Method Development and Scale-up for Peptide Purification

Case Studies for a GLP-1 Agonist and a Peptide Hormone

25.02. 2025

11-12 am (CET)

Speakers

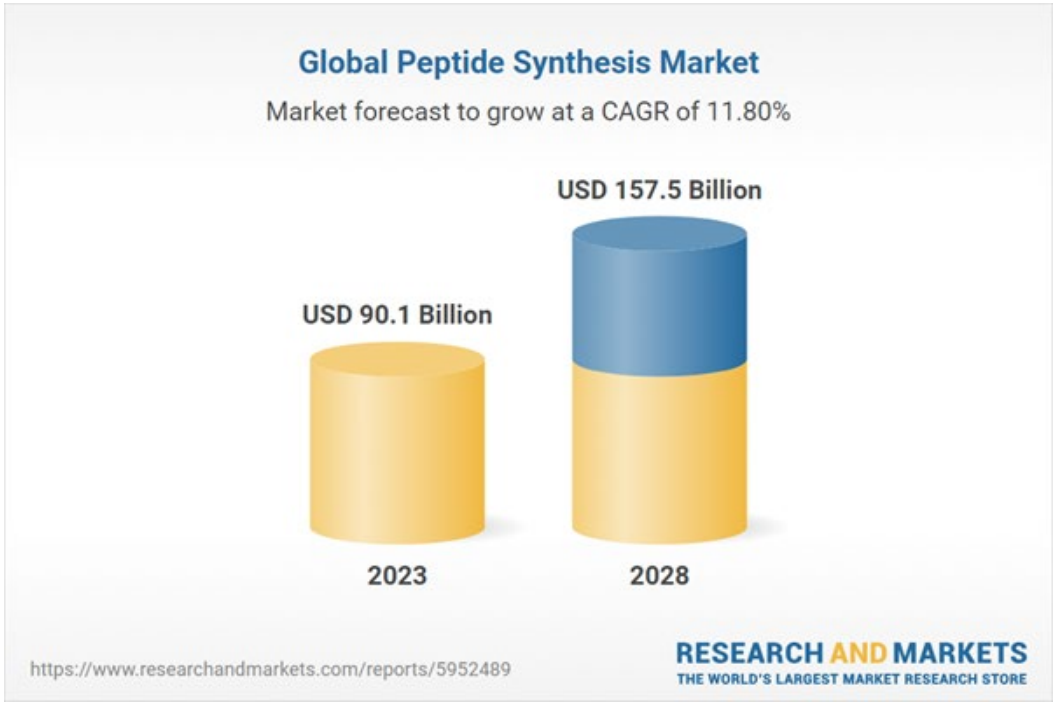
Julia Bartmann, **YMC Europe GmbH**
Dr. Yannick Krauke, **KNAUER Wissenschaftliche Geräte GmbH**

Great Potential of Peptides

Advantages of peptides as therapeutic agent

- highly specific
- well tolerated
- biodegradable

- cancer therapies
- diabetes management
- neurological disorders
- cardiovascular diseases
- rare genetic conditions



Continuous Chromatography for Liraglutide

BACHEM YMC ChromaCon

at TIDES 2024:

Liraglutide presentation available as webinar:

https://www.bachem.com/webinar/peptides-nce/a-second-generation-process-for-the-manufacturing-of-liraglutide/

Preparative Method Development

1. Method development at analytical scale

2. Loadability studies at analytical scale

3. Linear scale-up to preparative process

Analytical method needed:
- As basis for preparative process development
- For analysis of collected fractions

- Stationary phase selection
- Pore size requirements
- Selection of particle size
- Mobile phase composition
- Gradient optimisation

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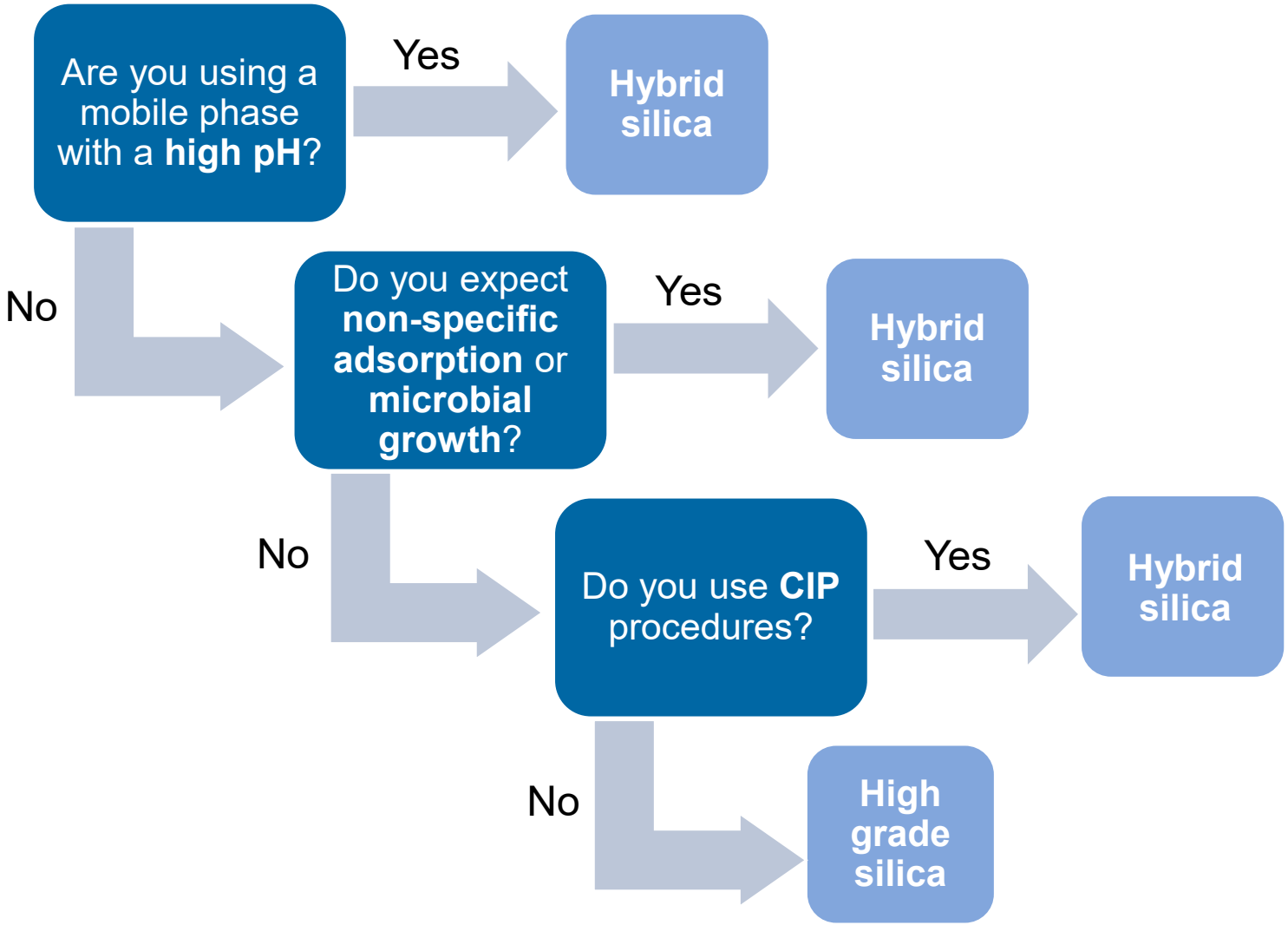
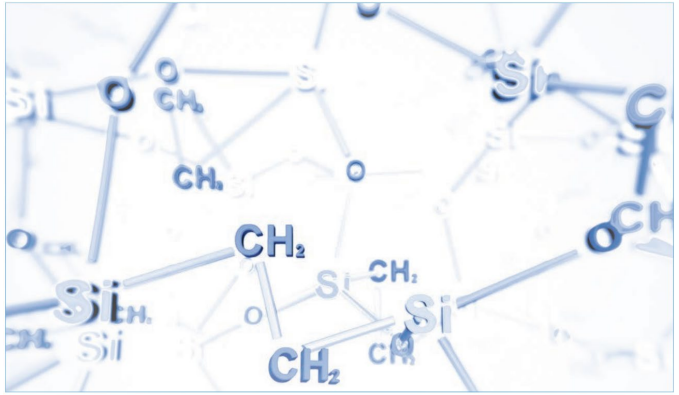
The Choice of the Base Particle

Which considerations should be made?

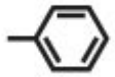


Hybrid silica



organic ethylen bridges between the silica

Stationary Phase Selection

	C18	C8	Phenyl	C4
Functional group	$-C_{18}H_{37}$	$-C_8H_{17}$		$-C_4H_9$
Hydrophobicity	High	←	←	Low
Hydrogen bonding capacity	Low	→	→	High
Surface recognition ability	High	←	←	Low
Suitable MW of the peptide	Low	→	→	High

Most commonly used selectivities for peptides

For peptides with aromatic systems

For very large peptides

→ **Screening of different selectivites**

Find the Best Selectivity by Column Screening

YMC EUROPE GMBH
The Selectivity Company

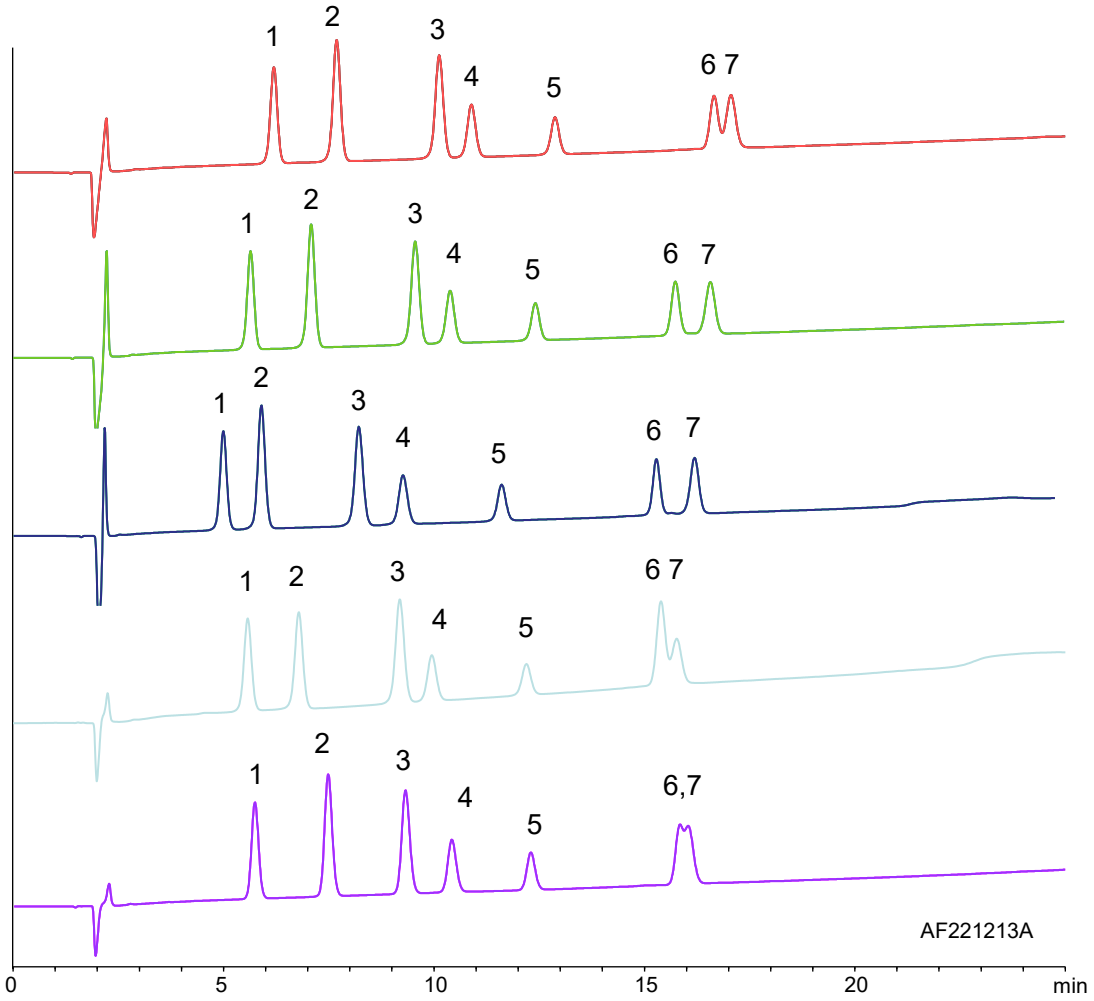
YMC-Triart Prep C18-S

YMC-Triart Prep C8-S

YMC-Triart Prep Bio200 C8

YMC-Triart Prep C4-S

YMC-Triart Prep Phenyl-S



Peptide Sample

1. Oxytocin	(MW 1,007)
2. Met-Enkephalin	(MW 574)
3. Leu-Enkephalin	(MW 556)
4. Neurotensin	(MW 1,673)
5. γ-Endorphin	(MW 1,859)
6. Insulin (Bovine)	(MW 5,733)
7. β-Endorphin	(MW 3,465)

Optimal Selectivity: Aromatic Peptides

YMC EUROPE GMBH
The Selectivity Company

YMC-Triart Prep C18-S

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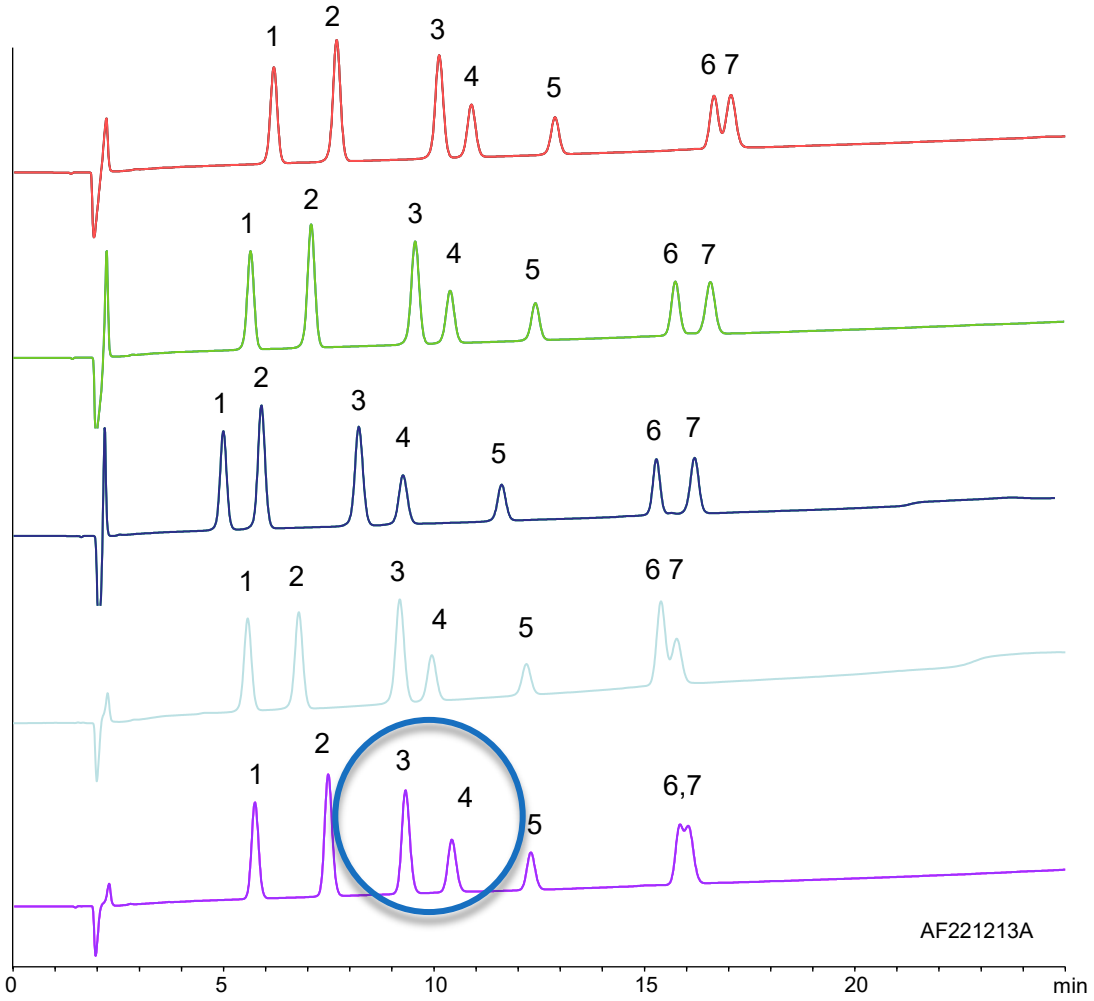

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Phenyl modification for π- π-interactions of aromatic groups

Optimal Selectivity: Large Peptides

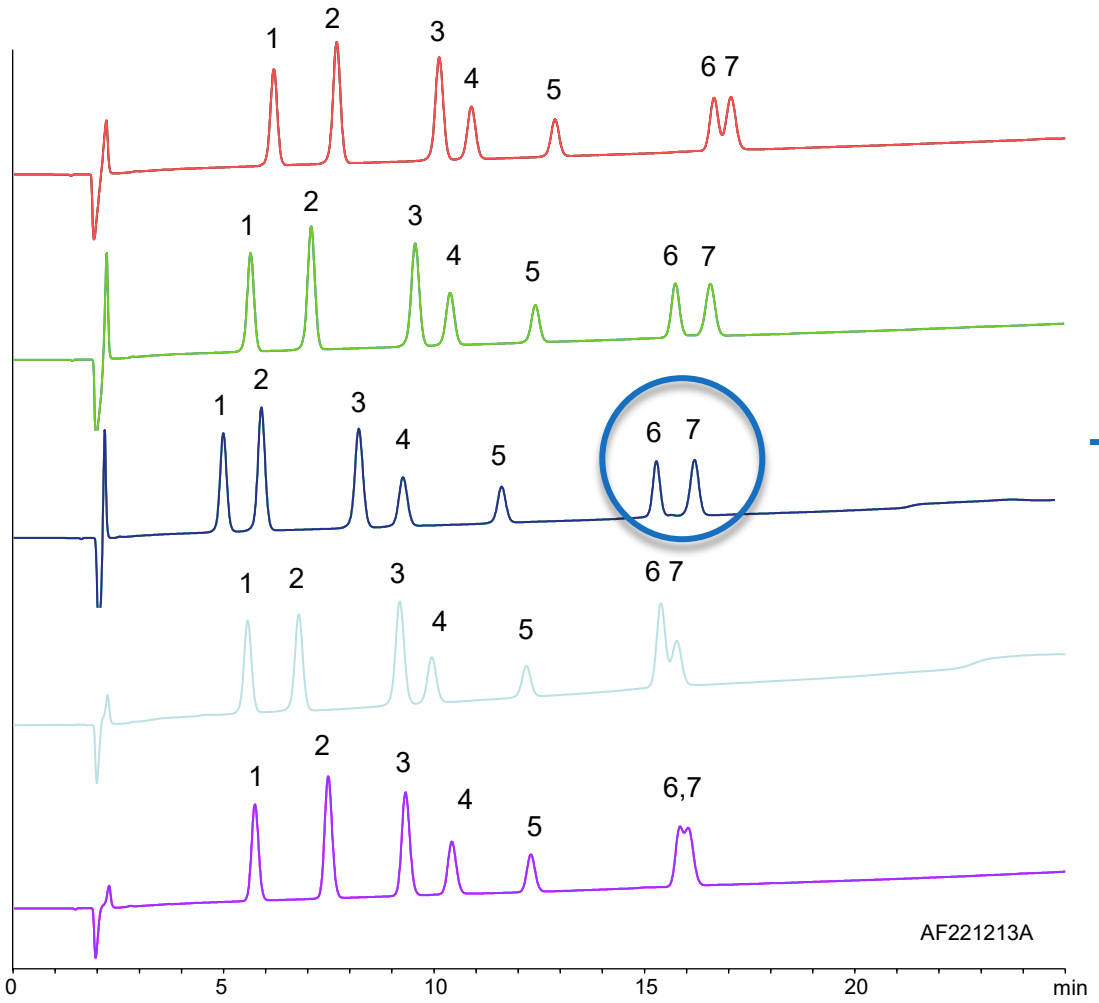
YMC-Triart Prep C18-S
120 Å

YMC-Triart Prep C8-S
120 Å

YMC-Triart Prep Bio200 C8
200 Å

YMC-Triart Prep C4-S
120 Å

YMC-Triart Prep Phenyl-S
120 Å



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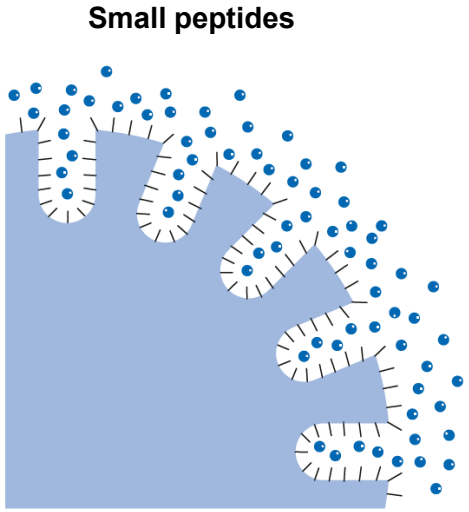
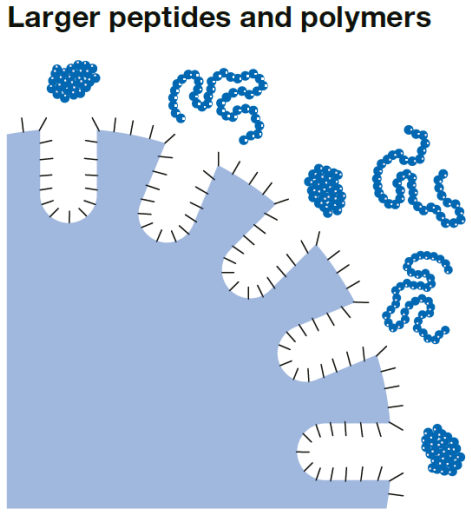
Larger pore size needed!

Selection of Pore Size

Peptide Size

Consider steric exclusion of the compound from pores and densely modified stationary phase surfaces



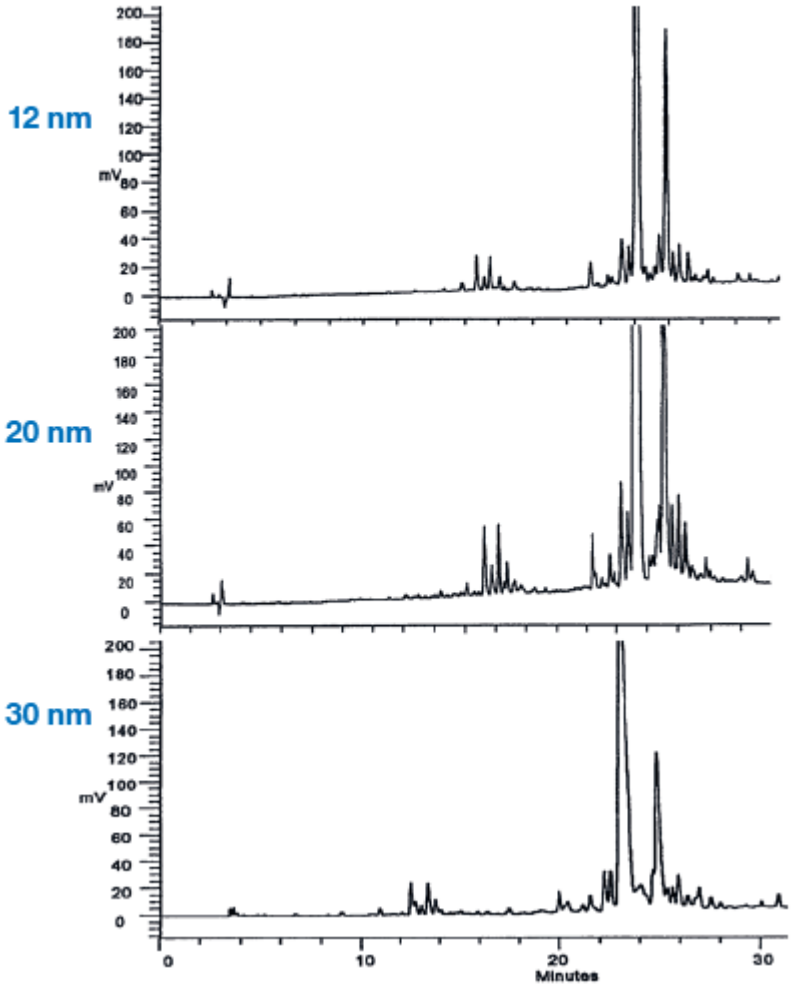


MW		C18	C8	C4
5,000	12 nm	+++	++	+
20,000	20 nm	++	+++	++
100,000*	30 nm	+	++	+++

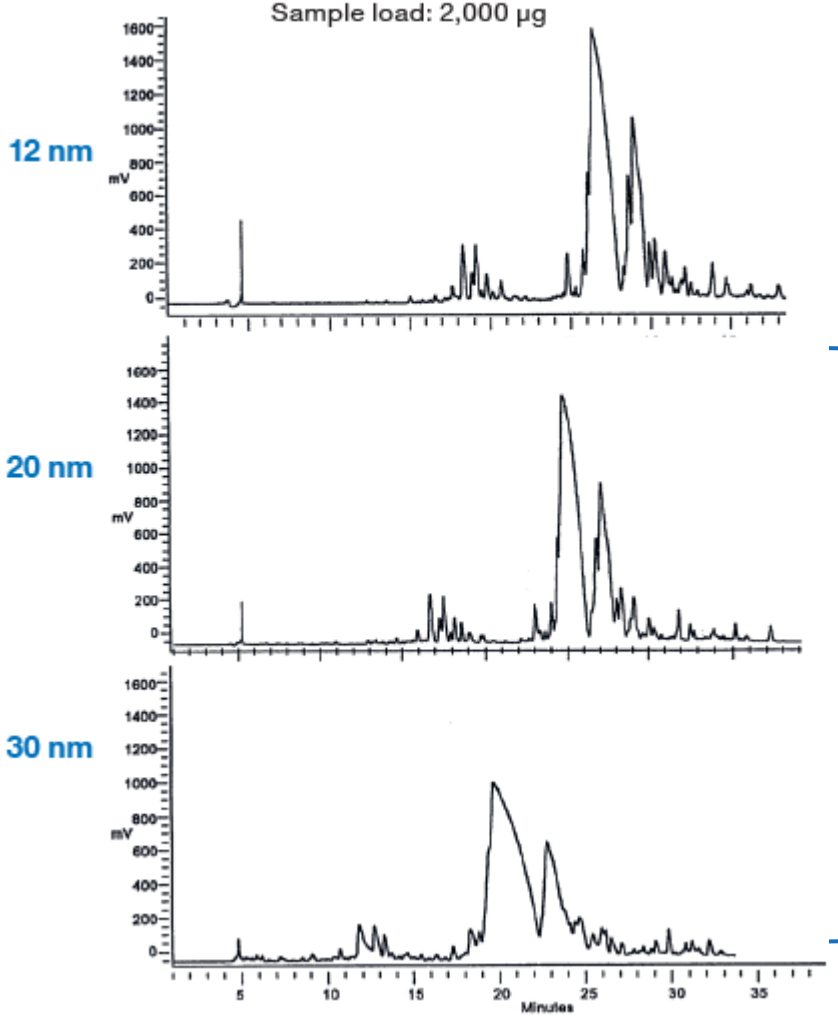
The pore size of the stationary phase should be selected as large as necessary and as small as possible.

Influence of the Pore Size on Loadability

Analytical separation of a peptide
(17 AA, MW 3,400)
Sample load: 50 µg

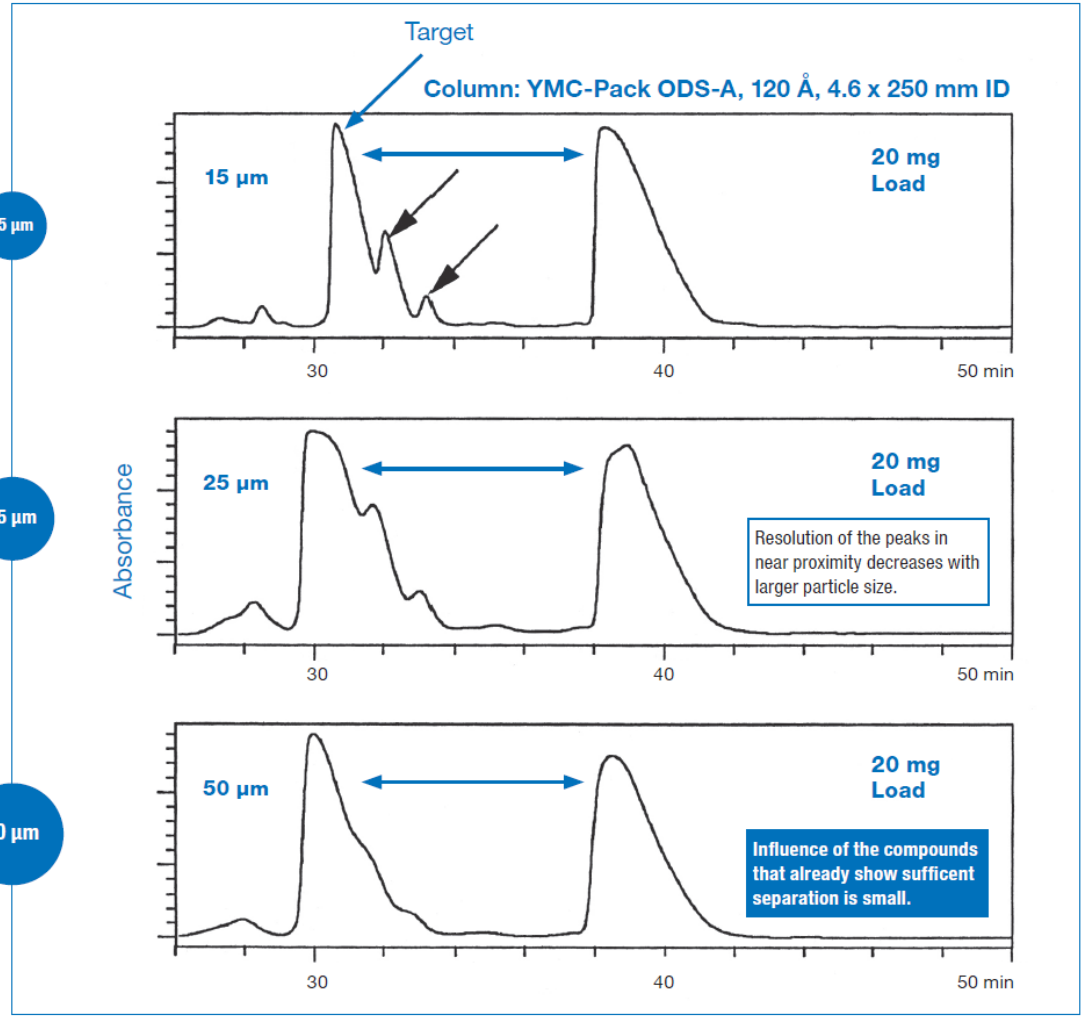


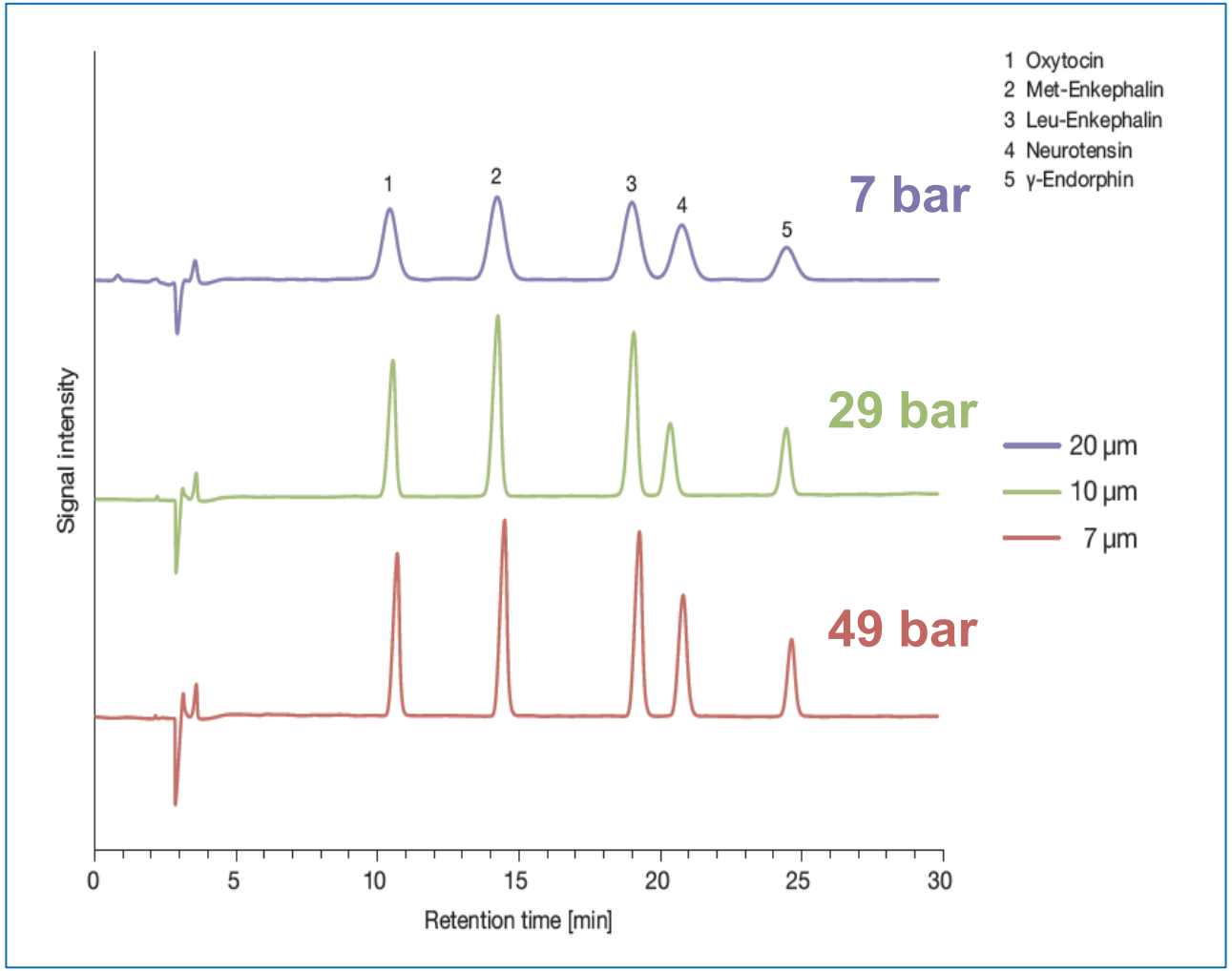
Preparative separation of a peptide
(17 AA, MW 3,400)
Sample load: 2,000 µg



Overloading Effects!

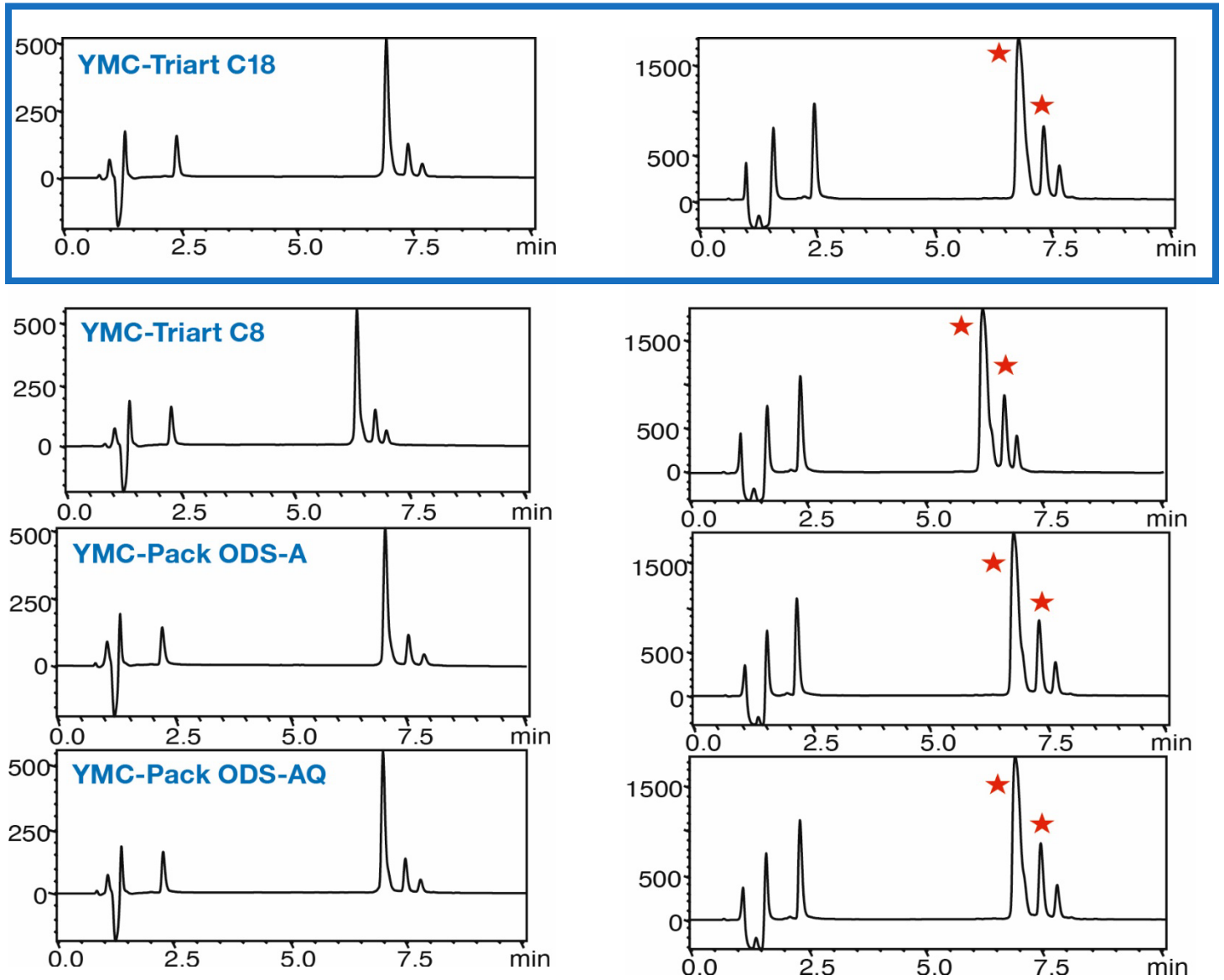
High Resolution Separations: Optimal Particle Size





Phase Screening for Liraglutide

Loading amount 3 µg → x 8 Loading → 24 µg



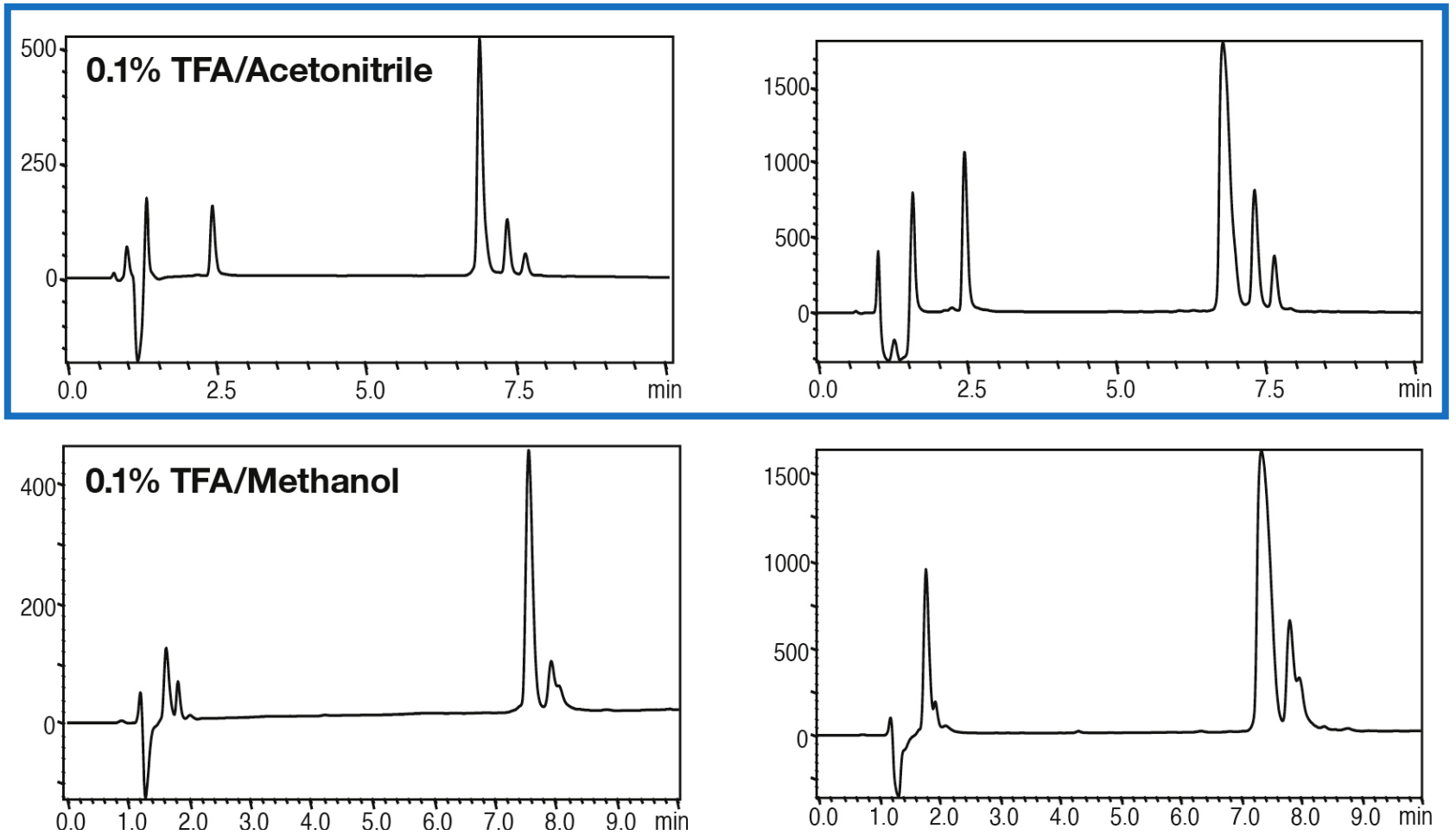
Not only the resolution is important, but also the pH stability!

Higher flexibility in method development

★critical pair

Selection of Organic Solvent for Liraglutide

Loading amount 3 µg → x 8 Loading → 24 µg

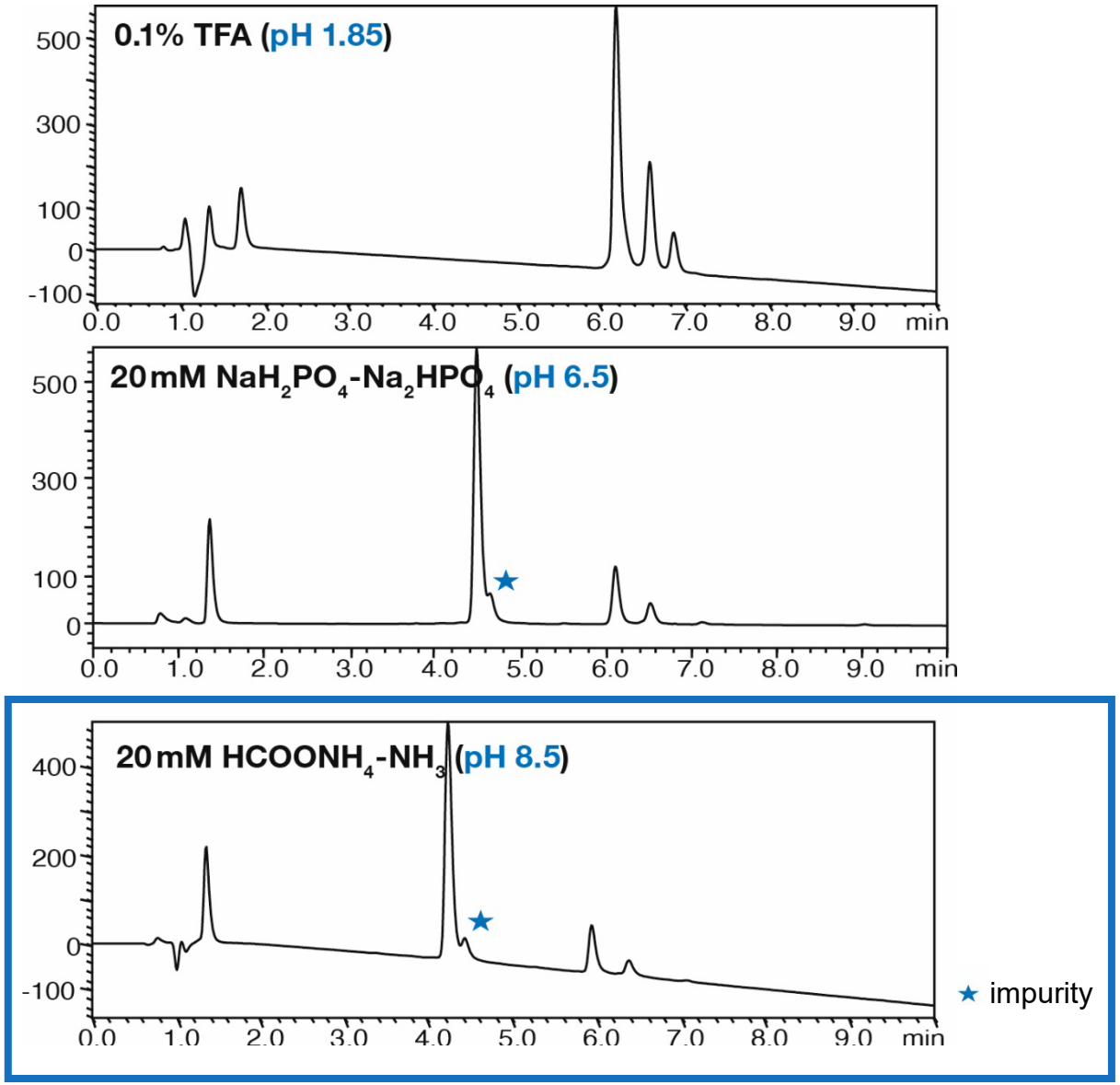


Choice of organic solvent influences the selectivity

Improvement of resolution and loadability

Check the solubility of the peptide in different solvents!

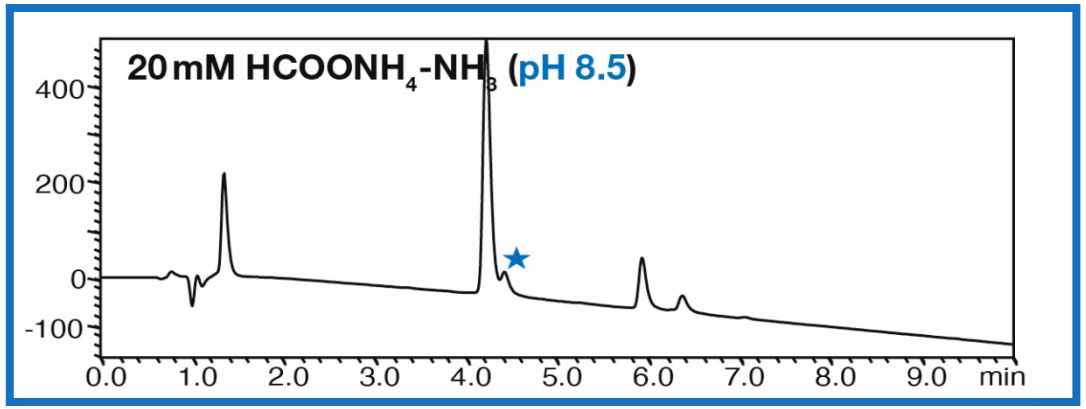
pH Optimisation for Liraglutide

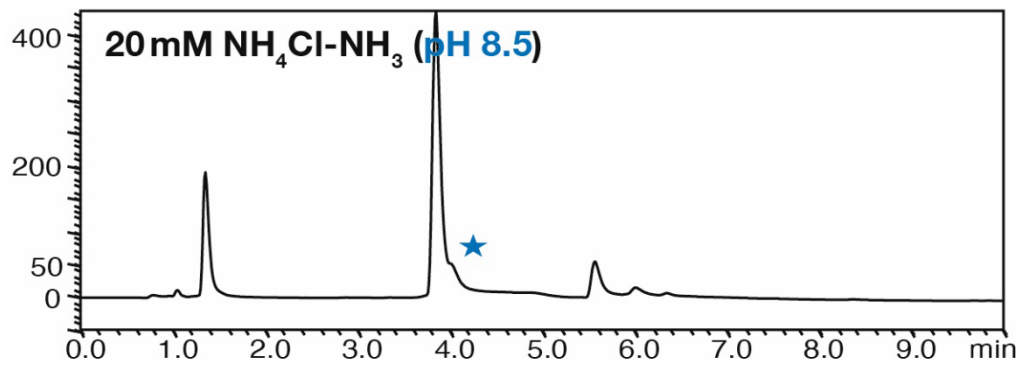


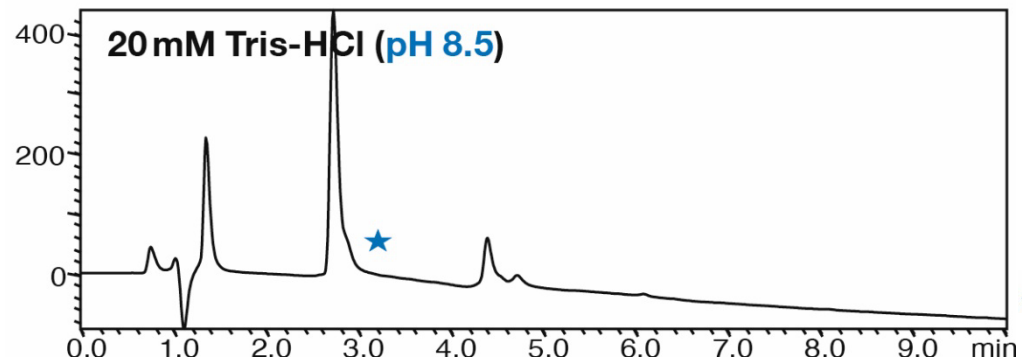
Conditions	Resolution of main peak and impurity peak
20 mM $HCOONH_4$-NH_3 (pH 8.5)	0.96
20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.5)	0.50
0.1% TFA (pH 1.85)	–

Maximum flexibility in method development requires highly stable stationary phases!

Buffer Type Selection for Liraglutide







★ impurity

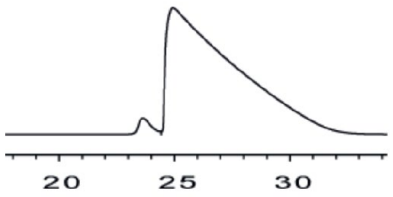
Conditions	Resolution of main peak and impurity peak
20 mM $HCOONH_4$-NH_3 (pH 8.5)	0.96
20 mM NH_4Cl-NH_3 (pH 8.5)	–
20 mM Tris-HCl (pH 8.5)	–

Buffer salts have a strong influence on the peptide separation
→ Screening is recommended

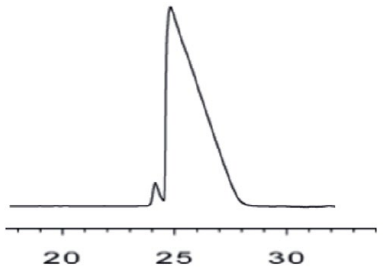
Gradient Optimisation for Liraglutide

Influence on peak shape

Isocratic elution vs. gradient elution

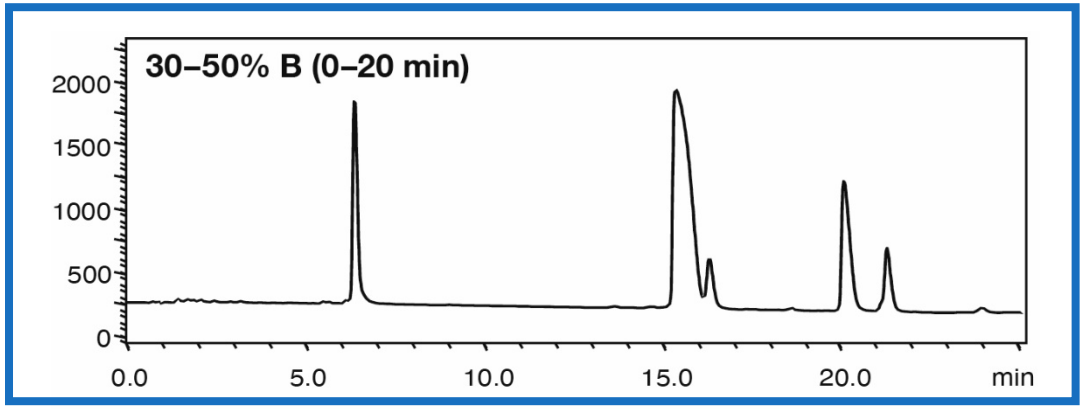


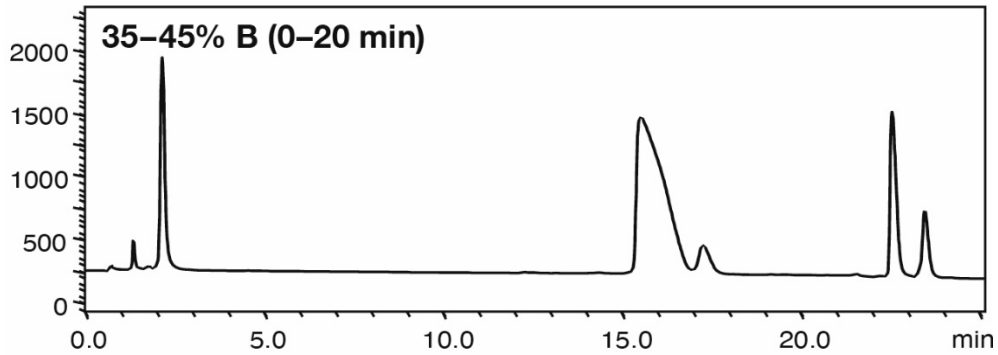
isocratic

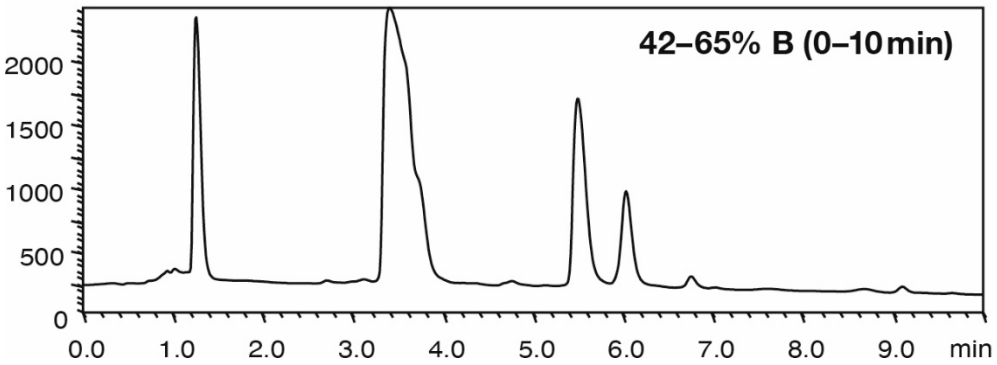


gradient

The slope of the gradient has to be adapted to give an optimal balance of resolution and productivity







Conclusion

Development of a purification method is performed in three steps:

1. **Method development at analytical scale**
2. **Loadability studies at analytical scale**
3. **Linear scale-up to preparative process**

Practical considerations for the purification of **Liraglutide**:

- ✓ **pH stable hybrid silica** as base particle for maximum flexibility in method development
- ✓ **Alkaline pH** for highest resolution
- ✓ **Acetonitrile** as organic modifier
- ✓ **Optimised gradient** to increase the loadability