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Scale-up for Peptide Purification

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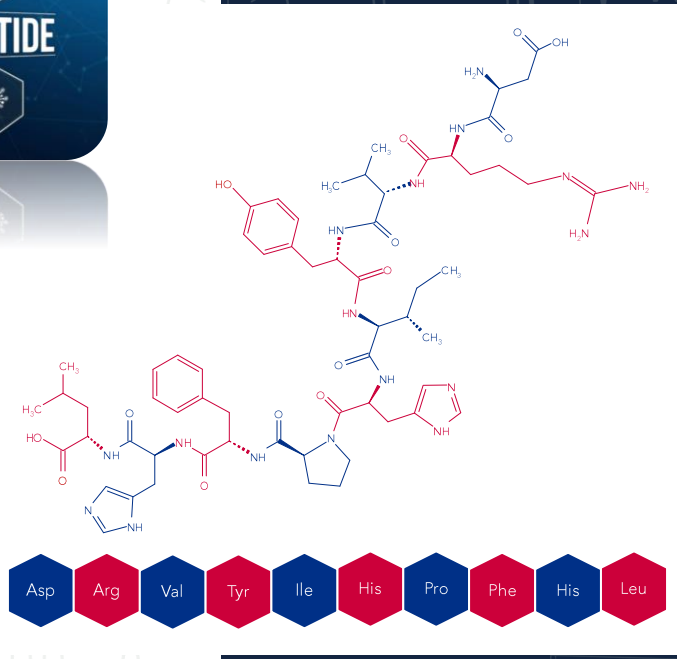
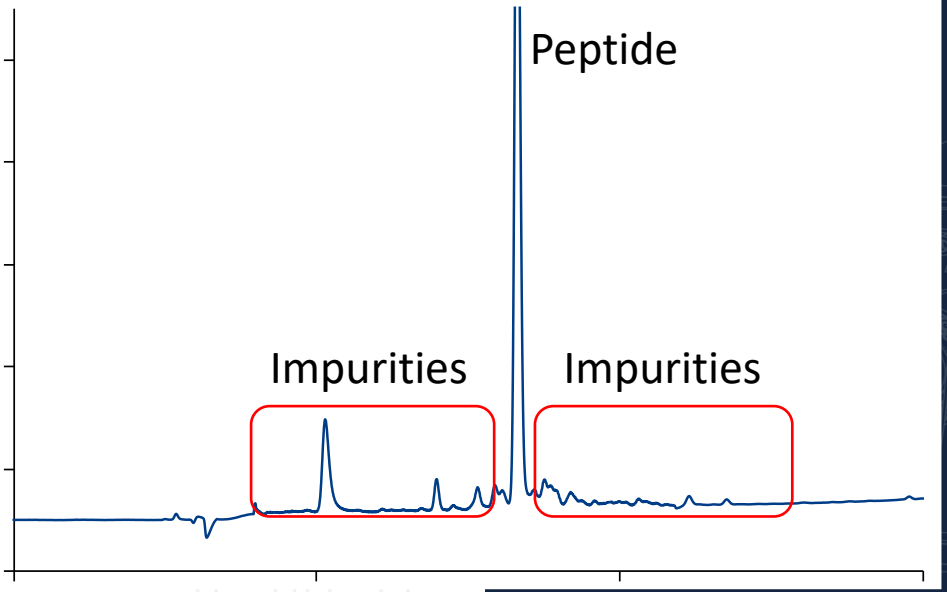
Angiotensin I as model

- Decapeptide
- Prohormone
  - Precursor to Angiotensin II
- Part of the renin-angiotensin-aldosterone system (RAAS)
  - Hormone system
  - Regulates blood pressure, fluid and electrolyte balance, and systemic vascular resistance



Peptide synthesis

- Formed by condensation of single amino acid derivates
- **S**olid **P**hase **P**eptide **S**ynthesis (Merrifield)
  - Amino acid sequence chained to resin beads
  - Beads are filtrated
  - Peptides are cleaved off
- Crude is purified and analyzed
  - Analytical HPLC
  - Preparative HPLC



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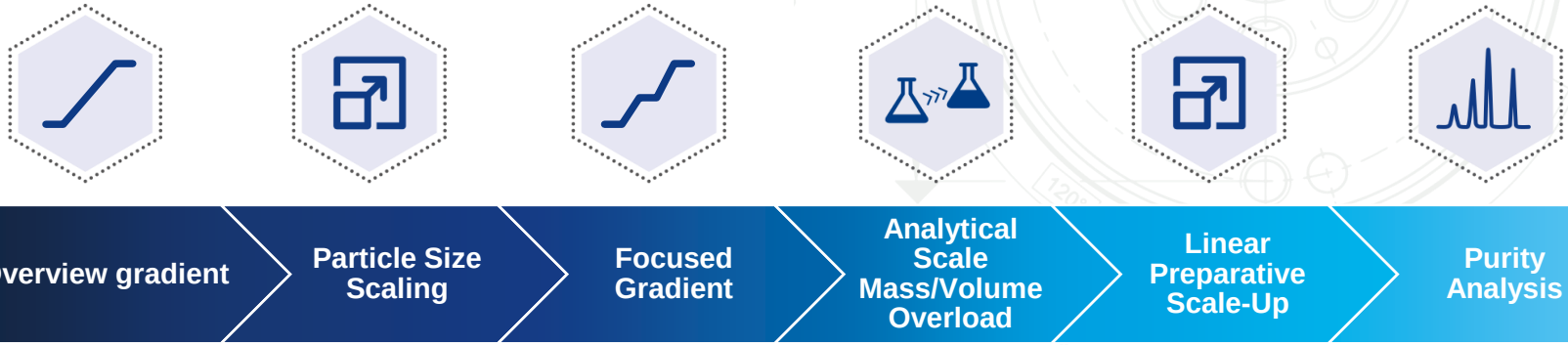
Purification method development



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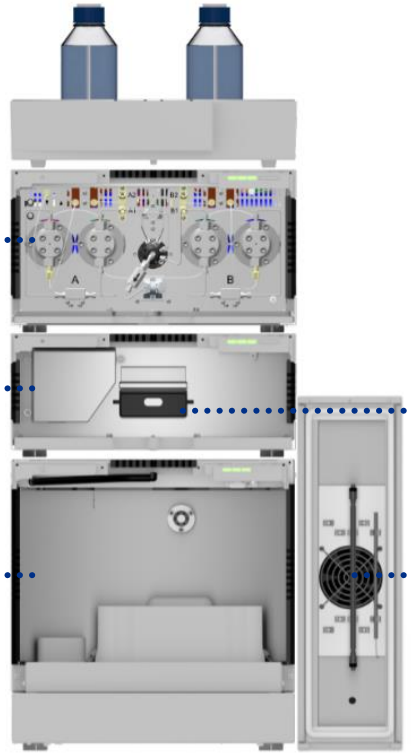
AZURA Analytical (U)HPLC



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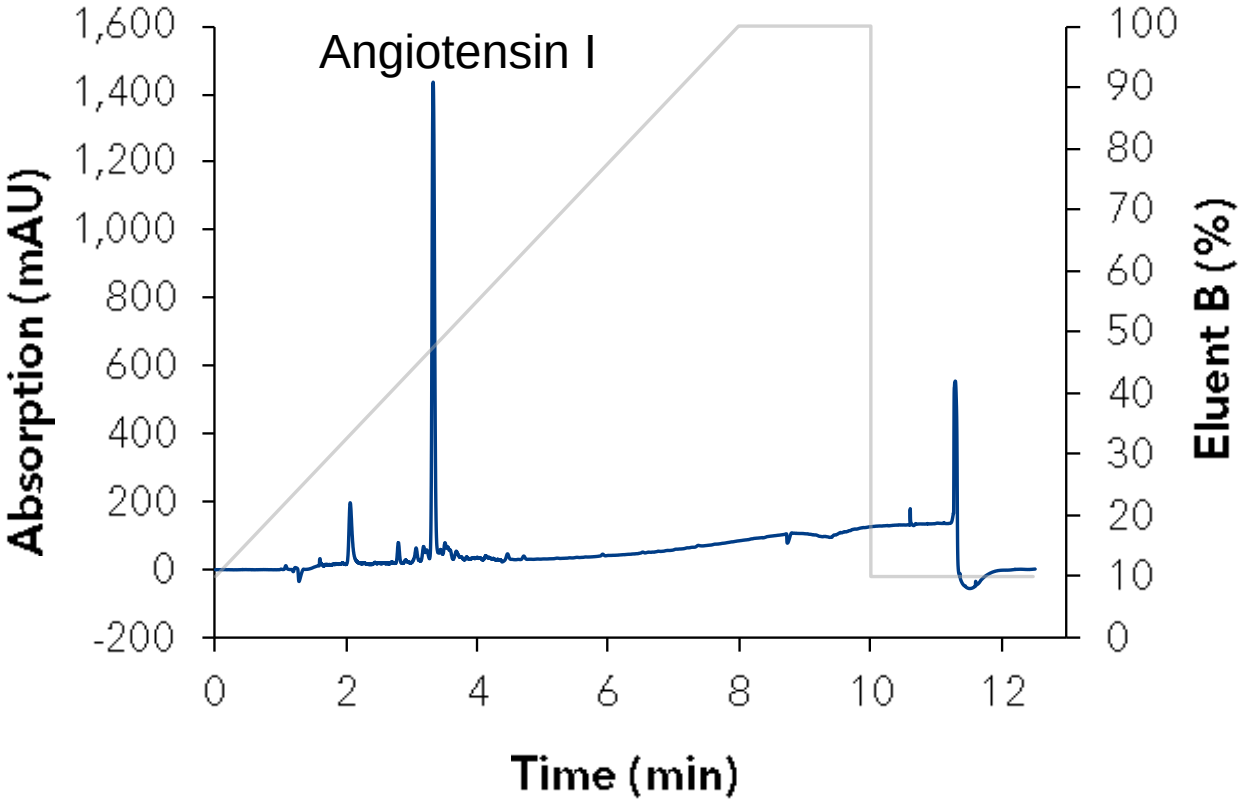
Analytical method

Parameter	Settings	
Run time	12.5 min	
Flowrate	1.2 ml/min	
Eluent A	H_2O_{dd}+ 0.1% TFA	
Eluent B	Acetonitrile + 0.1% TFA	
Gradient	Time	B %
	0 min	10
	8 min	100
	10 min	100
	10.02 min	10
	12.5 min	10

Parameter	Settings
Injection volume	10 µl
Detection mode	UV
Wavelength	220 nm
Data rate	10 Hz
Column	Eurospher II 100-5 C18 150 x 4 mm
Column temperature	25 °C

C18, 100 Å → short peptide
TFA → acidic IEP



150 x 4.6 mm, 5 µm

Overview Gradient

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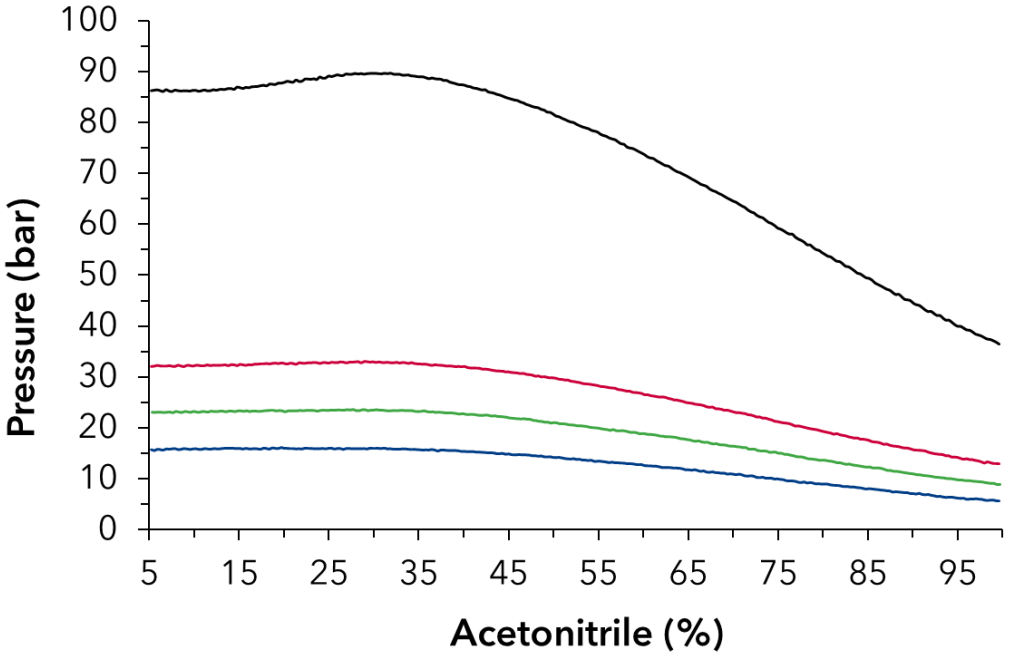
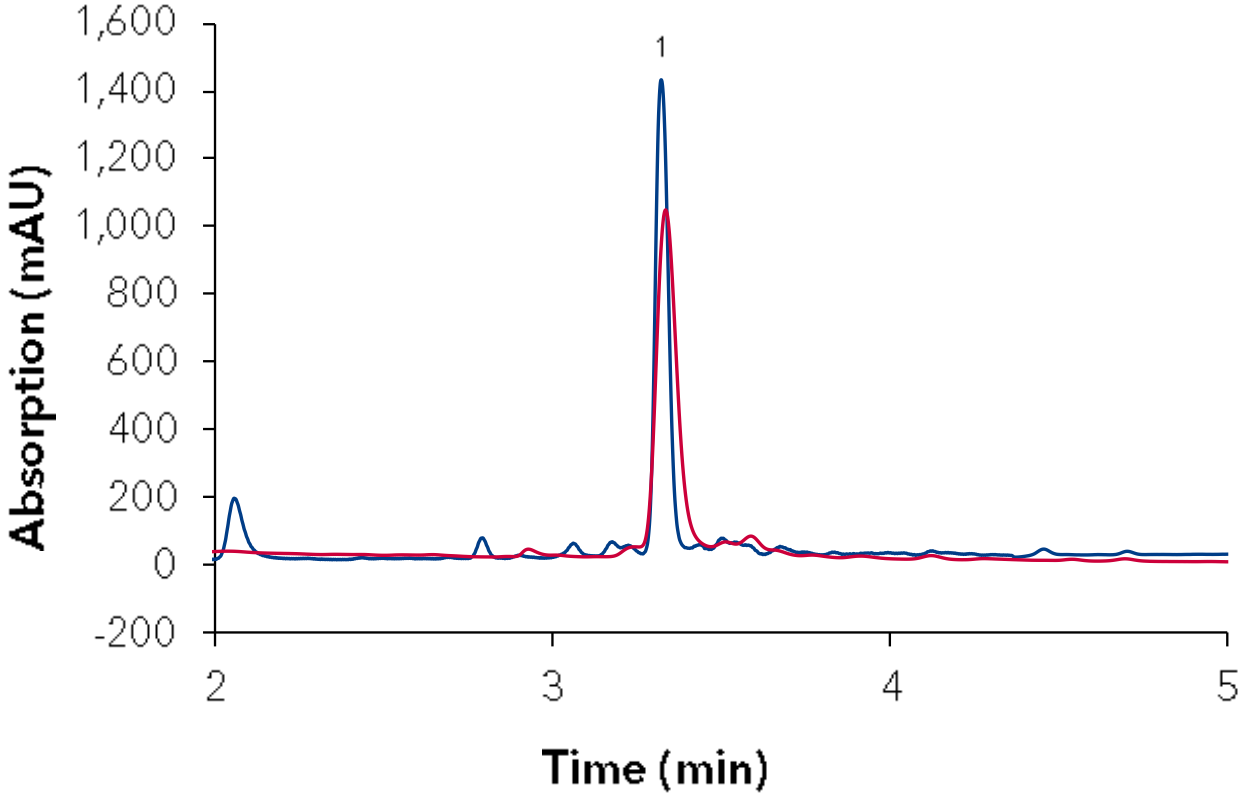
Backpressure



1.2 ml/min

5 µm
10 µm
15 µm
20/45 µm

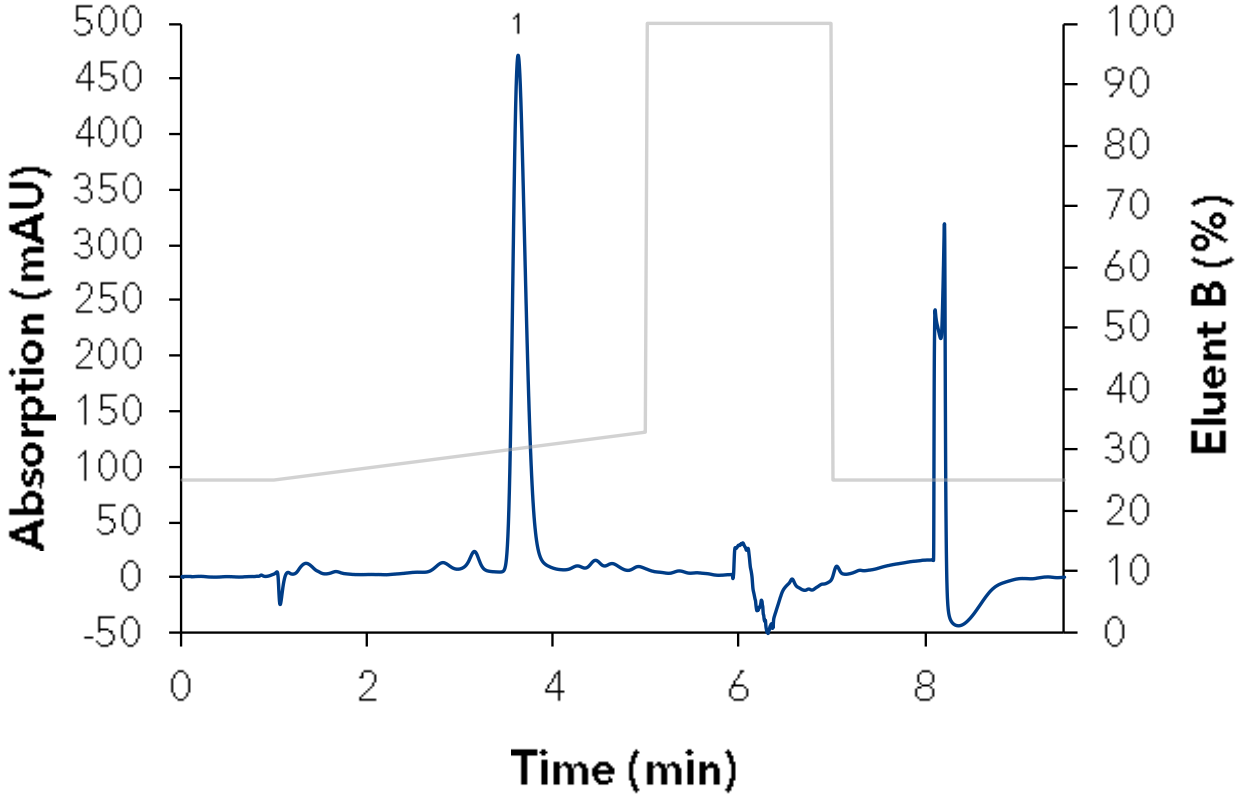
Larger particles
- Lower bp
- Lower costs
- Lower resolution



150 x 4.6 mm, 5 µm

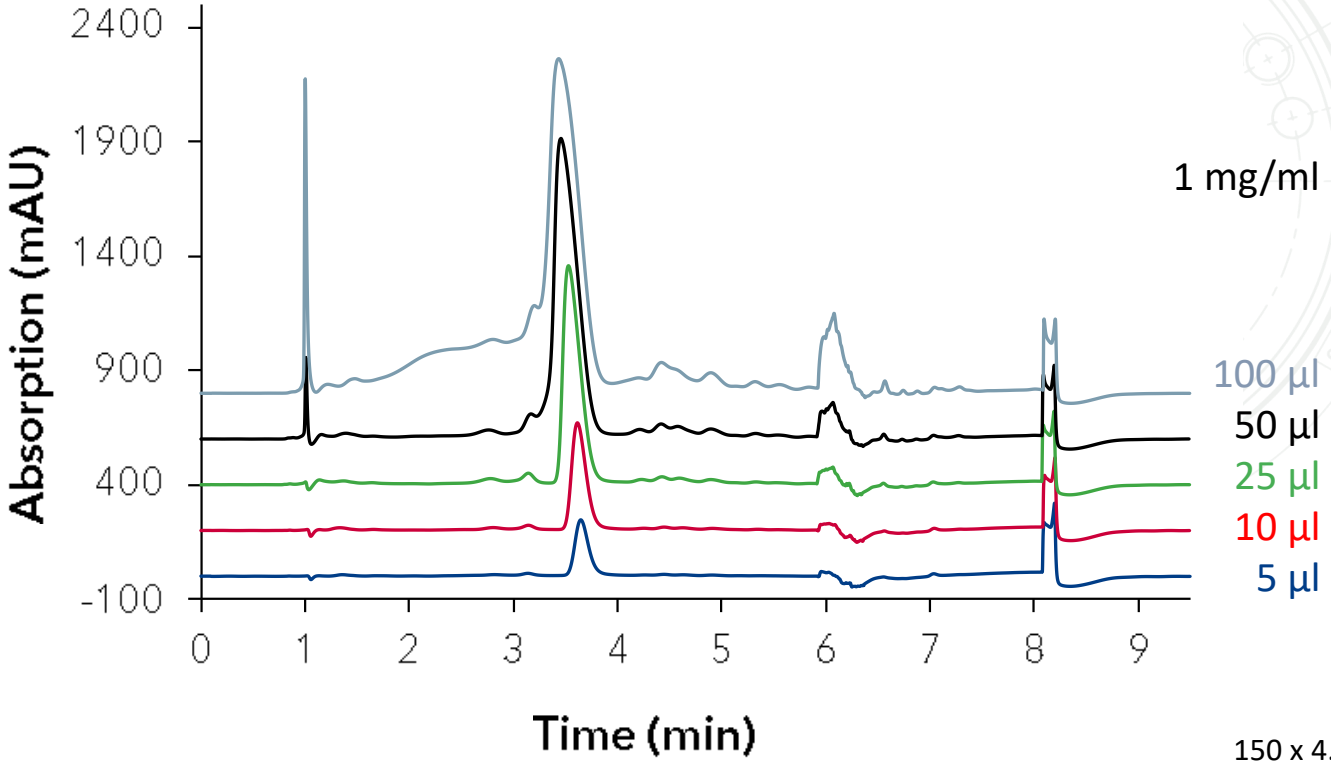
Particle size scaling 5 → 10 µm

150 x 4.6 mm, 10 µm



150 x 4.6 mm, 10 µm

Focus gradient



Volume overload

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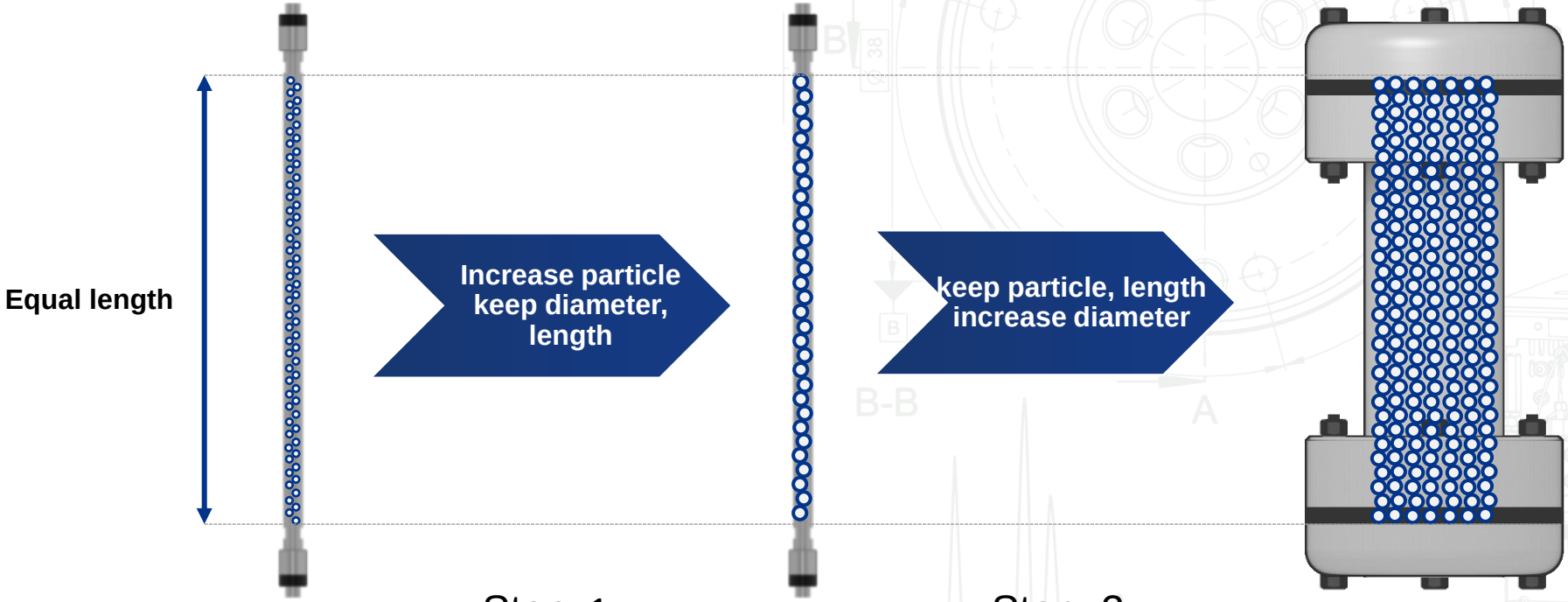
Linear Scale-up concept



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Linear Scale-up

Linear scale up: Scale up factor = $(ID^2_{preparative} / ID^2_{analytical})$

Scale up factor = $(20\ mm^2 / 4.6\ mm^2) = 18.9$

Column Dimensions [mm]	Scale Up Factor	Loadability [mg]	Flow Rate [ml/min]
150 x 4.6	1	1-4	1
150 x 20	**18.9**	20-80	19
150 x 30	**42.5**	40-160	43
150 x 50	**118**	80-350	118

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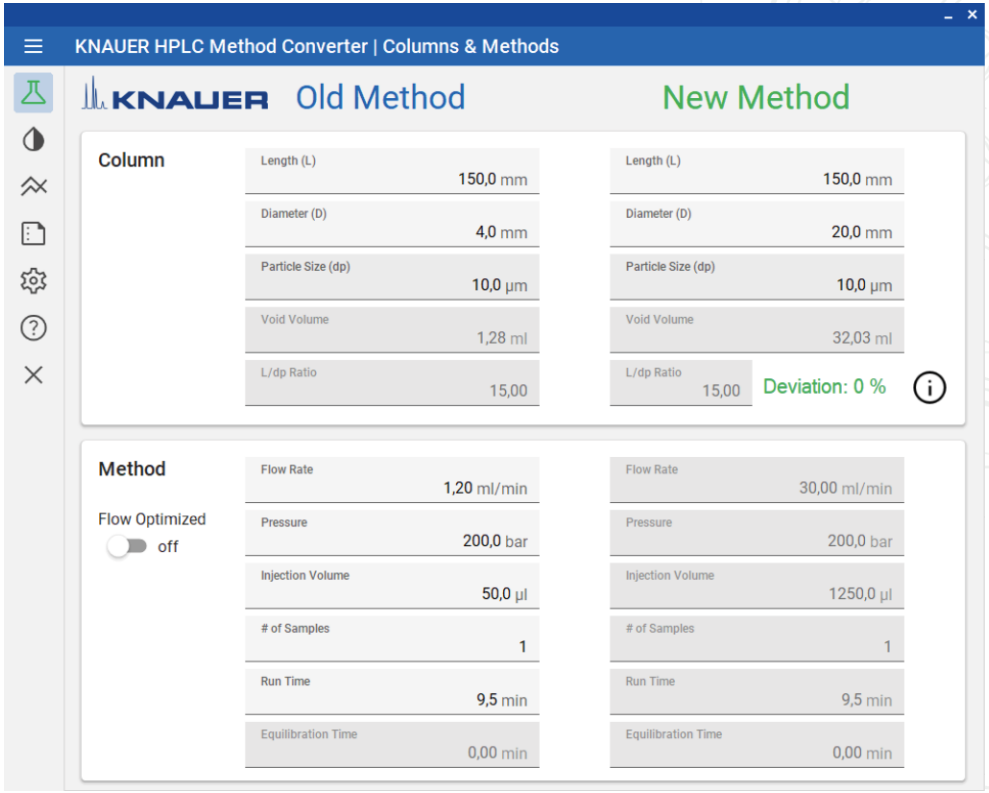
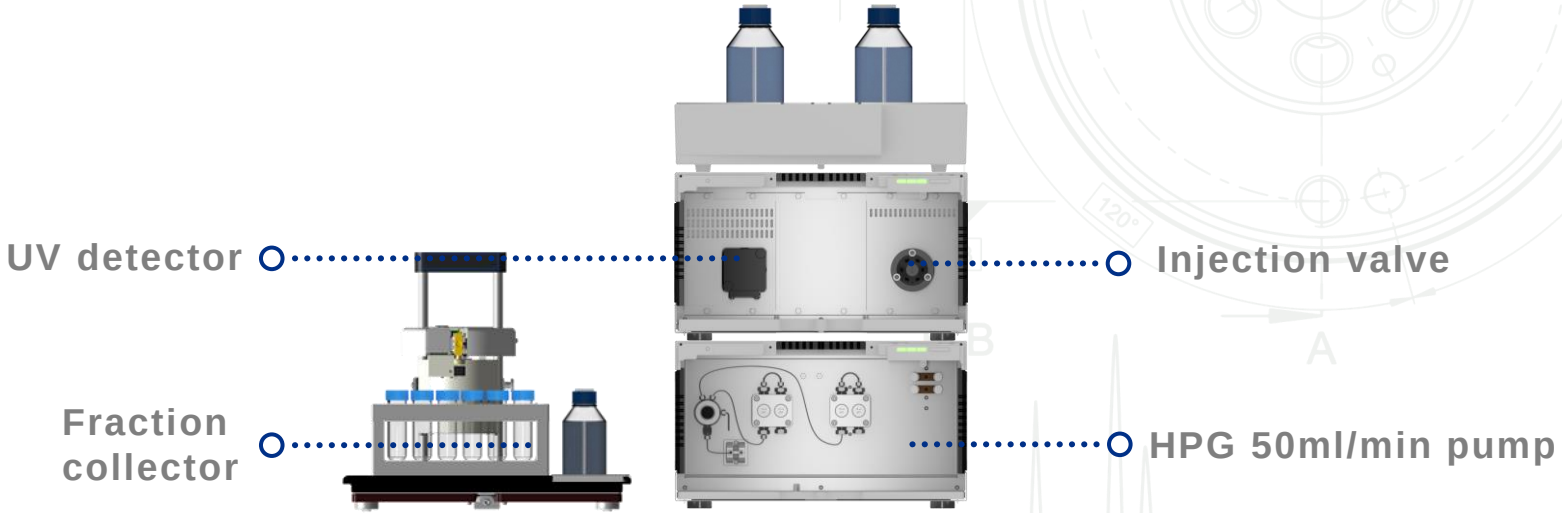
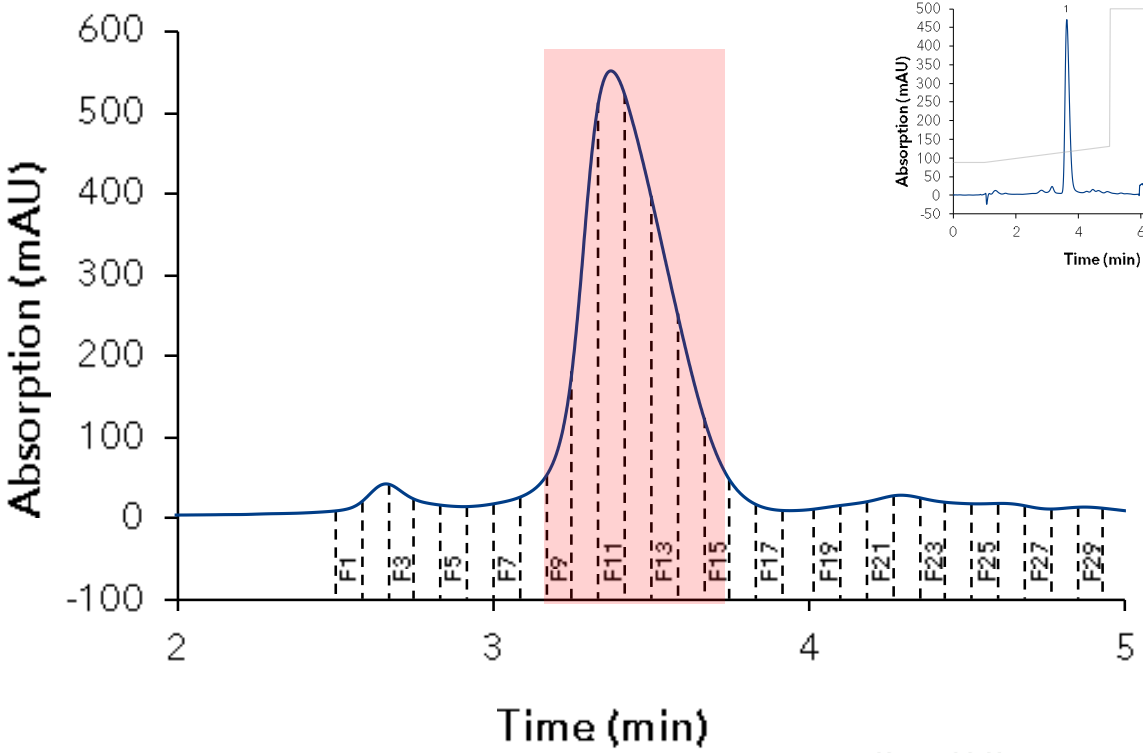
KNAUER HPLC Method Converter

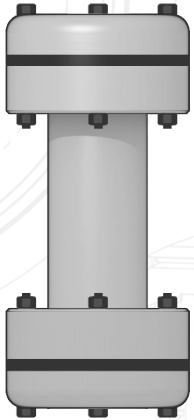


Free download

AZURA Semi - Preparative system

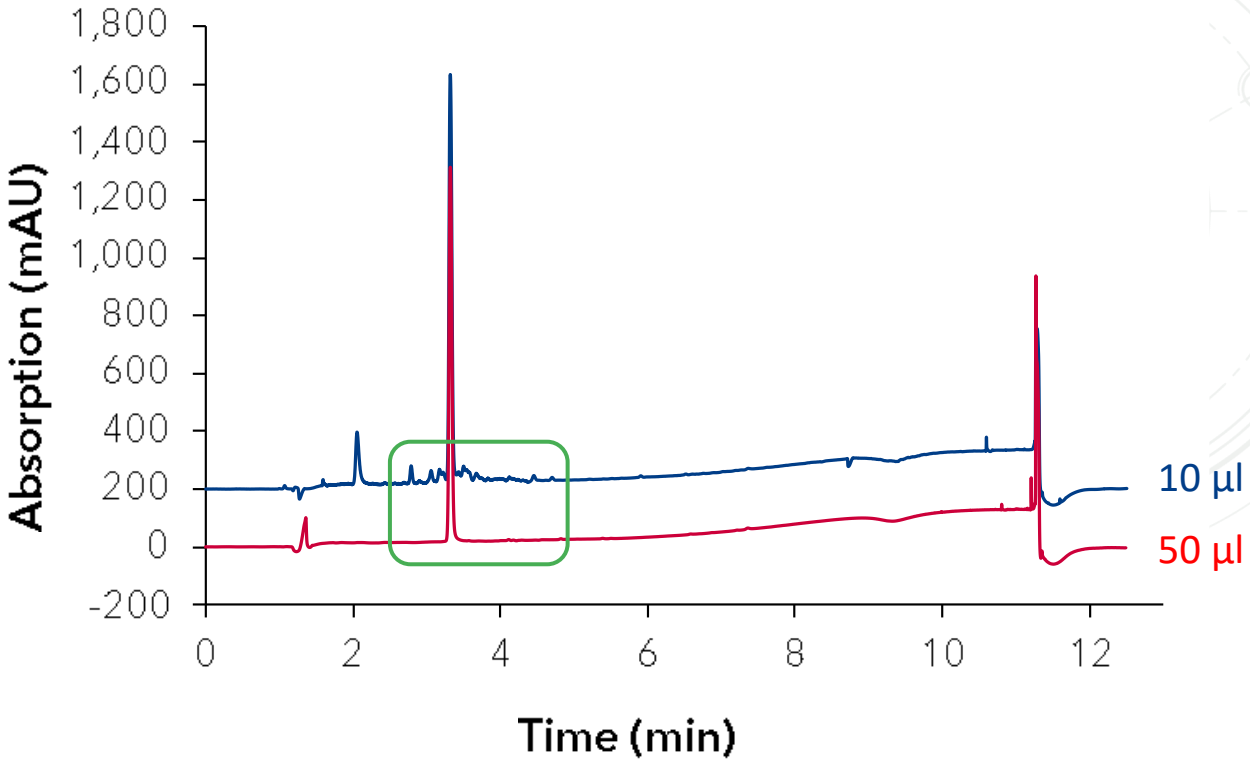






Preparative run

- ID 20 mm
- 30 ml/min
- 2 ml sample



150 x 4.6 mm, 5 µm

Analysis pooled fractions

Purity 70% → 99 %

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Conclusion

- Purified peptide crude from 70 to 99% purity
- Processed 2 mg sample in under 10 min. 1.4 mg product
- KNAUER provides various, scalable solutions for peptides purification

Scale-Up

Semi – preparative scale
Up to ID 20 mm, 30 ml/min

Conclusion

- Purified peptide crude from 70 to 99% purity
- Processed 2 mg sample in under 10 min; 1.4 mg product
- KNAUER provides various, scalable solutions for peptides purification

High flow rate
Up to ID 100 mm, 800 ml/min

High sample throughput
Automated injection/collection

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YMC EUROPE GMBH

Purification and Quality Control of Oligonucleotides

Applying AEX and IP-RP

SAVE THE DATE

- How to perform a large scale AEX purification: Design of preparative system and useful features
- Benefits and limitations using AEX for preparative purification
- What to consider for developing an IP-RP method: selection of stationary phase, ion-pair reagent, and solvent conditions
- How to improve analytical results by utilizing bioinert column hardware

01.04. 2025

11-12 am (CEST)

Further Information and Registration

https://ymc.eu/YMC_Knauer_Webinars.htm

Speakers

Dr. Ulrike Krop, **KNAUER Wissenschaftliche Geräte GmbH**
Dr. Daniel Eßer, **YMC Europe GmbH**