Focus: CHROMATOGRAPHY

Issue 2 2023

Welcome to FOCUS: Chromatography

Welcome to the second edition of the FOCUS: Chromatography magazine 2023

In this latest edition of our FOCUS: Chromatography magazine we consider the analysis of proteins and peptides and those tools available to improve your analytical workflows. Whether you are looking to separate peptides, recombinant proteins, or monoclonal antibodies, we have something for you.

Analytical scale separations of these biopharmaceuticals are run on fully porous and solid-core particles. Scale up from lab scale to bulk is also demonstrated using Tosoh Bioscience SkillPak™ BIO pre-packed columns. For those challenging peptide mapping applications, the correct solvent grades are critical. Learn the benefits of using VWR® UHPLC-MS grade solvents and how Merck LC-MS reagents and additives can be utilized to improve MS signals.

As the biotherapeutic industry grows, we also look at ways to be more sustainable and discuss the use of returnable barrels for HPLC solvents.

We have a wide range of chromatography webinars available on demand to supplement the magazine content at vwr.com/webinar and more information about products and services for your analytical lab at **vwr.com/chromatography**

If you have any questions or need application advice, please contact our industry-leading technical support team at **chromsupport@avantorsciences.com**

Enjoy reading and exploring!
Avantor Chromatography Workflow Team

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HiperSolv CHROMANORM® HPLC solvents in returnable barrels

Chromatography methods are continually improving to offer better sensitivity to meet today's demands. In the last 5 years, many improvements have been made to answer the strict requirements of the apparatus or detection methods, especially with impurities which can generate many issues. So, with their high degree of transmittance, low evaporation residue level, low particle count, low acidity and alkalinity, Hipersolv CHROMANORM® solvents are ideal for reproducible and accurate chromatographic results in HPLC. They are produced from specially selected raw materials that undergo a number of purification steps prior to final packaging.

Another challenge was also to work with the introduction of stainless steel returnable barrels. With such packaging we anticipate, in the future, minimising the environmental impact of packaging waste and health risks for your employees.

HPLC is now a key technique in research and development, quality control and purification used in different applications. Now, each of your demands can be covered with different pack sizes from 1 to 1400 L and materials from glass, aluminium and stainless steel. Depending on your annual consumption or application, choose the right packaging.

BENEFITS

- High quality and high UV transmission
- Low residue on evaporation
- Optimised peak baseline separation
- High resolution and sensitivity
- Excellent batch-to-batch reproducibility

WHY PUT HIGH PURITY SOLVENTS IN RETURNABLE BARRELS?

As one of the global market leaders in laboratory reagents and production, we still try to improve our quality and safety standards to our reagents themselves, and also to the containers we supply. That's why we continuously develop and improve packaging and tailor-made solvent withdrawal systems that meet the requirements of our customers. After the first launch of 185 L stainless steel barrels and 1000 and 1400 L containers for production, today we are able to provide the complete range with two additional sizes (10 and 30 L) with different withdrawal systems. These two new barrels sizes will certainly be used more often in analytical laboratories.

This new solvent management solutions will help to minimise the environmental impact of packaging waste and health risks for your employees.

BENEFITS

- Maximum user safety – minimise fire risks, incidents of spillage, and reduce the risk of staff being exposed to hazardous solvents
- Less storage space - our solvents take up to 50% less space than glass bottles, reducing storage costs by half
- Easy and contamination-free solvent handling – be confident in your results. Our barrels are filled with high quality solvents that have an excellent batch-to-batch reproducibility
- Easier to transport - with easy to grip handles and shatter proof stainless steel construction, our returnable barrels are simple to transport
- Application-oriented solutions and individual installations
- Ecological and economic benefits - with virtually no packaging waste, no hazardous disposal costs, no rising done on site, your lab will be greener and more environmentally friendly
- Time savings - save time testing solvent batches, handling solvent bottles and connecting and replacing bottles with your instrument

Technical data for stainless steel barrels

Parameter	10 L	30 L	185 L
Height (mm)	315	435	1085
Diameter (mm)	278	363	550
Wall thickness (mm)	1.5	1.5	1.5
Volume (L)	12	32	205
Filling quantity (L)	10	30	185
Weight (empty) (kg)	5.5	9.6	38
Number per pallet	11	6	
Working pressure (bar)	Max. 5	Max. 5	
Material	Stainless steel 1.4301		
Openings	2" centrally with Tri-Sure screw cap		None - dry brake coupler Type M and K

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SPECIFICATIONS OF THE SOLVENTS AVAILABLE IN STAINLESS STEEL BARRELS

Description	Pk (L)	Assay min. %	Residue max. %	Water max.%	Min. % UV transmittance/wavelength (nm) (1 cm quartz cell, distilled water)	Cat. No.
Acetonitrile super gradient grade	10	99.95	0.0002	0.003	80/195 95/200 96/210 97/220 98/230 99/240 99/250	83639.910
Acetonitrile super gradient grade	30	99.95	0.0002	0.003	80/195 95/200 96/210 97/220 98/230 99/240 99/250	83639.9030
Acetonitrile super gradient grade	185	99.95	0.0002	0.003	80/195 95/200 96/210 97/220 98/230 99/240 99/250	83639.500
Acetonitrile Isocratic grade	10	99.9	0.0005	0.03	80/200 85/210 90/220 98/230 99/250	20048.9010
Acetonitrile Isocratic grade	30	99.9	0.0005	0.03	80/200 85/210 90/220 98/230 99/250	20048.9030
Methanol super gradient grade	10	99.9	0.0001	0.02	45/210 65/220 70/225 85/235 90/240 95/250 98/260	85681.9010
Methanol super gradient grade	30	99.9	0.0001	0.02	45/210 65/220 70/225 85/235 90/240 95/250 98/260	85681.9030
Methanol isocratic grade	10	99.8	0.0005	0.05	60/210 80/220 90/230 92/235 95/240 98/250 98/260	20837.9010
Methanol isocratic grade	30	99.8	0.0005	0.05	60/210 80/220 90/230 92/235 95/240 98/250 98/260	20837.9030

New high purity HiPerSolv CHROMANORM® solvents for use in UHPLC-MS

New

NOW AVAILABLE!

These **NEW** VWR Chemicals HiPerSolv CHROMANORM® UHPLC-MS solvents provide consistent, ultra-pure quality for your discovery-driving results.

For highly sensitive UHPLC-MS analyses, how do they perform?
With our high purity solvents, will reduce noise and keep additional signals to a minimum, will also raise the standard for low baseline noise and clean mass spectra.

Our new range of advanced UHPLC-MS solvents have been developed to exceed all expectations, providing rapid and reliable results in both ESI/APCI positive and negative ionisation modes, ensuring low formation of metal ion adducts and improving peak profiles.

Our solvents offer real advantages when performing UHPLC-MS methods

- Suitability tested and specified for UHPLC-MS and UHPLC-UV
  Benefit: For analytical flexibility
- <0,1 µm ultrafiltration for demanding UHPLC-MS applications
  Benefit: Time and money savings - less issues due to clogging of instrument, columns and check valves
- LC-UV and LC-MS gradient suitability test
  Benefit: Minimal UV absorbing impurities combined with minimal MS ionising impurities, providing smooth baselines with extremely low interference, regardless of detector used
- Packed in borosilicate bottles
  Benefit: Significant reduction of contamination risks with metal cations (Na+ and K+)
- ESI/APCI (+) <2 ppb - ESI/APCI (-) <10 ppb
  Benefit: Specified quality in positive and negative ESI and APCI MS for lowest detection limits and confidence in analyses in all important MS modes
- Metal content impurities <10 ppb
  Benefit: For minimised metal ion adduct formation - <10 ppb
- Lowest level of polyethylene glycol (PEG)- (PEG S/N signal-to-noise ratio <50)
  Benefit: Impurities in our entire UHPLC-MS solvent line up to give you confidence in your results.

Our advanced VWR Chemicals UHPLC-MS HiPerSolv CHROMANORM® solvents have been designed to meet the highest requirements of UHPLC-MS in research and quality control, including proteomics and metabolomics as well as environmental, clinical, food or industrial testing applications.

Description	Pk	Cat. No.
Acetonitrile HiPerSolv CHROMANORM® UHPLC-MS	1 L	**83647.290**
Methanol HiPerSolv CHROMANORM® UHPLC-MS	1 L	**85802.290**
Water HiPerSolv CHROMANORM® UHPLC-MS	1 L	**83646.290**

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How Merck LC-MS reagents and additives can improve your analyte signal

INTRODUCTION

It is common practice in LC-MS to add certain reagents to the mobile phase, or to introduce them post-column prior to the interface to influence analyte ionisation. Most often, the goal is for an improvement in the analyte signal. In addition, some additives may be used to suppress unwanted signals, or selectively enhance the signal of particular compounds in a mixture. For example, glycosidic species in a mixture of peptides. To help you obtain the highest quality analysis, we offer a wide range of high purity mobile phase additives for LC-MS applications. The LC-MS portfolio includes the most commonly used acids, bases and volatile salts of high purity tested for LC-MS applications. Impurities, such as alkali ions, plasticisers or surfactants, that can be commonly found in lower grade solvents are particularly problematic as they interfere strongly with LC-MS, resulting in higher background noise and formation of adducts. Only ultra-pure reagents enable high signal-to-noise ratios, which results in the highest and most reliable performance for small and large molecule applications.

RESERPINE TEST

All of our LC-MS solvents and reagents are specified using the standard reserpine test. Reserpine (608.68) is used as the reference substance to quantify possible impurities in the LiChropur® LC-MS reagents. It is performed by diluting 2,5% (v/v) acid, base or 2,5% (w/v) salt in 50/50 (v/v) acetonitrile/water. Every lot produced is analysed via flow injection analysis mass spectrometry (FIA-MS). The dissolved reagent and the appropriate reserpine reference solutions are introduced into the MS ion source syringe pumps. In 3 minutes the total ion chromatogram (TIC) is accumulated. The relative intensities of the detected masses are compared with the reserpine signal. For electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) in the positive mode, the specified amount of reserpine is 2 ppb for acids and bases, and 20 ppb for salts. In the negative mode, the specified amount of reserpine is 20 ppb for both.

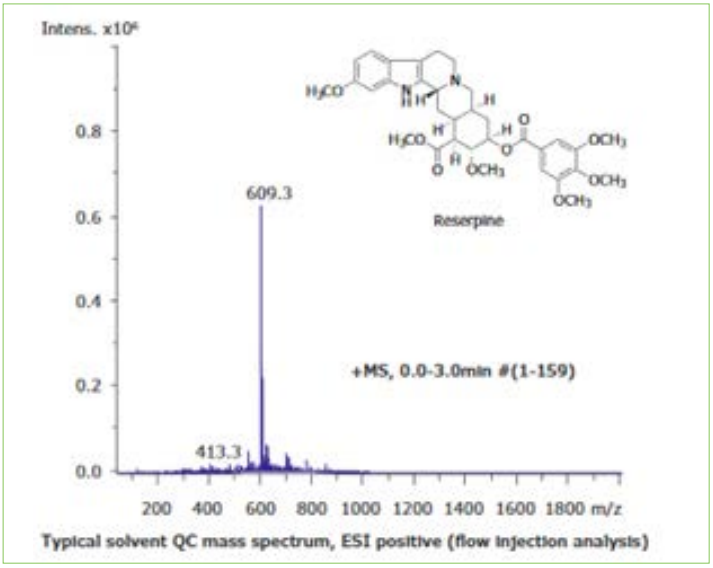

FIGURE 1: Reserpine test example.

Acid additives

Volatile, low molecular weight organic acids such as formic and acetic acid or novel difluoroacetic acid (DFA) improve ionisation and resolution of a wide range of molecules. Addition of organic acids to the mobile phase can help to overcome the ionisation-suppressing effect of trifluoroacetic acid (TFA) present in the mobile phases used for the analysis of proteins and peptides.

Neutral salts

Neutral volatile salts, such as ammonium acetate or ammonium formate are typically used as buffer compounds to control the ionisation state of the analytes (and phases), which have a strong influence on the LC-MS separation and performance.

Sodium adduct formation

Alkali adducts diminish instrument sensitivity. When adduct formation tendency is strong, often the addition of defined amounts of sodium ions (mostly pre-column) can help to obtain uniform and stable molecular ions for detection in LC-MS.

EXTENSIVE QC TESTING ENSURES HIGHEST SPECIFICATION

Residue on ignition (evaporation residue) tests show the low content of insoluble matter in the reagent. This provides confidence that your eluents have the low particle content needed for accurate LC-MS measurement. Sodium and potassium ions are particularly likely to form adducts with the analyte molecules. This leads to complex mass spectra leading to time-consuming data evaluation. The content of trace metals is in the low ppb range for LiChropur® LC-MS reagents to minimise the risk of adduct formation in the ion source for cleaner results. Our LiChropur® LC-MS reagents are stored in borosilicate bottles to prevent leaching of alkali ions out of the glass. The content of the potentially complex forming ions aluminium, copper and iron is also specified. Full specification can be found in the certificate of analysis for each of our LC-MS grade products.

Ordering information

Substance	Description	Pk	Cat. No.
Acetic acid	100% for LC-MS LiChropur®	50 ml	**5.33001.0050**
Ammonia solution	25% for LC-MS LiChropur®	50 ml	**5.33003.0050**
Ammonium acetate	For LC-MS LiChropur®	50 g	**5.33004.0050**
Ammonium hydrogen carbonate	For LC-MS LiChropur®	50 g	**5.33005.0050**
Sodium formate solution	Suitable for LC-MS LiChropur®	100 ml	*
Difluoroacetic acid	For LC-MS LiChropur®	1 ml, 10x1 ml, 50 ml	*
Trifluoroacetic acid	Eluent additive for LC-MS LiChropur®	10x1 ml, 10 ml, 50 ml	*
Difluoroacetic acid	Eluent additive for LC-MS LiChropur®	1ml, 5ml, 10ml	**74564***
1,1,1,3,3,3-Hexafluoro-2-propanol	Eluent additive for LC-MS LiChropur®	10 ml, 50 ml	*

* Coming soon

Your benchmark for quality

You're getting more than just a vial when you purchase an official USP Reference Standard. You're getting the confidence of knowing that you have a trusted entity that offers a unique combination of standards, process and service to help you on your journey to regulatory compliance. Our quality standards are the industry benchmark because of the robust scientific process, the unparalleled service and knowledgeable, expert advice that USP offers you throughout the development process.

We offer the largest collection of 3,700 highly characterized Reference Standards, most directly tied to documentary standards. USP creates official, quality standards for medicines that are enforceable in the United States and used in more than 140 countries.

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3,700+

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Rigorously tested and highly characterised - USP Reference Standards undergo a rigorous process of scientific characterisation by multiple independent laboratories around the world. Our standards provide more than scientific guidelines; they give you confidence in your approach throughout the product life cycle.

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Where USP or NF tests or assays call for the use of a USP Reference Standard, only those results obtained using the specified USP Reference Standard are conclusive

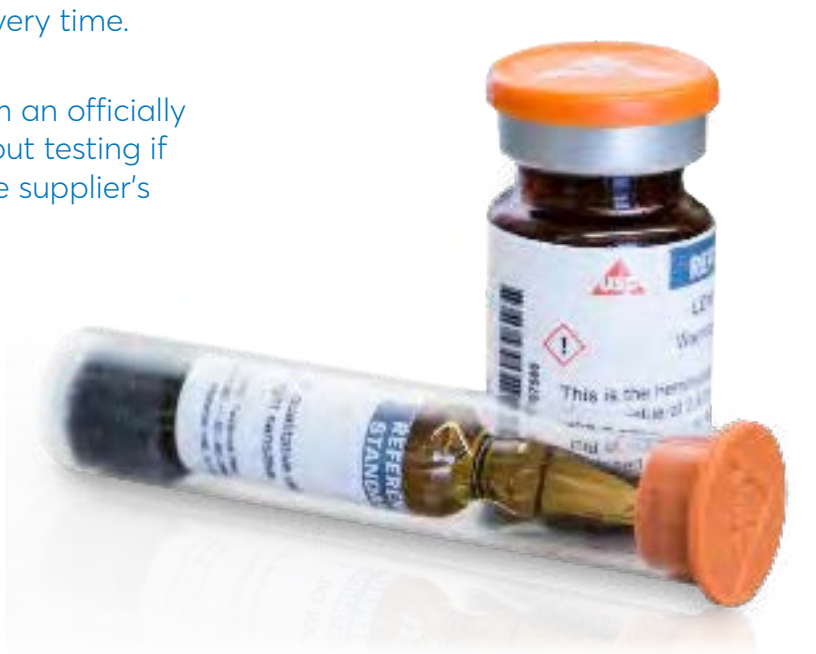
– USP–NF General Notices Section 5.80

USP standards:
Quality you can trust

Using USP official Reference Standards can reduce the time and resources that you need to invest in developing in-house standards and can facilitate compliance with regulatory requirements. Our standards provide precise testing and validation guidelines, as well as reference samples for testing, so that drugs can be made consistently, every time.

Primary reference standards obtained from an officially recognized source are normally used without testing if stored under conditions consistent with the supplier's recommendations.

– ICH Q7



Authorized Distributor

Reference Standard development process

MULTIPLE LAB TESTING
USP Reference Standards are thoroughly characterized by a variety of methods. Tests conducted by multiple laboratories improve the confidence in the assigned value by addressing real-life inter-lab variability in measurements.

COMPREHENSIVE ANALYSIS
Committees of scientific experts from government, industry and academia independently review and evaluate USP standards to help ensure accuracy and provide a trusted reference for analytical testing.

CONTINUED SUITABILITY FOR USE
Ongoing testing eliminates the need for an expiration date and helps ensure the quality of your product over time.

Standards tied to monographs

- Antivirals
- Antimicrobials
- Antibiotics
- Cardiovascular
- Cough & cold
- Analgesic
- Gastrointestinal
- Impurities
- Endocrine
- Ophthalmology
- Pulmonary
- Dermatology
- Excipients
- Oncology
- Steroids
- OTC
- Renal

4,900
number of official monographs that link directly with a Reference Standard

Only USP Reference Standards are linked to 4,900+ official USP–NF monographs that provide specifications for the identity, purity and potency to meet compliance requirements for FDA-approved drugs. Monographs for drugs, excipients, biologics and compounded preparations include specifications for identity, strength, quality, purity, packaging and labeling.

USP Reference Standards have official status when used with the compendial standard. They do not need to be compared or evaluated against any other standard; a CoA is not required for use.

Expert, independent volunteers

Independent volunteers, who are selected based on their knowledge and experience, review laboratory analyses and develop monographs and general chapters before they are published for public review and comment. These independent experts consider the public feedback and finally approve the monographs and general chapters before they are added to the USP–NF.

350+

general chapters with clear, step-by-step direction for assays, tests and procedures.

875+

volunteer experts from academic, commercial and government institutions, health practitioners, science associations and consumer organizations around the world.

USP

is the only organization that can produce Reference Standards linked to USP–NF monographs to help meet compliance requirements.

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Adding value to your drug manufacturing process

USP standards can be a strategic advantage as part of your pharmaceutical production process. From raw materials to product development, to manufacturing, to finished products and beyond, these are just some of the ways you can leverage our products, services and support to efficiently bring your products to market.

Raw materials – Suppliers may declare their raw materials conform to USP standards when they meet compendial specifications. Such indications of a supplier's quality program can simplify your supplier selection process.

In process – The compatibility of the ingredients that comprise the final dosage form is critical to its quality. USP Reference Standards help minimize the risk of poor-quality materials affecting in process testing, by improving the accuracy of blend uniformity and helping to ensure consistency from batch to batch.

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Chromatography Solutions

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For over a century, professionals around the world have chosen the J.T.Baker® brand for quality and performance they can trust. J.T.Baker products consistently meet the needs of the most demanding applications, and their premium, high-performance syringe filters are no exception, providing efficient filtration with minimal extractables to maximize sample purity.

Every batch is delivered with the assurance of a chromatogram and a certificate of quality and is rigorously tested for burst pressure of housing, bubble point, flow rate performance and extractables.

These syringe filters are specifically designed for chromatography sample preparation applications and are optimized to provide the most consistent results with minimal extractables.

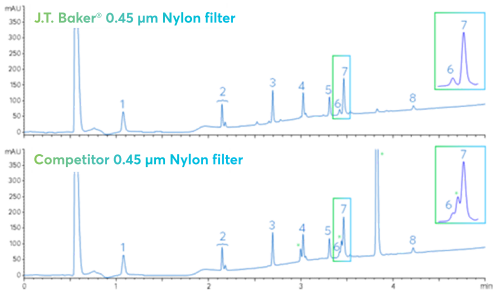


FIGURE 1: Comparison of chromatograms obtained from filtering a sample of valsartan spiked with eight nitrosamines with J.T.Baker® and competitor syringe filters.

Enhanced LC sample preparation with J.T.Baker® high performance syringe filters

J.T.Baker®

Charge variant analysis workflow

QC ANTIBODY CHARGE VARIANT ANALYSIS ON PROPAC ELITE WCX COLUMNS

Monoclonal antibodies (mAbs) are a preferred class of protein therapeutics used for the treatment of various diseases because of their ability to target specific tissues. The structure of monoclonal antibodies is often heterogeneous due to post-translational modifications and degradation during cellular production and downstream processing. Modifications can impart additional cationic and anionic charges, increasing the charge heterogeneity of the mAb. To analyse variants based on their charge, ion exchange chromatography (IEX) is a commonly used technique to separate analytes including proteins based on dierences in charge. Thermo Scientific™ ProPac™ Elite WCX columns (5 µm) possess weak cation exchange functionality designed for the analysis of proteins. Thermo Scientific CX-1 pH gradient buffers generate a linear pH gradient that simplifies method optimization for high resolution separation of the main product and impurities.

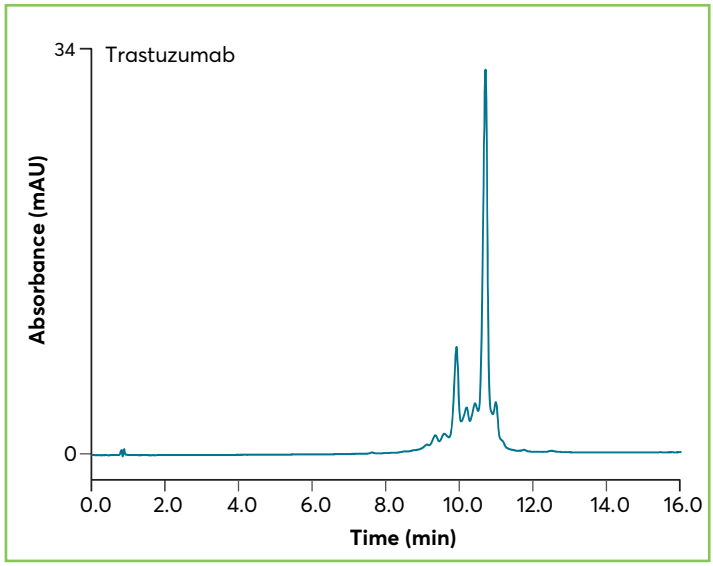


FIGURE 1: Separation of IgG1 therapeutic mAbs using a 20 to 70% B pH gradient on the 4 x 150 mm column

ProPac Elite WCX column

CX-1 pH gradient buffer

SureSTART vial and cap

Workflow solution

Descrption	Cat. No.
Thermo Scientific columns and guard columns	
Thermo Scientific™ ProPac Elite WCX column	**554-6585**
Thermo Scientific™ ProPac™ WCX-10 guard column	**554-5305**
Thermo Scientific gradient buffers	
Thermo Scientific™ CX-1 pH gradient buffer A	**555-0138**
Thermo Scientific™ CX-1 pH gradient buffer B	**555-0139**
Thermo Scientific vials and caps	
Thermo Scientific™ SureSTART™ 2 ml GOLD-Grade clear glass vial	**HYPE6PSV9-1PG**
Thermo Scientific™ SureSTART™ 9 mm screw cap	**HYPE6PSC9ST1**

This workflow includes the newest recommended products

Avantor® ACE® Wide Pore HPLC Columns for the Separation and Purification of Proteins in Biopharmaceuticals

By Matt James, Senior Research Scientist, Mark Fever, R&D Manager, Tony Edge, R&D Leader & Scientific Advisor

INTRODUCTION

The analysis of peptides and proteins is critical for ensuring the safety and efficacy of biopharmaceuticals. The comprehensive characterisation of protein-based therapeutics, is a highly complex challenge and typically involves a combination of complimentary analyses, targeting different critical quality attributes. Chromatography plays a crucial role in confirming product characterisation and the determination of related impurities. Figure 1 summarises the key chromatographic techniques that are typically employed. Determination of the target protein in its intact and aggregated forms, the assessment of charge variants, along with detailed analysis of various enzymatically cleaved fragments and characterisation of non-proteinaceous components (e.g. glycan distributions of glycoproteins) are commonplace.

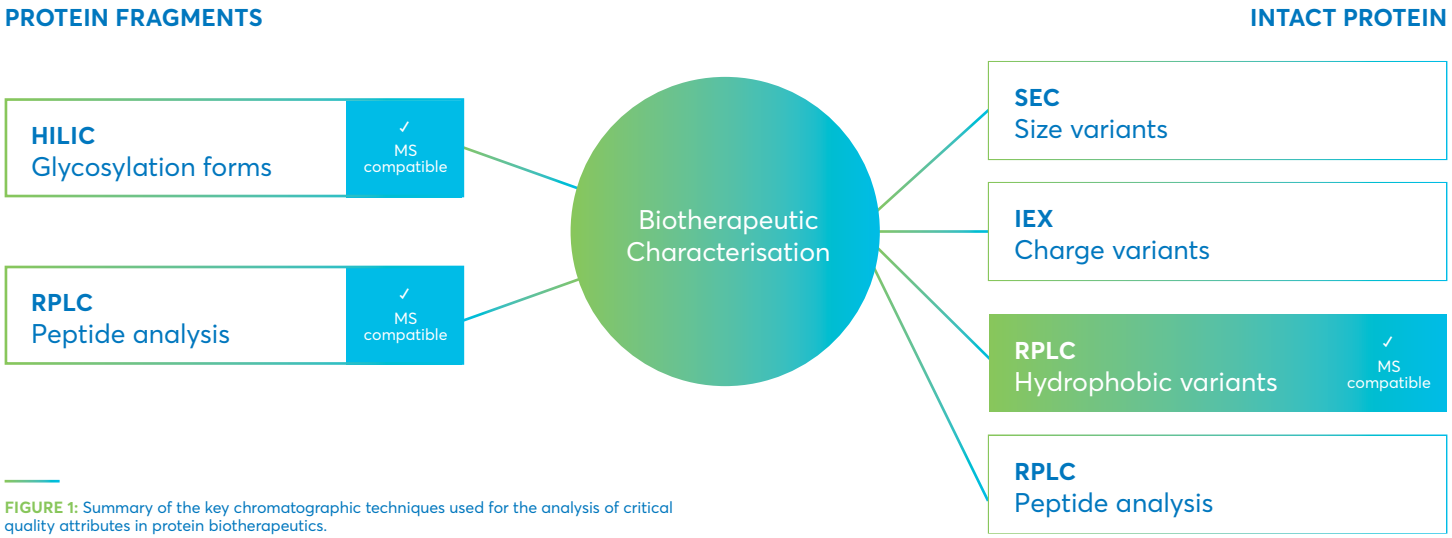


FIGURE 1: Summary of the key chromatographic techniques used for the analysis of critical quality attributes in protein biotherapeutics.

Reversed-phase liquid chromatography (RPLC) is a powerful and widely applied tool for the separation and characterisation of both intact proteins and peptides. The technique shows excellent robustness, reproducibility and, crucially, is readily coupled to mass spectrometry. Additionally, RPLC is a high-resolution technique and is therefore ideally suited to the analysis of complex samples, such as those produced by enzymatic digestion in peptide mapping[1], but is also valuable for intact protein analysis. In the latter case, the high resolving power can be used to resolve hydrophobic variants, including modified proteins that are similar in structure to the target protein, such as single amino acid post-translational modifications.

Whilst high resolution separations of peptides and complex protein digests can be readily achieved using columns packed with high-purity, high-performance silica-based particles with a pore size of approximately 80-100 Å (e.g. Avantor® ACE® Excel® C18), intact proteins require a modified approach. Proteins have considerably higher molecular weights and are therefore physically much larger. For example, a monoclonal antibody (mAb) has a molecular weight of ~150,000 Da compared to ~150-1,000 Da for typical small molecule drugs. Protein RPLC separations therefore require a stationary phase with a much larger pore size, typically of the order 300-500 Å. Using a 100 Å phase for a protein separation results in poor accessibility of the analyte into the stationary phase pores, thereby restricting diffusion and increasing peak broadening and potentially tailing. The use of wider-pore materials was found to solve this issue by improving pore accessibility to large molecules. Avantor® ACE® wide pore columns have been specifically engineered to produce highly reproducible separations for a wide range of peptides, proteins and other high molecular weight biomolecules. Based on ultra-high purity silica, they provide exceptional chemical stability, peak shape, sensitivity and column lifetime, allowing highly efficient separations of proteins to be achieved. Both fully porous (FPP) and superficially porous or solid-core silica particles (SPP) are available, as summarised in Table 1. Multiple column chemistries are available to fully explore column selectivity during method development.

Phase	Particle type	USP listing	Functional group	End capped	Particle size (µm)	Pore size (Å)	Surface area (m²/g)	Carbon load (%)	pH range
Fully porous phases									
C18-300	FPP	L1	Octadecyl	Yes	3, 5, 10	300	100	9	2-8
C8-300	FPP	L7	Octyl	Yes	3, 5, 10	300	100	5	2-8
C4-300	FPP	L26	Butyl	Yes	3, 5, 10	300	100	2.6	2-8
CN-300	FPP	L10	Cyano	Yes	3, 5, 10	300	100	2.8	2-8
Phenyl-300	FPP	L11	Phenyl	Yes	3, 5, 10	300	100	2.6	2-8
Solid core phases									
UltraCore BIO C18	SPP	L1	Octadecyl	Yes	3.5	300	16	1	1-8
UltraCore BIO C18	SPP	L1	Octadecyl	Yes	2.5	500	23	1.4	1-8
UltraCore BIO C4	SPP	L26	Butyl	Yes	3.5	300	16	0.4	2-9
UltraCore BIO C4	SPP	L26	Butyl	Yes	2.5	500	23	0.6	2-9
UltraCore BIO Phenyl2	SPP	L11	Diphenyl	Yes	3.5	300	16	0.7	2-9
UltraCore BIO Phenyl2	SPP	L11	Diphenyl	Yes	2.5	500	23	1	2-9

TABLE 1: Specifications of fully porous and solid-core wide pore columns.

ANALYSIS OF INTACT PROTEINS BY RPLC USING AVANTOR® ACE® FULLY POROUS PARTICLES (FPP)

Intact protein separations are typically achieved using an acetonitrile/water gradient, with the addition of TFA as an ion-pairing agent. Generally, separations are run at elevated temperatures up to 90 °C to improve analyte diffusivity and mass transfer, thereby improving peak shape, resolution and reducing protein adsorption[2]. Figure 2 demonstrates the high efficiency separation of a set of peptides and proteins with varying molecular weights using a broad scouting gradient run on a fully porous Avantor® ACE® 3 C4-300 column. Of particular note is the ability to separate the three insulin variants of human, bovine and porcine origin. These insulins have molecular weights of approximately 5,800 Da with only slight variation in their amino acid sequences. Despite this, the ACE 3 C4-300 provides sufficient chromatographic performance to separate these sample components.

0.1% TFA has historically proved popular for use as a mobile phase additive for RPLC protein analysis, however other non-ion-pairing additives such as 0.1% formic acid are gaining popularity, as they avoid potential issues with ion-suppression when coupled with mass spectrometry. Modern columns based on type B ultra-pure silica, such as Avantor® ACE® columns offer distinct advantages in terms of efficiency and peak shape for biomolecule analysis. The presence of metal ion impurities can result in undesirable secondary interactions with the target analytes and also increases the acidity of residual silanols at the silica surface. This can result in excessive peak tailing, low performance and reproducibility challenges. These effects are dramatically reduced for type B ultra-pure silica based columns and it is often possible to significantly reduce the amount of mobile phase additive (Figure 3). This is highly beneficial for both UV (reduced background adsorption and better sensitivity) and MS detection (reduced ion suppression and increased sensitivity).

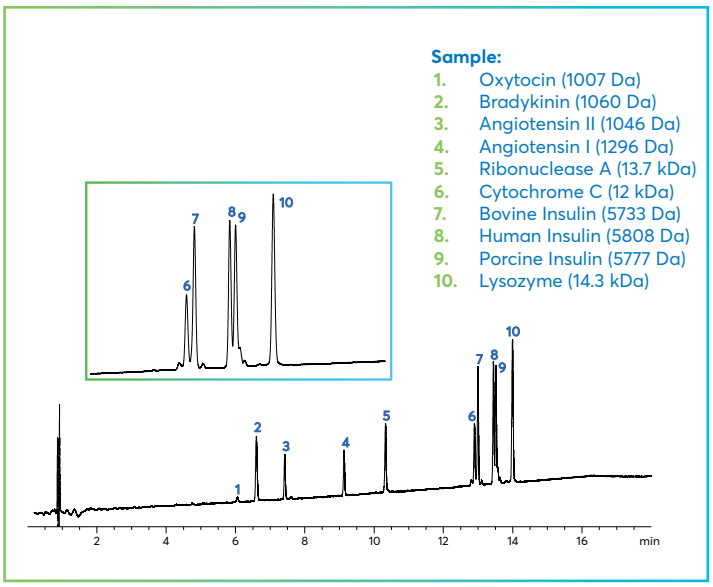
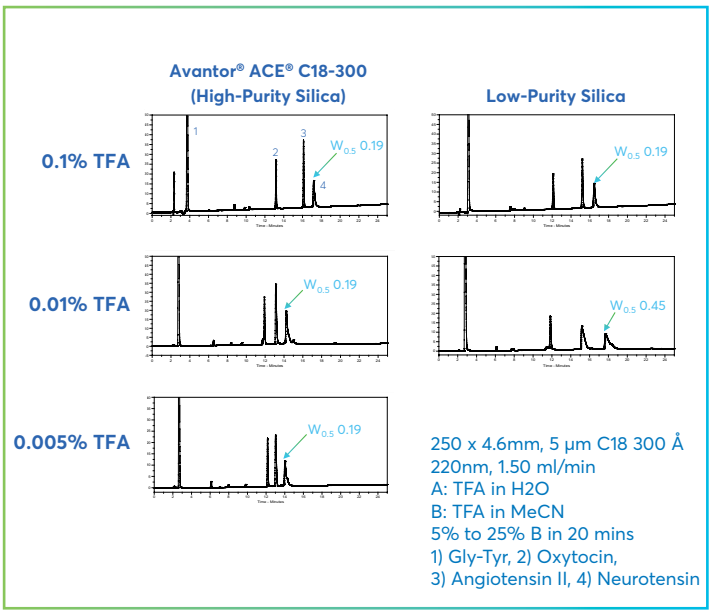


FIGURE 2: Reversed-phase separation of a range of peptides and proteins with varying molecular weights on an Avantor® ACE® 3 C4-300 (150 x 2.1 mm). Mobile phase A: 0.1% TFA in H2O, B: 0.1% TFA in MeCN/H2O 80:20 v/v; Gradient: 10 to 50% B in 15 minutes; Flow rate: 0.5 ml/min; Temperature: 60 °C; Detection: UV (220 nm).



FGURE 3: The effect of lowering mobile phase modifier (TFA) concentration with low- and high-purity silica base columns.

The separation of closely related protein variants is a common theme in protein characterisation. In this situation, shallower gradient profiles are often employed to provide enhanced resolution of closely related proteins. Figure 4 shows how this approach can be used for the analysis of milk proteins. A much shallower acetonitrile/water gradient was employed on an Avantor® ACE® 5 C18-300 column to elute a series of casein and lactoglobulin proteins between 20 and 30 minutes. The high efficiency and excellent peak shape of the ACE column allows for the successful separation of casein variants which differ in their structure by as little as one amino acid substitution. In this example, the concentration of TFA in the mobile phase has been reduced by a factor of 10 to just 0.01%, which would dramatically improve sensitivity if MS detection was employed. This is possible because of the high inertness of the ACE silica surface, which allows excellent peak shape to be obtained for proteins, even at low concentrations of TFA.

Time (mins)	% B
0	33
5	33
9	35
18	37
22	40
27.5	41
28	41
43	43

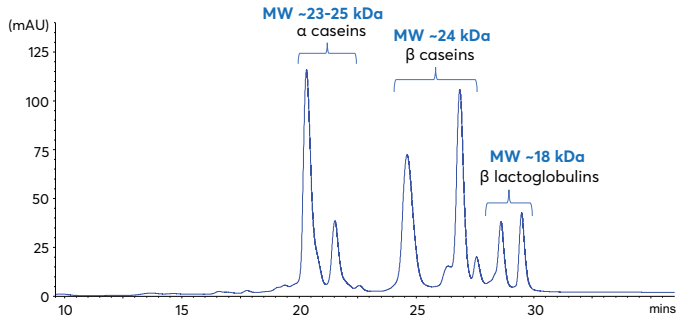


FIGURE 4: Separation of milk proteins using the Avantor® ACE® 5 C18-300. Column dimension: 150 x 2.1 mm; Mobile phase A: 0.01% TFA in H2O, B: 0.01% TFA in MeCN; Gradient: as shown above; Flow rate: 0.2 ml/min; Temperature: 45 °C; Detection: UV 214 nm. Reproduced with permission of The Chemical Analysis Facility, University of Reading, UK.

INTACT PROTEIN ANALYSIS USING AVANTOR® ACE® ULTRACORE SOLID-CORE COLUMNS

One of the key performance limitations of RPLC analysis of proteins is that the large analyte molecular weights give rise to slow molecular diffusion, resulting in broadening of chromatographic peaks. RPLC separation of intact proteins tend to be run at elevated temperature to counteract this. An alternative resolution could be to use non-porous stationary phase particles to reduce mass transfer, however, non-porous particles suffer from poor loadability[3]. More recently, solid-core (or superficially porous, SPP) particles were introduced to the market, so called as they are comprised of a solid, non-porous core, surrounded by a porous outer shell (Figure 5).

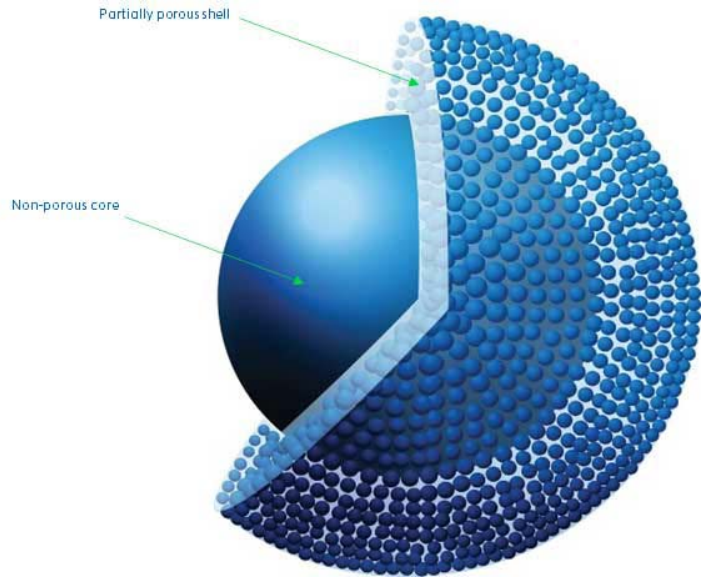


FIGURE 5: Schematic representation of a solid-core particle.

This particle morphology provides several benefits over traditional porous particles for the analysis of therapeutic proteins. Firstly, the monodispersed nature of solid-core particles results in more uniformly, "better" packed columns, reducing intra-column flow path variation (eddy-diffusion, van Deemter A-term)[4]. Additionally, longitudinal dispersion is reduced because of a reduction in column dead volume provided by the non-porous core (B-term). Finally, detrimental mass transfer effects are reduced as the analyte diffusion path length is reduced by the thin, porous shell compared to fully porous particles (C-term). This final effect provides a particular benefit to the analysis of larger molecular weight analytes, such as proteins, due to their inherently slower diffusion rates. This improved mass transfer is also exaggerated at higher mobile phase flow rates. The improved kinetic performance for solid-core particles therefore means that they have great potential to provide higher throughput and efficiency for intact protein separations, compared to fully porous particles.

Avantor® ACE® UltraCore BIO columns have been developed to offer multiple solutions for the high-efficiency separation of large biomolecules, such as proteins (Table 1). The range features solid-core particles in two different particle size/pore size combinations to ensure that the optimum pore size can be determined for any separation. These wide-pore columns are suitable for the analysis of proteins and peptides with molecular weights over 5 kDa. Three different stationary phases are available to help fine tune the selectivity of protein separations. The C4 phase provides less hydrophobicity than a C18 phase, whilst the Phenyl2 phase can provide additional pi-pi interactions with the aromatic amino acids phenylalanine, tryptophan and tyrosine, and can therefore provide alternative selectivity and retention to the C4 and C18 phases[5].

Figure 6 demonstrates the use of a wide-pore 500 Å Avantor® ACE® UltraCore BIO column to provide a high efficiency RPLC separation of a mixture of proteins and peptides. High performance and resolution are obtained for this broad range of analytes, which ranged in mass from 5.8 to 45 kDa, demonstrating the broad applicability of wide-pore UltraCore BIO phases for the analysis of intact proteins.

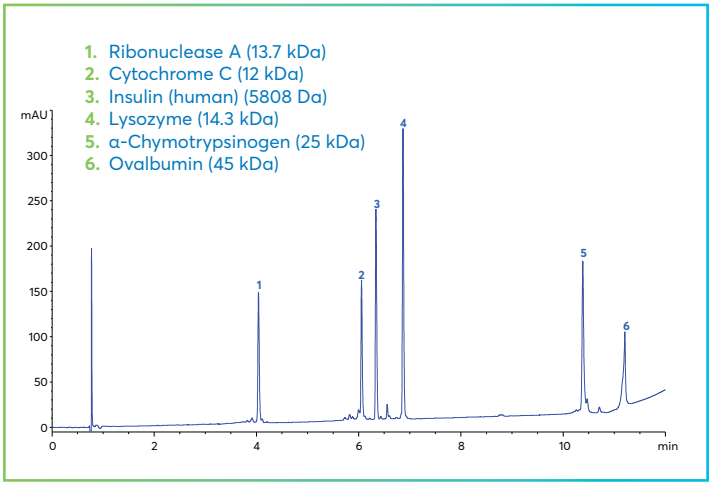


FIGURE 6: Separation of a range of peptides and proteins on an Avantor® ACE® UltraCore BIO C4-500 column. Column dimensions: 100 x 3.0 mm; Mobile phases: A: 0.1% TFA in H2O, B: 0.1% TFA in MeCN/H2O 90:10 v/v; Gradient: 20 to 50% B in 10 minutes, then 50 to 100% B in 2 minutes; Flow rate: 0.6 ml/min; Injection volume: 5 µl; Temperature: 60 °C; Detection: UV, 220 nm.

The high molecular weight of mAbs means that achieving high-resolution, high-throughput analyses of higher molecular weight proteins such as mAbs can be challenging, due to slow molecular diffusion and hindered pore access. The particle morphology of Avantor® ACE® UltraCore BIO means that highly efficient separations can be achieved for these analytes. Figure 7 demonstrates how the 500 Å C4 phase can achieve a similarly high-resolution separation of a mAb. In this case the NIST mAb analysed has a much greater molecular mass of 150 kDa. Using 0.1% TFA as the modifier, along with an elevated temperature of 80 °C, a rapid, high-resolution separation is readily achieved, with sample impurities clearly discernible from the main API peak.

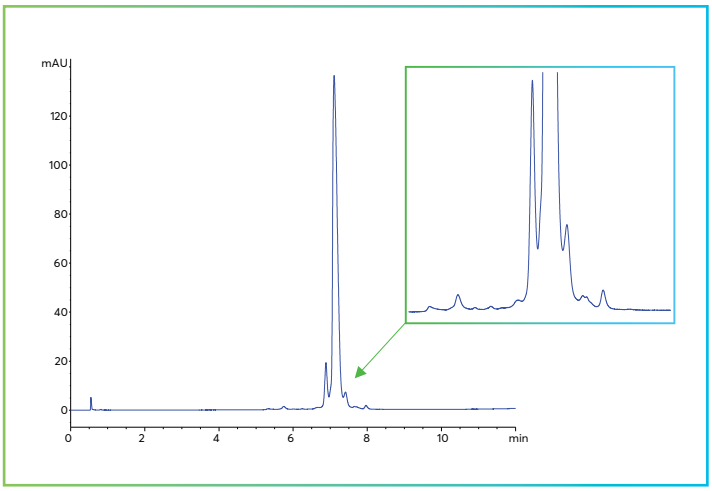


FIGURE 7: Analysis of NIST mAb on an Avantor® ACE® UltraCore BIO C4-500 column. Column dimensions: 100 x 3.0 mm; Mobile phases: A: 0.1% TFA in H2O, B: 0.1% TFA in MeCN/H2O 90:10 v/v; Gradient: 36 to 45% B in 10 minutes, then 45 to 80% B in 2 minutes, hold at 80% B for 2 minutes; Flow rate: 0.8 ml/min; Injection volume: 1 µl; Temperature: 80 °C; Detection: UV, 280 nm.

CONCLUSION

The analysis and characterisation of intact protein biotherapeutics is a complex analytical challenge requiring application of a range of analytical techniques. The high resolving power of reversed-phase liquid chromatography and compatibility with mass spectrometry makes it a valuable tool for the separation of intact proteins and their closely related variants. The successful analysis of such compounds requires the use of wide pore (300-500 Å) stationary phase, operated at elevated temperatures, low pH and with relatively shallow gradients. Avantor® ACE® wide pore columns offer a comprehensive range of options for the high resolution analysis of intact proteins. Both fully porous and solid-core particles are available, bonded with a variety of complementary stationary phase chemistries, enabling the optimisation of stationary phase selectivity during method development. The use of ultrapure and highly inert base silica means that lower concentrations of mobile phase modifiers can be used without compromising analyte peak shape, which is potentially highly beneficial for LC-MS applications.

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HPLC Analysis of Letrozole and its Impurities Using a Purospher® STAR RP18e Column

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ABSTRACT

This application note follows the USP method for analysis of Letrozole and its related impurity-A by HPLC-UV. The analytes were all separated on a Purospher® STAR RP-18 endcapped column with an excellent peak shape and good detection limits. The method validation results have met the acceptance criteria cited in USP43-NF38 monograph suggesting robust method performance and increased confidence in results.

INTRODUCTION

Letrozole is an oral non-steroidal aromatase inhibitor used in treatment of hormonally-responsive breast cancer after surgery. It prevents the aromatase from producing estrogens by competitive, reversible binding to the heme of its cytochrome P450 unit 1. In this work, we present an HPLC method with UV detection for the simultaneous quantification of letrozole and its impurities by following USP43-NF38 Guidelines[2].

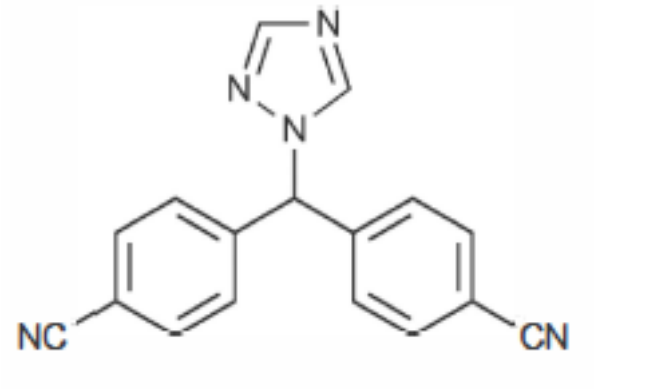


FIGURE 1: Chemical Structure of Letrozole

INSTRUMENTATION

- Shimadzu Prominence UFLC XR System
- Milli-Q® Integral 3 Water purification system
- Ultrasonic bath from PCI analytics
- Millex® HV Durapore membrane filter (PVDF) 0.45 µm

Parameter	Value		
Mobile phase	Solution-A: Milli-Q® or Ultrapure water (HPLC grade)		
	Solution-B: Acetonitrile (HPLC grade)		
	Gradient Program:		
	Time(min)	**A(%)**	**B(%)**
	0.01	70	30
	25	30	70
	26	70	30
	30	70	30
Analytical Column	Purospher ® STAR RP-18e, 125x4.6 mm, 5 µm		
Diluent	Solution-A and Solution-B (7:3) v/v		
Back pressure	72- 28 Bar (1044- 410 psi)		
Injection volume	20 µl		
Column temperature	25 °C		
Flow rate	1 ml/min		
UV detection	230 nm		
Standard	Dissolve 10 mg of Letrozole USP in 30 ml of diluent, mix well and dilute to 100 ml with the diluent.		
System suitability (SST) solution	Dissolve 10 mg of Letrozole USP and 20 mg of Letrozole related impurity-A containing 50 ml of diluent, mix well and dilute to 100 ml with the diluent.		
Sample	Weigh accurately about 25 mg of Letrozole to a 250 ml volumetric flask containing 75 ml of acetonitrile. Dilute with water to volume, mix well and filter the resulting solution using a 0.22 µm syringe filter for HPLC analysis.		

TABLE 1: Experimental conditions for the analysis

CHROMATOGRAPHIC DATA OF LETROZOLE AND ITS RELATED IMPURITY ANALYSIS

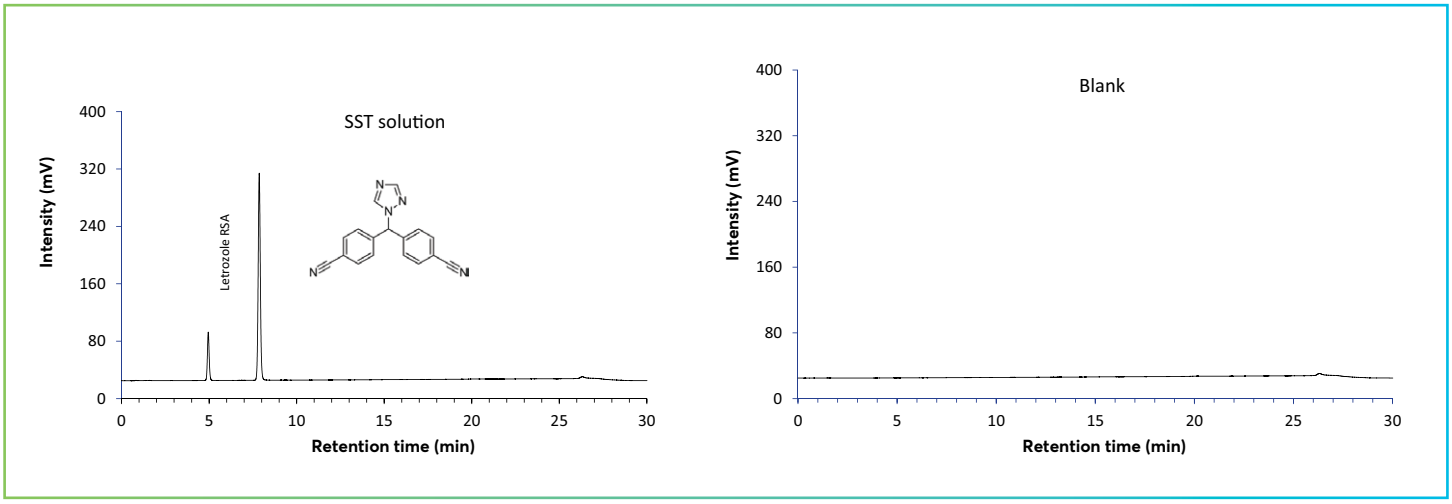


FIGURE 2: Chromatograms of (Left) Letrozole related impurity-A and Letrozole USP; (Right) Blank solution (diluent)

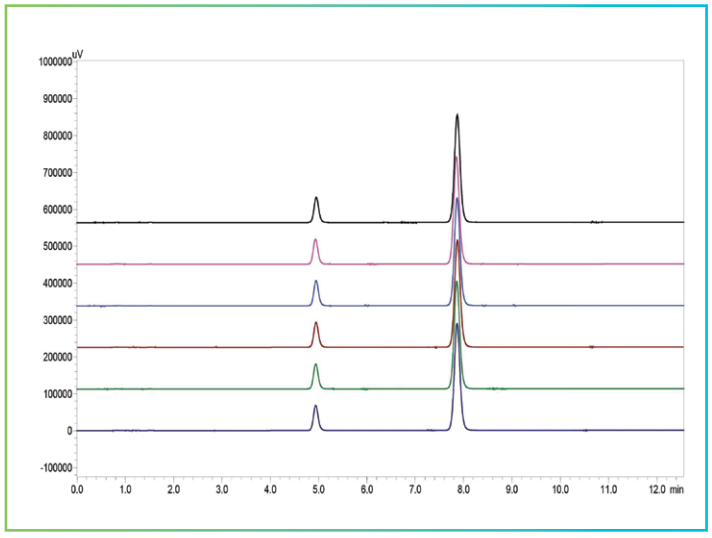


FIGURE 3: Overlaid Chromatogram of Letrozole and its related impurity-A using SST solution; 1). Letrozole (4. 9 min); 2). Letrozole related impurity-A (7.9 min)

#	Compound	Retention Time (min)	RRT	RRT per USP43	Resolution	Resolution per USP43	Theoretical plates
1	Letrozole related compound A(RCA)	4.9	0.62	0.67	-	-	**1.1**
2	Letrozole	1	1		14.3	2	**1.1**

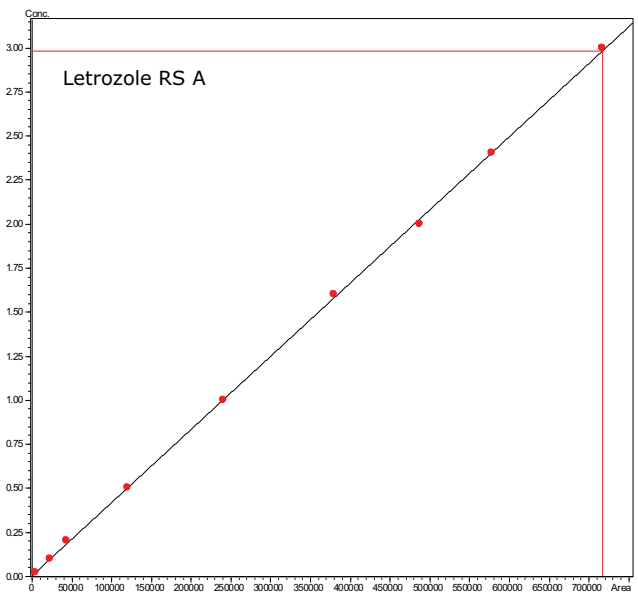
TABLE 2: System Suitability criteria of Letrozole and its Impurity-A

Identity	Letrozole RS-A	Letrozole
1	474836	2457649
2	474364	2458516
3	474541	2450935
4	474747	2449480
5	474431	2450485
6	473816	2451989
Average	174456	2453176
Standard Deviatiopn	362	3895
RSD(%)	<0.1	0.2

TABLE 3: Repeatability of Letrozole and its related impurity-A(SST Solution)

"Concentration (1,1g/mL)"	Letrozole RS-A (Mean Area)	"Concentration (1,1g/mL)"	Letrozole (Mean Area)
0.02	4394	0.1	22557
0.1	22630	0.5	116715
0.2	43882	1.0	229577
0.5	120464	2.5	631260
1	240666	5.0	1231279
1.6	380178	8.0	1976461
2	487146	10.0	2519018
2.4	578138	12.0	3001958
3	717196	15.0	3722293
Line equation	y = 240765x-1154.2	y = 250992x-10622	
R2	0.9998	0.9998	

TABLE 4: Linearity of Letrozole and its related impurity - A



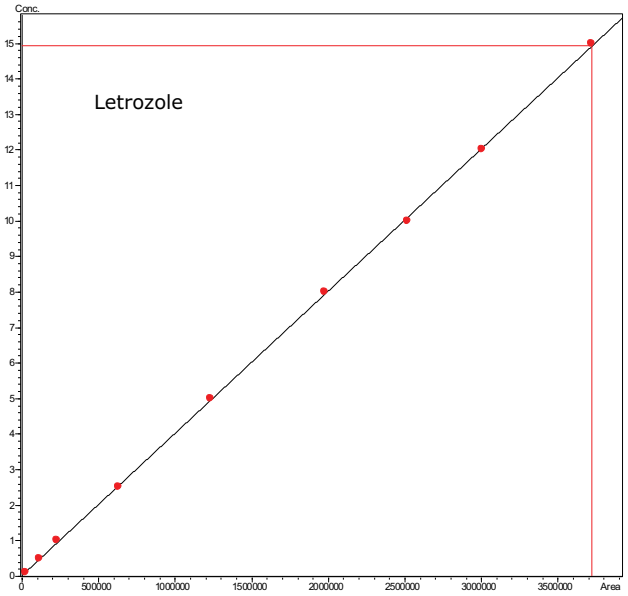


FIGURE 4: Linearity plot of (Left) Letrozole Related impurity-A; (Right) Letrozole

RESULTS AND DISCUSSION

An isocratic RP-HPLC method for the determination of Letrozole and its related impurity-A was tested using Purospher® STAR RP-18e column, referring to USP43-NF38. The experimental data indicated an excellent linearity with an r2 value of 2: 0.998 (Figure 4) for the selected concentration range (Table 4). The LOD & LOQ values for Letrozole related impurity-A were 0.03 & 0.09 µg/ml, respectively.

CONCLUSION

A HPLC-UV method was tested for the reliable estimation of Letrozole and its related impurity-A using Purospher® STAR RP-18 endcapped HPLC column. The data from linearity, system suitability, repeatability of the method suggests that the method provided better retentivity and specificity for the assay of Letrozole and its related impurity-A by following USP43-NF38 monograph.

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2. Letrozole. https://doi.org/10.31003/uspnf_m44500_04_01

RELATED PRODUCTS

Description	Product No.
Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 125-4.6 suitable for HPLC	1.51914
Letrozole United States Pharmacopeia (USP) Reference Standard	1356971
Letrozole Related Compound A United States Pharmacopeia (USP) Reference Standard	1356982
Acetonitrile gradient grade for liquid chromatography LiChrosolv® Reag. Ph. Eur.	1.00030
Water for chromatography (LC-MS Grade) LiChrosolv®	1.15333
Millex Syringe Filter, Durapore® (PVDF), Non-sterile 0.45 µm pore size, 13 mm diameter, Millex-HV Durapore® (PVDF) membrane, hydrophilic	SLHVX13NK

Analysis of Therapeutic Monoclonal Antibody Trastuzumab Using BIOshell™ A400 Protein C4 Column

Fast, high resolution analysis of intact monoclonal antibodies (mAbs)

Sundaram Palaniswamy, Segment Lead, Pharma QC; Aditya Pratihast, Application Scientist.

ABSTRACT

Although the majority of small molecules analysed by reversed phase have a mass below 1500 Da, there is a growing need to improve the performance of HPLC columns for the separation of therapeutic proteins and protein drug conjugates. This application note demonstrates a fast, reproducible reversed-phase method with high resolution for the analysis of intact therapeutic monoclonal antibody, trastuzumab. Separation and quantification were achieved using a BIOshell™ A400 Protein C4 column in less than 5 minutes, and more importantly, the optimised method was able to monitor degradation compounds created by heat stress studies.

INTRODUCTION

Over the past few years, monoclonal antibodies (mAbs) have become the best selling drugs in the pharmaceutical market, and in 2018, eight of the top 10 best selling drugs worldwide were biologics. The global therapeutic monoclonal antibody market was valued at approximately $115 billion in 2018 growing up to $300 billion by 2025. Although, as of December 2019, 79 therapeutic mAbs have been approved by the US FDA for sales worldwide, there is a significant potential for the number to increase[1]. HPLC is a well established method for the analysis of intact mAbs by size exclusion and ion exchange chromatography. However, technological advancements in the field of Reversed Phase (RP) have made them promising tools for the analysis on intact proteins[2]. Intact mAbs are yet analysed with limited success using wide pore, fully porous particles due to their large molecular size leading to slow mass transfer and long analysis times. Superficially porous particles have overcome these challenges, but have lower load ability and still a rather limited offering of different stationary phases. Moreover, highly resolving core-shell columns easily separate intact mAbs quickly and with high efficiency.

Here, we have demonstrated the suitability of the BIOshell™ A400 Protein C4 column for a fast and high resolution separation of intact trastuzumab using RP-HPLC. Retention time and area precision of the method were excellent, demonstrating the suitability of the column. In addition, we also showcase quantification and robustness that is ideal for biopharmaceutical QC applications.

EXPERIMENTAL

EQUIPMENT AND SAMPLE

The study was performed on a Shimadzu LC-2010CHT HPLC system. The therapeutic trastuzumab was purchased from a local pharmacy.

METHODS

Chromatographic parameters for intact trastuzumab using a BIOshell™ A400 Protein C4 column are shown in Table 1.

LC Parameters	
Column:	BIOshell™ A400 Protein C4, 100 x 2.1 mm I.D., 3.4 µm (66825-U)
Mobile Phase:	[A] Water + 0.1% TFA; [B] Acetonitrile + 0.09% TFA
Gradient Program:	Time %A %B 0 95 5 1 95 5 2 80 20 6 50 50 8 5 95 8.1 95 5
Post Time:	2 minutes
Flow rate:	1 ml/min
Autosampler Temp.:	5 °C
Column Temp.:	80 °C
Detector:	UV 280 nm, 20 Hz
Injection volume:	10 µL
Sample:	1 mg/ml trastuzumab (1:10 dilution of formulation in mobile phase A)

TABLE 1: Chromatographic parameters used for RP HPLC analysis of trastuzumab.

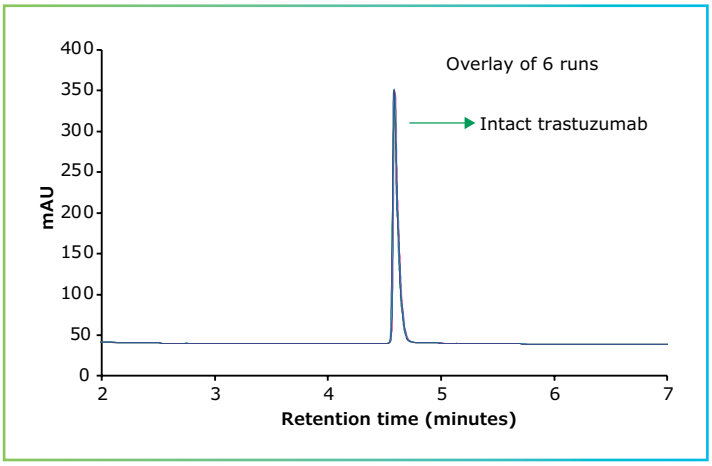

FIGURE 1: RP-HPLC analysis of trastuzumab on a BIOshell™ A400 Protein C4, 100x2,1 mm; 3,4 µm HPLC column.

LINEARITY, LIMIT OF QUANTITATION (LOQ) AND LIMIT OF DETECTION (LOD)

The calibration curve was constructed with nine standard concentrations of trastuzumab from 1 to 25 µg/ml. The mAb concentration that provided a signal-to-noise ratio (S/N) >3 was considered as LOD and S/N >10 was considered as LOQ.

FORCED DEGRADATION STUDIES

We compared the chromatographic profiles of native and heat-stressed trastuzumab for monitoring degraded products. For the forced degradation studies, 1 mg/ml of trastuzumab was exposed to 10 ppm hydrogen peroxide (H_2O_2) followed by heating at 80 °C for 60 minutes. An aliquot of 10 µl was used for RP HPLC analysis.

RESULTS AND DISCUSSION

INTACT TRASTUZUMAB ANALYSIS

For the HPLC analysis, a BIOshell™ A400 Protein C4, 3,4 µm HPLC Column with core-shell particles and 400 Å pore size delivered reproducible, fast and high resolution separation of intact trastuzumab, making it suitable for biopharma development and QC applications. Figure 1 demonstrates excellent peak shape and overlays of six replicates in less than 5 minutes under the chromatographic conditions.

PRECISION OF RETENTION TIME AND AREA

Table 2 shows the average Retention Time (RT) and Area RSDs from six replicates of trastuzumab injections. The Retention Time and Peak Area RSDs were less than 0.1% and 0.29%, respectively, which demonstrates excellent reproducibility of the method and thus its precision.

	Mean	RSD (%)
Retention time (min)	4.58	0.1
Peak area	987268	0.29

TABLE 2: Retention time and peak area precision (n = 6) for trastuzumab (1 mg/ml).

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

The LOD and LOQ were 0.125 µg/ml and 0.25 µg/ml, respectively, for trastuzumab, indicating that the method was sensitive. Observed LOD and LOQ values of trastuzumab are reported in Table 3. Representative chromatograms on same scale for two calibration runs and blank are shown overlayed in Figure 2.

	Concentration (µg/ml)	Mean area (n=3)	Retention time (min)
LOD	0.125	9562	4.58
LOQ	0.25	21977	0.29

TABLE 3: LOD, LOQ and mean area and retention time (n = 3).

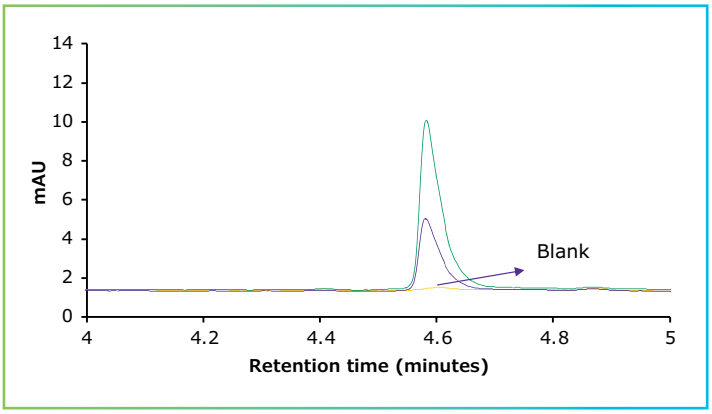

FIGURE 2: Overlay of representative chromatograms on same scale for two calibration runs and blank.

LINEARITY

Linearity curves for trastuzumab were constructed from 1 µg/ml up to 25 µg/ml in this study using area response and concentration of trastuzumab. The average peak areas are listed in Table 4. The linearity curve for trastuzumab is shown in Figure 3.

Trastuzumab conc. (µg/ml)	Average area
1	95,961
2	194,821
4	394,886
6	593,986
8	791,940
10	984,370
15	1,480,051
20	1,940,216
25	2,447,554

TABLE 4: Summary of linearity range (n = 3) for trastuzumab.

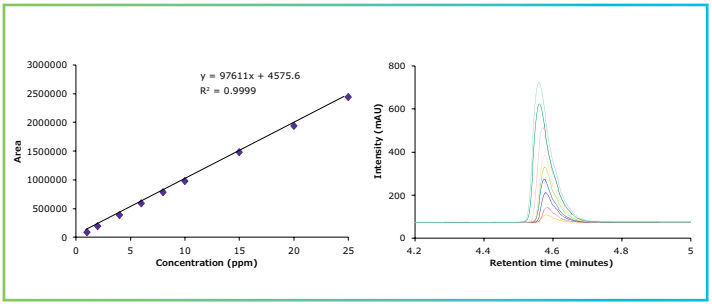

FIGURE 3: Linearity curve with nine standard concentrations of trastuzumab ranging from 1 to 25 µg/ml showing excellent coefficient values. Also shown are chromatogram overlays for the linearity ranges.

TRASTUZUMAB DEGRADATION STUDIES

We compared the intact and stressed trastuzumab using RP-HPLC to evaluate if this method is stability indicating. Any deviations in peak RT or area as a result of stress were considered degradation products.

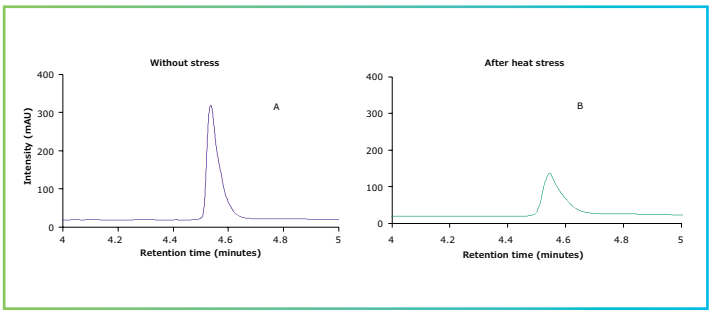

FIGURE 4: BIOshell™ A400 Protein C4, 100x2,1 mm; 3,4 µm RP-HPLC profiles of unstressed (A) and heat stressed trastuzumab sample (B).

CONCLUSION

Analysis of intact mAbs provides a first level of interrogation of size, post translational modification and heterogeneity. RP-HPLC analysis of mAbs requires large pore sizes, a hydrophobic stationary phase and appropriate chromatographic methods. In this application note a simple LC-UV method for the analysis of intact trastuzumab was showcased. Using a BIOshell™ A400 Protein C4 column, a high resolution and rapid separation of intact trastuzumab was developed. Area and RT precision of the method were excellent and showed the reliability of the method. The calibration curves with nine standard concentrations of trastuzumab had excellent coefficient of linearity values displaying that the method was quantitative and accurate. The LOD and LOQ for trastuzumab were found to be 0.125 µg/ml and 0.25 µg/ml, respectively, indicating the method was sensitive. In addition, heat stressed studies demonstrated that the BIOshell™ A400 Protein C4 column was able to monitor degraded mAbs and the method could be used for stability studies.

FEATURED PRODUCTS

Description	Cat. No.
BIOshell™ A400 Protein C4, 100x2.1 mm I.D.; 3.4 µm	66825-U
LiChrosolv® acetonitrile isocratic grade	1.14291
Trifluoroacetic acid HPLC grade	1.08262
LiChrosolv® water for chromatography	1.15333

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Increasing Gradient Peak Capacity for the Analysis of Protein Digests and Other Complex Samples

Complex samples, such as protein digests, may potentially contain hundreds of sample components. Characterisation of these challenging samples therefore requires the application of chromatographic techniques that can deliver exceptionally high resolving power. The structural diversity of sample components typically means that gradient analysis by reversed-phase is employed as the method of choice. For complex samples, it is important to optimise the separation peak capacity, to enable as many analyte peaks as possible to be separated within the specified gradient time. This article briefly examines how assessing and optimising key parameters, including gradient time and flow rate, to optimise the peak capacity for the gradient separation of a complex sample. Additionally, an approach using multiple solid core columns coupled in tandem is demonstrated, an approach that can be used to further enhance peak capacity for the high resolution separation of complex samples.

INTRODUCTION

Peak capacity (PC) can be used to assess the separation efficiency provided by a gradient method. It is defined as the maximum theoretical number of components that can be separated, with a resolution of 1, within the specified gradient time. The peak capacity can be estimated by the following equation, where tG is the gradient time and w the average 4σ peak width (measured at 13.4% of the peak height)1:

$$P_C = \frac{t_G}{\overline{w}} + 1$$

For complex samples, it is desirable to maximise the peak capacity by optimising the analytical conditions, to achieve separation of the maximum number of sample components. For gradient analyses, the peak capacity is affected by the gradient time, along with parameters such as flow rate and temperature[1,2]. Peak capacity can also be raised by increasing the efficiency of the column i.e., by increasing column length and/or decreasing the particle size. It is therefore highly beneficial to assess the influence of these parameters when developing methods for samples that require very high separation efficiency. This article examines how investigation of gradient time and flow rate can be used to optimise peak capacity for peptide mapping experiments and demonstrates potential benefits that can be achieved through column coupling. The approaches discussed are also applicable to the analysis of other complex samples demanding high separation efficiency, such as natural products, complex biological mixtures and environmental samples.

ASSESSMENT OF GRADIENT TIME AND FLOW RATE

Generally, increasing the gradient time will result in higher peak capacity, although at longer gradient times the increase becomes less significant as the peak capacity reaches a maximum[2]. To demonstrate this, a bovine serum albumin (BSA) protein digest sample was run on an Avantor® ACE® UltraCore 2.5 SuperC18 150 x 2.1 mm column packed with high efficiency 2.5 µm solid-core particles using a 10-40% B gradient. Various gradient times ranging between 5 and 120 minutes were assessed at a flow rate of 0.21 ml/min. Solid-core particles were selected, as they can provide high column efficiency at a modest backpressure; they are therefore ideally suited to the column coupling approach discussed later in this article[3]. Peak capacities for each gradient time were calculated using the average peak width for 10 resolved peptides. Figure 1 demonstrates how the measured peak capacity increases with increasing gradient time,

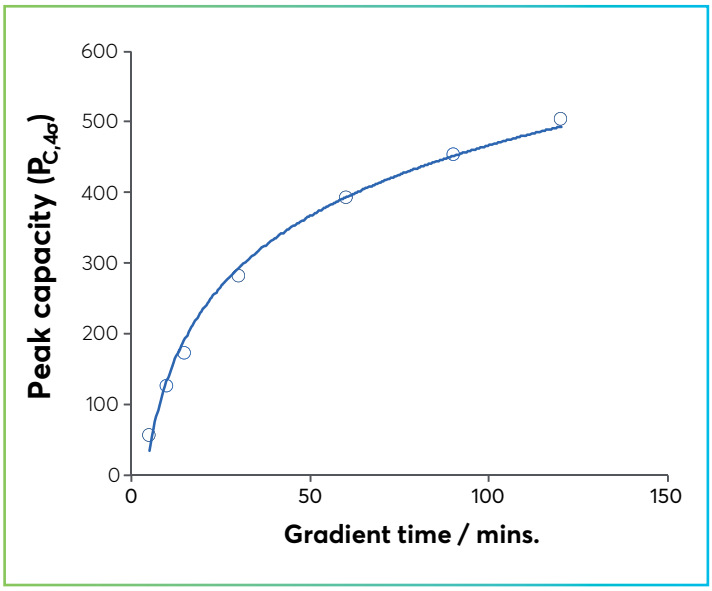

FIGURE 1: Effect of gradient time on measured peak capacity for the analysis of a BSA tryptic digest sample using a 10-40% B gradient at a flow rate of 0.21 mL/min on an Avantor® ACE® UltraCore 2.5 SuperC18, 150 x 2.1 mm column. Mobile phase: A = 0.05% TFA in H_2O, B = 0.05% TFA in MeCN; Temperature: 60 °C; Detection: UV, 214 nm; Injection volume: 20 µl.

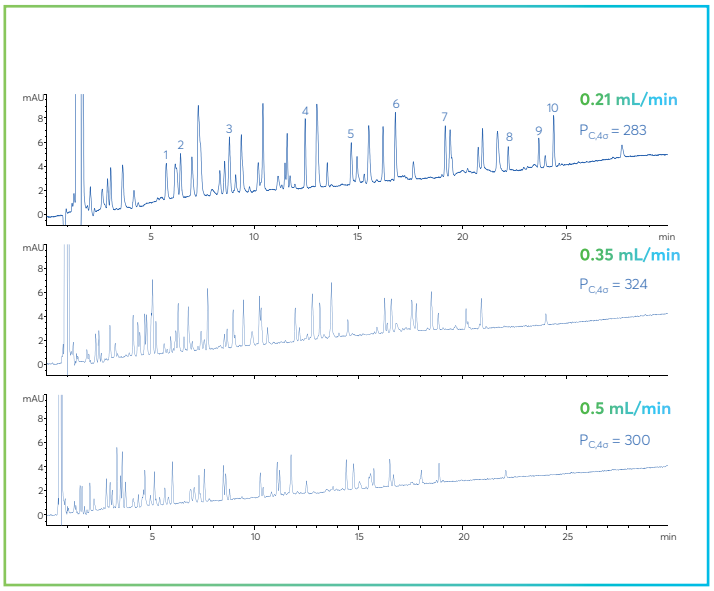

FIGURE 2: Comparison of peak capacity obtained for the BSA digest sample using a 30 minute gradient at three different flow rates on an Avantor® ACE® UltraCore 2.5 SuperC18, 150 x 2.1 mm column. Mobile phase: A = 0.05% TFA in H_2O, B = 0.05% TFA in MeCN; Gradient 10-40% B in 30 minutes; Flow rate: 0.35 ml/min; Temperature: 60 °C; Detection: UV, 214 nm; Injection volume: 20 µl. The 10 peptides used for peak capacity measurement are numbered.

although the gains become less significant at longer gradient time. There is therefore typically a trade-off between obtaining optimum peak capacity and the use of an acceptable gradient time, based on the specific requirements of the application.

To examine the effect of flow rate, a 30-minute gradient time was selected as a good compromise between performance and speed (doubling the gradient time to 60 minutes would only result in a 39% increase in peak capacity). The gradient separation was then assessed at 0.21, 0.35 and 0.50 ml/min (Figure 2). The maximum peak capacity value (324) was observed at a flow rate of 0.35 ml/min for this separation. The optimum flow rate is often application dependent and may be influenced by the mobile phase composition, column temperature and analyte physicochemical properties. These results demonstrate that by evaluating flow rate and gradient time for a new gradient separation, significant gains in peak capacity can be obtained. It is worth mentioning that the selectivity of the separation may change when using this approach of varying flow rate and gradient time independently. Therefore, a higher peak capacity may not always provide better resolution of a specific peak pair[4].

COUPLING MULTIPLE COLUMNS FOR FURTHER PEAK CAPACITY GAINS

Increasing column efficiency by decreasing particle size or increasing the column length is an additional approach that can be used to further increase peak capacity. Due to the maximum operating pressures of 400-600 bar offered by conventional HPLC instrumentation, both these options have practical limitations. However, the higher pressure capabilities of UHPLC instrumentation (up to 1,400 bar) opens up the possibility of coupling together multiple UHPLC columns, packed with highly efficient sub-2 µm or solid-core particles, to obtain ultra-high performance separations. This results in higher peak capacity values with subsequent higher average resolution and reveals low-level analyte details in complex samples that may not otherwise be attained. Columns packed with solid-core particles offer a particularly attractive option for column coupling, as they generate comparable efficiencies to sub-2 µm particles at lower back pressure3. Coupling together more than two columns packed with sub-2 µm fully porous particles is also possible and can be facilitated by using low viscosity mobile phases containing acetonitrile and working at higher column temperatures[5].

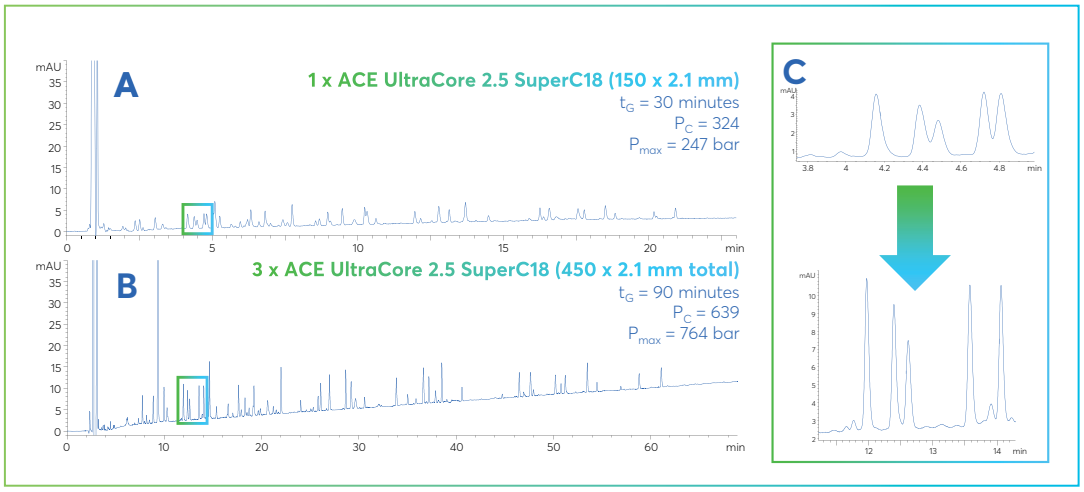

FIGURE 3: Gradient analysis of BSA protein digest sample on (A) one single Avantor® ACE® UltraCore 2.5 SuperC18 150 x 2.1 mm column packed with 2.5 µm solid-core particles and (B) three 150 x 2.1 mm columns connected in series. (C) Zoomed-in partial chromatogram comparing the regions highlighted in (A) and (B). Mobile phase: A = 0.05% TFA in H_2O, B = 0.05% TFA in MeCN; Gradient 10-40% B; Flow rate: 0.35 ml/min; Temperature: 60 °C; Detection: UV, 214 nm; Injection volume: 20 µl.

To use this approach, the method requires translation so that the gradient time is scaled with the column length. This ensures that the selectivity of the separation is replicated using the longer column format. This can be easily achieved using freely available translation tools[6] or fundamental gradient theory[7]. To demonstrate this approach, the optimum conditions for the BSA digest separation in Figure 2 (30-minute gradient and flow rate of 0.35 ml/min) were translated from the single 150 x 2.1 mm column to three Avantor® ACE® UltraCore columns coupled together. The three 150 mm columns were coupled, using low-dead-volume UHPLC connectors and 0.1 mm ID stainless steel capillaries, to give a total column length of 450 mm. Translation of the gradient method to the new column dimensions gives a gradient time of 90 minutes. The resulting separation is shown in Figure 3. By coupling three columns in series, the peak capacity was almost doubled from 324 to 639, with a maximum pressure of 764 bar; well within the limits of modern UHPLC instruments. The impact of this increase in performance can be readily appreciated by examining the zoomed-in region of Figure 3. Enhanced resolution of analyte peak pairs is apparent, along with enhanced sensitivity. A notable increase in the level of baseline detail, with the resolution of additional sample components, demonstrates the value of the column coupling approach for the analysis of complex samples.

CONCLUSIONS

Reversed-phase gradient separations are a powerful tool for the analysis of highly complex samples such as protein digests. This article has demonstrated how evaluation of gradient time and flow rate can help to optimise gradient separations and obtain higher peak capacities; to aid chromatographers in separating as many sample analytes as possible. The optimum analytical conditions are likely to be application dependent; therefore, it is advisable to assess these parameters during the development of new analytical methods. The availability of UHPLC instrumentation means that coupling multiple columns, packed with high efficiency sub-2 µm or solid-core particles to obtain even higher peak capacities, is now a feasible option for many laboratories. Although this approach may require long analysis times, the potential advantages that are realised can make this an attractive option for the characterisation of complex samples.

Article first published in Chromatography Today, Volume 12, Issue 4, 2019

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SkillPak™ BIO Pre-packed Columns - Simplify your MCC Process Development

SkillPak BIO pre-packed columns are part of Tosoh Bioscience's holistic solution for the development of multicolumn chromatography (MCC) processes. SkillPak BIO pre-packed columns are the perfect complement to the Octave™ BIO MCC system and TOYOPEARL® resins, offering a unique and comprehensive solution for developing MCC processes. Designed with dedicated dimensions and comprehensive TOYOPEARL resins for MCC applications, SkillPak BIO is designed to unlock the best MCC performances.

SkillPak BIO column design and packing procedures are based on Tosoh Bioscience's decades of expertise in packing chromatography media. We employ tightly controlled methods to pack the TOYOPEARL resins into the MCC-dedicated columns, which take into account the varying compressibility of each resin. This ensures that each column is consistently packed to the same level of performance, providing reproducible results for the batch process development work of biopharma experts. Available in kits that cover different MCC use cases, each kit contains a single resin lot with low column-to-column variability, ensuring optimal MCC performance. Additionally, customers benefit from short lead times thanks to Tosoh Bioscience's complete control of the media and hardware supply chain.

This overview summarizes the specifications, benefits, and features of SkillPak BIO pre-packed columns and how they can enhance the productivity and efficiency of your MCC process development work.

	SkillPak 1 BIO	SkillPak 5 BIO	SkillPak 10 BIO	SkillPak 25 BIO	SkillPak 100 BIO
Approx. volume	**1 ml**	**5 ml**	**10 ml**	**25 ml**	**100 mL**
Maximum flow rate in 1 mol/L NaCl (mL/min)	2	10	20	50	200
Storage temperature	see labels and Certificate of Analysis				
Frit porosity	10 µm				
Connections	10-32 Standard fitting			1/4-28 Standard fittings with flat- bottom geometry for 1/8" OD or 1/16" OD capillary	
Shipping buffer	20% ethanol for TOYOPEARL and TSKgel resins				
Asymmetry factor (As)	0.8 - 1.4 for TOYOPEARL and TSKgel resins				
Plate count	see resin-related specifications on the Certificate of Analysis				
Estimated shelf life	12 months				
Hardware pressure stability	10 bar				
Resin pressure stability	see resin documentation				

TABLE 1: Specifications of SkillPak BIO Columns.

SHORT LEAD TIMES

Tosoh Bioscience recognizes the importance of prompt delivery for process development products and has implemented a vertically integrated supply chain to ensure quick turnaround times for our SkillPak BIO pre-packed columns. With most screening/method development dimensions available off the shelf, customers can initiate their process development work in a matter of days. For SkillPak BIO pre-packed columns not available off the shelf, we have a lead time of 4-6 weeks, as we established a stock of resin at our production facility. By utilizing our vertically integrated supply chain, customers can focus on their process development without delay or concern over the availability and consistency of SkillPak BIO pre-packed columns.

Step	Dimensions	Volume	"Flow rate at 0.5 min residence time"
Screening / Method development	0.8 cm ID x2.0 cm	1 ml	2 ml/min
	1.6 cm ID x 2.5 cm	5 ml	10 ml/min
	1.6 cm ID x 5.1 cm	10 ml	20 ml/min
Technique adaption / Scale-up	2.5 cm ID x 5.1 cm	25 ml	50 ml/min
	5.0 cm ID x 5.1 cm	100 ml	200 ml/min

TABLE 2: Dedicated Dimensions for MCC Applications

Resin name	Application mode	Recommended to purifying
TOYOPEARL AF-rProtein A HC-650F	Capture with Protein A	Monoclonal antibodies
TOYOPEARL AF-rProtein L-650F	Capture with Protein L	Antibody fragments
TOYOPEARL NH2-750F	Polish with Anion exchange	Antibodies, antibody fragments, and other biomol-ecules
TOYOPEARL Sulfate-650F	Polish with Anion exchange	Antibodies, antibody fragments, and other biomol-ecules
TOYOPEARL GigaCap S-650S	Polish with (strong) Anion exchange	Viruses, vaccines, and other bio- molecules
TOYOPEARL SuperQ-650S	high throughput capture, intermedi- ate purification, and polishing	Therapeutic nucleic acids

TABLE 3: Dedicated Dimensions for MCC Applications

	Screening/ Method de- velopment (1, 5, 10 ml)	Technique adaptation / Scale-up (25, 100 ml)
AFC	6 columns for standard processes 10 columns for high- titer processes	1, 6, and 10 columns to adapt to any process setup
IEC	4 columns to cover all IEC MCC processes	1 and 4 columns to adapt to any process setup

TABLE 4: Kits with Single Resin Lot and Low Columnto- Column Variability for Optimal MCC Performance

Product description	Cat. No,
SkillPak 1 BIO AF-rProtein A HC-650F, x6	0045346
SkillPak 1 BIO AF-rProtein A HC-650F, x10	0045347
SkillPak 5 BIO AF-rProtein A HC-650F, x6	0045348
SkillPak 5 BIO AF-rProtein A HC-650F, x10	0045349
SkillPak 10 BIO AF-rProtein A HC-650F, x6	0045350
SkillPak 10 BIO AF-rProtein A HC-650F, x10	0045351
SkillPak 25 BIO AF-rProtein A HC-650F, x1	0045352
SkillPak 25 BIO AF-rProtein A HC-650F, x6	0045353
SkillPak 25 BIO AF-rProtein A HC-650F, x10	0045354
SkillPak 100 BIO AF-rProtein A HC-650F, x1	0045355
SkillPak 100 BIO AF-rProtein A HC-650F, x6	0045356
SkillPak 100 BIO AF-rProtein A HC-650F, x10	0045357
SkillPak 1 BIO AF-rProtein L-650F, x6	0045358
SkillPak 1 BIO AF-rProtein L-650F, x10	0045359
SkillPak 5 BIO AF-rProtein L-650F, x6	0045360
SkillPak 5 BIO AF-rProtein L-650F, x10	0045361
SkillPak 10 BIO AF-rProtein L-650F, x6	0045362
SkillPak 10 BIO AF-rProtein L-650F, x10	0045363
SkillPak 25 BIO AF-rProtein L-650F, x1	0045364
SkillPak 25 BIO AF-rProtein L-650F, x6	0045365
SkillPak 25 BIO AF-rProtein L-650F, x10	0045366
SkillPak 100 BIO AF-rProtein L-650F, x1	0045367
SkillPak 100 BIO AF-rProtein L-650F, x6	0045368
SkillPak 100 BIO AF-rProtein L-650F, x10	0045369
SkillPak 1 BIO Sulfate-650F, x4	0045370
SkillPak 5 BIO Sulfate-650F, x4	0045371
SkillPak 10 BIO Sulfate-650F, x4	0045372
SkillPak 25 BIO Sulfate-650F, x1	0045373
SkillPak 25 BIO Sulfate-650F, x4	0045374
SkillPak 100 BIO Sulfate-650F, x1	0045375
SkillPak 100 BIO Sulfate-650F, x4	0045376
SkillPak 1 BIO GigaCap S-650M, x4	0045377
SkillPak 5 BIO GigaCap S-650M , x4	0045378
SkillPak 10 BIO GigaCap S-650M, x4	0045379
SkillPak 25 BIO GigaCap S-650M, x1	0045380
SkillPak 25 BIO GigaCap S-650M, x4	0045381
SkillPak 100 BIO GigaCap S-650M, x1	0045382
SkillPak 100 BIO GigaCap S-650M, x4	0045383
SkillPak 1 BIO NH2-750F, x4	0045384
SkillPak 5 BIO NH2-750F, x4	0045385
SkillPak 10 BIO NH2-750F, x4	0045386
SkillPak 25 BIO NH2-750F, x1	0045387
SkillPak 25 BIO NH2-750F, x4	0045388
SkillPak 100 BIO NH2-750F, x1	0045389
SkillPak 100 BIO NH2-750F, x4	0045390
SkillPak 1 BIO SuperQ-650S, x4	0045391
SkillPak 5 BIO SuperQ-650S, x4	0045392
SkillPak 10 BIO SuperQ-650S, x4	0045393
SkillPak 25 BIO SuperQ-650S, x1	0045394
SkillPak 25 BIO SuperQ-650S, x4	0045395
SkillPak 100 BIO SuperQ-650S, x1	0045396
SkillPak 100 BIO SuperQ-650S, x4	0045397

mRNA sequencing workflow

QC MRNA HIGH THROUGHPUT AND HIGH ROBUSTNESS SEQUENCING

Large RNA including mRNA (messenger RNA) has emerged as an important new class of therapeutics. Recently, this has been demonstrated by two highly ecacious vaccines based on mRNA sequences encoding for a modified version of the SARS-CoV-2 spike protein. There is currently significant demand for the development of new and improved analytical methods for the characterisation of large RNA including mRNA therapeutics. This workflow is an automated, high throughput workflow for the rapid characterisation and direct sequence mapping of large RNA and mRNA therapeutics. Partial RNase digestions using RNase T1.

Characterisation and sequence mapping of large RNA and mRNA therapeutics using mass spectrometry

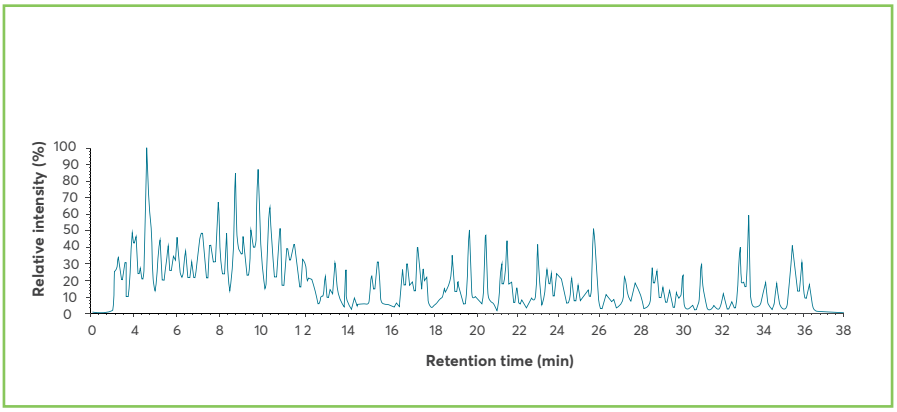


FIGURE 1: RNA sequence mapping of mRNA therapeutics and long RNA

DNAPac RP column

SMART Digest RNase kit

SureSTART vial and cap

Workflow solution

Descrption	Cat. No.
Thermo Scientific columns and guard columns	
Thermo Scientific™ DNAPac™ RP HPLC column	**555-7404**
Thermo Scientific™ DNAPac™ RP guard column	**555-7406**
Thermo Scientific™ Acclaim™ guard holder and coupler	**554-5295**
Thermo Scientific digest solutions	
Thermo Scientific™ SMART Digest™ RNase T1 mag bulk kit	**549-1071**
Thermo Scientific vials and caps	
Thermo Scientific™ SureSTART™ 2 mL polypropylene vial	**549-1072**
Thermo Scientific™ SureSTART™ 9 mm screw cap	**549-1073**

This workflow includes the newest recommended products

Seamless Scale-Up of an Antibody Platform From 1 to 200 ml Using SkillPak® Pre-Packed Columns

The demand for purified monoclonal antibodies (mAb) for therapeutic applications has increased drastically in the clinical pipeline over the last few years. To enable the implementation of such efficient therapies in a larger patient cohort, the biopharmaceutical industry needs to reduce the costs of such treatments. As a partner of the industry, Tosoh Bioscience is constantly working on new ways to improve the productivity of purification processes, reduce the development time, and make such development easier for biopharma professionals.

In this application note, we share how we developed a 2-step platform to purify a specific mAB, Pertuzumab, using SkillPak 1 and 5 ml pre-packed columns. The 2-step platform consists of a Protein A capturing and a single polishing step on a salt-tolerant anion exchanger (AEX). Subsequently, using the SkillPak family, we demonstrate how this new pre-packed family enables an easy and direct scale-up from 1 to 200 ml.
In a nutshell, SkillPak pre-packed columns allow scientists to develop and optimise chromatographic processes on a small scale with low sample and material consumption while enabling a robust and safe transfer to larger scales.

MATERIAL & METHODS

All experiments were done using Toyopearl® AF-rProtein A HC-650F and Toyopearl NH2-750F in SkillPak pre-packed columns with column volumes of 1 ml (7 mm ID x 2.5 cm L), 5 ml (8 mm ID x 10 cm L), 50 ml (25 mm ID x 10 cm L) and 200 ml (50 mm ID x 10 cm L) on an ÄKTA Avant 150 system.

DBC – Toyopearl AF-rProtein A HC-650F
DBC measurement was performed using a 1 ml SkillPak Toyopearl AF-rProtein A HC-650F column. After equilibration (5 CV) with 100 mM sodium phosphate buffer pH 7, the column was loaded with Pertuzumab Protein A eluate adjusted to 2 g/L mAb with a residence time of 4 minutes (39 cm/h). After 10% breakthrough of the maximum UV absorbance, DBC was calculated via sample volume.

Capture – Toyopearl AF-rProtein A HC-650F
Toyopearl AF-rProtein A HC-650F was equilibrated with 100 mM sodium phosphate pH 7.0 and loaded with 8 CV of clarified cell culture fluid with 4.4 mg/ml Pertuzumab. The first washing step was performed with 100 mM sodium phosphate pH 7.0 for 5 CV, and the second washing step was performed with 100 mM sodium acetate, pH 6.0 for 6 CV. Elution was carried out with 100 mM sodium acetate pH 3.0 (8 CV). The cleaning of the column (CIP) was performed with 200 mM NaOH + 500 mM NaCl for 5 CV. After cleaning, the column was re-equilibrated with 100 mM sodium phosphate pH 7.0. The flow rate in the equilibration, washing, CIP, and re-equilibration steps was 180 cm/h and 150 cm/h during the load.

Polishing – Toyopearl NH2-750F

To find the best process and separation conditions for the flow-through process, a 'bind and elute' purification with a 20 mM Tris-HCl pH 8; 8.5 and 9 was carried out on a 5 ml SkillPak column. The column was equilibrated for 3 CV before loading 55 mg of the Protein A eluate sample. Afterwards, a washing step with equilibration buffer for 5 CV was performed. The elution was performed with a linear gradient (20 CV) to 20 mM Tris-HCl pH 8; 8.5 and 9 + 1 M NaCl. Afterward, CIP was carried out with 500 mM NaOH. The flow rate during the entire process was 300 cm/h.

After an ideal pH value was determined, flow-through experiments were carried out at different conductivities to find the best separation conditions.

Scale-up of the 2-step process

The Protein A purification and flow-through polishing were transferred from 5 ml to 50 ml and 200 ml columns, keeping loading (mg/ml), linear flow rate (cm/h), and column volumes per step constant.

Analytical SEC

The feed and the flow-through of Toyopearl NH2-750F were analysed by size exclusion chromatography (SEC) using a TSKgel® UP-SW3000 to determine the content of monomers and aggregates of mAb.

RESULTS & DISCUSSION

Process development

The first experiments on AEX were performed in 'bind and elute' mode with a linear salt gradient at pH 8,0; 8.5 and 9.0 to determine the best pH value for the separation of Pertuzumab. Due to the lower net charge, the monomer elutes before the aggregates, which allows for the development of a flow-through process when setting the conductivity between the monomer and aggregate elution range.

The pH had an impact on the binding behaviour based on the isoelectric point (pI) of mAb. The best separation conditions of the mAb were found at pH 9 with two separated sample peaks (Figure 1).

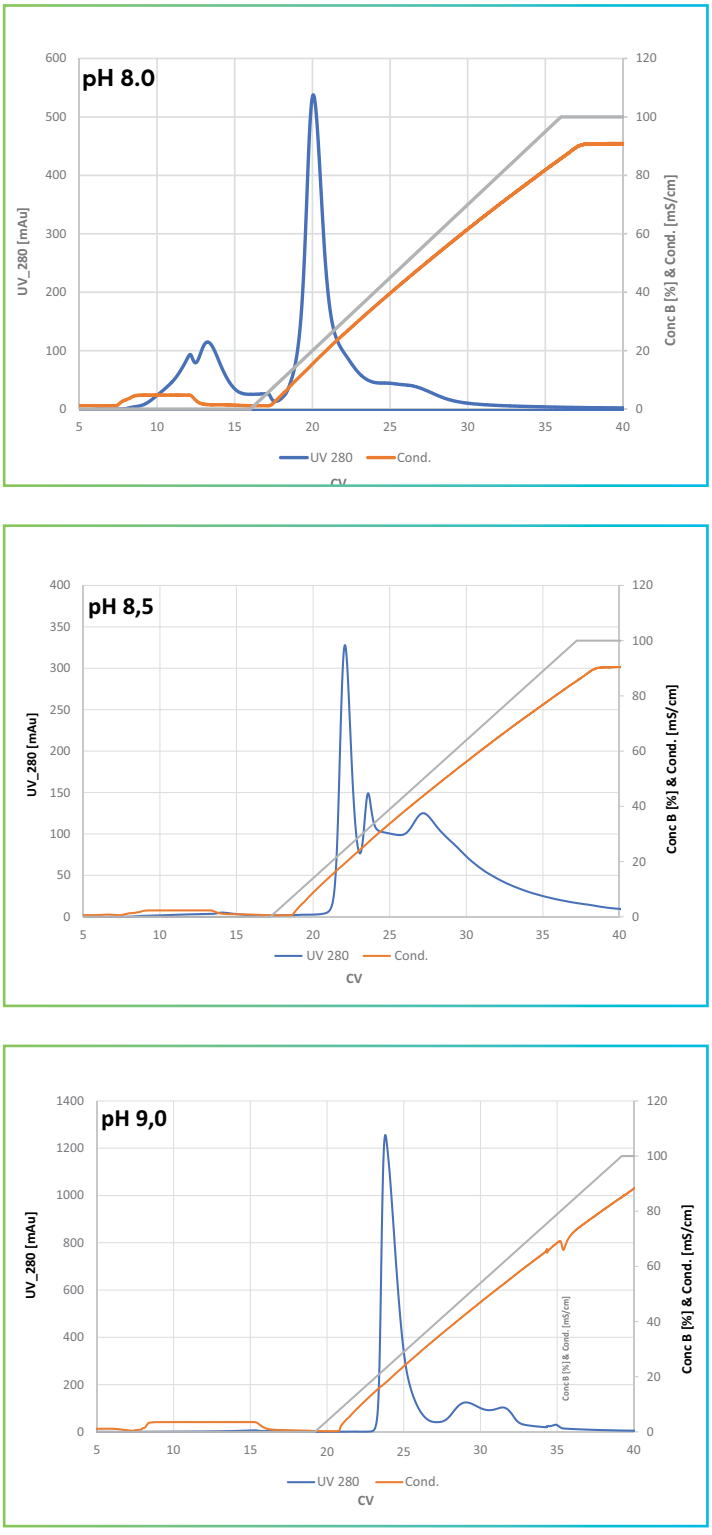


FIGURE 1: Bind/elute chromatograms of Toyopearl NH2-750F at different pH values.

From the experiment with AEX in 'bind and elute' mode at pH 9, we determined which conductivity range allows the elution of monomer from aggregate. Based on this range, we carried out flow-through tests at three different conductivities (17 mS/cm, 20 mS/cm and 25 mS/cm). The purities and yields at those conductivities are listed in Table 1.

Conductivity (mS/cm)	Aggregates (%)	Recovery (%)
17	0.08	87.76
20	0.27	94.17
25	1.47	98.41

TABLE 1: Aggregate content and recovery after polishing on Toyopearl NH2-750F at different conductivities during flow-through process development.

A 17 mS/cm conductivity was chosen for the platform design to achieve the desired purity without a subsequent chromatography step. The corresponding chromatogram is shown in Figure 2.

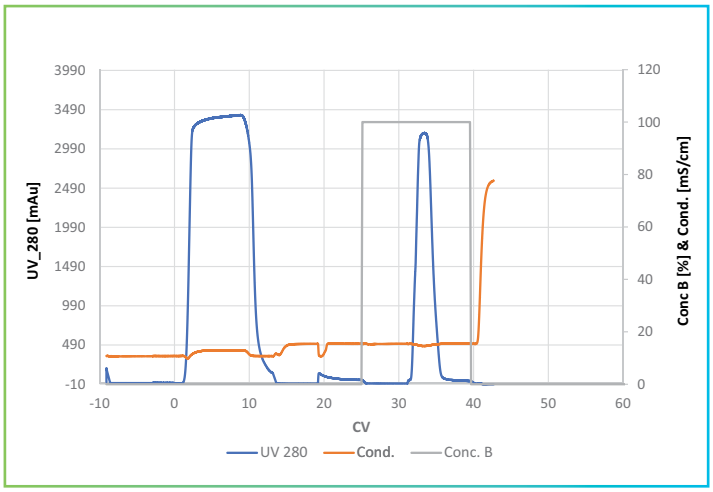


FIGURE 2: Flow-through chromatogram of the Protein A eluate on Toyopearl NH2-750F at pH 9 and 17 mS/cm.

Scale-up

The process generated on the 5 ml scale was then transferred to SkillPak columns with volumes of 50 ml (2,5 cm ID x 10 cm L) and 200 ml (5,0 cm ID x 10 cm L) packed with Toyopearl AF-rProtein A HC-650F and Toyopearl NH2-750F in.

The results are summarised in Table 2.

CV (ml)	Loaded mAb (mg)	Recovery Pro A (%)	Recovery NH2 (FT) (%)	Overall recovery (%)	Monomer Purity (%)
5	176	98.14	87.76	86.13	99.92
50	1932	98.58	94.97	93.62	99.69
200	7728	97.58	92.63	90.38	99.75

TABLE 2: Summary of 2-step scale up from 5 to 50 to 200 ml using Toyopearl AF-rProtein A HC-650F and Toyopearl NH2-750F in SkillPak pre-packed column.

Table 2 shows the individual recoveries for TOYOPEARL AF-rProtein A-650F- and NH2-750F - step as well as the combined overall recovery of the process in the different scales. The last column states the monomer purity at the end of the process. Overall recoveries of around 90% and a high monomer purity of up to 99,9% could be achieved. When comparing the performance at different scales, some deviations become apparent, especially regarding recovery of the flow-through AEX step. A likely explanation for these deviations is that the fractionation volume was not consistent with regards to column volume. This means that depending on where the last fraction ended, some more product with lower purity went into the product pool, as the later fractions in the flow-through AEX step contain increasing amounts of high molecular weight impurities. For another run, where the cut-off might have been earlier due to how the different factions spaced out, there was more product lost, which ultimately lead to a higher overall purity. This theory is confirmed by the observation that a higher purity coincides with a lower recovery and vice versa. Following this explanation, it can confidently be stated that the desired purity and recovery could be dialled in on by selecting the appropriate point for the flow-through cut-off.

These results are sufficient as a scale-up experiment. The next step before moving into pilot production would be the optimisation of fraction collection to ensure optimal recoveries and purities while maintaining maximum efficiency and avoiding implementing a third chromatography step.

CONCLUSION

In this application note, we highlighted the efficient development of a 2-step antibody purification platform with TOYOPEARL AF-rProtein A HC-650F and TOYOPEARL NH2-750F using 1 and 5 ml SkillPak pre-packed columns. Development at these small scales allows for low resin, feedstock and buffer consumption. Using 50 and 200 ml SkillPak columns, this process was effortlessly scaled up without any adjustments to the process parameters determined in the previous small-scale experiments. This seamless scale-up using SkillPak pre-packed columns allows scientists to move from process development to pilot scale quickly, safely and efficiently.

The Hitachi LA8080 high speed amino acid analyser

Useful in the monitoring of amino acids in cell culture fluids, either during research and development, or production of antibodies

Many biomedicines are protein-based drugs manufactured by cell culture. In the cell culture process to produce proteins, it is extremely important to monitor and control various process parameters such as dissolved oxygen, pH and temperature.

In addition, amino acids in culture fluid are important nutrient sources and thus monitoring the amino acids is useful for the understanding of cell conditions, the study of culture conditions and the optimisation of culture fluid compositions.

In this publication, the concentrations of various amino acids in a culture fluid under different culture conditions were monitored by using the Hitachi LA8080 amino acid analyser and the results are introduced here. The amino acid analyser, which allows simultaneous multi-component analysis with high resolution capability and excellent quantification accuracy, can provide process information useful for research and development of culture processes as well as quality control.

Amino acid analysis requires lab-scale cell culture / purification and culture production process

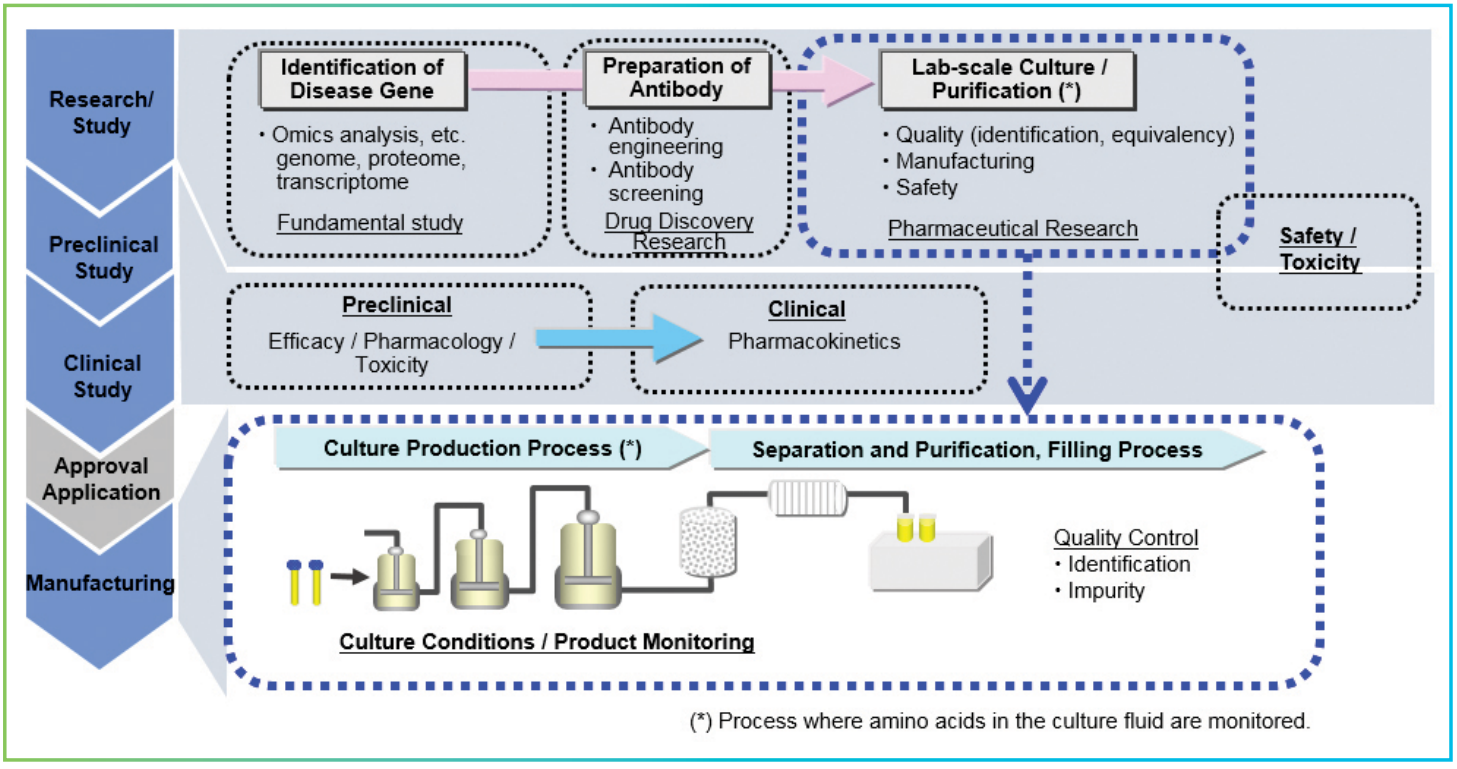


FIGURE 1: Work/time flow of biomedicine development.

Analysis example of amino acids in culture fluid

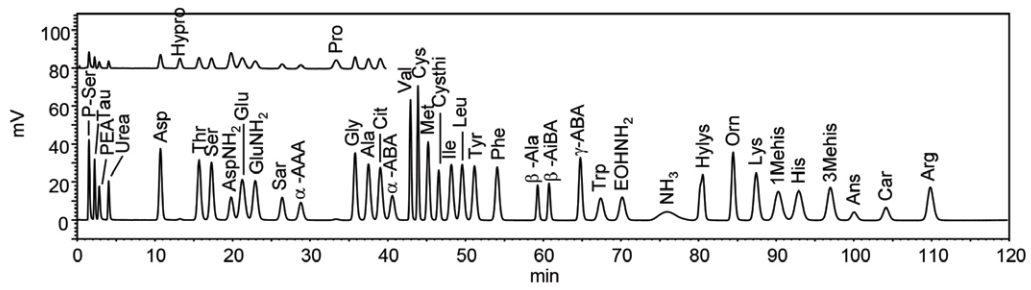


FIGURE 2: Analysis example of amino acid standards by biological fluid analysis method (2 nmol/20 µl). (However, some amino acids are at to 40 nmol/20 µl).

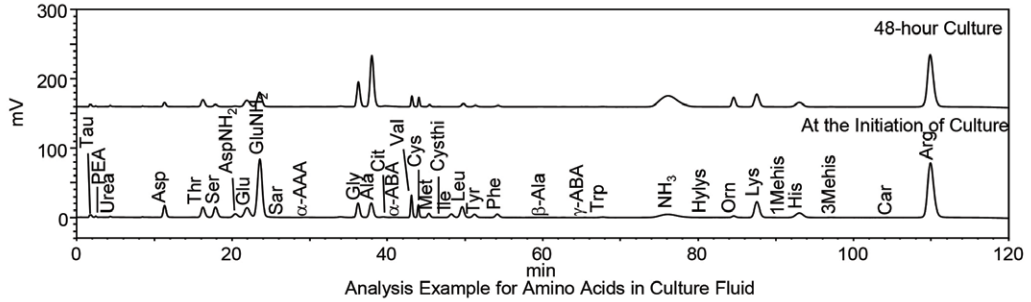
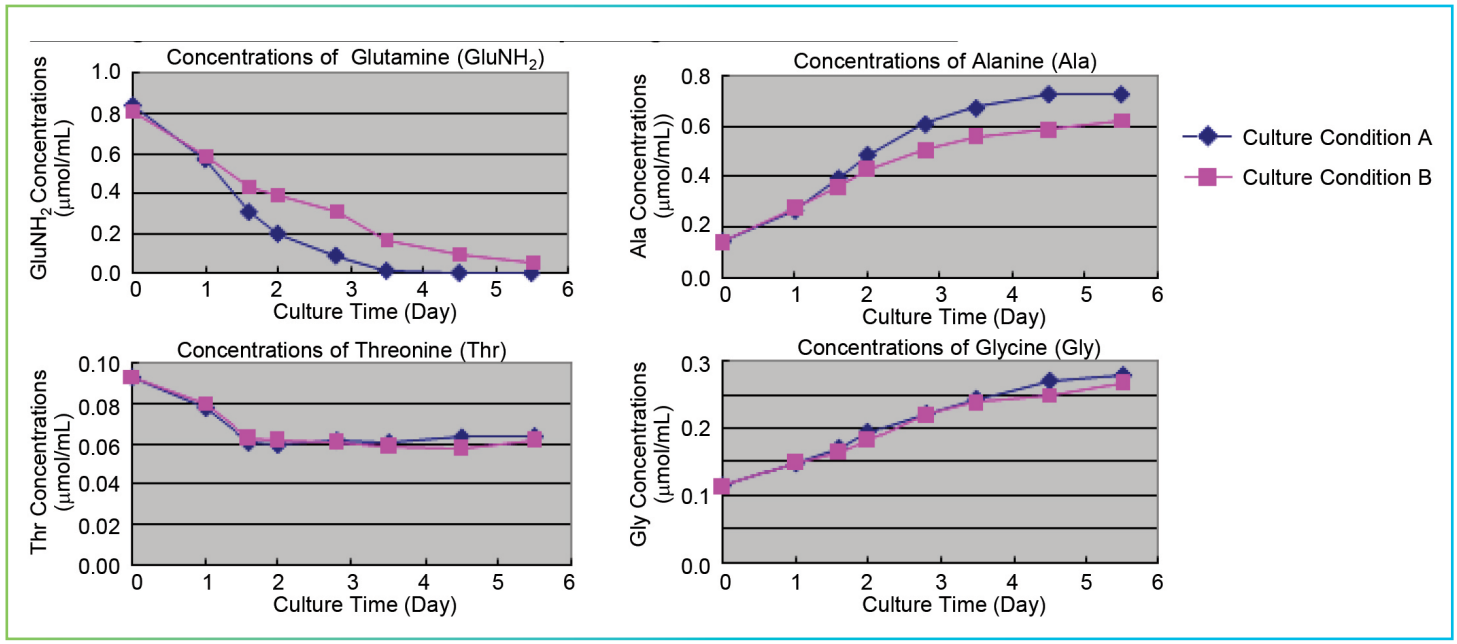


FIGURE 3: Analysis example for amino acids in culture fluid.

Change of amino acids over time depending on culture conditions



- In the analysis of the standards, among all the amino acids introduced here, the repeatability for the retention times of Thr, Gly, and Ala were RSD = 0.05 to 0.07% and those for the peak areas were RSD = 0.15 to 0.19% and thus, the high repeatability of the post-column ninhydrin method was confirmed (n = 3 for all). The product specifications are as follows: Retention time repeatability: NMT 0.5% (Ala), NMT 0.3% (Arg), area repeatability: NMT 1.5% (Gly, His).
- The results obtained by monitoring the concentrations of various amino acids in the culture fluid indicate the excess or deficiency of each component during the culture process, and thus the monitoring is useful in the optimisation study of culture fluid compositions.
- For some components, the amount varied depending on the culture conditions. It is thought that these variations reflect changes in intracellular processes, and it is possible that the protein produced may also change. Therefore, the simultaneous analysis of amino acids in the culture fluid is considered useful as an index for production control in antibody drug manufacturing.

ABOUT THE LA8080 HIGH SPEED AMINO ACID ANALYSER

As the ion exchange column developed by Hitachi is used to improve peak resolution, and owing to the precise construction of the instrument, results with good reproducibility can be obtained. The LA8080 is designed for trouble-free operation based on an 'engineered for humans' concept, and can easily be installed on a standard lab desktop.

Sample and analysis conditions for amino acid monitoring

	Up to day 1 of culture	After day 1 of culture
Culture condition A	Dissolved oxygen concentration	Standard dissolved oxygen concentration
Culture condition B	Standard dissolved oxygen concentration	Low dissolved oxygen concentration

Hitachi LA8080 - high speed amino acid analyser.

LA8080 amino acid analyser by VWR Hitachi

The bench top VWR Hitachi LA8080 AAA, combining the robustness of VWR Hitachi LC products with post-column ninhydrin application, has a range of purposes - from measurements of foodstuffs to quality control for biopharmaceuticals

DOWNLOAD THE LA8080 BROCHURE

Notes

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CHROMATOGRAPHY
Issue 2 2023

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