Specimen QA HR Method Guide

Introduction

Specimen unit is small volume device shipped together with CIMmultus QA HR chromatography column. Specimen use is not a prerequisite for running the CIMmultus QA HR column, but it can be primarily utilized as a control to follow column lot-to-lot variability.

Method Guide offers thorough explanations on how to use Specimen unit as a testing device for chromatography columns. Our internal Specimen testing method is given.

NOTICE
Specimen unit use is a **non-mandatory step** prior to use of CIMmultus QA HR columns. Instructions for use can be found **here**

NOTICE
To obtain reliable and repeatable results, all parameters must be kept constant – variabilities of the AAV sample, chromatographic parameters (buffer preparation, system setup, ...) and external factors (temperature, void volumes, ...) affect chromatographic performance.

NOTICE
Method below is our internal method operated with a specified AAV standard sample. Acceptance criteria need to be tailored to client's method, sample, and system setup.

For more information, contact
help.BIA@sartorius.com

Abbreviations

BTP	Bis-tris propane
CIM	Convective Interaction Media
CIP	Cleaning in Place
CV	Column volume
E/F	Separation of empty and full AVV capsids
FLD	Fluorescence detector
HR	High Reproducibility
ID	Internal diameter
MPA	Mobile Phase A
MPB	Mobile Phase B
Nm	Newton-meter
PES	Polyethersulfone
RSD	Relative Standard Deviation

What is Specimen?

Dear Reader!

Chromatography is frequently employed for purification and concentration of therapeutic products, and it represents a critical step in the production process. Monoliths are a type of chromatography columns typically used for Advanced Therapy Medicinal Products, or ATMPs production. As opposed to chromatography resins, which are packed into a housing from bulk slurry, monoliths are a solid, single-piece structure provided in a ready to use cartridge.

Testing a resin or column before a purification run is a safety measure to ensure the success of a production campaign. Column efficiency testing is used to evaluate packed bed chromatography columns. Beside packing, batch testing can be used to ensure consistency of supply. Resin can be tested by packing a smaller column with the same batch of material. Monoliths, due to their structure, historically provided limited testing options.

Precise control over manufacturing conditions ensures that a monolith is polymerized homogeneously throughout its structure. With advanced manufacturing and material handling solutions, bulk monolith can be sectioned to extract small units. These small units become testing Specimen, which provide monoliths with a chromatographic batch testing solution.

Specimens are 200 µL units are extracted from large GMP compliant monolithic columns (40 mL and larger) and thus belong to the same material batch. Their chromatographic properties are matching properties of parental column they were extracted from. They can be used to perform batch testing of a monolithic column before use to ensure an even more robust manufacturing processes.

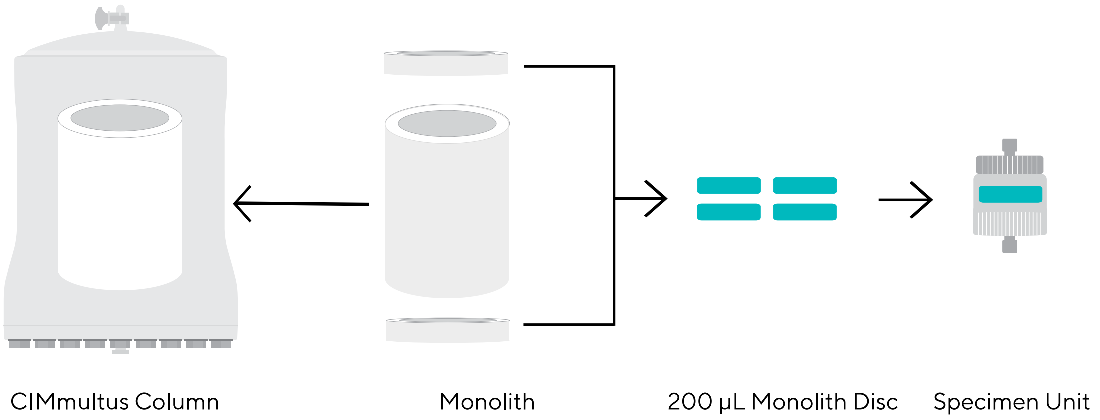


Figure 1: *Specimen and column packing.*

Technical Data for Specimen QA HR

Table 1: Technical Data for Specimen QA HR

Column chemistry	QA (strong anion exchanger; quaternary amine)
Channel radius	1050 nm (950 nm – 1150 nm)
Support matrix	Poly(glycidyl methacrylate -co- ethylene dimethacrylate)
Monolith dimensions	Diameter: 7.8 mm; height: 4.2 mm; bed volume (CV): 0.2 mL
Connector	10-32 UNF coned port, 1/16″ OD tubing connection; for manual operation with syringe Luer Lock Female to Male 10-32 connector can be used
Operating flow rates	0.2–3 mL/min; 0.42–6.3 cm/min; 1–15 CV/min
Maximum post-column pressure	2 MPa, 20 bar, 290 psi
Operating temperature	4 °C (39 °F) to 40 °C (104 °F)
Operating direction	Device can be operated in both directions
Chemical stability	All commonly used aqueous buffers, 0.1 M NaOH, 0.1 M HCl, 8 M urea, 6 M guanidine hydrochloride and 20% ethanol solution. Avoid oxidizing agents. Avoid prolonged use of concentrated acids (more than 0.1 M) like hydrochloric, sulphuric or acetic acid. Avoid prolonged exposure in NaOH and unnecessary exposure in more than 0.1 M NaOH solutions.
Recommended pH	Working range 2–13, cleaning in place 1–13.7
Storage conditions	2 °C (36 °F) to 25 °C (77 °F); 20% EtOH in 20 mM sodium acetate pH 5.5
Shelf life	N. D.

Specimen QA HR evaluation method

Laboratory equipment:

- Analytical weighing scale, accuracy 0.1 mg (e.g., Sartorius BCA3241-1CEU)
- Laboratory weighing scale, accuracy 0.1 g (e.g., Sartorius BCE6202-1S)
- Automatic pipette, various volumes (e.g., Sartorius)
- pH meter, accuracy 0.01 pH unit (e.g., pH Sensor InLab Expert Pro, Mettler Toledo, SevenExcellence)
- Conductivity meter, accuracy 0.01 mS/cm (e.g., Cond probe InLab 731, Mettler Toledo, SevenExcellence)
- Magnetic stirrer (e.g., Tehtnica Železniki, Rotamix 550 MMH)
- Vortex mixer (e.g., Grant-bio PV-1 Vortex mixer)
- PES 0.2 µm membrane filter (e.g. TPP syringe filter PES, TPP 99505, 99745)
- PES 0.22 µm vacuum filter (e.g., Sartorius 180E15---------E)
- Conical vial, 1 mL (e.g., Screw neck vial, N 8, 11.6x32.0 mm, 1.1 mL, conical, clear, Macherey-Nagel 702860)
- Vial caps (e.g., Screw closure, N 8, PP, black, center hole, Silicone white/PTFE red, 1.3 mm, Macherey-Nagel 70245)
- Conical vial support (e.g., PTFE Support for 1.1-STVG and 1.1-CTVG, Thermo Fisher Scientific TTS-312)
- Plastic PEEK fitting: Fitting, PEEK, for 1/16" O.D. tubing, one-piece fingertight hex-head, 10-32 (e.g. VICIAG International JR-5508-5 to JR-55025 or JR-5502)
- Auxiliary laboratory pump (e.g., Knauer, AZURA P 2.1S)
- Mechanical torque wrench (e.g., Stahlwille, Manoskop 714/6, Measuring range 0.1-10 Nm)

Chemicals

- L-Tryptophan (≥ 99.0%, e.g Merck T8941, M = 204.23 g/mol)
- D-(+)-sucrose (≥ 99.5%, e.g., Merck 84097, M = 342.20 g/mol)
- Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) (≥ 99.0%, e.g., Merck M2670, M = 203.30 g/mol)
- Poloxamer 188 (e.g. Merck 137079, M = 162.23 g/mol)
- Sodium hydroxide (NaOH) (≥ 98,0%, e.g., Merck 106498, M = 40.00 g/mol)
- Sodium acetate (CH3COONa) (≥ 99%, e.g Sigma-Aldrich S5636, M = 82.03 g/mol)
- Ethanol (e.g. 95.1 – 96.9%, e.g. Solchem 17925, ρ = 0.808 mg/mL @ 25 °C, M = 46.00 g/mol)
- ddH2O (resistance 18 MΩ cm, nuclease free)
- Sample: AAV2/8 standard, Sartorius BIA Separations

PATfix® system with configuration:

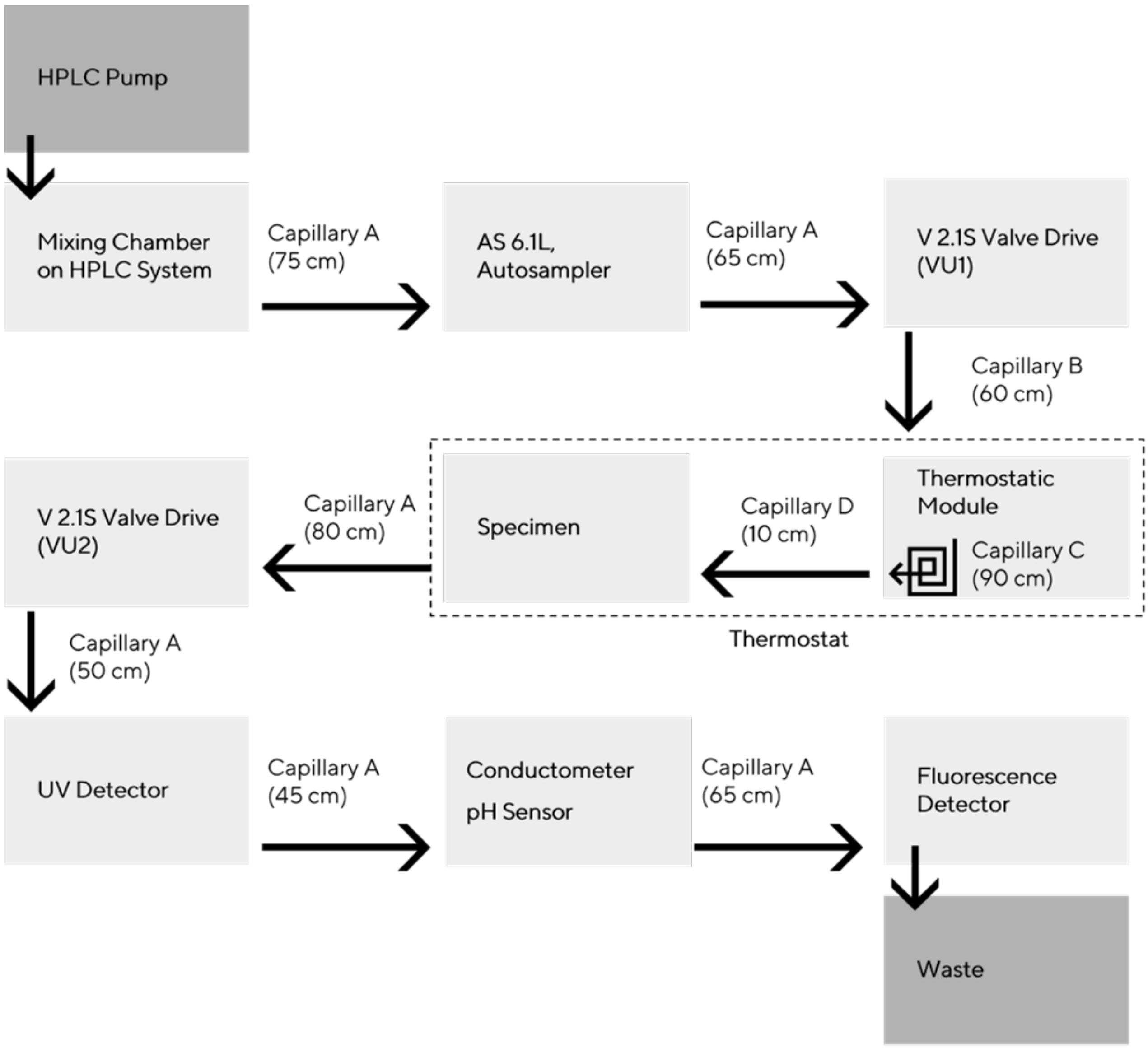
- High pressure gradient pump module (flow rate up to 10 mL/min) with a static mixing chamber (volume 0.25 mL) (Pump P6.1L HPG)
- Autosampler module (Autosampler AS 6.1L)
- Injection loop (V = 200 µL)
- Thermostat (CTO-40AC, Shimadzu)
- Conductivity monitor with pH electrode (Monitor CM 2.1S)
- Fluorescence (FLD) detector (Detector RF-20A, Shimadzu)
- UV detector with a 10 mm flow path cell, values of 260 nm and 280 nm (Detector MWD 2.1L)
- 6 or 8 port automatic valve drive (Valve Unifier VU 4.1)
- Thermostatic module (Tubing winding, SS, 1/16" OD x 0.18 mm ID)
- For the correct connection between the components and type and length of capillaries look at Figure 2.
- Only use a properly qualified PATfix system for analysis.

Chemicals – analytical grade:

- BTP (Bis-Tris-Propane) (≥ 99.0%, e.g., Merck B6755, M = 282.34 g/mol)
- Potassium chloride (KCl) (≥ 99.5%, e.g., Merck 104936, M = 74.55 g/mol)

- Standardized 1 M HCl (e.g., Titripur® Merck 1.09057)
- Hydrochloric acid (HCl) (≥37%, e.g., Honeywell 30721, M = 36.46 g/mol)
- Sodium chloride (NaCl) (≥ 99,0%, e.g., Honeywell 31434, M = 58.44 g/mol)

PATfix System Setup



	Specifications	Example
Capillary A	1/16" OD X 0.25 mm ID, stripped blue	VICI Jour JR-T-5710-M3
Capillary B	Pre-cut Stainless Steel Flexible Tubing 1/32" OD x 0.18 mm ID	VICI Jour JR-T-96130
Capillary C	Stainless Steel Tubing, 1/16" OD x 0.18 mm ID. Capillaries are coiled through thermostatic module.	VICI Jour JR-T-625-05-M10
Capillary D	1/16" OD x 0.18 mm, stripped yellow	VICI Jour JR-T-6000-M3

Figure 2: *Correct connection between the HPLC components.*

Composition and preparation of solutions

Table 2: *Solutions used in separation of empty and full AAV2/8 capsids.*

	Composition	Compound	Weight/Volume	pH at 23 ± 0.5 °C	Conductivity at ref. 25 °C
Mobile phase A	25 mM BTP, 51 mM KCl, 0.1% poloxamer-188, 1% sucrose, 2 mM $MgCl_2$, pH 9.00	BTP	7.06 ± 0.07 g	9.00 ± 0.05	7.90-8.10 mS/cm
		KCl	3.728± 0.037 g		
		$MgCl_2$	0.407 ± 0.004 g		
		D-sucrose	10.0 ± 0.1 g		
		Poloxamer 188	1.00 ± 0.01 g		
		Standardized 1 M HCl	14.46 ± 0.14 g		
		Final mass	1000 ± 1 g		
Mobile phase B	25 mM BTP, 153 mM KCl, 0.1% poloxamer-188, 1% sucrose, 2 mM $MgCl_2$, pH 9.00	BTP	7.06 ± 0.07 g	9.00 ± 0.05	19.80-20.20 mS/cm
		KCl	11.18 ± 0.11 g		
		$MgCl_2$	0.407 ± 0.004 g		
		D-sucrose	10.0 ± 0.1 g		
		Poloxamer 188	1.00 ± 0.01 g		
		Standardized 1 M HCl	15.37 ± 0.15 g		
		Final mass	1000 ± 1 g		
Equilibration solution	100 mM BTP, 1M KCl, pH 9.00	BTP	28.2 ± 0.3 g	9.00 ± 0.05	No need for measurement
		KCl	74.6 ± 0.7 g		
		1 M HCl	Titrate to pH 9.00		
		Final mass	1049 ± 1 g		
Cleaning in place (CIP) solution	0.1 M NaOH, 2 M NaCl	NaOH	4.0 ± 0.04 g	/	No need for measurement
		NaCl	116.9 ± 1.2 g		
		Final mass	1079 ± 1 g		
1 M sodium acetate, pH 5.50	1 M sodium acetate, pH 5.50	CH_3COONa	82.0 ± 0.80 g	5.50 ± 0.05	No need for measurement
		HCl	Titrate to pH 5.5		
		Final mass	1036 ± 1 g		
25 mM sodium acetate, pH 5.50	25 mM sodium acetate, pH 5.50	CH_3COONa	2.05 ± 0.02 g	5.50 ± 0.05	No need for measurement
		Standardized 1 M HCl	3.05 ± 0.03 g		
		Final mass	1000 ± 1 g		
Storage solution	20% EtOH in 20 mM sodium acetate pH 5.50	96% EtOH	200 ± 2 mL	/	No need for measurement
		25 mM sodium acetate, pH 5.5	800 ± 8 mL		
		Final volume	1000 ± 10 mL		
L-Tryptophan 1 g/L	L-Tryptophan 1 g/L	L-Tryptophan	50.0 ± 0.5 mg	/	No need for measurement
		dH_2O	50 mL		
L-Tryptophan 0.01 g/L	L-Tryptophan 0.01 g/L	L-Tryptophan 1 g/L	500 µL	/	No need for measurement
		dH_2O	49.5 mL		
Offset	L-Tryptophan 0.0001 g/L in MFB	L-Tryptophan 0.01 g/L	3 µL	/	No need for measurement
		MFB	297 µL		

Mobile phases A and B (1 L):

Add approximately 80% of the total mass (e.g., 800 g when preparing 1 L of buffer) of deionized water into the 1 L glass beaker with a magnetic stirrer. Add all weighted chemicals, except 1 M HCl, one by one. After all the components are completely dissolved in water add weighted 1 M HCl and remaining water to reach the final mass and pH. Mix and filter the solution through 0.22 µm PES filter. The mobile phases are stable up to 5 days at room temperature. Filter the mobile phases before each use and recheck their pH and conductivity.

Cleaning in place (CIP) solution (1 L):

CIP solution is prepared by adding approximately 80% of the total mass (e.g., 800 g when preparing 1 L of buffer) of deionized water into the 1 L glass beaker with magnetic stirrer. Add all weighted chemicals one by one. After all the components are completely dissolved, add remaining water to reach the final mass. Filter the solution through 0.22 µm PES filter. The CIP solution is stable up to 3 months at room temperature. Filter the solution before each use.

100 mM BTP, 1 M KCl, pH 9.00 (Equilibration solution) (1 L):

Add approximately 90% of the total mass (e.g., 900 g when preparing 1 L of buffer) of deionized water into the 1 L glass beaker with a magnetic stirrer. Add all weighted chemicals, one by one. After all the components are completely dissolved in water add 1 M HCl until pH reaches 9.00 ± 0.05. Add remaining water to reach a final mass of 1049 ± 1 g. Filter the solution through 0.22 µm PES filter. The equilibration solution is stable up to 1 month at room temperature. Filter the solution before each use and recheck its pH.

1 M sodium acetate, pH 5.50:

Add approximately 80% of the total mass (e.g., 800 g when preparing 1 L of buffer) of deionized water into the 1 L glass beaker with a magnetic stirrer. Add CH_3COONa. After CH_3COONa is completely dissolved in water, add HCl until pH reaches 5.50 ± 0.05. Add remaining water to reach a final mass of 1036 ± 1 g. Mix and filter the solution through 0.22 µm PES filter. Mobile phase is stable up to 1 month at room temperature. Filter the solution before each use and recheck its pH.

25 mM sodium acetate, pH 5.50:

Add approximately 80% of the total mass (e.g., 800 g when preparing 1 L of buffer) of deionized water into the 1 L glass beaker with a magnetic stirrer. Add CH_3COONa. After CH_3COONa is completely dissolved in water add weighted 1 M HCl and remaining water to reach a final mass and pH. Mix and filter the solution through 0.22 µm PES filter. The mobile phase is stable up to 1 month at room temperature. Filter the solution before each use and recheck its pH.

Storage solution:

Add 200 ± 2 mL of 96% EtOH in a 1 L measuring cylinder, add 25 mM sodium acetate, pH 5.50 to the 1000 mL mark in the measuring cylinder. Mix and filter the solution through a 0.22 µm PES filter. Storage solution is stable for 1 month at room temperature. Filter the solution before each use.

AAV2/8 standard test solution (AAV-UR-QA-01):

AAV-UR-QA-01 is a ready-to-inject test solution, containing AAV2/8 and uracil mixed in mobile phase A. The standard must be stored at -80 °C. Thaw the standard before use and mix it with the vortex mixer. Only use a freshly thawed standard test solution for analysis. After use, dispose of the remaining standard.

L-Tryptophan 1 g/L:

Add L-Tryptophan to a 50 mL volumetric flask, add approximately 30 mL of deionized water. Use 10 mL of deionized water to wash the neck of volumetric flask. Leave it for approximately 1 h for the L-Tryptophan to completely dissolve. After L-Tryptophan is completely dissolved fill with water to the 50 mL mark.

L-Tryptophan 0.01 g/L:

Add 500 µL of L-Tryptophan 1 g/L to a 50 mL volumetric flask. Fill with water to the 50 mL mark. Mix and filter the solution through 0.22 µm PES filter. L-Tryptophan 0.01 g/L is stored at -80 °C for 3 months or at -20 °C for 1 week.

Sample for offset:

In a conical vial add 297 µL of Mobile phase B and 3 µL of L-Tryptophan 0.01 g/L. Close the conical vial with the vial cap and mix it with the vortex mixer. The volume is enough for two injections.

NOTICE
Measure and note the end conductivity and pH of the mobile phases. Use only solutions with pH and conductivity values within acceptable range, where prescribed.

NOTICE
Buffer preparation, pH measurement and conductivity measurement should be performed at 23 °C. Conductivity values are calculated based on reference at 25 °C.

NOTICE
Solutions are filtered through a 0.22 µm PES filter.

NOTICE
Prior to use check conductivity and pH of mobile phases again. Use only solutions with pH and conductivity values within acceptable range.

System setup

Check system performance before connecting QA HR Specimen unit to a HPLC system.

Measure the exact flow rate φ (mL/min) on the system (flow rate defined in Table 3) by collecting dH_2O for 4 minutes in an appropriate container, e.g., measuring cylinder. Determine the flow rate by weighing the collected dH_2O. The measured flow rate must be in the range of the defined flow rate for the testing procedure given in Table 3.

Check the system for any leakages.

Determine the system pressure and post-column pressure, which should not exceed the maximum pressure specified in Table 3. No column or Specimen should be connected to the system during this measurement.

To determine the system pressure, apply the defined flow rate and record the system pressure generated when all detectors are connected (P *system*). The system pressure should not exceed 40 bar.

Next, disconnect the column inlet tube from the rest of the system and apply the defined flowrate with the flow going directly to waste from the column inlet tube. Record the pre-column pressure generated this way (P *pre-column*). The post-column pressure is the difference between P *system* and P *pre-column*. The post-column pressure should not exceed 10 bar.

Table 3: *Required system parameters.*

Parameter	
Flow rate (mL/min)	0.95-1.05
P *system* (bar)	≤ 40
P *post-column* (bar)	≤ 10

Offset determination

Offset is used to determine the delay of FLD signal in relation to the conductivity signal. More accurate comparison of Specimen testing results is achieved if the d*t* correction is considered.

Determination of the offset is done by setting up the system as shown in Table 4 and injecting the sample for offset using the method listed in Table 5. The analyte containing a Fluorescence (L-Tryptophan) and a Conductivity (KCl) tracer is used to measure the delay between conductivity and fluorescence readings, abbreviated as d*t* (Figure 3).

To determine the offset, set up the system as described in Table 4 and run the method listed in Table 5.

Table 4: *PATfix system setup.*

Component	**Setting**
Column thermostat temperature	23 °C – turn on the thermostat at least 30 min before use.
Autosampler temperature:	8°C.
Injection volume:	100 µL.
UV detector:	260 nm and 280 nm
Fluorescence (FLD):	ex 280 nm, em 348 nm; RF-20A: sensitivity: low (3), gain: x 4 (2).

Table 5: *Offset determination method.*

Time [min]	**Mobile phase A [%]**	**Mobile phase B [%]**	**Flow rate [mL/min]**
0.0	100	0	1.00
3.0	100	0	1.00

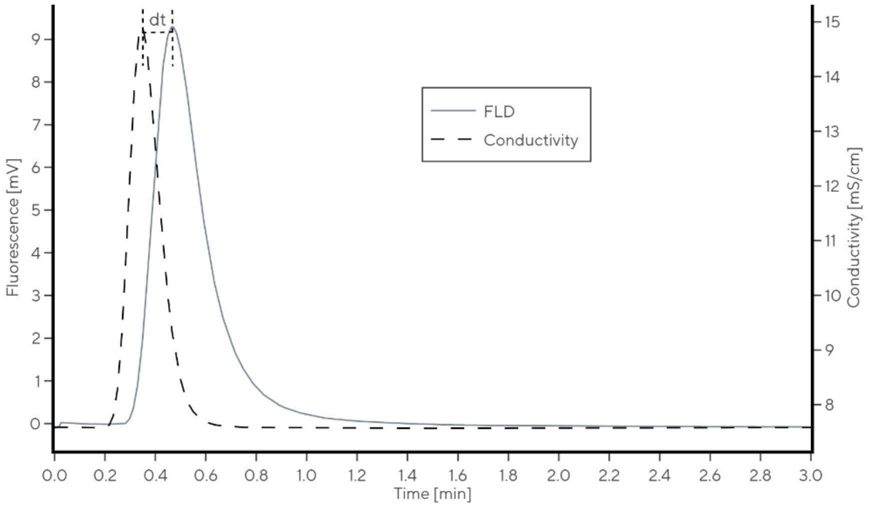


Figure 3: *Example chromatogram to determine delay in retention times (offset) given by conductivity monitor and FLD.*

Specimen QA HR preparation

Cleaning in Place and Equilibration

Cleaning in place (CIP) should be performed before equilibration and separation run.

NOTICE
For robust end reliable results Cleaning in place and Equilibration protocol listed below should be followed diligently.

Specimen preparation can be carried out manually with syringe or on auxiliary laboratory pump at a flow rate of 1-2 mL/min.

- Use the wrench included in the box with the Specimen unit to loosen and carefully remove the blind fittings on both sides of the Specimen unit. **Note**: The wrench may only be used in the direction in which the blind fittings are unscrewed, as indicated on the label on the wrench.
- Connect one side of the Specimen with the inlet tubing of an auxiliary laboratory pump or with a syringe via Luer Lock Female to Male 10-32 connector.
- Wash the Specimen with 2 mL (10 column volumes; CV) of deionized water.
- Wash the Specimen with 2 mL (10 CV) of the CIP solution containing 0.1 M NaOH, 2 M NaCl.
- Immediately wash the Specimen with 2 mL (10 CV) of dH_2O
- Immediately wash the Specimen with 6 mL (30 CV) of Equilibration Solution 100 mM BTP, 1 M KCl, pH 9.0
- Wash the Specimen with 2 mL (10 CV) of dH_2O
- Connect the Specimen unit to the PATfix system tubing inside the thermostat. Connecting the Specimen to the system is possible with an inlet placed either at the top or at the bottom (see Figure 2). The system settings are listed in Table 4.
- Before running the experiment, keep the Specimen in the thermostat at 23 °C for at least 15 min.
- Wash the Specimen with 4 mL (20 CV) of Mobile phase A
- Wash the Specimen with 4 mL (20 CV) of Mobile phase B
- Wash the Specimen with 8 mL (40 CV) of Mobile phase A

Analysis

The test method for analysis is defined in Table 6. Start with a blank run injecting only mobile phase A. Record and inspect the UV signal. The blank should be smooth without ghost peaks or deformations which can be confused with a sample peak in later injections.

Table 6: *Chromatography method gradient setup for QA HR Specimen evaluation.*

Time [min]	Mobile phase A [%]	Mobile phase B [%]	Flow rate [mL/min]
0.0	100	0	1.00
0.5	100	0	1.00
6.5	0	100	1.00
8.0	0	100	1.00
8.02	100	0	1.00
13	100	0	1.00

Continue with AAV sample injection and run the test procedure again according to the test method described in Table 6. Observe and record the signal from the system. Note the retention time (tr) at the maximum of the empty and full capsids peak. Note the conductivity of mobile phase A (MPA) and mobile phase B (MPB) at the beginning and at the end of gradient (see marks on the reference chromatogram on Figure 4). Note or calculate the resolution (R) between eluted peaks.

Integration, calculations, and sample chromatogram

Chromatographic peaks are integrated manually as shown in the figure below.

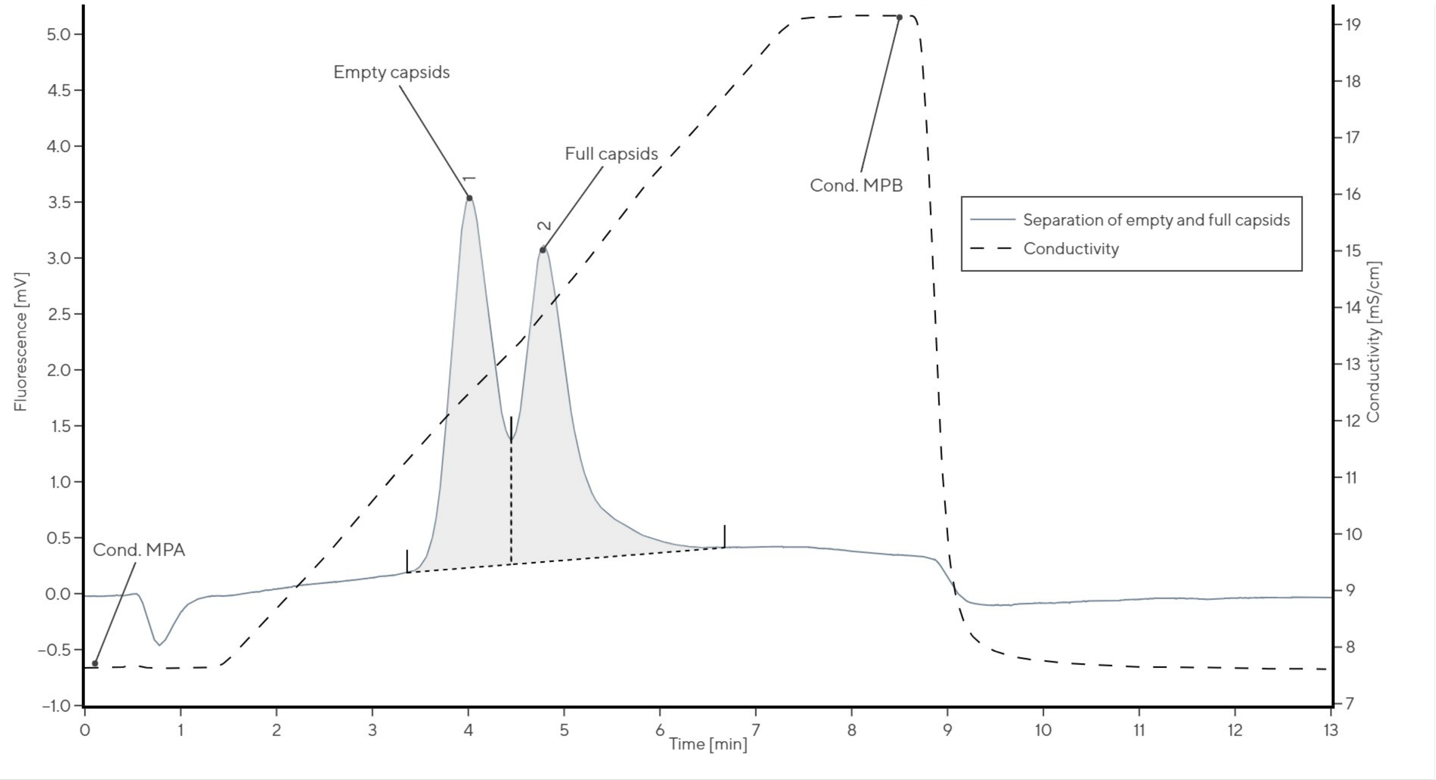


Figure 4: *Example chromatogram of empty/full AAV2/8 separation on Specimen QA HR with indicated peak integration.*

Calculations:

Calculate the difference in retention times (offset) given by conductivity and FLD detector.

$$\mathrm{d}t = t_{\mathrm{FLD}} - t_{\mathrm{cond}}$$

$\mathrm{d}t$	difference in retention times for cond. and FLD (offset) (min)
t_{FLD}	retention time FLD signal (min)
t_{cond}	retention time conductivity signal (min)

Calculate the normalised retention time of empty capsids:

$$t_{\mathrm{empty,\ norm.}} = t_{\mathrm{empty}} - \mathrm{d}t$$

$t_{\mathrm{empty,\ norm.}}$	Retention time of empty capsids, normalised (min)
t_{empty}	Retention time of empty capsids (min)
$\mathrm{d}t$	Offset (min)

Obtain the empty capsid elution conductivity ($\mathrm{cond}_{\mathrm{empty,\ norm}}$) at normalised retention time of empty capsids ($\mathrm{t}_{\mathrm{empty,\ norm.}}$) – see Figure 5.

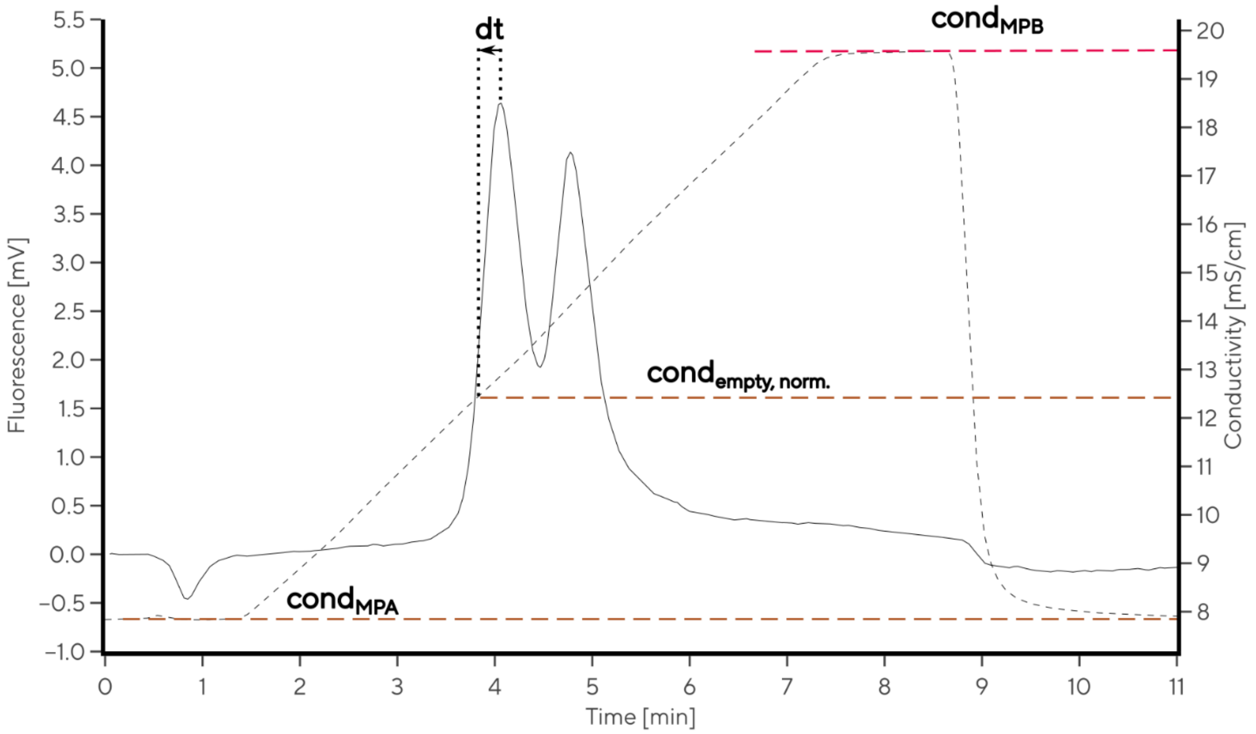


Figure 5: Obtaining $cond_{empty,norm.}$, $cond_{MPA}$ and $cond_{MPB}$ from a chromatogram.

Calculate the concentration of KCl at empty capsid elution ($c(KCl)_{empty}$):

$$c(KCl)_{\text{empty}} = \frac{cond_{\text{empty,norm.}} - cond_{\text{MPA}}}{cond_{\text{MPB}} - cond_{\text{MPA}}} \times f1 + f2$$

$c\ (KCl)_{empty}$	KCl concentration at empty capsid elution (mM)
$cond_{empty,\ norm.}$	Empty capsid elution conductivity (mS/cm)
$cond_{MPA}$	Mobile phase A conductivity (mS/cm)
$cond_{MPB}$	Mobile phase B conductivity (mS/cm)
$f1$	102 mM (difference in KCl conc. between MPB and MPA)
$f2$	*51 mM* (concentration of KCl in MPA)

If necessary, calculate resolution *(R)* between empty and full elution peaks.

$$R = \frac{1.18 \cdot (t_{\text{full}} - t_{\text{empty}})}{W_{\text{empty}} + W_{\text{full}}}$$

R	resolution between empty and full capsids
t_{empty}	retention time of empty capsids (min)
t_{full}	retention time of full capsids (min)
W_{empty}	width at half maximum of empty peak (min)
W_{full}	width at half maximum of full peak (min)

The release criteria for QA HR Specimen with the current batch of AAV2/8 internal standard corresponds to KCl concentration at empty capsid elution of 92.3 mM ± 3%. Changing the AAV2/8 standard may influence the average KCl concentration for column release and requires bridging of column release criteria. User should set up their own in-house method with the respective criteria.

Following instructions listed in this Guide enables repetitive and accurate comparison of different Specimen units and their comparison.

NOTICE

For relevant Specimen evaluation and comparison between units the same standard sample should be used.

QA HR Specimen regeneration and storage

Specimen regeneration and storage can be performed manually with syringe or on an auxiliary laboratory pump at a flowrate of 1-2 mL/min.

After the analysis:

- Carefully remove the Specimen from the system and connect one side of the Specimen with the inlet tubing of an auxiliary laboratory pump or with a syringe via Luer Lock Female to Male 10-32 connector.
- Wash the Specimen with 2 mL (10 CV) of deionized water.
- Wash the Specimen with 2 mL (10 CV) of the CIP solution containing 0.1 M NaOH, 2 M NaCl.
- Immediately wash the Specimen with 2 mL (10 CV) of deionized water.
- Wash the Specimen with 3 mL (15 CV) of 1 M sodium acetate pH 5.5.
- Wash the Specimen with at least 4 mL (20 CV) of 25 mM sodium acetate pH 5.5.
- Wash the Specimen with 2 mL (10 CV) of storage solution.
- Seal the Specimen with blind fittings:
  - Short-term storage (i.e. up to 1 week): Seal blind fittings manually
  - Long-term storage (i.e. more than 1 week): Use new, unused blind fittings (not provided) and tighten to 0.30 Nm using a mechanical torque wrench (not provided). **Note:** Blind fittings may be damaged if more than 0.30 Nm is used, allowing storage solution to evaporate.
- Store at a temperature of 2-25 °C.

Responsibilities

The user is responsible to perform AAV empty/full separation with Specimen QA HR in compliance with this document. Parameters that can influence Empty-Full AAV separation should be considered.

Ordering Information

Cat No.	**Product Name**
BIA-311.5213-2	CIMmultus® QA HR 1 mL Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-414.5213-2	CIMmultus® QA HR 4 mL Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-411.5213-2	CIMmultus® QA HR 8 mL Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-914.5213-2	CIMmultus® QA HR 40 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-911.5213-2	CIMmultus® QA HR 80 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-924.5213-2	CIMmultus® QA HR 400 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-921.5213-2	CIMmultus® QA HR 800 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-934.5213-2	CIMmultus® QA HR 4000 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-931.5213-2	CIMmultus® QA HR 8000 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
656 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

Slovenia
Sartorius BIA Separations
Mirce 21
5270 Ajdovscina
Phone +386 596 995 00

For more information, visit
www.sartorius.com

www.biaseparations.com

Status: Specimen-QA-HR-2310-vr1