PATfix™ - At-line monitoring of impurities and critical quality attributes in biopharmaceutical up- and downstream processes using HPLC fingerprinting

S. Peljhan[1], T. Kostelec[1], R. Žabar[1], B. Goričar[1], V. Skvarča[1], A. Štrancar[1],
1 BIA Separations d.o.o., Mirce 21, 5270 Ajdovščina, Slovenia

INTRODUCTION

Production of high value biological therapeutics usually involves complex manufacturing processes with high process variability. Additionally, development of robust and reliable bioprocesses can be challenging. PAT aims to enhance bioprocess understanding and implies a holistic approach to ensure that quality is built into products by design. Efficient PAT therefore calls for fast and robust analytical techniques which enables to asses high quality information about critical quality attributes and key performance indicators as parallel as possible to the manufacturing process.

PATfix™ is unique HPLC system for routine gradient separations that enables every analytical task. Equipped with bio-inert ceramic pump heads is deliberately tailored to meet the demands of analytical applications covering wide range of biomolecules. Highly sensitive and fast multi-wavelength detector enables to detect component peaks even in very fast gradients.

inCyght Chromatography Data Science Software

Setup
- Validation
- Information acquisition

Data and system management
- User friendly database functionality
- Complete management of chromatographic data
- Seamless integration with existing data management environment
- Preconfigured chromatographic system characterization
- Test chromatographic system based on industrial best-practices
- Automatically detect system malfunction and alerts

Analyze
- Visualization
- Detection of changes
- Prediction

Act
- Optimal time-point of harvest
- Cell lysis monitoring
- Pooling decisions
- Development

Quantification and tracking of key process parameters
- Follow impurity and product formation and clearance in upstream and downstream processes
- Unique fingerprinting methodology to quantify CQAs and KPIs in complex and changing sample matrices
- Simple to use multivariate and univariate model building tools that tailored for chromatographic data

Report
- Mass visualization of data

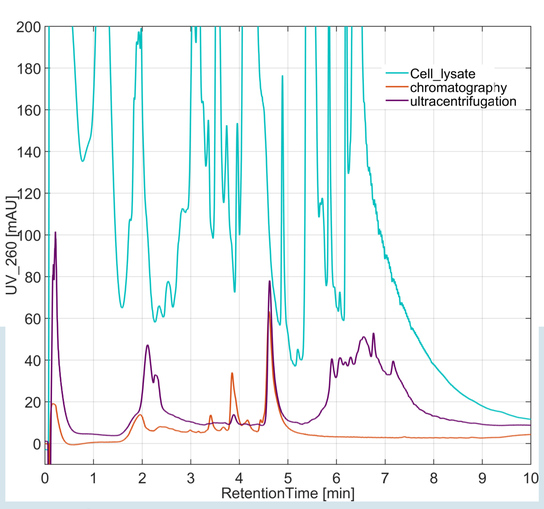
Analysis and visualization of chromatograms
- Automatic chromatogram alignment
- Automatic extraction of chromatographic features
- Automatic detection of chromatogram segments with highest variance
- Automatic correction of peak and chromatogram artefacts
- Generate scientific plots in publication quality
- Plotting templates

CASE STUDY 1

In-process control for Ad3 VLPs using CIMac™ QA Analytical Column

Virus like particles (VLPs) are particles that structurally resemble viruses, but do not contain any genetic material. Extracts from expressing cells contain not only VLPs, but also cellular DNA and proteins. These need to be removed in order to obtain pure VLPs, which are then applied for the production of vaccines, as delivery systems, as well as in other fields of nanotechnology applications (for the application on DSP of Ad3 VLPs check the Application Note A029). The purity of the final VLPs product is evaluated by methods like SDS-PAGE, agarose electrophoresis, PicoGreen analysis, BCA or Bradford assay.

CIMac™ QA Analytical Column was used for in-process control of the adenovirus serotype 3 dodecahedric virus-like particles (Ad3 VLPs). Samples obtained from different purification steps were injected on the CIMac™ QA Analytical Column and elution profiles were compared.



Column CIMac™ QA

Buffer A: 20 mM TRIS, 1 mM EDTA, 5 % glycerol pH 7.5
Buffer B: 20 mM TRIS, 1 mM EDTA, 5 % glycerol, 1 M NaCl pH 7.5
Flow rate : 1 mL/min
Gradient elution method
- Wash after load: 1 min buffer A
- Linear gradient: 0 – 1 M NaCl, 8 min
- High salt wash: Buffer B, 1 min

Sample: 60 µL of three times diluted Ad3 VLP lysate sample
Sample loop: 100 µL
Detection: UV detection, 280 nm

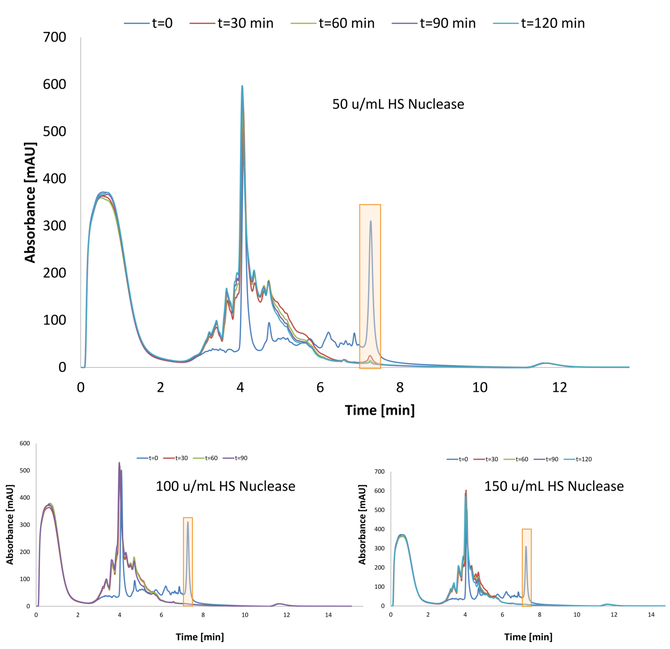
Results

By comparing elution profile of samples injected on a CIMac™ QA Analytical Column one can monitor the purity of the samples. The presence of DNA and proteins can be examined as well as the amount of Ad3 VLPs. The column can be therefore applied for in process control of the purification protocol as well as for determining the purity in the final product. This information can be obtained in less than 10 minutes.

CASE STUDY 2

Adenovirus purification: online nuclease treatment monitoring and process optimization

Determining the concentration of viruses and sample impurities is a crucial step in any production process. The most commonly used methods for sample qualification and quantification are either based on the infectivity of the virus (plaque assay, TCID50), determination of 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. An at-line fingerprinting method was used to track nuclease treatment of a cell lysate sample and to optimize this nuclease treatment step.



Column CIMac™ Adeno

Buffer A: 50 mM TRIS pH 8.0
Buffer B: 50 mM TRIS, 1 M NaCl pH 8.0
Flow rate : 1 mL/min

Gradient elution method
- Wash after load: 1 min buffer A
- Linear gradient: 0 – 1 M NaCl, 7 min
- High salt wash: Buffer B, 2 min

Sample: Nuclease treated cell lysate, 3 x diluted with buffer A, filtered using 0.22 µm membrane filter
Sample loop: 1 mL
Detection: UV detection, 280 nm

Results

- Fast fingerprinting method enables almost real-time measurement of nuclease treatment
- Fingerprint method can be simultaneously used as a quantification method for DNA
- Nuclease treatment unit operation can be optimized/modified and implemented in a matter of hours if necessary.