Increased lifetime of RPC resins in insulin production by clean-up using WorkBeads 40S

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Introduction

Purification of recombinant insulin requires very high purity, often achieved by high-resolution reversed phase chromatography (RPC) using silica-based resins. Impurities from the feed often cause fouling that is difficult to remove since cleaning-in-place using sodium hydroxide is limited for silica resins. These issues result in shortened lifetime of the RPC columns. By introducing a capture step with WorkBeads™ 40S (cation exchanger), a standard purification process of recombinant human insulin precursor involving two RPC steps was improved. WorkBeads 40S is an agarose-based resin with sulfonate ligands, that is stable in high concentration of sodium hydroxide thus allowing efficient cleaning-in-place. The use of WorkBeads 40S can thus significantly extend the lifetime of the subsequent RPC column.

Results

The feed for the purification process contained 72.5% pure human insulin precursor (Met-Lys-Human insulin), (Fig 1A). Each purification step was optimized and fractions were pooled to give a yield of 90% with highest possible purity.

In the first purification step the extract was applied to a column with WorkBeads 40S. A comparison with Capto™ SP ImpRes (GE Healthcare) as the capture step was performed, Table 1. A slightly higher purity could be obtained by using WorkBeads 40S.

Table 1. Purity comparison between WorkBeads 40S and Capto SP ImpRes.

Eluted pool	Purity (%)
WorkBeads 40S	88.0
Capto SP ImpRes	85.0

The cleaned-up feed minimized impurities to be applied on the second and third purification steps based on RPC. The same RPC column was used in both steps, while the conditions were different.

The optimized conditions and chromatograms are shown in Table 2 and Figure 1. The final purity of the three-step process was 99.7%. Table 3 shows the purity after each step in the process.

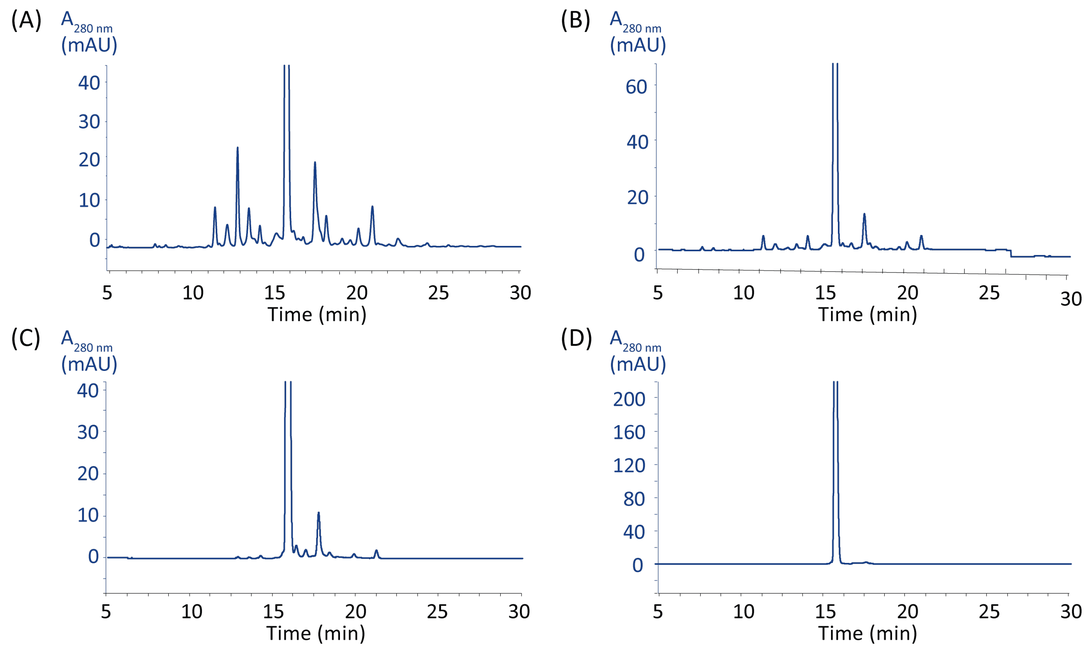

Figure 1. RPC analyses of purified pools from the optimized three-step purification. Feed (A), step 1: eluted pool from WorkBeads 40S (B), step 2: eluted pool from RPC1 (C) and step 3: eluted pool from RPC2, final product (D).

Table 3. Purity obtained during the optimized three-step purification process of recombinant human insulin precursor.

Step	Purity (%)
Feed	72.5
Step 1: WorkBeads 40S	88.0
Step 2: PK-C8-10µm-100Å (condition 1)	97.0
Step 3: PK-C8-10µm-100Å (condition 2)	99.7

Conclusion and comments

The added capture step using WorkBeads 40S reduced the loaded impurities on the first RPC column from 27.5% to 12%. This improvement significantly reduced the fouling of the RPC column and the need for cleaning-in-place, prolonging the lifetime of the RPC resin. The final purity for the process was 99.7%, well above the target of 99%.

WorkBeads 40S gave higher purity than Capto SP ImpRes in the introduced capture step due to that WorkBeads 40S often gives different selectivities compared to other cation exchange resins.

Table 2. Three-step purification process of a recombinant human insulin precursor.

	Step 1: IEX	Step 2: RPC	Step 3: RPC
Resin	WorkBeads 40S (Bio-Works, Sweden)	PK-C8-10µm-100Å (Osaka Soda, Japan)	PK-C8-10µm-100Å (Osaka Soda, Japan)
Bed height	24 cm	25 cm	25 cm
Feed	Crude human insulin precursor	Pooled elution fraction from step 1	Pooled elution fraction from step 2
Loading	20 g product/L resin	15 g product/L resin	15 g product/L resin
Eluent A	50 mM NaAc, pH 4.0 : EtOH (7:3)	100 mM $(NH_4)_2SO_4/H_2SO_4$, pH 3.2	200 mM NH_4Ac/AcOH, pH 5.5
Eluent B	50 mM NaAc, 1 M NaCl, pH 4.0 : EtOH (7:3)	Acetonitrile	Acetonitrile
Gradient	0 - 20% B in 2 column volumes (CV), 20% B in 1 CV, 20 - 70% B in 6.5 CV, 100% B in 20 CV	5 - 21% B in 1 CV, 21 - 27% B in 6 CV, 60% B in 2 CV	5 - 25% B in 1 CV, 25 - 28% B in 6 CV, 60% B in 2 CV
Flow	150 cm/h	180 cm/h	180 cm/h

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