Efficient purification of *Haemophilus influenzae* type b capsular polysaccharide in a one-step chromatography workflow

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This application note demonstrates the purification of *Haemophilus influenzae* type b (Hib) capsular polysaccharide using chromatography. The described process constitutes a viable alternative to the traditional process for purification of Hib capsular polysaccharide to improve manufacturability and facilitate process scaling. Using the suggested process, similar results could be achieved at three different scales.

Introduction

Haemophilus influenza is a Gram-negative rod-shaped bacterium. Although the bacterium can cause illness in people of all ages, ranging from mild ear infections to more severe infections of the bloodstream, the risk of disease is highest in young children between 6 months and 2 years of age.

Haemophilus influenza can occur in unencapsulated and encapsulated form. There are six types of encapsulated strains (a, b, c, d, e, and f), of which type b is the most familiar. Available Hib vaccines are either polysaccharides vaccines or conjugate vaccines, where the capsular polysaccharide is covalently coupled to a carrier antigen.

Hib vaccines are commonly based on the capsular polysaccharide polyribosyl-ribitol phosphate (PRP). Purification of PRP for vaccine production is traditionally performed by phenol extractions and dialysis to remove protein, followed by ethanol precipitation and centrifugation to remove endotoxins. This purification strategy is time-consuming, and the phenol handling constitutes environmental and health risks. In addition, the process is difficult to scale to meet market demands.

In this work, we propose an alternative strategy for purification of PRP (Fig 1). In the described process, phenol extraction is replaced by a simple sample pretreatment followed by a chromatography step to improve manufacturability and scaling abilities. Capto™ adhere resin was used for the chromatography step. Capto adhere is a multimodal anion exchange resin designed to remove key contaminants such as DNA, host cell proteins, larger aggregates, and viruses in a single step. To demonstrate scalability of the suggested purification process, parallel experiments were performed in HiScreen™ (9.3 mL), HiScale™ 16/20 (40 mL), and AxiChrom™ 50/20 (400 mL) columns. The objective for the development of the chromatography step was a recovery of more than 50% in all three scales.

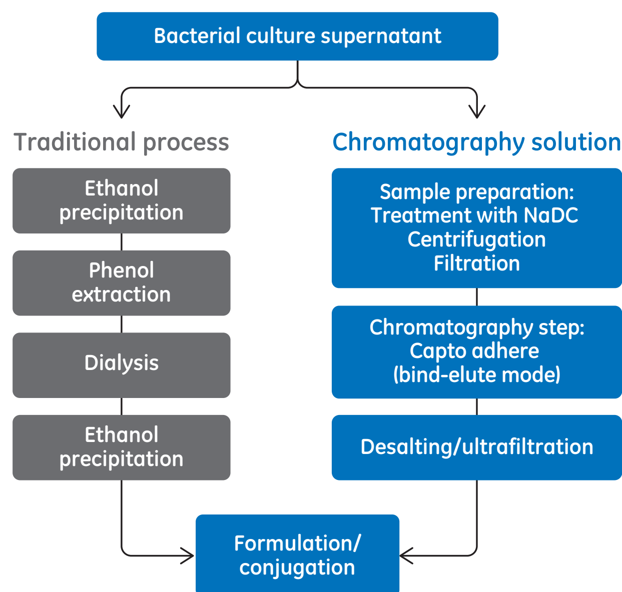

Fig 1. Traditional versus proposed PRP purification process.

Materials and methods

Sample preparation

Crude extract containing PRP was added sodium dicarboxylate cotransporter (NaDC), pH-adjusted to 6.1, and incubated at 4°C for 4 h before centrifuged at 12 000 × g for 30 min and filtered to remove precipitate before loaded onto the chromatography columns.

Purification process

Capto adhere resin was packed in HiScreen (9.3 mL), HiScale 16/20 (40 mL), and AxiChrom 50/20 (400 mL) columns. The columns were equilibrated with 15% buffer B (50 mM phosphate buffer, 1.0 M NaCl, pH 7.6) in buffer A (50 mM phosphate buffer, pH 7.6) before 2 column volumes (CV) of PRP-containing sample was injected onto the column. Bound particles were step-wise eluted with 2 CV of 15% buffer B, 3 CV of 40% buffer B, and 3 CV of 70% buffer B. At 70% running buffer, fractions containing PRP were collected. Pooled PRP-containing fractions were desalted and subjected to ultrafiltration before analyzed for PRP recovery and purity.

Analysis

Quantification of PRP was performed by nucleic magnetic resonance (NMR). Detection of host cell protein (HCP) and DNA (hcDNA) as indictors of impurity was conducted according to methods and standards in the Chinese and European Pharmacopoeias (Table 1). As required by both the Chinese and European Pharmacopoeias, HCP and hcDNA content should be less than 10 mg/g PRP, respectively.

Table 1. Control tests of final polysaccharide preparations

Step	PRP content (µg/mL)	HCP content (µg/mL)	HCP removal (%)	HCP/ PRP (%)	hcDNA content (µg/mL)	hcDNA removal (%)	hcDNA/ PRP (%)	PRP recovery (%)
Crude	2016	1757	0		830	0		100
NaDC treatment	2134	307	82.5		115	86.1		100
Centrifugation/filtration	1030	75	4.3		26.9	10.7		48.2
HiScreen (9.3 mL)	868	3.98	11.9	0.45	2.65	2.9	0.30	31.2* (65.5†)
HiScale 16/20 (40 mL)	532	2.39	12	0.45	1.2	3.0	0.23	32.4* (61.7†)
AxiChrom 50/20 (400 mL)	770	2.74	12	0.36	1.35	3.1	0.17	29.6* (61.5†)

*Total recovery, including pretreatment and chromatography.

†Recovery over the chromatography step.

Columns:	Capto adhere packed in (A) HiScreen (9.3 mL) (B) HiScale 16/20 (40 mL) (C) AxiChrom 50/20 (400 mL)
Sample load:	2 CV
Buffer A:	50 mM phosphate buffer, pH 7.6
Buffer B:	50 mM phosphate buffer + 1.0 M NaCl, pH 7.6
Equilibration:	15% Buffer B in Buffer A
Elution:	15% Buffer B in Buffer A 40% Buffer B in Buffer A 70% Buffer B in Buffer A (target eluent)

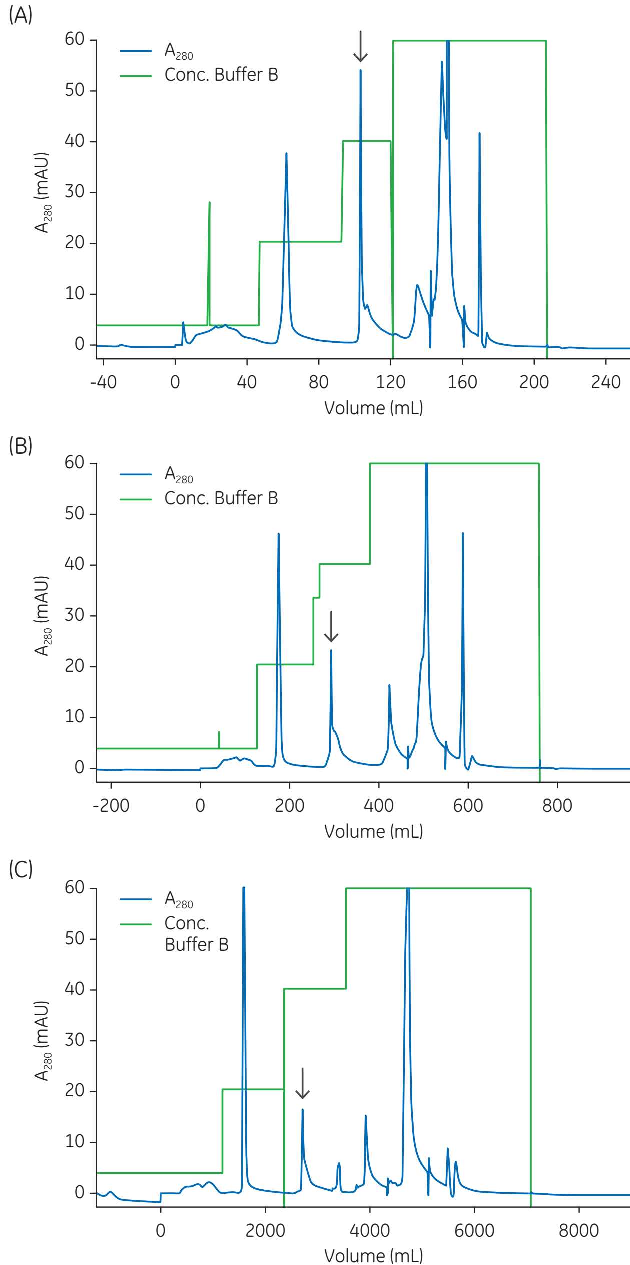

Fig 2. Chromatogram from (A) HiScreen, (B) HiScale, and (C) AxiChrom column experiments. Arrows show target eluent.

Results

The Capto adhere chromatography step offered good separation of PRP from impurities in bind-elute mode. Recovery over the chromatography step was determined to more than 60%. Total process recovery was determined to about 30%. As shown in Figure 2 and Table 1, results were comparable between all three scales. Levels of HCP and hcDNA were found to meet the requirements of both the Chinese and European Pharmacopoeias.

Conclusion

Here, we describe the purification of Hib PRP in an easily scalable process based on modern Capto adhere multimodal chromatography resin. Using the described process, similar results, meeting the quality requirements of the Chinese and European Pharmacopoeias for HCP and hcDNA content, were achieved in three scales ranging from 9.3 to 400 mL. Eliminating the need for multi-step phenol extractions, the suggested process allowed PRP to be successfully purified with high recovery in a way that benefits the environment and operator health.

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