Comparison of three different screening techniques for predicting step elution conditions for a cation exchanger

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Introduction

Screening of chromatographic conditions can be done in several ways, such as 96-well plates, mini-columns and tips. It is important that the screening can give an indication of the optimal conditions for binding and/or elution in a chromatographic column run.

The aim of this study was to investigate conditions for reduction of host cell proteins (HCP) on a strong cation exchanger, Capto™ S ImpAct. Three different screening techniques using PreDictor™ plates are compared and discussed. Selected conditions were applied in column step elution experiments and antibody yield, pool volume and reduction of HCP was compared.

Material and methods

Binding study (SBC)

Equilibration: 3 × 200 µL binding buffer, 1 min incubation for each addition
Sample load: 200 µL approx. 3 g/L sample, 60 min incubation
PreDictor plate: PreDictor Capto S ImpAct, 2 µL
Phase ratio: 200/2 = 100

Flow through was collected and concentration of antibody and HCP analyzed and SBC was calculated according to Eq 1 and 2.

$$SBC\ (static\ binding\ capacity) = \frac{V_{liq}}{V_{resin}}(C_0 - C_{eq}) - \frac{V_{holdup}}{V_{resin}} C_{eq} \qquad \textbf{Eq 1}$$

$$V_{holdup} = 0.6\ V_{resin} + 6\ \mu L \qquad \textbf{Eq 2}$$

Elution study

Equilibration: 3 × 200 µL binding buffer, 1 min incubation for each addition
Sample load: 60 g sample/L resin (50 mM sodium acetate, pH 5.5, 50 mM NaCl, 60% of SBC), 60 min incubation
Wash: 3 × 200 µL binding buffer, 1 min incubation for each addition
Elution: 200 µL elution buffer, 1 min incubation
PreDictor plate: PreDictor Capto S ImpAct, 20 µL
Phase ratio: 200/20 = 10

Only one elution was performed. Antibody yield was calculated.

K_p screen

Equilibration: 3 × 200 µL binding buffer, 1 min incubation for each addition
Sample load (K_p HCP): 40 g/L resin, 60 min incubation
Sample load (K_p Antibody): 5 g/L resin, 60 min incubation
PreDictor plate: PreDictor Capto S ImpAct, 20 µL
Phase ratio: 200/20 = 10

To be able to look at K_p-values in the whole design space, K_p-values were predicted as described by McDonald *et al.* (*J. Chromatogr. A* 1433 (2016) 66–74).

$$K_p\ (partitioning\ coefficient) = \frac{C_{bound}}{C_{eq}} \qquad \textbf{Eq 3}$$

$$\alpha\ (separation\ factor) = \frac{K_p^{Impurity}}{K_p^{Antibody}} \qquad \textbf{Eq 4}$$

Column runs

Column: 2-mL Tricorn™ 5/100
Resin: Capto S ImpAct
Equilibration: 5 CVs 50 mM sodium acetate, pH 5.5
Load: 70 g sample/L resin (50 mM sodium acetate, pH 5.5)
Wash: 5 CVs 50 mM sodium acetate, pH 5.5
Step elution: 1) pH 5.5, 135 mM NaCl, or 2) pH 5.5, 180 mM NaCl

Sample: *Protein A purified mAb*

Separation of phases: *Centrifugation, 500 g, 1 min*

Incubation: *With agitation (1100 rpm) for specified incubation time*

Conditions studied: *40 conditions were screened. Five different pH values (5–7) at 8 different NaCl concentrations (0–350 mM)*

Results and discussion

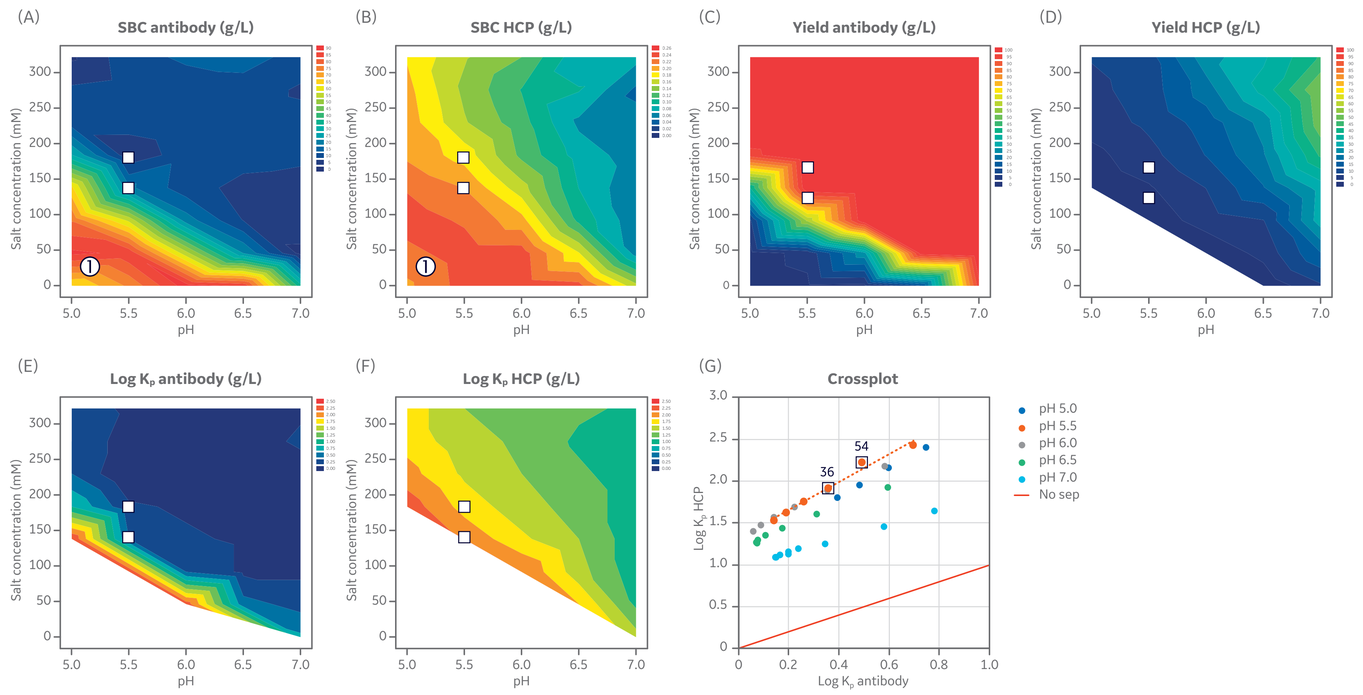

Fig 1. SBC, yield, and Log K_p for antibody and HCP, respectively. Crossplot, Log K_p HCP vs Log K_p Antibody, illustrating separation of antibody and HCP in terms log K_p-values. ① denotes areas where non-traditional binding behaviour is observed. (☐) Indicates conditions applied in column experiments (Fig 2). Inserted values in the crossplot are the separation factors calculated for the two conditions used in the column experiments.

SBC plots

- Gives the maximum capacity at given conditions. For this antibody and the studied conditions, the maximum capacity is about 90 g/L resin. HCP binding was less affected than antibody binding by the conditions.
- Both the antibody and the HCP show non-traditional behaviour due to steric hindrance at low pH and salt concentration (① in Fig 1). A little salt or increase in pH is needed to reach the resins' potential maximum capacity.

Elution plots

- The yield plots show the same trends as the SBC plots, except reversed. Good elution conditions at higher pH and higher salt concentration.

K_p screen

- The separation of antibody and HCP is studied by looking at log K_p values for the two species. High K_p values correlate with good binding conditions or poor elution conditions, while low K_p values correlated with lower binding and good elution conditions. Log K_p values above 2.5 were not evaluated as obtained values are unreliable due to measurement uncertainties.
- Crossplot explains the separation of the two components in terms of their log K_p-values. For all of the studied conditions good separation was obtained as expected.

Column experiments

- Step elution was performed at two conditions (pH 5.5, 135 mM NaCl and pH 5.5, 180 mM NaCl) which should give good reduction of HCP and good yield. The chosen conditions were based on multiple criteria, of which only HCP reduction is presented here. As expected, good yield and HCP reduction was obtained at both conditions. The pool volumes differed considerably (Table 1, Fig 2). As indicated by the plate experiments, elution with 135 mM NaCl gave larger elution pool volume than elution with 180 mM NaCl. This was expected as 135 mM NaCl was in the transition zone between binding and non-binding conditions.

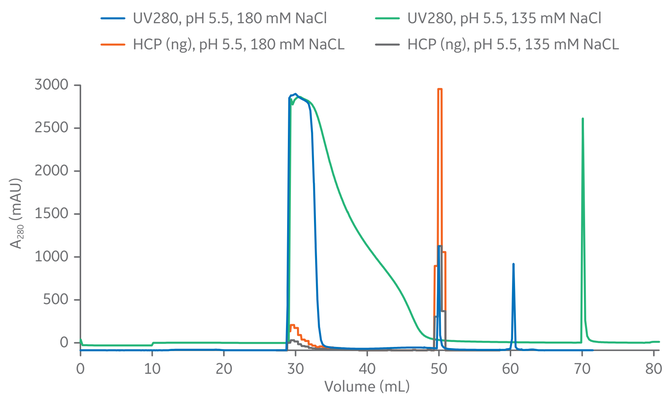

Fig 2. Comparison of step elution profiles of antibody and HCP at pH 5.5, 135 mM NaCl and pH 5.5, 180 mM NaCl.

Table 1. Antibody yield, elution pool volume, and HCP reduction

Step elution condition	Antibody yield (%)	Elution pool volume (CV´s)	HCP reduction factor
pH 5.5, 135 mM NaCl	90	9.3	~5
pH 5.5, 180 mM NaCl	96	2.4	~5

Conclusions

Screening of conditions in plates gives a good picture of the performance that can be expected in terms of capacity and selectivity. Different approaches are good complements to each other.

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