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A fast and reproducible HPLC method for quantification of Adenoviruses

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. In addition to that, the data obtained is very reproducible and accurate.

For any kind of quantification, a calibration curve obtained with a virus standard is needed. The work presented in this application note shows the excellent performance of the CIMac™ Adeno Analytical Column – a monolith based anion exchange column, designed for fast and reproducible analyses of adenoviruses.



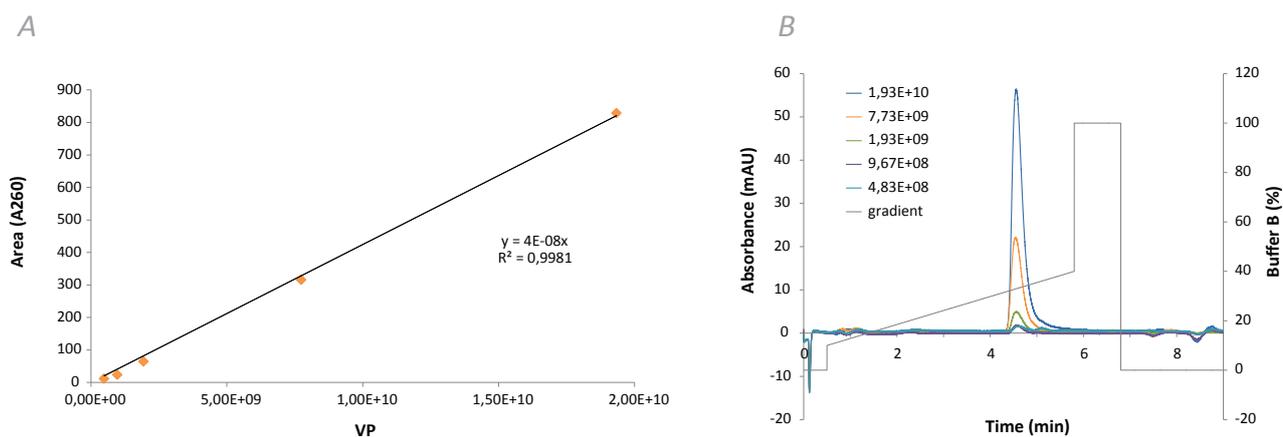
SAMPLE AND METHODS:

The performance of the CIMac™ Adeno-0.1 Analytical Column was examined with a viral standard which was the Adenovirus Serotype 5, purified by a CsCl gradient. The concentration of the virus was determined by a method based on absorbance [method by Mittereder et al (2006). Evaluation of the concentration and bioactivity of adenovirus vectors for gene therapy. J Virol 70, 7498-7509]. Various dilutions of the viral standard were prepared in order to determine the linear range of the method and basic analytical parameters. Linearity was determined by plotting the concentration as a function of the peak area. Samples were injected in triplicates. LOD and LOQ were obtained by injecting progressively more diluted samples into the column. In order to achieve proper binding of the virus, samples were diluted at least 3 times with the equilibrating mobile phase (Buffer A).

<i>Column:</i>	CIMac™ Adeno-0.1 Analytical Column (CV: 0.1 mL); Catalog number: 150.0002
<i>System:</i>	Agilent series 1100 (Agilent, Waldbronn, Germany) HPLC system equipped with degasser, quaternary pump, autosampler, thermostated column compartment, multiwave UV/vis detector and fraction collector
<i>Mobile phases:</i>	Buffer A: 20 mM Tris, pH 7.4 + 5% glycerol Buffer B: 20 mM Tris, pH 7.4 + 2 M NaCl, pH 7.4 + 5% glycerol
<i>Flow rate:</i>	1 mL/min
<i>Method:</i>	100% buffer A (0.5 min), linear gradient from 10% buffer B to 40% buffer B in 5.8 min (58 CV), 100% buffer B (1 min), and 100% buffer A (2.2 min), method depicted in Figure 1
<i>Sample loop:</i>	500 µL
<i>Sample:</i>	Ad5 wild type purified with CsCl (replication genes deleted) with a CsCl gradient concentration determined by qPCR: 2.23E+12 VP/mL, diluted with buffer A
<i>UV Detection:</i>	260 nm

> **Figure 1**

[A] Chromatograms of the calibration curve; [B] Calibration curve obtained by UV detection.



RESULTS

A fast analytical method for determining the Ad5 wild type Adenovirus was developed using a CIMac™ Adeno-0.1 Analytical Column. Using 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, and LOQ was $1.93E+09$ VP.

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