ResiPure™ ADVANCED

SOLUTION FOR PEPTIDE PURIFICATION

SiliCycle
A Company of the ZEOCHEM Group

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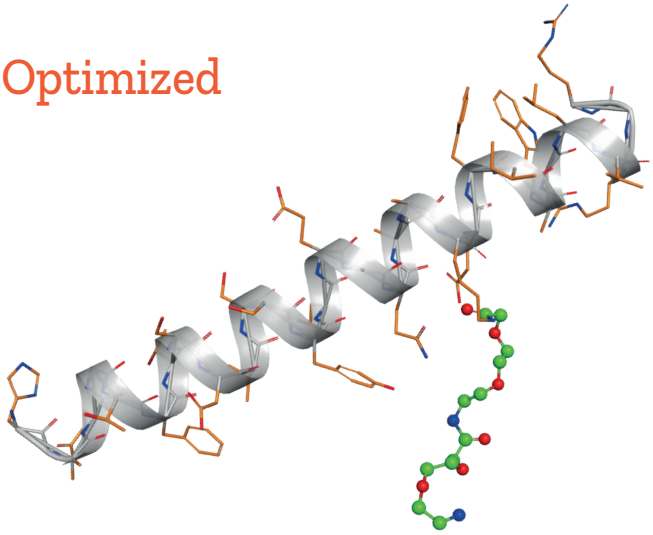
ResiPure™ ADVANCED

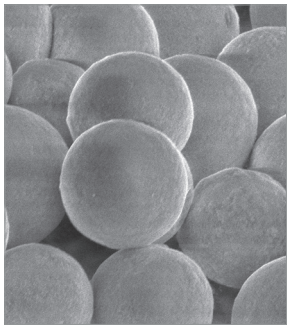
Optimize the Purification of your GLP-1 Agonists and Peptide Analogs

ResiPure™ ADVANCED

High Resistance Silica for Optimized Peptide Purification



ResiPure ADVANCED is a high-performance functionalized spherical silica specifically designed to meet the rigorous demands of peptide purification.



Engineered for both analytical and preparative chromatography, ResiPure ADV (ADVANCED) offers unparalleled robustness, efficiency, reproducibility, and scalability, making it the ideal solution for applications in pharmaceutical, biotechnology, and research settings.

ResiPure ADV is built to perform under challenging conditions. Its wide pH range tolerance for alkaline wash (pH 1–13) and compatibility with organic solvents, including trifluoroacetic acid (TFA), enable flexibility across diverse purification workflows. Moreover, its exceptional chemical and mechanical stability ensures durability over multiple cycles, reducing costs and downtime while maintaining high-quality results.

ResiPure ADVANCED Silica Bead

At the core of ResiPure ADV's performance is its optimized particle design.

The perfectly spherical silica particles ensure uniform packing, minimal back-pressure, and consistent flow, resulting in sharp and symmetrical peaks even for complex peptide mixtures. Its dense functionalization and advanced endcapping provide robust hydrophobic interactions, allowing for efficient separation of hydrophilic and hydrophobic peptides with superior resolution.

- Perfectly Spherical
- High Purity & Low Metal Content
- High Loading Capacity
- Dense Functionalization
- High Mechanical & Chemical Resistance
- Uniform Pore Size & Density

Why Choose ResiPure ADVANCED

Optimal Separation Performance
- Excellent selectivity and efficiency

Up to pH 13 for alkaline wash

Material Stability & Robustness
- High mechanical strength
- High chemical resistance, with a wide pH stability

Batch-to-Batch Reproducibility
- Material and performance consistency, batch after batch

Combines all benefits of other high performance gels on the market

Excellent ROI
- High performance purification
- Long lifetime, resulting in more cycles

Compliance with Regulatory Requirements
- Meets all pharmaceutical standards
- Full traceability and documentation: technical data sheets and certificates of analysis (CoA)

Renowned technical support

Manufactured by a Silica Specialist
- 30 years expertise in chromatography, purification and material science
- Product designed, developped and supported by our own R&D team
- Renowned technical and customer support

Metric tons available

Easily Scalable
- Industrial capacities: from 10 g to multi-ton quantities

Ordering Information

Bulk spherical silica gels

ResiPure ADV silica gels are offered from 10 g up to multi-ton scale.

ResiPure ADV C18	
SKU	Quantity
S03107H-B-100G	100 g
S03107H-B-250G	250 g
S03107H-B-1KG	1 kg
S03107H-B-5KG	5 kg
S03107H-B-10KG	10 kg
S03107H-B-25KG	25 kg

ResiPure ADV C8	
SKU	Quantity
S30907H-B-100G	100 g
S30907H-B-250G	250 g
S30907H-B-1KG	1 kg
S30907H-B-5KG	5 kg
S30907H-B-10KG	10 kg
S30907H-B-25KG	25 kg

ResiPure ADV C4	
SKU	Quantity
S32807H-B-100G	100 g
S32807H-B-250G	250 g
S32807H-B-1KG	1 kg
S32807H-B-5KG	5 kg
S32807H-B-10KG	10 kg
S32807H-B-25KG	25 kg

HPLC columns

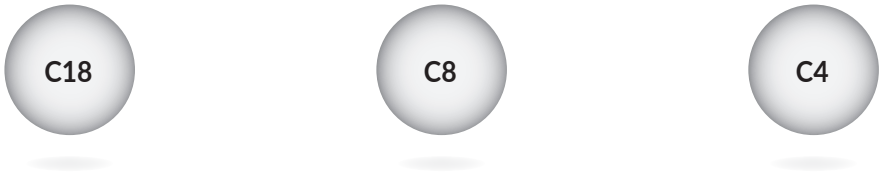
For analytical needs, HPLC columns packed with ResiPure ADV are also offered: contact us!

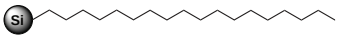
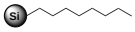
Available sizes:

- length: 50 mm, 100 mm, 150 mm, and 250 mm.
- diameter: 4.6 mm, 10 mm, 21.2 mm, 30 mm, and 50 mm.

Phases Portfolio

To meet all purification needs, ResiPure ADV is offered in three functionalizations:



		C18	C8	C4
PN		S03107H-B	S30907H-B	S32807H-B
Structure				
Material		Silica	Silica	Silica
Particle Size		10 µm	10 µm	10 µm
Pore Diameter		150 Å	150 Å	150 Å
Specific Surface Area		250 m^2/g	250 m^2/g	250 m^2/g
Endcapping		Yes	Yes	Yes
pH Stability	Mobile Phase	1 - 10	1 - 10	1 - 10
	Washing	up to pH 13	up to pH 13	up to pH 13
Description		Most hydrophobic, best for small to medium-sized peptides, provides high retention and resolution.	Moderate hydrophobicity, suitable for larger or moderately hydrophobic peptides, offering shorter retention times than C18.	Least hydrophobic, ideal for large or highly hydrophobic peptides and proteins, reducing retention and peak broadening. Generaly requires more aqueous eluents.

Batch-to-Batch Reproducibility

The spherical shape and uniform particle size distribution minimize back-pressure and ensure smooth column packing, reducing the risk of channeling or uneven flow. This uniformity translates to consistent performance, maintaining separation efficiency and peak resolution over multiple cycles.

Hydrophobic retention profiles on 3 different batches of ResiPure ADV

CONDITIONS

Columns: C18, 250 × 4.6 mm, 10 µm

Mobile phase: acetonitrile/water (80/20)

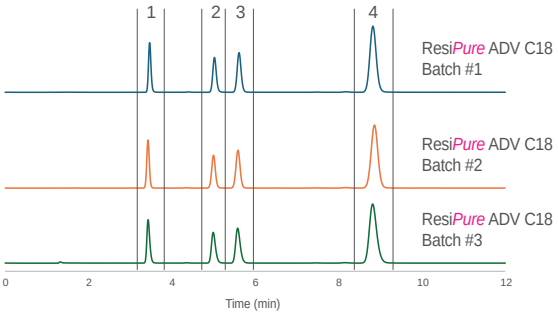
Flow rate: 1.000 mL/min

Column temperature: 25°C

Detection: UV at 254 nm

Injection volume: 5 µL

Sample: acetophenone (1), toluene (2), naphtalene (3), and anthracene (4)



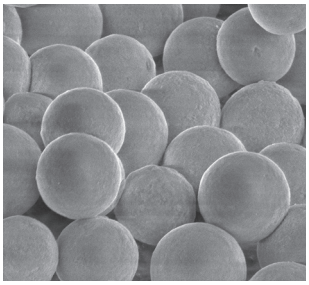
1- Acetophenone 2- Toluene 3- Naphtalene 4- Anthracene

Efficiency and retention were similar for the hydrophobic markers analysed on 3 different batches of ResiPure ADV.

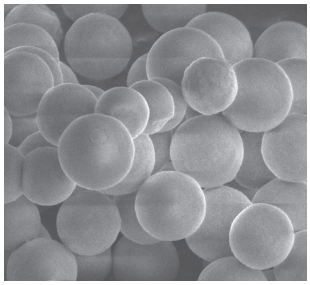
Morphology

Scanning Electron Microscope (SEM) particles pictures of 3 batches of ResiPure ADV.

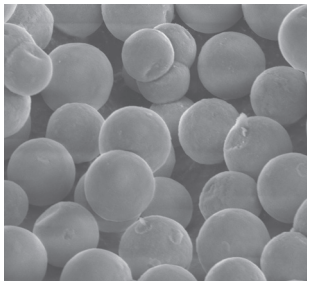
Batch #1



Batch #2



Batch #3



Peptide Analysis on ResiPure ADV C18

Sigma-Aldrich H2016 Peptide Mix

CONDITIONS

Columns: C18, 250 × 4.6 mm, 10 µm

Mobile phases

- Mobile phase A (MPA): 0.1 % TFA in water (v/v)
- Mobile phase B (MPB): 0.1 % TFA in acetonitrile (v/v)

Gradient

1. Hold 3 minutes at 90/10 (MPA/MPB)
2. Increase to 65/35 (MPA/MPB) during 27 minutes
3. Hold 5 minutes at 65/35 (MPA/MPB)

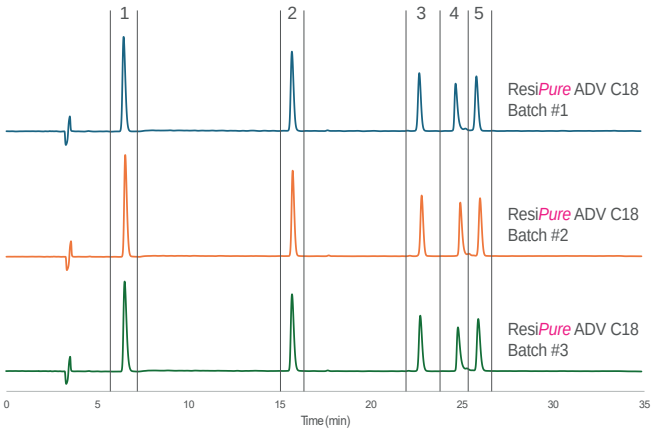
Flow rate: 1.000 mL/min

Column temperature: 25°C

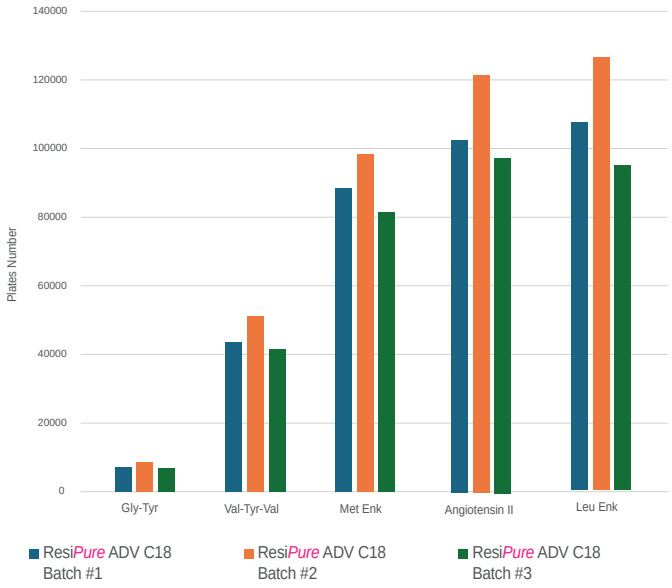
Detection: UV at 220 nm

Injection volume: 10 µL

Sample: calibration peptide mixture (Sigma Aldrich H2016) diluted in 1 mL of water.



1- Gly-Tyr 2- Val-Tyr-Val 3- Met Enk 4- Angiotensin II 5- Leu Enk



From one batch to the other, retention, selectivity and efficiency were found to be the same for the different compounds in a peptide mixture.

Brunauer-Emmett-Teller (BET) Analysis

BET Analysis				
Batch	Surface area (m^2/g)	Pore size (Å)	Pore volume (mL/g)	Carbon Load (%)
1	250	147	0.92	17.5
2	239	154	0.92	16.5
3	248	149	0.93	17.0

BET analysis shows uniformity in material and chemical properties of ResiPure ADV silica particles from batch to batch.

ResiPure ADV offers reproducibility and consistency of its particles, batch after batch.

Superior Mechanical Resistance

By choosing our robust silica, you benefit from a reduced cost of operation, as fewer replacements and less downtime mean optimized productivity. The durability of our material ensures that you get the most out of every batch, making it a cost-effective solution.

Test: Packing – Repacking of a Preparative Column

CONDITIONS

Method

Procedure: 20 consecutive cycles of column packing / unpacking

Column: C18, 150 × 21.2 mm, 10 µm

Mobile phase: acetonitrile/water (70/30)

Flow rate: 15 mL/min

Detection: UV at 254 nm

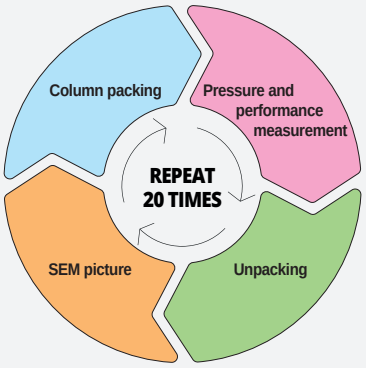
System: Shimadzu Preparative HPLC

Injection volume: 500 µL

Sample: mixture of acetophenone, toluene, naphthalene, and anthracene (marker)

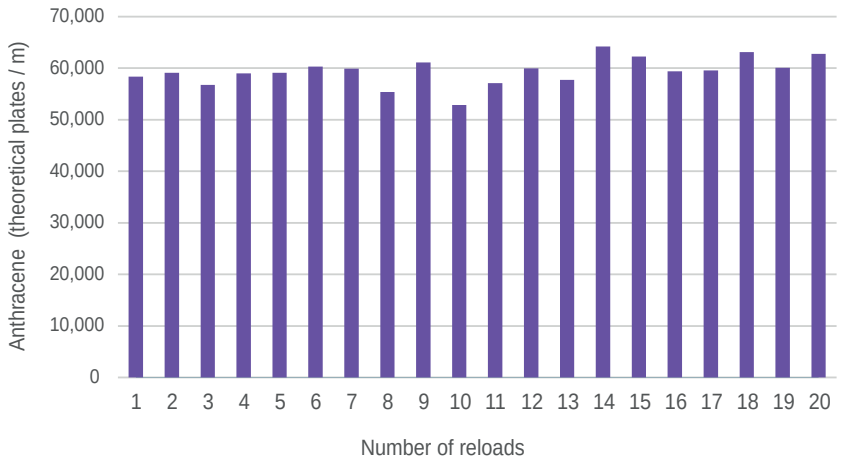
Procedure

20 consecutive cycles of column packing / unpacking



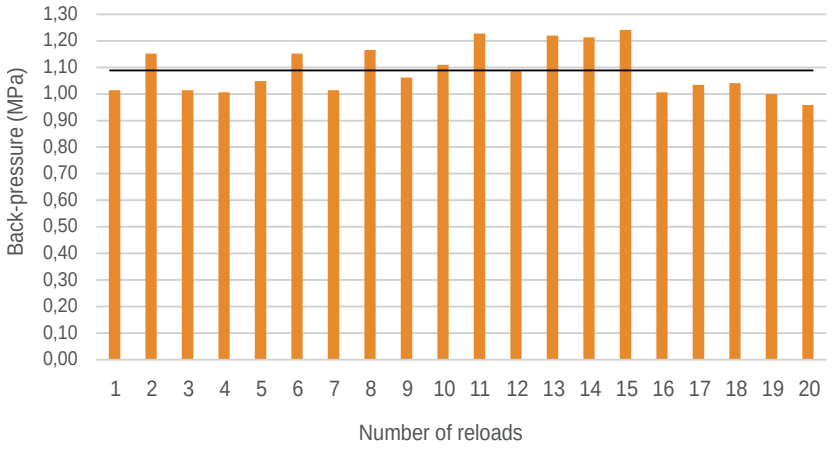
Results

Column performance vs number of reloads



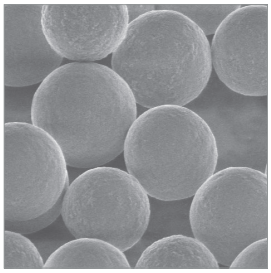
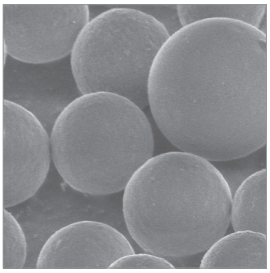
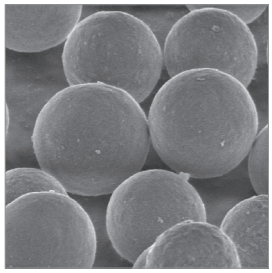
Results

Back-pressure vs number of reloads



SEM Pictures

ResiPure ADV particles SEM pictures at the start, and after several repacking processes:

Before	After 10 packings	After 20 packings
		

The silica beads are still perfectly spherical, with smooth surfaces free of any cracks or cavities, even after packing and unpacking 20 times a column of 21.2 mm ID.

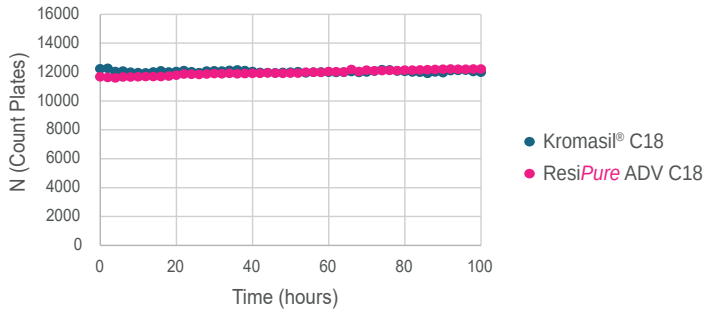
pH Stability in Mobile Phase

pH stability in the mobile phase is essential for consistent and reproducible peptide separation. Fluctuations in pH can lead to changes in peptide charge, affecting their interaction with the stationary phase and thus the separation process.

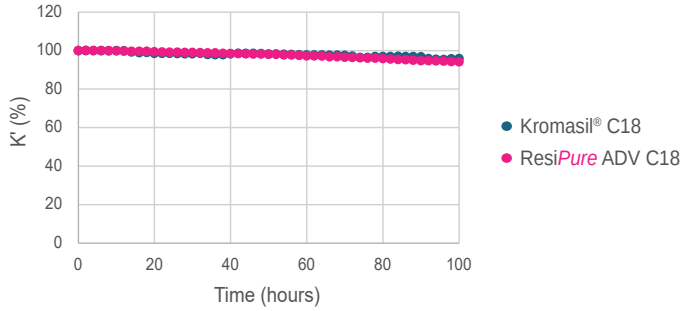
A stable pH ensures that peptides maintain their desired charge state throughout the chromatographic process, optimizing separation efficiency.

At pH 1.0 (1 % TFA at 70°C)

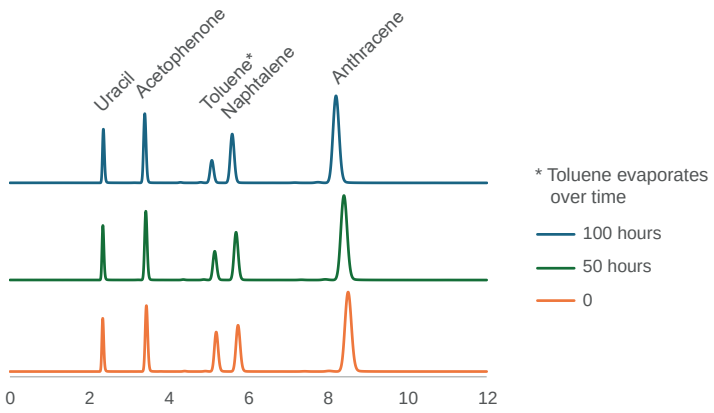
No efficiency loss after 100 hours (retention marker: anthracene):



Minimum retention factor loss after 100 hours (retention marker: anthracene):



Chromatograms at 0, 50 and 100 hours are almost identical:



CONDITIONS

pH 1.0 conditions

Columns: C18, 250 × 4.6 mm, 10 µm

Flow rate: 0.400 mL/min

Mobile phase: acetonitrile/water/trifluoroacetic acid (9.5/89.5/1)

Column temperature: 70 °C

Chromatographic test conditions

Columns: C18, 250 × 4.6 mm, 10 µm

Flow rate: 1.000 mL/min

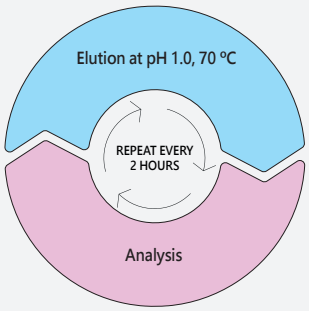
Mobile phase: acetonitrile/water (70/30)

Column temperature: 70 °C

Detection: UV at 254 nm

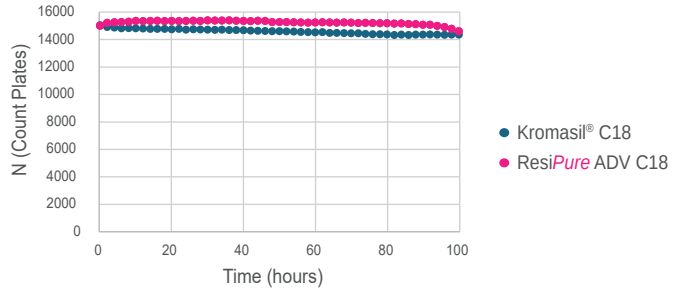
Sample: uracil, acetophenone, toluene, naphtalene, anthracene

Test Cycle

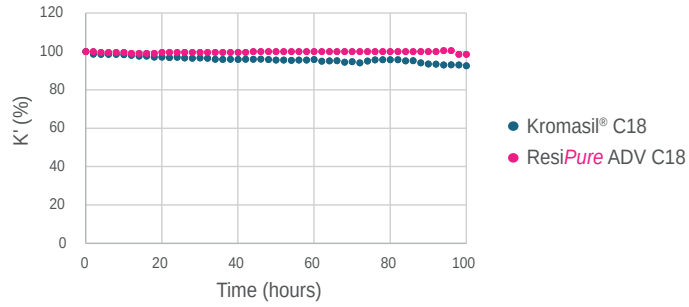


At pH 10.0 (Ammonium carbonate at RT)

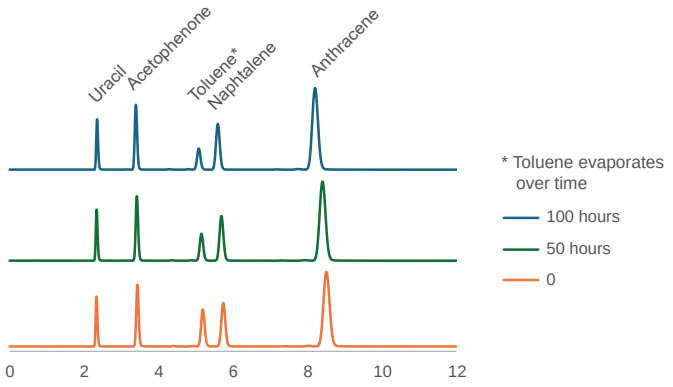
No efficiency loss after 100 hours:



No retention factor loss after 100 hours:



Chromatograms at 0, 50, and 100 hours are perfectly identical:



CONDITIONS

pH 10 conditions

Columns: C18, 250 × 4.6 mm, 10 µm

Flow rate: 0.400 mL/min

Mobile phase: 10 mM ammonium carbonate in 90/10 (acetonitrile/water), adjusted to pH 10.00

Column temperature: 23 °C

Chromatographic test conditions

Columns: C18, 250 × 4.6 mm, 10 µm

Flow rate: 1.000 mL/min

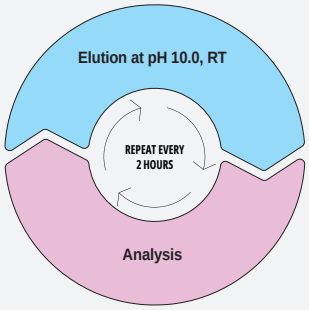
Mobile phase: acetonitrile/water (80/20)

Column temperature: 23 °C

Detection: UV at 254 nm

Sample: uracil, acetophenone, toluene, naphtalene, anthracene

Test Cycle



ResiPure ADV can be run from pH 1 to 10 without compromising your results.

pH Stability through Washing Cycles

pH stability in the mobile phase is essential for consistent and reproducible peptide separation

Fluctuations in pH can lead to changes in peptide charge, affecting their interaction with the stationary phase and thus the separation process.

A stable pH ensures that peptides maintain their desired charge state throughout the chromatographic process, optimizing separation efficiency.

Up to pH 13 for alkaline wash

Test: Alkaline Washing Cycles (pH 13)

CONDITIONS

Alkaline washing cycle conditions

Column: C18, 250 × 4.6 mm, 10 µm

Flow rate: 1.000 mL/min

Cycles

- Step 1 - **Alkaline wash** (pH 13): ACN/0.1 M NaOH (50/50), 6 CV
- Step 2 - **Neutralisation** (pH 3.5): ACN/water adjusted to pH 3.5 with glacial acetic acid (50/50), 6 CV
- Step 3 - **Wash**: 100 % acetonitrile, 6 CV
- Step 4 - **Column performance analysis** (marker: anthracene)

Chromatographic test conditions

Column: C18, 250 × 4.6 mm, 10 µm

Mobile phase: acetonitrile/water (80/20)

Flow rate: 1.000 mL/min

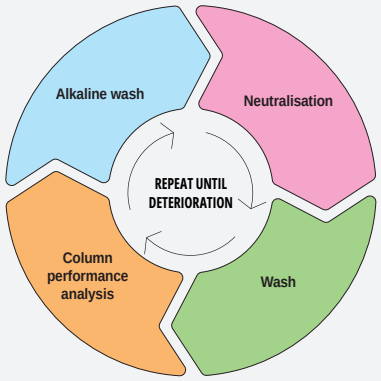
Column temperature: 25°C

Detection: UV at 254 nm

Test compound: anthracene

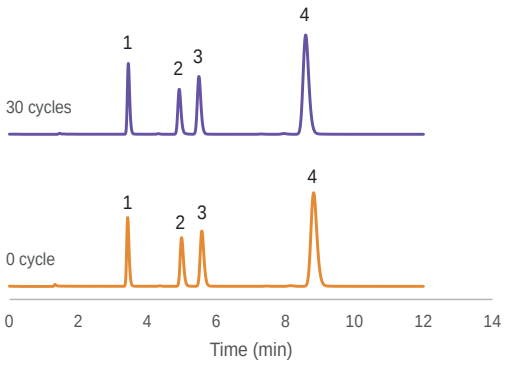
Sample: acetophenone (1), toluene (2), naphtalene (3), and anthracene (4)

Washing cycles procedure



Results

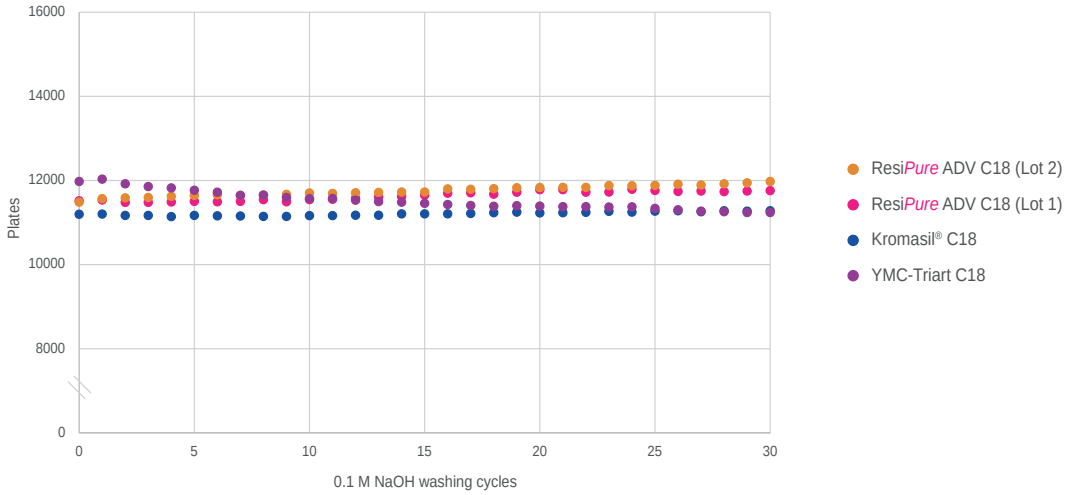
Performance after 30 cycles



Results

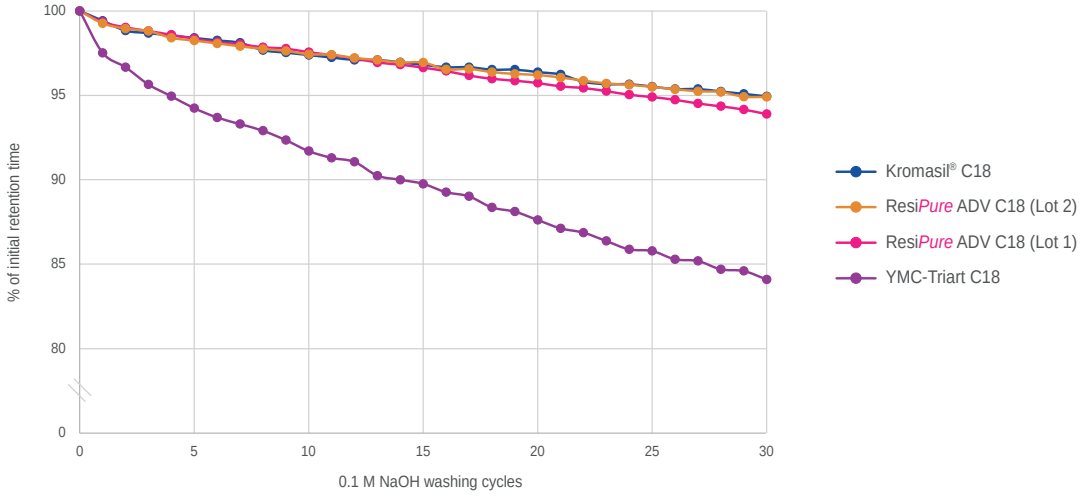
Loss of efficiency after alkaline washing cycle (pH 13)

Retention marker: Anthracene



Loss of retention after alkaline washing cycle (pH 13)

Retention marker: Anthracene



ResiPure ADV for washing cycles:

- **alkaline resistance, alkaline durability, alkaline-tolerant**
- **wide pH tolerance range, up to pH 13**
- **enduring more than 30 cycles of NaOH washing without showing any meaningful back-pressure increase / performance loss**

ResiPure ADV C18 vs Kromasil® C18

Analysis of Thymalfasin from a synthetic crude product by C18 liquid chromatography

Known for its immunomodulatory properties, Thymalfasin is a synthetic 28-amino acid peptide derived from thymosin alpha-1. It has a linear structure with an N-terminal acetylation, which enhances its stability.

With a molecular weight of approximately 3108.3 g/mol, it is a hydrophilic peptide, highly soluble in water due to its multiple acidic residues, such as aspartic and glutamic acids.

CONDITIONS

Columns: 250 × 4.6 mm, 10 µm

Mobile phases

- Mobile phase A (MPA): 50 mM ammonium formate in water, buffered at pH 3.8
- Mobile phase B (MPB): Acetonitrile

Flow rate: 1.000 mL/min

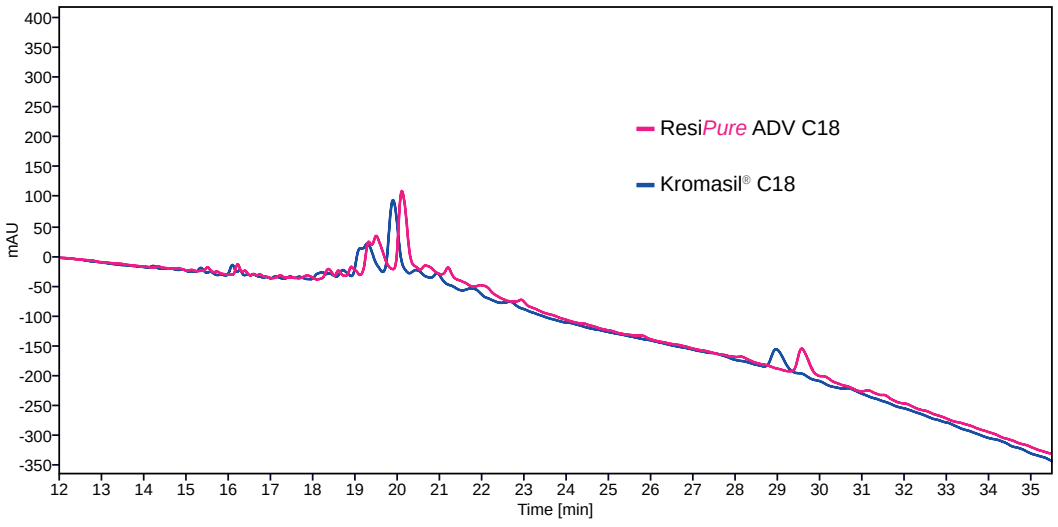
Column temperature: 25°C

Detection: UV at 215 nm

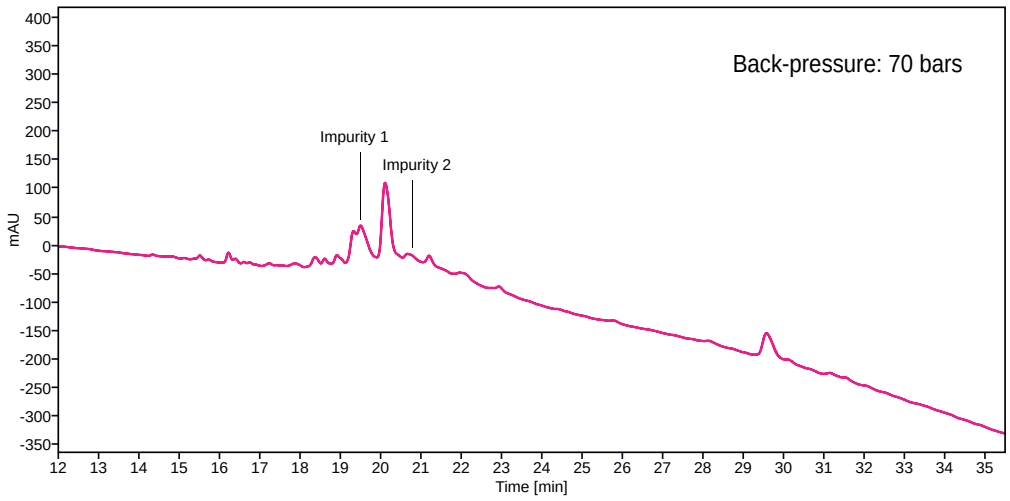
Sample load: 40 µL of 50 mg crude product diluted in 10 mL of 1 % of acetic acid in water (v/v)

Gradient		
Time (min)	MPA (%)	MPB (%)
0.01	95	5
5.00	95	5
65.00	20	80
70.00	20	80
70.01	95	5
80.00	95	5

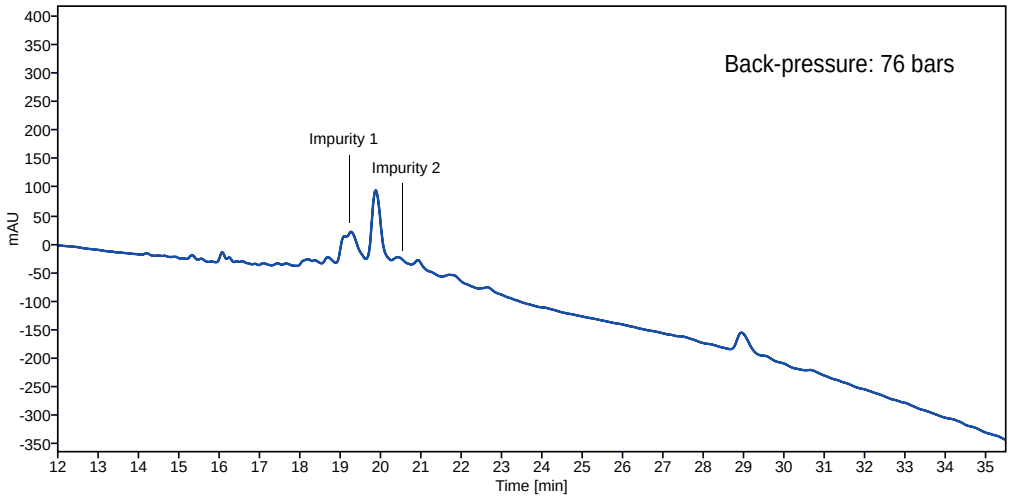
Impurities profiles comparison: ResiPure ADV C18 vs Kromasil® C18



ResiPure ADV C18



Kromasil® C18



Comparison of the two chromatograms						
Stationary phase	Retention time (min)	Plate (Thymalfasin)	Selectivity (Thymalfasin-Impurity 1)	Selectivity (Thymalfasin-Impurity 2)	Resolution (Thymalfasin-Impurity 1)	Resolution (Thymalfasin-Impurity 2)
Kromasil® C18	19.87	45 133	1.04	1.03	1.27	1.57
ResiPure ADV C18	20.10	55 355	1.04	1.03	1.30	1.63

ResiPure ADV is more efficient than the Kromasil® C18 to separate Thymalfasin from the main impurities in a crude product, while generating less back-pressure.

ResiPure ADV C18 vs Kromasil® C18

Analysis of Tirzepatide from a synthetic crude product by C18 liquid chromatography

Tirzepatide is a synthetic peptide consisting of 39 amino acids, designed as a dual GIP and GLP-1 receptor agonist. It has a linear structure with a fatty acid moiety, enhancing plasma stability and albumin binding.

CONDITIONS

Columns: 250 × 4.6 mm, 10 µm

Mobile phases

- Mobile phase A (MPA): 10 mM ammonium acetate in water
- Mobile phase B (MPB): acetonitrile

Flow rate: 1.000 mL/min

Column temperature: 25°C

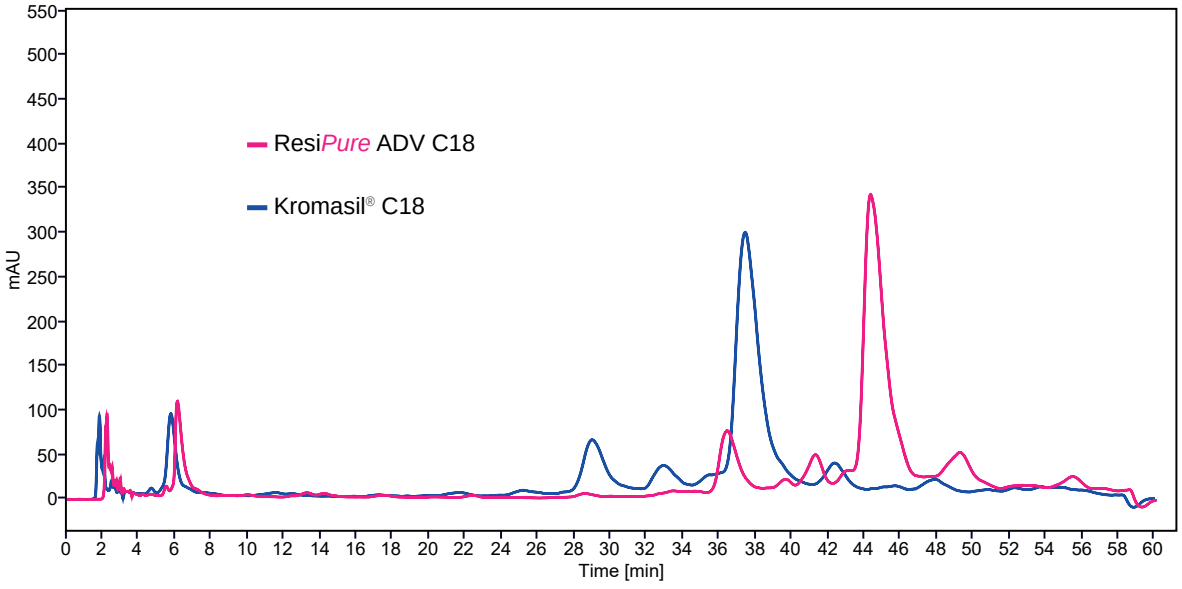
Detection: UV at 220 nm

Sample load: 5 µL of 120 mg of crude product diluted in 5 mL of dissolving solution

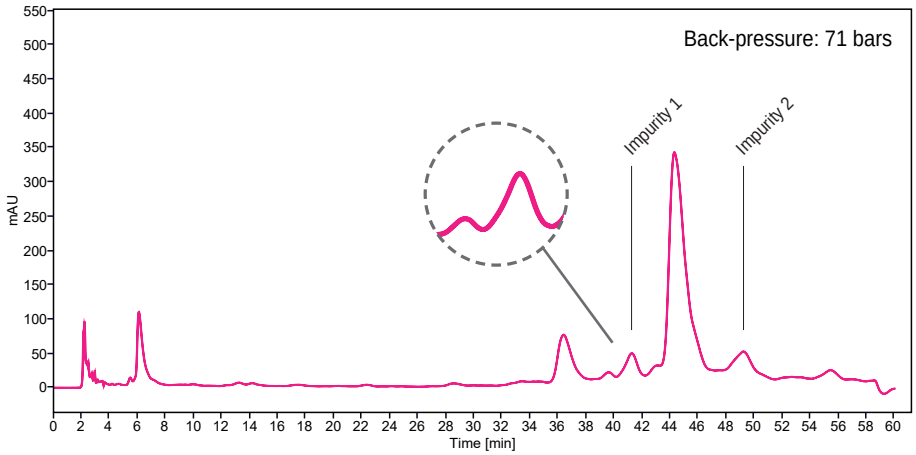
Dissolving solution: 50/50 acetonitrile/water

Gradient		
Time (min)	MPA (%)	MPB (%)
0.01	60	40
50.00	53	47
55.00	53	47
55.01	60	40
60.00	60	40
80.00	95	5

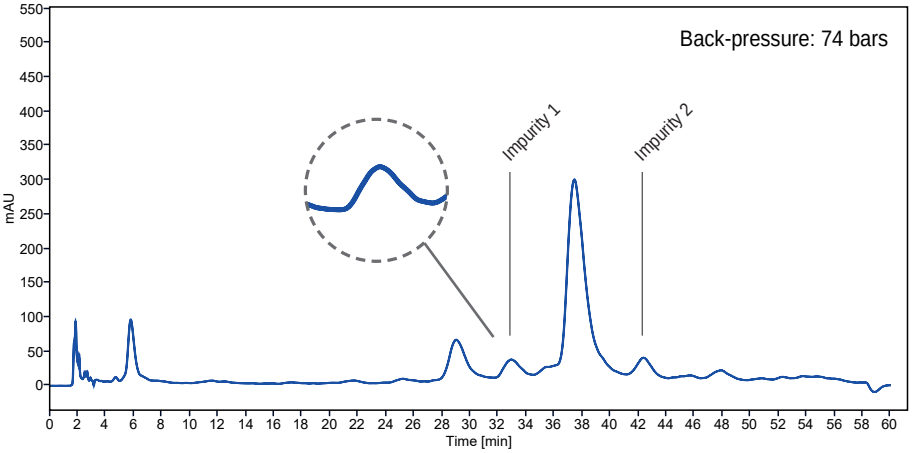
Impurities profiles comparison: ResiPure ADV C18 vs Kromasil® C18



ResiPure ADV C18



Kromasil® C18



Comparison of the two chromatograms						
Stationary phase	Retention time (min)	Plate (Tirzepatide)	Selectivity (Tirzepatide-Impurity 1)	Selectivity (Tirzepatide-Impurity 2)	Resolution (Tirzepatide-Impurity 1)	Resolution (Tirzepatide-Impurity 2)
Kromasil® C18	37.44	4 715	1.15	1.14	2.16	2,29
ResiPure ADV 18	43.10	8 486	1.08	1.12	1.81	2.38

A slightly different selectivity is observed when using ResiPure ADV C18 compared to Kromasil® C18.

ResiPure ADV C18 allows a better selectivity with Tirzepatide than Kromasil® C18 for some impurities. On the other hand, Kromasil® C18 allows a better selectivity for other impurities.

Retention profile for a peptides mixture

A calibration peptides mixture, Sigma-Aldrich H2016, composed of Angiotensin II, Gly-Tyr, Leu enkephalin, Met enkephalin and Val-Tyr-Val, has been tested with ResiPure ADV C18 to evaluate its retention efficiency.

CONDITIONS

Columns: C18, 250 × 4.6 mm, 10 µm

Mobile phases

- Mobile phase A (MPA): 0.1 % TFA in water (v/v)
- Mobile phase B (MPB): 0.1 % TFA in acetonitrile (v/v)

Gradient

1. Hold 3 minutes at 90/10 (MPA/MPB)
2. Increase to 65/35 (MPA/MPB) during 27 minutes
3. Hold 5 minutes at 65/35 (MPA/MPB)

Flow rate: 1.000 mL/min

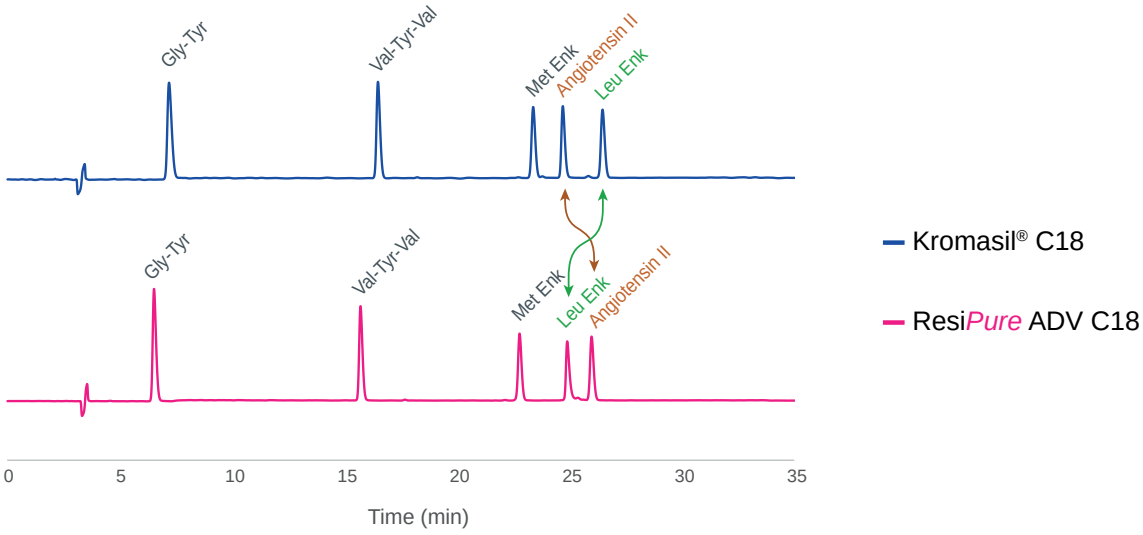
Column temperature: 25°C

Detection: UV at 220 nm

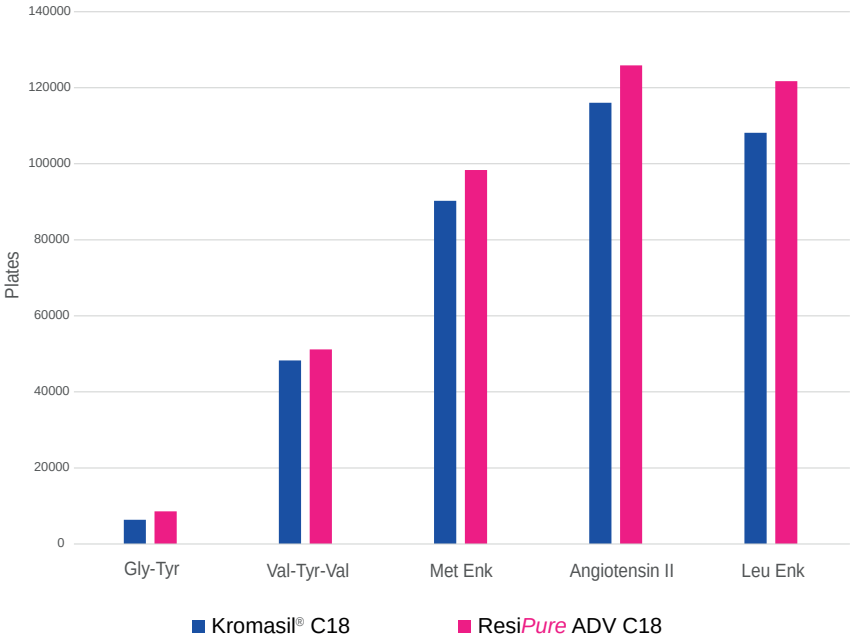
Injection volume: 10 µL

Sample: calibration peptide mixture (Sigma Aldrich H2016) diluted in 1 mL of water.

Chromatogram



Efficiency for each peptide component



ResiPure ADV can offer different selectivity compared to Kromasil®, while providing high efficiency.

pH Effect

The pH of a solution plays a crucial role in peptide separation. Adjusting pH helps optimize the interaction between peptides and the stationary phase, improving purification outcomes.

pH variation effect on Tirzepatide

CONDITIONS

Column: C18, 250 × 4.6 mm, 10 µm

Mobile phases

- Mobile phase A (MPA):
  - Test 1: 50 mM ammonium formate in water, adjusted to pH 3.5
  - Test 2: 10 mM potassium phosphate dibasic in water, adjusted to pH 8.0
- Mobile phase B (MPB): acetonitrile

Gradient

1. Hold 10 minutes at 68/32 (MPA/MPB)
2. Increase to 55/45 (MPA/MPB) during 50 minutes
3. Hold 10 minutes at 55/45 (MPA/MPB)
4. Reequilibrate at 68/32 (MPA/MPB) for 10 minutes

Flow rate: 1.000 mL/min

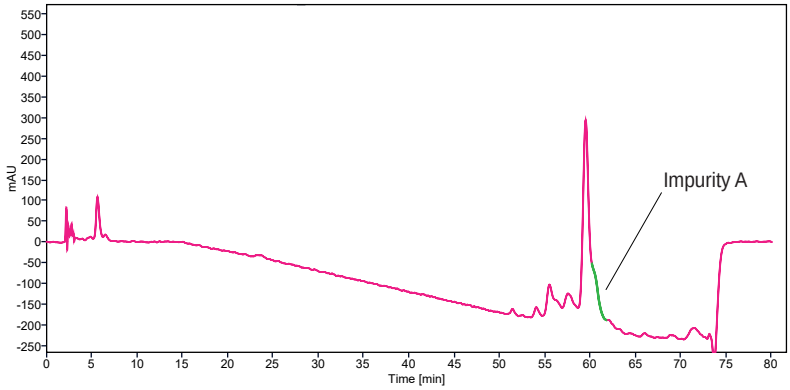
Column temperature: 25°C

Detection: UV at 220 nm

Injection volume: 5 µL

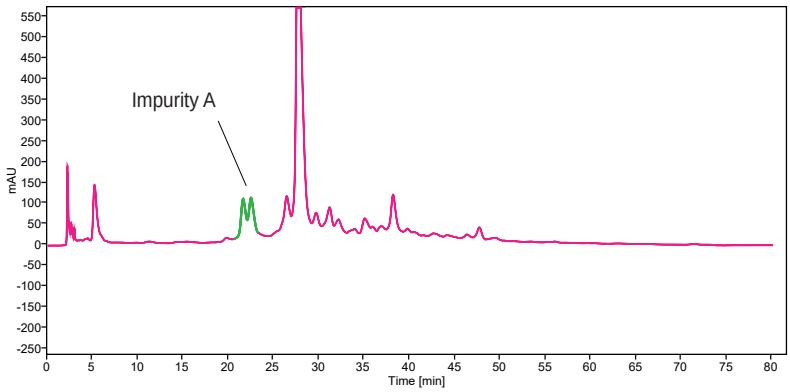
Sample: 125 mg of crude material diluted in 5 mL of acetonitrile/water (50/50)

pH 3.5



Tirzepatide elutes at 59.4 minutes, with some impurities clustered before and after the main peak.

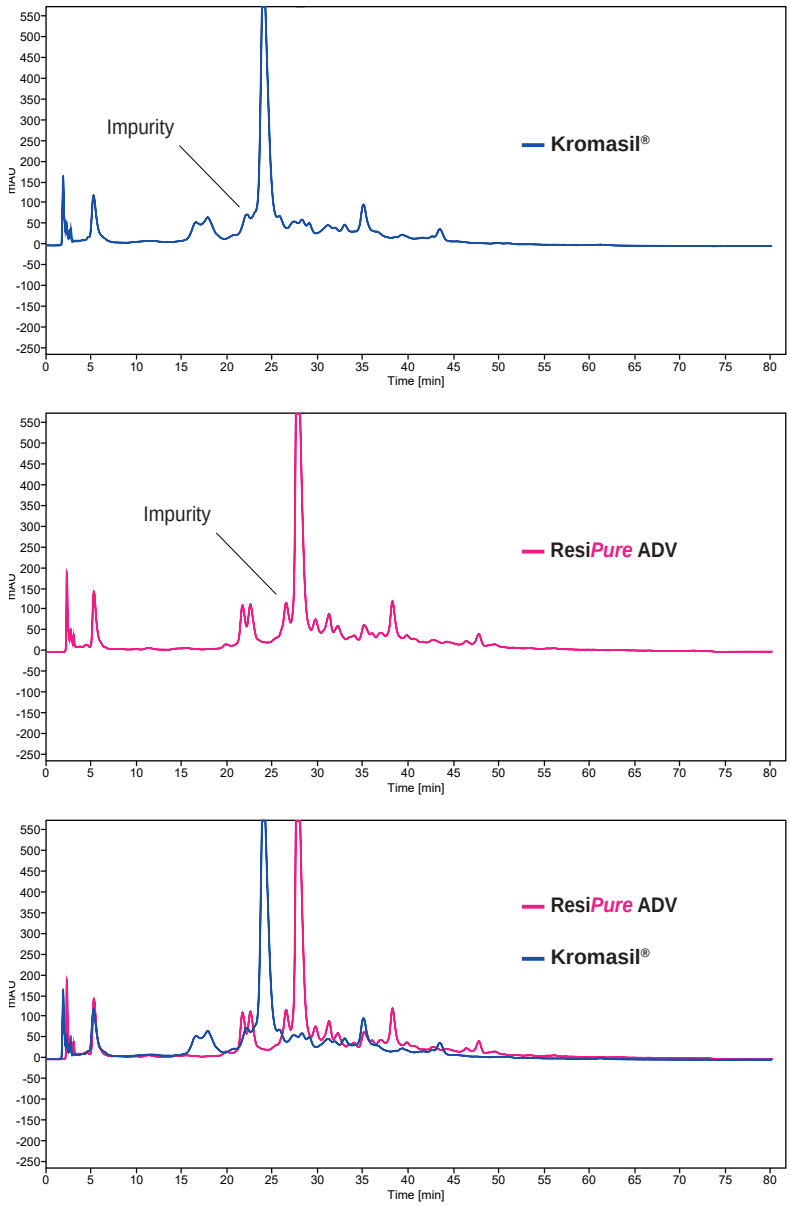
pH 8.0



Tirzepatide elutes at 27.6 minutes, with a greater number of well-resolved impurities appearing afterwards.

Comparison with competitor at pH 8.0

Kromasil® vs ResiPure ADV



Comparison of the two chromatograms				
Stationary phase	Retention time (*min*)	$N_{Tirzepatide}$	$\alpha_{Tirzepatide\text{-}Impurity}$	$R_{Tirzepatide\text{-}Impurity}$
Kromasil® C18	23.914	4113	1.08	0.78
ResiPure ADV C18	27.612	8301	1.08	1.61

ResiPure ADV provides better separation of Tirzepatide from its impurities compared to Kromasil®.

A pH change in eluting conditions on reverse chromatography can lead to a favorable selectivity, and thus obtain the desired separation between impurity and targeted peptide.

ResiPure ADV C18 vs ResiPure ADV C8

Change of stationary phase selectivity

For peptide purification applications, it is strongly recommended to verify the selectivity of both C8 and C18. Depending on the hydrophobicity of the peptide, using a C8 instead of a C18 can improve selectivity under the same elution conditions. As shown here with Tirzeparide purification.

CONDITIONS

Columns: C18 & C8, 250 × 4.6 mm, 10 µm

Mobile phases

- Mobile phase A (MPA): 10 mM potassium phosphate dibasic in water, adjusted to pH 8.0
- Mobile phase B (MPB): acetonitrile

Gradient

1. Hold 10 minutes at 68/32 (MPA/MPB)
2. Increase to 55/45 (MPA/MPB) during 50 minutes
3. Hold 10 minutes at 55/45 (MPA/MPB)
4. Reequilibrate at 68/32 (MPA/MPB) for 10 minutes

Flow rate: 1.000 mL/min

Column temperature: 25°C

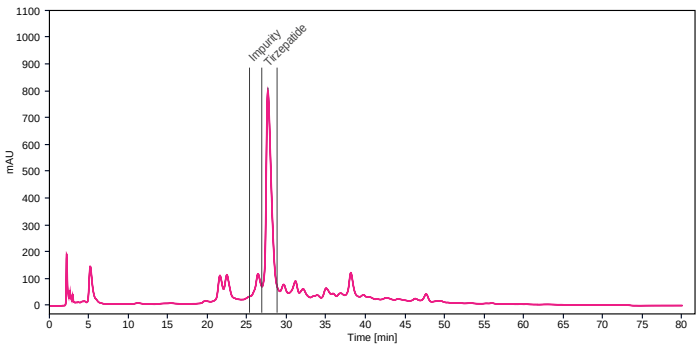
Detection: UV at 220 nm

Injection volume: 5 µL

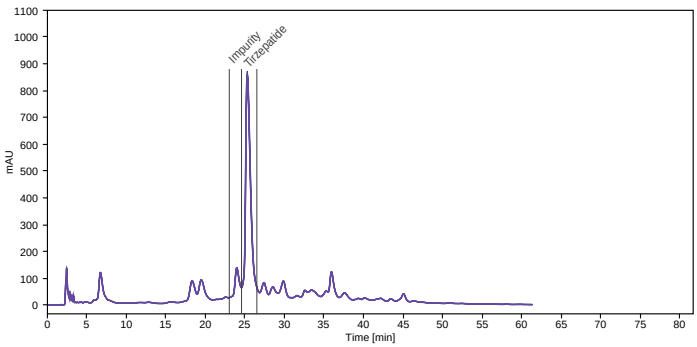
Sample: 125 mg of crude material diluted in 5 mL of acetonitrile/water (50/50)

Gradient		
Time (min)	MPA (%)	MPB (%)
0.01	68	32
10.00	68	32
60.00	55	45
70.00	55	45
70.01	68	32
80.00	68	32

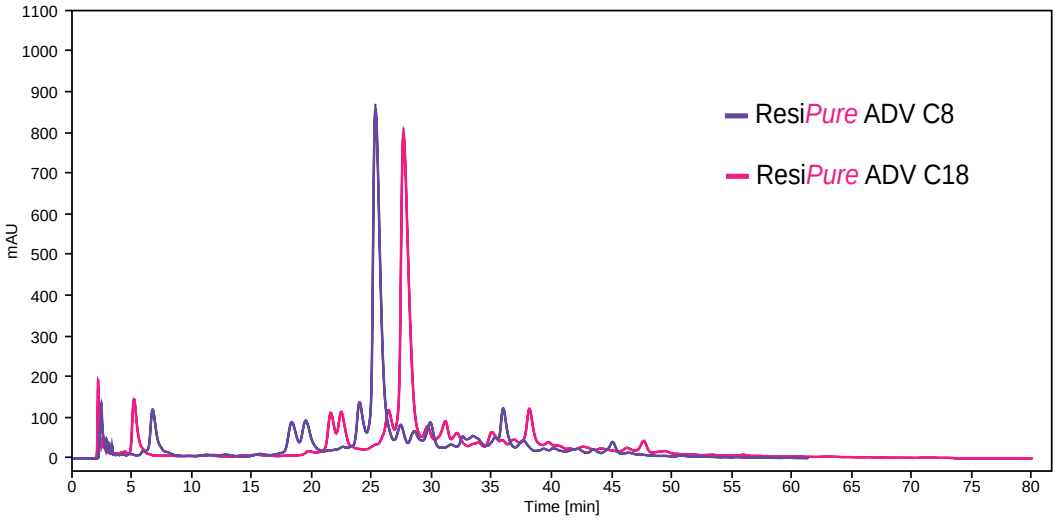
C18



C8



Comparison of the two chromatograms



C8 vs C18 for Tirzepatide Analysis		
Separation parameters	ResiPure Stationnary Phase	
	C8	C18
$\alpha_{Tirzepatide\text{-}Impurity}$	1.06	1.05
$R_{Tirzepatide\text{-}Impurity}$	1.27	1.01

This application shows that ResiPure ADV C8 stationary phase is more selective than ResiPure ADV C18 for purifying Tirzepatide from crude material, with a lower retention time under the same eluent conditions.

Gradient Effect

A gradient optimizes separation by gradually changing the mobile phase composition. This process enhances selectivity, improves peak resolution, and minimizes overlap with impurities.

By precisely adjusting the gradient conditions, process chemists can efficiently obtain high-purity peptides, ensuring superior results.

Here, we compare two gradients of the same duration but with different percentages of acetonitrile.

Comparison of two gradients

CONDITIONS

Column: ResiPure ADV C8, 250 × 4.6 mm, 10 µm

Mobile phases

- Mobile phase A (MPA): 10 mM buffer phosphate pH 8.0
- Mobile phase B (MPB): 100 % acetonitrile

Flow rate: 1.000 mL/min

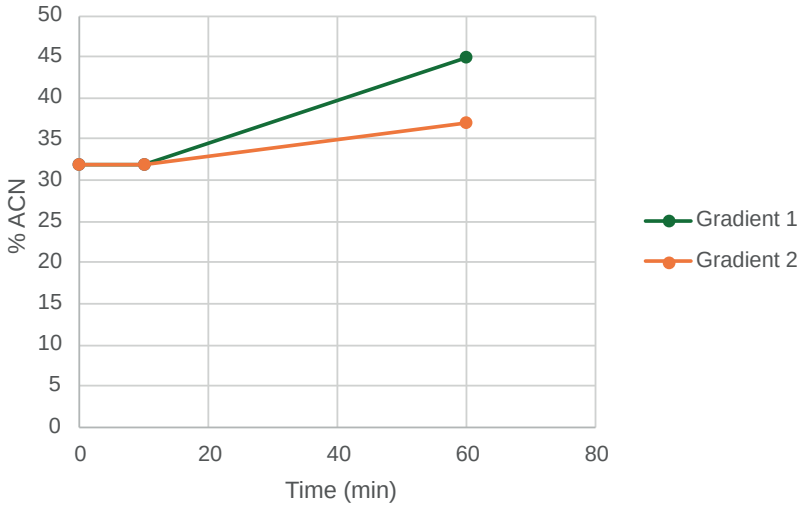
Column temperature: 25°C

Detection: UV at 215 nm

Injection volume: 5 µL

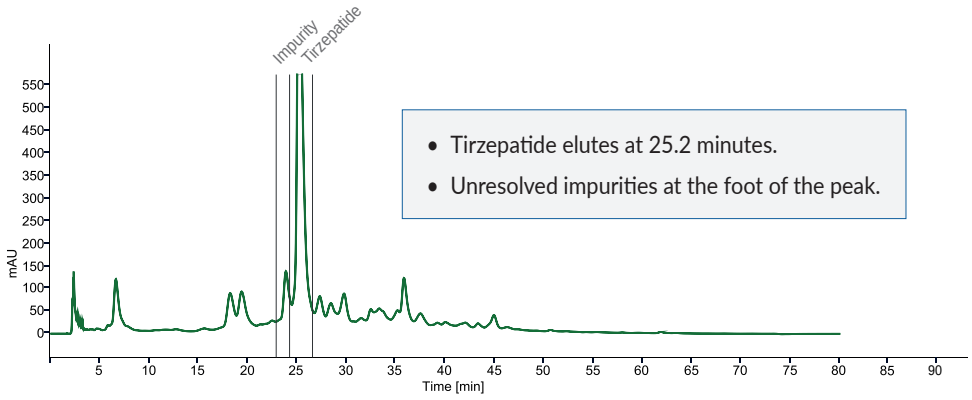
Sample: 125 mg of crude material diluted in 5 mL of acetonitrile/water (50/50)

Different percentages of acetonitrile



Gradient 1

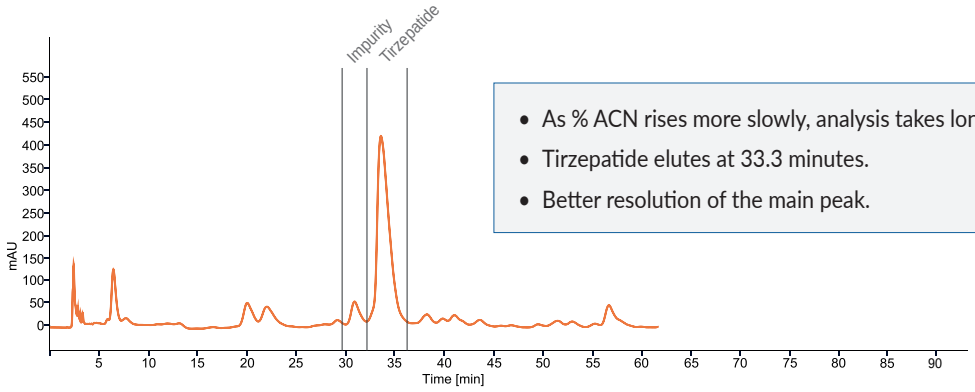
- 0 min: 32 % ACN
- 10 min: 32 % ACN
- 60 min: 45 % ACN



- Tirzepatide elutes at 25.2 minutes.
- Unresolved impurities at the foot of the peak.

Gradient 2

- 0 min: 32 % ACN
- 10 min: 32 % ACN
- 60 min: 37 % ACN



- As % ACN rises more slowly, analysis takes longer.
- Tirzepatide elutes at 33.3 minutes.
- Better resolution of the main peak.

Gradient Effect		
Separation parameters	Gradient	
	1	2
$\alpha_{\text{Tirzepatide-Impurity}}$	1.06	1.09
$R_{\text{Tirzepatide-Impurity}}$	1.27	1.49

Modifying conditions of a gradient can improve selectivity, allowing for better separation of an API from its impurities.

Purification of Tirzepatide

The crude material is about 22.3 % pure Tirzepatide. A multi-step purification process must be developed to achieve the final target purity of > 99 %.

Analytical purity test

The purity of each extract was determined using the following HPLC conditions.

HPLC CONDITIONS

Column: Silia*Chrom* dt C18, 250 × 4.6 mm, 3 µm

Flow rate: 0.800 mL/min

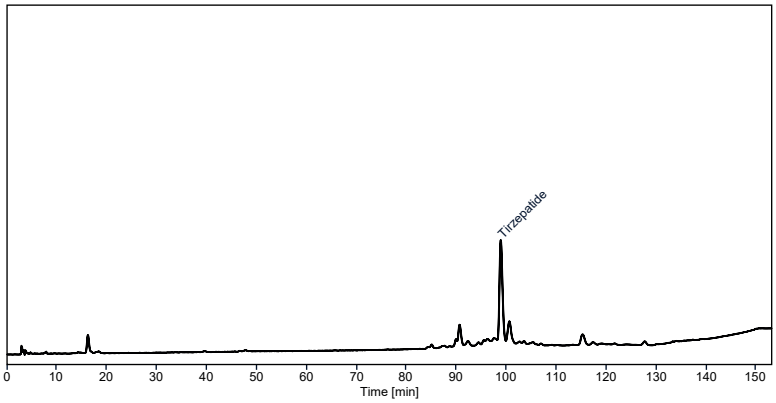
Column temperature: 30 °C

Mobile phases

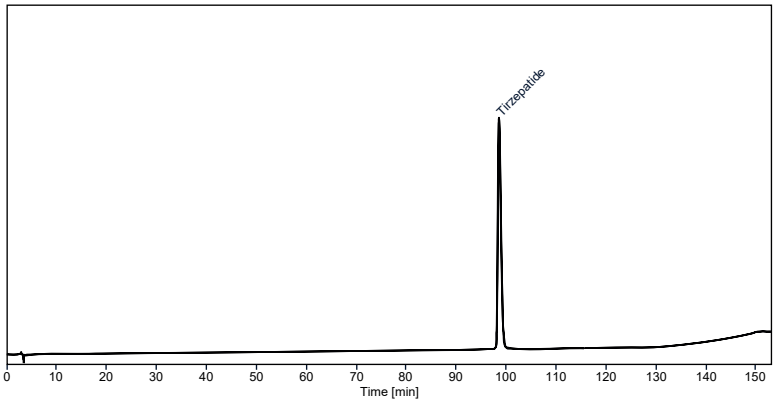
- Mobile phase A (MPA): 0.1 % trifluoroacetic acid in water (v/v)
- Mobile phase B (MPB): 0.1 % trifluoroacetic acid in acetonitrile (v/v)

Detection: UV at 220 nm

Gradient		
Time (min)	MPA (%)	MPB (%)
0.01	70	30
120.00	45	55
125.00	45	55
145.00	20	80
150.00	20	80
150.01	70	30
160.00	70	30



HPLC profile of Tirzepatide for the crude material: 20 mg/mL diluted in 75/25 (water/acetonitrile), injection volume: 3 µL.



HPLC profile of Tirzepatide for the reference standard: 1 mg/mL diluted in 50/50 (water/acetonitrile), injection volume: 20 µL.

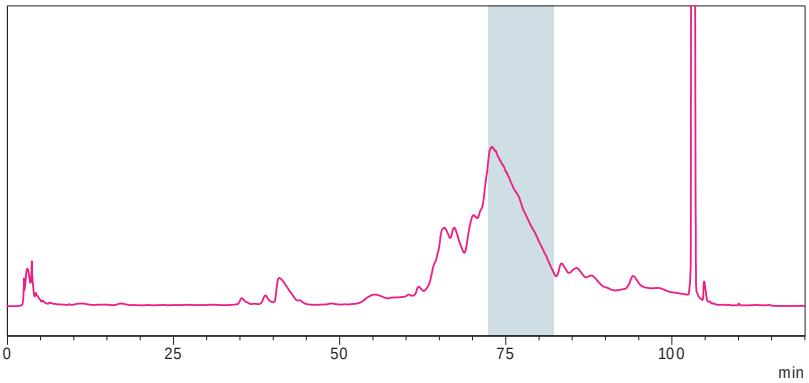
Purification process summary

Two successive purification steps were necessary to purify the Tirzepatide from the crude material and achieve the targeted purity of > 99 %.

Both steps were performed using ResiPure ADV C8. The first step was performed under basic conditions (pH 8.0), and the second under acidic conditions (pH 3.5).

Please refer to Figures 1 and 2 for the complete purification process of Tirzepatide.

Step 1: crude purification



Step 2: final polishing

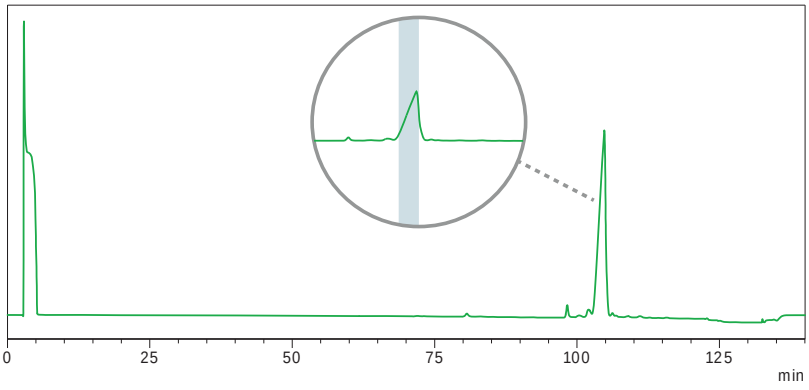


Figure 1: Tirzepatide purification process summary

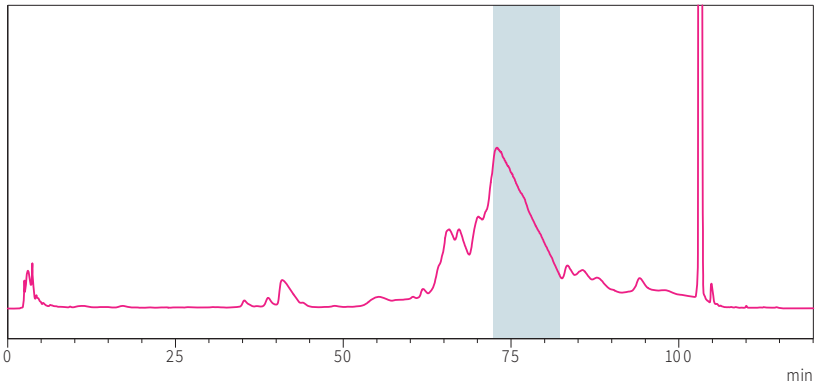
Purification step 1: removal of bulk impurities

The initial purification step was performed using the crude Tirzepatide product. The purity of the crude material was approximately 22.3 %.

Method development was performed at the analytical scale by screening all ResiPure ADV stationary phases. The best results in terms of separation efficiency, target purity, and recovery rate were obtained using ResiPure ADV C8 with elution conditions fixed at pH 8.0.

The final fractionation was optimized to achieve a target purity of 91 % and a yield of 95 %.

This chromatographic purification step at pH 8.0 with ResiPure ADV led to a significant improvement in Tirzepatide purity.



The purification step 1 conditions are described below:

CONDITIONS

Column: ResiPure ADV C8, 250 × 10 mm, 10 µm

Mobile phases:

- Mobile phase A (MPA): 20 mM ammonium bicarbonate, pH 8.0
- Mobile phase B (MPB): acetonitrile

Flow rate: 4.75 mL/min

Column temperature: 25 °C

Detection: 220 nm

Injection volume: 2 mL

Sample: 40 mg/mL Tirzepatide crude in 75/25 (water/acetonitrile)

Collection: 72.7 minutes to 82.0 minutes

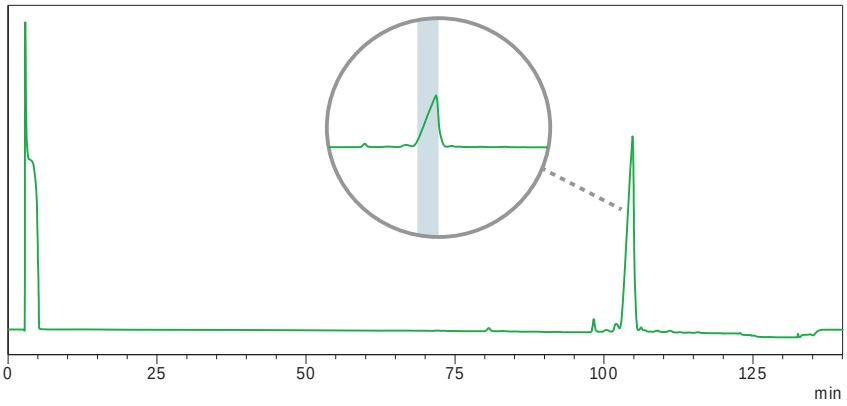
Gradient		
Time (min)	MPA (%)	MPB (%)
0.01	75	25
10.00	75	25
80.00	65	35
100.00	65	35
110.00	20	80
120.00	75	25

Purification step 2: final polishing

The second chromatography step in this purification process was a polishing step.

This step was also carried out using the ResiPure ADV C8. Purification was performed at pH 3.5, which led to excellent separation results.

The final product purity was 99.8 %, with a yield of about 75 %.



The purification step 2 conditions are described below:

CONDITIONS

Column: ResiPure ADV C8, 250 × 10 mm, 10 µm

Mobile phases:

- Mobile phase A (MPA): 50 mM ammonium formate, pH 3.5
- Mobile phase B (MPB): acetonitrile

Flow rate: 4.75 mL/min

Column temperature: 25 °C

Detection: 220 nm

Injection volume: 1 mL

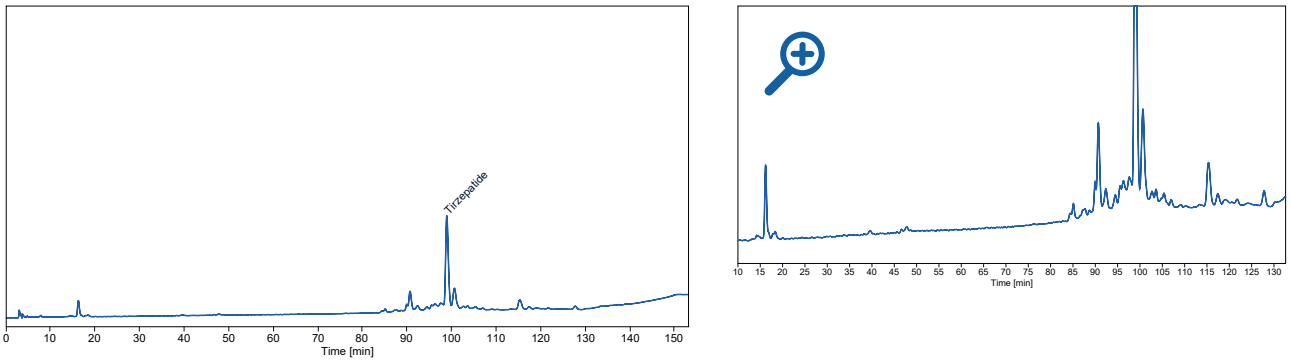
Sample: Tirzepatide crude after the purification step 1, 25 mg/mL in DMSO

Collection: 103.5 minutes to 105.0 minutes

Gradient		
Time (min)	MPA (%)	MPB (%)
0.01	80	20
10.00	80	20
60.00	70	30
120.00	45	55
130.00	20	80
140.00	80	20

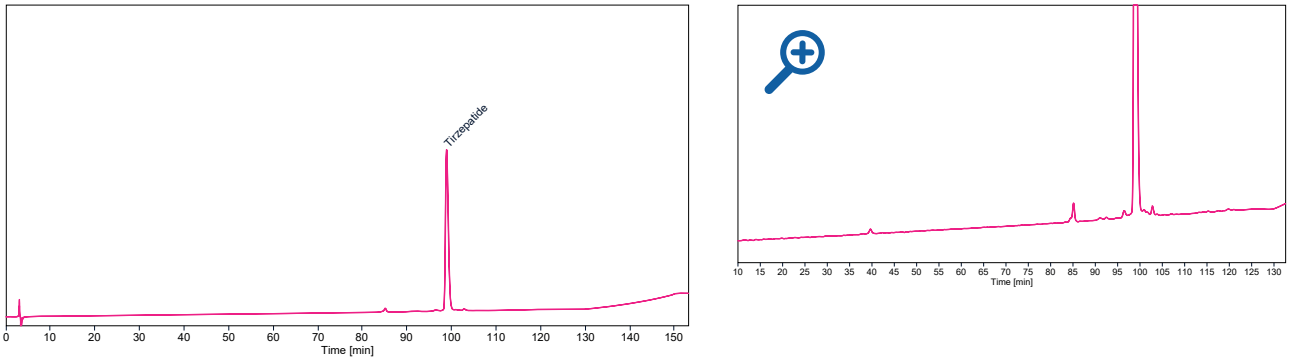
Tirzepatide purity analysis by HPLC-UV for each purification step

Initial purity: 22.3 %



Purification Step 1

Purity: 91 %
Yield: 95 %



Purification Step 2

Purity: 99.8 %
Yield: 72 %

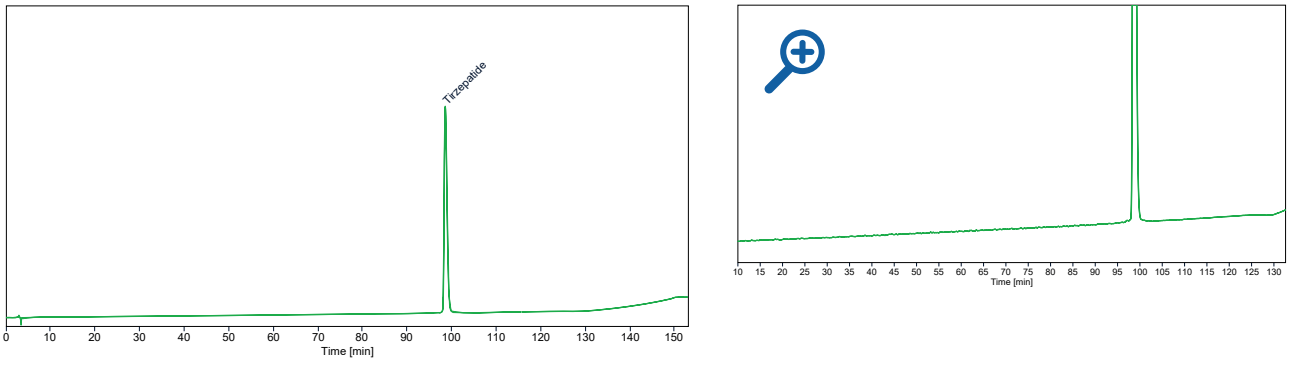


Figure 2: Chromatographic profiles of Tirzepatide, for each purification step, using Silia*Chrom* dt C18

Conclusion

Two successive purification steps were required to purify the Tirzepatide crude material to a purity level greater than 99.5 %. Both steps used ResiPure ADV C8.

- The first step was performed under basic conditions (pH 8.0) to remove bulk impurities from the crude.
- The second step was performed under acidic conditions (pH 3.5) to refine the extract from the first purification step.

The purity of Tirzepatide in the final extract was 99.8 %, compared to 22.3 % in the initial crude.

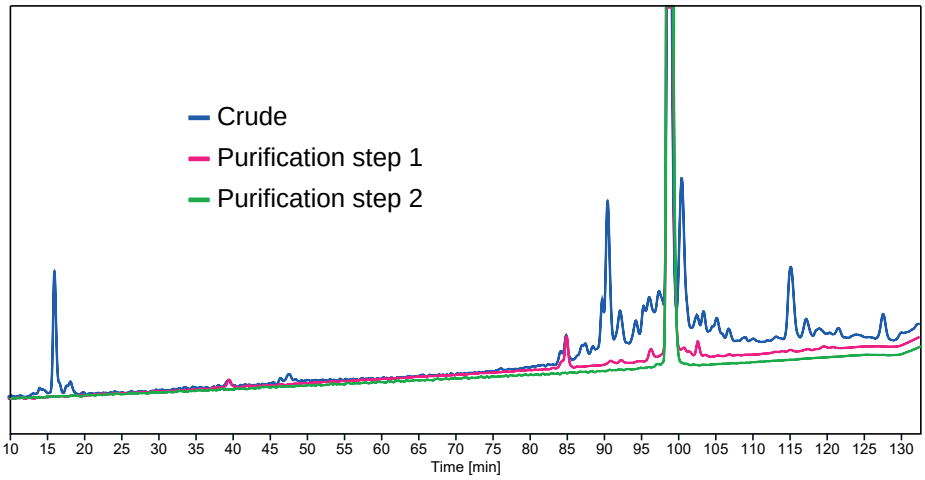


Figure 3: Overlay of the different chromatographic profiles of Tirzepatide after each purification step

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