Purification of peptides

Increasing the purity and recovery by salt addition

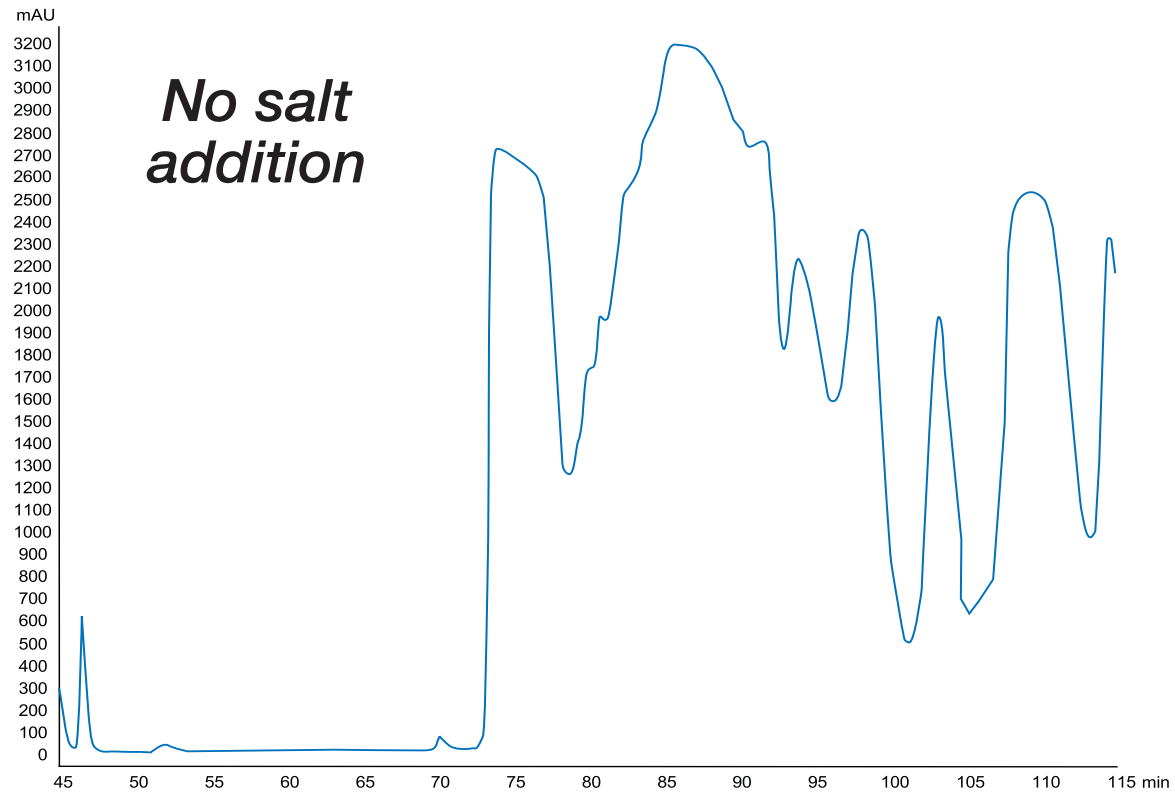
The purification of peptides can be very challenging. Depending on the complexity of the sample feed, the use of regularly used methods may not be sufficient. In this example, the positive effect of adding a salt to the mobile phase is described for the purification of insulin. This data is based on a joint project with an insulin manufacturer searching for improved process parameters.

In the screening for potential optimisation parameters, minimum purity and recovery values were defined. The purity must be a minimum of 99.5 % and the recovery must be higher than 80 %.

The crude sample had a purity of 75.8%. During the screening, the following parameters were considered.

- Stationary phase screening
- Mobile phase screening
  - Influence of organic solvent
  - Influence of pH
  - Influence of buffer type
  - Influence of additives

Out of all these parameters, the decisive step was to add ammonium chloride to the mobile phase. Without using the salt as additive, no fraction was found with the required purity of 99.5 %.



Column Size:	YMC-Triart Prep Bio200 C8, 250 x 4.6 mm ID
Eluent:	A) buffer*/acetonitrile (90/10)
	B) buffer*/acetonitrile (10/90)
Gradient:	0%B (0–10 min), 0–20%B (10–15 min), 20–25%B (15–45 min)
Detection:	UV at 214 nm
Flow:	0.74 mL/min
Temperature:	ambient
Sample:	Crude insulin human recombinant (Purity 75.8%)
*buffer:	20 mM CH_3COONH_4-CH_3COOH (pH 4.3)

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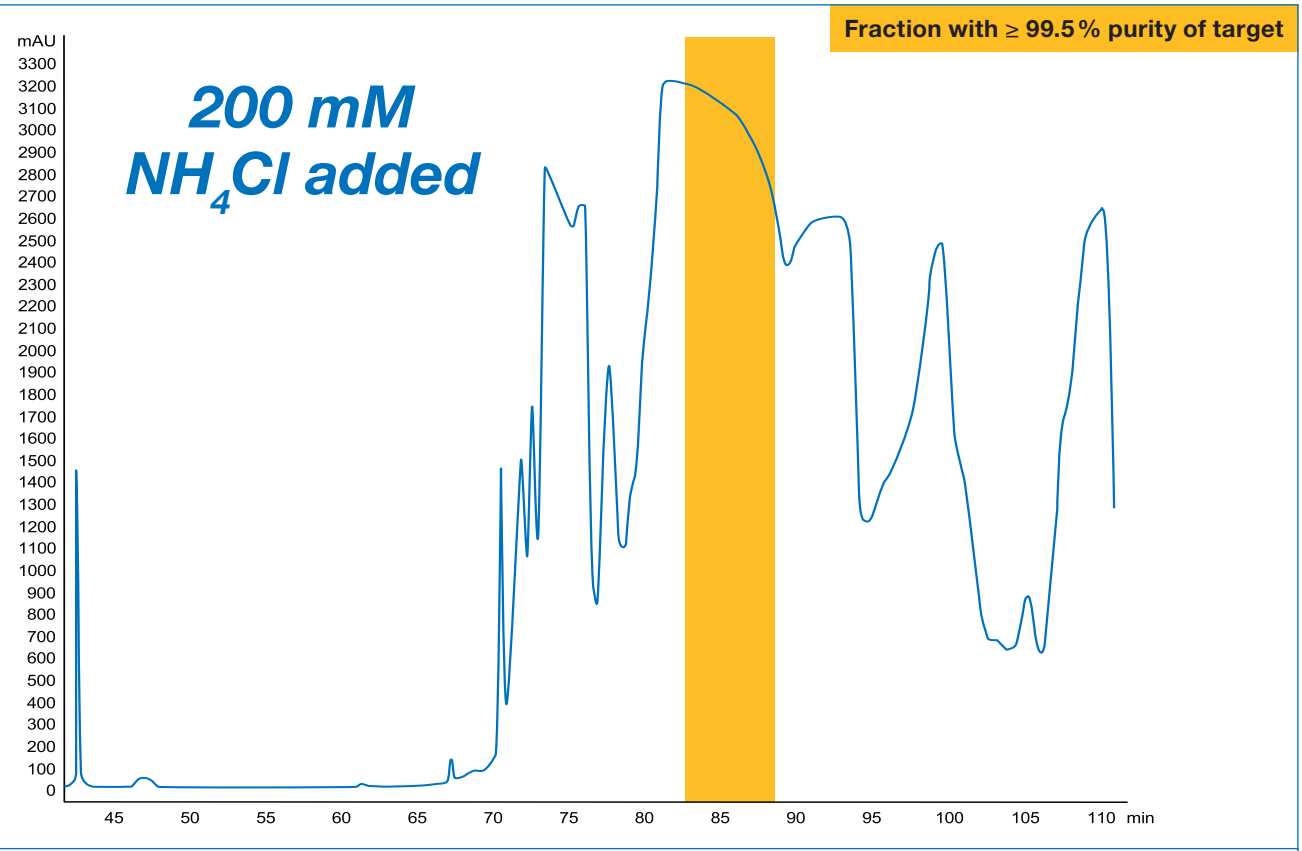
Expert tip

With the salt added to the mobile phase, the results were greatly improved. The set criteria were exceeded in terms of purity and recovery. The results show this process allows a purity of 99.7 % based on a crude with a purity of 75.8 % to be achieved! The recovery reached a level of 87 %.



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Detection:	UV at 214 nm
Flow:	0.74 mL/min
Temperature:	ambient
Sample:	Crude insulin human recombinant (Purity 75.8%)
*buffer:	20 mM CH_3COONH_4-CH_3COOH, 200 mM NH_4Cl (pH 4.3)

	Purification Criteria	Achieved Results
Fraction volume (≥99.5%)	**99.5%**	**99.7%**
Insulin concentration in recovered fraction (≥99.5%)	**80.0%**	**87.0%**

Conclusions

Adding salt to the mobile phase greatly improved the separation for the isolation of insulin. Without the salt added to the mobile phase, the set criteria for the purification were not fulfilled. However, with the salt added to the mobile phase, the purity and recovery easily fulfilled the set criteria. This effect of an improved separation has also been seen for other peptide- and oligonucleotide-based compounds. It extends the range of possible options available for process optimisation.

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