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Fast Purification and Analysis of *Influenza A* Virus Using CIMac Monolith Columns

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Introduction

New vaccines against *Influenza A* are required each year to keep up with the most virulent evolving strains. This highlights a need for predictive analytical tools that can aid purification process development and validation. Rapid and reliable quantification of *Influenza A* virus is therefore of the utmost importance for enabling good yields and controlling the costs of the downstream processing. Here we demonstrate the ability of monolithic chromatography media to produce process-predictive profiles that can document ability to remove impurities and obtain high product recoveries.

CIMac Analytical Columns are short bed high performance monolithic columns offering all the advantages of CIM® monolithic technology. Their small volume and short column length allow the operation at high volumetric flow rates enabling to receive the information about the product quantity and purity in just a few minutes. Hence, the CIMac Analytical Columns can be effectively used for the in-process and final control of various samples from different purification process steps.

Figure 1: *CIMac SO3*

Results

An aliquot of clarified harvest of *Influenza A* virus was diluted in mobile phase A and processed on PATfix® HPLC system. Separation was achieved by using a CIMac SO3 Analytical Column (strong cation exchanger). Several experiments were performed to determine reproducibility, LOD and LOQ of selected analytical method.

Chromatographic Conditions

Column	CIMac SO3-0.1 Analytical Column (Sulfonyl. Pores 1.3 µm)
HPLC system	PATfix®
Mobile phases	Buffer A: 50 mM HEPES. pH 7.50 Buffer B: 50 mM HEPES + 1 M NaCl. pH 7.50
Flow rate	1 mL/min
Gradient method	Wash: 20 CV Buffer A Elute: 25 CV to Buffer B Hold: 15 CV Buffer B Equilibrate 20 CV Buffer A
Sample	Purified *Influenza A* virus in Buffer A
Injection volume	500 µL
Detection	UV (260 and 280 nm) 50 mm optical path length Fluorescence: Ex: 280 nm, Em: 335 nm Conductivity detector

TCID50 and Hemagglutination Assays

Fraction	TCID50		Hemagglutination	
	TCID50	Yield (%)	HA Titre	Yield (%)
Load	5.00E+06	100%	8.00E+07	100%
FT	0.00E+00	0%	0.00E+00	0%
E1	**6.00E+06**	> 100%	**6.40E+07**	80%

Chromatogram for *Influenza A* Virus Using Cimac

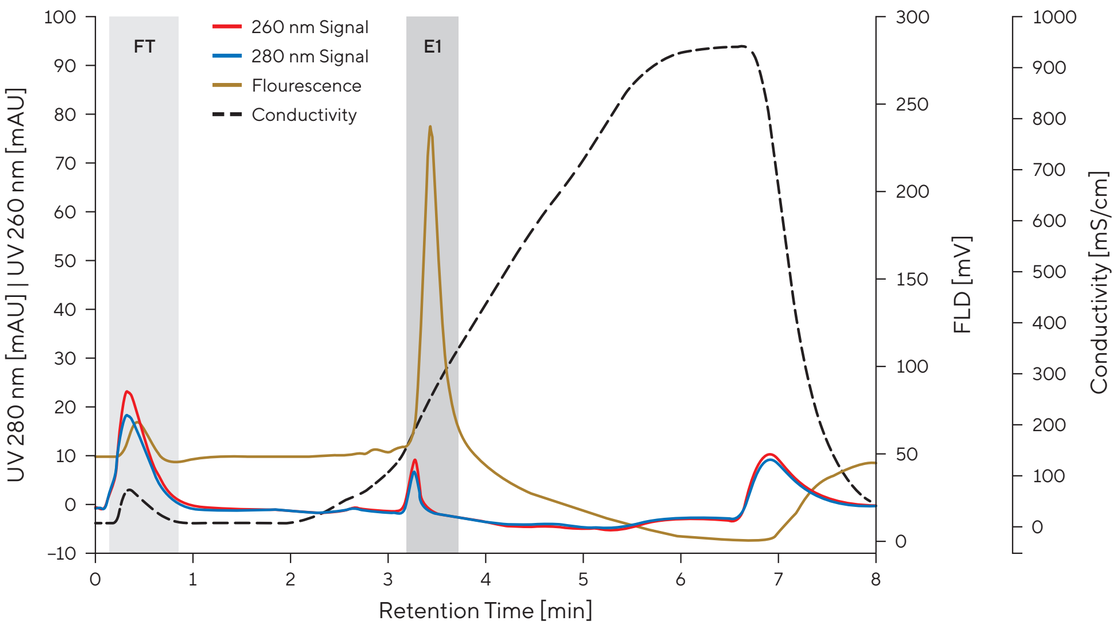


Figure 2: *Chromatogram for Influenza A Virus Separation on CIMac SO3-0.1 Analytical Column*

Moreover, to estimate the capture efficiency of *Influenza A* virus by the monolith column and to assess the information about its distribution during elution in salt gradient, several fractions of chromatogram were collected and further analyzed by TCID50 and hemagglutination assays. Both assays documented complete binding of *Influenza A* virus on CIMac SO3 analytical column monolith column, outstanding recovery, and conservation of infectivity.

Validation

In order to properly evaluate the developed analytical method for *Influenza A* virus quantification on CIMac analytical monolith column, several analytical and statistical parameters were validated:

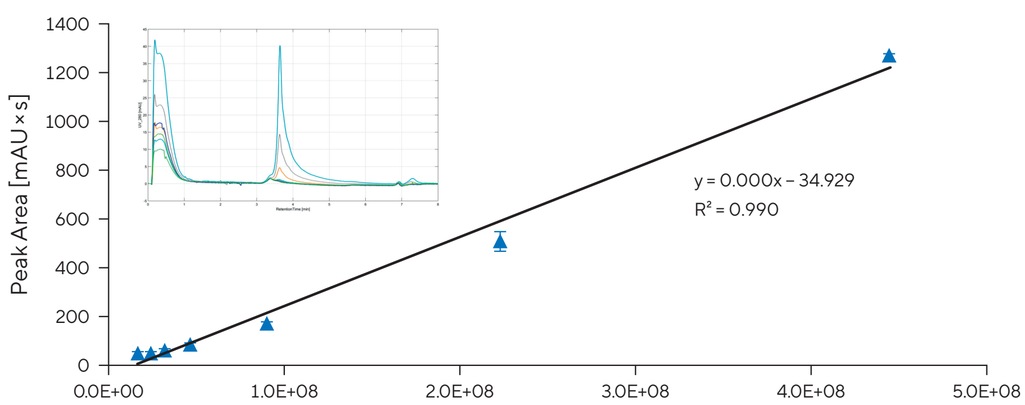
- Limit of detection (LOD)
- Limit of quantification (LOQ)
- Linearity of the method (y = kx + n)
- Precision of the method (RSD)

The calibration curves were extracted from corresponding peak areas of fluorescence, UV 260 nm and UV 280 nm signals. The linearity of the method was confirmed with all three signals and the R^2 were acceptable, with mean RSD of 11%.

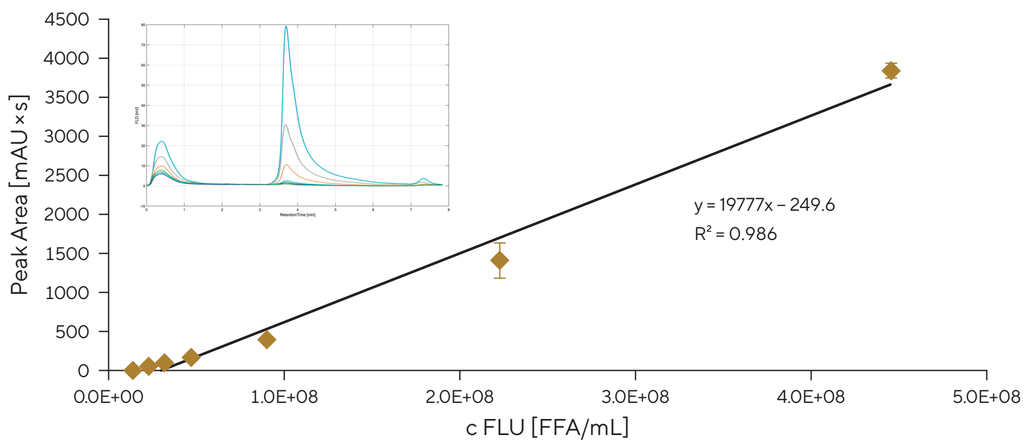
	LOD (1.12E+07 FFU*/mL)				LOQ (1.50E+07 FFU*/mL)				Sample (8.97E+07 FFU*/mL)			
	Rt FLD min	Area FLD mV×s	Area 260 mAU×s	Area 280 mAU×s	Rt FLD min	Area FLD mV×s	Area 260 mAU×s	Area 280 mAU×s	Rt FLD min	Area FLD mV×s	Area 260 mAU×s	Area 280 mAU×s
1	3.70	12.6	91.5	78.0	3.70	12.6	91.5	78.0	3.81	393.0	254.7	173.8
2	3.89	13.4	112.7	81.1	3.89	13.4	112.7	81.1	3.94	459.5	229.8	155.1
3	3.48	11.1	94.9	85.2	3.48	11.1	94.9	85.2	3.90	492.4	252.2	203.2
4	3.85	11.8	97.7	82.0	3.85	11.8	97.7	82.0	3.82	503.1	296.8	189.3
5	3.85	11.8	104.6	52.8	3.85	11.8	104.6	52.8	3.91	497.2	252.3	156.5
stdev	0.17	0.9	8.4	13.1	0.17	0.9	8.4	13.1	0.06	45.8	24.4	20.8
RSD %	**4.57**	**7.2**	**8.4**	**17.3**	**4.57**	**7.2**	**8.4**	**17.3**	**1.52**	**9.8**	**9.5**	**11.9**

* Focus Forming Units

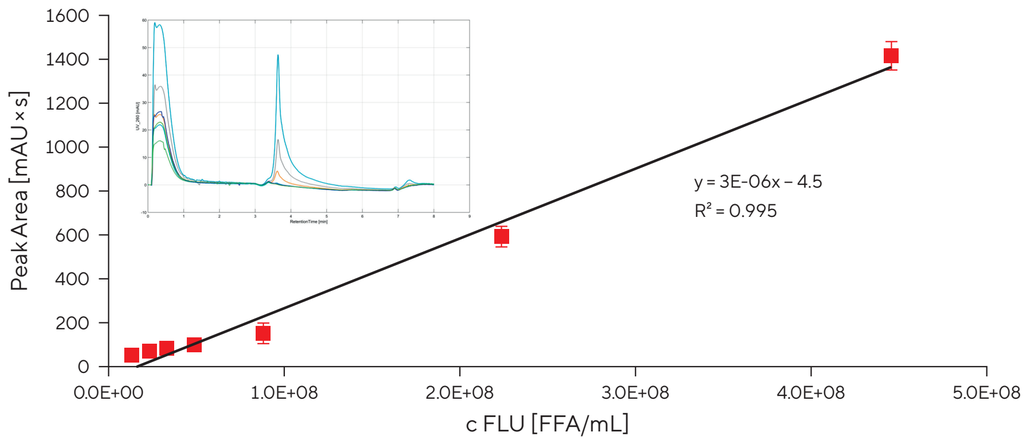
Calibration Curve for UV 280 Area



Calibration Curve for FLD Area



Calibration Curve for UV 260 Area



Conclusion

The chromatographic method proved to be fast and efficient analytical tool for quantitative evaluation of *Influenza A* titre. The analytical method could in principle replace traditional and time consuming hemagglutination assay.

- *Influenza A* virus was successfully captured on CIMac SO3-0.1 Analytical Column.
- No virus was detected in flowthrough fraction with either TCID50 and hemagglutination assays.
- *Influenza A* virus was present only in fraction E1 with high yields of infective virus particles.
- LOD, LOQ showed great sensitivity of the method and the linearity of the calibration curve was acceptable. The precision of multiple injections resulted in mean RSD of 11%.

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