:	5,97 ml
to:	10,99 ml
Solvent:	Water, Sodium azide 0.5g/L
Flow rate:	1,00 ml/min
Injection volume:	20 µl
Detector(s):	I1: RID 1, RI Signal
	I1: IsoPump 1, Pressure

Validation results

	Reference value	*Allowed range*	*Value*	*Deviation [%]*	*Status*
Results for detector: I1: RID 1, RI Signal					
Mn [Da]	38800	35700 - 41900	39405	1,560	passed
Mw [Da]	59200	54460 - 63940	59553	0,597	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 follwing the instructions in the user documentation.
If the validation still fails please contact:

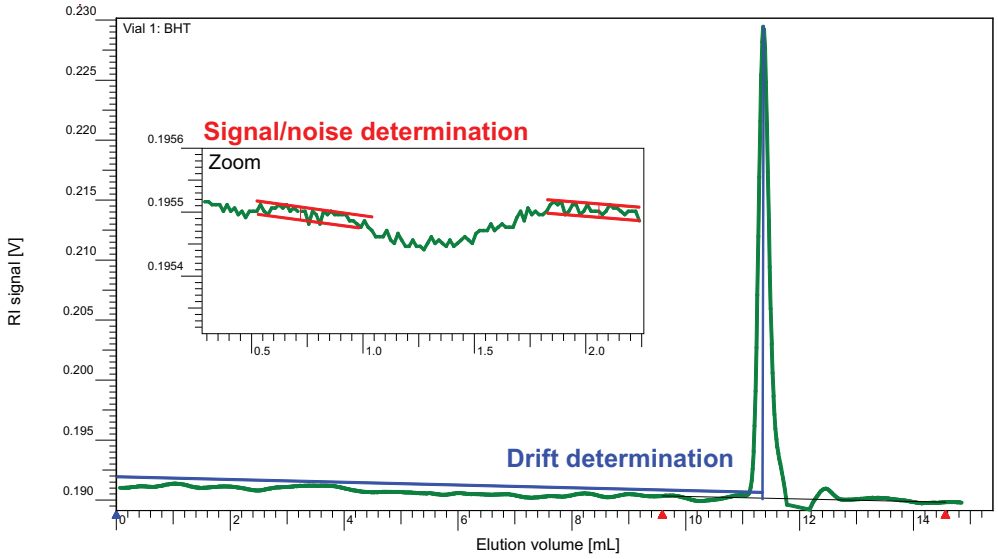
Print date (Month, Day, Year): 01-25-2024 Page 1 of 1

Validation Report dxt50-ev2 Version2.1

Enhanced Validation (Optional)

Determination of detector drift for one concentration detector

Example: RI detector



The injection for Dextran DXT50-EV2 and Dextran DXTB60-EV2 shows the status “passed”. This means that the validation has passed.

D
= Detector signal peak maximum – detector signal injection
= 0.1910 V – 0.1920 V = –0.001 V

H
= Detector signal peak height – detector signal baseline at intersection
= 0.2295 V – 0.1905 V = 0.039 V

DR
= D/H ×100
= –0.001 V / 0.039 V × 100% = –2.56%

If the drift ratio is below the maximum drift ratio of ± 5%, the test passed.

Determination of the S/N ratio for one concentration detector, example: RI detector

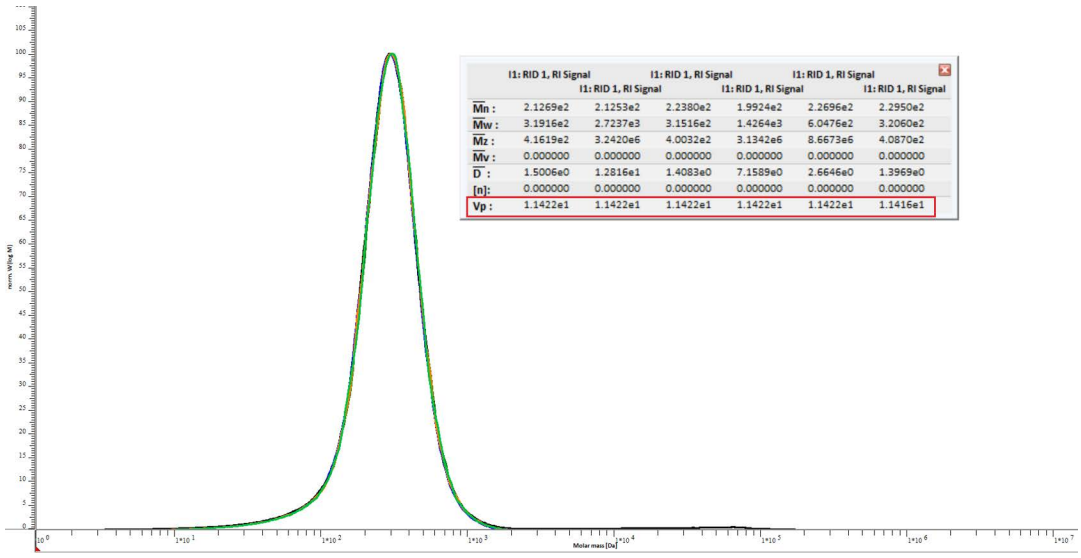
R = Signal 2 – Signal 1 = 0.00001 V

S/N = R/H × 100 = 0.00001 V / 0.039 V × 100% = 0.25%

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

Determination of the repeatability peak maximum

Example: six injections of Dextran dxtp1



D = (Maximum volume at the peak maximum – minimal volume at the peak maximum) / maximum volume at the peak maximum

Repeatability RI detector:

D(RI) = (11.422 mL – 11.416 mL) / 11.422 mL × 100% = 0.05%

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

NOTE **Internal standard correction is not applied.**

5 Possible Reasons for a Failed System Validation

Possible Reasons for a Failed General System Performance Test

The following (partial) list shows possible explanations why a system fails the general system performance test and plate counts below 15,000 [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 micro leakage 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 reports the same result, 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 micro leakages 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

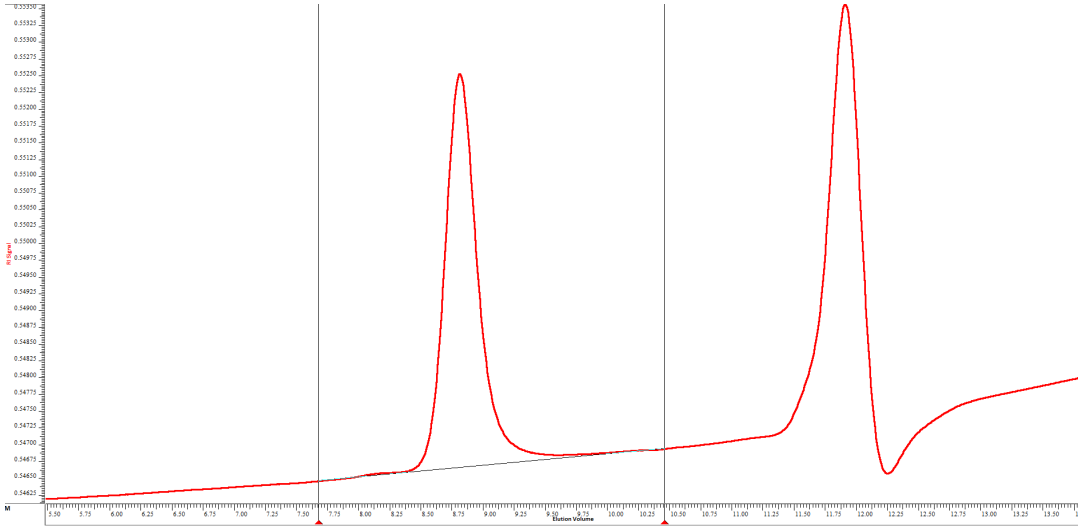
The following figures show two examples of insufficient raw data quality leading to a failed validation.

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.

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.

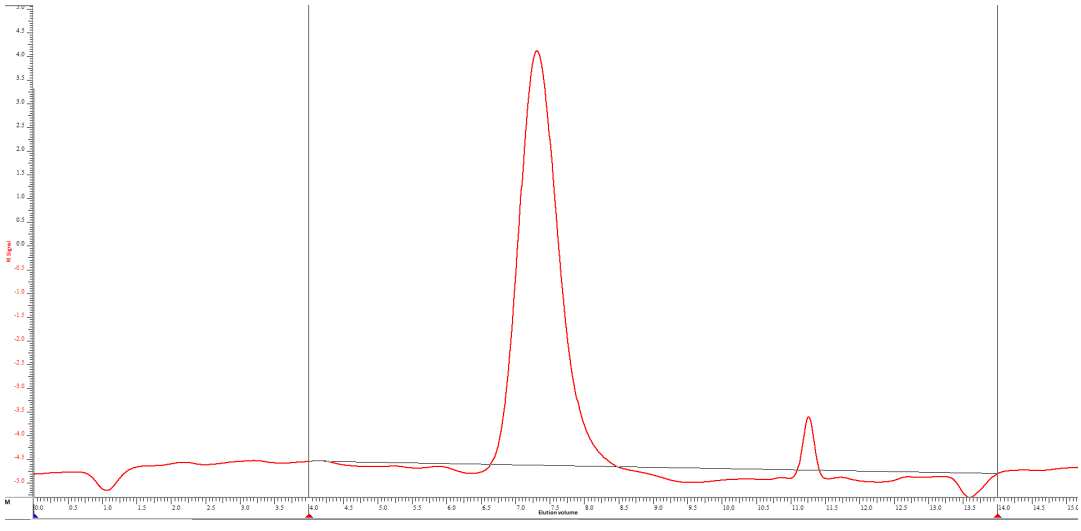


Detector wander

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.



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 water 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 cell 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 Aqueous.

EasyValid Validation Kit Aqueous — Results Overview

Results for system: _____

Pump: _____	S/N: _____
Injection system: _____	S/N: _____
RI detector: _____	S/N: _____
Additional components: _____	S/N: _____
_____	S/N: _____
_____	S/N: _____
_____	S/N: _____

Results validation step 3.2.1:

Plate count:
Expected: N ≥ 15,000 [1/m] Measured: N = _____
☐ Passed ☐ Failed

Results validation step 3.2.3:

Results sample Dextran DXT50-EV2:

Expected: molar mass averages for all three injections within the allowed range.

☐ Passed ☐ Failed

Results sample Dextran DXT60-EV2:

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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DE36493007

First edition, February 2024

5994-7069EN