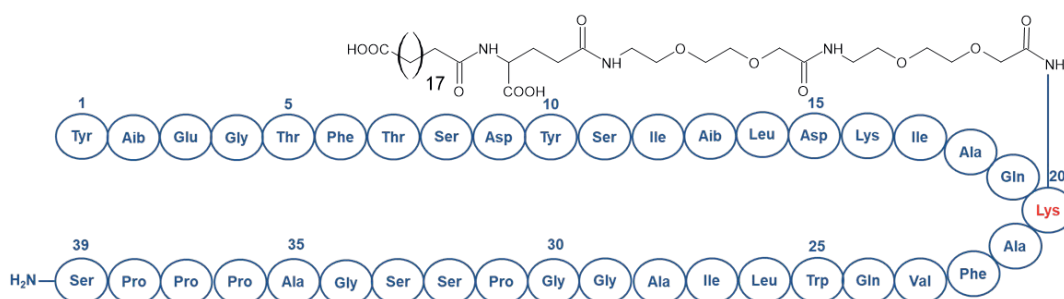


# Two-Step RP Purification of Tirzepatide: The Benefit of High pH in GLP-1 Purification Processes

## Background

GLP-1 Agonists are the most important peptide therapeutics nowadays. Their revolutionary potential as antidiabetic and anti-obesity drug led to an extensive demand of the peptide drugs worldwide. Besides Semaglutide and previously Liraglutide, Tirzepatide is the most important GLP-1 derived peptide. It consists of a polypeptide chain with 31

amino acids and a long fatty acid chain. The lipopeptide is challenging regarding efficient purification strategies and is required in excessive amounts. Here, we demonstrate a purification strategy using RP that can easily be scaled-up based on the YMC-Triart Prep family.

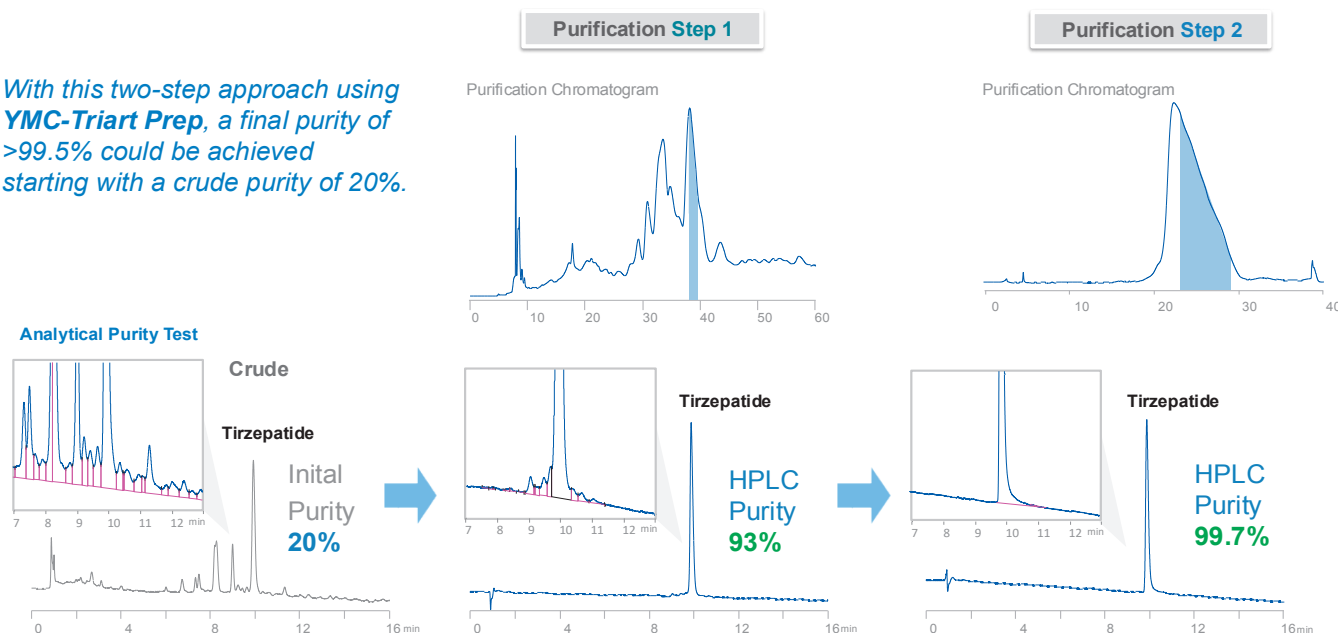


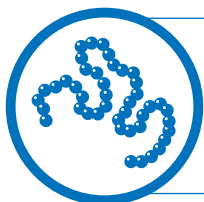
## Purification Process: A Two-Step RP Purification Approach

In this approach, a two-step purification procedure was applied. The crude material has a purity of 20% which requires a multi-step purification to achieve the final target purity of >99%. The first step was performed with

YMC-Triart Prep Bio200 C8 and the subsequent one with YMC-Triart Prep C4-S. The combination of these different modifications led to a successful purification.

*With this two-step approach using YMC-Triart Prep, a final purity of >99.5% could be achieved starting with a crude purity of 20%.*

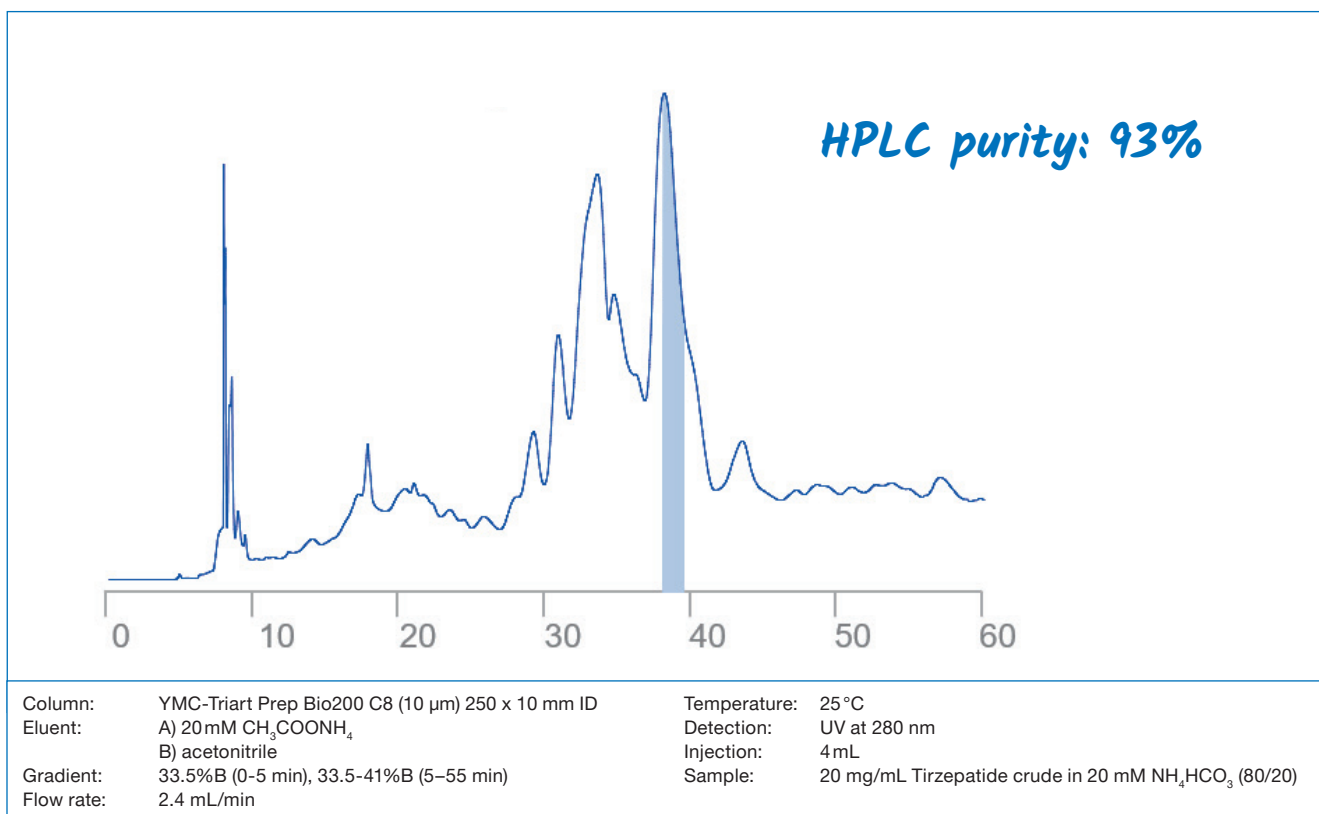




## Purification Step 1: Removal of Bulk Impurities

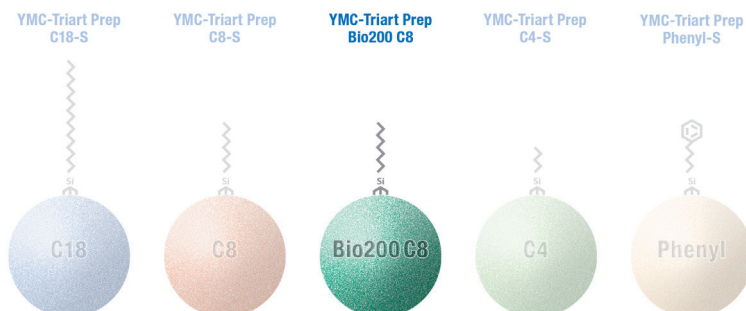
The initial purification step was carried out with the crude Tirzepatide material. The crude had a purity level of 20%. The method development was performed at analytical scale screening the complete YMC-Triart Prep set-up. The best results regarding separation efficiency, target purity and recovery rate were obtained with YMC-Triart Prep Bio200 C8, a C8-modified stationary phase with lower hydrophobicity

and optimised pore for crude peptide purifications. During method development the chromatographic parameters like mobile phase composition and organic modifier were adapted to improve the overall separation results. Subsequently, the loading was increased to improve the productivity of the purification.



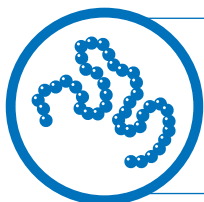
The final fractionation was optimised and yielded in a target purity level of 93% starting from a very low purity of initially 20% of the crude. This chromatographic purification step with YMC-Triart Prep Bio200 C8 leads to a massive improvement

of the purity level of Tirzepatide. To achieve the required final purity of >99.5%, a polishing step is required. This can also be performed with YMC-Triart Prep.



Get your complete screening set-up!

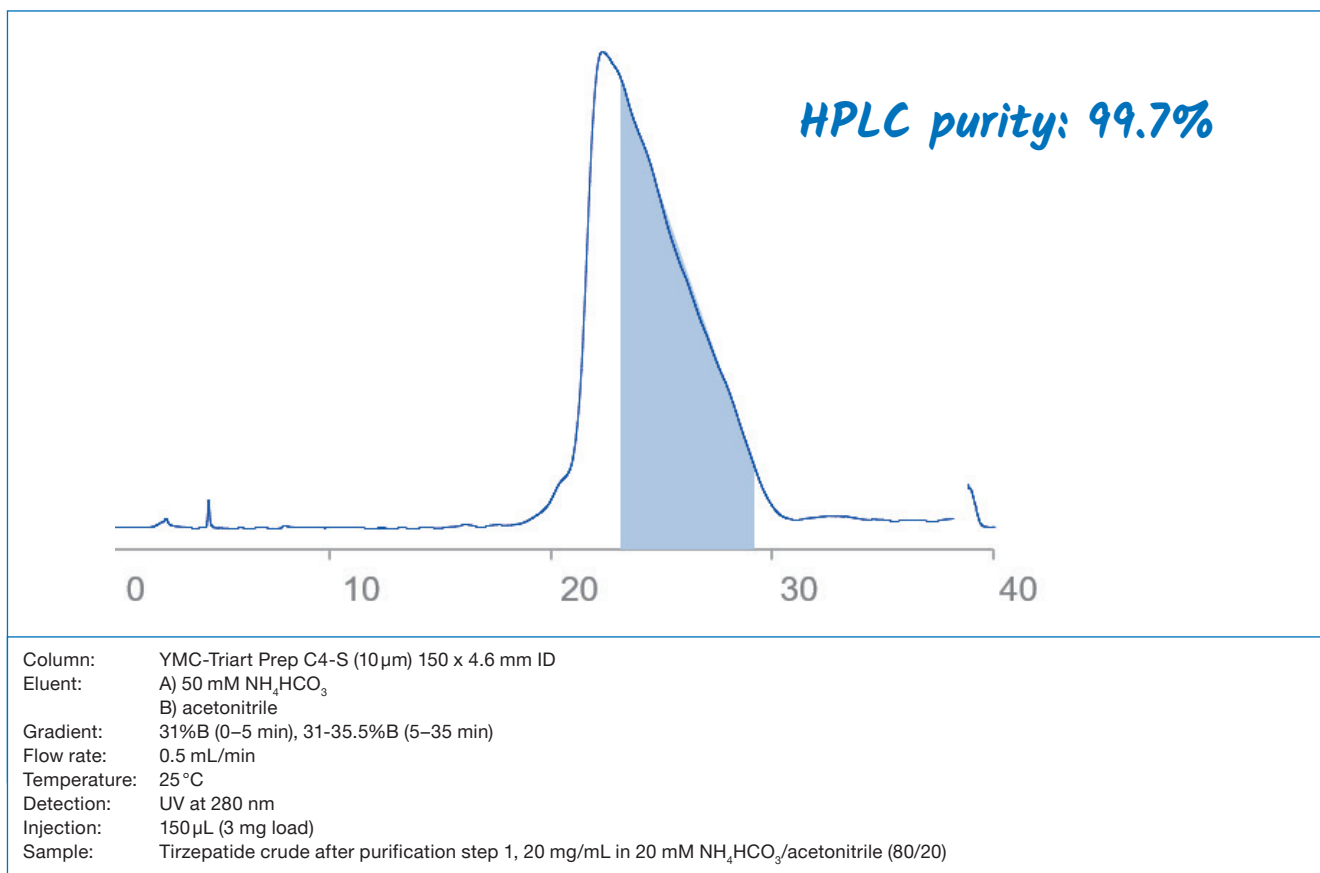




## Purification Step 2: Final Polishing

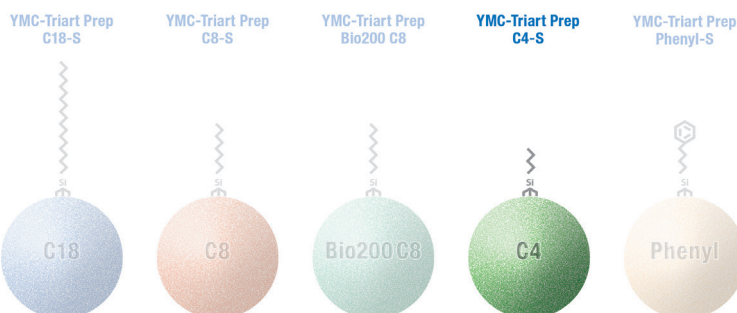
The second chromatography step in this purification process was a polishing step via RP. This step was carried out with YMC-Triart Prep C4-S which gave the best results in the stationary phase screening in combination with adjusting the method parameters. The purification was performed at pH 8.0 leading to great separation results. This is possible due

to the innovative hybrid-silica base of the YMC-Triart Prep stationary phases. The C4 modification seems to be the ideal modification for a polishing purification of Tirzepatide and also other GLP-1 peptides as it provides the ideal hydrophobicity for these challenging peptides.



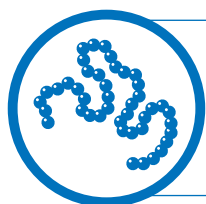
This second step yielded in a final product purity of 99.7%. With this second step, a highly efficient purification process could be established using high pH as a key factor for

GLP-1 purification especially for the polishing step. With conventional silica-based RP phases, this is not possible.



*YMC-Triart Prep: first choice for GLP-1 derived peptides!*

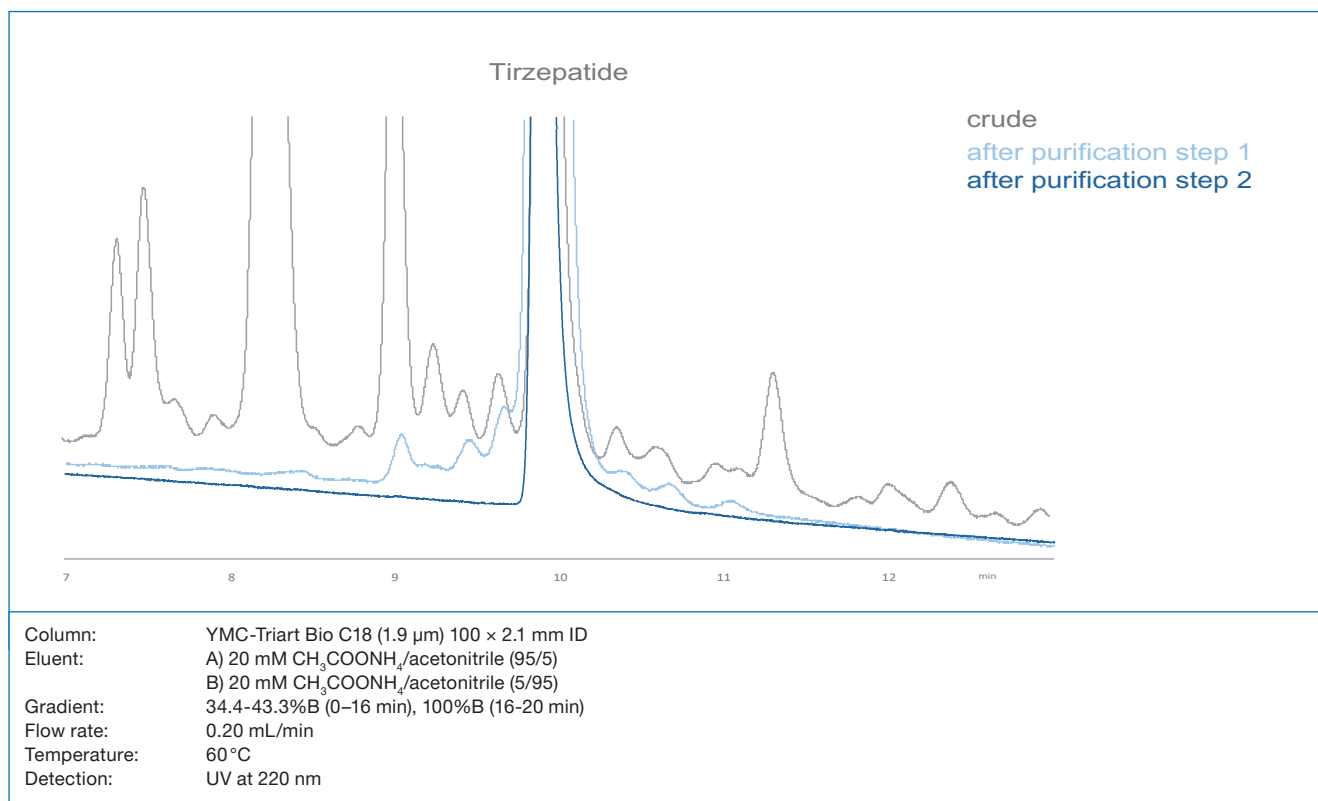




### Analytical Process Control: YMC-Triart at Small Scale

The analytical runs were also performed with a YMC-Triart material at UHPLC scale. Here, YMC-Triart Bio C18 (1.9  $\mu\text{m}$ ) in analytical dimensions was used. The YMC-Triart series is also available as analytical material leading to full flexibility in method development. Beside the purification process

development, the process control can also be done with the highly stable YMC-Triart series. In this case, the analytical control perfectly monitors the purity level of the Tirzepatide crude and purification fractions.



### Conclusion

This two-step process approach is perfectly suited for an efficient purification of Tirzepatide. The YMC-Triart Prep series offers a broad spectrum of modern process RP phases. A screening of these phases leads to the identification of the ideal stationary phase modification for GLP-1- derived peptides. In this case, YMC-Triart Prep Bio200 C8 is the best stationary phase for the initial purification of crude Tirzepatide

with low purity levels. For a polishing step, YMC-Triart Prep C4-S gave the best results leading to a final purity level of >99% measured by HPLC. The possibility to increase the pH of the mobile phase is a key factor in the purification of these type of peptides and is possible with the highly stable YMC-Triart series.

**Learn more about method development for peptides such as GLP-1 agonists in the YMC Whitepaper**  
Including a practical example of the GLP-1 peptide Liraglutide

