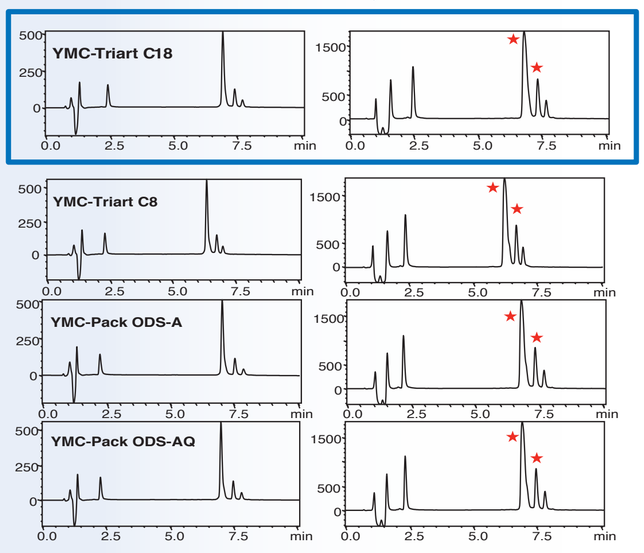
Purification method development for Liraglutide

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%.

Step 1 Column Screening

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



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

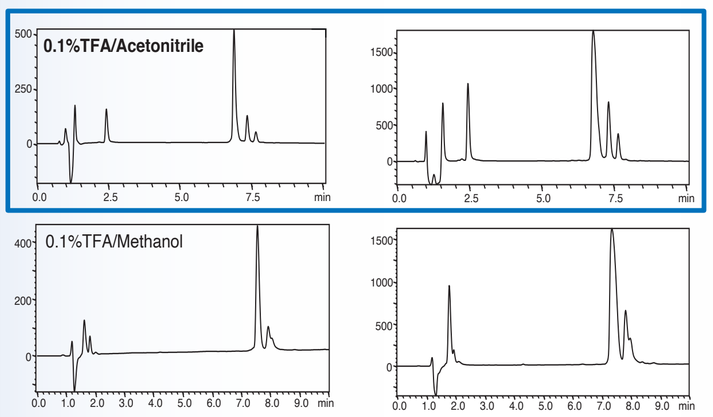
No significant difference in resolution was found between columns.
▶ Triart C18 was selected, as alkaline CIP might be conducted at the scaled-up purification.

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)

Critical pair: ★ marked

Step 2 Influence of Organic Solvent

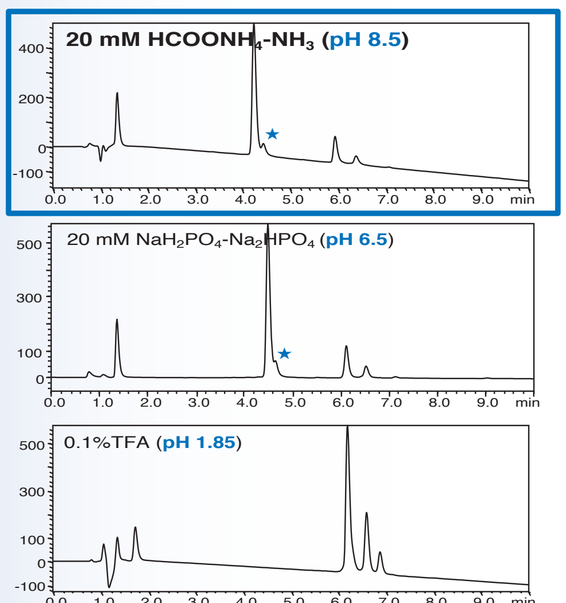
Loading amount: 3 µg → x 8 Loading → 24 µg



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 _____
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 0.5mg/mL)

Acetonitrile gave better resolution of the critical pair than methanol. Furthermore, longer retention when using methanol is not good for prep purpose from the stand point of post-chromatography step.
▶ Acetonitrile was selected.

Step 3 Influence of pH



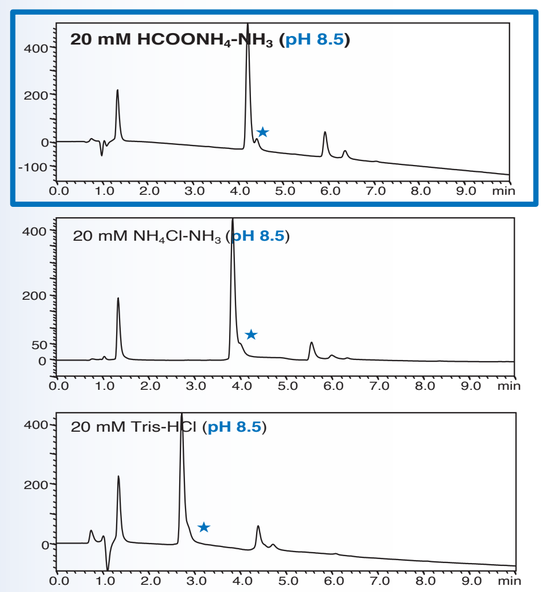
Conditions	Resolution of main peak and impurity peak
20 mM $HCOONH_4$-NH_3 (pH 8.5)	0.96
20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.5)	0.50
0.1% TFA (pH 1.85)	–

Higher pH gave better resolution, and a new impurity peak appeared and separated at higher pH.
▶ pH 8.5 was selected, by considering chemical durability of the packing material and resolution.

Column: YMC-Triart C18 (3 µm, 12 nm), 100 x 3.0 mm ID
Eluent: A) _____ 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

Impurity: ★ marked component

Step 4 Influence of Buffer Type



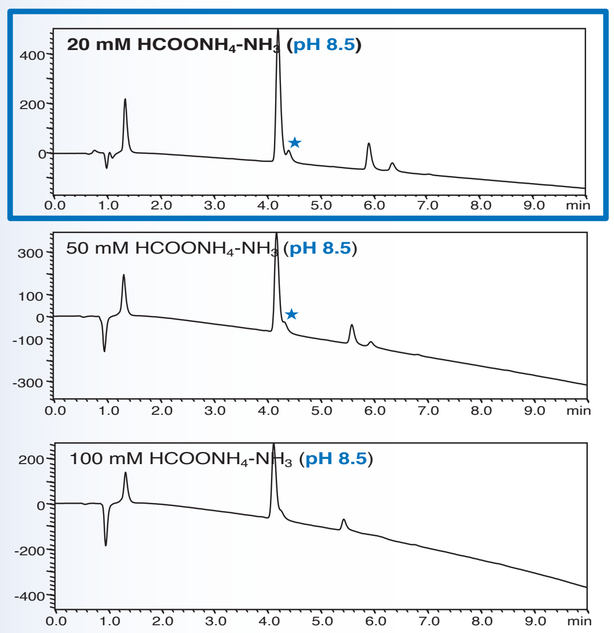
Conditions	Resolution of main peak and impurity peak
20 mM $HCOONH_4$-NH_3 (pH 8.5)	0.96
20 mM NH_4Cl-NH_3 (pH 8.5)	–
20 mM Tris-HCl (pH 8.5)	–

Only $HCOONH_4$-NH_3 system showed a fair separation of the main peak and impurity peak.
▶ $HCOONH_4$-NH_3 was selected.

Column: YMC-Triart C18 (3 µm, 12 nm), 100 x 3.0 mm ID
Eluent: A) _____ 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

Impurity: ★ marked component

Step 5 Influence of Buffer Concentration



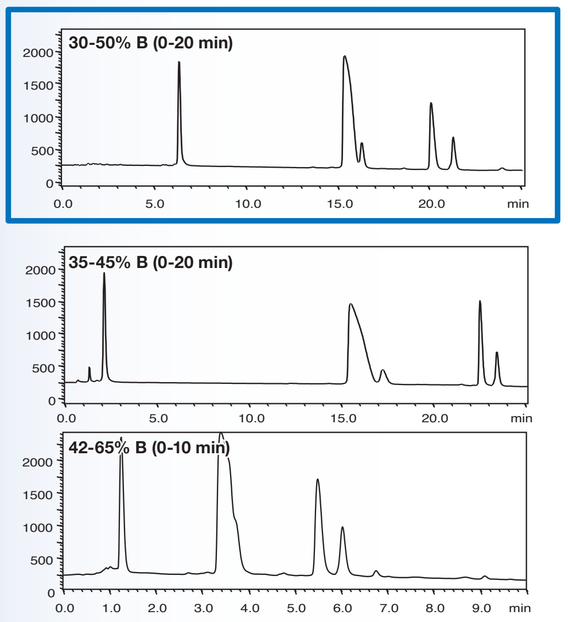
Conditions	Resolution of main peak and impurity peak
20 mM $HCOONH_4$-NH_3 (pH 8.5)	0.96
50 mM $HCOONH_4$-NH_3 (pH 8.5)	0.13
100 mM $HCOONH_4$-NH_3 (pH 8.5)	–

A lower buffer concentration showed better resolution than higher concentration.
▶ 20 mM $HCOONH_4$-NH_3 (pH 8.5) was selected.

Column: YMC-Triart C18 (3 µm, 12 nm), 100 x 3.0 mml.D.
Eluent: A) _____ 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

Impurity: ★ marked component

Step 6 Gradient Optimization

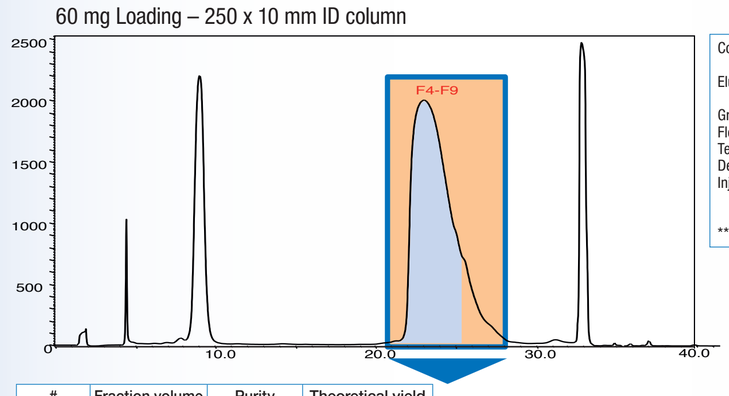


At increased loading condition, influence of gradient curve was evaluated.
▶ 30-50%B (0-20 min) condition was selected when considering balance of resolution of critical pair and fraction volume.

Column: YMC-Triart C18 (3 µm, 12 nm), 100 x 3.0 mm ID
Eluent: A) 20mM $HCOONH_4$-NH_3(pH8.5)
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**

Result Purification Run

60 mg Loading – 250 x 10 mm ID column



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

*** Column is washed with 90%B after elution

#	Fraction volume (mL)	Purity	Theoretical yield
F4	4.7	99.6%	19.5%
F5	4.7	100%	33.3%
F6	4.7	99.0%	18.7%
F7	4.7	79.6%	9.4%
F8	4.7	49.8%	2.3%
F9	4.7	11.9%	0.3%

#	Fraction volume (mL)	Purity	Theoretical yield	Estimated Recovery yield
F4-F6	4.1	99.5%	71.5%	26.3%

▶ Fraction with desired purity was obtained with 71.5% theoretical recovery. ◀

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

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