APPLICATION NOTE

Purification of Peptides with Full Flexibility

Purification is the most critical step in the manufacturing process of **peptide therapeutics**. The right choice of chromatography media is crucial for **cost-effective production**. With its **wide pH range** (pH 2-10), YMC-Triart Prep C18-S provides you with **full flexibility** in the method development of peptide purification. **Simple scale-up** procedures ensure the reproducible result at manufacture-scale. A method for the purification of liraglutide with high resolution (antidiabetic peptide therapeutic, marketed by Novo Nordisk as Victoza®.) was successfully developed with YMC-Triart Prep C18-S under alkaline condition. The purity obtained for the target compound was 99.5%.

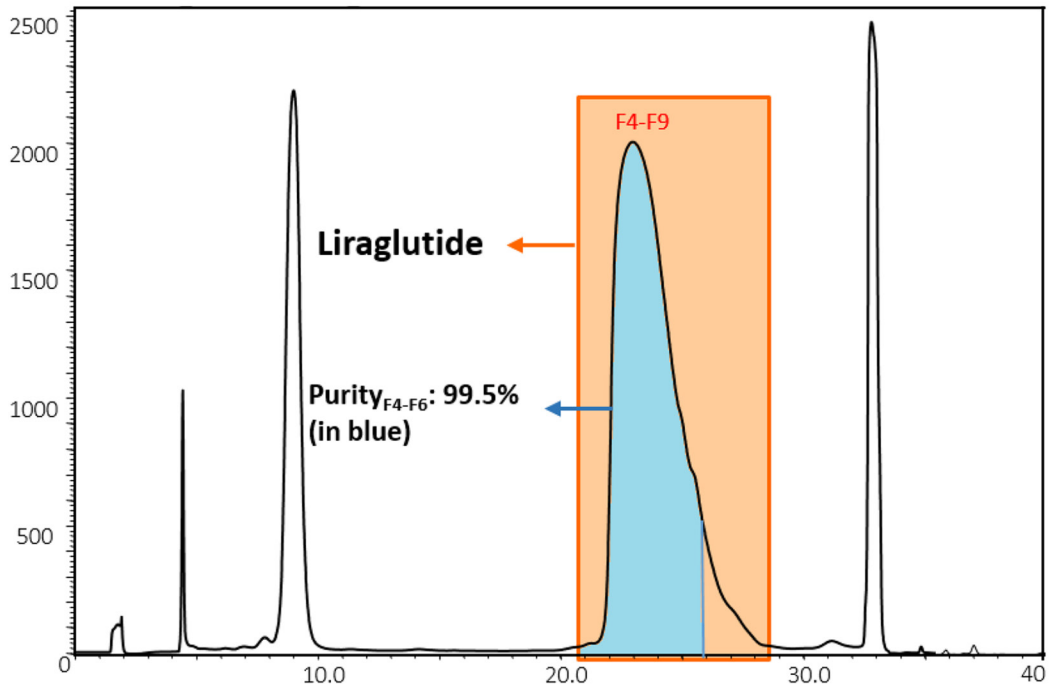


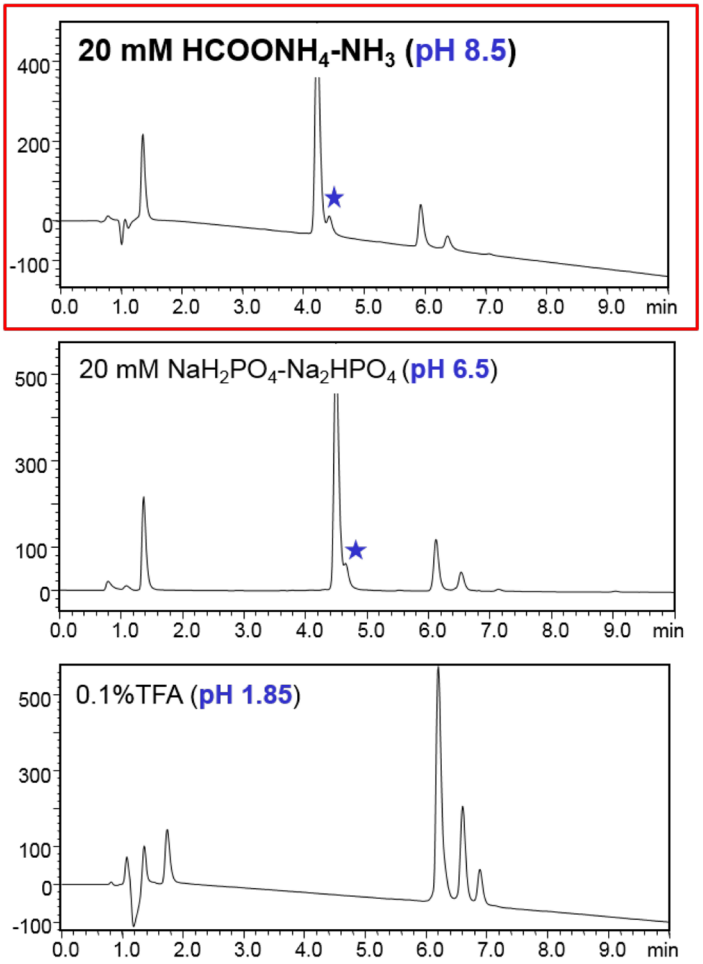
Figure 1:
The purification of liraglutide with YMC-Triart Prep C18-S (10 µm, 12 nm, 250 x 10 mm ID).

Column:	YMC-Triart Prep C18-S (10 µm, 12 nm, 250 x 10 mm ID)
Eluent:	A) 20 mM $HCOONH_4$-NH_3 (pH 8.5)
	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)

Purification of Peptides with Full Flexibility

Improved Resolution under alkaline condition

During the scouting process of liraglutide purification, under alkaline condition (pH 8.5), the chromatogram shows an obvious better separation of the peaks with a new impurity peak also appearing.



* Impurity: ★ marked component

Figure 2:
Optimization of pH for the purification of liraglutide.

Column:	YMC-Triart C18 (3 µm, 12 nm, 100 x 3.0 mm ID)
Eluent:	A) Different buffer showed in the figure 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

Purification of Peptides with Full Flexibility

Easy Scale-up with YMC-Triart Prep

The developed method with YMC-Triart Prep can easily be scaled-up. Below is an example of the theoretical scale-up calculation for the developed method for liraglutide purification with YMC-Triart Prep C18-S. With an YMC-Triart C18-S (250 x 600 mm I.D.) column, up to 800 g liraglutide can be purified per day.

Table 1: Scale-up calculations for liraglutide purification.

Column	YMC-Triart C18-S (10 µm, 12 nm)		
Eluent	A) 20 mM $HCOONH_4$-NH_3 (pH 8.5)	B) Acetonitrile	
Gradient	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

Conclusions

Benefits of YMC-Triart Prep for liraglutide purification:

- **An optimized method at high pH with improved resolution**
- **Up to 4-fold longer lifetime than conventional silica materials**
- **High loadability and high productivity**
- **Easy scale-up procedures**