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Excellence in Peptide and Oligonucleotide Purification: Advanced Process Solutions

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Abstract

The therapeutic success of peptides and oligonucleotides has sparked renewed interest, creating a demand for advanced downstream processing solutions. Among these, GLP-1 receptor agonists, known for their potential to treat diabetes and obesity, represent a significant breakthrough in peptide-based therapies.

In this white paper, we will explore established and robust technologies tailored to peptide production, with a special focus on GLP-1 receptor agonists. We share how these high-performing technologies—supported by advanced digital solutions and data analytics—can drive process optimization and operational excellence by enhancing downstream process efficiency, streamlining and accelerating development and scale-up, and enabling real-time troubleshooting. We present case studies demonstrating how these technologies can be assembled to optimize GLP-1 receptor agonist purification processes and stay ahead in this competitive field.

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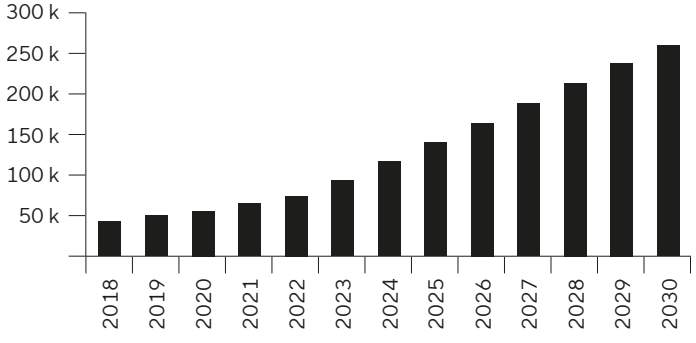
Introduction

Peptides and oligonucleotides are complex molecules with significant potential for treating a wide range of illnesses, including metabolic disorders, various cancers, and genetic conditions. Consequently, interest in their therapeutic applications is growing.

However, therapeutic peptides have historically faced limitations. Rapid degradation by enzymes and swift elimination by the kidneys results in short durations of activity within the body (i.e., short in vivo half-life), which ultimately hampers their clinical effectiveness.[1] Fortunately, advancements in peptide synthesis, synthetic chemistry, and oligonucleotide manufacturing have revolutionized the production and design of these intricate compounds, unlocking their vast clinical potential.

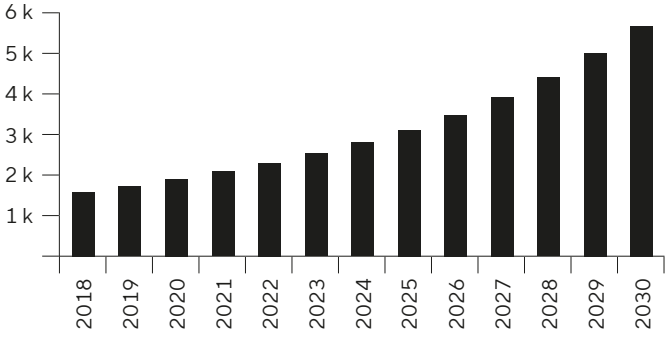
This revolution has spurred the development and approval of numerous peptide-based drugs. Globally, over 110 peptides have received regulatory approval for therapeutic use to date, contributing to an estimated market share of USD 93.97 billion in 2023. This market is predicted to experience further growth, reaching an estimated value of USD 260 billion by 2030, with a compound annual growth rate (CAGR) of 15.7% worldwide (Figure 1), out of which metabolic disorders accounted for a revenue of USD 55.87 billion in 2023.2 Globally, over 20 oligonucleotides were approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) by March 2024.[2]

Figure 1: *Global Peptides Market (2018-2030)*



This revolution has spurred the development and approval of numerous peptide-based drugs. Globally, over 110 peptides have received regulatory approval for therapeutic use to date, contributing to an estimated market share of USD 93.97 billion in 2023. This market is predicted to experience further growth, reaching an estimated value of USD 260 billion by 2030, with a compound annual growth rate (CAGR) of 15.7% worldwide (Figure 1), out of which metabolic disorders accounted for a revenue of USD 55.87 billion in 2023.[2] Globally, over 20 oligonucleotides were approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) by March 2024 contributing to an estimated market share of USD 5.8 billion in 2024 and is predicted to reach an estimated value of USD 34 billion by 2030 at an annual growth rate of ~28% worldwide.[3] Market research indicates that the global peptide and oligonucleotide CDMO market reached a value of USD 2.53 billion in 2023 and is projected to expand at a CAGR of 12.5% from 2024 to 2030 (Figure 2).[4]

Figure 2: *Global Peptide & Oligonucleotide CDMO Market (2018–2030)*



The remarkable expansion of the peptide and oligonucleotide market has fueled the growing demand for specialized contract development and manufacturing organizations (CDMOs) with expertise in this field. These CDMOs act as crucial partners for pharmaceutical and biotech companies, providing the specialized skills and infrastructure needed to navigate the complexities of developing and manufacturing these next-generation therapeutics. CDMOs will play in facilitating the development and production of these therapeutics. As the field of peptide and oligonucleotide therapeutics continues to evolve, CDMOs with expertise in this domain will be well-positioned to capitalize on this exciting market opportunity, ultimately accelerating the delivery of these life-saving treatments to patients in need.

Oligonucleotides

Oligonucleotides are short, synthetic nucleic acid polymers, typically 20 nucleotides long. They can be single- or double-stranded and are designed to modulate gene expression by targeting specific RNA sequences.[5] Oligonucleotides hold the potential to revolutionize the treatment of diseases traditionally resistant to conventional pharmaceutical approaches. By targeting undruggable disease-causing genes and patient-specific genetic sequences, these innovative molecules offer hope for patients with rare diseases. Unlike traditional drugs, oligonucleotides can directly modulate gene expression, providing a targeted and precise therapeutic approach. This capability opens new avenues for treating diseases that were previously considered intractable.
For example, oligonucleotides can be designed to silence specific genes that contribute to disease development. By inhibiting the production of disease-causing proteins, these molecules can alleviate symptoms and improve patient outcomes. Additionally, oligonucleotides can be tailored to address the unique genetic variations associated with rare diseases, offering personalized therapies that are more effective and less likely to cause adverse side effects.
Since the FDA's approval of the first oligonucleotide drug in 1998, the oligonucleotide therapeutics landscape has significantly expanded. Currently, a robust pipeline of candidates is in advanced clinical development stages.[6] The commercial success of drugs like Nusinersen | Spinraza has solidified the clinical potential and market viability of oligonucleotide therapies.[7]

Peptides

Therapeutic peptides and polypeptides are molecules typically composed of fewer than 100 amino acids and are the fundamental building blocks of proteins.[6] They represent a unique class of pharmaceutical compounds, molecularly poised between small molecules and proteins, yet biochemically and therapeutically distinct from both.[8] They have emerged as a pivotal class of biopharmaceuticals, offering diverse therapeutic applications due to their ability to target specific biological pathways with high selectivity and potency. The production of peptides can be challenging due to their complex structures and susceptibility to degradation. However, advancements in peptide manufacturing sciences, such as solid-phase peptide synthesis (SPPS) and recombinant DNA technology, have helped enable the efficient and scalable production of these valuable biomolecules. Efficiency and high yields are vital for the optimal economy of any industrial manufacturing process.

Methods for purifying peptides and peptide-like molecules usually use various principles of chromatography, such as ion-exchange chromatography, medium- or high-pressure reversed-phase chromatography, and tangential flow filtration (TFF).[9,10]

Manufacturing Technology

The production of peptides can be achieved through a variety of methods, each with its own advantages and limitations. One common approach is microbial recombinant technology, where bacteria such as *E. Coli* or other microorganisms are genetically engineered to produce the desired peptide. This method is particularly well-suited for larger peptides, such as those with multiple amino acid chains, often referred to as polypeptides. Notable examples include insulin, a 51-amino acid hormone, and teduglutide, a 33-amino acid therapeutic agent. Another method for peptide synthesis is synthetic chemistry, which involves chemically linking amino acids together one by one. This approach is more versatile and can be used to produce a wide range of peptides, including those that are difficult to produce using recombinant technology. Synthetic chemistry is particularly well-suited for smaller peptides, such as octreotide, leuprolide, and vasopressin, which have fewer than 10 amino acids.

In recent years, there has been a growing trend towards using hybrid approaches, which combine elements of both recombinant and synthetic methods. For example, one peptide fragment may be produced recombinantly while another is chemically synthesized. The two fragments can then be joined together using synthetic chemistry to form the final peptide. This approach can be helpful in producing complex peptides that are difficult to synthesize using a single method. While microbial recombinant technology and synthetic chemistry are the two primary methods for peptide production, synthetic chemistry has become increasingly dominant in recent years. This is partly due to advances in synthetic chemistry techniques, which have made it possible to produce peptides more efficiently and at lower cost.[11]

SPPS has traditionally been preferred over liquid-phase peptide synthesis (LPPS) because excess reagents and reaction byproducts in solution are easily washed from the solid resin. LPPS can produce a material with higher purity and lower reagent use; however, isolating the peptide after each step by precipitation is burdensome.

Regardless of the peptide synthesis route, the presence or formation of impurities in the crude product is inevitable, requiring different purification strategies for their removal. Unit operations such as chromatography and membrane filtration can be implemented to ensure higher purity. The impurities could be product- or process-related and are structurally related to the main chain.[12, 13]

Membrane filtration is an efficient method to either improve peptides assay purity or concentrate the eluates coming from the chromatography step. For any membrane-based synthetic strategy, the membrane must be compatible with the organic solvents used in peptide synthesis, i.e., acetone (ACN), dimethylformamide (DMF) or trifluoroacetic acid (TFA).[10] Regenerated cellulose membranes have increased compatibility with organic solvents and are often used in purifying peptides. Some peptides are prone to hydrogel formation or fibrillization. Gelation can foul the membrane or the stationary phase in the chromatography column, and irreversible gelation or fibrillization can make the peptide unsuitable for downstream processing. Therefore, peptides should be carefully screened during process development to understand if they are at risk for gelation during the conditions encountered in TFF.[14, 15]

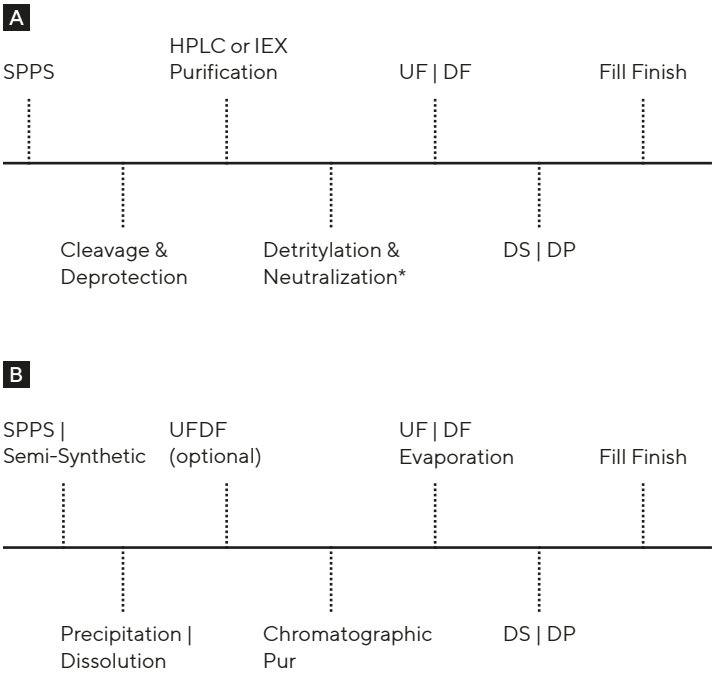
Table 1: *US FDA-Approved GLP-1 Drugs on the Market*

Generic Name	Manufacturer	Brand Name	FDA Approved Year, Use
Semaglutide injection	Novo Nordisk	Ozempic®	2017, Type 2 diabetes
Semaglutide injection	Novo Nordisk	Wegovy®	2021, Weight loss
Semaglutide tablets & oral	Novo Nordisk	Rybelsus®	2019, Type 2 diabetes
Liraglutide	Novo Nordisk	Victoza®	2010, Type 2 diabetes
Liraglutide	Novo Nordisk	Saxenda®	2014, Weight loss
Tirzepatide	Eli Lily	Mounjaro®	2022, Type 2 diabetes
Tirzepatide	Eli Lily	Zepbound®	2023, Weight loss
Dulaglutide SC	Eli Lily	Trulicity®	2014, Type 2 diabetes
Exenatide	Astra Zeneca	Byetta®	2005, Type 2 diabetes
Exenatide extended release	Astra Zeneca	Bydureon®	2017, Type 2 diabetes

Recent market analysis shows that most of the top-selling non-insulin peptides belong to a category of drugs known as Glucagon-like peptide-1 (GLP-1) agonist molecules[11] (Table 1). GLP-1 molecules are relatively short gut peptide hormones (consisting of ~30 – 35 amino acids) produced in the L cells of the small intestine. Pharmaceutical companies are actively developing next-generation GLP-1 agonists with extended-release profiles or combination therapies, offering patients more convenient and potentially more effective treatment options. This continuous innovation keeps the market dynamic and fosters future growth.

The purification of peptide and oligonucleotide molecules can be challenging due to their size, hydrophilicity | hydrophobicity, and potential aggregation. Figure 3 depicts a generic process flow for manufacturing and purifying peptides and oligonucleotides. Typically, organic solvents are utilized for production and purification, and the materials used for purification should be compatible with these solvents. In this white paper, we delve into the strategies and solutions that can be implemented for peptide purification process e.g. chromatography, digital solution tools, filtration and TFF technology.

Figure 3: *Generic Purification Processes for* ***(A)*** *Oligonucleotides and* ***(B)*** *Peptides (e.g., a GLP-1 Agonist)*



* - Detritylation and neutralization can be interchangeable

Note. HPLC = high-performance liquid chromatography, IEX = ion-exchange chromatography, UF | DF = ultrafiltration

Introduction to Chromatography

Small molecules synthesized by organic chemistry can often be purified by crystallization, but peptides and oligonucleotides will always require one or several chromatography steps to achieve high purity requirements. Depending on the molecular structure and the mode of production — SPPS or the recombinant route — purification processes could be based on low-pressure liquid chromatography (LPLC), high-performance liquid chromatography (HPLC), or a combination of both.

Table 2 illustrates the differences between LPLC and HPLC in polymer or silica-based processes. The main differences are the much smaller size of the particle beads (10 – 20 µm) and the use of solvents in the mobile phase in HPLC. Solvents introduce additional interactions with impurities and target products, enhancing selectivity and improving resolution. Resolution is also improved by the small beads, as a better mass transfer will lead to the elution of narrower peaks. As a trade-off, higher pressure is created when the mobile phase flows through the media: HPLC chromatography systems will typically be rated at 70 or 100 bar vs. ~5 bar for LPLC.

Table 2: *Differences Between Low-Pressure and High-Performance Liquid Chromatography*

Parameters	LPLC	HPLC
Operating pressure	< 5 bar	~40 to 100 bar
Mean particle size	> 45 µm	10 – 20 µm
Chromatography modes (not exhaustive)	Affinity (Protein A), IEX, HIC, SEC	▪ Adsorption normal phase ▪ Adsorption reversed phase ▪ Chiral chromatography ▪ IEX and ion pairing (oligonucleotides)
Consumable type	Resins, monoliths, membranes	Silica-based media, resins
Key words	▪ Feed loading ▪ Buffers ▪ In-line dilution ▪ Resins	▪ Feed injection ▪ Solvents ▪ Gradient ▪ Stationary phases
Mechanism	Affinity, Ionic interactions (strong), size exclusion, hydrophobic interactions	Ionic interactions (strong), hydrophobic interactions, polar interactions
Elution control	pH, salt content, solvent system	Solvent content in mobile phase (reverse mode) or polar solvent content in mobile phase (normal mode)

Note. IEX = ion-exchange chromatography, HIC = hydrophobic interaction chromatography, SEC= size-exclusion chromatography.

In the case of peptides and HPLC, the elution of product and impurities will be controlled mainly by the proportion of solvent in the mobile phase. Those molecules will be adsorbed through weak hydrophobic interactions with the stationary phase (reverse phase liquid chromatography; RPLC) and desorbed by the solvent, which also competitively interacts with the matrix. Regarding peptides, the matrix will be mostly amorphous silica bonded with organic ligands based on aliphatic chains like C18, C8, or C4. The other component in the mobile phase will be a buffer of the correct pH and conductivity to achieve the optimum ionic state of the peptide, which will also impact its retention. Polymers or resins can also be used as a stationary phase for peptides but mainly as an intermediate step to improve the crude quality before the final silica-based purification step(s). Polymers can be based on polystyrene | divinylbenzene (PS | DVB) or polymethacrylate, and their superior chemical resistance will support the processing of crudes of lower purity containing strongly adsorbed contaminants that will be desorbed by strong acid or alkaline conditions that would not be applicable on silica (for example, NaOH solutions).

Unlike peptides, for which the primary sequence and 3D arrangements determine their physico-chemical properties, oligonucleotides are always acidic due to the phosphate bonds present between all nucleotide monomers. Oligonucleotides are highly negatively charged, and the main mode of interaction involved in their retention on a chromatography matrix will be anion exchange. However, hydrophobic interactions with the matrix may also play a role in their separation (when direct interactions with the polymer or the ligands of bonded silica are involved). Table 3 describes three different chromatography processes, with the most common involving a single anion exchange step on a polymer-based matrix in either LPLC or HPLC mode, depending on the bead size (Process 1).

Table 3: *Chromatography Processes for Oligonucleotide Purification*

Process 1	Process 2	Process 3
AEX - LPLC or HPLC	RP - HPLC	HIC + AEX - LPLC
Solvent or aqueous	Solvent	100% aqueous
▪ HPLC: Cationic Polystyrene | DVB with buffers and solvent in mobile phase (RNAs) ▪ LPLC: Agarose or polymethacrylate (DNAs)	▪ HPLC used for polishing at high-pressure ▪ Organic solvents, notably acetonitrile, are often used as elution buffer ▪ Hydrophobic salts added in the mobile phase for ion-pairing chromatography	▪ Two-step, solvent-free process implemented by Biogen* ▪ HIC and a high salt buffer to decrease the solvation of the sample and achieve retention ▪ Salt gradient is used for elution to increase hydrophobicity. Elution can also be assisted by the addition of modifiers or detergents
UF | DF	Precipitation	UF | DF
Freeze drying	Freeze drying	Liquid drug substance

Note. AEX = anion-exchange chromatography, HIC = hydrophobic interaction chromatography, HPLC = high-performance liquid chromatography, LPLC = low-pressure liquid chromatography, RP = reverse phase, SEC= size-exclusion chromatography, UF | DF = ultrafiltration | diafiltration.

*Biogen, "Hydrophobic Interaction Chromatography for Purification of Oligonucleotides," US patent 20190248823A1, 2019

Process 2 also involves anion interactions but with ion–exchange ligands created in situ by adding ion pairing agents in the mobile phase that will stay adsorbed on the matrix and interact with product and impurities. The drawback of this method is the need to remove the hydrophobic salts from the purified product. A third, less common process involves a purification platform with two LPLC steps: Hydrophobic interaction chromatography (HIC) followed by anion-exchange chromatography (AEX)*. Oligonucleotides are isolated in their protected form after synthesis, with a trityl group linked to the last nucleotide monomer added to their sequence. This trityl group is highly hydrophobic; its deprotection is performed after chromatography in the case of reverse-phase HPLC or HIC, as the trityl group participates in retention by interacting with the hydrophobic matrix.

Oligonucleotides are also highly negatively charged molecules and chromatography systems must, therefore, be able to withstand the high salt concentrations required in the buffer solutions used for desorption in an AEX step and adsorption in a HIC step. Furthermore, oligonucleotides may require denaturing to ensure their retention on chromatography matrices. Increasing process temperatures will help modify their secondary or tertiary molecular structures, as they contribute to increasing the number of chemical functions available for the interactions involved in their separation. Equipment should allow operation at temperatures as high as 60 °C and enable cooling down of the collection fractions to recover the oligomers in their initial active form.

Combining high salt concentration and high temperature may lead to pitting and corrosion of the stainless-steel process parts on the system and, in the worst case, loss of system integrity. A sanitary-grade design is preferred where possible and is achieved by minimizing dead legs, selecting components recognized for their sanitary purpose (notably diaphragm valves and metric pumps), and applying strict surface finishing controls for all stainless-steel wetted parts.

Chromatography for Peptides

Most peptide manufacturing processes consist of a series of steps that generate desired products and byproducts that cannot be separated easily. Some byproducts are closely related to the desired product and cannot be separated by standard methods, e.g., crystallization or extraction processes. Preparative chromatography, especially HPLC, can remove these challenging byproducts. Such techniques help separate impurities from peptides, e.g., during GLP-1 RA purification.

Figure 4: *Primary Structure of GLP-1 and GLP-1 Receptor Agonists*

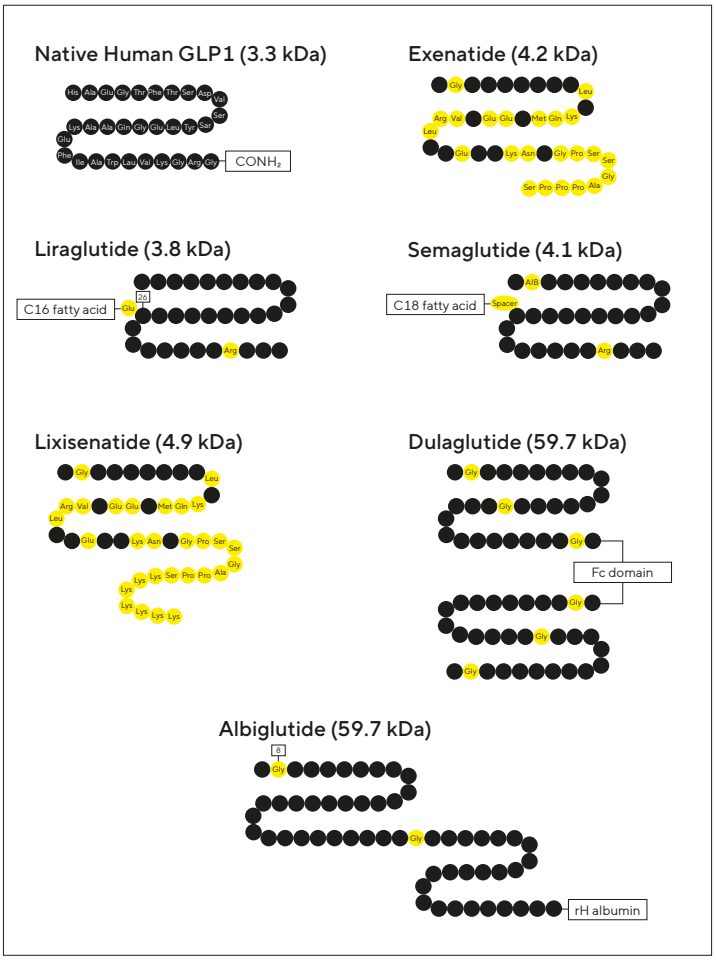


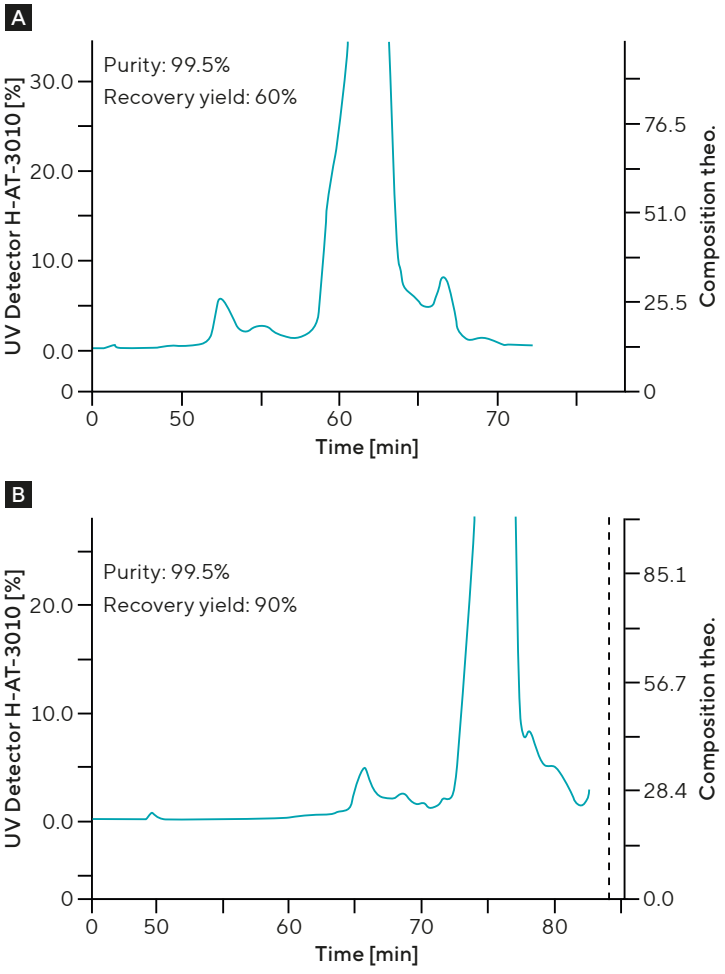
Figure 4 shows the polymeric sequence of the endogenous GLP-1 polypeptide and GLP-1 receptor agonists. The native GLP-1 has a very short half-life (~2–3 min), and pharmaceutical companies have developed products of similar structure to mimic GLP-1 biological activity but with structural modifications for better resistance to proteases and longer stability in the bloodstream. Those modifications may translate into a higher hydrophobic character for some of them, with liraglutide and semaglutide being linked to a fatty acid chain for better stability to proteases in plasma or lixisenatide and exenatide being fitted with some sequences of aliphatic amino acids like proline, alanine or glycine. In reverse-phase mode, this will lead to stronger retention and, as a consequence, the need for a higher solvent content to desorb them. As a reference, the polypeptide hormone insulin of 51 amino acids requires ~25 % of acetonitrile in the mobile phase for its elution on C8 silica, while the smaller size liraglutide or semaglutide will require ~35% of that same solvent for their desorption on C18.

Another approach to improve GLP-1 agonist pharmacological properties is to link the active GLP-1 sequence to a protein fragment, such as Fc (in dulaglutide) or albumin (in albiglutide). Both are produced via the recombinant route and consist of two GLP-1 units linked to the protein component. Their molecular size will be dramatically larger (15–20x times) than the smaller agonists, impacting their purification process; larger cut-off membranes will be required for TFF, and resins of larger pore size will be necessary for the chromatography steps.

Both recombinant and SPPS production methods will create a significant amount of impurities with chemical structures close to the target. A particular class of impurities, often difficult to separate from the target, are created from undesired enrichment or depletion of amino acids in the primary structure. These impurities are analogous to shortmers and longmers in oligonucleotide production and may be eluted before or after the primary target peak, depending on the solvent or buffer used in the mobile phase. Due to the wide range of possible chemical functions on the monomer side chains, peptides will also be subject to many potential residue modifications, giving rise to significant impurity levels. HPLC is the technology of choice to achieve the most stringent standards of final purity (as high as 99.5% UV purity).

Another challenge for peptide purification is the strong sensitivity of their retention to the composition of the mobile phase in terms of solvent percentage. A small variation in solvent content can lead to a significant shift in elution volume, which may impact the separation yield or final target purity.

Figure 5: *Chromatograms for GLP-1 Receptor Agonist Separation With an* ***(A)*** *35–40% and* ***(B)*** *33%–38% Acetonitrile Gradient Over 80 Minutes*



Note. Internal Sartorius data.

Figure 5 shows two chromatograms for GLP-1 receptor agonist separation obtained from almost identical conditions. All process parameters are the same, and a very shallow gradient is applied: 5% solvent variation over 80 minutes. The only difference is the starting point of the gradient (35% acetonitrile in Figure 5A compared to 33% in Figure 5B). For the same final purity (> 99.5%), a significantly higher yield is achieved with greater retention due to the lower acetonitrile initial content. As little as 2% variation in the initial gradient composition leads to a significant difference in recovery yield. As such, controlling the mobile phase composition with 1% accuracy—the typical value for gradient deviations guaranteed from HPLC systems vendors—may not be sufficient to guarantee a good yield.

Systems capable of a higher accuracy than 1% will ensure the expected performance or may even improve yields at scale-up. Such performance depends on the design of the HPLC system (Figure 6) and other factors linked to the system environment in the facility, such as the pressurization on inlet lines, which should be stable and controlled at ~0.5 bar.

Figure 6: *Hipersep® Flow Drive and Prochrom Columns*

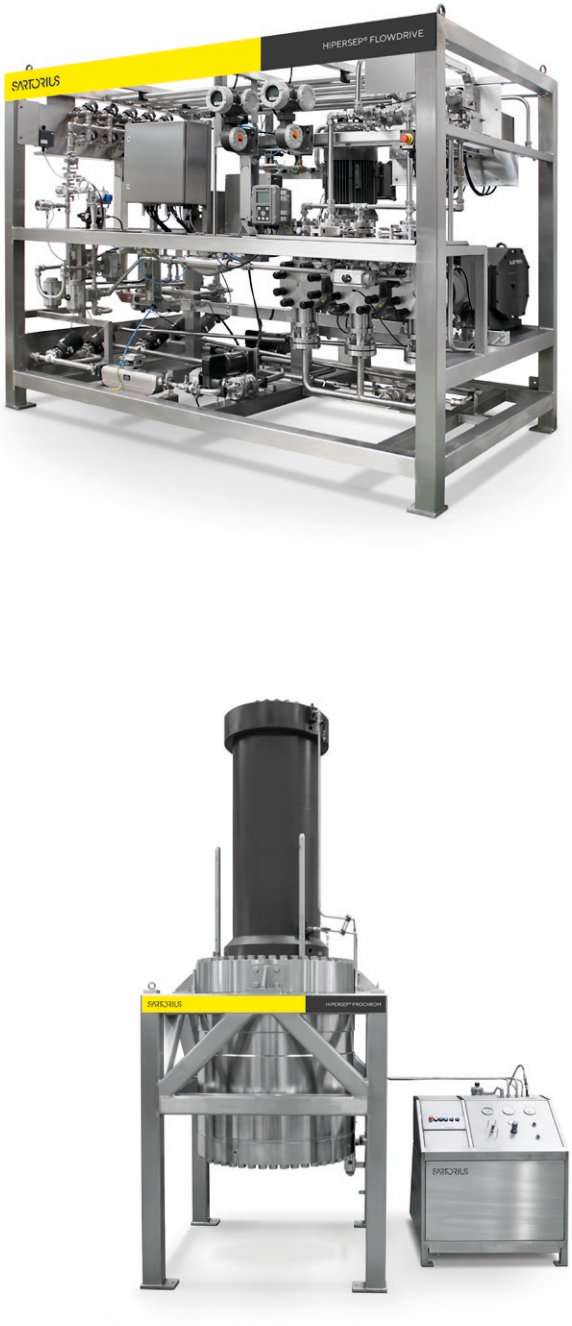
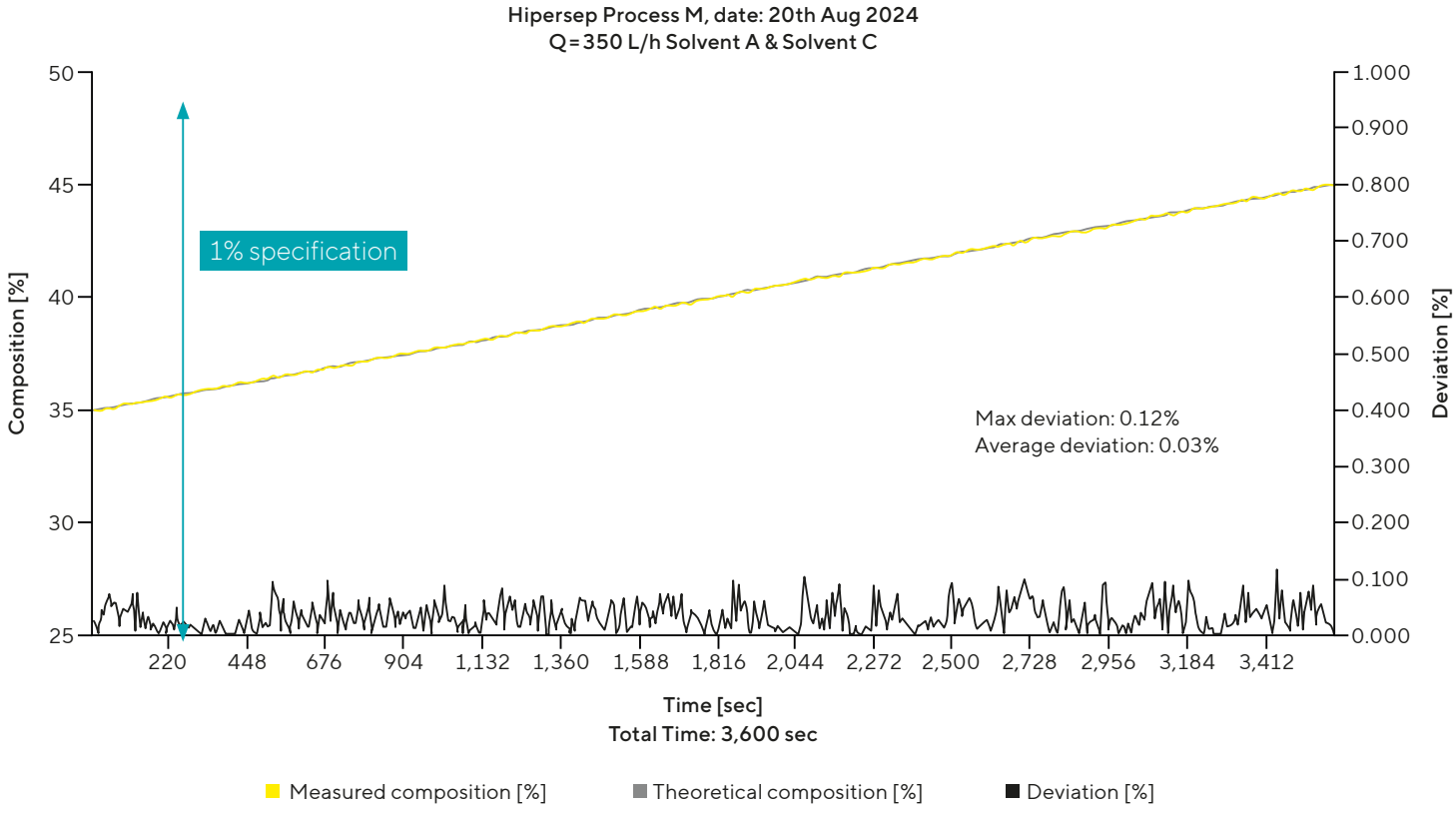


Figure 7: *Acceptance Test for Gradient Accuracy on the Hipersep® Flowdrive HPLC System*



Note. Analog gradient from 35-45% in 60 minutes.

Figure 7 shows the result of an acceptance test for gradient accuracy performed on a Hipersep® Flowdrive HPLC system. The green curve is the theoretical solvent content in the mobile phase. This linear curve is almost hidden by the overlaid red curve, which shows the actual composition measured using Coriolis mass flow meters. The black curve shows the difference between the theoretical and measured values at any time, also called the deviation. To guarantee 1% accuracy, any point of the black curve should be within the limit of those 1% deviations. Using the right system in the proper setup can achieve a much higher level of control than those 1% specifications, as shown in Figure 7, leading to better performances in terms of yield and purity than a system still compliant but with an accuracy closer to the specification 1%.

Besides the mobile phase composition, other factors may impact the robustness of the chromatography process. It is often more challenging to achieve reproducibility when purifying larger peptides, for example, polypeptides like the hormone insulin and its analogs (such as insulin glargine, lispro, aspart, and detemir) or GLP-1 and its agonists.

Such products tend to form some gels under conditions of their separation, a phenomenon also observed during TFF separations. As the gel forms on the matrix inside the column, it is challenging to desorb in RPLC mode, even with a high-solvent-content washing step. This will require the development of a cleaning step with harsh conditions that will affect the silica integrity in the case of C18,C8 or C4 matrix-based processes. The column performance will decrease over time, and the stationary phase will have to be changed as soon as the minimum number of plates required for the separation has been reached.

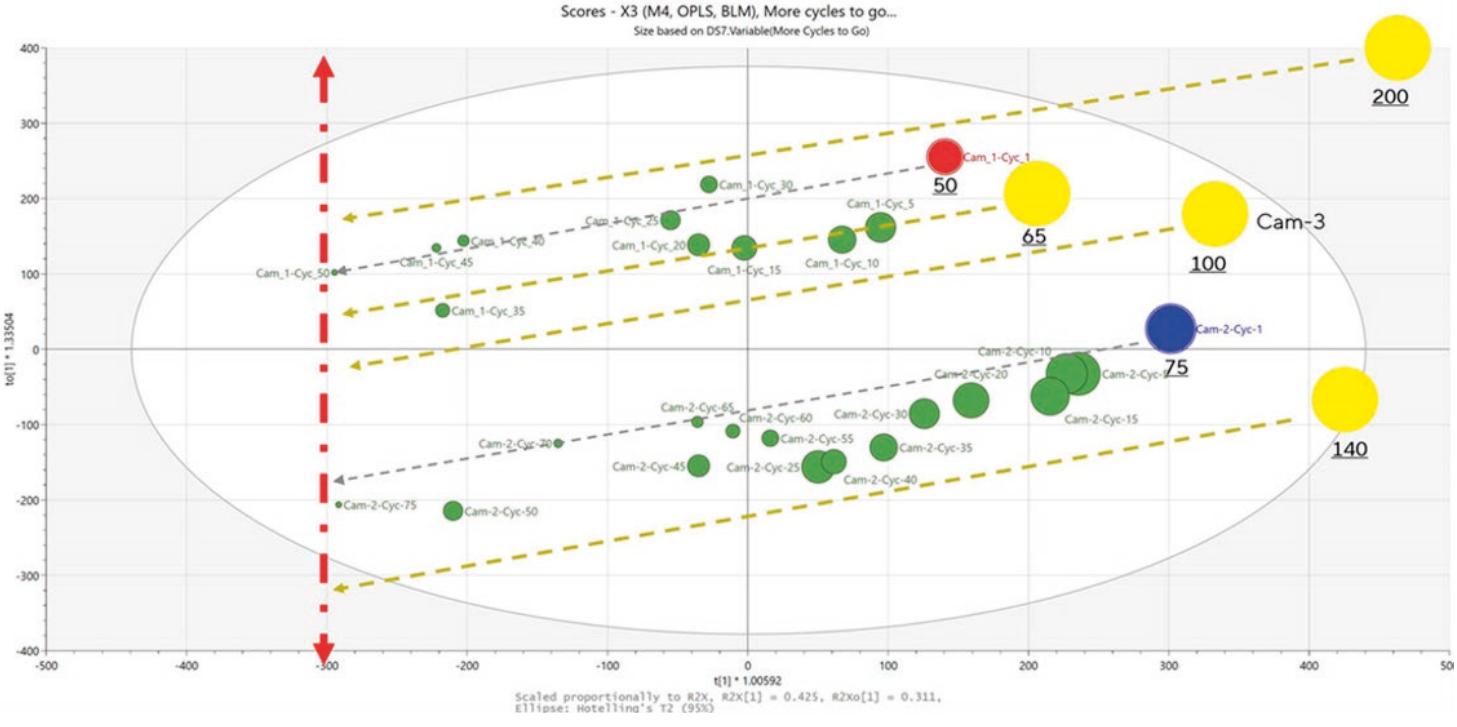
It is important to monitor the column's performance and predict when it will stop functioning effectively. Digital tools—such as multivariate data analysis (MVDA) and direct transition analysis (DTA)—are valuable for this evaluation, as they can measure the quality of a separation process without interrupting the production campaign for a column performance test.

Digital Solutions

We used the MVDA software SIMCA® to develop predictive models that significantly enhance the efficiency and precision of downstream processes. These models are designed to streamline workflows across development, scale-up, and production, ensuring faster, more accurate, and error-resilient operations. By enabling real-time monitoring and troubleshooting, SIMCA® empowers teams to identify and resolve issues proactively. This approach drives continuous process optimization, bolsters operational excellence, and ensures consistent process robustness and reliability, ultimately supporting a seamless path to achieving quality and efficiency goals.

Figure 8 highlights a powerful application of MVDA in resin health monitoring. We used SIMCA® to transform chromatography process data from each run into single points on a score plot. Each point represents all offline and online data captured during that run. As runs progress over a resin lot and runs increase, the points move from left to right, while the size of each dot reflects the number of cycles the resin lot can still be used for before it no longer performs effectively. The red boundary on the left indicates when the resin should be replaced.

Figure 8: *Score Graph: Real-Time Prediction of the Number of Additional Cycles for Which the Resin is Suitable*



This approach provides detailed insights into the resin condition, enabling reasonably accurate predictions of performance and lifespan. With SIMCA®'s predictive models, consistent product quality can be achieved, waste minimized, and resin inventory management optimized, driving process efficiency and reliability.

Building on the insights from resin health monitoring, SIMCA® also delivers significant value in downstream manufacturing through advanced tools like DTA. DTA is a robust feature in SIMCA® that is designed to evaluate column performance with precision.

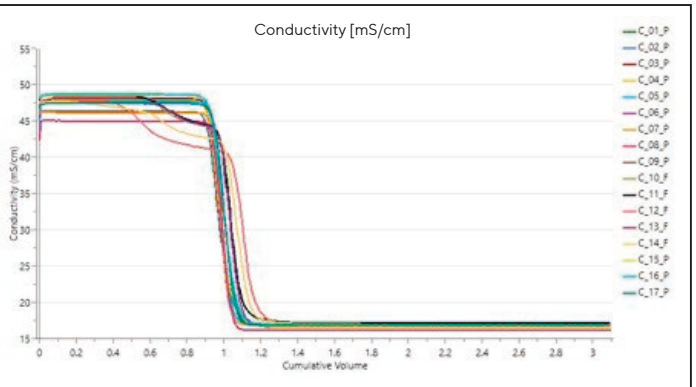
It captures chromatographic transitions, preprocesses the data, and calculates critical parameters such as TransWidth (analogous to height equivalent to a theoretical plate; HETP) and direct Af (equivalent to asymmetry).

This capability allows real-time monitoring of column efficiency and peak shape, empowering teams to make timely adjustments and maintain optimal separation performance. By refining process control, DTA helps achieve smoother transitions and greater overall efficiency in downstream processes. Below are three unique use cases showcasing the potential of SIMCA® and DTA to drive operational excellence.

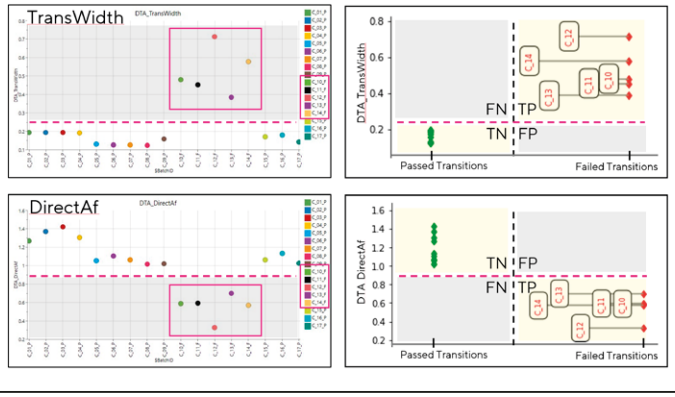
Use Case 1: Automated Classification Using DTA Metrics in SIMCA® Matches Expert Classification

DTA in SIMCA® streamlines chromatographic performance evaluation by automatically recording transitions, preprocessing data, and calculating key metrics. It measures TransWidth and direct Af and analyzes transition patterns in practice, ensuring they align with expert classifications (Figure 9). This enables more precise assessments and enhances decision-making in chromatographic analysis. To ensure precision, a confusion matrix is used to validate and cross-verify the results, delivering highly accurate insights into column efficiency and performance. This streamlined approach supports better decision-making and enhances downstream process reliability.

Figure 9: *DTA TransWidth and Direct Af Segregate Good and Bad Transitions With High Accuracy (Use Case 1)*



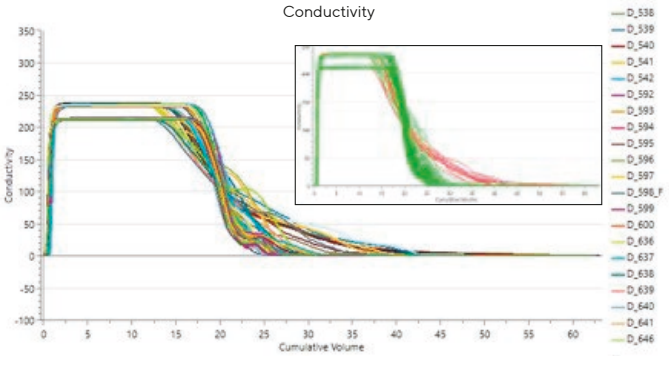
- 17 chromatography batches
- Phase: Wash
- Parameter: Conductivity
- 17 transitions: 12 normal vs. 5 abnormal



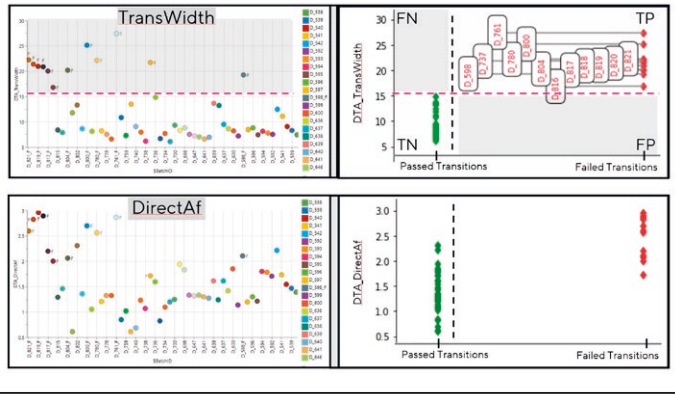
Use Case 2: Identification of Abnormal Column Behavior Matches Expert Evaluations

DTA in SIMCA® successfully identified abnormal column behavior during transitions from storage conditions in single-column chromatography runs (Figure 10). By analyzing conductivity data from 56 transitions (44 normal and 12 abnormal), DTA-predicted TransWidth closely aligned with expert evaluations. A validated confusion matrix further confirmed the accuracy of these predictions, highlighting SIMCA®'s powerful ability to deliver deeper process insights and enhance diagnostics for improved downstream performance.

Figure 10: *DTA TransWidth Can Identify Abnormal Column Behavior When Transitioning From Column Storage Conditions (Use Case 2)*



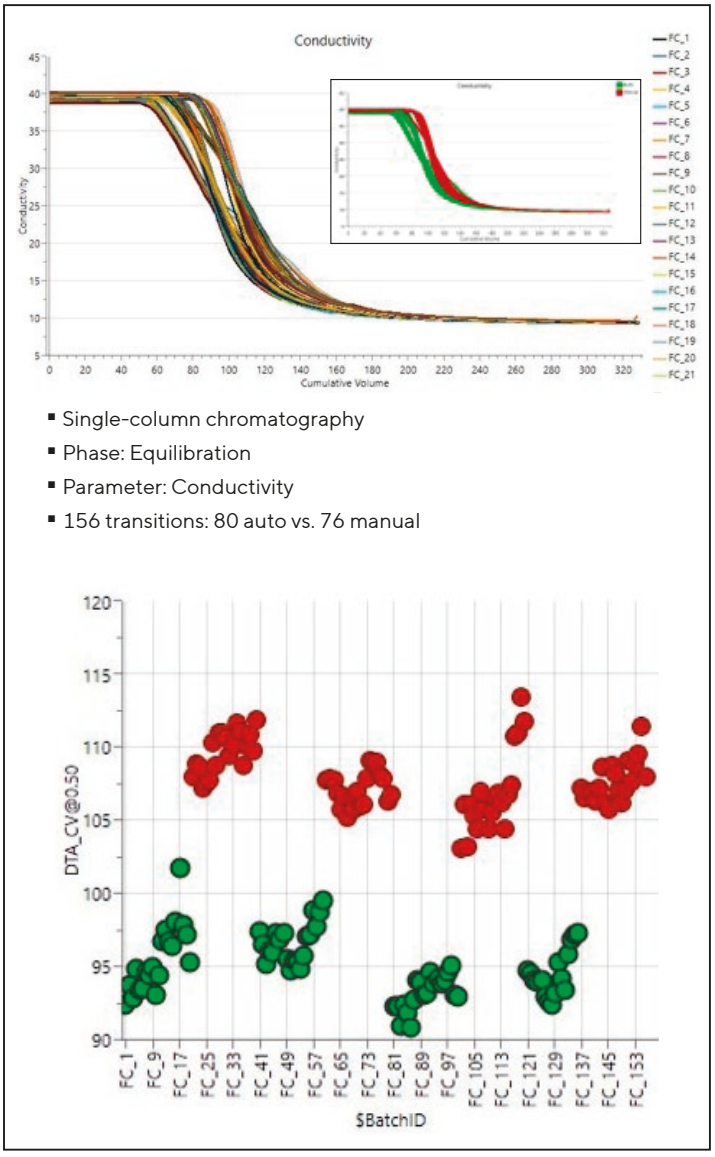
- Single-column chromatography
- Phase: Storage
- Parameter: Conductivity
- 56 transitions: 44 normal vs.12 abnormal



Use Case 3: Explorative Analysis With DTA—Column Packing Differences

DTA in SIMCA® facilitates detailed exploration of column packing variations during the equilibration phase in single-column chromatography (Figure 11). By analyzing conductivity data from 156 transitions (80 auto-packed and 76 manually packed), the DTA CenterPoint clearly distinguishes manual packing (red) from auto-packing (green). This analysis provides valuable insights into how different packing methods affect process performance, enabling better decision-making and optimization of column packing strategies.

Figure 11: *CenterPoint Can Distinguish Between Manually-Packed (Red) and Auto-Packed (Green) Columns (Use Case 3)*



- Single-column chromatography
- Phase: Equilibration
- Parameter: Conductivity
- 156 transitions: 80 auto vs. 76 manual

Filtration

Membrane-based normal flow filtration is generally employed to remove any particulate matter or for bioburden reduction and typically takes place before chromatography and TFF. Implementing filtration steps before these unit operations helps to enhance the life cycle and reusability of chromatography media and TFF membranes. The positioning of these filters depends on the peptide production method.

For SPPS, peptides are typically precipitated with diethyl ether or tert-butyl ether solvents (which are then removed by evaporation) or purified with a membrane-based process. Semi-synthetic production involves a membrane-based process or centrifuge for precipitation. The employment of a membrane-based process is generally governed by the condition of the feed material, which typically remains acidic at this stage and contains some quantities of organic solvent. Sartorius offers a range of filters that are compatible with acidic conditions and the presence of solvents. The key characteristics of some of these filters are listed in Table 4.

Table 4: *Characteristics of Sartorius Filters Used in GLP-1 Processes*

Sartorius Filters	MOC	Retention Rate [µm]	Intermediate Filtration	Sterilizing Filtration	Capacity	pH Compatibility	Organic Solvent Compatibility*
Sartopore® 2	PES	0.45 + 0.2 0.8 + 0.45	Yes	Yes	High	High	L
Sartopore® 2 XLM	PES	0.2 + 0. 1	Yes	Yes	High	High	L
Sartoguard	PES	1.2 + 0.2	Yes	No	High	High	C | L
Sartoguard	GF	0.8 + 0.2	Yes	No	High	High	C | L
Sartolon	Polyamide	0.45 + 0.2	Yes	Yes	Moderate	Low	C | L
Sartopure® PP3	Polypropylene	100 – 0.45	Yes	No	High	High	C
Sartofluor®	PTFE	0.2	Yes	Yes	Moderate	Moderate	C

* L = Limited, C = Compatible

Note. MOC = material of construction, PES = polyethersulfone, PTFE = polytetrafluoroethylene, GF = glass fiber, L = limited, and C = compatible.
For details, please refer to the validation guide of the respective filters.

It is important to optimize the parameters for filtration. Key parameters to consider are listed in Table 5.

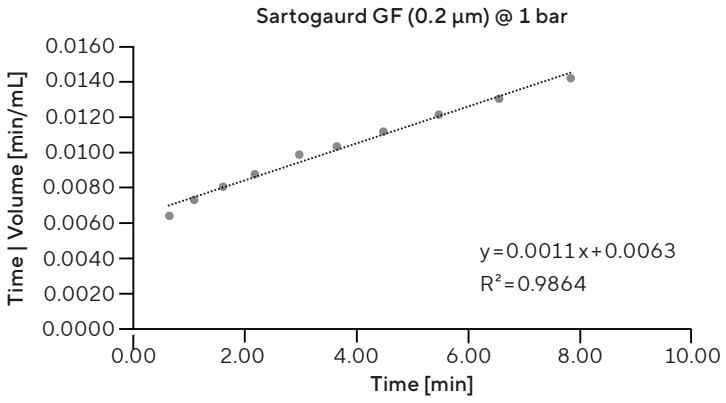
Table 5: *Filter Optimization Parameters*

Optimization Parameter	Flux	Yield	Capacity	Filter Integrity
Product Concentration	Y	Y	Y	–
Product Purity	Y	Y	Y	–
Product Composition	Y	Y	Y	Y
Membrane Type	Y	Y	Y	Y
Membrane Pore Size	Y	Y	Y	Y
Test Pressure	Y	–	Y	Y

Prefilters and Sterile Filters

Sartoguard and Sartopore® 2 are optimal filters that can be implemented before chromatography and TFF to reduce particulates and bioburden. No product loss is reported due to the adsorption of the peptide molecule. Figure 12 shows the high particle retention and bioburden reduction achieved with Sartoguard GF filters used during intermediate filtration, achieving more than 99% recovery.

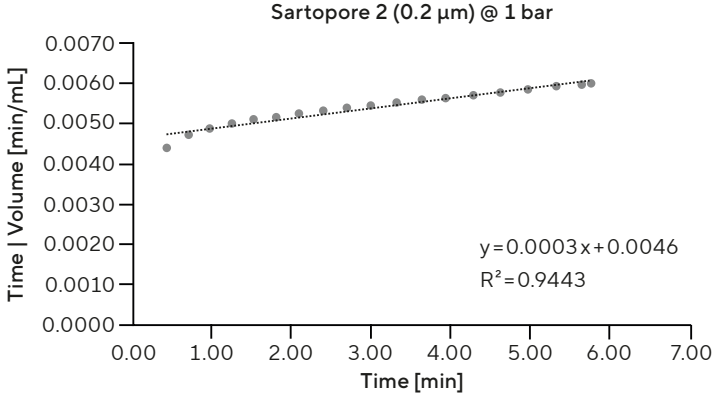
Figure 12: *Filterability of Sartoguard GF (Time vs. Time/Volume) for a Synthetic Peptide Molecule During Intermediate Filtration*



Note. 0.8 | 0.2 µm filters with >800 L/m² capacity and average flux of >3,000 LMH at 1 bar.

The Sartopore® 2 is ideal for critical unit operations where sterility is required, such as at the drug substance or final active pharmaceutical ingredient stage and the drug product stage in the final formulation. Figure 13 shows the performance of the Sartopore® 2 as the final filter for the drug product with more than 98% recovery.

Figure 13: *Filterability of Sartopore® 2 (Time vs. Time/Volume) for a Synthetic Peptide Molecule During Final Filtration of the Drug Product*



Note. Drug product with 6 mg/mL concentration, capacity >3000 L/m², average flux >8,000 LMH, and 1 bar pressure at 80% filter plugging.

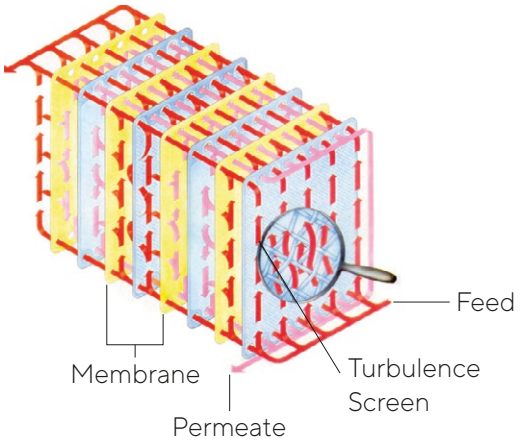
Parameters like flux, yield, and capacity (Table 5) can be obtained by running small, laboratory-scale studies and scaling up (using some degree of safety factor to account for variations). One key parameter, filter integrity, should be tested before and after use, especially for bioburden reduction or sterilization operations. The integrity of the filter or the entire setup can be tested with automatic instruments like the Sartocheck® 5 and Sartocheck® 5 Plus.

Tangential Flow Filtration

TFF, also known as cross-flow filtration, is employed in the peptide process to remove impurities, condition feed, concentrate feed, and exchange buffers, among other applications. Sartorius offers two membrane types for TFF processes: polyethersulfone (PES) and stabilized cellulose (Hydrosart®) membranes. Peptide therapeutics, e.g., GLP-1 products prepared by SPPS processes, generally contain solvents like acetonitrile or isopropyl alcohol. In contrast, products produced via semi-synthetic or recombinant processes are typically aqueous in nature. PES membranes have excellent compatibility with aqueous-acidic and basic environments but have limited compatibility with organic solvents. Hydrosart® cassettes have excellent compatibility with aqueous and organic solvents. For this reason, they are often the first choice for GLP-1 processes. The following are the key features of Hydrosart® cassettes:

- Non-adsorptive
- Non-product binding
- Wide pH and temperature stability
- High and sustained flux
- Caustic stability
- High mechanical strength
- Broad chemical resistance
- High biocompatibility
- Low fouling effect

Figure 14: *Structure of TFF Cassettes and Flow Channel* [13]



Unlike proteins, peptide molecules are generally low molecular weight species, with molecular weight cut-offs (MWCOs) ranging from 1 kDa to < 10 kDa (in some cases up to 60 kDa). Sartorius offers a wide range of TFF cassettes for applications requiring low MWCOs in two different materials of construction: Hydrosart® (Table 6) and PES (Table 7).

At Sartorius, membranes used for TFF cassettes are tested for water flow, membrane thickness, flux of model protein solution, and retention | rejection profiles for selected markers (e.g., 2 kDa Hydrosart®: ≥ 88% Vitamin B-12, 5 kDa Hydrosart®: > 96% insulin). Burst pressure is also measured to evaluate mechanical strength. Membrane sheets are visually inspected before installation. Each casted cassette is subject to testing for retentate and permeate flux, a diffusion test for integrity, a durometer (Shore A) test of the silicone frame, and a visual inspection of the final product.[14] Implementing TFF in any process requires thorough optimization and sizing.[13]

Some important parameters include:

- Feed composition: Particle content, turbidity, additives, etc.
- Feed concentration
- Feed flow rate
- Transmembrane pressure (TMP) and flux
- Concentration factor
- Diafiltration (batch vs. continuous)
- Mass loading or volumetric loading
- Recovery

Table 6: *Retention Rates and MWCO for Hydrosart® Cassettes*

Substance	Approximate Molecular Weight	2 kDA	5 kDA	10 kDA	30 kDA	100 kDA	300 kDA
Vitamin B12	1,200	≥ 88%	–	–	–	–	–
Insulin	5,000	–	> 96%	–	–	–	–
Cytochrome C	12,400	–	–	> 97.5%	–	–	–
Albumin	67,000	–	–	–	> 97.5%	≤ 60%	–
γ Globulin	169,000	–	–	–	–	> 96%	–
Blue dextran	200,000	–	–	–	–	–	< 90%

Table 7: *Retention Rates and MWCO for PES Cassettes*

Substance	Approximate Molecular Weight	1 kDA	5 kDA	8 kDA	10 kDA	PESUmax kDA	30 kDA	50 kDA	100 kDA	300 kDA
Vitamin B12	1,200	> 70%	50 – 80%	–	–	–	–	–	–	–
Cytochrome C	12,400	–	–	99%	> 95%	–	60 – 90%	–	< 80%	–
Albumin	67,000	–	–	–	–	> 99.6%	–	> 95%	≥ 98%	–
γ Globulin	169,000	–	–	–	–	–	–	> 99%	–	< 70%
Blue dextran	200,000	–	–	–	–	–	–	–	–	> 95%

Diafiltration is essentially a buffer exchange process. Batch mode diafiltration involves diluting the sample with buffer to a defined volume. The diluted sample is then concentrated back to its original volume by removing the same buffer volume. In continuous diafiltration, the buffer is added to the feed tank at the same rate that the buffer is removed from the permeate, ensuring efficient buffer use. The ionic strength, buffer composition, and stabilizer concentration generally remain in a defined range. Continuous diafiltration offers an advantage over batch diafiltration in that the retentate concentration remains constant. It is often seen as a more gentle process relative to the stability of the product.

TMP, feed flow and flux optimization are generally considered critical and vary with the nature of the feed. Optimizations should be performed before the start of actual sizing. TMP is the most critical parameter in TFF. TMP is the driving force for the flow through the membrane. The TMP is defined by the following equation:

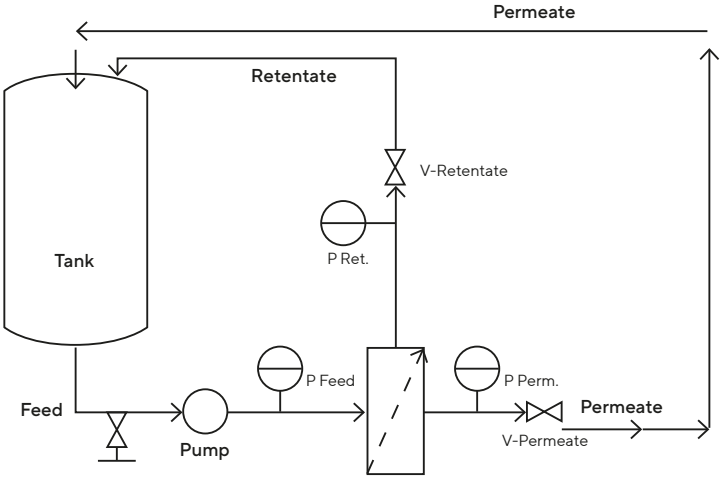
$$TMP = \frac{p_{Feed} + p_{Retentate}}{2} - p_{Permeate}$$

With:

TMP [bar]:	Transmembrane pressure
p_{Feed} [bar]:	Pressure in the feed stream
$p_{Retentate}$ [bar]:	Pressure in the retentate stream
$p_{Permeate}$ [bar]:	Pressure in the permeate stream

TMP and flux optimization are performed in total recycle mode, i.e., the feed, retentate, and permeate tubes are all directed into a single feed tank. Optimization should be performed at various feed flow rates or differential pressures, beginning with the highest flow rate or differential pressure to avoid membrane fouling during the study. Figure 15 depicts the setup of a TMP and flux optimization process.

Figure 15: *TMP and Flux Optimization Setup in Recycle Mode*

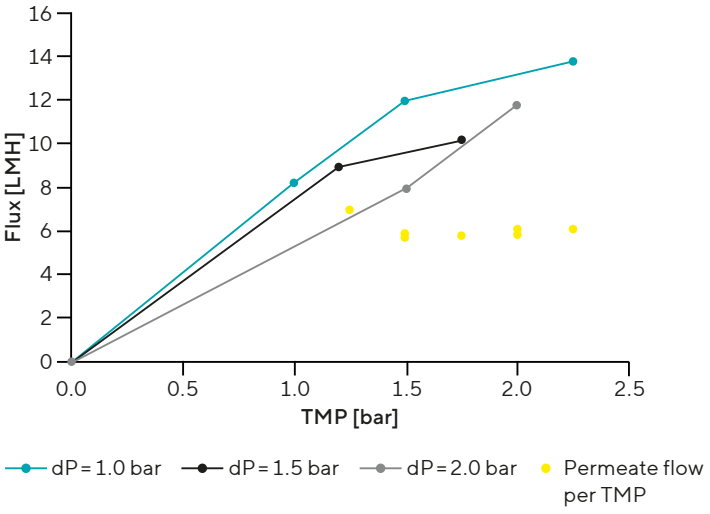


It is important to maintain a consistent feed volume throughout the study; if a sample must be withdrawn at a certain point, an equal quantity of fresh feed should be added to avoid variation in concentration.

TMP and flux optimization depend on the nature of the feed, feed concentration, feed buffer condition, applied feed flow rate or differential pressure, temperature, and other factors. It can even vary for the same molecule at different conditions. Optimization can be done using a manual process or with an automated system, e.g., the Sartoflow® Smart. Automated systems are generally preferred, as achieving a TMP greater than 2 bar is difficult in manual systems due to the pressure rating limitations of silicone tubing.

TMP and flux optimization using variable differential pressure will remove variability contributed by the pump. In this case, TMP and flux are derived as functions of differential pressure across the membrane. One such case study using a GLP-1 molecule and 2 kDa Hydrosart® cassette is presented in Figure 16. This study used a Sartoflow® Smart TFF system (Figure 17) with automated TMP control. There is an increase in flux with increasing TMP, indicating that the feed is non plugging in nature.

Figure 16: *TMP and Flux Optimization Case Study 1: TFF on the Sartoflow® Smart at Constant Feed Concentration*

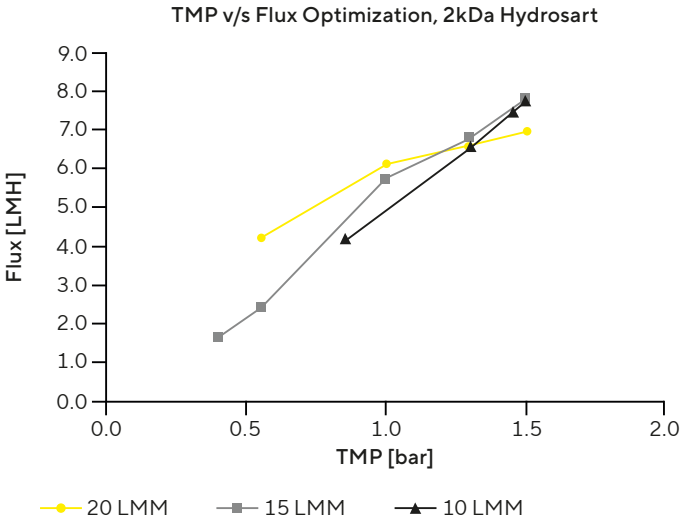


Note. Optimized TMP = 2.25 bar, dP = 1 bar, and flux = 14 LMH.

Figure 17: *Figure 17 Sartoflow® Smart: Fully Automated TFF System*

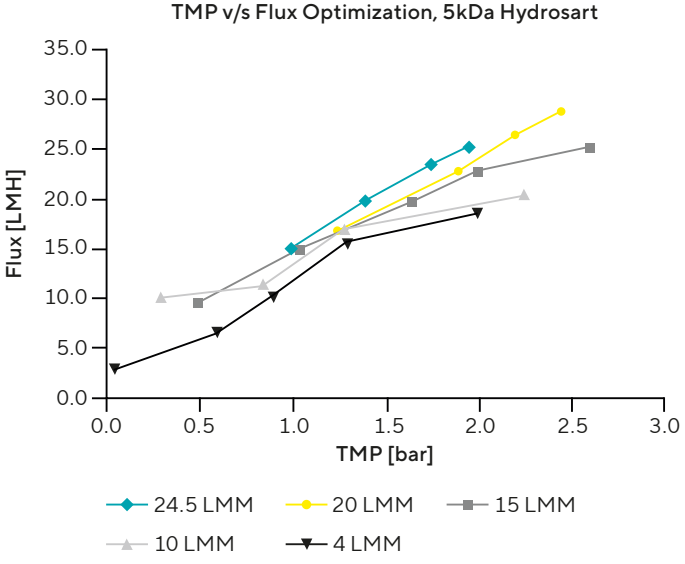
A traditional method of TMP flux optimization uses variable feed flow, which can be easily done using a manual setup. Figures 18 and 19 represent two such case studies.

Figure 18: *TMP and Flux Optimization Case Study 2: Constant Feed Concentration, Manual TFF Holder With a 2 kDa Hydrosart® Membrane*



Note. Optimized feed flow = 10 – 15 LMM, TMP = 1.0 – 1.4 bar, flux = 7 – 8 LMH at load challenge 10 L/m². Peptide = 4 – 5 kDa, no loss in permeate at any TMP.

Figure 19: *TMP and Flux Optimization Case Study 3: Constant Feed Concentration, Manual TFF Holder With a 5 kDa Hydrosart® Membrane*



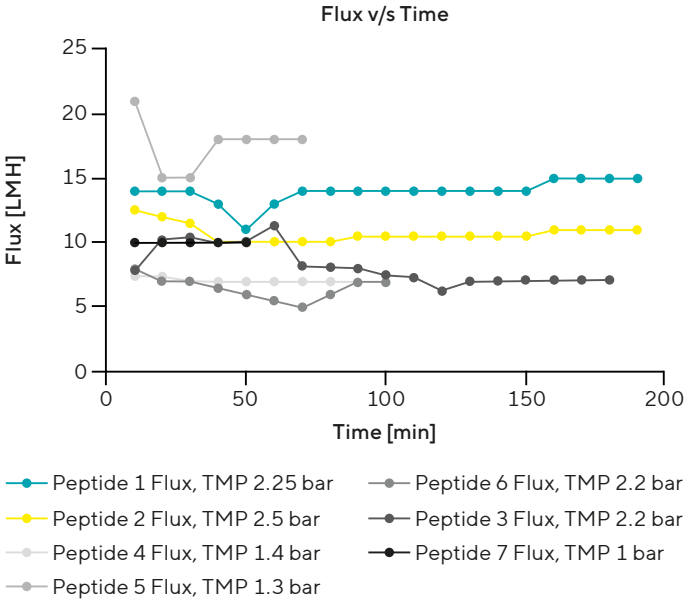
Note. Optimized feed flow = 10 – 15 LMM, TMP = 1.0 – 1.4 bar, flux = 15 – 22 LMP at load challenge 20 L/m². Peptide = 5.6 kDa, no loss in permeate at any TMP.

After the optimization study, it is recommended that the cassette be cleaned and the clean water flux checked.

The actual process should be run at a TMP lower than the optimized TMP, providing a safety factor. It generally has the following phases: equilibration, concentration, diafiltration or buffer exchange, final concentration, and recovery.

Examples of process parameters for purifying peptides containing GLP-1 agonist are listed in Table 8. Test feed concentration was upto 8 mg/mL. In most cases, the operational TMP was found to be between 1.3 and 2.2 bar, and the flux was between 6.5 and 17 LMH for both 2 and 5 kDa membranes. Once optimized, an entire process (including concentration and buffer exchange) can be completed within 200 minutes (Figure 20).

Figure 20: *TFF Process Data for Different Therapeutic Peptide Molecules*



Note. Peptides 1–4 were processed on a 2 kDa Hydrosart® membrane, whereas peptides 5 and 6 were processed on a 5 kDa Hydrosart® membrane.

Table 8: *Process Parameters for GLP-1-Containing Peptides Using 2 and 5 kDa Membranes*

Parameters	Peptide 1	Peptide 2	Peptide 3	Peptide 4	Peptide 5	Peptide 6	Peptide 7
Feed source	Chemical synthesis	Chemical synthesis	Chemical synthesis	Chemical synthesis	Chemical synthesis	Microbial	Chemical synthesis
Feed molecular weight [kDa]			4	4	4	5.6	4
Feed solvent system	25% acetonitrile	15–20% acetonitrile	35% acetonitrile	25% acetonitrile	20–40% acetonitrile	Aqueous buffer	25–30% acetonitrile
Product category	Oligopeptide	GLP-1	GLP-1	GLP-1	GLP-1	Peptide	GLP-1
Cassette	Hydrosart®	Hydrosart®	Hydrosart®	Hydrosart®	Hydrosart®	PESU	Hydrosart®
Cassette (MWCO)	2	2	2	2	5	5	2
Membrane loading [L/m^2]	17	11	25	10	10	12	5
Concentration factor	5	6	15	5	5	5	–
Number of diavolumes	–	14	6	5	5	3	5
Average process flux	14	10.7	8.2	7.1	17.6	6.5	10
Average process TMP [bar]	2.2	2.5	2.2	1.4	1.3	2.2	1.0
Recovery [%]	100	100	93	100	98	96	99

Note. MWCO = molecular weight cut-off

The peptide industry generally prefers cassettes, which can be reused, reducing the cost of goods and improving sustainability. Before reuse, cassettes need to be cleaned using a defined process. Table 8 shows the cleaning agents and conditions recommended by Sartorius for our membrane materials.

Table 9: *Recommended Cleaning Conditions for Hydrosart® and PES Membranes in TFF Processes*

Cleaning Agent	Concentration	pH	Time [min]	Temperature [°C]	Feed Pressure [bar]	Retentate Pressure [bar]
Sodium hydroxide	1 N	14	60	50	2	0
Phosphoric acid	2% w/w	1.3	30	50	2	0

Note. These are suggested procedures only; requirements may vary in individual cases.
The cleaning solution should be circulated with permeate valves open for 60 minutes and repeated as required based on clean water flux values.

Conclusion

To meet the growing demand for therapeutic peptides, it is essential to have robust production processes that ensure high purity and yield. Achieving precise control over critical process parameters, such as gradient and mobile phase composition, can enhance production throughput without sacrificing product quality. Our data demonstrates that the Hipersep® Flowdrive HPLC system offers gradient accuracy with deviations of less than 1%. Chromatography and TFF are key unit operations in the production of high-purity GLP-1 agonists. Optimizing TFF can improve performance while eliminating the challenges associated with complex solvent removal and concentration steps. Our data also shows that 2 kDa Hydrosart cassettes are compatible with organic solvents up to 45%, with a flux of ~18 LMH and a recovery rate greater than 99%. The use of prefilters and sterile filters can help reduce particulate load and minimize bioburden at intermediate and drug product stages, improving patient safety. Additionally, data analytics can provide deeper insights into the process, aiding in monitoring, control, and optimization of both productivity and process robustness.

Sartorius offers a range of technologies, including chromatography columns and systems, TFF cassettes and systems, sterile filters, fill finish solutions, and digital tools. Please contact your local field application specialist for more details.

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