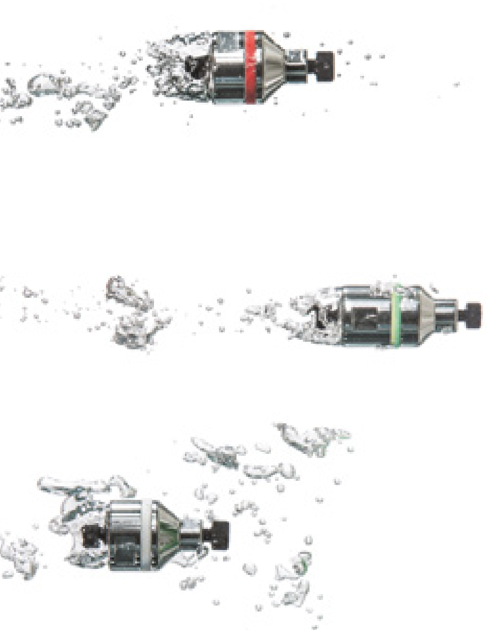


PATfix™

Lead the way of your process

www.biaseparations.com

Short description

Production of high value biological therapeutics usually involves complex manufacturing processes with high process variability. Additionally, development of robust and reliable bioprocesses can also 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 enable to assess high quality information about critical quality attributes and key performance indicators as parallel as possible to the manufacturing process.

Technical features

PATfix™ HPLC is the ideal system for routine gradient separations that enables you to do every analytical task. Equipped with bio-inert ceramic heads, it is deliberately tailored to meet the demands of analytical applications covering a wide range of biomolecules. Its highly sensitive and fast multi-wavelength detector enables detection of component peaks even in very fast gradients.

> High pressure gradient pump (up to 400 bar) with integrated degasser and mixer
> Bio-buffer compatible ceramic pump heads allowing flow rates of up to 10 mL/min also suitable for semi-preparative HPLC
> Highly sensitive multi-wavelength detector with intelligent temperature control to minimize signal drift
> Fluorescence detector
> Temperature controlled autosampler for maximal working efficiency
> Extensive safety features such as leak management sensors

exputec inCyght® Chromatography Data Science Software

Exputec inCyght® for Chromatography offers a user-friendly and powerful toolbox for the analysis of chromatographic data sets. Due to its fingerprint approach it allows the direct determination of CQAs and KPIs from complex and changing sample matrices.

SETUP
- Validation
- Information acquisition

ANALYZE
- Visualization
- Detection of changes
- Prediction

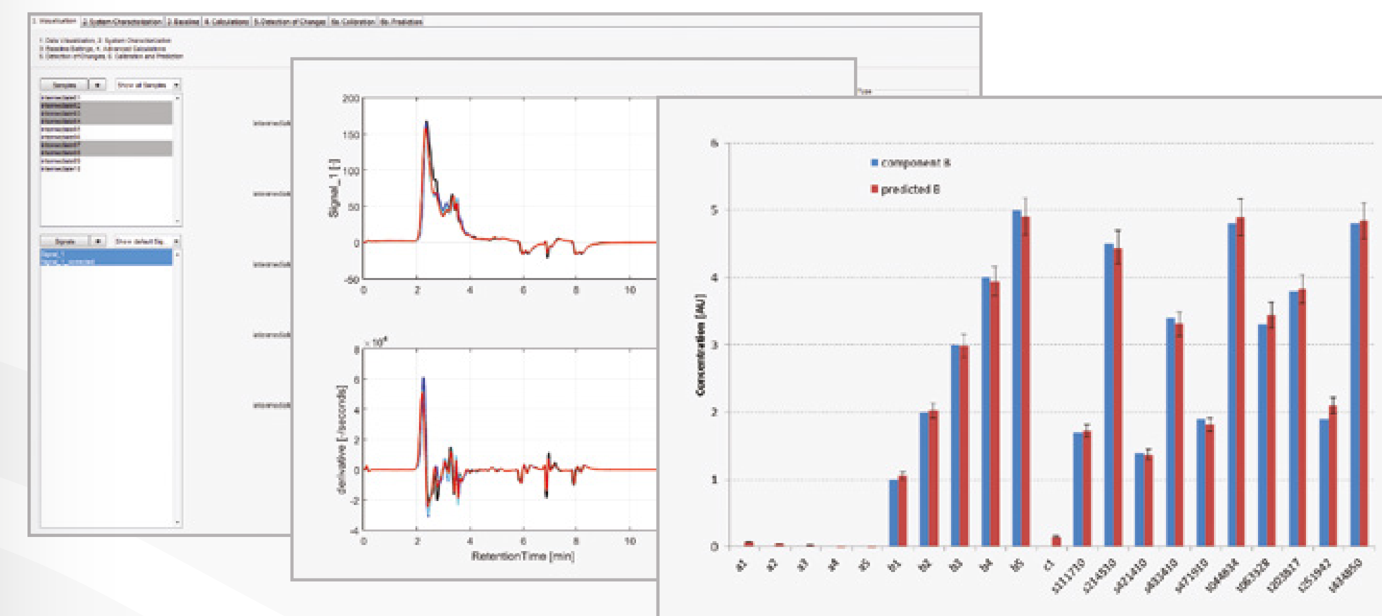
ACT
- Optimal time-point of harvest
- Cell lysis monitoring
- Pooling decisions
- Development

REPORT
- Mass visualization of data

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

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 are tailored for chromatographic 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



Raw material control: Production of Intra-venous IgG from Cohn I+II+III paste

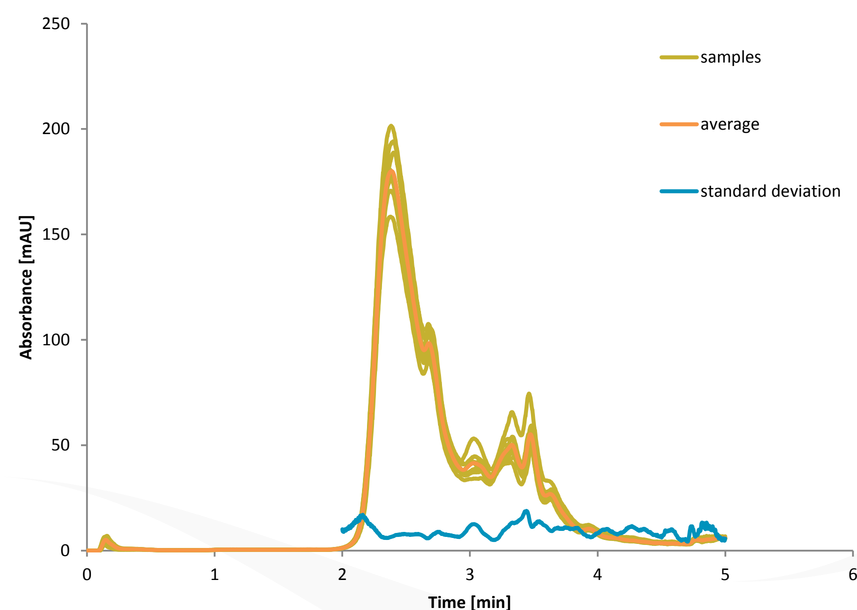
Plasma derived IgG used either for diagnostic purpose or intravenous application in various medical therapies is gaining more and more attention on annual basis. Different manufacturing processes are used to isolate immunoglobulins from human plasma. However, a search for efficient IgG isolation not only requires robust processes, but also rapid and reliable analytics to track the purification. A fast and reliable fingerprint-based method was used here to characterize raw materials entering the production process as well as the intermediate products of unit operations in less than 5 minutes. The ratio between IgG and impurities in each sample was easily assessed from differences in the chromatograms.

Method

Column	CIMac™ QA (catalog #: 110.5113-1.3)
Mobile phases	Buffer A: 50 mM TRIS pH 8.5 Buffer B: 50 mM TRIS, 1 M NaCl pH 8.5
Flow rate	1 mL/min
Gradient elution method	Wash after load: buffer A, 2 min Linear gradient: 0 – 500 mM NaCl, 3 min High salt wash: Buffer B, 1 min
Sample	1 g of Cohn I+II+III paste in 10 mL of 20 mM Na-acetate pH 5.0, dissolved for 12 h at 4 °C, centrifuged 30 min at 9500 G; 10 x diluted with Buffer A and filtered using 0.22 µm membrane filter
Sample loop	50 µL
Detection:	UV detection, 280 nm

Results

1. 10 different samples of Cohn I+II+III were analysed using HPLC fingerprinting method
2. IgG concentration deviation in samples was below 10 %
3. Impurity deviation in samples was up to 20 %
4. The amount of loading material can be tuned to account for the impurity/product ratio in the sample.



Raw material control: Production of Intra-venous IgG from Cohn I+II+III paste

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.

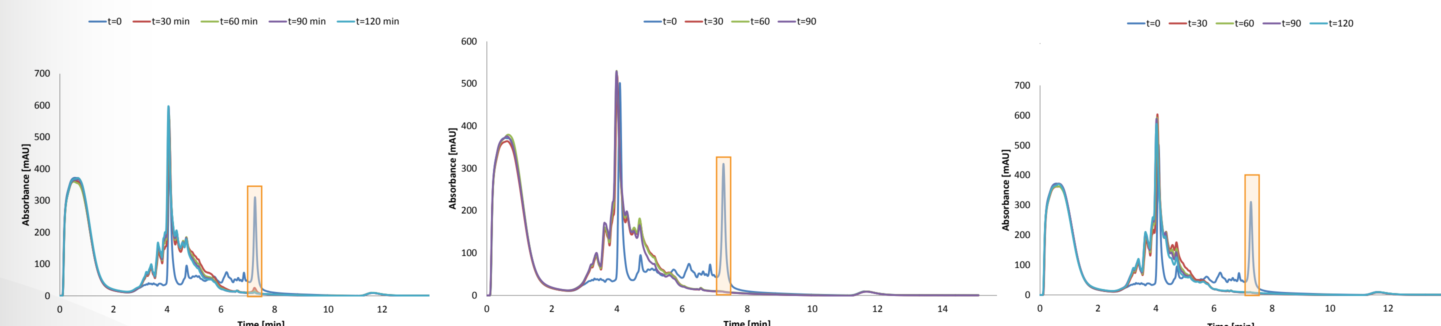
Method

Column	CIMac™ Adeno (catalog #: 110.8502-2)
Mobile phases	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: buffer A, 1 min Linear gradient: 0 – 1M 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

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

Adenovirus purification: online nuclease treatment monitoring and process optimization



Addition of 50u/mL HS Nuclease — Addition of 100u/mL HS Nuclease — Addition of 150u/mL HS Nuclease

APPLICATIONS

Monitoring of impurity formation in recombinant upstream processes

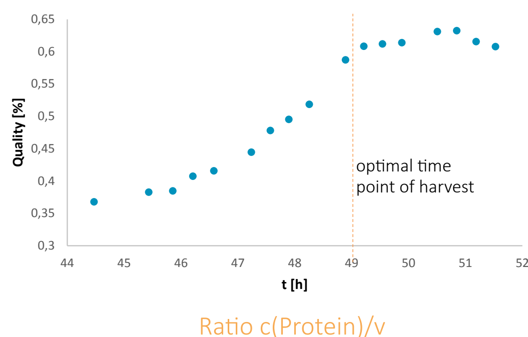
The optimal time point of harvest in a bioprocess plays a key role in preserving product quality and to aid subsequent downstream unit operations. In practice, time point of harvest for bioprocesses with extracellular protein production is usually defined based on tedious offline analytics comprising of sample preparation and incubation steps (e.g. Bradford or BCA assay). Our novel PAT toolbox methodology is aimed to circumvent these bottlenecks by monitoring the formation of impurities along a bioprocess. Furthermore, monitoring of impurity populations not only provides us with enhanced process knowledge but also helps us to derive key process information such as the optimal time point of harvest.

Method

Column	CIMac™ QA (catalog #:110.5113-1.3)
Mobile phases	Buffer A: 50 mM Tris-HCl, pH 8 Buffer B: 50 mM Tris-HCl + 1 M NaCl, pH 8
Flow rate	1 mL/min
Gradient elution method	Wash after load: buffer A, 1 min Linear gradient: 0 – 100 % buffer B, 2 min High salt wash: buffer B, 1 min
Sample	P. pastoris culture samples, filtered using 0.22 µm membrane filter, 3 x diluted with buffer A
Sample loop	50 µL
Detection:	UV detection, 280 nm, 260 nm

Results

1. Calibration models were established to predict impurity changes based on full information from chromatograms at multiple wavelengths
2. Prediction of optimal point of harvest.



Ratio c(Protein)/v

Raw material quality evaluation

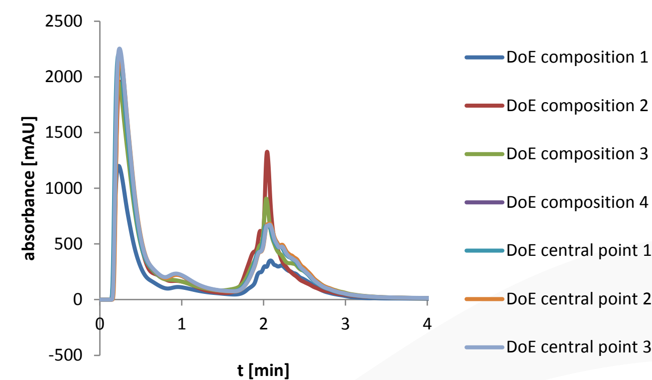
Reduction of variance in a process starting conditions would pave way for better process control and in accordance with quality by design (QbD) principles. Furthermore for proper modelling of a bioprocess, batch to batch variations should be taken into account as they directly affect process performance (e.g. productivity). In this study, chromatogram fingerprinting methodology is implemented for identifying changes in the quality of the raw materials. The changes in the concentrations of complex media components can be clearly seen using the chromatogram fingerprinting.

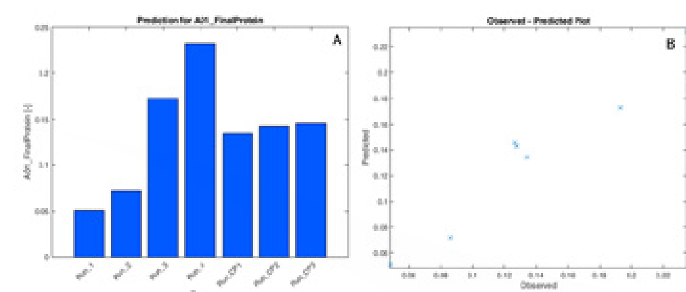
Method

Column	CIMac™ QA (catalog #: 110.5113-1.3)
Mobile phases	Buffer A: 50 mM Tris-HCl, pH 8 Buffer B: 50 mM Tris-HCl + 1 M NaCl, pH 8
Flow rate	1 mL/min
Gradient elution method	Wash after load: buffer A, 1 min Linear gradient: 0 – 100 % buffer B, 2 min High salt wash: buffer B, 1 min
Sample	P. pastoris culture samples, filtered using 0.22 µm membrane filter, 3 x diluted with buffer A
Sample loop	50 µL
Detection:	UV detection, 280 nm, 260 nm

Results

1.Fingerprinting information can be used to identify variations in raw materials for fermentation processes,
2.Characterization of raw materials is necessary to decrease process variations.





CIMac™ ANALYTICAL COLUMNS

CIMac™ QA-0.1

Analytical Column (Quarternary amine)
Strong anion-exchange monolithic analytical column
cat. Nr: 110.5113-1.3

CIMac™ DEAE-0.1

Analytical Column (Diethylamino)
Weak anion-exchange monolithic analytical column
cat. Nr: 110.5114-1.3

CIMac™ EDA-0.1

Analytical Column (Ethylene diamino)
Weak anion-exchange monolithic analytical column + activated chemistry for immobilizations
cat. Nr: 110.5116-1.3

CIMac™ SO3-0.1

Analytical Column (Sulfonyl)
Strong cation-exchange monolithic analytical column
cat. Nr: 110.6157-1.3

CIMac™ CM-0.1

Analytical Column (Carboxymethyl)
Weak cation-exchange monolithic analytical column
cat. Nr: 110.6170-1.3

CIMac™ r-Protein A-0.1

Analytical Column (Recombinant Protein A)
Immunoaffinity monolithic analytical column
cat. Nr: 110.1004-2

CIMac™ r-Protein G-0.1

Analytical Column
cat. Nr: 110.1011-1.3

CIMac™ Adeno-0.1

Analytical Column
Strong anion-exchange monolithic analytical column optimized for the HPLC analytics of 10
cat. Nr: 110.8502-2

CIMac™ pDNA-0.3

Analytical Column
Weak anion - exchange column optimized for the HPLC analytics of plasmid DNA
cat. Nr: 150.8501-1.4, 150.8501-2, 150.8501-6

CIMac™ AAV

Analytical Column-0.1
(empty & full)
cat. Nr: 110.8503-2

CIMac™ AAV

Analytical Column-0.1
(total viral particle)

Coming soon!

CIMac™ Lenti

Analytical Column-0.1

Coming soon!

OTHER PRODUCTS

CIMmultus™ Disposable Columns

- Multi-use disposal column
- Versatile player in DSP- from capture to polishing
- Strict process economics goes with gentle treatment of large moleculs

CIM® line

- Packed in polypropylene or stainless steel housing
- Versatile player in DSP- from capture to polishing
- First generation of monolithic columns

CIMac™ Analitical Columns

- Rapid, high resolution analysis achived in minutes
- PAT enabeling technology
- High sensitivity and reproducibility

CIM® Kits & Packs

- DSP with CIM® monoliths made easy
- CIM® HiP² Plasmid process Pack™- ready to use, adjustable pDNA purification
- CIMmultus™ Purification Screening Kit – Introducory kit with different chemistries

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