Purification of a 45-amino acid residue peptide using WorkBeads 40S

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Introduction

Purification of biomolecules from complex feeds is often best carried out using combinations of orthogonal separation methods. We have investigated the capture of a synthetic peptide with 45-amino acid residues using WorkBeads™ 40S, an agarose-based cation-exchange resin.

This resin has optimal flow-pressure properties and is stable towards efficient cleaning using strong alkaline conditions. This makes it an excellent tool for purification of therapeutic peptides from crude feeds following solid-phase synthesis or after recombinant expression. The capture step reduces the irreversible contamination of the downstream high-performance silica-based reversed phase chromatography (RPC) column.

The binding and elution conditions were optimized for WorkBeads 40S and Capto™ SP ImpRes (GE Healthcare), and the optimal performance of each resin was compared.

Results

The dynamic binding capacity (DBC) was analysed by frontal analysis at different flow rates using pure peptide (10 g/l) applied under optimized binding conditions, and determined at 10% breakthrough. Figure 1 shows the breakthrough curve for WorkBeads 40S, see the conditions below.

Resin: WorkBeads 40S
Column: 10 × 57 mm, 4.5 ml
Flow: 150 cm/h (2 ml/min)
Feed: 10 g pure peptide/l
Buffers: Mobile phases for binding and elution contained 15% acetonitrile in a proprietary buffers composition.
Elution: Step elution (see arrow in insert)

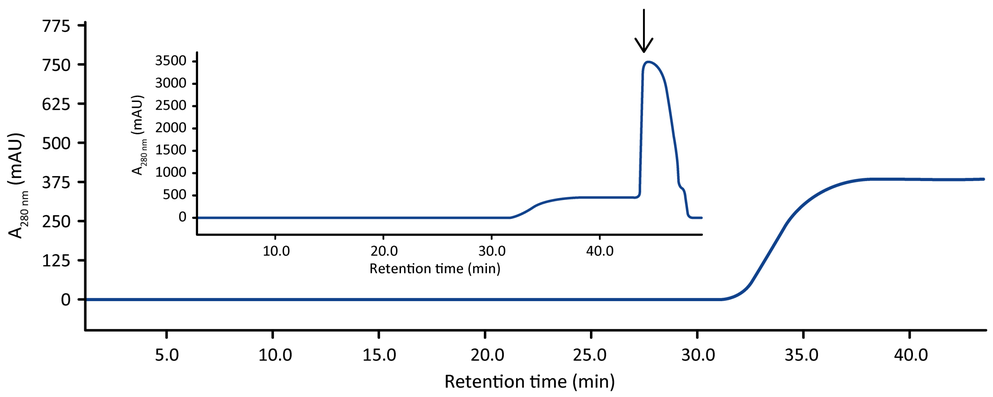

Figure 1. Dynamic binding capacity determined by frontal analysis.

DBC was 151 mg/ml at 75 cm/h, and 140 mg/ml at 300 cm/h (see Fig. 2). 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 (see Table 1). The values were 140 mg/ml and 123 mg/ml, respectively, at 1.1 minutes residence time, indicating excellent mass transport, also supported by the fast desorption kinetics.

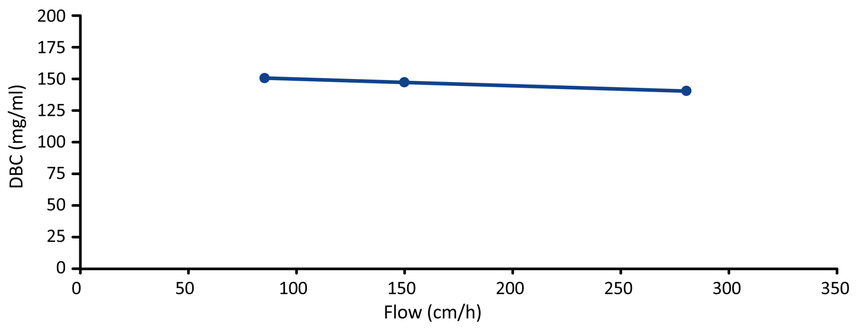

Figure 2. DBC vs linear flow determined for WorkBeads 40S in the column used in Figure 1.

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

Resin	DBC (mg/ml) at 2.0 min residence time	DBC (mg/ml) at 1.1 min residence time
WorkBeads 40S	150	140
Capto SP ImpRes	125	123

A 55% pure feed was purified with WorkBeads 40S and Capto SP ImpRes to establish which of the resins give the most optimal combination of purity and yield (Fig. 3).

Columns: WorkBeads 40 S (10 × 240 mm, 19 ml), Capto SP ImpRes (10 × 240 mm, 19 ml)
Flow: 50 cm/h (2 ml/min)
Feed: Crude, 30 g target peptide/l resin (55% pure)
Buffers: Mobile phases for binding and a combined pH- and salt-gradient for elution contained 15% acetonitrile in a proprietary buffers composition

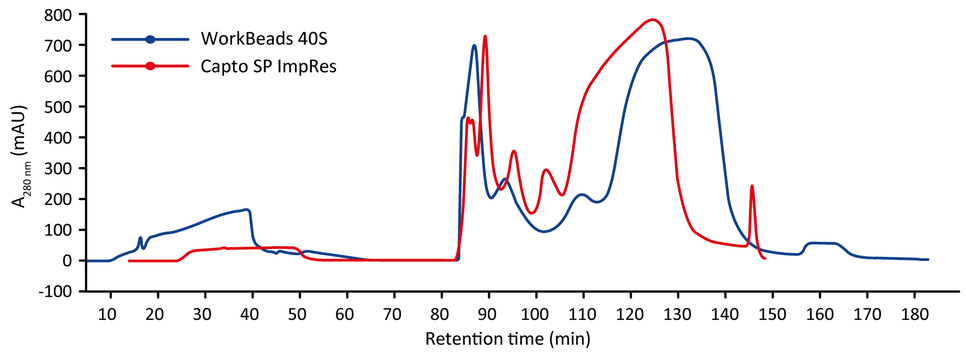

Figure 3. Purification of a 45-amino acid residue peptide from 55% crude feed. UV traces are shown for WorkBeads 40S (blue) and Capto SP ImpRes (red).

The target peptide was collected to keep the yield the same (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. The purity increased to 93.0 % 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 containing 55% target peptide.

Resin	Purity (%)
WorkBeads 40S	91.8
Capto SP ImpRes	85.2

The material prepared using WorkBeads 40S (Fig. 3) was subjected to a polishing step using a RPC silica column (not shown) to obtain the final purity of 99 % of this therapeutic peptide (Fig. 4).

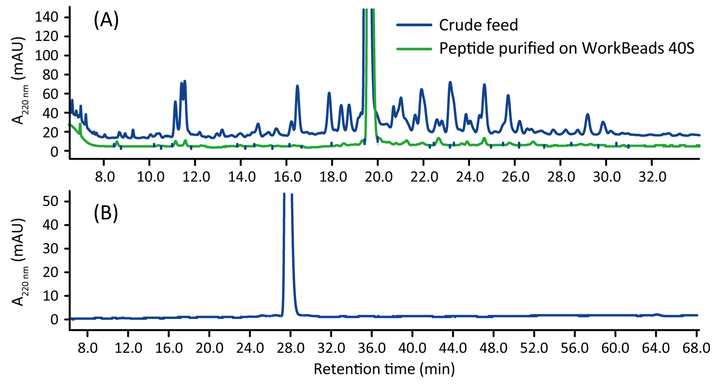

Figure 4. Purity analysis by RPC. (A) Crude feed (blue trace), peptide purified on WorkBeads 40S (green trace). (B) final product after RPC polishing.

Conclusions

The results demonstrate the significant enhancement of purity using an agarose-based orthogonal ion-exchange step before the final polishing by RPC. Further investigations will promote understanding the gain in productivity for the RPC step by being able to do larger loading of feed, reduced fouling and increased column lifetime.

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