

# STRATEGIC PEPTIDE PURIFICATION

## PRACTICAL AND THEORETICAL ASPECTS OF METHOD DEVELOPMENT USING REVERSED PHASE CHROMATOGRAPHY



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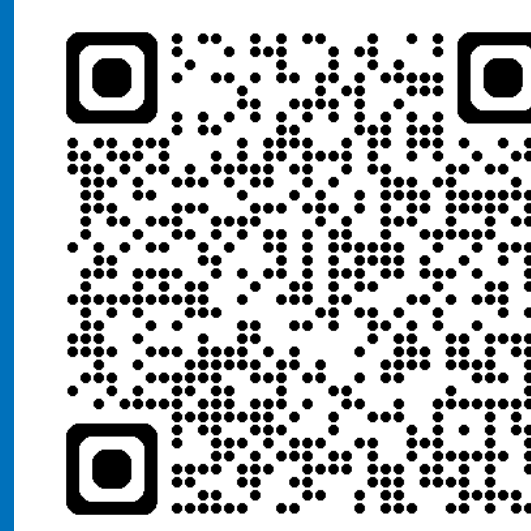
The broad functional spectrum of peptides opens up numerous opportunities for pharmaceutical applications. Chain length, amino acid composition, secondary structures, and modifications result in a wide range of sizes and physicochemical properties. A precise purification strategy that considers the complex impurity profile of a peptide is therefore essential. Regardless of whether the molecule is produced through chemical synthesis or recombinant methods, reversed-phase chromatography has established itself as the preferred method. It allows for the accurate separation of structural variants and even peptides with minimal sequence differences. The detailed guidelines presented here focus on maximising yield, reducing process times, and ensuring economic feasibility. While milligram to gram quantities are isolated in smaller scales, industrial production requires quantities ranging from kilograms to tons. The approach presented here is based on the linear scaling method.

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

- Step 1: Method development at analytical scale
- Step 2: Loadability studies at analytical scale
- Step 3: Scale-up to the preparative process

Read more about all steps in method development for peptide purification:

**Whitepaper: Strategic peptide purification**  
Including practical example: The complete method development for the purification of Liraglutide

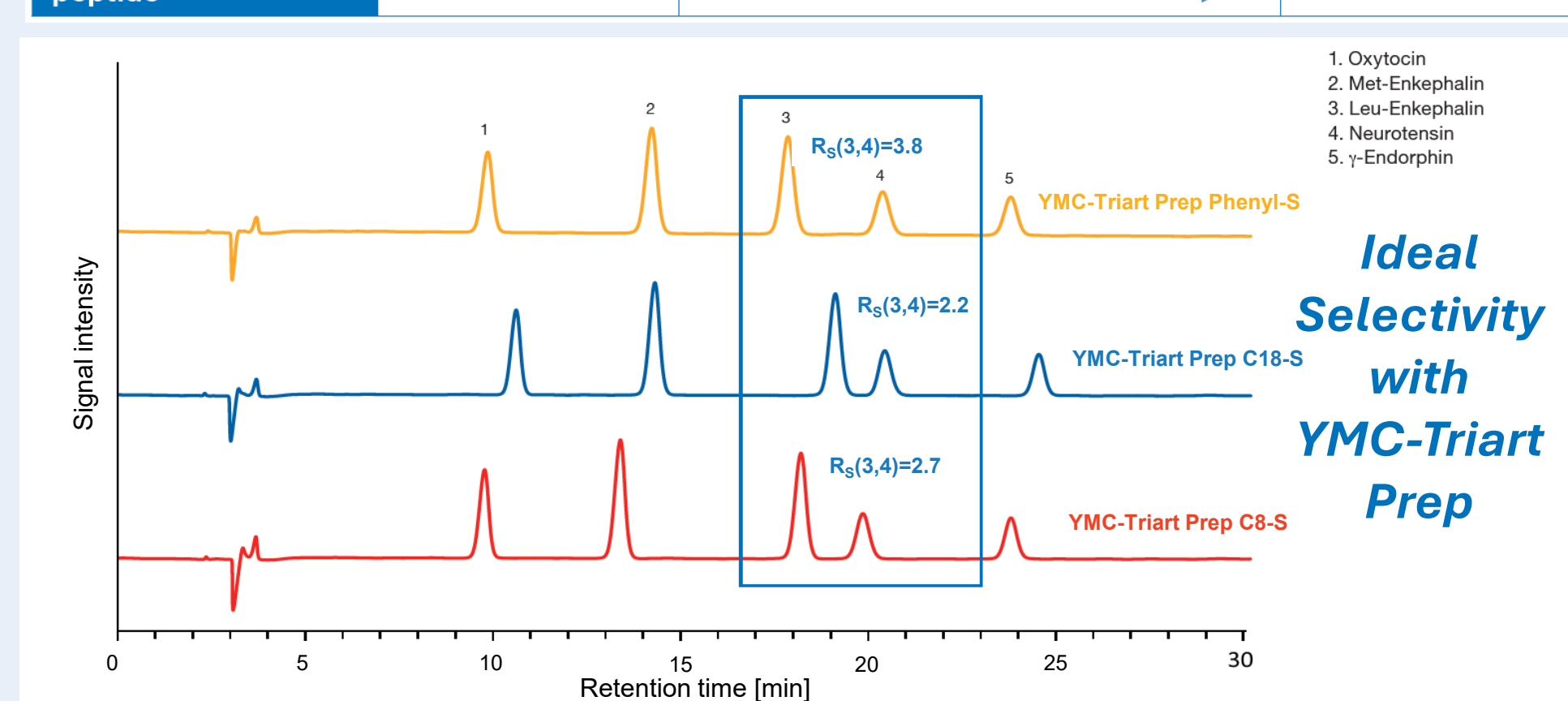


### Step 1: Method Development at Analytical Scale – Stationary Phase Selection

#### Phase Modification

The first step is the identification of an optimal stationary phase ligand. This mainly depends on the content of hydrophobic amino acid side chains, potential secondary structures of the peptide and additional modifications.

	C18	C8	Phenyl	C4
Functional group	-C <sub>18</sub> H <sub>37</sub>	-C <sub>8</sub> H <sub>17</sub>		-C <sub>4</sub> H <sub>9</sub>
Hydrophobicity	High	←		Low
Hydrogen bonding capacity	Low		→	High
Surface recognition ability	High	←		Low
Suitable MW of the peptide	Low		→	High

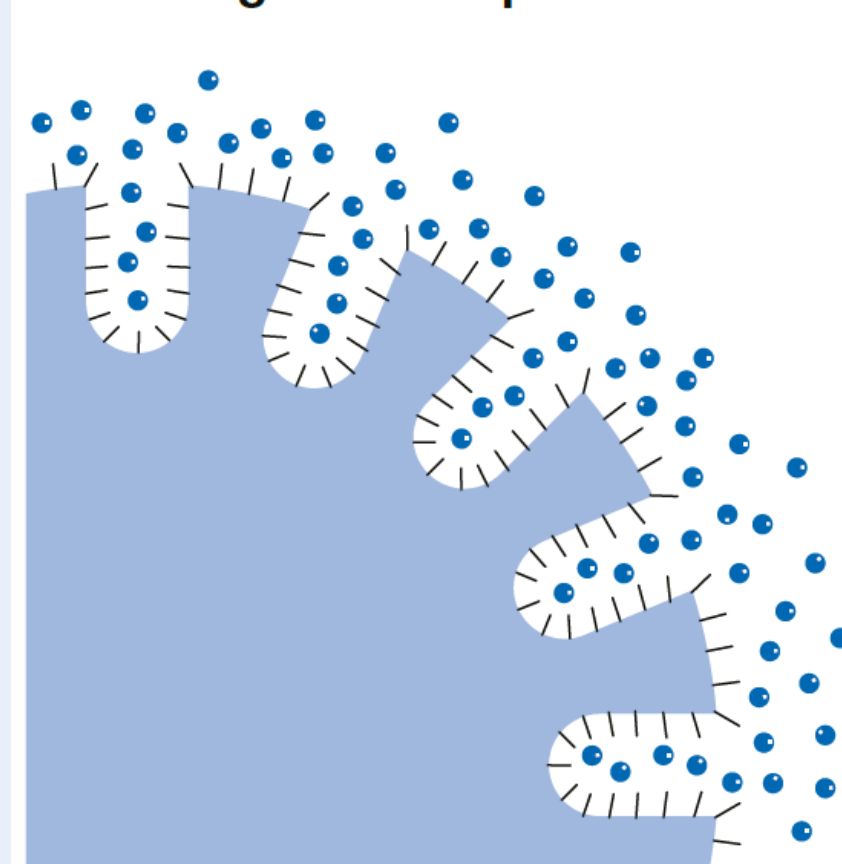


#### Pore Size

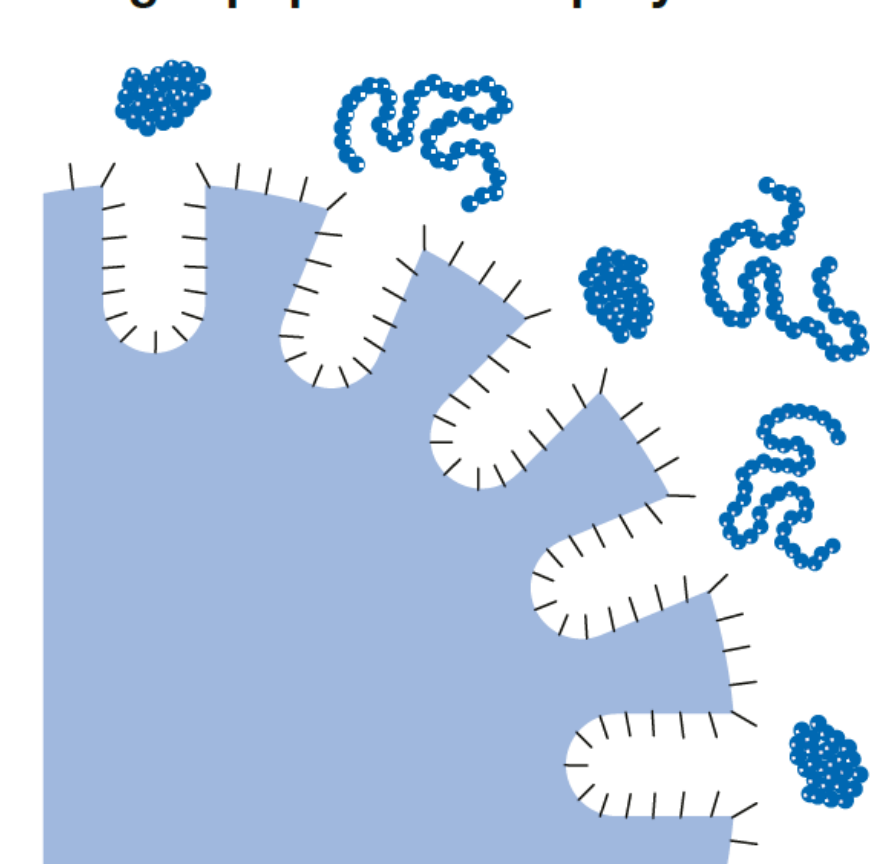
The pore size has to be chosen carefully to achieve an optimal separation effect. Generally, the pore size should be as small as possible but large enough for separation efficiency. Additionally, the pore size determines the loadability, because with increasing pore sizes, the surface area decreases.

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

Small organic compounds

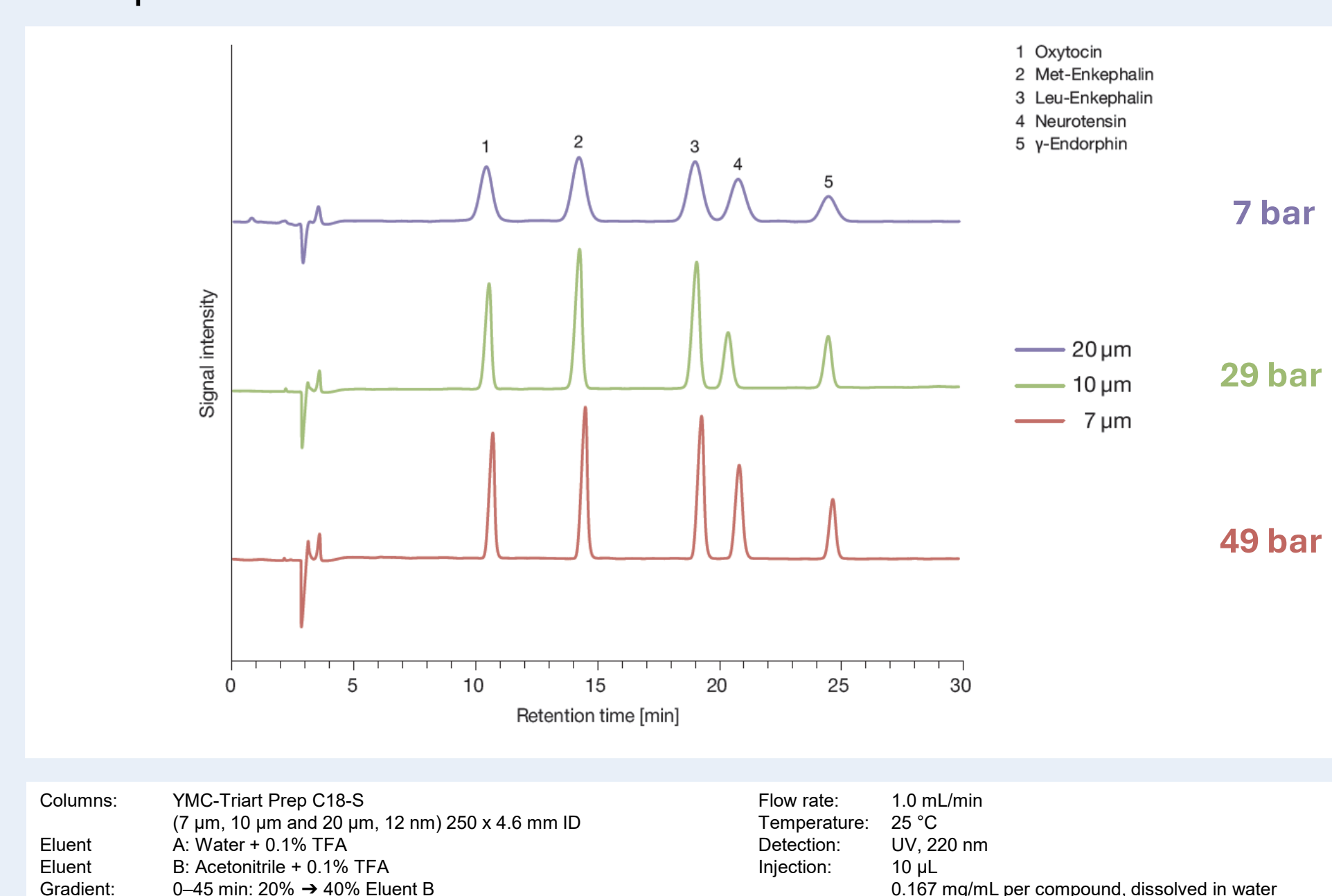


Larger peptides and polymers



#### Particle Size

The particle size of the stationary phase determines the resolution and also the productivity of the overall process. Smaller particles in general provide higher resolution. On the other side, they generate elevated backpressures. Therefore, the particle size selection is a compromise between sufficient resolution and resulting backpressure.



**Expert Tip: Easy selection of the right selectivity due to flexible stationary phases**

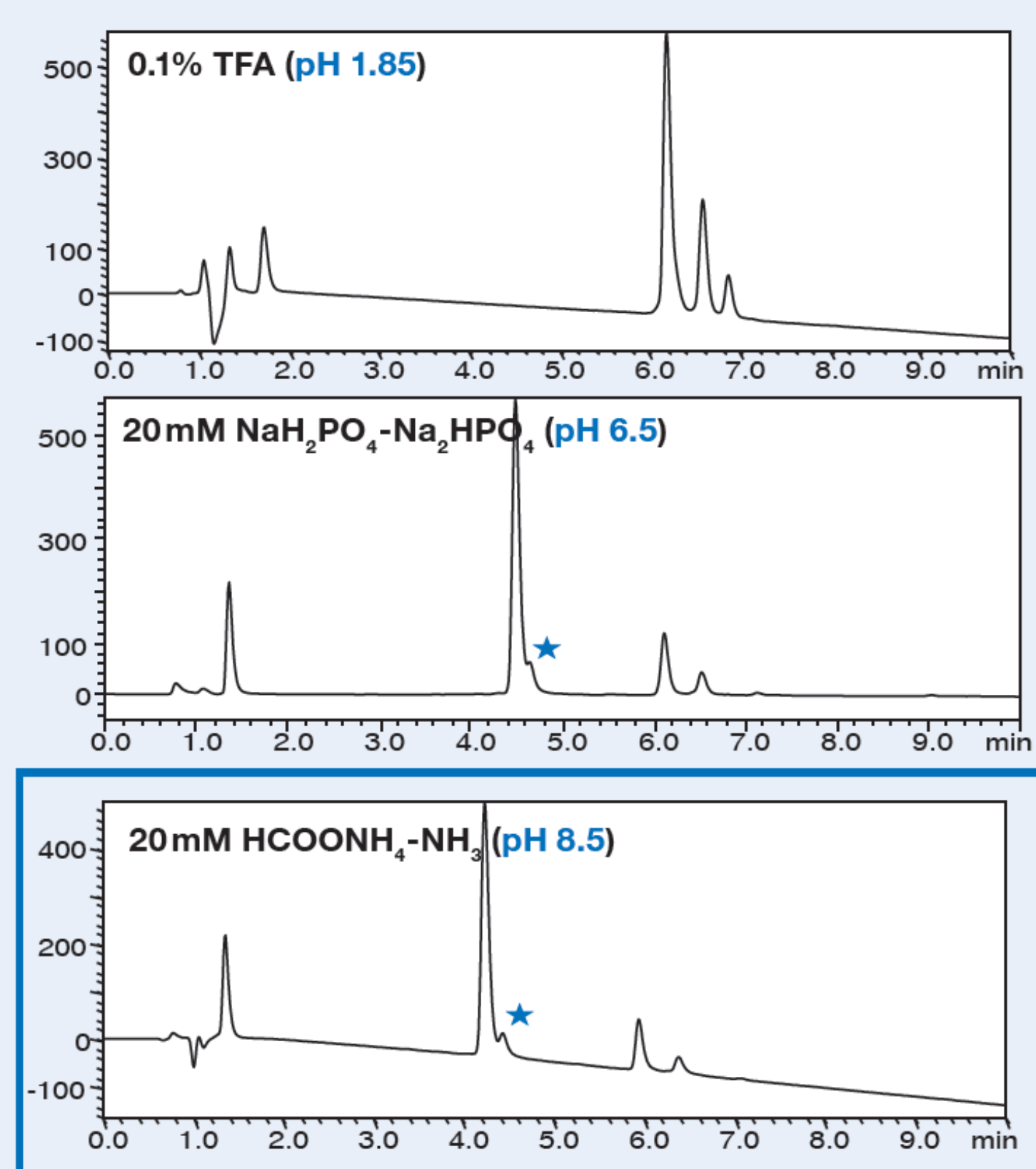
The innovative RP stationary phases YMC-Triart Prep are based on a hybrid-silica base and are available with different modifications from C4 to C18 covering all relevant ligands for peptide purification. With various combinations of pore and particle sizes, YMC-Triart Prep allows full flexibility in method development and therefore leads to tailored solutions for all types of purification processes.

### Mobile Phase Selection

The mobile phase composition influences chromatographic resolution. Therefore, determining the most appropriate elution conditions are critical steps in process development. The following parameters need to be adjusted to identify the best elution conditions

- organic solvent and amount
- pH considerations
- buffer type selection
- additives and salts
- gradient/isocratic elution optimisation

This example shows the separation of Liraglutide at different pH values. The alkaline elution conditions provide the best selectivity in this case.



**Expert Tip: Choose a stationary phase with alkaline stability for more flexibility**

YMC-Triart Prep provides high chemical stability even at elevated pH. Due to this stability, purifications at alkaline pH like in the example above are possible. This drastically increases the possible method set-ups.

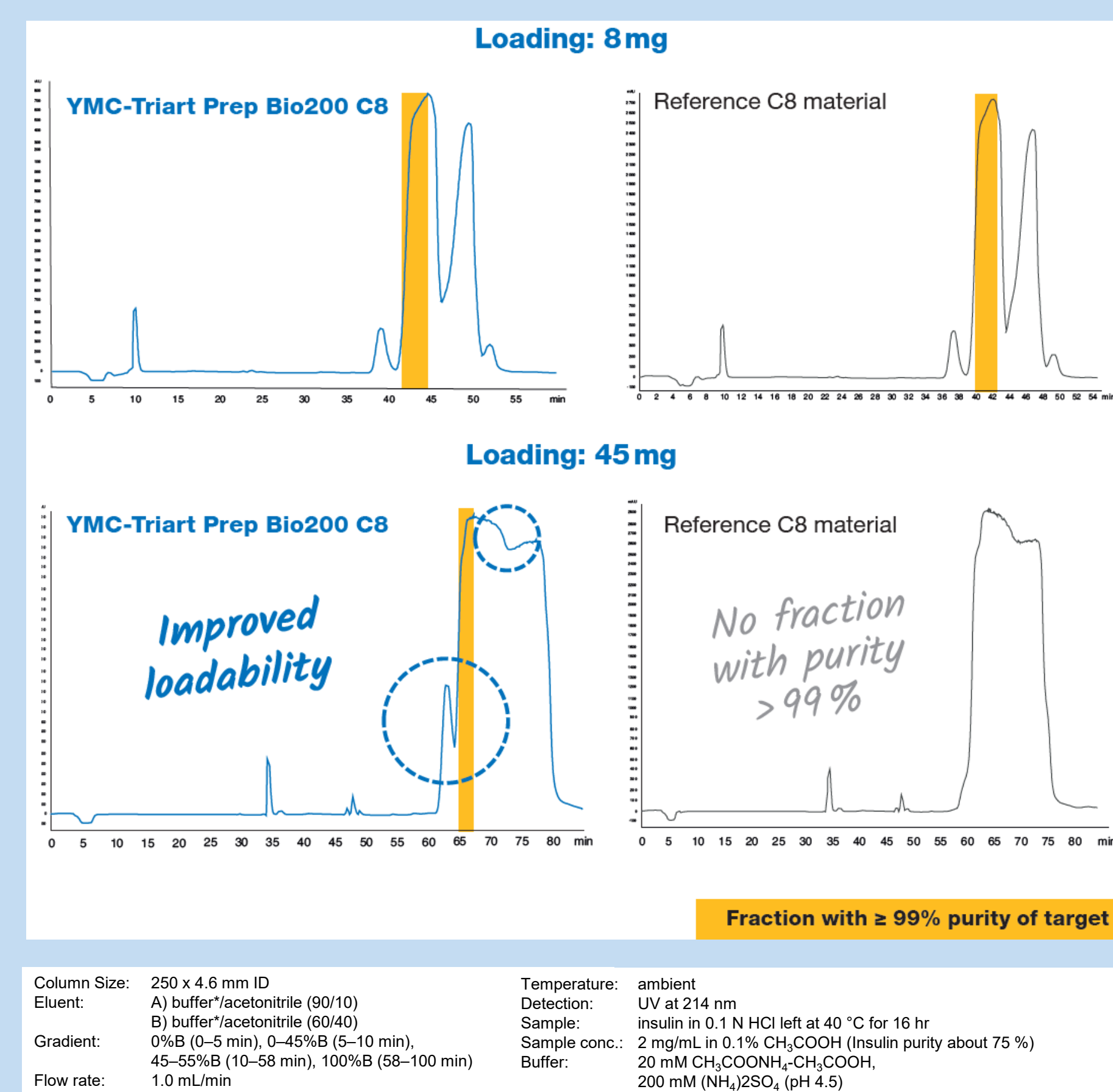
**Additional benefit: With YMC-Triart Prep, Cleaning-in-Place (CIP) procedures with 0.1 M NaOH are possible which restore the column performance and increase the column lifetime.**

### Step 2: Loadability Studies at Analytical Scale

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.

As preparative processes require high sample feed, loadability should also be evaluated during the phase screening.

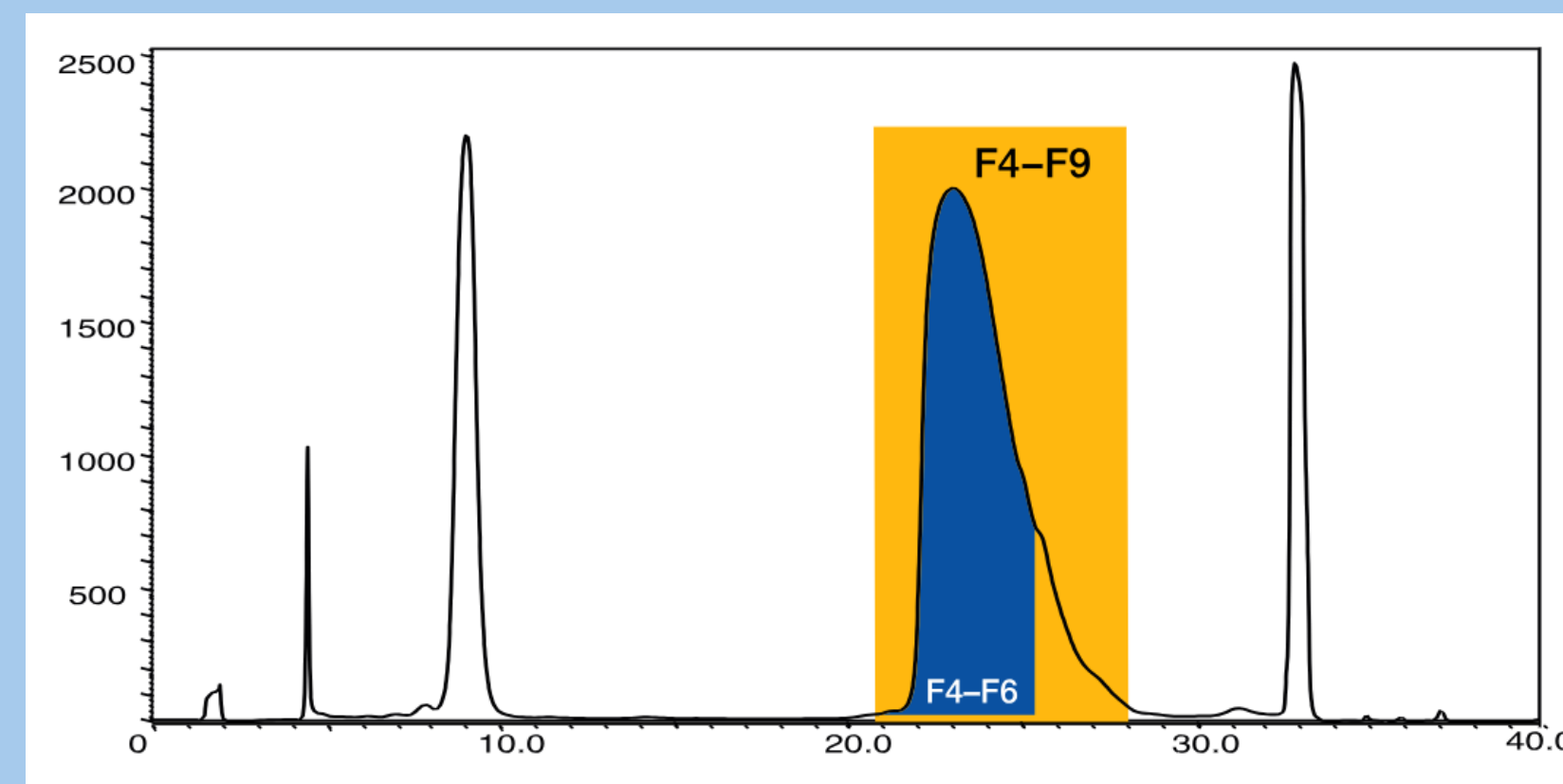
The loadability depends on the properties of the stationary phase as well as on the peptide itself and the impurity profile. In this case, a good separation of the critical peaks was achieved with both stationary phases. However, increased loading level shows that sufficient purity can only be obtained with YMC-Triart Prep and the optimized pore size and selectivity.



**Expert Tip: Choose a stationary phase that allows for high sample loadings and provides high resolution**

YMC-Triart Prep leads to high resolution results. Based on the large portfolio, the ideal stationary phase can be selected from the YMC-Triart Prep family identifying the optimal selectivity. Due to its optimised particle technology, high sample loadings are possible with YMC-Triart Prep.

### Step 3: Scale-Up to Final Process Scale



Column: YMC-Triart Prep C18-S (10 µm, 12 nm), 250 x 10 mm ID  
Eluent: A) 20 mM HCOONH<sub>4</sub>-NH<sub>3</sub> (pH 8.5), B) Acetonitrile (10-50 min)  
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)

The final step in process development is the scale-up to preparative or even industrial scale. At this point all relevant parameters were optimized leading to a final purification method that can be scaled up to large scale.

For detailed information regarding preparative scale-up including all relevant considerations and calculations and many further aspects, please refer to the YMC Technical Note on linear scale-up.

### Conclusion

- ✓ This efficient yet comprehensive concept makes it possible to easily achieve the goal of cost-effective purification of a target peptide. The method development is carried out on an analytical scale so that various stationary phases can be tested robustly and reliably.
- ✓ In addition, loadability studies are carried out on an analytical scale to reduce the process time. Since both steps are kept small, the costs also remain low.
- ✓ In the final step, the developed process is transferred to the required larger scale. The success of this development strategy was impressively demonstrated using a real peptide purification process!

**Expert Tip: Choose a stationary phase that is available in large scale quantities for a safe and reliable supply: YMC-Triart Prep is the ideal stationary phase for peptide purification. The innovative hybrid-silica base material makes this phase highly chemically and mechanically stable. Together with the long lifetime, this directly leads to increase productivity. Discover the YMC-Triart Prep family and get your free sample now!**

