# Purification of a therapeutic peptide using an orthogonal method to achieve high purity

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## Introduction

Purification of therapeutic peptides presents a number of major challenges, mainly related to the very stringent purity requirements of the end product. Exceptionally high resolution is required in the purification process due to the close similarity between product and associated impurities. To achieve optimal purity without sacrificing yield, it is therefore advisable to implement orthogonal modes of purification, such as ion exchange and RPC in combination, to maximize selectivity.

Additionally, with peptides of increasing length comes an increased burden of impurities in the form of failure sequences from the solid-phase peptide synthesis (SPPS) (Fig. 1). In a purely RPC–based purification regime, this typically leads to a build-up over time of tightly bound impurities on the expensive and sensitive silica column. Introducing ion exchange as a complementary step helps alleviate this issue by removing most smaller residues prior to RPC loading.

Challenge: Increasing peptide sequence length → increased amounts of synthesis-related impurities (Fig. 1)

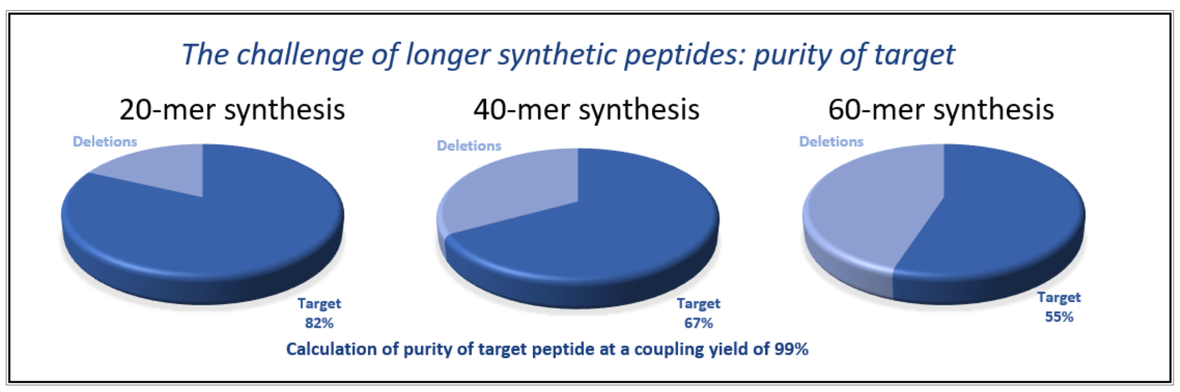


**Figure 1.** Visual representation of the increasing impurity levels from SPPS with increased peptide length.

Therapeutic peptide: 45 aa residues, pI 4.3-4.5, linear
WorkBeads™ 40S resin: 45 µm rigid agarose beads, optimized flow-pressure properties, alkali-tolerant

The binding and elution conditions were optimized for WorkBeads 40S and an industry-standard resin, Capto™ SP ImpRes (Cytiva). The optimal performance of each resin were compared.

## Results

The dynamic binding capacity (DBC) of the two resins was analyzed by frontal analysis at different flow rates using pure peptide (10 g/L) and determined at 10% breakthrough. DBC for WorkBeads 40S and Capto SP ImpRes was determined to be 150 mg/mL and 125 mg/mL, respectively, at 2 minutes residence time (Table 1), indicating excellent mass transport, supported by fast desorption kinetics.

**Table 1.** Dynamic binding capacities for WorkBeads 40S and Capto SP ImpRes.

| Resin | DBC (mg/mL) at 2 min residence time | DBC (mg/mL) at 1 min residence time |
|---|---|---|
| Workbeads 40S | **150** | **140** |
| Capto SP ImpRes | 125 | 123 |

A 55% pure crude synthesis feed with a load of ~20% of resin's DBC was purified with WorkBeads 40S and Capto SP ImpRes respectively, to establish optimal performance with regards to purity and yield (Fig. 2).

| | |
|---|---|
| *Feed:* | Crude, 30 g target peptide/L resin (55% pure) |
| *Columns:* | WorkBeads 40S (10 × 240 mm, 19 mL) |
| | Capto SP ImpRes (10 × 240 mm, 19 mL) |
| *Flow rate:* | 50 cm/h (2 mL/min) |
| *Binding buffer:* | 5 mM $NH_4Ac$, pH 4, 15% ACN |
| *Elution buffer:* | 250 mM $NH_4Ac$, pH 6, 15% ACN |
| *Gradients:* | 1. Linear: 0% - 40% elution buffer, 1 CV |
| | 2. Step: 40% elution buffer, 2 CV |
| | 3. Linear: 40 - 70% elution buffer, 5 CV |

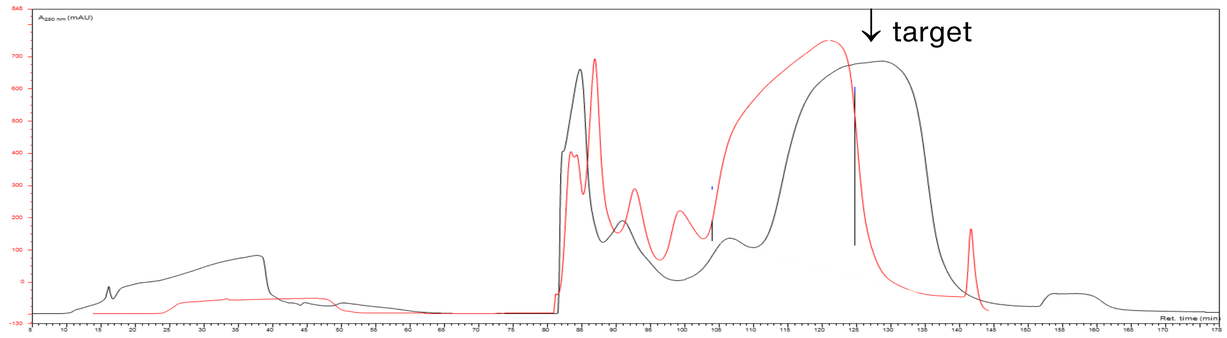


**Figure 2.** Purification of a 45-amino acid residue peptide. UV traces are shown for WorkBeads 40S (black) and Capto SP ImpRes (red).

The peptide fractions were collected with a target yield of 92% on both columns. The purity obtained with a loading of 30 g/L was 91.8% for WorkBeads 40S and 85.2% for Capto SP ImpRes (Table 2). The purity increased to 93% on WorkBeads 40S when the loading was decreased to 10 g/L resin.

**Table 2.** Purity obtained with load of 30 g/L of a crude feed.

| Resin | Purity (%) | Impurities (%) |
|---|---|---|
| Feed | 55 | 45 |
| Workbeads 40S | **91.8** | **8.2** |
| Capto SP ImpRes | 85.2 | 14.8 |

The material purified on WorkBeads 40S (Fig. 2) was further subjected to an RPC polishing step to obtain the final purity of 99 % (Fig. 3).

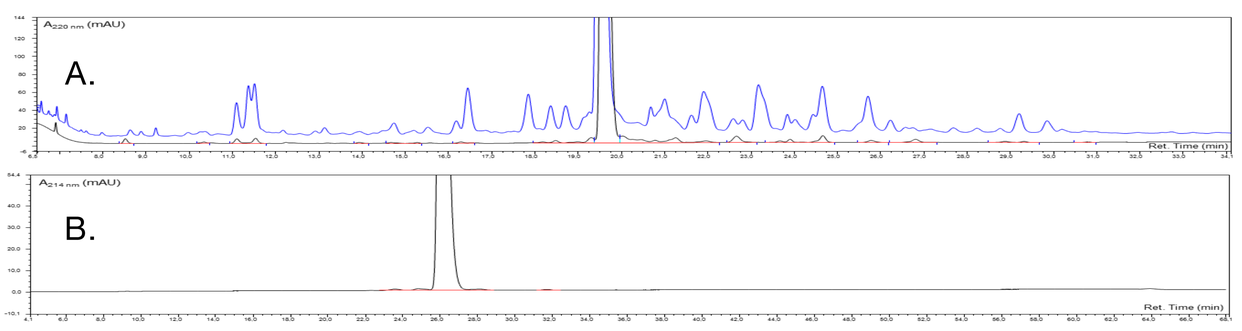


**Figure 3.** Purity analysis by RPC. A) Crude feed (blue trace), peptide purified on WorkBeads 40S (black trace). B) Final product after RPC polishing.

## Conclusions

The results demonstrate a significant enhancement of purity using an agarose-based orthogonal ion-exchange step prior to the final polishing by RPC. The observed decrease of sample impurities following IEX purification will protect the RPC column and minimize the risk of decreased performance due to fouling. Finally, due to the ease of scalability of agarose-based resins, introduction of IEX as a pre-step for process scale can help concentrate the sample prior to RPC and decrease both purification time and process cost.

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