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Technical Note

Release Criteria for CIM® QA HR AAV Test

Introduction

The most selective QC test for the release of CIM® QA HR chromatographic columns is a specific AAV test based on the results of AAV E/F separation performed in an ascending linear KCl elution gradient [1]. This gradient ranges from 100 % MPA to 100 % MPB, corresponding to the range from 51 mM to 153 mM KCl over 30 CV following iso-conductivity scale up [2]. AAV elutes at a specific KCl concentration within the linear gradient, regardless of the CIM QA HR column volume. To meet the requirements for highly reproducible CIM QA HR line, KCl concentration at elution peak apex of empty AAV2/8 capsids ($[KCl]_E$) must fall within the interval of 4.6 mM KCl.

Chromatographic column release parameter is best determined directly from the chromatograms, therefore the retention time of empty AAV2/8 capsids was chosen as the parameter for column release. Unlike $[KCl]_E$, retention time depends on column volume.
In this document the correlations between the retention time of empty AAV2/8 capsids and $[KCl]_E$ for 0.1 mL, 0.2 mL and 1 mL column volumes is described. Based on the derived equations column release criteria were calculated.

Abbreviations

AAV	Adeno-Associated Virus
AAV E/F	Empty and full AAV capsids
HR	High Reproducibility
CV	Column volume
MPA	Mobile phase A
MPB	Mobile phase B
$[KCl]_E$	KCl concentration at elution of empty AAV2/8 capsids
FLD	Fluorescence detector
QC	Quality control

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

Method

The method is based on the separation of specific AAV2/8 capsids in an ascending KCl gradient from 100 % MPA to 100 % MPB over 30 CV.

The column release criteria for CIM QA HR line was set to 90.06 - 94.64 mM $[KCl]_E$ for specific AAV2/8 batch samples following the chromatographic conditions noted in Table 1.

Table 1: Chromatographic conditions

QA HR column volume & type	0.1 mL CIMac	0.2 mL Specimen	1 mL CIMmultus		
System	PATfix system [1]				
Column thermostat (°C)	23				
MPA	25 mM BTP, 51 mM KCl, 2 mM $MgCl_2$, 1 % sucrose, 0.1 % poloxamer 188, pH 9.0				
MPB	25 mM BTP, 153 mM KCl, 2 mM $MgCl_2$, 1 % sucrose, 0.1 % poloxamer 188, pH 9.0				
Batch AAV sample	AAV-UR-QA-01-230316				
Flow rate (mL/min)	1.0		2.0		
Injection volume (µL)	100		250		
Autosampler temperature (°C)	8				
UV detector (nm)	260 and 280				
FLD	ex 280 nm, em 348 nm; RF-20A: sensitivity: low (3), gain: x 4 (2)				
Chromatography gradient	0.1 mL CIMac Time [min]	0.2 mL Specimen Time [min]	1 mL CIMmultus Time [min]	Mobile Phase A [%]	Mobile Phase B [%]
	0	0	0	100	0
	0.5	0.5	0.50	100	0
	3.5	6.5	15.50	0	100
	7	9	18	0	100
	7.02	9.02	18.02	100	0
	12	14	25	100	0

The detailed protocol for 0.2 mL column volume is published in the document Specimen QA HR Method Guide [1].

Determination of Correlation Between KCl Concentration and Time in Gradient

The chromatographic method described in Table 1 was performed with nine different 0.2 mL monolithic columns. KCl concentration ([KCl]) was calculated from recorded conductivity values during each run (Equation 1).

Equation 1: Calculation of KCl concentration in linear gradient

$$[\mathrm{KCl}] = \frac{\sigma_{c\,(\mathrm{KCl})} - \sigma_{\mathrm{MPA}}}{\sigma_{\mathrm{MPB}} - \sigma_{\mathrm{MPA}}} \times f1 + f2$$

[KCl]	Calculated KCl concentration in linear gradient from 100 % MPA to 100 % MPB (mM)
$\sigma_{c\,(KCl)}$	Recorded conductivity at a chosen point in linear gradient from 100 % MPA to 100 % MPB (mS/cm)
σ_{MPA}	Mobile phase A conductivity (recorded on the conductometer on the system) (mS/cm)
σ_{MPB}	Mobile phase B conductivity (recorded on the conductometer on the system) (mS/cm)
f1	102 mM (difference in [KCl] between MPB and MPA)
f2	51 mM ([KCl] in MPA)

Figure 1 presents the correlation between calculated [KCl] and time in gradient. Each set of points was fitted with a linear regression model. Offset was used to determine the delay of FLD signal in relation to the conductivity signal [1].

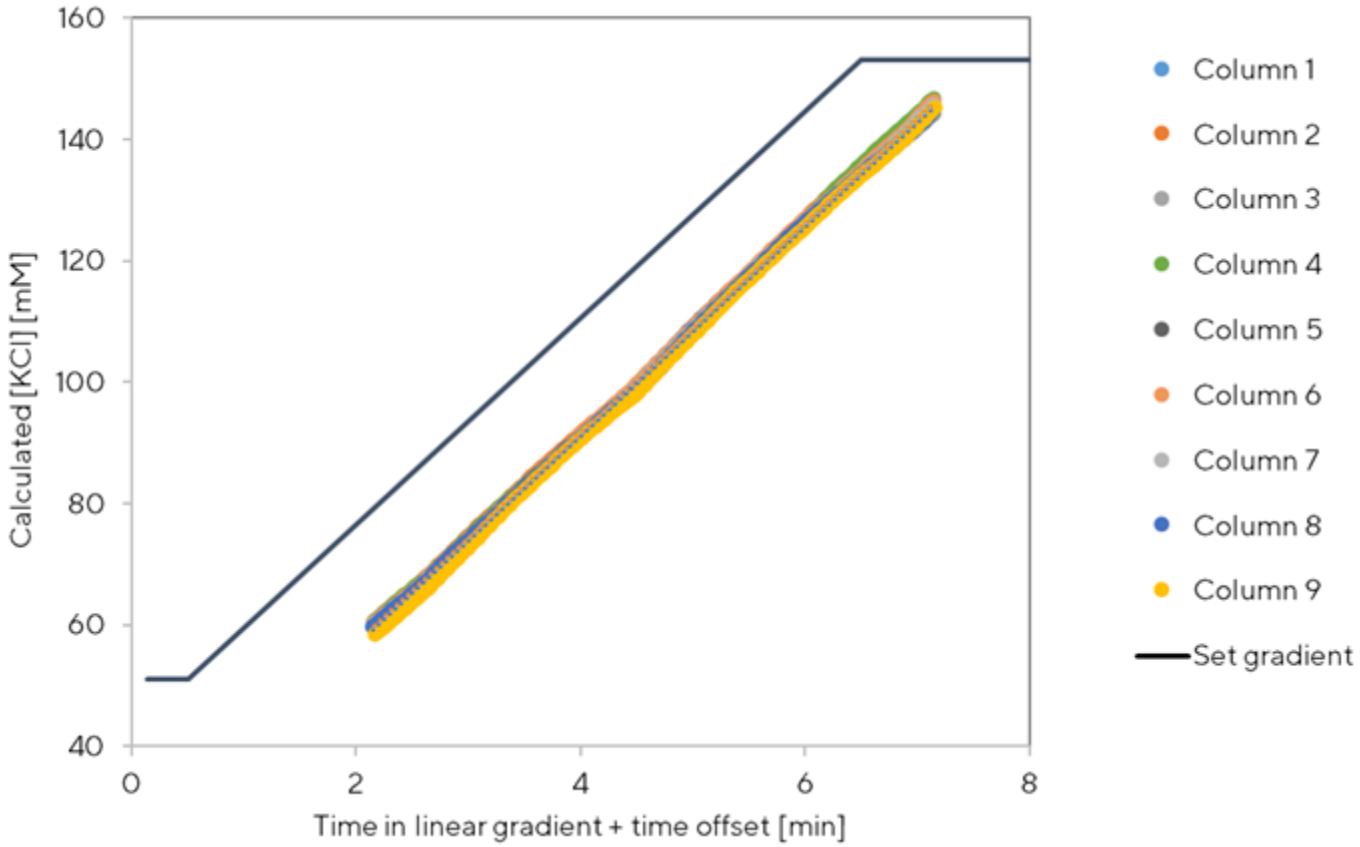

Fig. 1: Correlation between calculated [KCl] and time in linear gradients for nine different 0.2 mL specimen monolithic columns.

Similar tests were performed on CIMac 0.1 mL and CIMmultus 1 mL columns.

Based on the results from these experiments the following equations were derived for the three model column volumes (Table 2).

Table 2: The averaged line equations and R^2 for 0.2, 0.1 and 1 mL columns.

Column volume (mL)	Equation	R^2
0.2	$[KCl] = 17.29 * t_{linear\ gradient} + 22.10$	0.9998
0.1	$[KCl] = 33.70 * t_{linear\ gradient} + 1.45$	0.9996
1	$[KCl] = 6.84 * t_{linear\ gradient} + 39.76$	0.9997

Calculation of Column Release Criteria

The criteria for the retention time of empty capsids of specific AAV2/8 sample batch for model column volumes using the selected HPLC system were determined using the equation in Table 2.

For the defined interval of $[KCl]_E$ (between 90.06 and 94.64 mM for the specific AAV2/8 sample) the column release criteria are presented in Table 3.

Table 3: Column release criteria for retention time of empty AAV2/8 capsids for model column volumes

Column volume (mL)	Retention time of empty capsids - $t_{linear\ gradient}$ (min)
0.2	3.93 – 4.20
0.1	2.63 – 2.77
1	7.35 – 8.02

Changing AAV2/8 sample and/or HPLC configuration may alter the column release criteria. Therefore, conducting bridging experiments would be necessary to establish new criteria.

References

[1] Specimen QA HR Method Guide: https://www.biaseparations.com/library_items/specimen-qa-hr-method-guide/
[2] Yamamoto, S., Kita, A. *Journal of Chromatography. A.* 2005, *1065*, 45–50. DOI: 10.1016/j.chroma.2004.12.090

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

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