# ResiPure™ ADVANCED

## SOLUTION FOR PEPTIDE PURIFICATION

SILICYCLE

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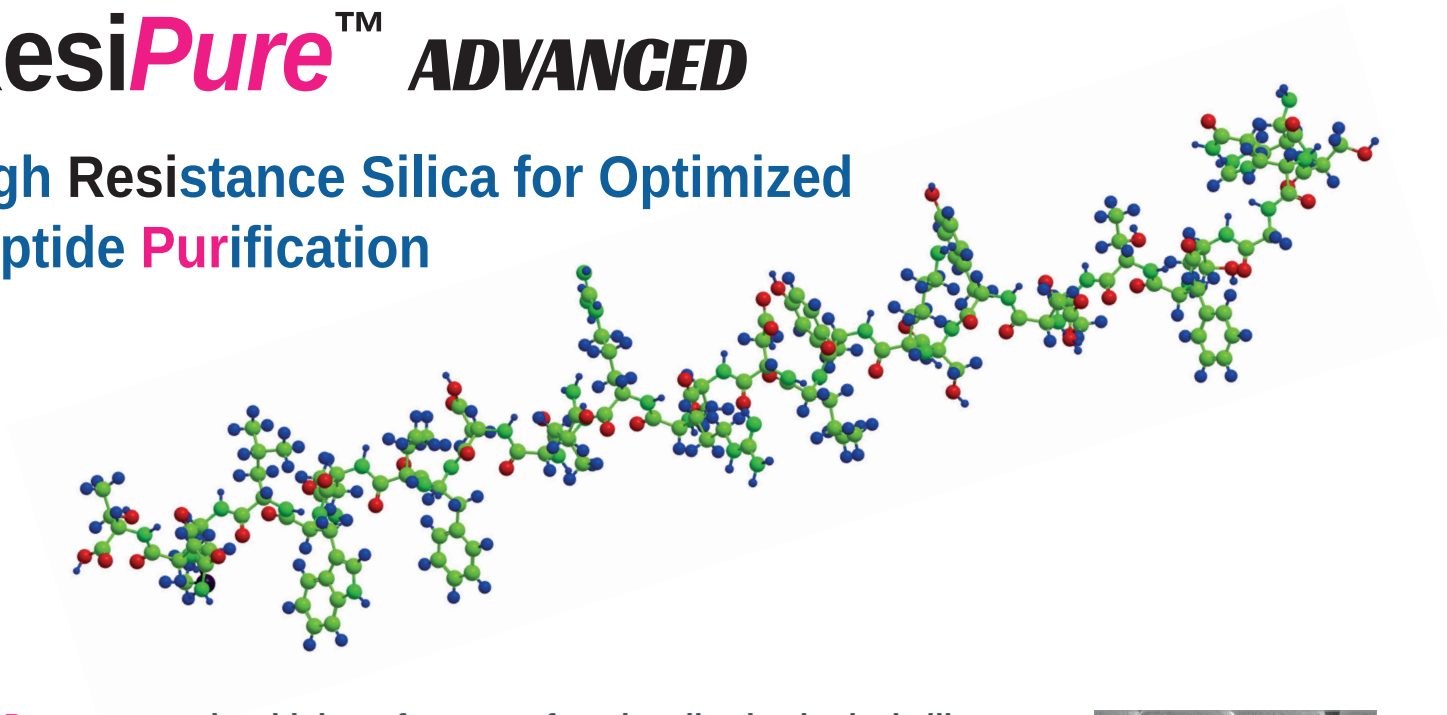
# ResiPure™ ADVANCED

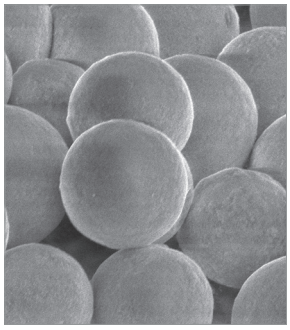
## Optimize the Purification of your GLP-1 Agonists and Peptide Analogs

# 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

pH 1 to 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 gels on the market

## Excellent ROI

- High performance purification
- Particle offering long lifetime

## 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

Resi*Pure* ADV silica gels are offered from 10 g up to multi-ton scale.

| Resi*Pure* 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 |

| Resi*Pure* 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 |

| Resi*Pure* 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

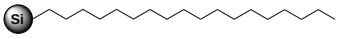
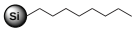
For analytical needs, HPLC columns packed with Resi*Pure* ADV are also offered: contact us!

**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, Resi*Pure* 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

**CHROMATOGRAPHIC TEST CONDITIONS**

**Column**: C18, 250 × 4.6 mm, 10 µm

**Mobile phase**: acetonitrile/water (80/20)

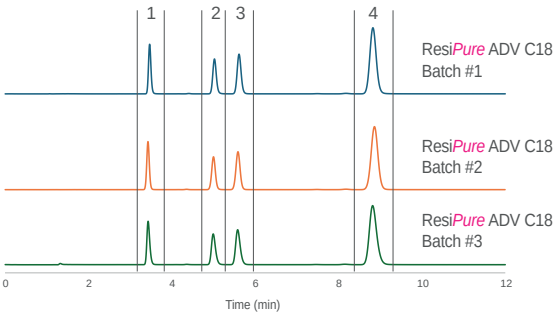
**Flow rate**: 1.000 mL/min

**Column temperature**: 25°C

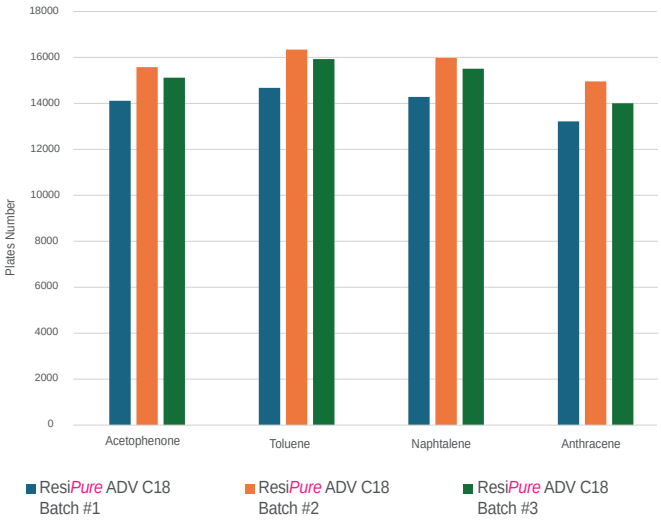
**Detection**: UV at 254 nm

**Injection volume**: 5 µL

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




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

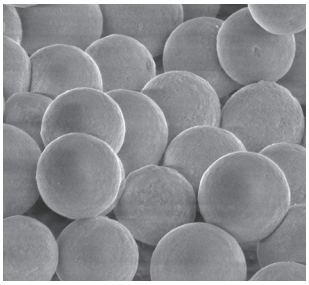



Efficiency and retention were similar for the hydrophobic markers analysed on 3 different batches of ResiPure ADV.

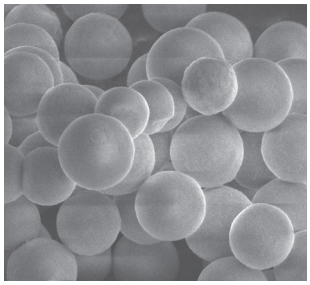
## Morphology

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

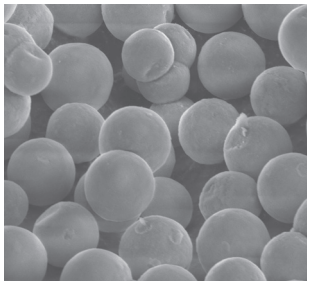
**Batch #1**



**Batch #2**



**Batch #3**



# Peptide Analysis on ResiPure ADV C18

Sigma-Aldrich H2016 Peptide Mix

**CONDITIONS**

**Column**: 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)

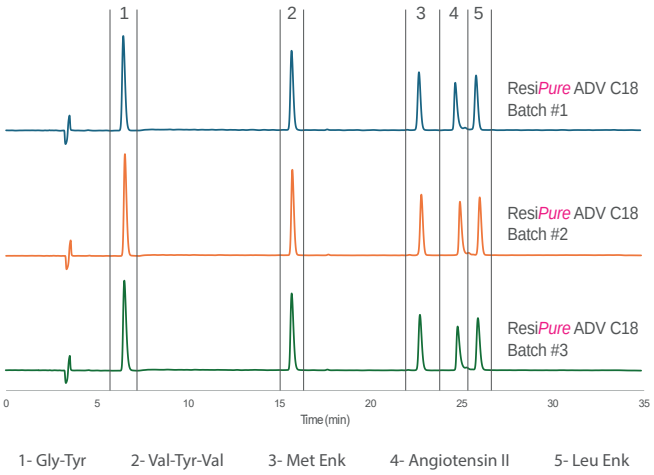
**Flow rate**: 1.000 mL/min

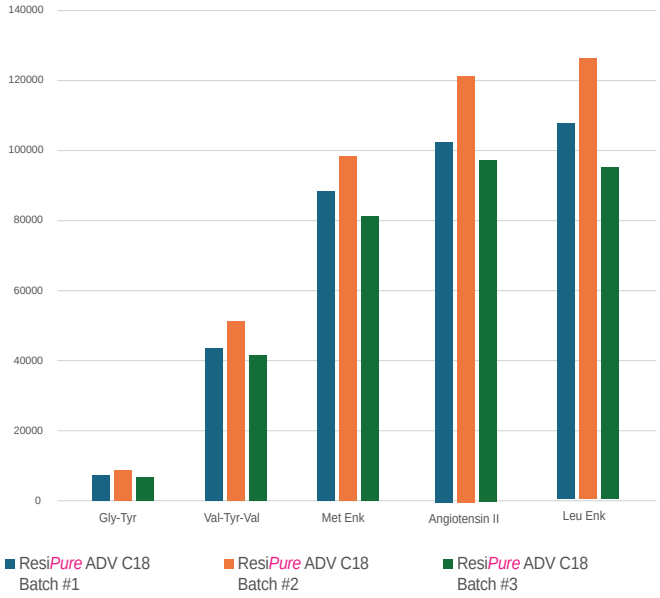
**Column temperature**: 25°C

**Detection**: UV at 220 nm

**Injection volume**: 10 µL

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




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 (A) | 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**

**Test 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

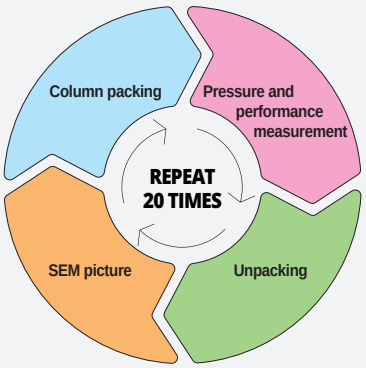
**System**: Shimadzu Preparative HPLC

**Injection volume**: 500 µL

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

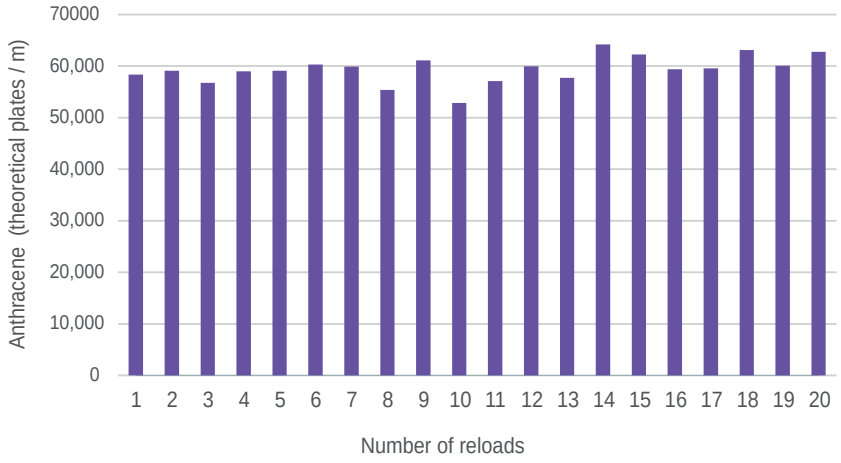
**Test Procedure**

20 consecutive cycles of column packing / unpacking



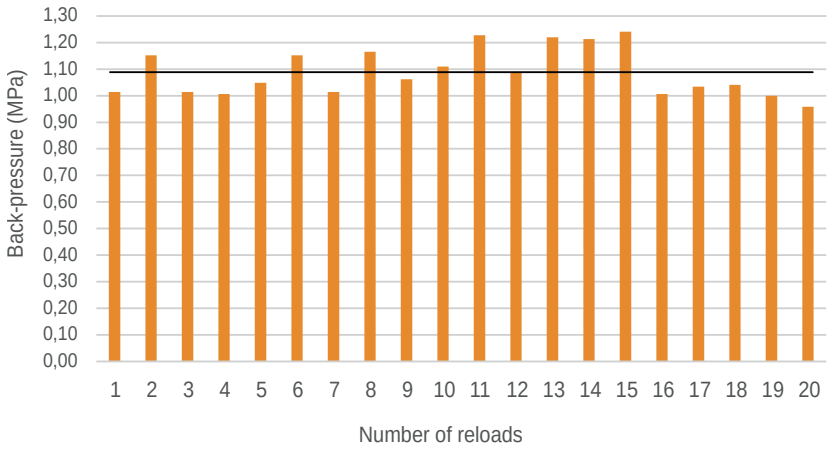

### Results

**Column performance vs number of reloads**



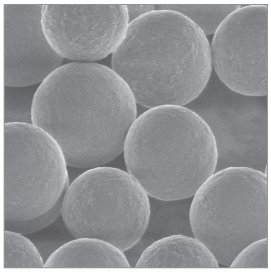
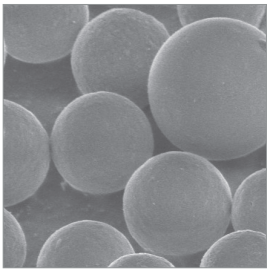
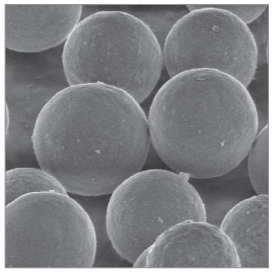
## Results

### 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.**

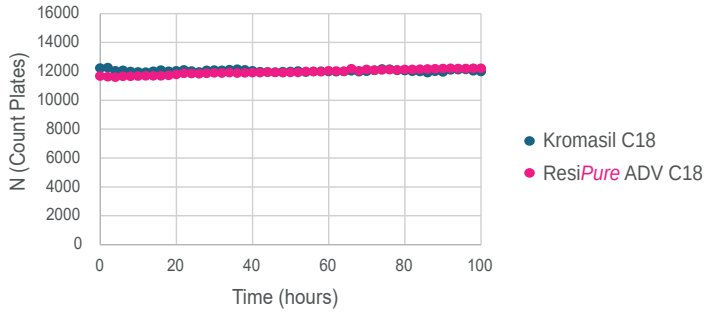
# pH Stability in Mobile Phase

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.

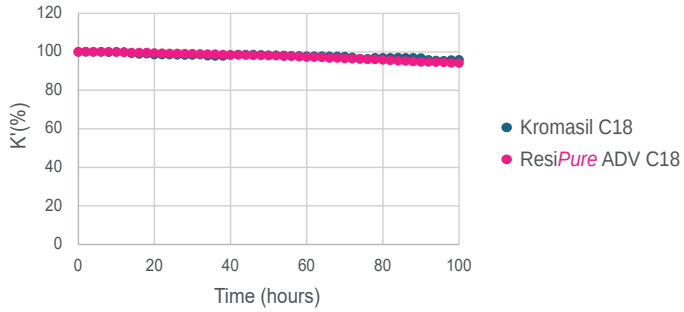
A stable pH ensures that peptides maintain their desired charge state throughout the chromatographic process, optimizing separation efficiency.

## At pH 1.0 (1% TFA at 70°C)

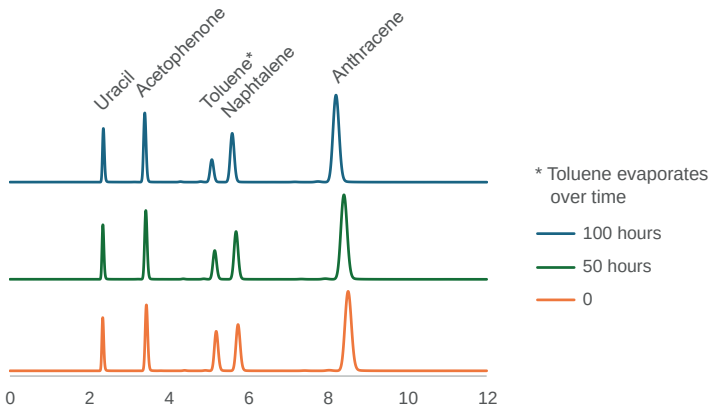
No efficiency loss after 100 hours (retention marker: anthracene):




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




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




**CONDITIONS**

**Low pH conditions (1% TFA at 70°C)**

**Column size**: 250 × 4.6 mm

**Flow rate**: 0.400 mL/min

**Mobile phase**: acetonitrile/water/trifluoroacetic acid

**Column temperature**: 70 °C

**pH** = 1.00 (9.5/89.5/1)

**Chromatographic test conditions**

**Column size**: 250 × 4.6 mm

**Flow rate**: 1.000 mL/min

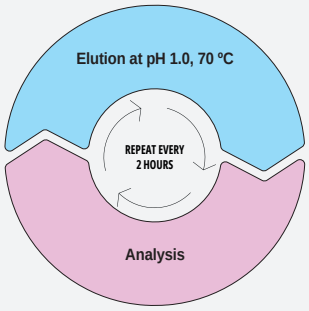
**Mobile phase**: acetonitrile/water (70/30)

**Column temperature**: 70 °C

**Detection**: UV at 254 nm

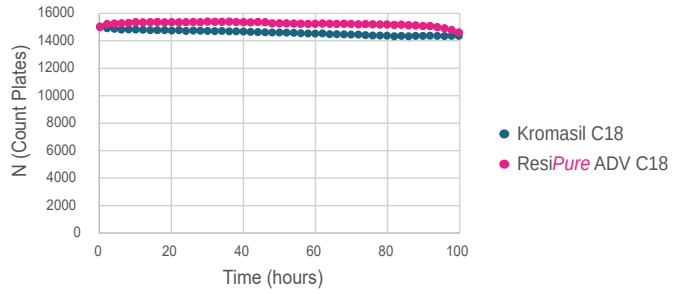
**Sample**: uracil, acetophenone, toluene, naphtalene, anthracene

**Test Cycle**

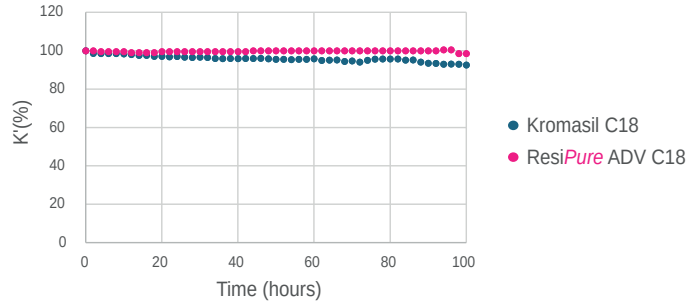


## At pH 10.0 (Ammonium carbonate at RT)

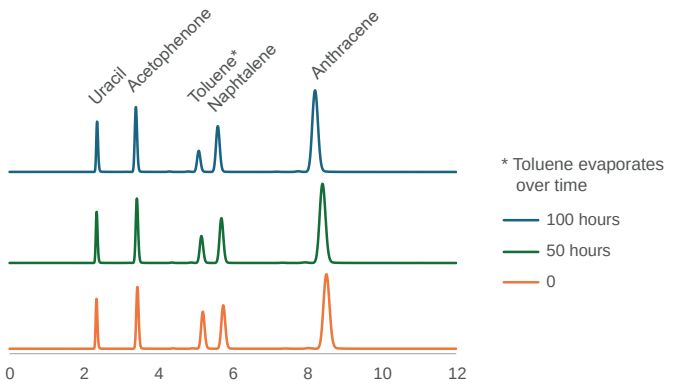
No efficiency loss after 100 hours:




No retention factor loss after 100 hours:




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




**CONDITIONS**

**pH 10 conditions**

**Column size**: 250 × 4.6 mm

**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**

**Column size**: 250 × 4.6 mm

**Flow rate**: 1.000 mL/min

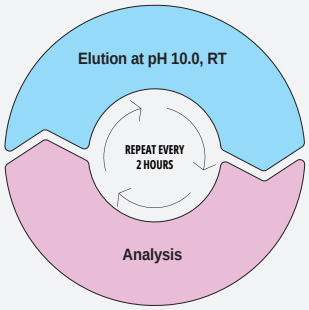
**Mobile phase**: acetonitrile/water (80/20)

**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.

**pH 1 to 13 for alkaline wash**

## Test: Alkaline wash cycles (pH 13)

**CONDITIONS**

**Alkaline washing cycle conditions**

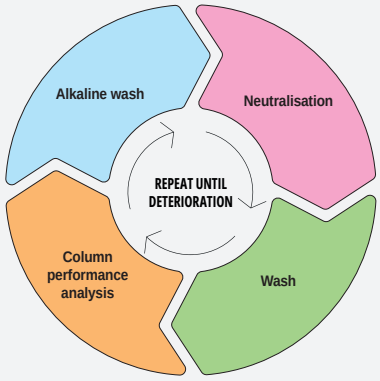
**Column**: C18, 250 × 4.6 mm, 10 µm

**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)

**Washing cycles procedure**




**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

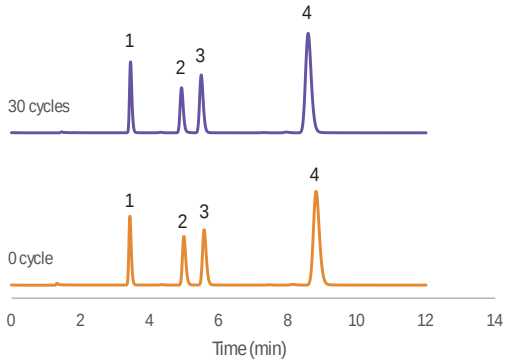
**Detection**: UV at 254 nm

**Test compound**: anthracene

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

## Results

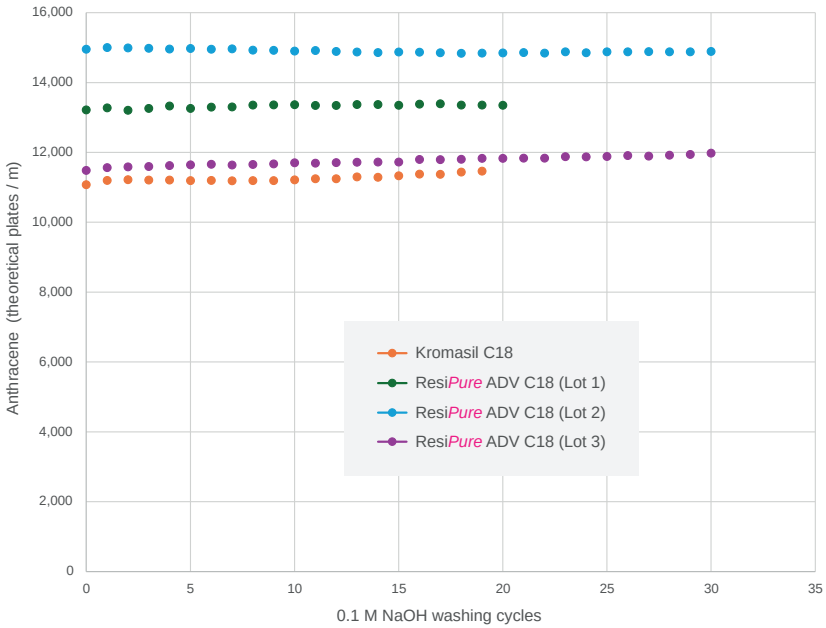
### Performance after 30 cycles



# Results

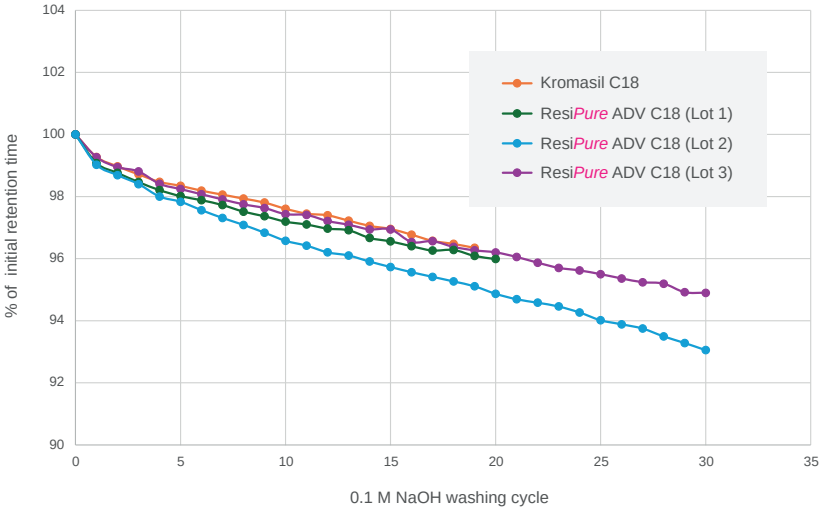
## Loss of efficiency after alkalin washing cycle (pH 13)

Retention marker: Anthracene




## Loss of retention after alkalin 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**

Column: 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

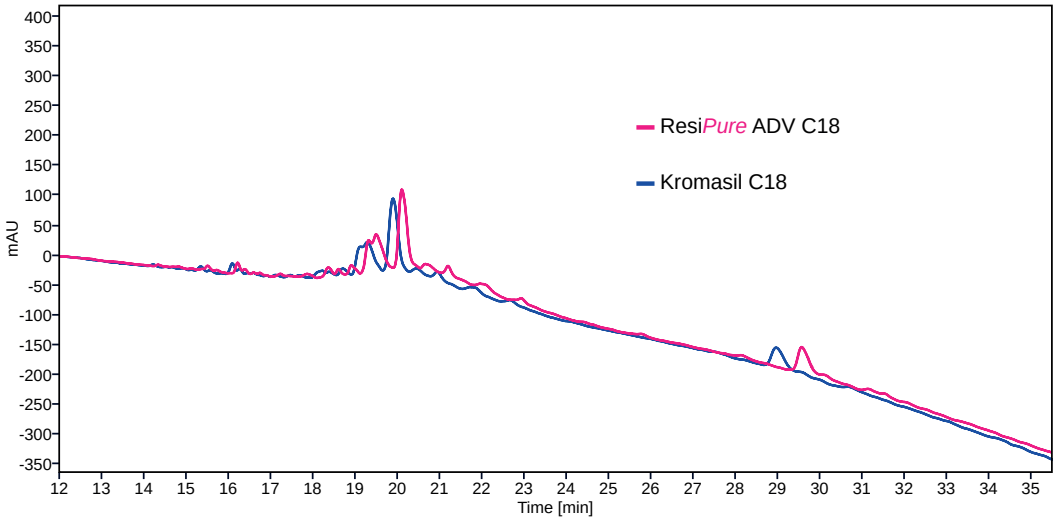
**Column temperature**: 25°C

**Detection**: UV at 215 nm

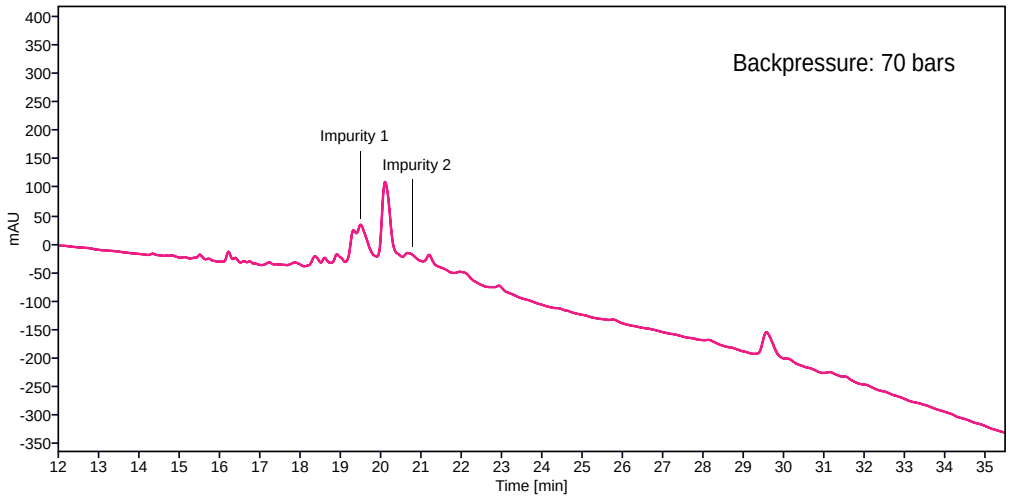
**Sample load**: 40 µL of 50 mg crude product diluted in 10 mL of 1% of acetic acid in water (v/v)

| 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 |

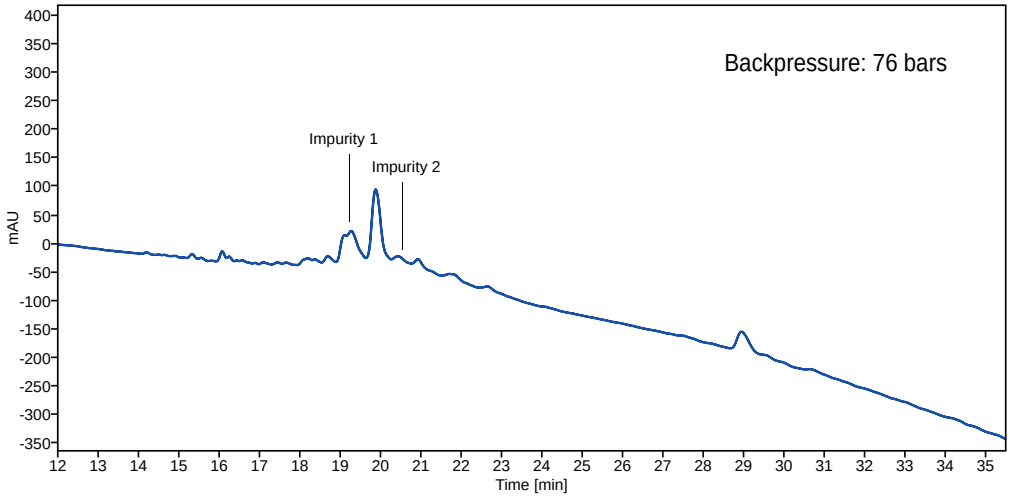
## Impurities profiles comparison: ResiPure ADV C18 vs Kromasil® C18



## 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

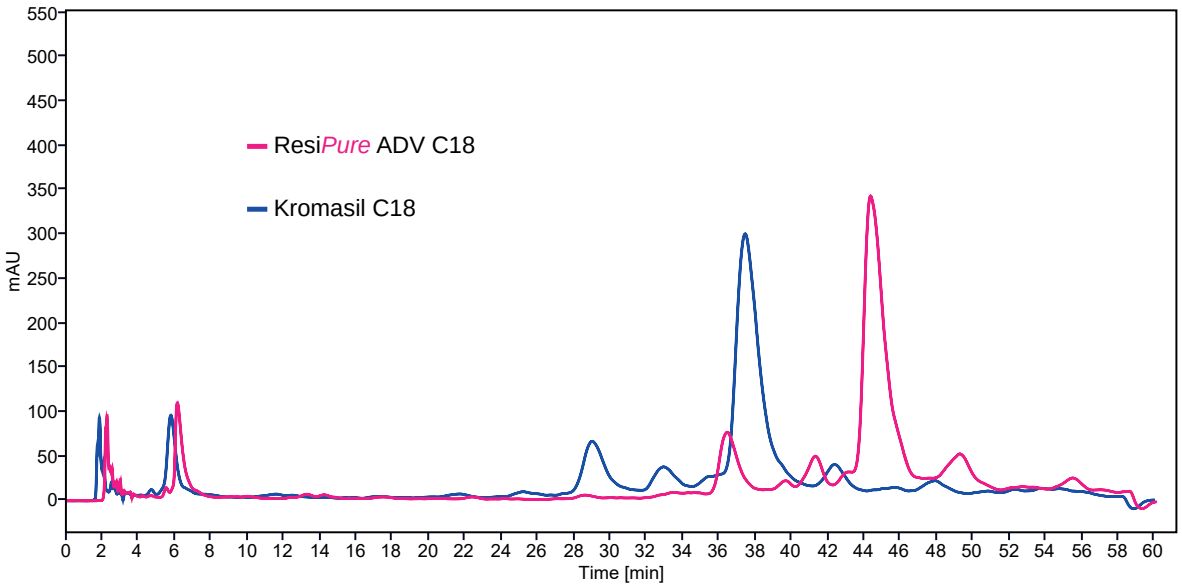
**Detection**: UV at 220 nm

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

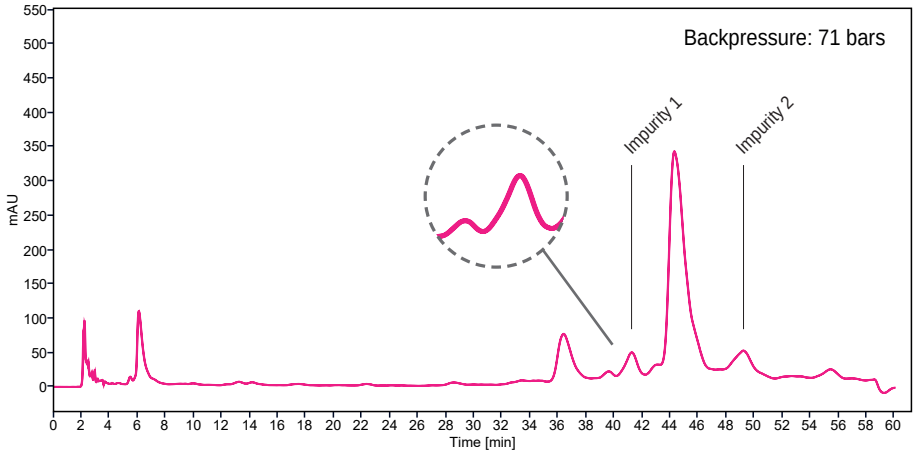
**Dissolving solution**: 50/50 acetonitrile/water

| 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 |

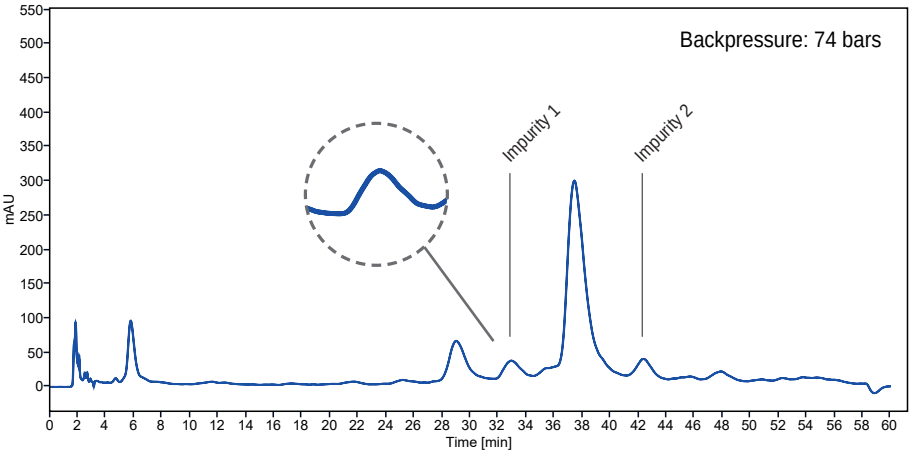
## Impurities profiles comparison: ResiPure ADV C18 vs Kromasil® C18



## 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 peptide mixtures

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**

**Column**: 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

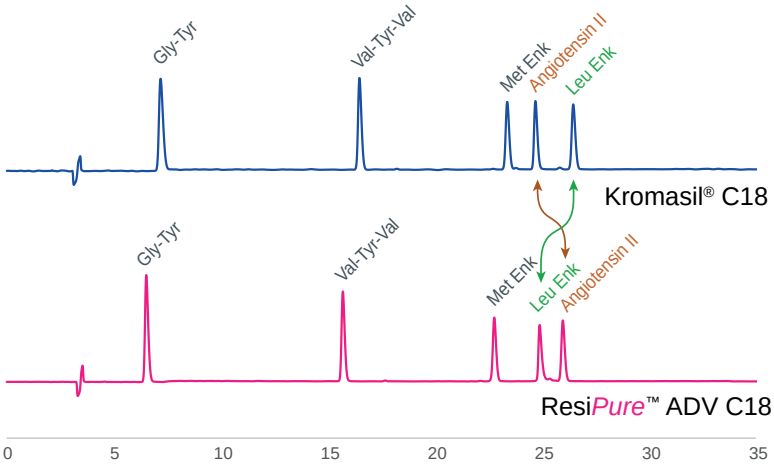
**Column temperature**: 25°C

**Detection**: UV at 220 nm

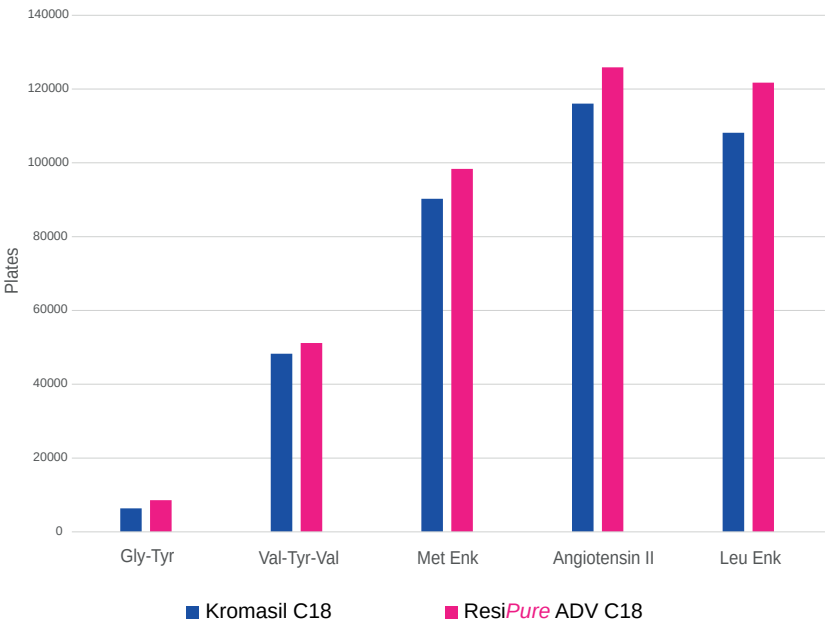
**Injection volume**: 10 µL

**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 ammoniun 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**: 1.000 mL/min

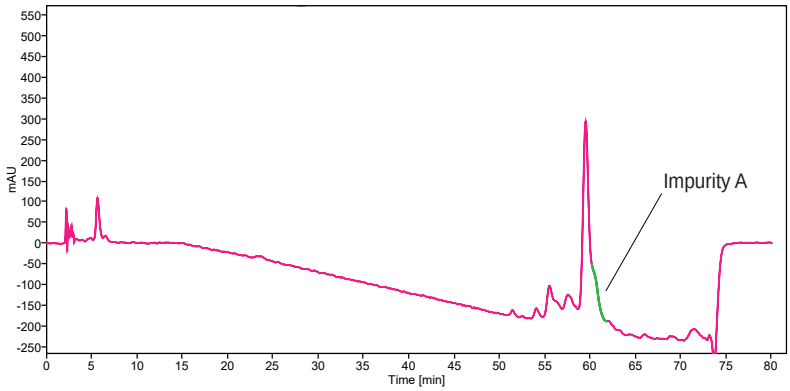
**Column temperature**: 25°C

**Detection:** UV at 220 nm

**Injection volume**: 5 µL

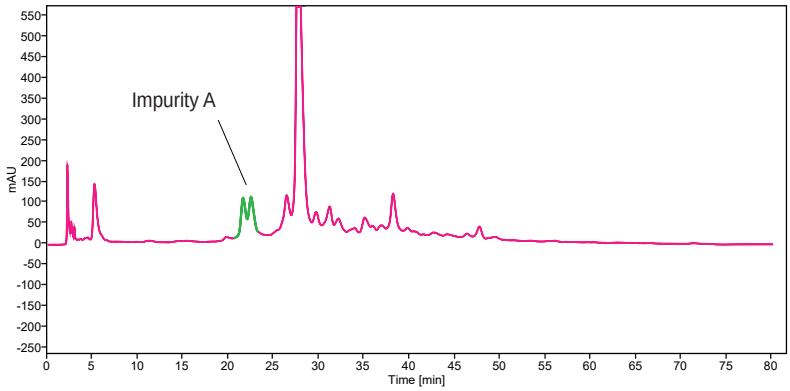
**Sample**: 125 mg of crude material diluted in 5 mL of acetonitrile/water (50/50)

### pH 3.5




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

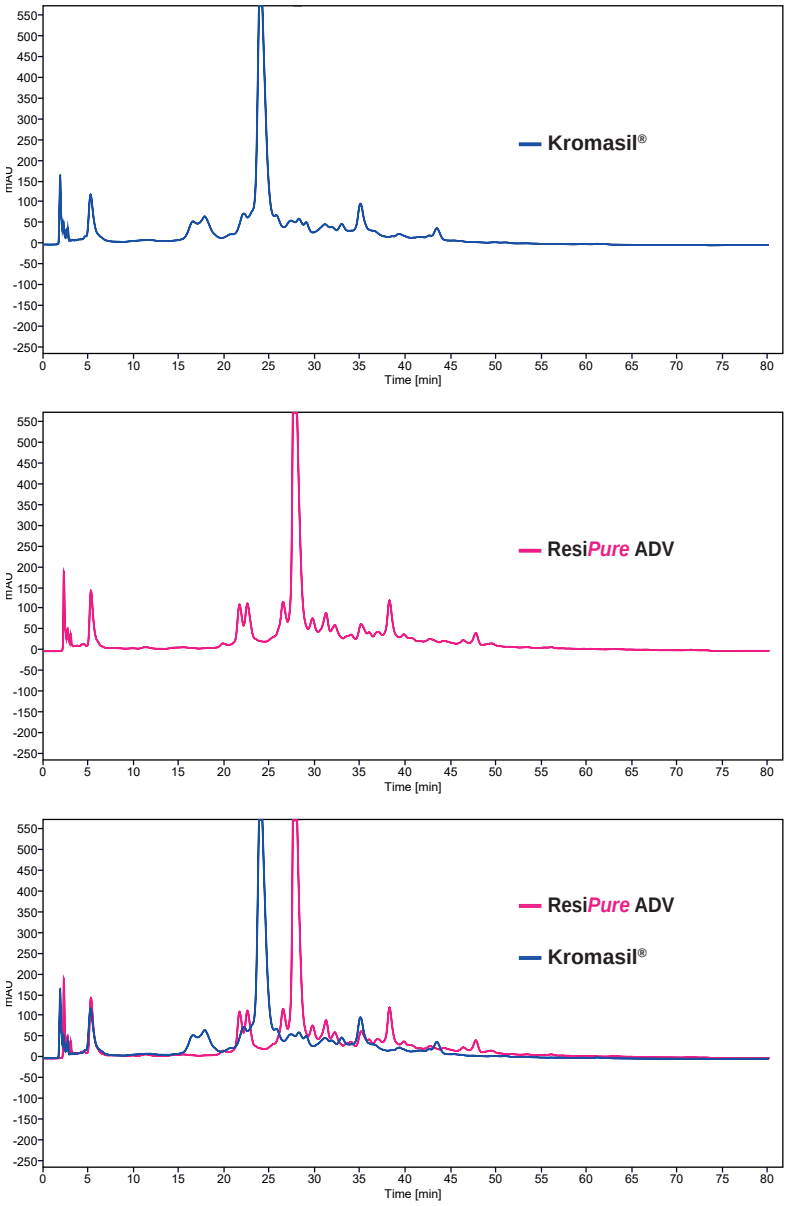
### pH 8.0




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.

**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

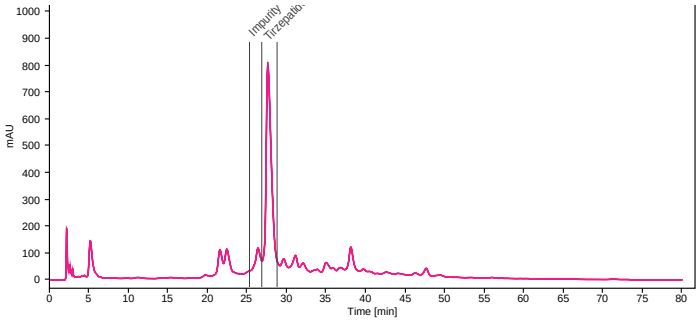
**Detection**: UV at 220 nm

**Injection volume**: 5 µL

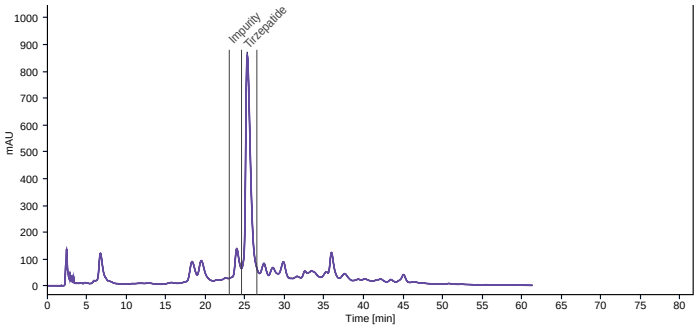
**Sample**: 125 mg of crude material diluted in 5 mL of acetonitrile/water (50/50)

| 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 |

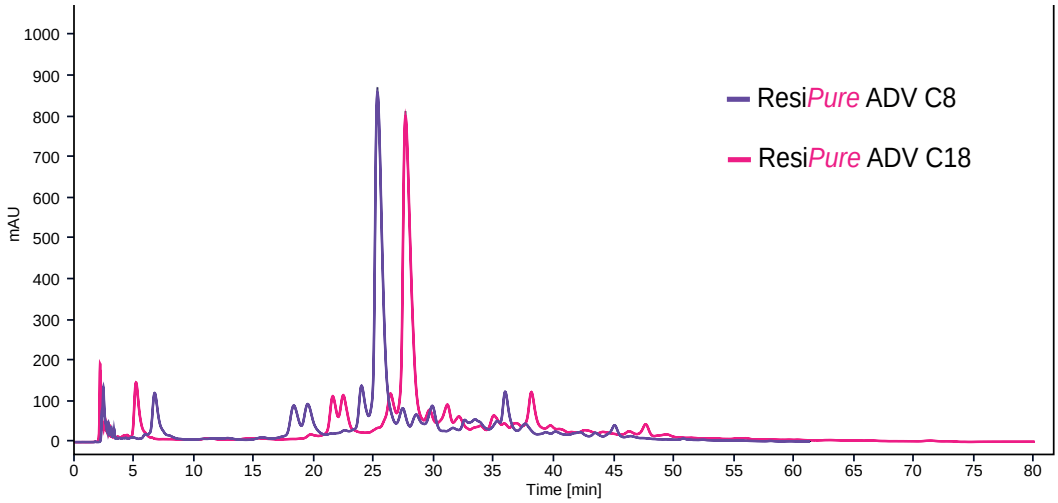
## C18




## C8



## Comparison of the two chromatograms




**C8 vs C18 for Tirzepatide Analysis**

| Separation parameters | ResiPure Stationnary Phase | |
|---|---|---|
| | C8 | C18 |
| $\alpha_{\text{Tirzepatide-Impurity}}$ | 1.06 | 1.05 |
| $R_{\text{Tirzepatide-Impurity}}$ | 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

The gradient effect optimizes separation by gradually modifying the mobile phase composition. This enhances selectivity, improves peak resolution, and minimizes impurities.

By fine-tuning gradient conditions, process chemists achieve high-purity peptides efficiently, ensuring superior results.

**Here, we compare two gradients, of the same duration but varying ACN percentage.**

## Comparison of two gradients

**CONDITIONS**

**Column**: Resi*Pure* ADV C8, 250 × 4.6 mm, 10 µm

**Mobile phases**

- Mobile phase A (MPA): 10 mM buffer phosphate pH 8.00
- Mobile phase B (MPB): 100% acetonitrile

**Flow**: 1.000 mL/min

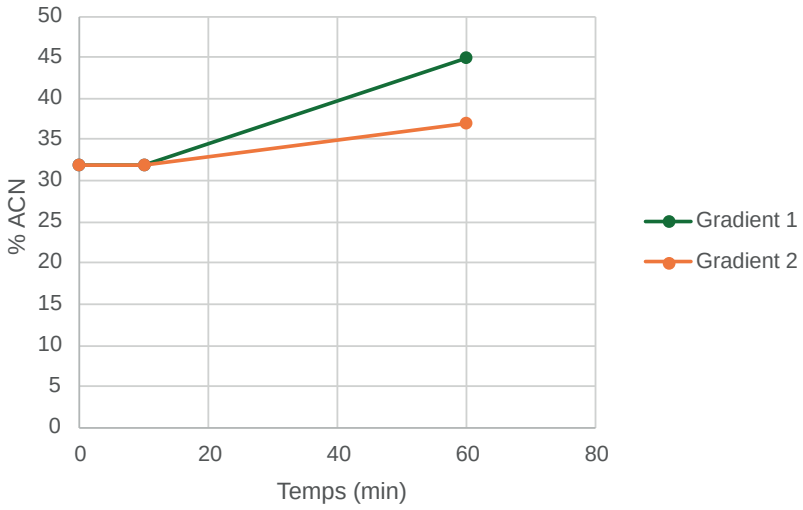
**Column temperature**: 25°C

**Detection**: UV at 215 nm

**Injection volume**: 5 µL

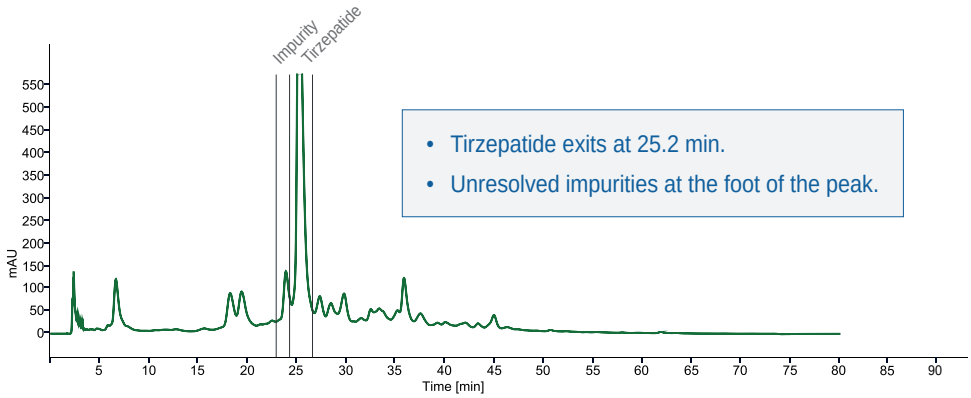
**Sample**: 125 mg of crude material diluted in 5 mL of acetonitrile/water (50/50)

### Varying ACN percentage



## Gradient 1

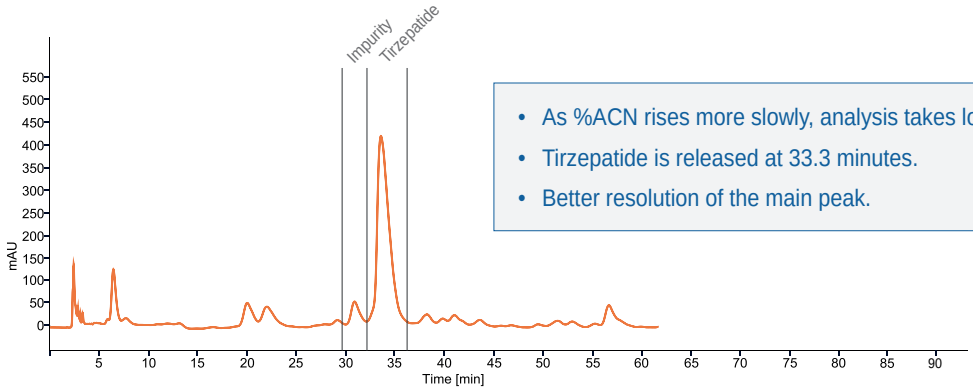
- 0 min: 32% ACN
- 10 min: 32% ACN
- 60 min: 45% ACN




- Tirzepatide exits at 25.2 min.
- 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 is released 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 |

**Change of linear gradient conditions can lead to an improvement in selectivity to separate an API from the other impurities.**

# Purification of Tirzepatide

The crude material has a purity about 22.3% in Tirzepatide. A purification process must be developed which requires a multi-step purification to achieve the final target purity of >99%.

## Analytical purity test

The purity of the different extract was determined according to the following HPLC conditions.

**LIQUID CHROMATOGRAPHY CONDITIONS**

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

**Flow**: 1.000 mL/min

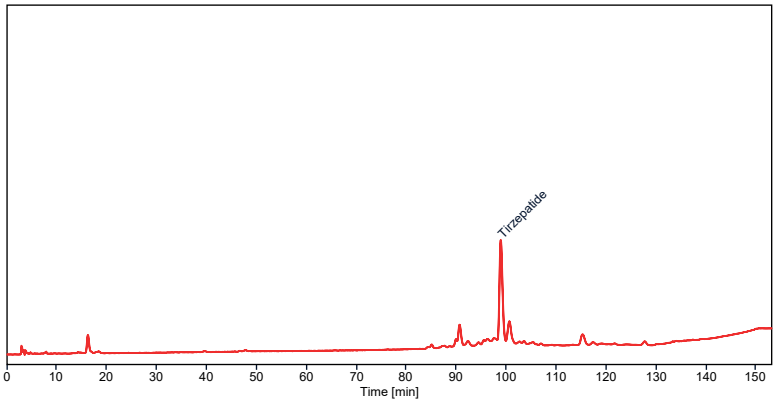
**Column Temperature**: 30 °C

**Gradient**

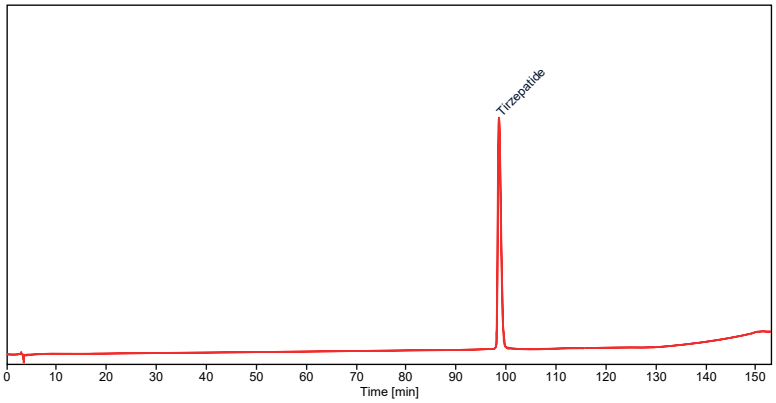
- MPA: 0.1% of trifluoroacetic acid in water (v/v)
- MPB: 0.1% of trifluoroacetic acid in Acetonitrile (v/v)

**Detection**: UV at 220 nm

| 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 |




Liquid chromatographic profile of Tirzepatide for the crude material: 20 mg/mL diluted in 75/25 (Water/Acetonitrile), injection Volume: 3 µL.




Liquid chromatographic 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 to get the targeted purity (>99%).

- Both purification steps were performed in using the Resi*Pure* ADV C8.
- The first purification step was performed under the basic conditions (pH 8.00).
- The second purification step was performed under the acidic conditions (pH 3.50)

Refer to figure 2 and 3.

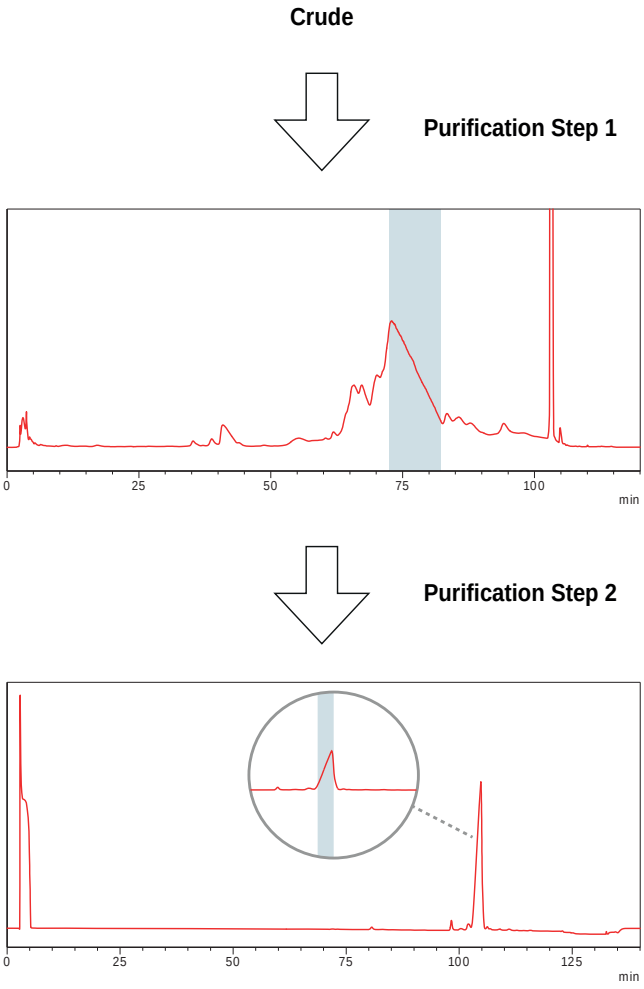
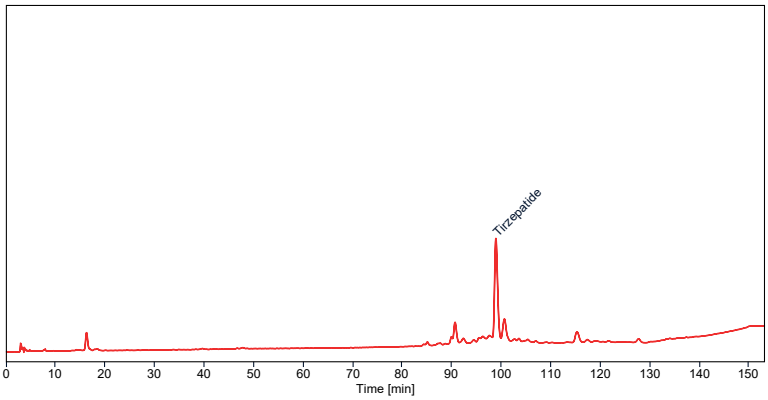
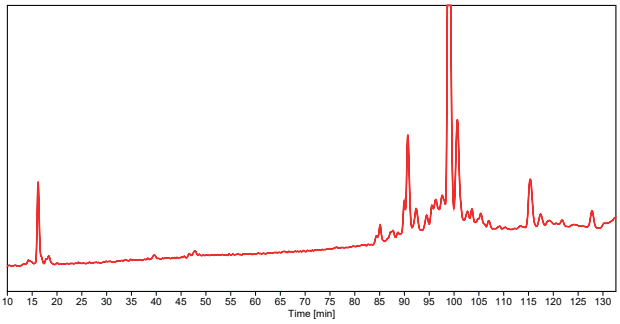



**Figure 2**: Purification process summary of the Tirzepatide

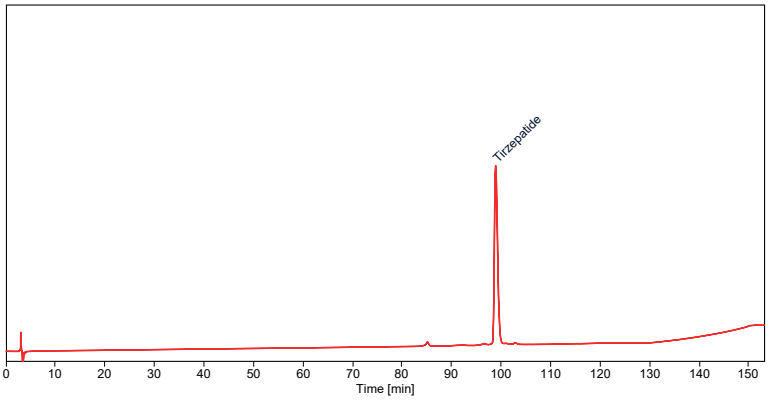
Initial purity: 22.3%

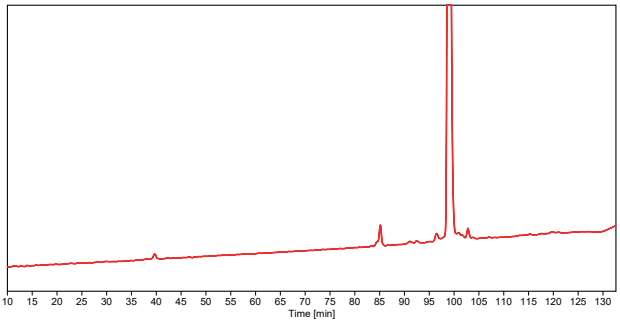



**Purification Step 1**

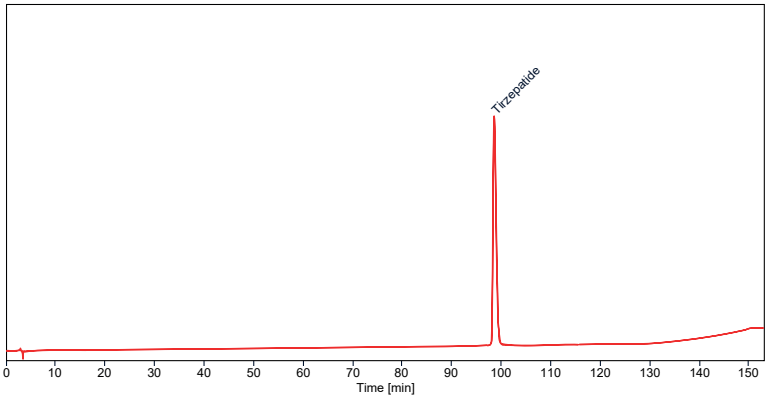
Purity: 91%
Yield: 95%




**Purification Step 2**

Purity: 99.8%
Yield: 72%



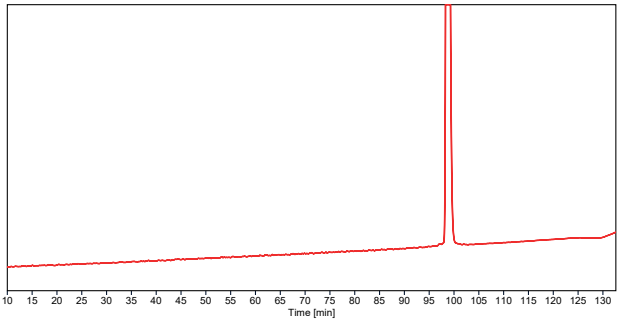




**Figure 3**: Tirzepatide purity analysis by liquid chromatography for each purification step.

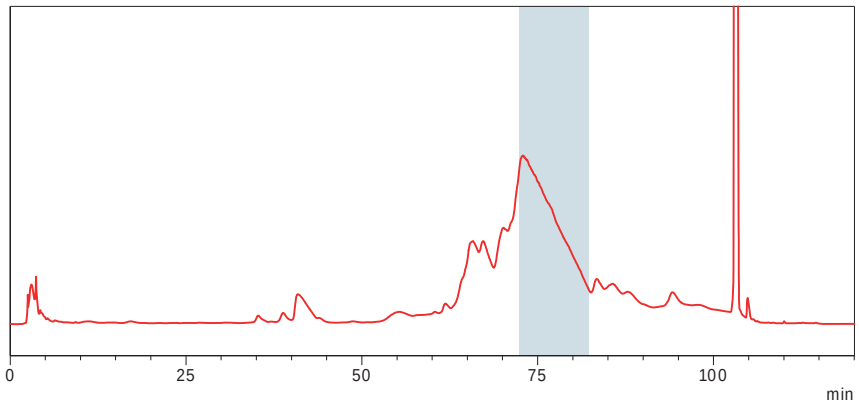
## Purification step 1: removal of bulk impurities

The initial purification step was carried out with the crude Tirzepatide product. The purity of the crude material was about 22.3%.

The method development was performed at analytical scale screening the complete Resi*Pure* ADV stationary phases. The best results regarding separation efficiency, target purity and recovery rate were obtained with Resi*Pure* ADV C8 in using the elution conditions fixed to pH 8.00.

The final fractionation was optimised to get a target purity of 91% and a yield of 95% starting from a very low purity initially 22.3% of the crude. This chromatographic purification step at pH 8.00 with the Resi*Pure* ADV leads to a massive improvement of the purity level of Tirzepatide.

The conditions of the purification step 1 are described below:




**CONDITIONS**

**Column**: Resi*Pure* ADV C8, 250 × 10 mm, 10 µm

**Eluent**

A) 20 mM $NH_4HCO_3$, pH 8.00

B) Acetonitrile

**Gradient**: 25%B (0-10 min), 25-35%B (10-80 min), 35%B (80-100 min), 80%B (100-110 min), and 25%B (110-120 min)

**Flow rate**: 4.75 mL/min

**Column temperature**: 25°C

**Detection**: UV at 220 nm

**Injection volume**: 2mL

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

**Collection**: 72.7 minutes to 82.0 minutes

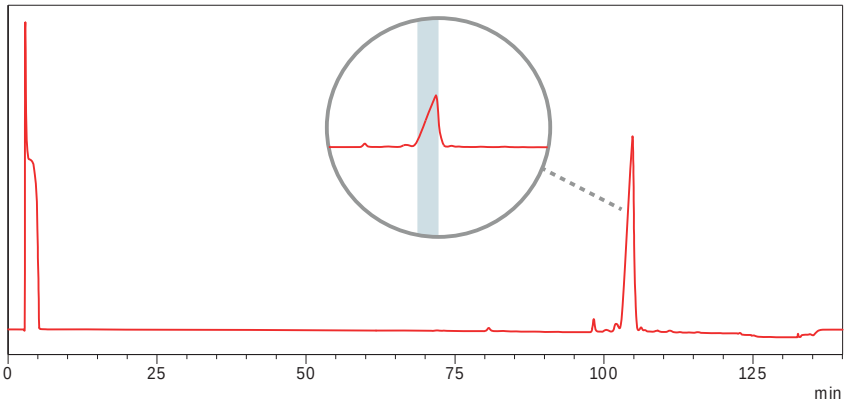
## Purification step 2: final polishing

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

This step was also carried out with the ResiPure ADV C8. The purification was performed at pH 3.5 leading to great separation results.

This second step leads to a final product purity of 99.8% with a yield about 75%.

The conditions of the purification step 2 are described below:




**CONDITIONS**

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

**Eluent**

A) 50 mM $NH_4HCO_3$, pH 3.5

B) Acetonitrile

**Gradient**: 20%B (0-10 min), 20-30%B (10-60 min), 30-55%B (60-120 min), 80%B (120-130 min) and 20%B (130-140 min)

**Flow rate**: 4.75 mL/min

**Column temperature**: 25°C

**Detection**: UV at 220 nm

**Injection volume**: 1 mL (25 mg load)

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

**Collection**: 103.5 minutes to 105.00 minutes

## Conclusion

Two successive purification steps were necessary to purify the Tirzepatide from the crude material to the targeted purity (>99.5%).

- Both purification steps were performed in using the Resi*Pure* ADV C8.
- The first purification step was performed under the basic conditions (pH 8.00) to remove bulk impurities from the crude.
- The second purification step was performed under the acidic conditions (pH 3.50) to polish the extract coming from the first purification step.

In using this process, the purity of the Tirzepatide in the final extract was 99.8%. The purity of Tirzepatide in the starting material was 22.3 %.

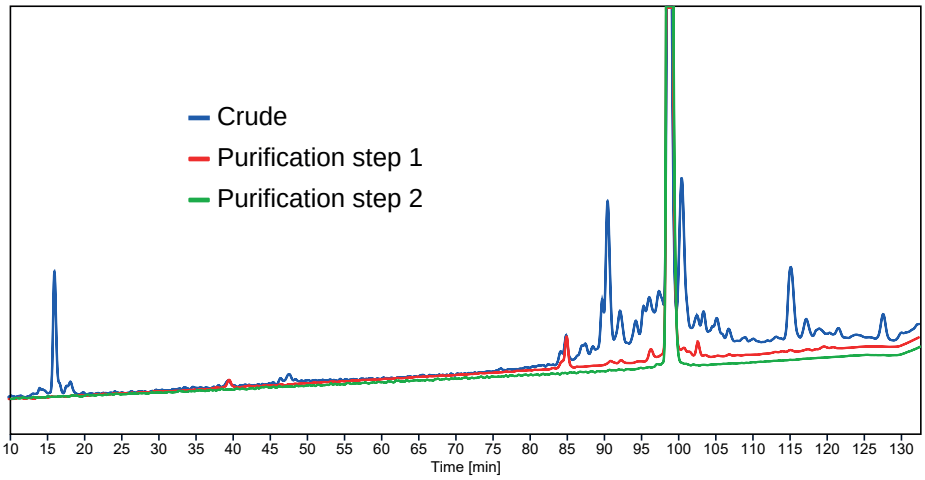



**Figure 4**: Overlay of the different chromatographic profiles for Tirzepatide analyzed after each purification step.

# About SiliCycle

## We define purity

**Founded in 1995 in Quebec City, Canada, SiliCycle is specializing in the development, manufacturing, and commercialization of silica-based products and services for chromatography, purification, synthesis and analysis.**

With a strong commitment to innovation and quality, SiliCycle offers a coherent portfolio of products:

- **Bare irregular and spherical silica**, for chromatographic and analytical purposes ;
- Functionalized silica:
  - **Chromatographic phases** ;
  - **Metal and organic scavengers** ;
  - **Heterogeneous catalysts** and **reagents for synthesis** ;
- **E-PAK® flow purification cartridges** ;
- **Flash cartridges** ;
- **TLC plates** ;
- **SPE** and **sample preparation** cartridges and well plates ;
- **HPLC columns**.

## Renowned silica experts

**For 30 years SiliCycle has developped a unique expertise and know-how about silica and chromatography.**

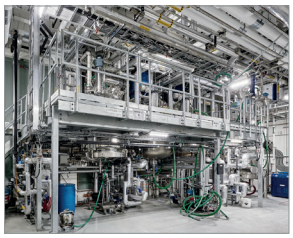
Its R&D team brings together scientists from diverse disciplines, including material chemistry, analytical chemistry, organic and organometallic chemistry, process chemistry, and chemical engineering.

They not only design and develop SiliCycle products but also provide specialized R&D services. Additionally, they work closely with customers to offer dedicated technical support.

## A trusted partner

**SiliCycle's modern facilities ensure high manufacturing standards and industrial production capacity,**

enabling the company to provide high quality products, and ramp up production according to customers requirements.



## Why choose SiliCycle ?

- Consistent portfolio
- Efficient products & turnkey solutions
- Excellent customer service & technical support
- Renowned quality & purity
- Customized solutions

# Contact us

## Email and phone

- Email: **info@silicycle.com**
- Phone: **+1 418.874.0054**
- Toll Free: **+1 877.745.4292** (*North America only*)

## Canadian headquarters

SiliCycle Inc.
2500, Parc-Technologique Blvd,
Quebec City (Quebec) G1P 4S6, Canada

## Overseas offices

SiliCycle Europe / europe@silicycle.com

SiliCycle Shanghai / contact@silicyclechina.com

## Learn more about SiliCycle

- Visit www.silicycle.com
- Get all information and documentation: https://linktr.ee/silicycle

## Follow us

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# We Redefine Purity

High Value Silica-based and Specialty Products for Organic Chemistry, Chromatography and Purification

## DISCOVER AND DOWNLOAD OUR BROCHURES

### METAL AND ORGANIC SCAVENGING

**SiliaMetS®** / **SiliaBond®** – Metal and Organic Scavengers
**StreamXtract™** – Industrial Grade Metal Scavenger
**E-PAK®** – Fixed Bed Flow-Through Purification Cartridges



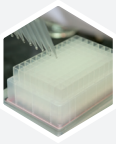
### CHROMATOGRAPHY AND PURIFICATION

**SiliaFlash®** – Irregular Silica Gels | **SiliaSphere™ PC** – Spherical Silica Gels
**SiliaBond®** – Chromatographic Phases
**SiliaSep™** – Flash Cartridges | **SiliaPlate™** – TLC Plates



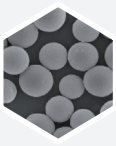
### SAMPLE PREPARATION

**SiliaPrep™** – Silica-based SPE Cartridges and Well Plates
**SiliaPrepX™** – Polymeric SPE Cartridges and Well Plates


### ANALYTICAL AND PREPARATIVE CHROMATOGRAPHY

**SiliaSphere™** – Spherical Silica Gels
**SiliaChrom®** – HPLC Columns

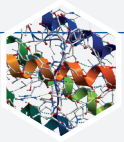

### ORGANIC SYNTHESIS

**SiliaBond®** – Reagents and Oxidants
**SiliaCat®** – Heterogeneous Catalysts



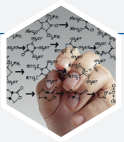
### PEPTIDE SYNTHESIS AND PURIFICATION

**ResiPure™ ADVANCED** – Phases for Peptide Purification
Phases for Peptide Synthesis
Amine Free Basing and TFA Removal



### R&D SERVICES

Metal and Organic Scavenging Screenings | Organic Synthesis
Chromatography and Purification | Material Science
Method Development, Optimization, and Transfer



### Technical Support

**At SiliCycle, we are committed to providing the best technical support possible.**

Our worldwide Technical Support Group of highly qualified M. Sc., Ph. D. Chemists and Engineers will answer your questions and provide solutions to your most advanced chemistry and purification needs. Contact us at **support@silicycle.com** or **call us**.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