Development of a Two-Step Chromatographic Process for the Purification of a Dual GIP / GLP-1 Receptor Agonist

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Introduction

GLP-1 agonists are a class of medications that mimic the natural hormone GLP-1. These medications help regulate blood sugar and promote feelings of fullness. They are primarily used to treat type 2 diabetes and can aid weight management. A dual GIP and GLP-1 receptor agonist has gained significant attention in the pharmaceutical industry as a breakthrough therapy for type 2 diabetes and obesity. The structural complexity of this peptide necessitates an advanced purification strategy to achieve optimal purity and yield. To address this, a two-step chromatographic process was developed utilizing two different YMC Triart media, in preparative formats. The first purification step employs Triart Prep Bio200 C8, which effectively removed the majority of the impurities present in the initial crude, improving purity from 20% to 93%. A secondary polishing step using Triart Prep C4-S, further refined the purification, ultimately achieving 99.6% purity to meet the desired pharmaceutical-grade criteria.

This study describes column screening and method development for a two-step chromatographic purification process for a dual GIP and GLP-1 receptor agonist. The combination of a YMC Triart Prep Bio200 C8 and a Triart Prep C4-S were chosen for this purification process. The applicability of this two-step process is demonstrated by starting with low purity crude and producing material that is greater than 99.6% pure. The initial material contained 20% of the target material. The first step and second step provided optimal recoveries of 58.3% and 71.3% respectively. These findings reinforce the importance of method development, including the stationary phase screening and selection, for preparative chromatography of complex biopharmaceutical molecules.

Target Compound

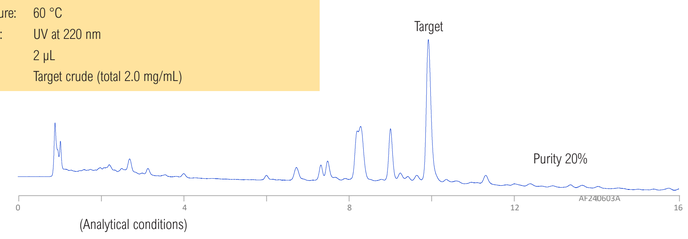
Dual GIP and GLP-1 receptor agonist

The test sample for this work is a glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) receptor agonist and is considered one of the most promising drugs approved for the treatment of type 2 diabetes and obesity.

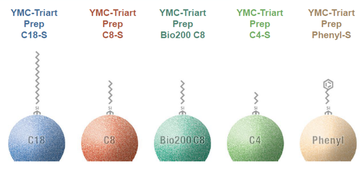
It provides benefits that controls blood glucose levels, stimulates insulin release, suppresses appetite, and weight loss.

- Molecular weight: Between 4500 and 5000 Da
- Structure: Structure: 39 AA sequence conjugated to a fatty acid moiety

Column:	YMC-Triart Bio C18 (1.9 µm, 30 nm) 100 X 2.1 mm ID
Eluent:	A) 20 mM CH_3COONH_4/acetonitrile (95/5) B) 20 mM CH_3COONH_4/acetonitrile (5/95) 34.4–43.3%B(0–16 min), 100%B(16–20 min)
Flow rate:	0.20 ml/min
Temperature:	60 °C
Detection:	UV at 220 nm
Injection:	2 µL
Sample:	Target crude (total 2.0 mg/mL)



Chromatography Media



Specifications

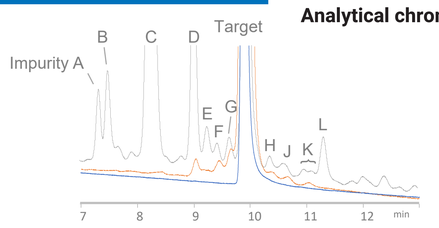
	YMC-Triart Prep C18-S	YMC-Triart Prep C8-S	YMC-Triart Prep Bio200 C8	YMC-Triart Prep C4-S	YMC-Triart Prep Phenyl-S
Functional Group	C18	C8	C8	C4	phenyl (butyl)
Base Material	organic / inorganic hybrid silica				
Particle Size [µm]	7, 10, 15, 20	10, 15, 20	10	10	10
Pore Size [nm]	12	12	20	12	12
Carbon Load [%]	20	17	14	14	17
Endcapping	yes				
pH Range	2–10 for regular use, 2–12 for column cleaning				

More Opportunities with YMC-Triart Prep

- ✔ High chemical stability
- ✔ Versatile selectivity
- ✔ High productivity and reliability
- ✔ Long column lifetimes
- ✔ Large-scale supply for industrial purifications

Impurity Tracking

Analytical chromatogram with impurities labeled

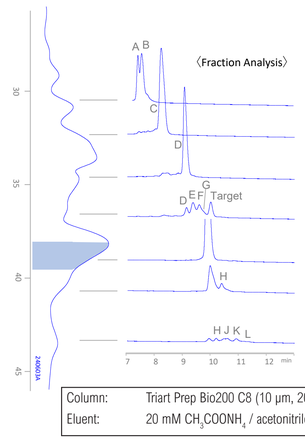


- Crude
- After Purification Step1
- After Purification Step2

Chromatograms aligned with fraction analysis

Purification Step 1

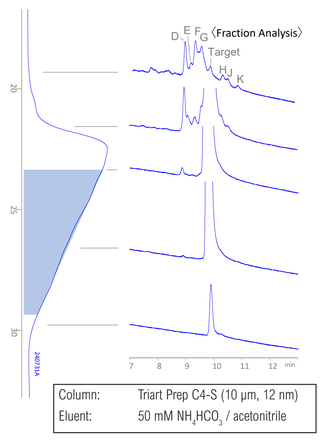
Preparative Chromatogram



Column:	Triart Prep Bio200 C8 (10 µm, 20 nm)
Eluent:	20 mM CH_3COONH_4 / acetonitrile

Purification Step 2

Preparative Chromatogram

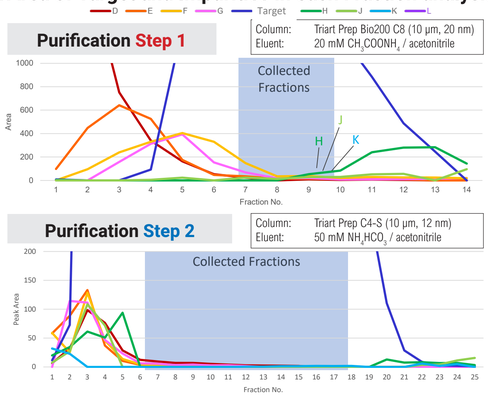


Column:	Triart Prep C4-S (10 µm, 12 nm)
Eluent:	50 mM NH_4HCO_3 / acetonitrile

- Chromatograms of fraction analysis in Purification Step 1 and 2 were shown.
- Purification Step 1 and 2 resulted in a change in impurity behavior.
- In Purification Step 1, impurities H, J, and K elute after the Target, whereas in Purification Step 2 they elute earlier than the Target.

Peak Area of Target and Impurities in each Fraction analysis

- Peak area of the Target and impurities in each fraction analysis were shown.
- In the Purification Step1, removal of the major impurities A, B, and C was achieved.
- In Purification Step 2, impurities H, J, and K moved from behind to in front of the Target and most of the remaining impurities were removed by changing the mobile phase conditions and column type from Purification Step 1.



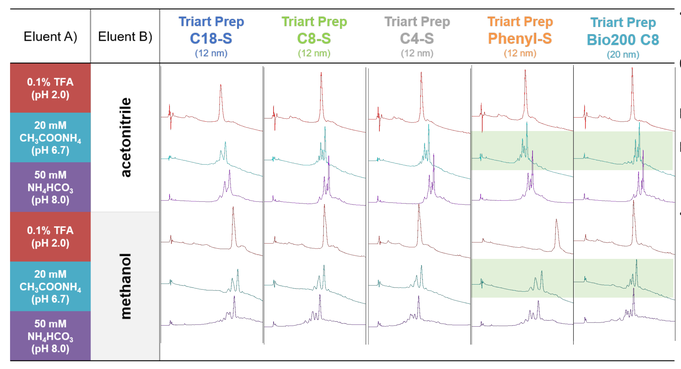
Step-1 Development

Outline for Screening of Step-1 Components

Eluent		Organic solvent		Column
TFA (pH 2.0) Ammonium Acetate (pH 6.7) Ammonium Bicarbonate (pH 8.0)	X	Acetonitrile Methanol	X	YMC-Triart Prep C18-S YMC-Triart Prep C8-S YMC-Triart Prep C4-S YMC-Triart Prep Phenyl-S YMC-Triart Prep Bio200 C8

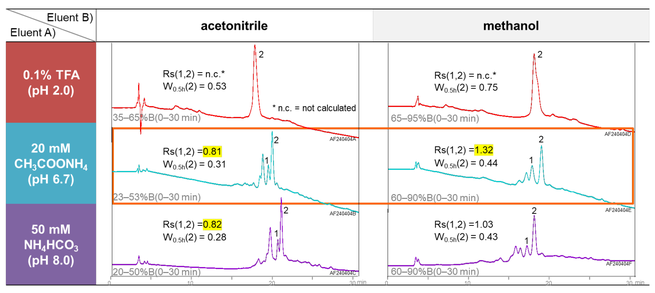
Column:	YMC-Triart Prep series (10 µm) 150X4.6 mm ID
Eluent	A) aqueous solution B) acetonitrile or methanol gradient slope 1.0%B/min
Flow rate:	0.50 mL/min
Temperature:	25 °C
Detection:	UV at 220 nm
Injection:	5 µL
Sample:	Crude Peptide (2.0 mg/mL in 20 mM NH_4HCO_3)

Initial Screening Chromatograms



- The following conditions were selected as candidates.
  Column:
  » Triart Prep Phenyl-S
  » Triart Prep Bio200 C8
  Eluent A):
  » 20 mM CH_3COONH_4
  Eluent B):
  » acetonitrile
  » methanol
- Reasons for selection of candidate preparative conditions
  » Separation from impurities (large number of detected peaks, resolution)
  » Peak sharpness (peak width at half height)

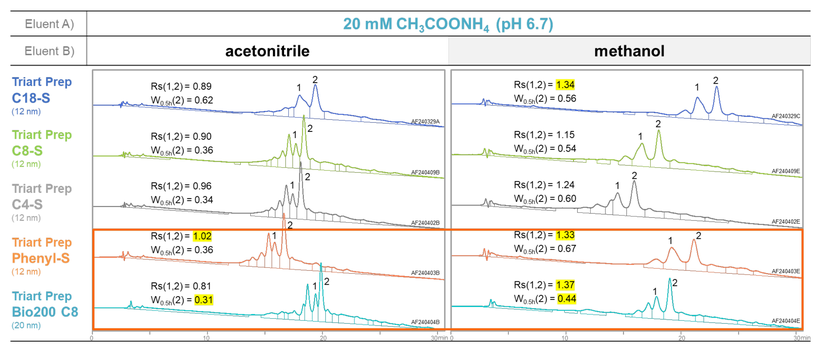
Mobile Phase Screening (Triart Prep Bio200 C8)



- The number of detected peaks was larger in neutral and weak alkaline conditions than in acidic condition. In acidic conditions, the target and impurity peaks were considered to overlap.
- Under methanol conditions, the peak width was larger than that of acetonitrile, but the resolution tended to be higher.
- Neutral conditions were selected because it showed good separation between the target product and impurities.

Peak 1: Impurities before Target
Peak 2: Target
Rs = Resolution
$W_{0.5h}$ = peak width at half height [min]

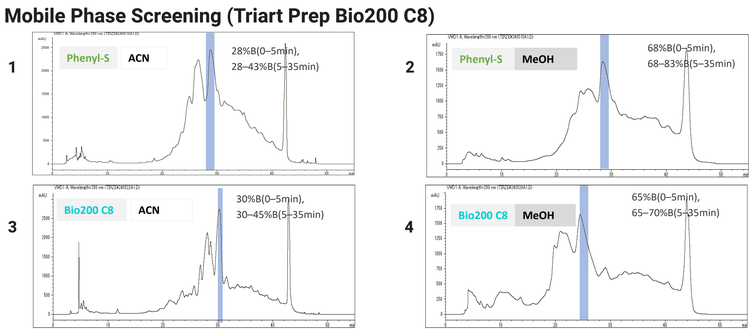
Column Screening (Triart Prep Bio200 C8)



- Triart Prep Phenyl-S showed good separation of the target product from front impurities in both organic solvents.
- The 20 nm pore size (Triart Prep Bio200 C8) had a narrower peak width than 12 nm columns.

Peak 1: Impurities before Target
Peak 2: Target
Rs = Resolution
$W_{0.5h}$ = peak width at half height [min]

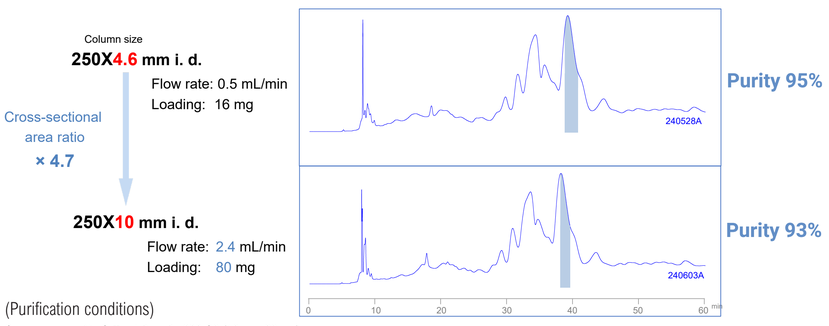
Mobile Phase Screening (Triart Prep Bio200 C8)



Method	1	2	3	4
Column	Triart Prep Phenyl-S		Triart Prep Bio200 C8	
Eluent B)	acetonitrile	methanol	acetonitrile	methanol
HPLC purity	83.8%	76.6%	88.7%	84.3%
Recovery	55.9%	43.7%	58.3%	40.2%
Fraction volume [ml]	0.75	0.75	0.50	0.75

- ✓ Both columns showed higher purity and recovery with acetonitrile than with methanol.
- ✓ Triart Prep Bio200 C8 column and 20 mM CH_3COONH_4/acetonitrile eluent (Method 3) were selected as preparative conditions.

Optimized Conditions and Scale-up



(Purification conditions)

Column:	YMC-Triart Prep Bio200 C8 (10 µm, 20 nm)
Eluent:	A) 20 mM CH_3COONH_4 B) acetonitrile
Gradient:	33.5%B(0–5 min), 33.5–41%B (5–55 min)
Temperature:	25 °C
Detection:	UV at 280 nm
Sample:	20 mg/mL Target crude in 20 mM NH_4HCO_3/acetonitrile (80/20)

In column scale-up, equivalent separation was obtained by proportionally increase the flow rate and sample loading amount to match the cross-sectional area ratio of the column.

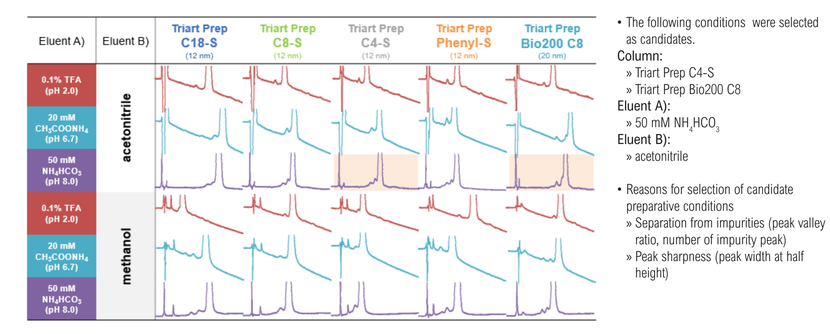
Step-2 Development

Outline for Screening of Step-2 Components

Eluent		Organic solvent		Column
TFA (pH 2.0) Ammonium Acetate (pH 6.7) Ammonium Bicarbonate (pH 8.0)	X	Acetonitrile Methanol	X	YMC-Triart Prep C18-S YMC-Triart Prep C8-S YMC-Triart Prep C4-S YMC-Triart Prep Phenyl-S YMC-Triart Prep Bio200 C8

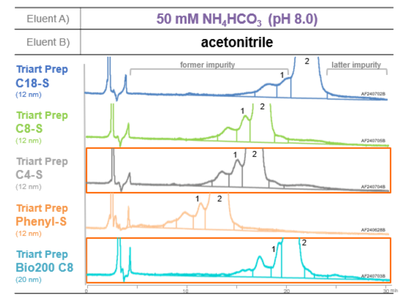
Column:	YMC-Triart Prep series (10 µm) 150X4.6 mm ID
Eluent:	A) aqueous solution B)acetonitrile or methanol gradient slope 0.25%B/min
Flow rate:	0.50 mL/min
Temperature:	25 °C
Detection:	UV at 220 nm
Injection:	5 µL
Sample:	Target crude (after Step 1 purification, 1.0 mg/ml in 20 mM NH_4HCO_3)

Initial Screening Chromatograms with Step-1 Product



- The following conditions were selected as candidates.
  Column:
  » Triart Prep C4-S
  » Triart Prep Bio200 C8
  Eluent A):
  » 50 mM NH_4HCO_3
  Eluent B):
  » acetonitrile
- Reasons for selection of candidate preparative conditions
  » Separation from impurities (peak valley ratio, number of impurity peak)
  » Peak sharpness (peak width at half height)

Selectivity Comparison



W0.5h(2) = 1.09
p/v(1,2) = 1.00
Area%(latter impurity) = 1.9%

W0.5h(2) = 0.70
p/v(1,2) = 1.21
Area%(latter impurity) = 3.0%

W0.5h(2) = 0.69
p/v(1,2) = 1.31
Area%(latter impurity) = 3.0%

W0.5h(2) = 0.70
p/v(1,2) = 1.32
Area%(latter impurity) = 1.6%

W0.5h(2) = 0.57
p/v(1,2) = 1.00
Area%(latter impurity) = 2.6%

Peak 1 : Impurities before Target
Peak 2 : Target

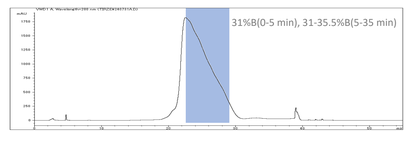
$W_{0.5h}$ = peak width at half height [min]

p/v = peak valley ratio (a/b)

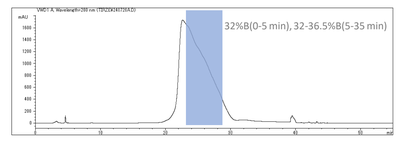
- The 20 nm pore size (Triart Prep Bio200 C8) had a narrower peak width than 12 nm columns.
- Triart Prep C4-S and Triart Prep Phenyl-S showed higher peak valley ratio of Target to former impurities. Furthermore, the Area% of latter impurity was higher for Triart Prep C4-S, which suggests that there is less overlap between the target product and the impurity.

Step-2 Column Comparison

1 Triart Prep C4-S



2 Triart Prep Bio200 C8



Method	1	2
Column	Triart Prep C4-S	Triart Prep Bio200 C8
HPLC purity	99.7%	99.3%
Recovery	71.3%	58.9%
Fraction volume [ml]	6.0	5.0

Triart Prep C4-S column and 50 mM NH_4HCO_3/acetonitrile eluent (Method 1) were selected as preparative conditions.

Summary

A single step purification process is certainly preferred, but a two-step process is often necessary to achieve the required purity with complex samples. Developing the first-step methodology is an iterative process, in a similar way as a single step process.

- A plan is designed to evaluate possible eluent and stationary phase combinations.
- A general gradient is used to screen the eluent and column combinations.
- The most promising column is then tested with different eluents and a more optimized gradient.
- The most promising eluent is then used to screen the columns again with the most promising eluent.
- The optimized conditions are scaled up and determined if a two-step process is needed.
- The second step is developed similar to the first step but starts with the product of the first step.

>99.5% purity for the Target was achieved by a reversed-phase two-step purification that started with 20% purity crude.