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%.

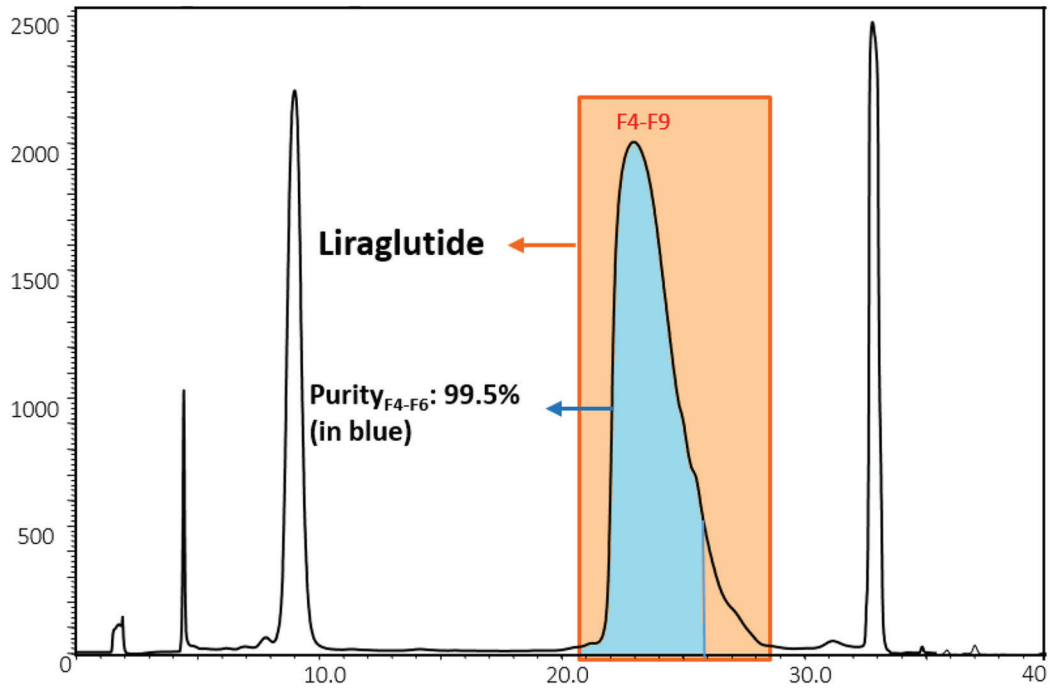

Figure 1:
The purification of liraglutide with YMC-Triart Prep C18-S (10 µm, 12 nm, 250 x 10 mm ID).

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)

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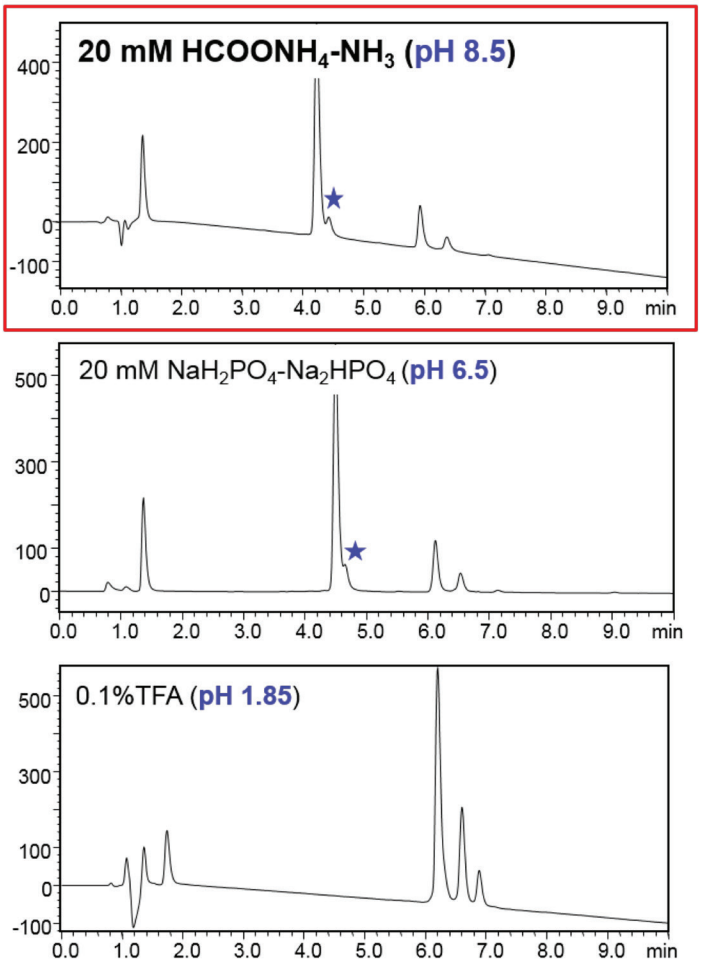
Improved Resolution under alkaline condition

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

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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**