The use of an agarose-based ion-exchange resin in the purification of a 45-amino acid residue peptide

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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™ 40 S, an agarose-based cation-exchange chromatographic resin. This resin has optimal flow-pressure properties and is stable towards efficient cleaning using strong alkaline conditions. This make it an excellent tool for the capture and purity enhancement of therapeutic peptides from crude feeds following solid-phase synthesis or after recombinant expression. The capture step reduces the irreversable contamination of the downstream high-performance silica-based reversed phase chromatography (RPC) column.

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

Results

The dynamic binding capacity 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 40 S at 150 cm/h in column with 57-mm bed height.

Column: WorkBeads 40 S (10 × 57 mm, 4.5 ml)
Flow: 150 cm/h (1.96 ml/min)
Feed: 10 g pure peptide/l
Mobile phases for binding and step desorption contained 15% acetonitrile in a proprietary buffers composition.
Elution: Step desorption (see arrow in insert)

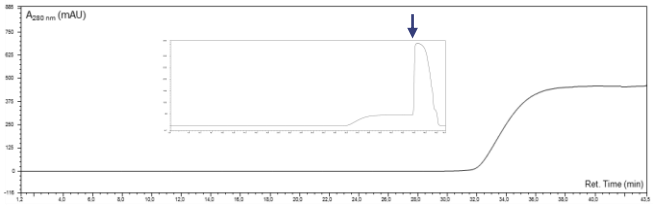

Figure 1. Frontal analysis determination of binding capacity

The dynamic binding capacity was 151 mg/ml packed bed at 75 cm/h, and decreased to 140 mg/ml at 300 cm/h (see Figure 2). The binding capacity for WorkBeads 40 S and Capto SP ImpRes was determined to be 150 mg/ml and 125 mg/ml, respectively, at 2.0-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 fast the desorption kinetics.

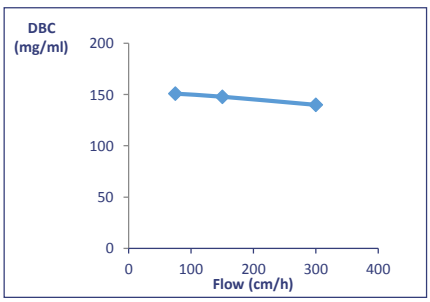

Figure 2. DBC *vs* linear flow determined for WorkBeads 40 S in the column used in Fig. 1.

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

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

A 55-% pure feed was purified using proprietary mobile phases with acetonitrile on columns with WorkBeads 40 S and Capto SP ImpRes to establish which of the resins gives the most optimal combination of purity and yield (Figure 3).

Columns: WorkBeads 40 S (10 × 240 mm, 19 ml)
Capto SP ImpRes (10 × 240 mm, 19 ml)
Flow: 150 cm/h (1.96 ml/min)
Load: Crude feed with 30 g target peptide/l resin
Mobile phases for binding and a combined pH- and salt-gradient desorption contained 15% acetonitrile in a proprietary buffers composition.

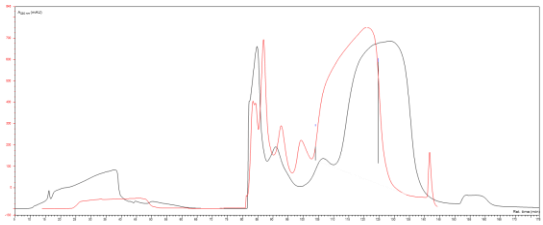

Figure 3. Purification of the 45-amino acid residue peptide from 55% crude feed. UV traces are shown for WorkBeads 40 S (black line) and Capto SP ImpRes (red line).

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 40 S and 85.2 % for Capto SP ImpRes. The purity increased to 93.0 % on WorkBeads 40 S when the loading was decreased to 10 g/l resin.

Table 2: Purity obtained with a load of 30 g/l of a crude feed containing 55% target peptide.

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

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

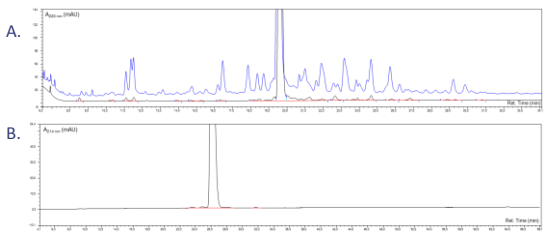

Figure 4. Purity analysis by RPC. A) Crude feed (blue trace) and peptide purified on WorkBeads 40 S (black trace), and B) final product after RPC polishing.

Conclusions and comments

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