

A man with a beard and dark hair is holding a young child with blonde hair. They are in a hardware store aisle, with shelves of tools and materials visible in the background. The man is holding a flashlight and looking at it, while the child looks on. The man is wearing a dark t-shirt and jeans, and the child is wearing a white t-shirt with red stripes and blue jeans. The background shows various tools and materials on shelves, including a stack of red pipes on the right.

Kromasil®

GLP-1 agonist purification toolbox

Methodology to maximize purity and throughput in
Liraglutide and Semaglutide purification

Nouryon

A toolbox to reach your purification goals

GLP-1 agonists are a family of peptides that have become popular in the early treatment of diabetes type II. Achieving the right purity for these peptides is however quite challenging.

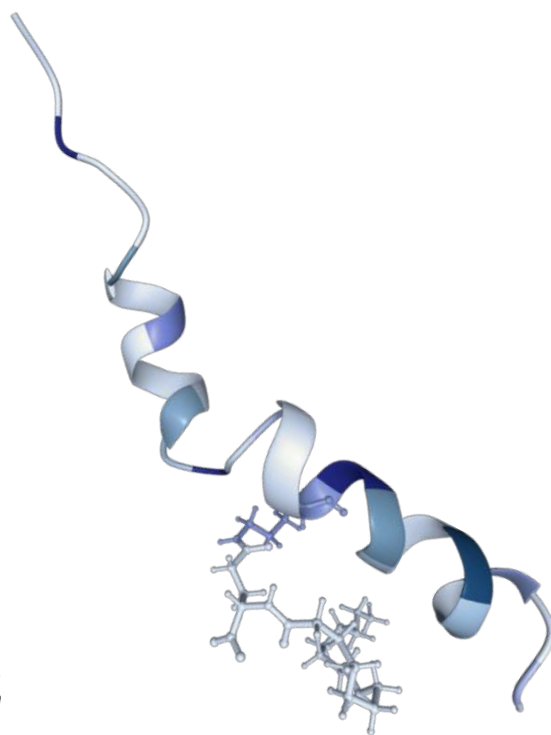
A set of purification methods have been developed using Kromasil® stationary phases for removal of impurities in peptide crudes that can be combined in a systematic way to improve purity from crude to 99.5%. This set is called the **GLP-1 agonist purification toolbox**.

With a set of well adapted purification methods and routines in place, high efficiency and long column lifetime can be maintained to maximize throughput and productivity.

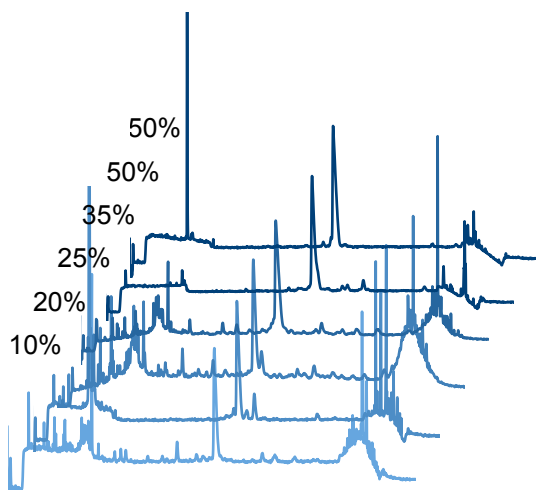
Liraglutide and Semaglutide

Liraglutide and Semaglutide are a couple of the main GLP-1 agonists commercially produced on the market. They have a couple of amino acid substitutions compared to human GLP-1 to slow down peptidase degradation and are both acetylated with a hydrocarbon side chain.

However, if inadequately handled during purification, they easily degrade or aggregate. Most of these issues are avoided when using the toolbox methods.



Structure representation of Liraglutide
(pdb id 4apd) using Mol* Viewer (D. Sehnal et al (2021)
Nucleic Acids Research. doi: 10.1093/nar/gkab314) and RCSB PDB.



Reaching for purity

Crudes of Liraglutide are typically anything in the range from 10 to 50% in purity when entering downstream processing. The goal of the toolbox is to offer purification methods that will bring purity up to 99.5%.

Typical crude purity profiles for Liraglutide

Tools selection

Operating the major factors affecting chromatography efficiency in HPLC, many different combinations of the following properties were screened:

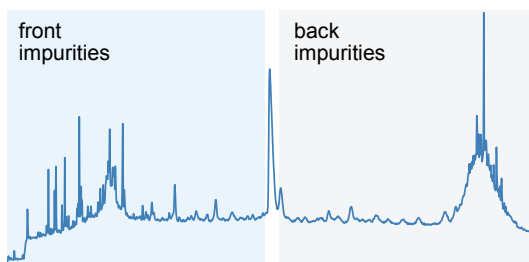
- Kromasil® stationary phase
- Mobile phase organic solvent
- Mobile phase buffer

The screening compilation resulted in set of methods combinations with focus on the relative location of the impurities removed from the crude: back or front. The most efficient combinations have been compiled in the table below.

Toolbox methodology

The methodical approach is to select two methods from the toolbox (tables on next page) that will target front and back impurities from the crude, respectively. In some cases this can be achieved in a single step.

In a typical crude, impurities can be grouped as front and back impurities



Toolbox combinations for the purification of Semaglutide

Targeted impurities	Stationary phases	Organic solvents	Buffer salts
front	Kromasil® C4	acetonitrile	potassium phosphate
			trifluoroacetic acid
	Kromasil® C8	acetonitrile	potassium phosphate
		propanol	ammonium acetate
	Kromasil® Phenyl	acetonitrile	ammonium acetate
			potassium phosphate
	Kromasil® diC4	2-propanol	ammonium acetate
		acetonitrile	potassium phosphate
ethanol		ammonium acetate	
back	Kromasil® C4	acetonitrile	potassium phosphate
	Kromasil® C8	acetonitrile	potassium phosphate
	Kromasil® Phenyl	acetonitrile	ammonium acetate
			ammonium carbonate
	Kromasil® diC4	acetonitrile	potassium phosphate

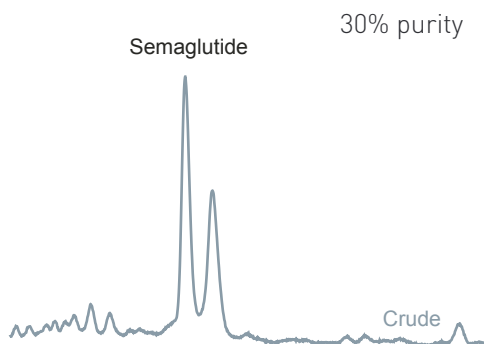
Toolbox combinations for the purification of Liraglutide

Targeted impurities	Stationary phases	Organic solvents	Buffer salts
front	Kromasil® C4	ethanol	ammonium formate
		acetonitrile	ammonium acetate
	Kromasil® C8	ethanol	ammonium acetate
	Kromasil® Phenyl	acetonitrile	ammonium carbonate
	Kromasil® diC4	acetonitrile	ammonium carbonate
			ammonium acetate
back	Kromasil® C4	ethanol	ammonium carbonate
			citric acid
		2-propanol	ammonium acetate
	Kromasil® C8	acetonitrile	ammonium acetate
			ammonium carbonate
	Kromasil® Phenyl	ethanol	ammonium acetate
		acetonitrile	ammonium carbonate
	Kromasil® diC4	ethanol	ammonium acetate
	Kromasil® diC4	acetonitrile	ammonium carbonate
			ammonium acetate

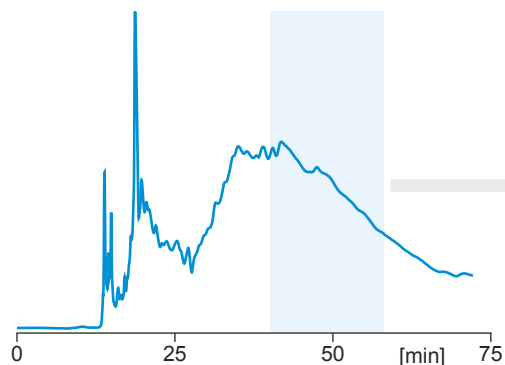
Examples with Semaglutide

In this example, starting with a 30% purity crude of Semaglutide, the two-steps method uses Kromasil® C4 in both steps, but at different pH, obtaining 92.2% purity after the first step and then 99.5% after the second step.

Starting crude with focus around the product peak. Other impurities are present further away, before and after the focus area.

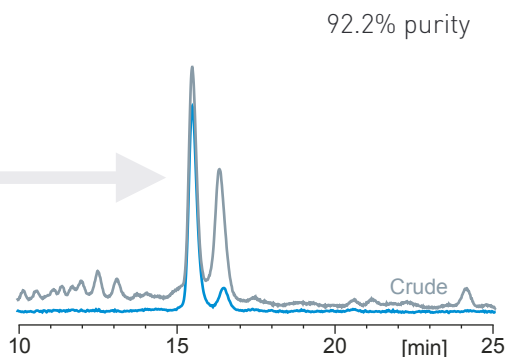


Purification step 1

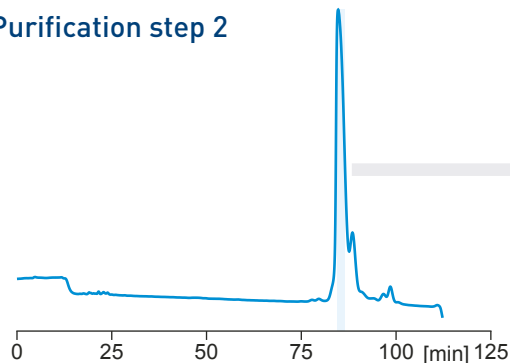


Conditions

Stationary phase: Kromasil 100-10-C4
Mobile phase organic solvent: acetonitrile
Mobile phase buffer: trifluoroacetic acid (TFA)

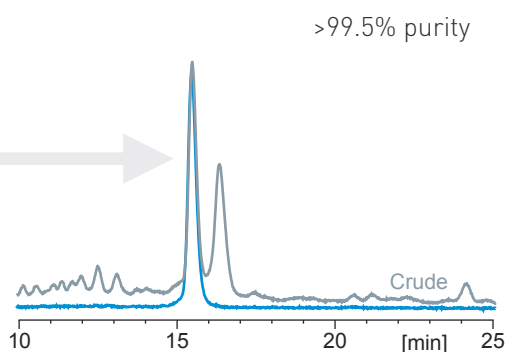


Purification step 2



Conditions

Stationary phase: Kromasil 100-10-C4
Mobile phase organic solvent: acetonitrile
Mobile phase buffer: ammonium acetate

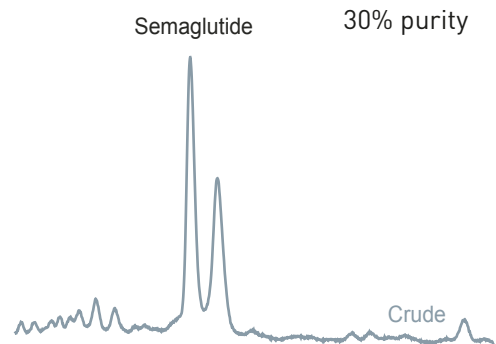


Analytical conditions (all above)

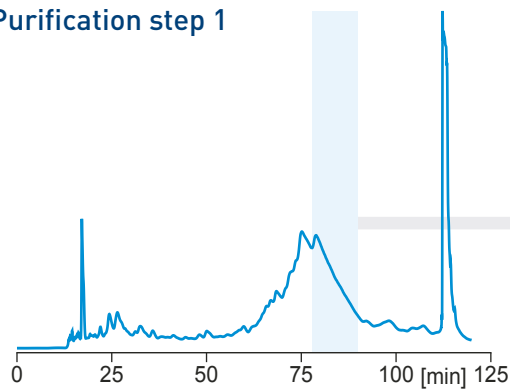
Stationary phase: Kromasil 100-3.5-C4
Mobile phase organic solvent: acetonitrile
Mobile phase salt: trifluoroacetic acid

Example with Semaglutide using phosphate buffers at different pH on the same C4 packing media. Also starting with the 30% purity crude, the first steps at low pH reaches a 90.5% purity. The second step is run at neutral pH achieving the final 99.5% purity.

Starting crude with focus around the product peak. Other impurities are present further away, before and after the focus area.

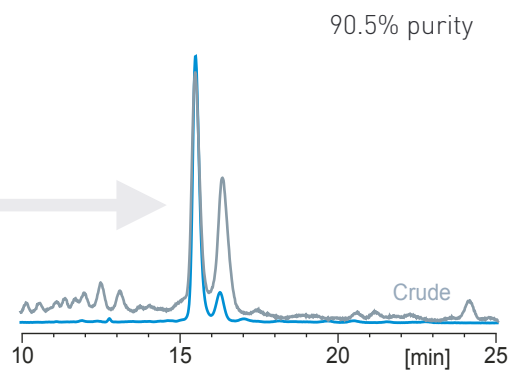


Purification step 1

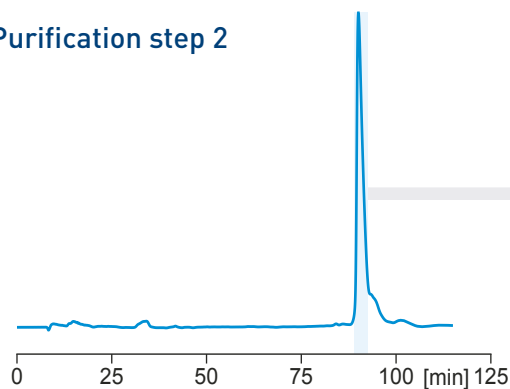


Conditions

Stationary phase: Kromasil 100-10-C4
 Mobile phase organic solvent: acetonitrile
 Mobile phase salt: potassium phosphate, low pH

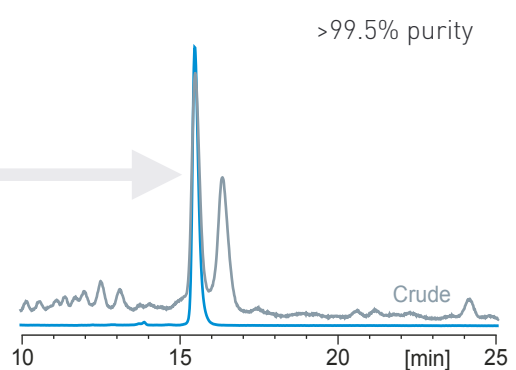


Purification step 2



Conditions

Stationary phase: Kromasil 100-10-C4
 Mobile phase organic solvent: acetonitrile
 Mobile phase salt: potassium phosphate, neutral pH

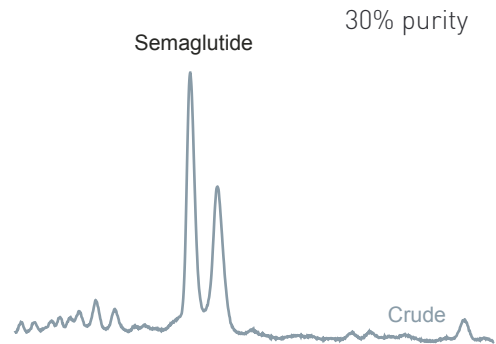


Analytical conditions (all above)

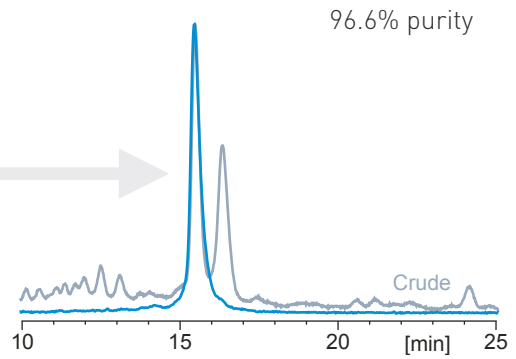
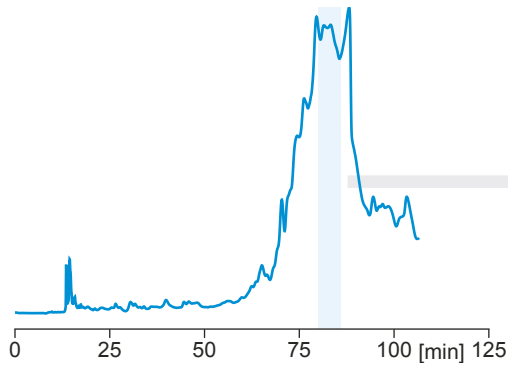
Stationary phase: Kromasil 100-3.5-C4
 Mobile phase organic solvent: acetonitrile
 Mobile phase salt: trifluoroacetic acid

Example using the new Kromasil® diC4 phase for GLP-1 receptor agonists and Kromasil® Phenyl with 2 fairly neutral buffer steps with potassium phosphate and ammonium acetate, respectively

Starting crude with focus around the product peak. Other impurities are present further away, before and after the focus area.



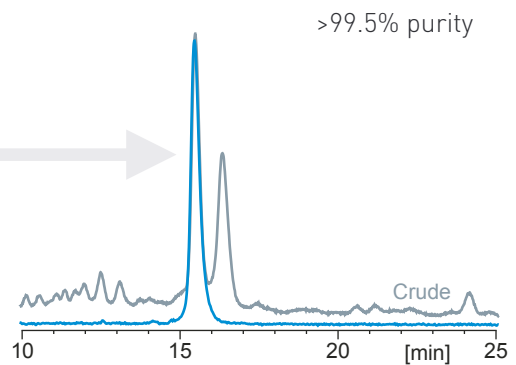
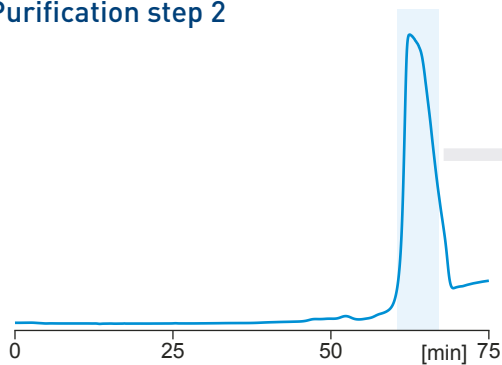
Purification step 1



Conditions

Stationary phase: Kromasil 100-10-diC4
 Mobile phase organic solvent: acetonitrile
 Mobile phase buffer: potassium phosphate, neutral pH

Purification step 2



Conditions

Stationary phase: Kromasil 100-10-Phenyl
 Mobile phase organic solvent: acetonitrile
 Mobile phase buffer: ammonium acetate, neutral pH

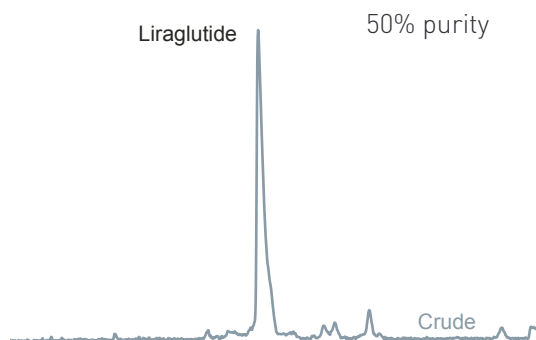
Analytical conditions (all above)

Stationary phase: Kromasil 100-3.5-C4
 Mobile phase organic solvent: acetonitrile
 Mobile phase salt: trifluoroacetic acid

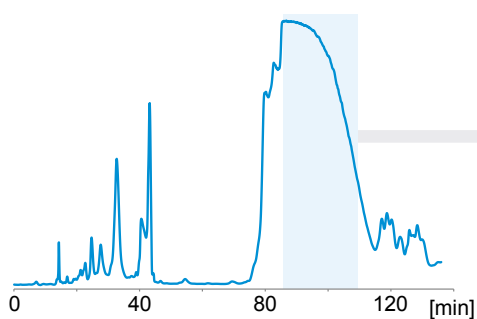
Examples with Liraglutide

In this first example, starting with a 50% purity crude, a first method using Kromasil® Phenyl targets front impurities, obtaining a 94% purity. To reach a final purity of above 99.5%, a second step on Kromasil® C4 is run, removing remaining back impurities.

Starting crude with focus around the product peak. Other impurities are present further away, before and after the focus area.

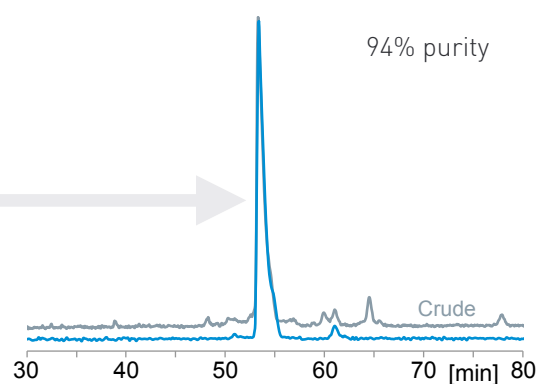


Purification step 1

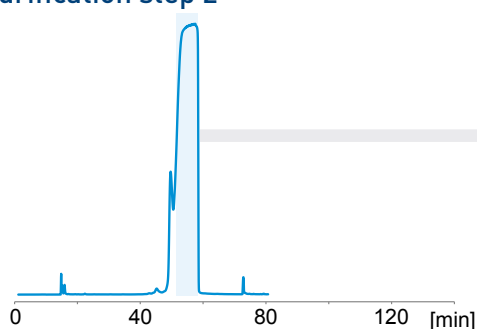


Conditions

Stationary phase: Kromasil 100-10-Phenyl
Mobile phase organic solvent: acetonitrile
Mobile phase buffer: ammonium carbonate

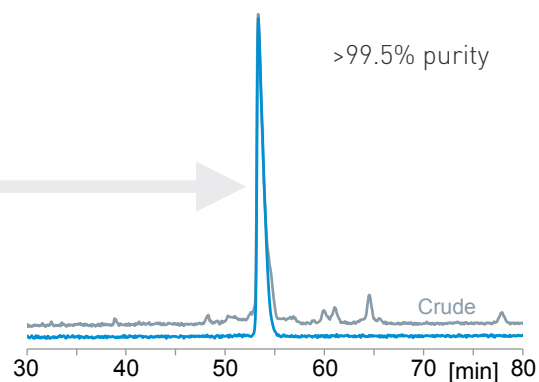


Purification step 2



Conditions

Stationary phase: Kromasil 100-10-C4
Mobile phase organic solvent: acetonitrile
Mobile phase buffer: ammonium acetate

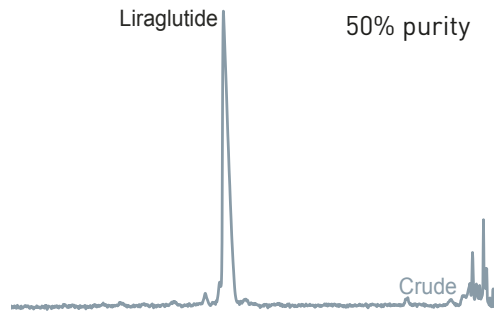


Analytical conditions (all above)

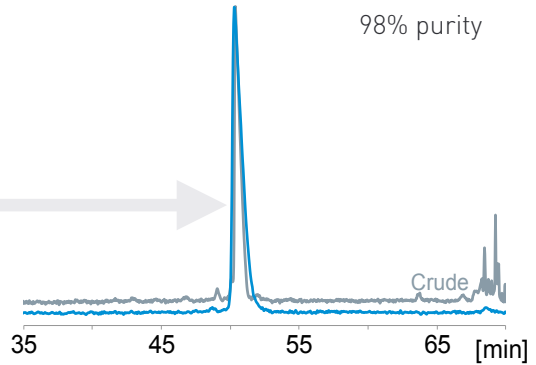
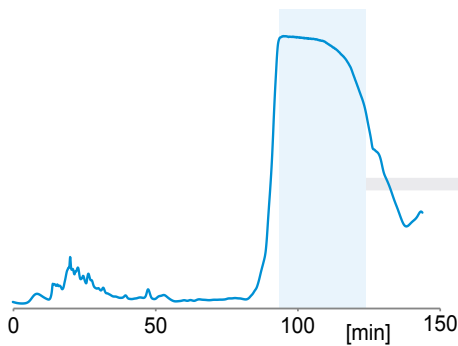
Stationary phase: Kromasil 100-3.5-C4
Mobile phase organic solvent: acetonitrile
Mobile phase salt: trifluoroacetic acid

In this second example, starting with another 50% purity crude, a first method using Kromasil® C8 targeting both front and back impurities, reaching a 98% purity. To achieve the final purity of above 99.5%, the second step is run on Kromasil® C4, removing the remaining impurities.

Starting crude with focus around the product peak. Other impurities are present further away, before and after the focus area.



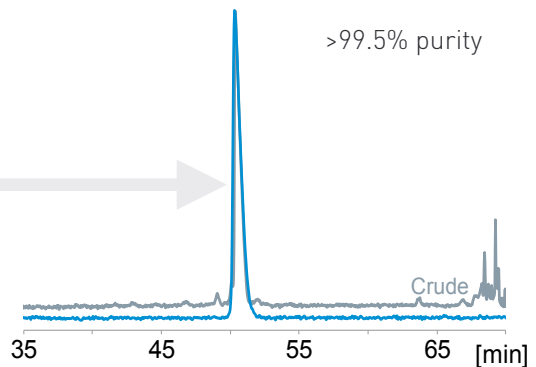
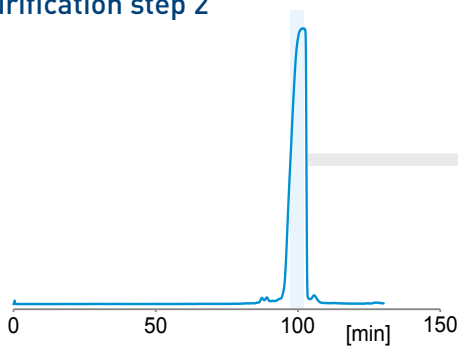
Purification step 1



Conditions

Stationary phase: Kromasil 100-10-C8
 Mobile phase organic solvent: ethanol
 Mobile phase buffer: ammonium acetate

Purification step 2



Conditions

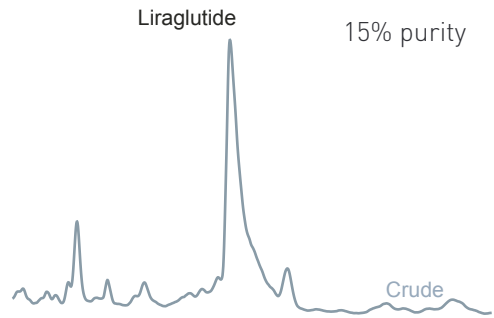
Stationary phase: Kromasil 100-10-C4
 Mobile phase organic solvent: acetonitrile
 Mobile phase buffer: ammonium acetate

Analytical conditions (all above)

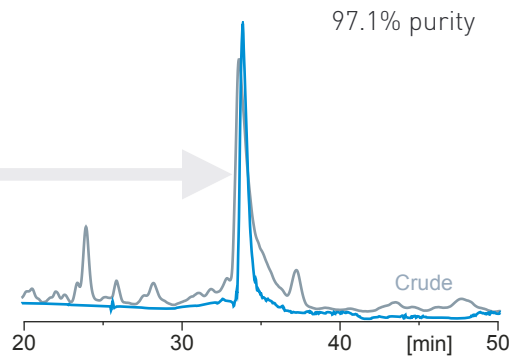
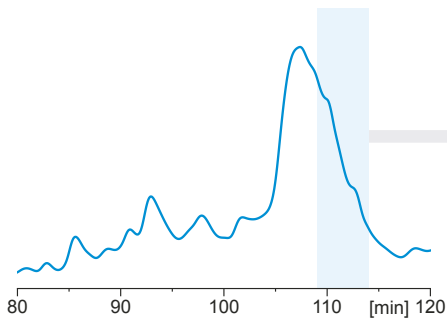
Stationary phase: Kromasil 100-3.5-C4
 Mobile phase organic solvent: acetonitrile
 Mobile phase salt: trifluoroacetic acid

In this third example, starting with a 15% purity liraglutide crude, and running both steps on Kromasil® diC4, stepping up purity to 97.1% and finally 99.5%.

Starting crude with focus around the product peak. Other impurities are present further away, before and after the focus area.



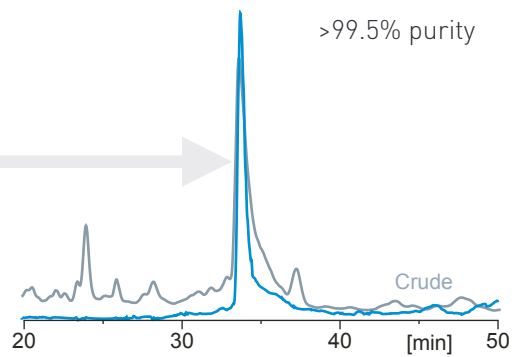
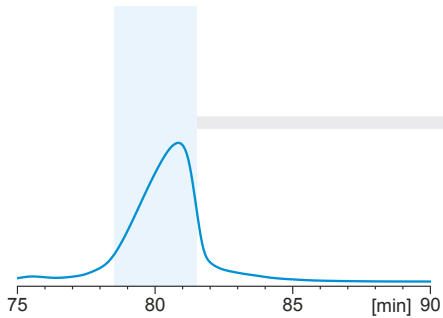
Purification step 1



Conditions

Stationary phase: Kromasil 100-10-diC4
 Mobile phase organic solvent: acetonitrile
 Mobile phase buffer: ammonium carbonate, neutral pH

Purification step 2



Conditions

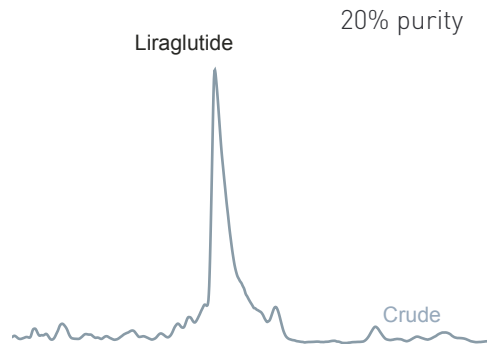
Stationary phase: Kromasil 100-10-diC4
 Mobile phase organic solvent: acetonitrile
 Mobile phase buffer: ammonium acetate, mild basic pH

Analytical conditions (all above)

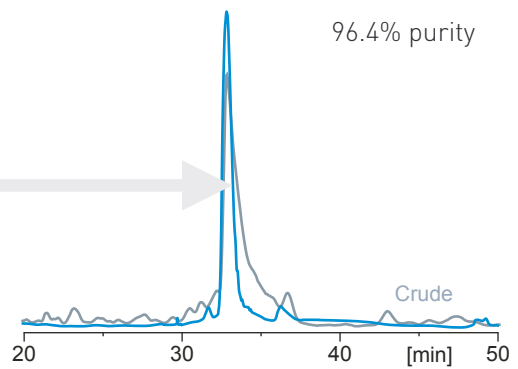
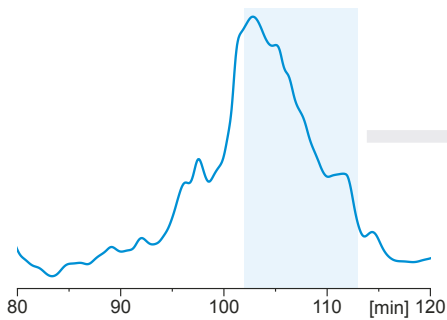
Stationary phase: Kromasil 100-3.5-C4
 Mobile phase organic solvent: acetonitrile
 Mobile phase buffer: potassium phosphate, 10 mM pH 7.5

This fourth example is similar to the previous one but with a different crude with 20% liraglutide purity. Both steps are also run on Kromasil® diC4, stepping up purity to 96.4% and, finally, virtually 100%.

Starting crude with focus around the product peak. Other impurities are present further away, before and after the focus area.



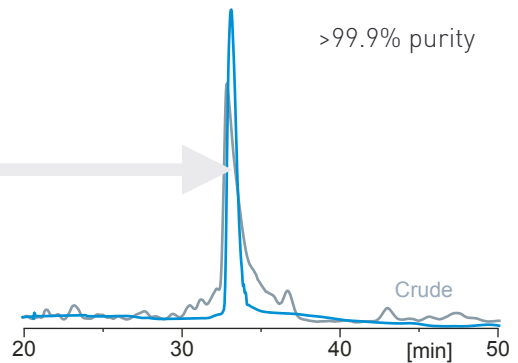
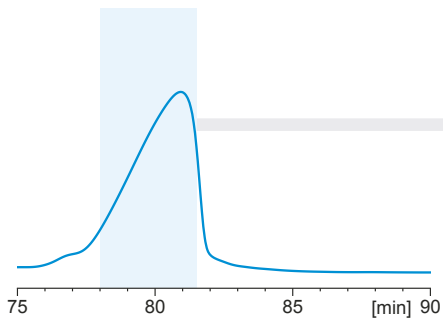
Purification step 1



Conditions

Stationary phase: Kromasil 100-10-diC4
 Mobile phase organic solvent: acetonitrile
 Mobile phase buffer: ammonium carbonate, neutral pH

Purification step 2



Conditions

Stationary phase: Kromasil 100-10-diC4
 Mobile phase organic solvent: acetonitrile
 Mobile phase buffer: ammonium acetate, mild basic pH

Analytical conditions (all above)

Stationary phase: Kromasil 100-3.5-C4
 Mobile phase organic solvent: acetonitrile
 Mobile phase buffer: potassium phosphate, 10 mM pH 7.5

Doing it right

While peptide crudes can be challenging when repeatedly injected on an HPLC column, having a regeneration routine can be beneficial to maintain a good column lifetime and efficiency and thereby process productivity.

Troubleshooting back-pressure increase

When purifying demanding peptide crudes like Liraglutide, the column quickly gets fouled by absorbed impurities with repeated injections, even when performing a traditional 2 column volume wash between injections.

We propose here to perform an additional wash under mild conditions after a sequence of injections to quickly and fully recover column capacity and efficiency, optimizing productivity and throughput.

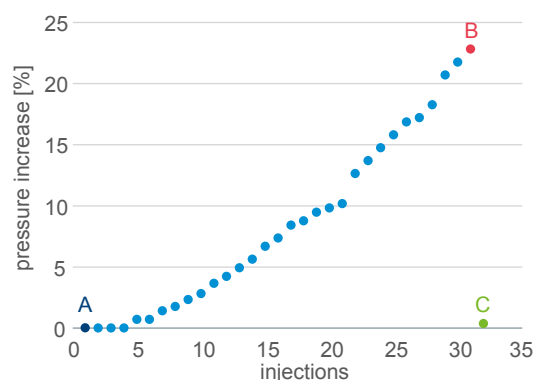
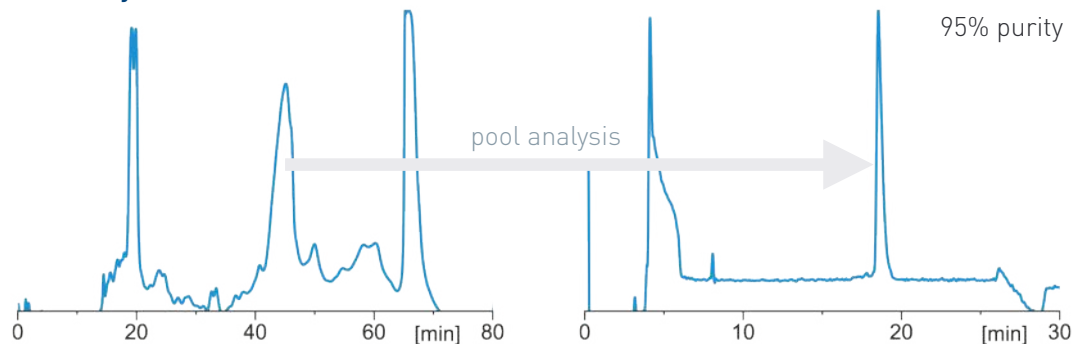


Figure showing the back-pressure over the column increasing between 30 consecutive crude injections from A to B. C shows restored normal back-pressure after the additional column wash. Chromatograms illustrating the status at these three points are shown in the following figures.

A Initial injection



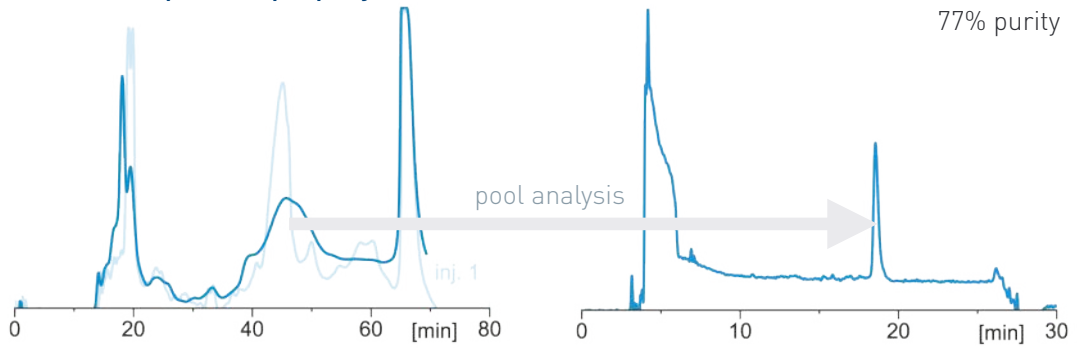
Prep conditions (in A, B and C)

Stationary phase: Kromasil 100-10-C4
Mobile phase organic solvent: acetonitrile
Mobile phase buffer: ammonium acetate

Analytical conditions (in A, B and C)

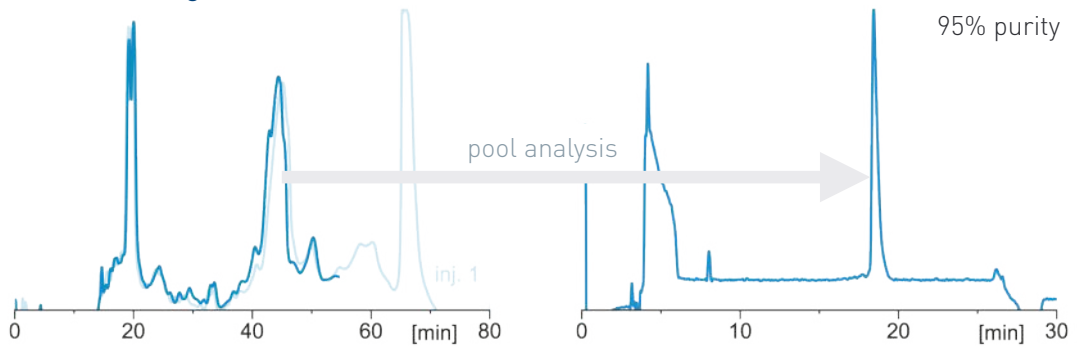
Stationary phase: Kromasil 100-3.5-C4
Mobile phase organic solvent: acetonitrile
Mobile phase salt: trifluoroacetic acid

B After 30 sequential prep injections

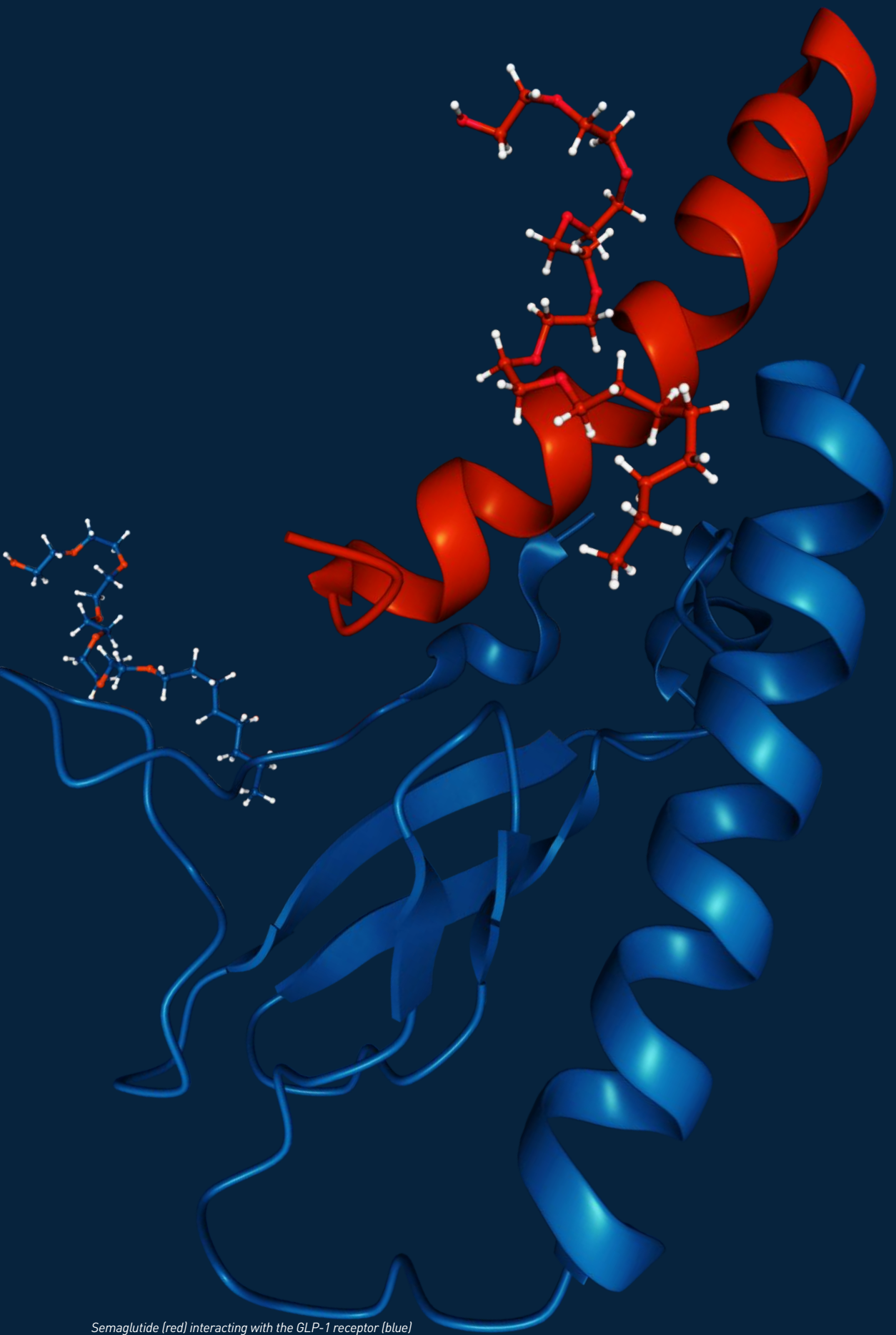


After 30 preparative injections, column efficiency is impaired resulting in lower purity in extracted fractions. In the preparative chromatogram, the initial peak shape is shown in light blue as reference.

C After mild regeneration wash



After the regeneration wash, peak shape and thereby efficiency and purity has been practically restored. The mild wash allows for a quick turn around to proceed with new injections. In the preparative chromatogram, the initial peak shape is shown in light blue as reference.



Semaglutide (red) interacting with the GLP-1 receptor (blue)

Availability of Kromasil® Classic

Family	Phase	Particle size, [μm]									
		1.8	2.5	3	3.5	5	7	10	13	16	25
60 Å	SIL					●	●	●	●	●	
60 Å	CN					●		●		●	
60 Å	Diol					●		●			
60 Å	HILIC-D					●		●			
100 Å	SIL	●	●		●	●	●	●	●	●	
100 Å	C1					●					
100 Å	C4	●	●		●	●	●	●	●	●	
100 Å	diC4							●	●		
100 Å	C8	●	●		●	●	●	●	●	●	
100 Å	C18	●	●		●	●	●	●	●	●	
100 Å	C18(w)					●		●			
100 Å	NH2				●	●	●	●	●	●	
100 Å	Phenyl					●		●		●	
300 Å	SIL					●		●		●	
300 Å	C4					●		●		●	
300 Å	diC4							●		●	
300 Å	C8					●		●		●	
300 Å	C18					●		●		●	

- : standard product, available in bulk quantities
- : analytical product, only available in slurry-packed columns
- : bare silica product in analytical particle sizes available in bulk for contracted OEM producers

www.kromasil.com/classic

Contact us

Are you also working on some GLP-1 agonist peptides and is interested in how this toolbox can reduce your development time and boost your productivity? Don't hesitate to contact us and reach out to our team of skilled application scientists for more details:

www.kromasil.com/contact



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The moment you adopt our Kromasil® high performance concept, you join thousands of chromatographers who share a common goal: to achieve better separations when analyzing or isolating pharmaceuticals or other substances.

Not only will you benefit from our patented silica technology, but you gain a strong partner with a reliable track record in the field of silica products. For the past 70 years, we have pioneered new types of silica. Our long experience in the field of silica chemistry is the secret behind the development of Kromasil® stationary phases, and the success of our chromatography team.

Kromasil® media are available in bulk and in high-pressure slurry-packed columns. The production of Kromasil® silica is ISO 9001 and ISO 14001 certified.

Kromasil® is a brand of Nouryon, a global specialty chemicals leader. Markets and consumers worldwide rely on our essential solutions to manufacture everyday products. The dedication of more than 7 650 employees with a shared commitment to our customers, safety, sustainability and innovation has resulted in a consistently strong performance. We operate in over 80 countries around the world with a portfolio of industry-leading brands.

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