PALL Life Sciences

High Throughput Sorbent Screening Using AcroPrep™ ScreenExpert Plates and PRC Prepacked Columns for the Purification of a Fab Antibody Fragment

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INTRODUCTION

This study describes the development of a three-step purification process for a Fab expressed in *Pichia pastoris*.

High-throughput chromatography sorbent screening was carried out in AcroPrep 96-well filter plates using an automated platform combined with fast analytics (Figure 1). Transfer and optimization of a three-step process was conducted onto 1mL PRC prepacked columns (Figure 2).

Figure 1
Screening Strategy Principle

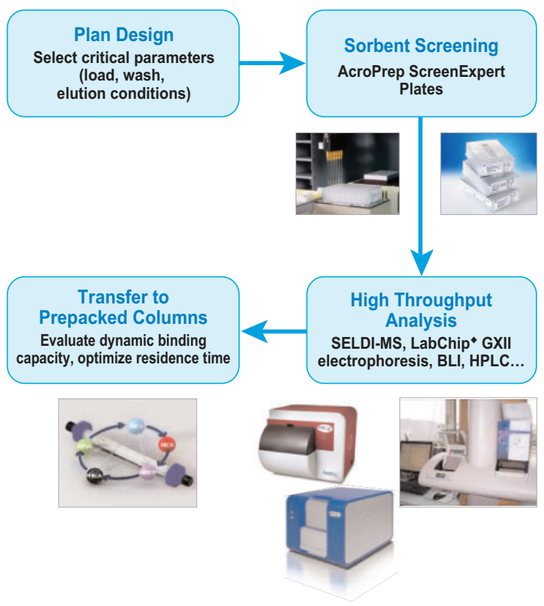
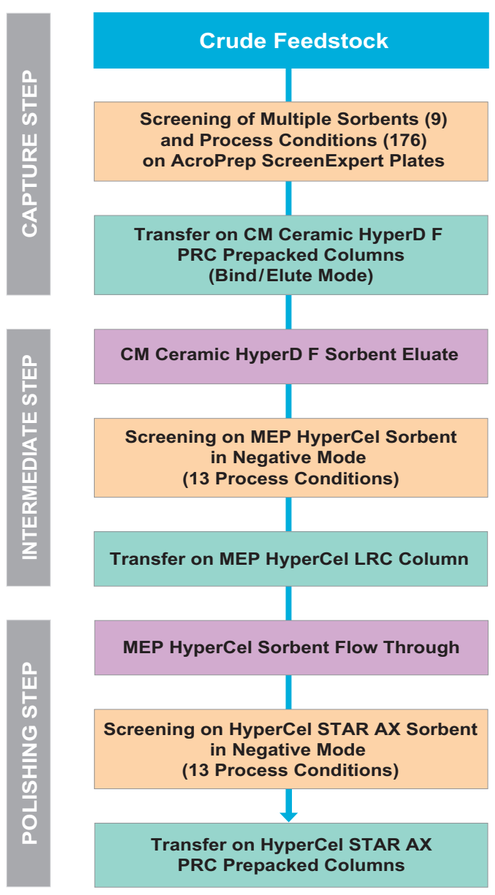


Figure 2
Process Development Approach Using AcroPrep ScreenExpert 96-Well Plates and PRC prepacked Columns

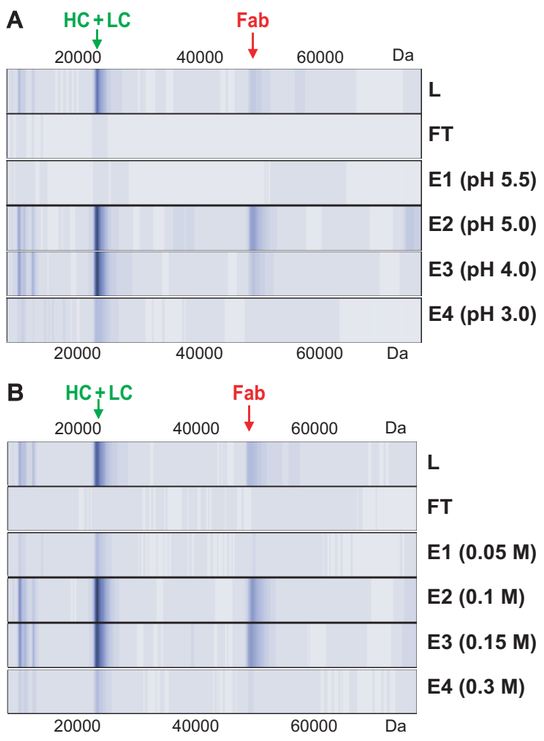


CAPTURE STEP

Screening in 96-Well Plate

176 conditions were tested on 9 different chromatography sorbents on a Freedom EVO◆ 150 robotic platform (TECAN). The screening on mixed-mode HyperCel™ and anion exchanger HyperCel STAR AX sorbents was carried out in AcroPrep ScreenExpert plates and on other sorbent types (hydroxyapatite and ion exchangers), in empty 1 mL plates filled manually with 50 µL of chromatography sorbent per well.

Figure 3
*SELDI-MS Profiles of the Fractions After Screening on **(A)** MEP HyperCel Sorbent or **(B)** CM Ceramic HyperD® F Sorbent in 96-Well Plate*



*Equilibration in **(A)** 20 mM Na phosphate, pH 6.0 or **(B)** 50 mM Na acetate, pH 5.0; **L**: Direct load; **FT**: Flow through; **E1** to **E4**: Successive elution steps*

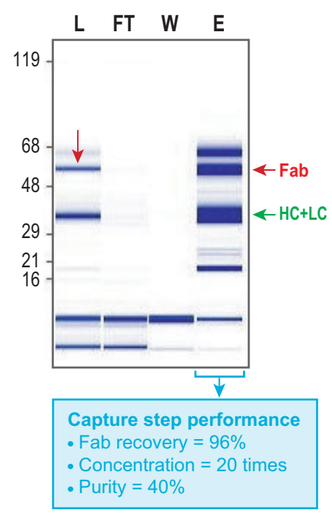
The proposed purification strategies resulting from the screening (Figure 3) were:

- Either direct loading of the feedstock on MEP HyperCel sorbent at pH 6.0 and recovery of the Fab at pH 5.0
- Or loading of the feedstock on CM Ceramic HyperD® F sorbent after adjustment at pH 5.0 and recovery of the Fab at pH 5.0 + 0.15 M NaCl.

Transfer of the Fab Capture Step on PRC Prepacked Columns

Based on the capacity and recovery yield at elution, cation exchange chromatography on CM Ceramic HyperD F sorbent was selected over mixed-mode chromatography as the capture strategy of the Fab (Figure 4).

Figure 4
Capture Step on CM Ceramic HyperD F PRC Prepacked Column at pH 5.0: LabChip GXII Analysis of Fractions

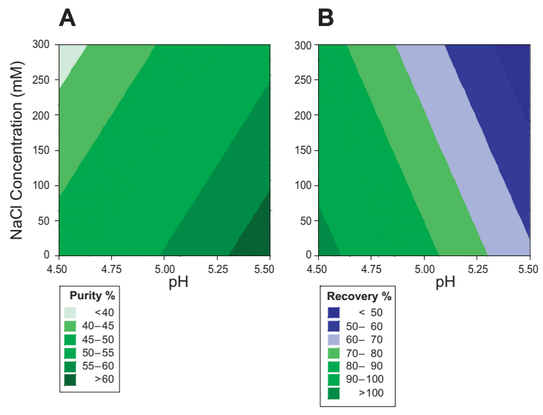


***L**: Crude feedstock (200 mL) adjusted at pH 5.0; **FT**: Flow through; **W**: Wash in Na acetate, pH 5.0; **E**: Elution in Na acetate, pH 5.0 + 0.3 M NaCl. Residence time: 3 min. **HC**: Heavy chain; **LC**: Light chain*

INTERMEDIATE PURIFICATION STEP

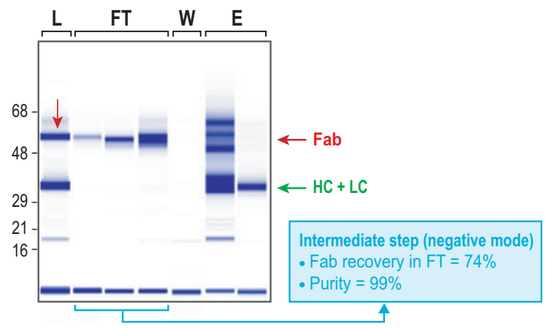
MEP HyperCel sorbent was considered in a negative mode for the intermediate purification step (to remove the main contaminants: free light and heavy chains). A full factorial design of experiments (DoE) revealed that optimal Fab recovery and purity were observed between pH 5.0 and 5.2 (Figure 5).

Figure 5
*Fab Purity **(A)** and Recovery **(B)** in the Flow Through Fraction After MEP HyperCel Sorbent Negative Mode Batch Chromatography*



Transfer of the optimal conditions from the 96-well plate to PRC prepacked columns at pH 5.0, 5.1 and 5.2 showed that optimal purity (99% based on LabChip◆ GXII analysis) and recovery (74%) of the Fab in the column flow through was reached at pH 5.2 (Figure 6).

Figure 6
Intermediate Step: Transfer of Conditions on MEP HyperCel PRC Prepacked Column

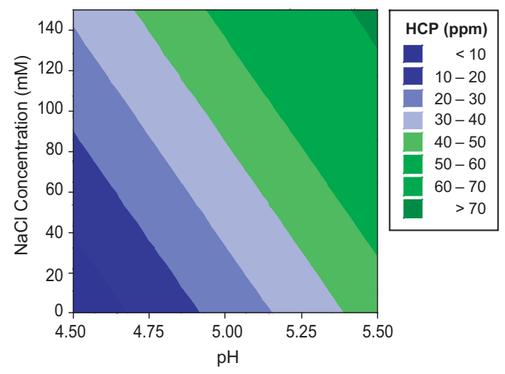


***L**: Load at pH 5.2; **FT**: Flow through; **W**: Wash; **E**: Elution at pH 3.0. Residence time: 5 min.*

POLISHING STEP

A polishing step to decrease further HCP content in the recovered Fab fraction was screened in an AcroPrep ScreenExpert plate filled with HyperCel STAR AX sorbent. ELISA anti-HCP assay of flow through fractions evidenced that the Fab was recovered in the column flow through and that the HCP content was below 30 ppm when the binding was comprised between pH 4.5 and 5.0 and 0 to 140 mM NaCl (Figure 7).

Figure 7
HCP Removal on HyperCel STAR AX Sorbent



HCP content in the flow through fraction after batch chromatography on HyperCel STAR AX sorbent in negative mode as a function of the binding pH and salt concentration

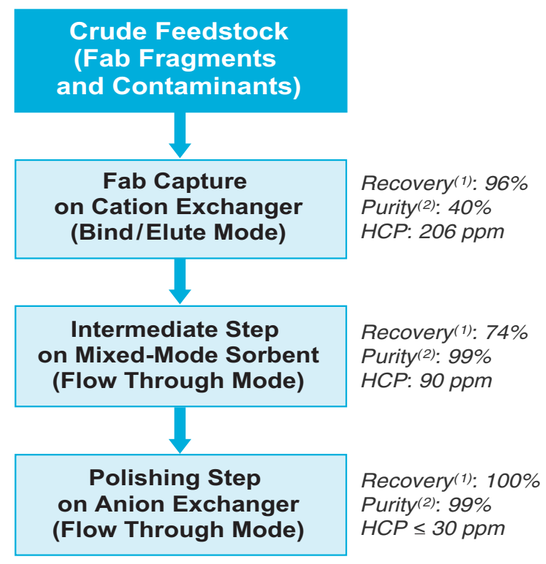
At the end of the three-step process, the Fab purity was estimated around 99% with not more than 30 ppm HCP. The overall process recovery was around 70%.

CONCLUSION

This example shows the benefits of the combination of high throughput screening using AcroPrep ScreenExpert 96 well filter plates and transfer of the process steps in prepacked columns for the fast development of a three step purification strategy of a Fab fragment:

- **80 chromatography conditions were screened and analyzed per day, significantly reducing process development duration.**
- **Conditions optimized in plates were checked and confirmed by running chromatography columns.**
- **Cation exchange capture on CM Ceramic HyperD F sorbent acted as a first concentration (about 20-fold) step in the process, with medium purity (40%).**
- **Used in flow through mode, mixed-mode chromatography on MEP HyperCel sorbent was a powerfull intermediate step to remove various contaminants, especially free light and heavy chains (Fab purity 99%).**
- **Anion exchange chromatography on HyperCel STAR AX final polishing step allows the decrease of the level of contaminants (HCP ≤ 30 ppm).**

Figure 8
Overall Purification Scheme



(1) Recovery evaluated using a Bradford assay
(2) Purity evaluated using the LabChip GXII system

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