CIMac™ ANALYTICAL COLUMNS FOR IN-PROCESS CONTROL OF ADENOVIRUSES

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INTRODUCTION

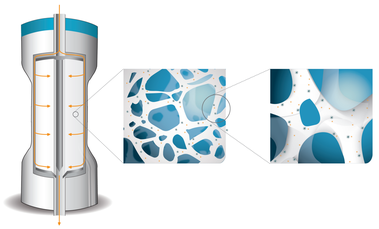
Determining the concentration of viruses is a crucial step in any production process. The most commonly used methods for virus quantification are either based on the infectivity of the virus (plaque assay, TCID50) determination of their genomic material (qPCR), or protein content (SRID, ELISA) and are very cumbersome and time consuming. HPLC analytical methods represent a fast alternative to these assays since they provide information on the virus content and purity in a matter of minutes. Due to the structural properties of the monolithic supports, monolithic analytical columns offer a great advantage over particle based HPLC columns in terms of time and their ability to separate large biomolecules, like viruses, VLPs, pDNA.

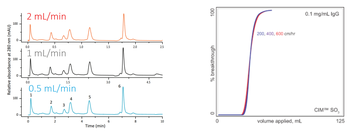
In this poster the performance of the CIMac™ Adeno Analytical Column – a monolith based anion exchange column, designed for fast and reproducible analyses of adenoviruses was evaluated. CIMac Adeno column can be used for designing a fast finger printing method that is applicable for monitoring the DSP production process of adenoviruses. Once the basic analytical parameters like linearity and sensitivity are determined using a purified adenoviral standard, the metod can be applied for quantitative determination of adenoviruses.

Monolith Chromatography

MONOLITH CHROMATOGRAPHY

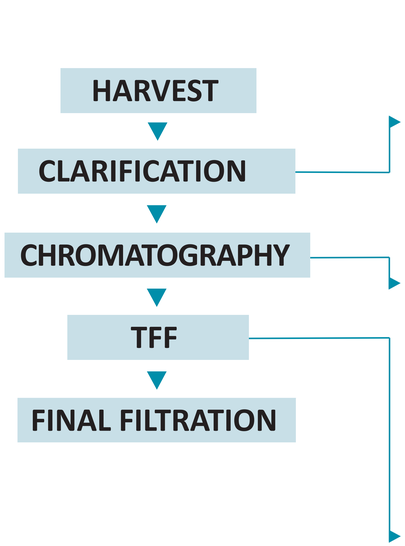
- Unlimited load volume of lysate
- Binding sites inside the channel
- No dead-end pores or void-volume
- No stagnant zones
- No diffusion limitations
- Performance-independent of flow rate
- Large channels (2 µm)- optimal for binding large molecules, such as viruses, VLPs and DNA
- Flow-independent resolution
- High flow rates (up to 10 CV/min)
- Purification within minutes
- Scale-up straightforward

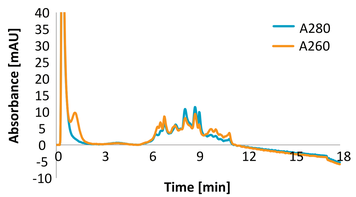


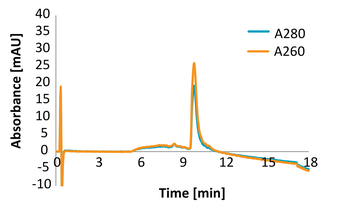


RESULT

MONITORING YOUR ADENOVIRUS DOWNSTREAM PROCESS - A QUICK FINGERPRINTING METHOD





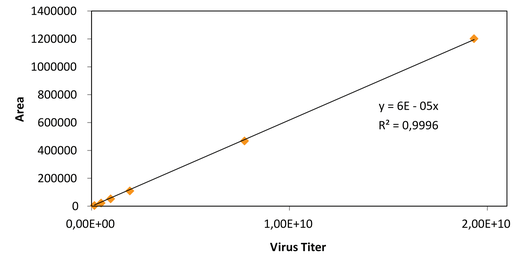


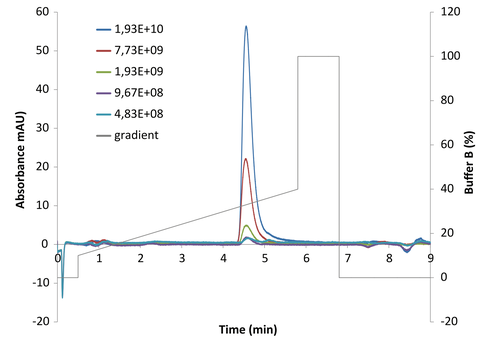
Chromatographic conditions:

The CIMac™ Adeno-0.1 Analytical Columns were run with an Explorer 10 FPLC System (GE Healthcare) under control of the Unicorn software. Sample: rAd5, Flow rate: 0.5 mL/min, sample volume: 50 µL, sample diluted in buffer A: 20 mM Tris, pH 8.0, method: a NaCl gradient from 0 to 1 M in Tris, pH 8 was applied to elute in 30 CV.

QUANTITATION OF YOUR ADENO SAMPLES

Using a CIMac™ Adeno-0.1 Analytical Column and UV detection at 260 nm, the method was linear in the range of 4.8E+08 VP to at least 1.9E+10 VP; LOD was 4.8E+08 VP. One order of magnitude lower sensitivity (4.8E+07 VP) can be achieved by using a fluorescence detector (fluorescence detection at λexc = 280 and λem = 335).





Chromatographic conditions:

The CIMac™ Adeno-0.1 Analytical Columns were run with an Agilent series 1100 (Agilent, Waldbronn, Germany) HPLC system, sample: Ad5 wild type purified with CsCl (replication genes are deleted), buffer A: 20 mM Tris 5% glycerol pH 7.4, buffer B: 20 mM Tris 2 M NaCl 5% glycerol pH 7.4, flow rate: 1 mL/min.

CONCLUSIONS

- The CIMac™ Adeno Analytical column can be used as a fast finger printing method, that gives you information about the production process of your Adenoviruses.
- Using a CIMac™ Adeno-0.1 Analytical Column and UV detection at 260 nm, the method was linear in the range of 4.8E+08 VP to at least 1.9E+10 VP; LOD was 4.8E+08 VP.
- One order of magnitude lower sensitivity (4.8E+07 VP) can be achieved by using a fluorescence detector (fluorescence detection at λexc = 280 and λem = 335).

Acknowledgments

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