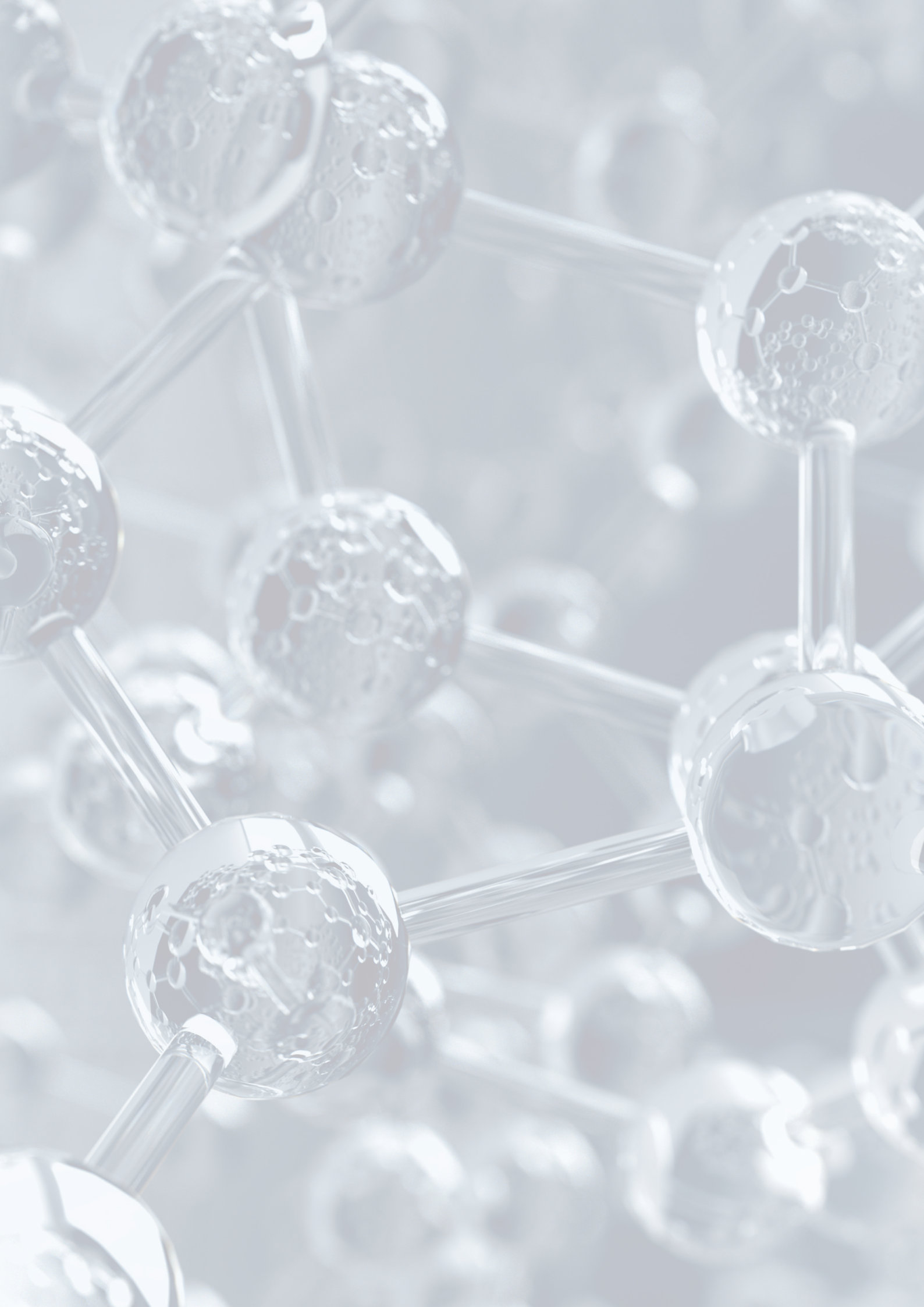
YMC

Strategic peptide purification

practical and theoretical aspects of method development using reversed phase chromatography



1. Introduction

Peptides have become one of the most important biopharmaceuticals. The broad functional spectrum of these molecules offers multiple pharmaceutical applications. The chemical properties of peptides directly depend on the length of the peptide chain, the composition of the amino acid side chains and potential secondary structures of the molecule and modifications. Therefore, peptides can strongly vary in their size and physicochemical properties. This leads to special demands regarding the production and preparative purification. Therefore, the most appropriate purification strategy has to be found that addresses the resulting varied impurity profile.

Despite the origin of the molecule – synthesis or recombinant production – purification via reversed phase is most commonly used because structural variants or even single amino acid differences of smaller peptides can be easily separated.

This document is a guide for developing preparative peptide purification strategies with the considerations of economic and productive aspects. This means: maximum sample recovery in the shortest cycle time within cost limitations. Whereas isolation focuses on the scale up of mg- to g-units, production scale needs to achieve up to kilograms or even tons.

The most comprehensive approach for method development in preparative LC in general is the so-called linear scale-up method, which consist of:

1. **Method development at analytical scale**
2. **Loadability studies on analytical scale**
3. **Scale-up to preparative process**
4. **Regulatory Affairs Support**

Practical example of process development: purification of liraglutide

Within this whitepaper, the important steps in method development for peptide purifications using RP chromatography are demonstrated on a real-life example – the purification of liraglutide, a therapeutic peptide for the treatment of diabetes. These practical examples are indicated by these boxes for the single chapters and steps of method development.

2. Method development at analytical scale

The optimal selectivity, i.e. the ideal combination of the most suitable stationary phase and elution conditions, is the basis of every economic preparative separation of peptides. Only the optimal selectivity will allow for the highest productivity; meaning the maximum loading capacity and yield in the shortest cycle time.

Hence, the method development reflects the choice of stationary phase and eluent conditions.

This chapter introduces to the following aspects of method development with the example of a real peptide purification process:

- **Phase selection**
- **Pore size**
- **Particle size**
- **Mobile phase composition**
- **Gradient optimisation**

Important Check List

Before choosing a phase for a method screening in preparative LC, the following check list might be helpful:

1. **Availability of particle sizes**
2. **Bulk or prepacked column?**
3. **Reproducibility**
4. **Mechanical stability**
5. **Supply guarantee**

2.1. Selection of the stationary phases

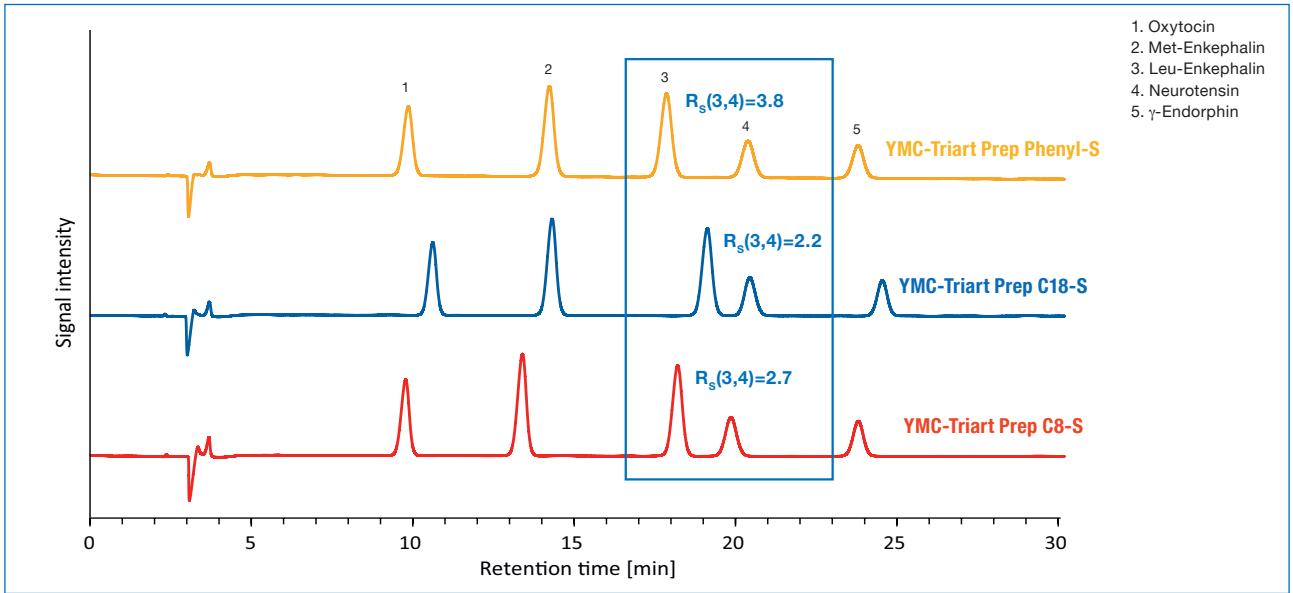
Peptides can vary very widely with regards to their molecular weight, but also in their chemical properties. This depends on the composition of the amino acids and directly on the length of the peptide chain. For a good separation and therefore purification of the peptide, it is necessary to select a stationary phase with an appropriate ligand. This mainly depends on the content of hydrophobic amino acid side chains, potential secondary structures of the peptide and additional modifications.

Table 1: Stationary phase selection tool

	C18	C8	Phenyl	C4
Functional group	$-C_{18}H_{37}$	$-C_8H_{17}$		$-C_4H_9$
Hydrophobicity	High			Low
Hydrogen bonding capacity	Low			High
Surface recognition ability	High			Low
Suitable MW of the peptide	Low			High

The higher the molecular weight, the longer the peptide chain and so the less hydrophobic should be the most suitable stationary phase. For peptides with a molecular weight up to 30 kDa, C18- or C8-modified stationary phases are the optimal choice. A C4-modification is a better choice for isolation of larger proteins. To select the most appropriate stationary phase for the peptide separation of interest, a phase screening is the best approach.

In the application shown in fig. 1, the separation of five different peptides was investigated using C18, C8 and phenyl modified stationary phases. The result: in this case the best separation of the critical peak pair (peaks 3 and 4) was achieved with the phenyl modification. The resolution between all other peak pairs is very good for all of the three phases. Therefore, an initial phase screening gives very useful insights into the selectivity for all relevant target and impurity peaks.



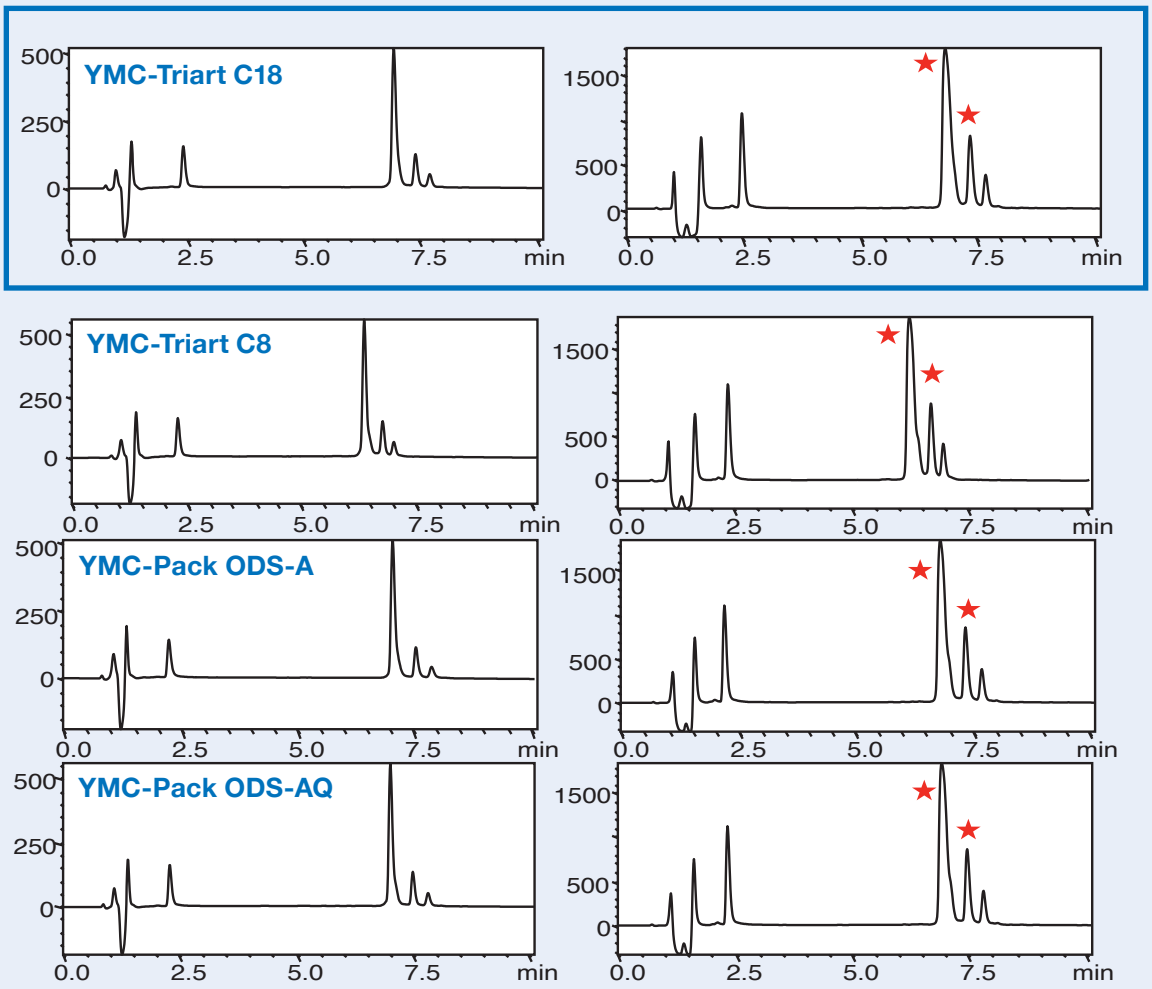
Columns: **YMC-Triart Prep Phenyl-S** (10 µm, 12 nm) 250 x 4.6 mm ID
YMC-Triart Prep C18-S (10 µm, 12 nm) 250 x 4.6 mm ID
YMC-Triart Prep C8-S (10 µm, 12 nm) 250 x 4.6 mm ID
Part. Nos.: (YMC) TPS12S11-2546WT, TAS12S11-2546WT, TOS12S11-2546WT
Eluents: A) Water + 0.1% TFA, B) Acetonitrile + 0.1% TFA
Gradient: 20–40% B (0–45 min)
Flow rate: 1.0 mL/min
Temperature: 25 °C
Injection: 10 µL
Sample: 0.167 mg/mL per peptide dissolved in water
Detection: UV at 220 nm

Figure 1: Selectivity Screening

Practical example: Stationary phase screening for liraglutide. In this case, YMC-Triart C18 was selected as the best suitable stationary phase. The important advantage for alkaline CIP-procedures when using YMC-Triart C18 combined with the good resolution was significant to the selection of YMC-Triart C18 as the most promising stationary phase for further process development.

Practical example of process development: Stationary phase screening

Loading amount 3 µg x 8 Loading 24 µg



Critical pair: ★ marked

Column:	(3 µm, 12 nm), 100 x 3.0 mm ID
Eluent:	A) 0.1% TFA in Water
	B) 0.1% TFA in Acetonitrile
Gradient:	40–75% B (0-10 min)
Flow rate:	0.43 mL/min
Temperature:	35 °C
Detection:	UV at 215 nm
Injection:	6 µL/48 µL (Crude 0.5 mg/mL)

Column	Resolution at 24 µg loading
YMC-Triart C18	2.6
YMC-Triart C8	2.4
YMC-Pack ODS-A	2.8
YMC-Pack ODS-AQ	2.9

2.2. Effect of the pore size on peptide purification

Good chromatographic performance leads to high purity and yield. As peptides generally can vary in their size, the pore size of the stationary phase has to be chosen carefully to achieve an optimal separation effect. Generally, the pore size should be as small as possible but large enough for separation efficiency. Table 2 illustrates a generally recommended relationship between the pore size and the modification of the stationary phase for different peptide sizes. Generally, the larger the peptide, the less hydrophobic should be the stationary phase modification. Of course, these values are only recommendations and the limits are only approximate. Therefore, the testing of different pore sizes with increased loadings might also be useful.

Table 2: Column pore size selection tool

MW		C18	C8	C4
5,000	12 nm	+++	++	+
20,000	20 nm	++	+++	++
100,000*	30 nm	+	++	+++

** up to 150,000 Da possible using e.g. higher temperature*

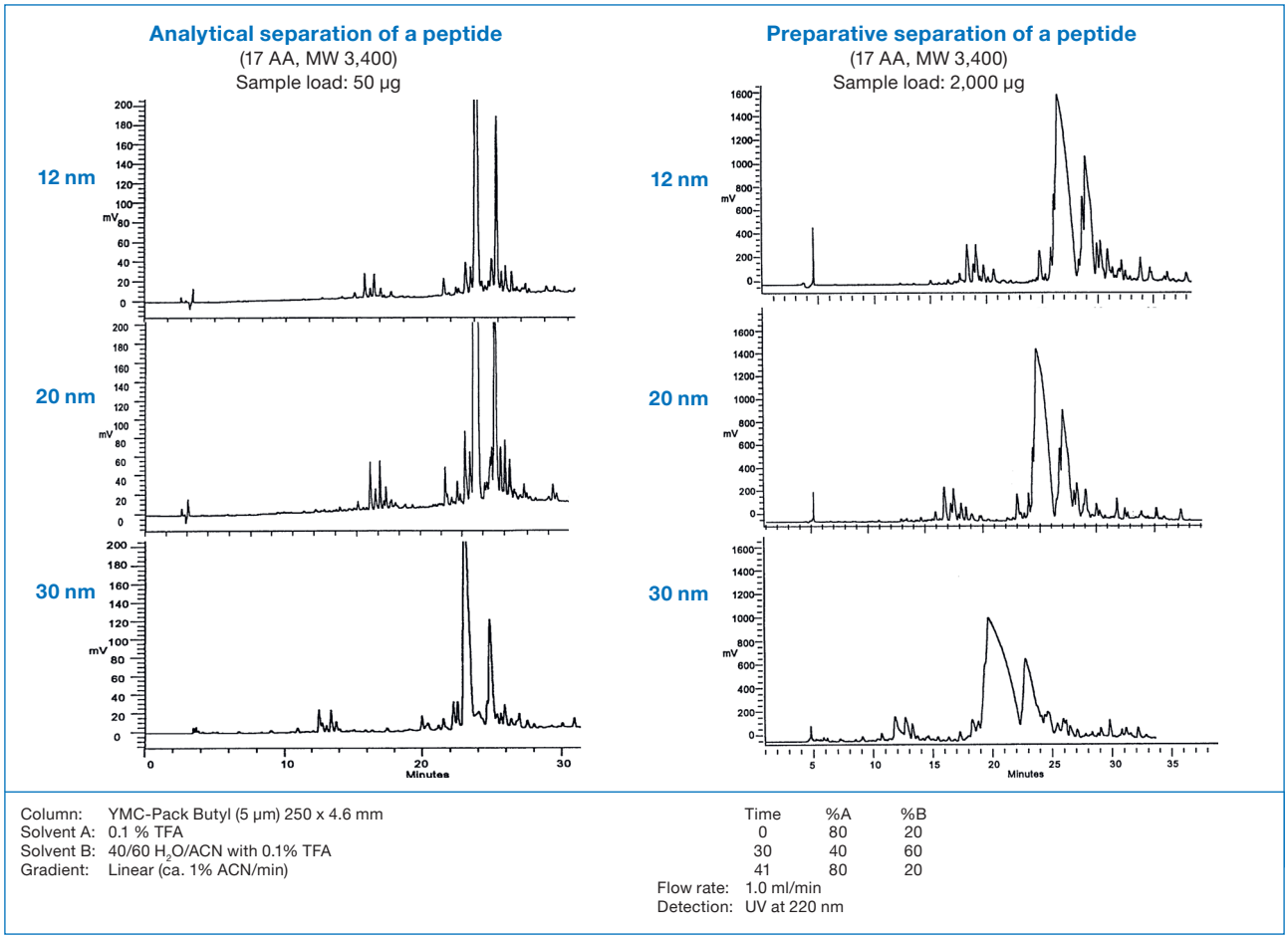


Figure 2: Influence of the pore size on a peptide separation at analytical and preparative scale.

The performance of the selected pore size should also be monitored with higher loading of the peptide. The most appropriate pore size for the peptide of interest leads to good chromatographic performance and therefore to an effective purification process. The combination of the pore size with particle size and hydrophobicity leads to the best separation and therefore purification success.

2.3. Effect of the particle size on peptide purification

The particle size of the stationary phase material determines the resolution and also the productivity of the overall process. Smaller particles in general provide better separation and therefore higher resolution. On the other side, smaller particle sizes generate elevated backpressures. Therefore, the particle size selection is a compromise between sufficient resolution and resulting backpressure being appropriate for the chromatographic system whilst allowing efficient flow rates.

Table 3: Influence of particle size on plate count and backpressure

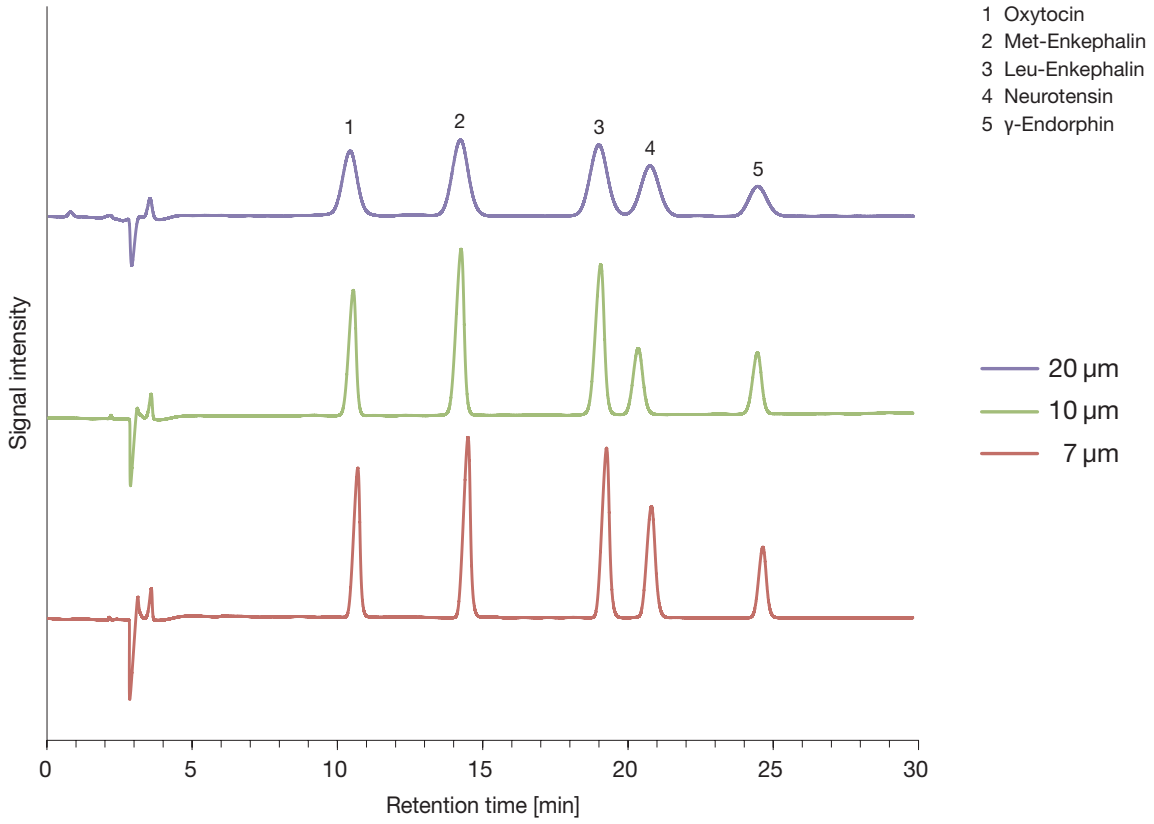
d_p	N	psi	bar
5	20,000	3,300	228
7	14,300	1,680	116
10	10,000	830	57
15	6,700	370	26
20	4,500	250	17
40	2,300	60	4
50	2,200	35	2.4

Stationary Phase: C18
Mobile Phase: Methanol / Water (50/50)

Expert Tip

Multiple particle sizes allow for easy process transfer

Choose a stationary phase material, which is available with multiple particle sizes having the identical selectivity. It allows process transfer directly from analytical to preparative particle size and vice versa. Fig. 3 illustrates the separation of five peptides on the same C18 modified base material with three different particle sizes. The elution profile remains unchanged whereas the resolution differs depending on the particle size.



Columns:	YMC-Triart Prep C18-S (7 µm, 10 µm and 20 µm, 12 nm) 250 x 4.6 mm ID
Eluent A:	Water + 0.1% TFA
Eluent B:	Acetonitrile + 0.1% TFA
Gradient:	0–45 min: 20% ➔ 40% Eluent B
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV, 220 nm
Injection volume:	10 µL
Sample concentration:	0.167 mg/mL per compound, dissolved in water

Figure 3: Effect of the particle size on the resolution of five peptides

2.4. Mobile phase composition

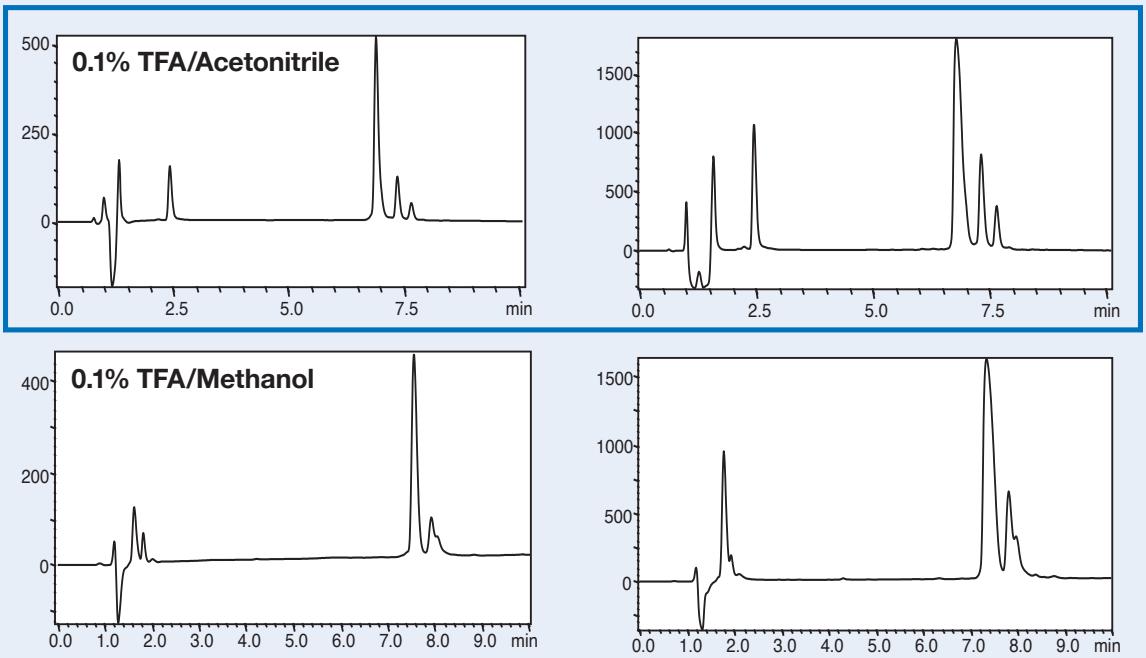
The organic phase influences chromatographic resolution. Therefore, determining the most appropriate organic solvent, buffer and pH-value are critical steps in process development.

2.4.1. Selection of organic solvent and amount

The selection of the organic solvent and its concentration determines the polarity of the mobile phase and therefore the strength of interaction of the peptide with the stationary phase. In our practical example, two of the most common organic solvents in preparative RPC – acetonitrile and methanol - are compared with different loading amounts. In this case, acetonitrile leads to higher resolution and therefore has a better separation.

Practical example of process development: screening of organic solvent

Loading amount: 3 µg → x 8 Loading → 24 µg



Column:	YMC-Triart C18 (3 µm, 12 nm), 100 x 3.0 mm ID
Eluent:	A) 0.1% TFA in Water B) 0.1% TFA in Acetonitrile/Methanol
Gradient:	Acetonitrile 40–75% B (0–10 min) Methanol 75–90% B (0–10 min)
Flow rate:	0.43 mL/min
Temperature:	35 °C
Detection:	UV at 215 nm
Injection:	6 µL /48 µL (Crude 0.5 mg/mL)

An important aspect to consider is the solubility of the target peptide. Small hydrophobic peptides may have to be dissolved in organic solvents whereas other peptides may precipitate if the content of the organic solvent increases. Therefore, solubility and stability have to be tested prior to choosing the most appropriate chromatographic conditions.

Expert Tip

Native purification of peptides – with 100% aqueous conditions

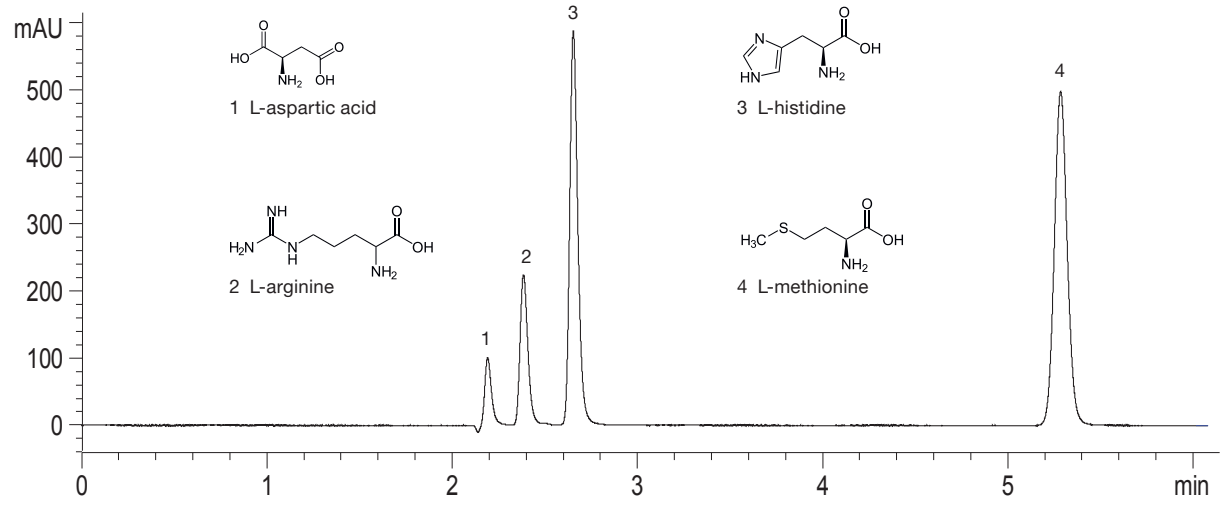
If the peptide of interest exhibits a secondary structure that is crucial for its function, the use of organic solvents has to be carefully considered. Organic solvents can disturb a peptides' structure and denaturation occurs.

For small peptides, these structures may be restored after the removal of the organic solvent or the structural integrity may not be important for the functionality. But in some cases, especially for very large peptides, these structures are crucial and the destruction of the secondary structure can be irreversible. Therefore, the use of organic solvents has to be avoided or kept to a minimum.

Stationary phases, which can tolerate 100 % aqueous conditions in a reproducible manner, are beneficial. For example, YMC-Triart Prep C18-S tolerates 100% aqueous buffer conditions and delivers reproducible separation performance.

Polar peptides as well as single amino acids already show good retention on the reversed phase materials when 100% aqueous conditions are used as the mobile phase. This can directly lead to better separation of the target molecules. In this application (Fig. 4), four single standard amino acids are separated with YMC-Triart C18 using 100% aqueous conditions. The result: good separation without the addition of any organic solvent.

Amino acids with 100% aqueous phase



Column:	YMC-Triart C18 (1.9 µm, 12 nm) 150 x 3.0 mm ID
Eluent:	40 mM K_2HPO_4 (pH 7.0)
Flow rate:	0.3 mL/min
Temperature:	20 °C
Detection:	UV at 210 nm
Injection:	2 µL (1 mg/mL)

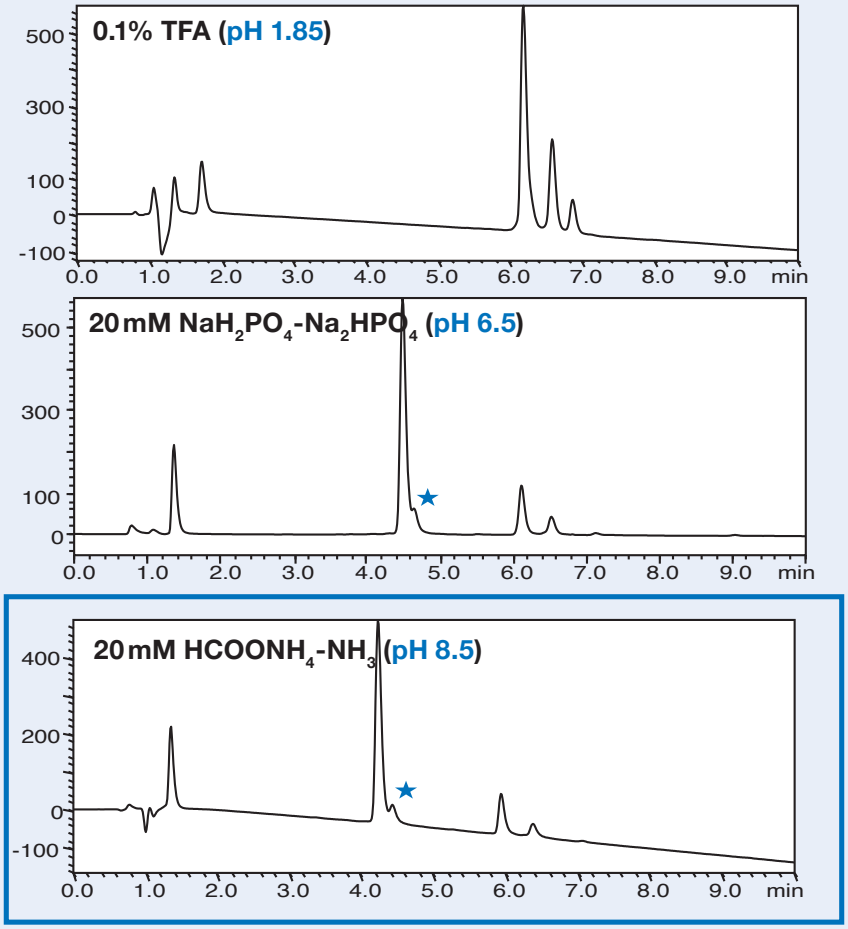
Figure 4: Separation of amino acids in 100 % aqueous conditions.

2.4.2. pH considerations

A polypeptide chain is composed of different amino acid residues with different chemical properties and also different isoelectric points. Therefore, the pH of the solvent has a strong effect on the peptide itself and on its interaction with the stationary phase. The overall isoelectric point of a peptide can be calculated from its amino acid sequence.

Practical example: the pH of the mobile phase has a strong influence on the retention of the peptide. Here, the Ammonium formate buffer with a pH value of 8.5 has increased the separation effect compared to the use of lower pH buffers. Furthermore, an additional impurity (marked) becomes visible and can be separated in the subsequent downstream process.

Practical example of process development: screening for optimal pH



Impurity: ★ marked component

Column:	YMC-Triart C18 (3 µm, 12 nm), 100 x 3.0 mm ID
Eluent:	A) Water/Buffer B) Acetonitrile
Gradient:	40–75% B (0–10 min)
Flow rate:	0.43 mL/min
Temperature:	35 °C
Detection:	UV at 215 nm
Injection:	6 µL (Crude 0.5 mg/mL) = 3 µg loading

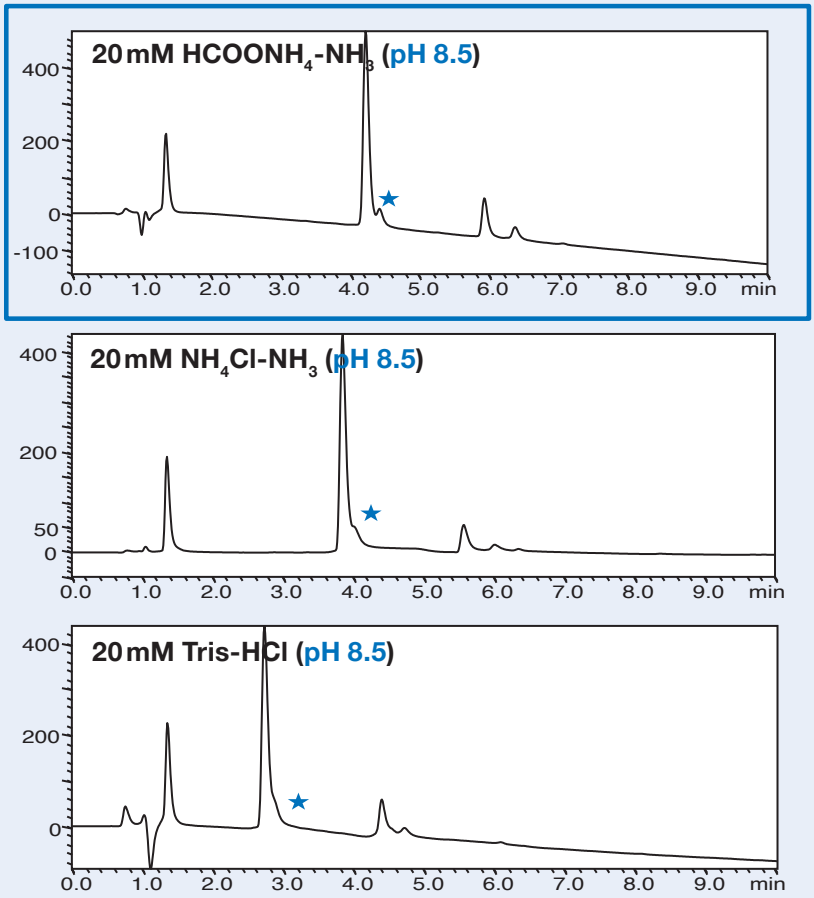
Conditions	Resolution of main peak and impurity peak
20 mM $HCOONH_4$-NH_3 **(pH 8.5)**	**0.96**
20 mM NaH_2PO_4-Na_2HPO_4 **(pH 6.5)**	**0.50**
0.1% TFA **(pH 1.85)**	**–**

2.4.3 Buffer type selection

Buffer salts also have a strong influence on the peptide separation. The **practical example** below shows results obtained with three different buffers, Ammonium formate-, Ammonia-ammonium chloride- and Tris-buffer. For the sake of comparison all buffers were adjusted to the same pH-value. The best separation was achieved with the Ammonium formate buffer.

The other buffers lead to no significant separation effect. Generally, the different buffer types have a strong influence on the retention of the target peptide. Using Tris buffer the retention time was significantly reduced. However, the resolution of the impurity was not sufficient.

Practical example of process development: screening of buffer types



Impurity: ★ marked component

Column:	YMC-Triart C18 (3 µm, 12 nm), 100 x 3.0 mm ID
Eluent:	A) Water B) Acetonitrile
Gradient:	40–75% B (0–10 min)
Flow rate:	0.43 mL/min
Temperature:	35 °C
Detection:	UV at 215 nm
Injection:	6 µL (Crude 0.5 mg/mL) = 3 µg loading

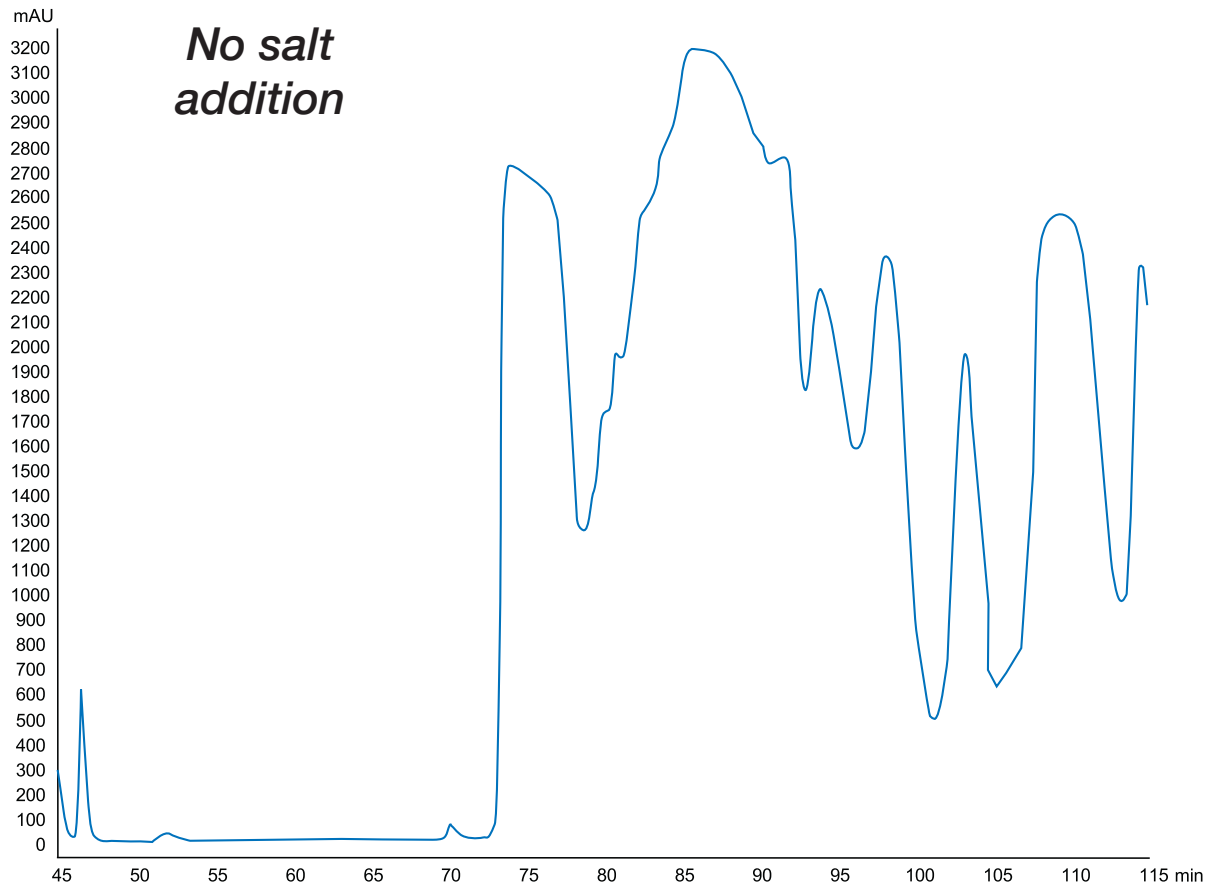
Conditions	Resolution of main peak and impurity peak
20 mM $HCOONH_4$-NH_3 (pH 8.5)	0.96
20 mM NH_4Cl-NH_3 (pH 8.5)	–
20 mM Tris-HCl (pH 8.5)	–

After determination of the most suitable buffer and pH, the concentration of the added buffer has to be optimised. In the **practical example**, different concentrations of the carbonate buffer, varying from 20–100 mM, were tested. The best separation of the main peak from the impurity was obtained with the lowest buffer concentration.

Expert Tip

Salt addition for increased resolution and recovery

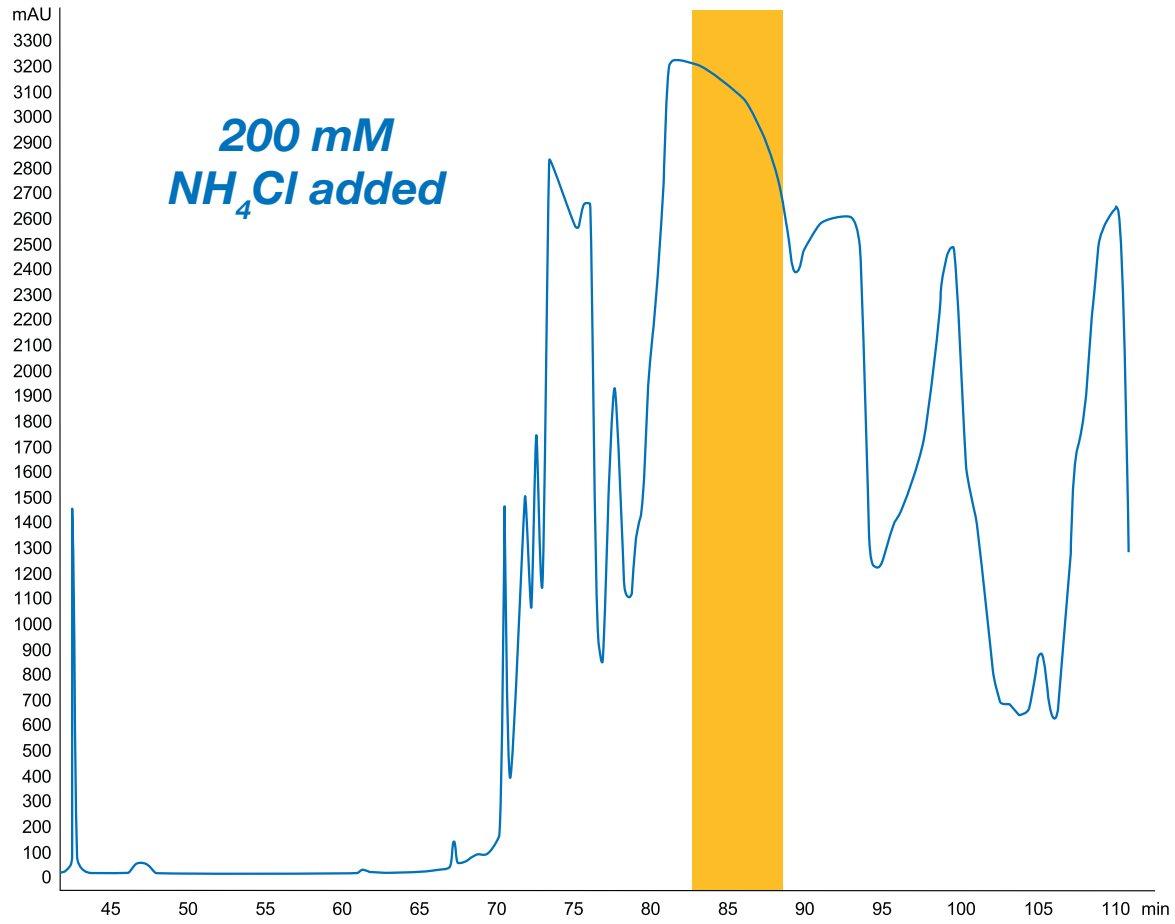
In many cases, the use of standard methods may not lead to sufficient separation of the peptides. In this example, the positive effect of adding a salt to the mobile phase is described for a peptide purification process. For this process, minimum purity and recovery values were defined. When the salt was not used as an additive, no fraction was found with the required purity in the following peptide purification process (Fig. 5).



Column Size:	YMC-Triart Prep Bio200 C8, 250 x 4.6 mm ID
Eluent:	A) buffer*/acetonitrile (90/10) B) buffer*/acetonitrile (10/90)
Gradient:	0%B (0–10 min), 0–20%B (10–15 min), 20–25%B (15–45 min)
Flow rate:	0.74 mL/min
Temperature:	ambient
Detection:	UV at 214 nm
Sample:	Crude insulin human recombinant (Purity 75.8%)

Figure. 5: Separation of crude insulin without salt addition.

When the salt was added to the mobile phase, the results were greatly improved (Fig. 6). The set criteria were exceeded in terms of purity and recovery. The results show this process allows a final purity of 99.7% (based on a crude sample with a purity of 75.8%) to be achieved! Additionally, high recovery values were achieved which exceeded the set criteria.



Column Size:	YMC-Triart Prep Bio200 C8, 250 x 4.6 mm ID
Eluent:	A) buffer*/acetonitrile (90/10)
	B) buffer*/acetonitrile (10/90)
Gradient:	0%B (0–10 min), 0–20%B (10–15 min), 20–25%B (15–45 min)
Detection:	UV at 214 nm
Flow rate:	0.74 mL/min
Temperature:	ambient
Sample:	Crude insulin human recombinant (Purity 75.8%)
*buffer:	20 mM CH_3COONH_4-CH_3COOH, 200 mM NH_4Cl (pH 4.3)

Figure 6: Separation of crude insulin with salt addition.

Adding a salt to the mobile phase greatly improved the separation in this peptide purification process. Without the salt added to the mobile phase, the set criteria for the purification were not fulfilled. However, with the salt added to the mobile phase, the purity and recovery easily fulfilled the set criteria for this process.

This effect of an improved separation has also been seen for other peptide-based compounds. It extends the range of possible options available for process optimisation. Therefore, the addition of additives such as a salt to the mobile phase is a good option in process development.

2.5. Gradient optimisation

After defining the optimum mobile phase for a peptide purification process, the elution conditions have to be fixed. Therefore, the choice between isocratic and gradient elution is the next critical step in method development. During isocratic elution, the same eluent composition is constantly used. The advantages of an isocratic elution are the possibility of stacked injections and that no re-equilibration time is needed between the purification cycles. Generally, isocratic elution is rarely used for peptides. Larger peptides such as insulin elute as broader peaks compared to small molecules and therefore isocratic elution would cause additional broadening of the peak resulting in increased fraction volume and cycle time.
A comparison of both elution types can be found in Fig. 7.

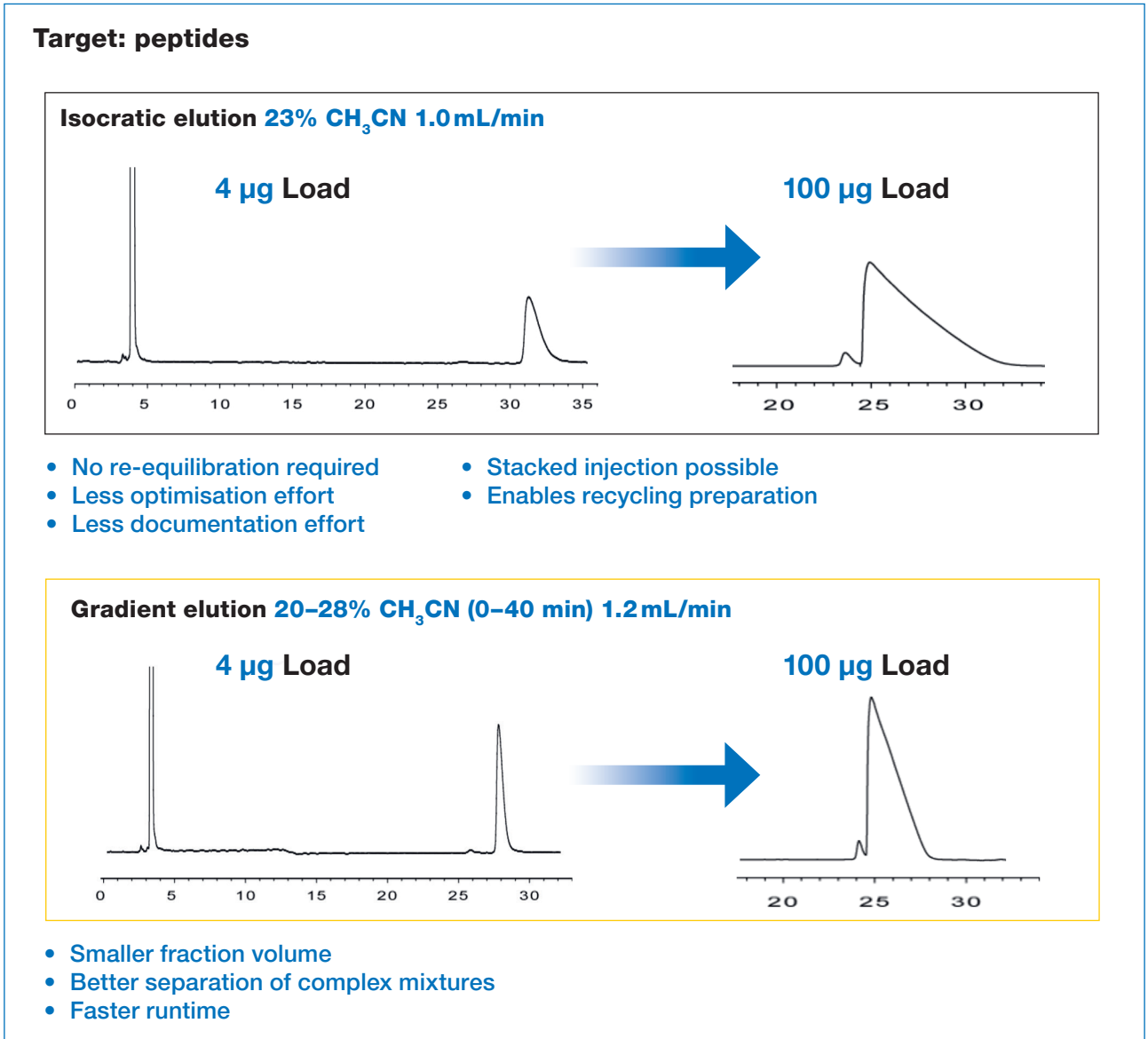
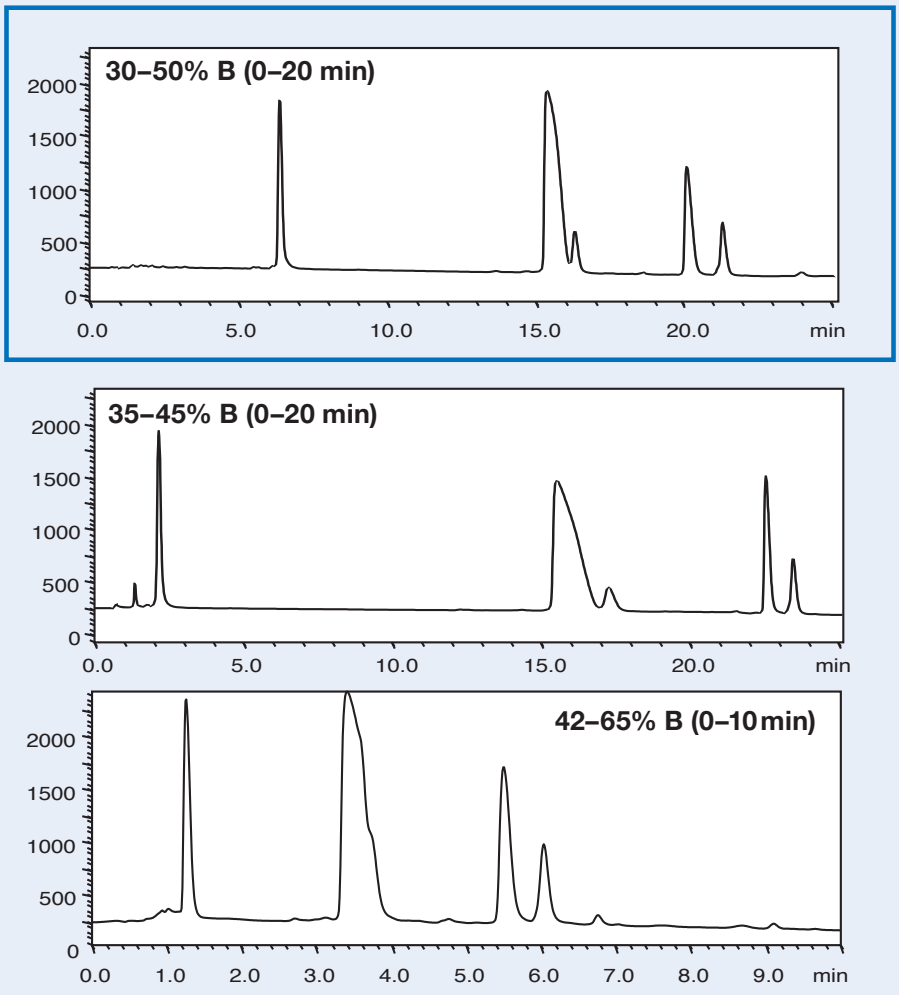


Figure 7: Differences between isocratic and gradient elution.

If the elution is performed using a gradient, the slope of the gradient has to be adapted to give an optimal balance of resolution and productivity.

In the **practical example** for process development shown below, the use of a gradient slope 30–50% B in 20 min was selected as the optimal balance between separation of the critical peak pair and fraction volume obtained.

Practical example of process development: gradient optimisation

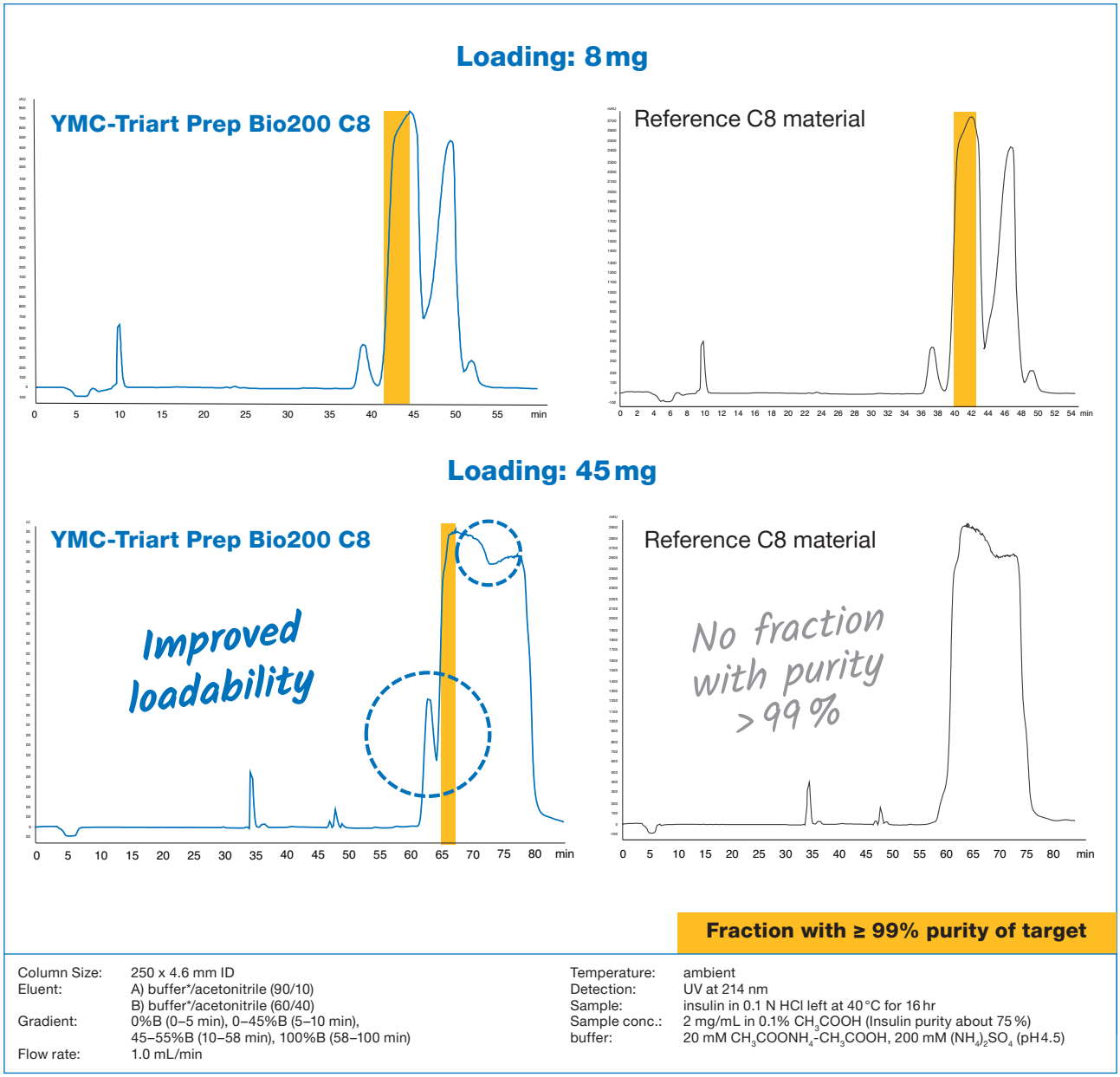


Column:	YMC-Triart C18 (3 µm, 12 nm), 100 x 3.0 mm ID
Eluent:	A) 20mM $HCOONH_4$-NH_3(pH 8.5)
	B) Acetonitrile
Flow rate:	0.43 mL/min
Temperature:	35 °C
Detection:	UV at 215 nm
Injection:	3 µL (Crude 20.5 mg/mL) = 61.5 µg loading

3. Loadability studies and interpretation of screening results

In addition to defining the process parameters such as mobile phase composition, loadability studies at analytical scale are the next step towards a highly productive preparative scale purification. Most phase screenings for preparative processes are carried out only using an analytical loading. Based on the results of this phase screening, the stationary phase offering the best resolution can be found. However, this may not necessarily be the best stationary phase for the preparative process. As preparative processes require high sample feed, loadability should also be evaluated during the phase screening. Only a combination of these parameters will lead to an economic and efficient process.

The loadability depends on the properties of the stationary phase as well as on the peptide itself and the impurity profile. In this case, a good separation of the critical peaks was achieved with both stationary phases. However, increased loading level shows that sufficient purity can only be obtained with a stationary phase material with an optimised pore size as illustrated in Fig. 8. Therefore, the addition of a study of increased loading to the phase screening is highly recommended.



Column Size:	250 x 4.6 mm ID	Temperature:	ambient
Eluent:	A) buffer*/acetonitrile (90/10) B) buffer*/acetonitrile (60/40)	Detection:	UV at 214 nm
		Sample:	insulin in 0.1 N HCl left at 40 °C for 16 hr
Gradient:	0%B (0–5 min), 0–45%B (5–10 min), 45–55%B (10–58 min), 100%B (58–100 min)	Sample conc.:	2 mg/mL in 0.1% CH_3COOH (Insulin purity about 75 %)
		buffer:	20 mM CH_3COONH_4-CH_3COOH, 200 mM $(NH_4)_2SO_4$ (pH 4.5)
Flow rate:	1.0 mL/min		

Figure 8: Loadability studies.

4. Scale up to large scale production

The final step in process development is the scale-up to preparative or even industrial scale. For detailed information regarding preparative scale-up including all relevant considerations and calculations and many further aspects, please refer to the YMC Whitepaper on method development strategies for prep LC!

Practical example: final purification separation of liraglutide

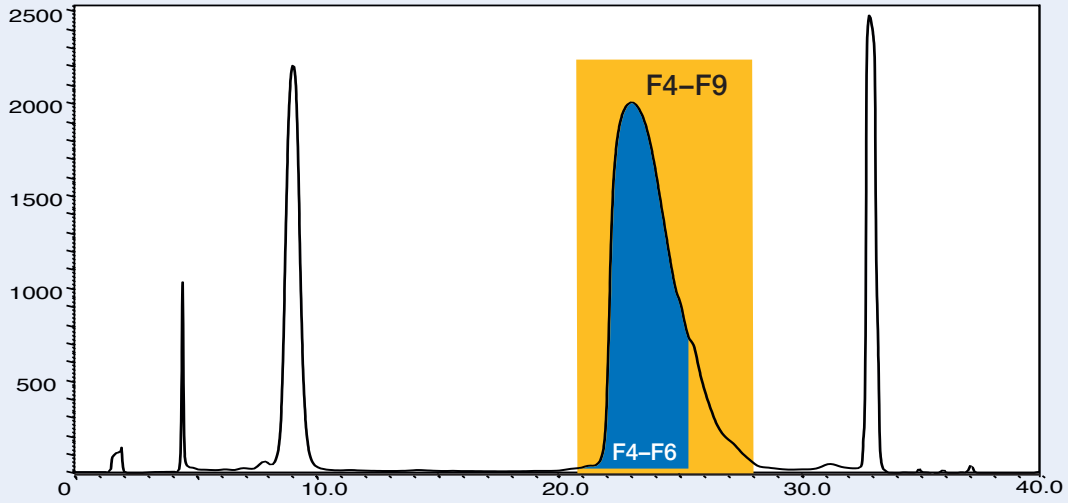
At this point all relevant parameters were optimised leading to a final purification method that can be scaled up to large scale.

Finally, a purification separation was performed with 60 mg loading using a 10 mm ID column leading to the desired purity with high theoretical recovery!

Additionally, the theoretical scale-up for a 600 mm ID DAC column was calculated.

Practical example of process development: final purification parameters

60 mg Loading – 250x10 mm ID column



Column: YMC-Triart Prep C18-S (10 µm, 12 nm), 250 x 10 mm ID
Eluent: A) 20 mM $HCOONH_4$-NH_3(pH8.5)
B) Acetonitrile
Gradient: 30–50%B (0–50 min)

Flow rate: 4.7 mL/min
Temperature: ambient
Detection: UV at 215 nm
Injection: 3 mL (Crude 20.0 mg/mL) = 60 mg loading (36.8 mg Liraglutide)

Theoretical Scaling Up Calculation

Column	YMC-Triart Prep C18-S (10 µm, 12 nm)		
Eluent	A) 20 mM $HCOONH_4$-NH_3 (pH 8.5) B) Acetonitrile 30–50% B (0–50 min)		
Detection	UV at 215 nm		
Temperature	Ambient		
Cycle time	60 min/run – 8 cycles/day		
Column dimension	250 x 100 mm ID	250 x 450 mm ID	250 x 600 mm ID
Flow rate	0.47 L/min	9.52 L/min	16.92 L/min
Loading/run	6.0 g	121.5 g	216.0 g
Fraction volume / run	1.4 L	28.6 L	50.8 L
Liraglutide recovery / run	2.6 g	53.4 g	94.9 g
Liraglutide recovery /day	20.8 g	427.2 g	759.2 g

Conclusion

The aim to purify and isolate any target peptide cost efficiently can be easily addressed with this very simple but comprehensive approach.
The method development is carried out at analytical scale allowing screening of different stationary phases in analytical format for a robust and reliable application.
Secondly, loading studies are also carried out at analytical scale, in order to reduce time. As both steps are kept to a small scale, the costs are also minimised.
The last step is to transfer the developed process to the required larger scale. The succes of such a process development strategy has been demonstrated using an actual peptide purification process!

Table 4: Correlation of column diameter, particle size, flow rate and loading for the scale up to preparative processes.

			Lab scale → Production scale								
Column inner diameter [mm ID]			**4.6**	10	20	30	50	100	200	500	1,000
Cross sectional area ratio			**1.0**	4.7	19	42	118	473	1,890	11,800	47,300
Example of calculation	**Flow rate** [ml/min]		**0.5**	2.4	9.5	21	60	235	950	6,000 (6 L)	24,000 (24 L)
			1.0	4.7	19	42	120	470	1,900	12,000 (12 L)	47,000 (47 L)
	Loading [mg]		**5**	25	100	220	600	2,500	10,000	60,000 (60 g)	240,000 (240 g)
Column efficency, Pressure, Costs HIGH / LOW	**Particle size** [µm]	**5**	+++	+++	+++	+++	++	+	+		
		10	++	+++	+++	+++	+++	++	++	++	++
		10–20	+	++	++	++	+++	+++	+++	++	++
		15–30		+	+	+	++	+++	+++	+++	++
		50~					+	++	++	+++	+++

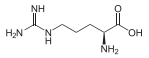
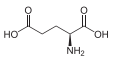
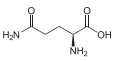
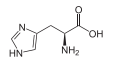
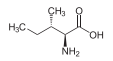
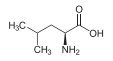
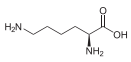
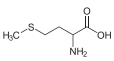
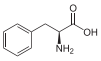
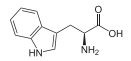
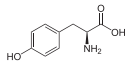
Flow rate equation (Use the same equation to calculate the sample load)

$F' = F \times (Dc'/Dc)^2$

F': Preparative column flow rate [mL/min]
F: Analytical column flow rate [mL/min]
Dc: Analytical column diameter [mm ID]
Dc': Preparative column diameter [mm ID]

+++ Most appropriate, ++ Appropriate, + Depending on purpose

Overview standard amino acids

Amino Acid	3 Letter Code	1 Letter Code	pKa1	pKa2	pKb	pI	MW (g/mol)	Structure
Alanine	ALA	A	2.34	–	9.69	6.00	89.10	
Arginine	ARG	R	2.17	12.48	9.04	10.76	174.20	
Asparagine	ASN	N	2.02	–	8.80	5.41	132.12	
Aspartic Acid	ASP	D	1.88	3.65	9.60	2.77	133.11	
Cysteine	CYS	C	1.96	8.18	10.28	5.07	121.16	
Glutamic Acid	GLU	E	2.19	4.25	9.67	3.22	147.13	
Glutamine	GLN	Q	2.17	–	9.13	5.65	146.15	
Glycine	GLY	G	2.34	–	9.60	5.97	75.07	
Histidine	HIS	H	1.82	6.00	9.17	7.59	155.16	
Isoleucine	ILE	I	2.36	–	9.60	6.02	131.18	
Leucine	LEU	L	2.36	–	9.60	5.98	131.18	
Lysine	LYS	K	2.18	10.53	8.95	9.74	146.19	
Methionine	MET	M	2.28	–	9.21	5.74	149.21	
Phenylalanine	PHE	F	1.83	–	9.31	5.48	165.19	
Proline	PRO	P	1.99	–	10.60	6.30	115.13	
Serine	SER	S	2.21	–	9.15	5.68	105.09	
Threonine	THR	T	2.09	–	9.10	5.60	119.12	
Tryptophan	TRP	W	2.83	–	9.39	5.89	204.23	
Tyrosine	TYR	Y	2.20	10.07	9.11	5.66	181.19	
Valine	VAL	V	2.32	–	9.62	5.96	177.15	

Regulatory Affairs Support

Detailed information regarding regulatory affairs support can be found in the YMC Whitepaper on method development strategies for preparative LC – request via the YMC homepage or directly get in contact with your YMC representative.

Training and Education Opportunities

It is recommended that continued training is carried out for all staff involved in preparative chromatography. Specifically in this complex and cost intensive field, improved skills will result in better efficiency, safety and reliability for the daily work. YMC training courses can be provided for all types of liquid chromatography, from analytical to process scale, and from basic principles to advanced application support. If you would like to address specific tasks, we will be happy to supply a tailored seminar schedule for you.


Online Seminar:
Introduction to Preparative HPLC

YMC EUROPE GMBH

Learn all the basics of preparative chromatography!

Contents and learning objectives
- Differences between analytical and preparative chromatography
- Important preparative process parameters: purity, yield and productivity – the "triangle" of preparative chromatography
- Outline of preparative method development: three steps to your preparative method

Target Group
All chromatography operators who are new to the field of preparative chromatography

Date
20.11.2024, 11 am (CET), duration 2 hours

Price (net)
235 € / 285 CHF per participant
including seminar documents and certificate of participation

Platform
You will receive your invitation to the online seminar platform (Cisco Webex) shortly before the seminar date.

Please contact us for further information and inquiries:
Dr. Mathias Hahn
Head of Laboratories
Phone: +49 2064 427-283
Fax: +49 2064 427-222
Email: m.hahn@ymc.eu

www.ymc.eu

We can also organise training sessions at your site:
- Duration and topics by agreement
- Flexible numbers of attendees
- Fixed costs

Target group
All staff involved in preparative chromatography
(laboratory assistants, technicians, engineers, chemists)

You can find actual dates for seminars in our seminar flyer at www.ymc.eu
or you can contact us directly:
Phone: +49 2064 427-0
Fax: +49 2064 427-222
Email: info@ymc.eu

Laboratory Services

If you are working on a tight schedule or you do not have any experience in column packing there is a solution! Rely on the experience of YMC and ask for a packed column. Our knowledge of packing is your benefit!

Our services include:

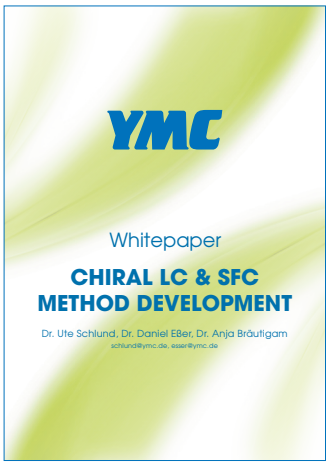
- Packing of analytical-scale columns with preparative bulk materials for convenient method development, phase screening and reliable, effortless scale-up (ID of up to 20 mm)
- Packing of semi-preparative YMC-Actus columns with axial compression technology for excellent column stability and efficiency (ID of 20, 30, or 50 mm)
- Packing of small-scale preparative columns (ID from 50 to 200 mm)
- Custom purifications according to your individual requirements

How it works:

Further information

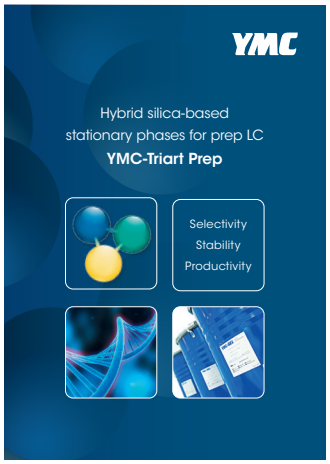
Whitepaper
Method development strategies

Whitepaper
Cleaning and Cleaning Validation
of Glass Columns



Whitepaper
Chiral LC/SFC

Also of interest



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YMC-Triart Prep

Brochure
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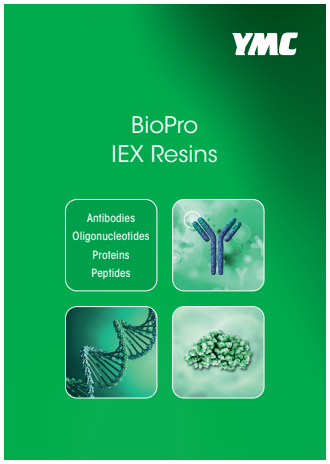
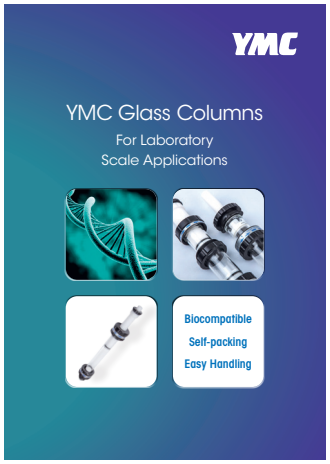
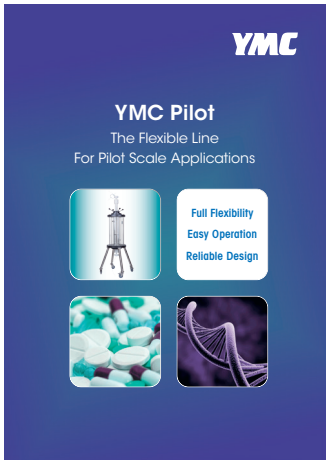


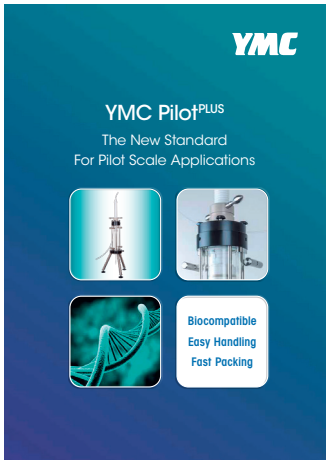
Brochure
BioPro IEX Resins



Brochure
YMC Glass Columns



Brochure
YMC Pilot



Brochure
YMC Pilot^{PLUS}

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Whitepaper prep_peptide_02/2025_Int_V1.0