High-throughput screening and optimization of a Protein A capture step in a monoclonal antibody purification process

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Key words: *PreDictor™, MabSelect SuRe™, process development, high-throughput screening, HiScreen™, monoclonal antibody, column optimization, Protein A, host cell protein*

Abstract

We have developed and optimized a capture step for the purification of a monoclonal antibody (MAb) on MabSelect SuRe, which is a Protein A-based affinity chromatography medium. The use of PreDictor prefilled 96-well filter plates allowed us to investigate a large experimental space in order to find the best conditions for the capture step. Once these conditions were identified, fine tuning and verifications were carried out with HiScreen prepacked columns on an ÄKTA™ design system. Finally, a scale-up protocol was developed and tested under robust production conditions. The procedure described in this application note represents an efficient and robust solution for high-throughput process development.

Introduction

The increasing demand for monoclonal antibodies as biopharmaceuticals has promoted the development of efficient processes such as higher titers and two-step purification platforms. However, the use of higher titers often introduces another obstacle into the purification process because the feed may contain an increased number of impurities that would have to be separated from the target. Meanwhile, the increasing demand for project throughput in development laboratories has led to constraints on research efforts into optimized and robust protocols. Introduction of high-throughput methods into the process development workflow have led to significant efficiency gains such as a reduction in both the time and amount of sample required for the development of different chromatographic steps.

PreDictor 96-well filter plates—prefilled with BioProcess™ chromatographic media from GE Healthcare—are suitable for efficient high-throughput screening of chromatographic conditions during process development. Defined conditions can then be verified and optimized with HiScreen prepacked columns. In the first of a series of four application notes, we describe the development and optimization of a capture step for the purification of a monoclonal antibody on MabSelect SuRe. The other application notes are:

- High-throughput screening and optimization of a multimodal polishing step in a monoclonal antibody purification process (28-9509-60)
- Scale-up of a downstream monoclonal antibody purification process using HiScreen and AxiChrom™ column formats (28-9403-49)
- A flexible antibody purification process based on ReadyToProcess™ products (28-9403-48)

High-throughput screening of the binding and elution conditions was performed using PreDictor plates to identify the most promising conditions. Based on the results from the screening experiments, the binding, wash, and elution conditions of the capture step were optimized using a Design of Experiments (DoE) approach on HiScreen columns. A workflow for process development is presented in Figure 1.

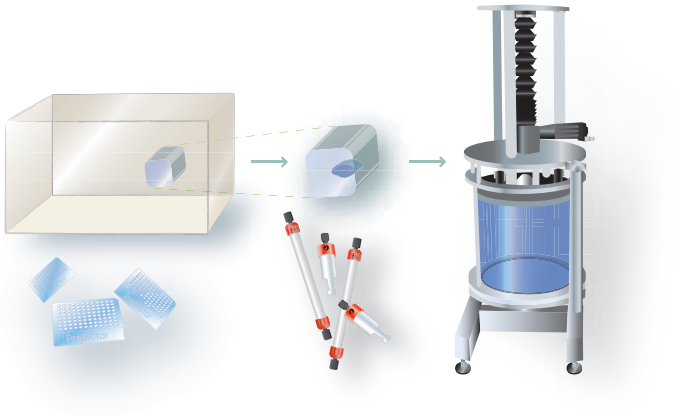


Fig 1. Conceptual visualization of a workflow for process development. Parallel screening using PreDictor plates makes it possible to explore a large experimental space (left). Once optimal conditions have been identified, fine tuning and verification are carried out on prepacked HiScreen or HiTrap columns using ÄKTA design systems (middle). The design space, shown in blue (middle), is identified and scaled up to a robust production scale process (right).

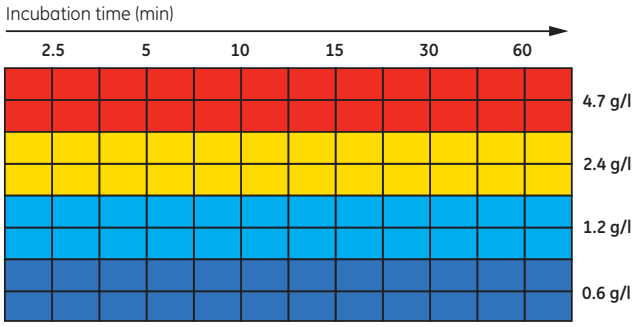


Fig 2. Plate layout for the uptake experiment. The sample (200 µl/well) was added to the PreDictor plate at 6 different incubation times (2.5, 5, 10, 15, 30, and 60 min) beginning with the longest duration.

Materials and methods

Screening with PreDictor plates

All the experiments with PreDictor plates were performed with fully automated protocols on a Tecan™ Freedom EVO-2 200 Robotic System, but they can also be carried out manually. Liquid removal was performed by vacuum or centrifugation throughout the study. The sample used in all the experiments was clarified cell culture supernatant (CCS) containing 1.1 mg/ml MAb. For convenience, prefilled buffer plates were used whenever possible. General recommendations for working with PreDictor plates are described in detail in the instruction manual (1).

Uptake experiments

Uptake experiments were performed to screen for binding conditions for the capture step (2) and the results were used for:

- Predicting the dynamic binding capacity (DBC) at 10% breakthrough as a function of residence time in a chromatographic column (2, 3). This was later verified on a 1 ml HiTrap™ MabSelect SuRe column
- Determining the incubation time for the subsequent elution study based on the rate of protein uptake
- Generating an adsorption isotherm that was used to determine the phase ratio for the elution study

The experiments were carried out in quadruplicate using PreDictor MabSelect SuRe, 6 µl plates. The protocol was based on three equilibration steps with 200 µl/well of 50 mM Na_2HPO_4 containing 0.15 M NaCl, pH 7.4 in each step. The CCS was concentrated to a final MAb concentration of 4.7 mg/ml. The concentrated CCS was diluted in the equilibration buffer to 4 different MAb concentrations (4.7, 2.4, 1.2, and 0.6 mg/ml). The different concentrations were distributed vertically according to the plate layout (Fig 2).

The wash step was performed with 3 × 200 µl of equilibration buffer and elution was performed with 3 × 200 µl of 50 mM sodium citrate, pH 3.5. The 3 elution fractions were pooled and 200 µl of the pool was transferred to a UV-readable 96-well plate. The UV absorbance of the elution pool as well as that of the elution buffer (blank) was measured at 280 nm.

Analysis of uptake experiments

The concentration of MAb in the elution pool, $C_{elution}$, was determined spectrophotometrically using Lambert-Beer's law. The static capacity, Q, of MAb bound to MabSelect SuRe was calculated using equation 1.

$$Q = \frac{C_{elution} \times V_{elution}}{V_{medium}} \qquad \textit{Equation 1}$$

where: $V_{elution}$ is the elution volume (ml) and V_{medium} is the volume of chromatography medium in the well (ml). Uptake curves were then obtained by plotting the static capacities against the incubation time for each MAb concentration.

Adsorption isotherm

The results from the uptake curves were used to generate an adsorption isotherm. The binding capacities, after 60 min of incubation, were plotted against the concentration in the liquid phase for the 4 different MAb concentrations using Equation 2. The graph was used to determine the phase ratio for the elution study (see "Results").

$$Q = (C_O - C)\frac{V_{liq}}{V_{medium}} \qquad \textit{Equation 2}$$

where: C_0 is the initial MAb concentration (mg/ml), V_{liq} is the volume of liquid added to the well (ml) and C is the concentration of MAb in the liquid phase after incubation (mg/ml).

Elution study

The elution conditions that were investigated are summarized in Table 1. The additives did not have any significant effect on either the yield or purity.

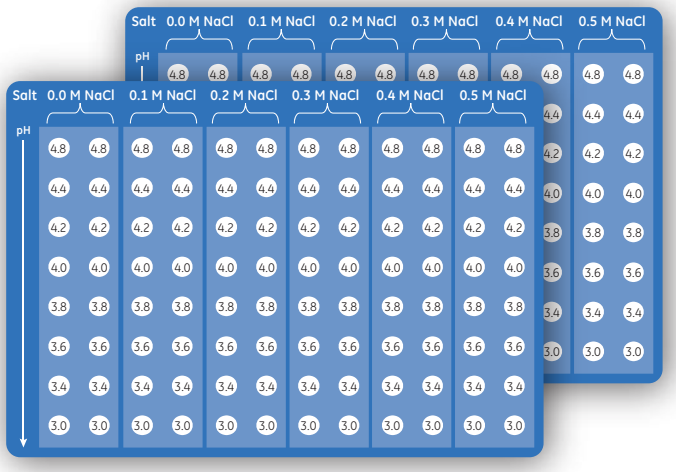
Table 1. Factors investigated in the elution study with PreDictor plates

Factor (A)	Range	Plate
Sodium citrate	20 and 100 mM	1 and 2
pH	3.0 to 4.8	1 and 2
NaCl concentration	0 to 0.5 M	1 and 2
Factor (B)		
Additives	Arginine (0*, 1 and 2 M)	3 and 4
Additives	Glycine (0*, 0.1 and 0.2 M)	3 and 4
Additives	Urea (0*, 1 and 2 M)	3 and 4
Additives	Sucrose (0*, 0.1 and 0.2 M)	3 and 4

* In plate 1 (20 mM sodium citrate) all additives were 0 mM.

Each condition was investigated in duplicate and plate layouts for the elution study are shown in Figure 3.

A) Schematic diagram of plate 1 containing 20 mM sodium citrate and plate 2 containing 100 mM sodium citrate.



B) Depiction of plates 3 and 4 containing 20 mM sodium citrate.

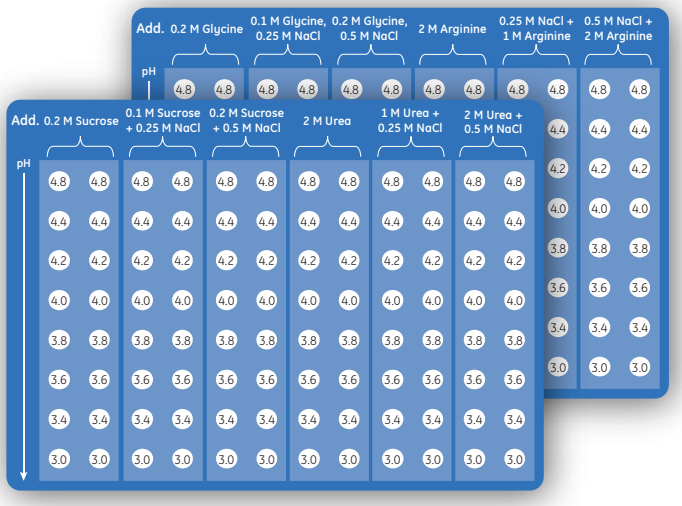


Fig 3. Plate layouts for the elution study. (A) Effect of NaCl; plate 1 with 20 mM sodium citrate; plate 2 with 100 mM sodium citrate; (B) Effect of additives.

The elution study was performed using PreDictor MabSelect SuRe, 20 µl. The chromatography medium was equilibrated with 3 × 200 µl of equilibration buffer (20 mM sodium phosphate, 0.15 M NaCl, pH 7.4). Uptake results were used to determine the phase ratio (see "Selection of a phase ratio for the elution study"). The sample (200 µl of CCS containing 1.1 mg/ml MAb) was added to all the wells and incubated for 30 min. After removing the sample, another 200 µl of CCS was added to the plate and incubated for another 60 min to achieve the desired loading challenge. The wash step was performed with 3 × 200 µl of equilibration buffer. The elution step was performed with 3 × 200 µl of elution buffer. A 0.5 M solution of Na_2HPO_4 was added to neutralize the eluted material to about pH 7 to prevent the aggregation of MAb that occurs under acidic conditions. The eluted fractions were pooled for subsequent analysis in UV-readable 96-well plates and the absorbance at 280 nm was measured spectrophotometrically.

Analytical approach to column optimization

The focus of the screening phase was to improve yield and remove aggregates. The concentration of MAb in the elution pool was measured spectrophotometrically (SpectraMax™ Plus 384, Molecular Devices) and the yields were determined. The monomer and aggregate content of the elution pools were analyzed via size exclusion chromatography (SEC) with an analysis time of 15 min per sample. Two 3 ml Superdex™ 200 5/150GL columns were coupled in series for the screening study. The monomer and aggregate peak areas were evaluated automatically for all the 96 wells at 215 and 280 nm using a batch run procedure within the UNICORN™ software v5.01.

Optimization in HiScreen columns

Based on the results from the high-throughput screening experiments in the PreDictor plates, the capture step was optimized on a HiScreen MabSelect SuRe column with a bed height of 10 cm and a column volume of 4.7 ml. A DoE using a Central Composite Circumscribed (CCC) design was set up with MODDE™ software v8 (Umetrics). The factors investigated are summarized in Table 2.

Table 2. Factors investigated during the optimization phase with HiScreen columns. Equilibration was performed with 20 mM Na_2HPO_4, pH 7.2. The sample (CCS at 1.1 mg/ml MAb) was loaded with a residence time of 4 min (150 cm/h). A wash step with 20 mM Na_2HPO_4, pH 7.2 containing NaCl at 245 cm/h was followed by a second wash with equilibration buffer at the same flow velocity. Elution was performed with 20 mM sodium citrate

Step	Factor	Range
Loading	Load	15 to 37 mg/ml (34 to 86% of DBC)
Wash	NaCl	60 to 840 mM
Elution	pH	3.4 to 4.0

A UV watch function was used to collect the elution pool. The elution pool was immediately neutralized to approximately pH 7 with 0.5 M Na_2HPO_4 solution.

Analytical approach to column optimization

The column optimization phase typically involves fewer experiments compared to high-throughput screening; where simplicity and speed of analysis, are critical to success due to the large number of samples involved. This allows you to collect more empirical data for each column experiment. The main focus of the column optimization phase was to improve yield and reduce the levels of impurity. The elution pools from the chromatographic runs were analyzed for yield via spectrophotometry, aggregate content via SEC, host cell protein (HCP) content via ELISA (on an automated Gyrolab™, Gyros), and ligand leakage from MabSelect SuRe via ELISA (Protein A from Repligen Corporation).

Results and discussion

Uptake experiments and DBC prediction

The data from the uptake curves (Fig 4) for MabSelect SuRe showed that the rate of protein uptake was much slower after 45 min, indicating that MabSelect SuRe had become saturated with MAb after 1 h. The isotherm obtained then becomes an approximiation of the real equilibrium.

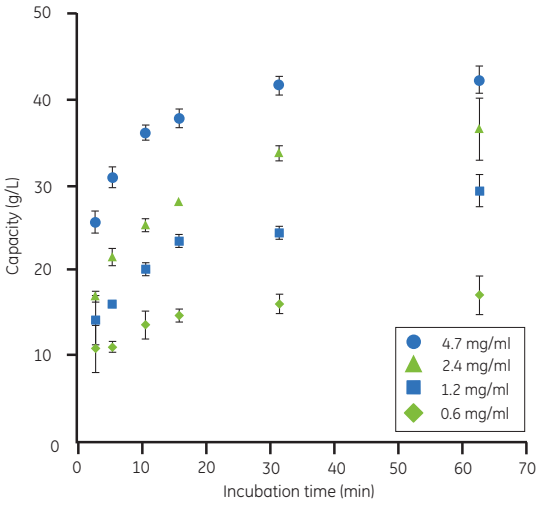


Fig 4. Uptake curves using Predictor MabSelect SuRe plates (6 µl) at four different antibody concentrations.

A mathematical model describing the adsorption process within the wells of the PreDictor plate was fitted to the data presented in Figure 4. The estimated model paramaters were subsequently used to model the same absorption process in a chromatography column in order to predict the dynamic binding capacites at different residence times (3). The predicted DBC values of MAb on MabSelect SuRe at 10% breakthrough (based on the results from PreDictor plates) were compared with DBC values obtained from experiments on a 1 ml HiTrap MabSelect SuRe prepacked column (Fig 5).

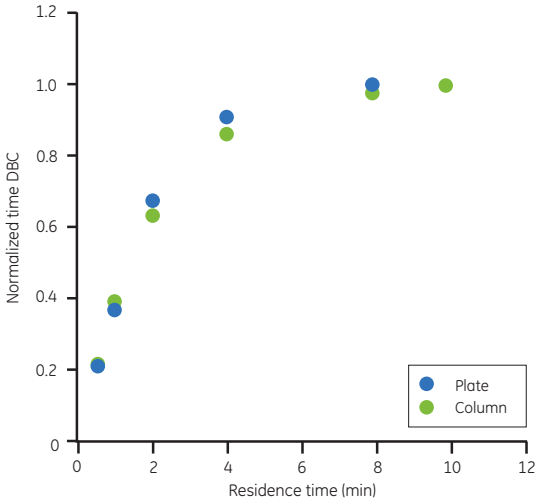


Fig 5. Dynamic binding capacities of MAb on MabSelect SuRe at various residence times (0.5, 1, 2, 4, 8, and 10 min residence times) determined with both PreDictor plates and 1 ml HiTrap columns.

The trend generated with PreDictor plates showed good correlation with that generated with chromatographic columns, thus making the plates an excellent tool for the initial screening of process conditions. The DBC began to level off at 4 min, therefore, the residence time for the subsequent optimization studies with HiScreen columns was set to 4 min in order to utilize the column capacity.

Selection of a phase ratio for the elution study

For the elution study, a particular sample load was chosen to attain a binding capacity of 80% of the DBC at 10% breakthrough. In this study, that sample load was 25 mg MAb/ml of medium. The isotherm (Fig 6) was used to determine the load needed, that is the phase ratio (V_{liq}/V_{medium}) for the sample concentration that represents the concentration of feed in the starting material.

In order to produce a sufficient amount of material for the analysis of impurities, a volume of 20 µl/well for the medium is recommended for elution studies (1). It is also recommended to use a maximum sample volume of 300 µl/well to avoid cross-contamination between wells. A phase ratio of 10 (200 µl of sample and 20 µl medium) was chosen to achieve the target capacity of 25 mg/ml. A phase ratio of 10 at a feed concentration of 1.1 g/l required 2 sample additions of 200 µl each to avoid overfilling the well.

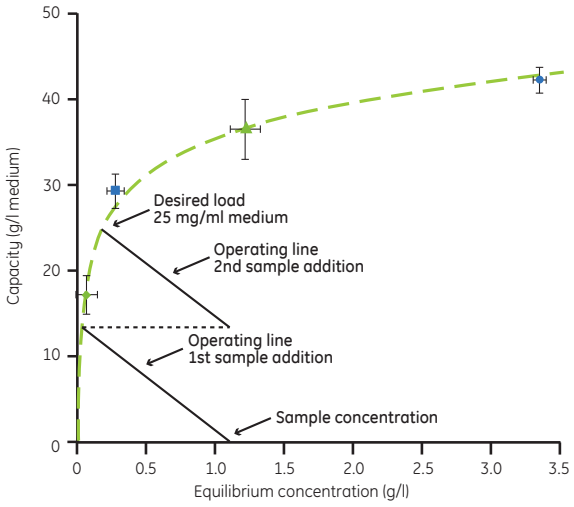


Fig 6. Schematic representation of operating lines for two theoretical sample additions. The operating line is a graphical representation of mass balance (equation 2) and it is used to determine the sample load. The operating line originates at the point representing the initial state of the system and it ends at the point representing the equilibrium state given by the adsorption isotherm. The slope of the operating line represents the phase ratio.

Elution study results

The response surfaces of monomer content in relation to total MAb content and total MAb yield in the capture step at different pH values and NaCl concentrations in the elution buffer are presented in Figure 7.

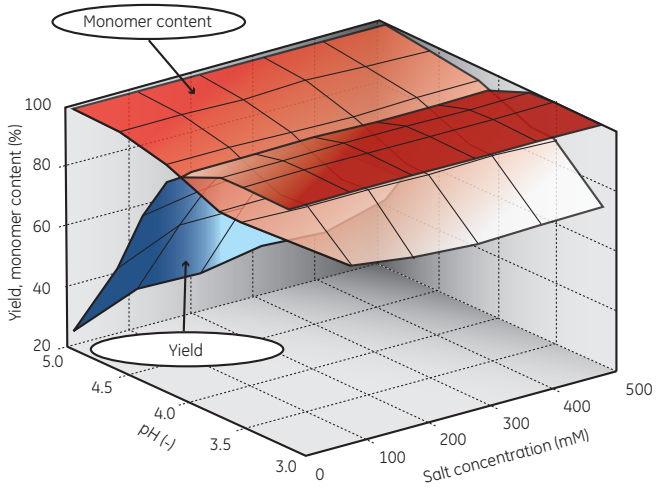


Fig 7. Monomer content and the total MAb yield at different NaCl concentrations and pH levels of the elution buffer obtained from the PreDictor plates.

A decrease in pH increased the total MAb yield but led to a decrease in monomer content. The additives for the elution buffer (see Table 1) were not used in the elution study because their inclusion did not enhance the yield or monomer content.

The response surfaces of monomer yield at the two different sodium citrate concentrations (20 and 100 mM) are shown in Figure 8.

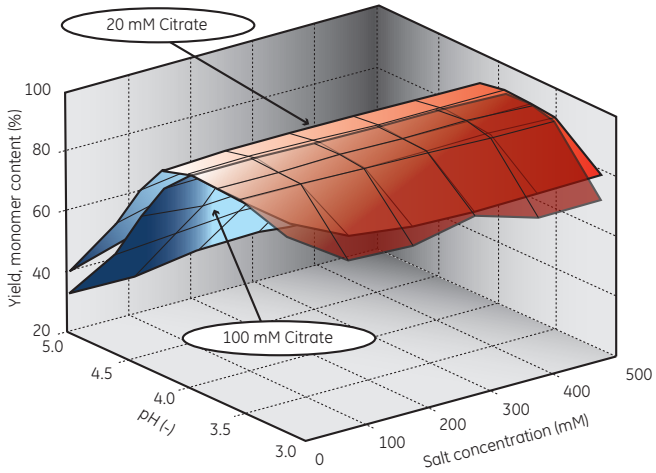


Fig 8. Monomer yield (%) at 20 and 100 mM sodium citrate with different pH levels and salt (NaCl) concentrations obtained using PreDictor plates.

Apart from a decrease in monomer yield at pH 3 that was caused by the aggregation of MAb, the shape of the monomer yield was similar to that shown in Figure 7. The monomer yield was slightly higher for the lower citrate buffer concentration.

The total MAb yield was verified on a 1 ml HiTrap MabSelect SuRe column for a selection of the conditions in the elution study (Fig 9).

There was a good correlation between the PreDictor plate and chromatographic column results.

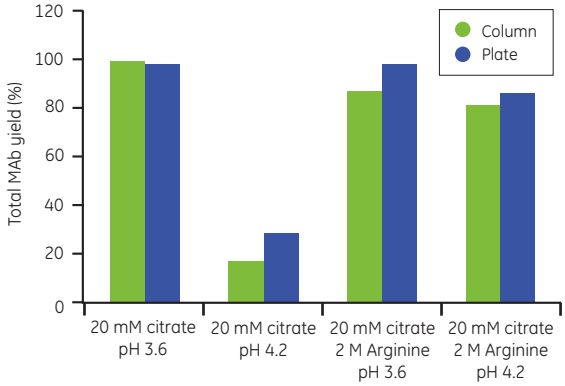


Fig 9. The total MAb yields (%) based on the results from columns (green) and plates (blue) at certain conditions using additives from the elution study (see Table 1).

Reduction of the experimental space for column optimization

The screening experiments in PreDictor plates led to a significant reduction in the number of experiments performed in the optimization stage with the chromatographic columns. For example, there was no need to consider factors such as the residence time for the loading step, concentration of the elution buffer, plus the inclusion or exclusion of NaCl, and the other additives in the elution buffer. In addition, the pH range for the elution step was narrowed.

Optimization with HiScreen columns

We used MODDE software v8 to investigate significant factors that affected yield, aggregates, HCP, and the presence or absence of Protein A during the optimization phase with HiScreen columns. The response surfaces for the factors that had the greatest effect on yield and aggregation are shown in Figure 10.

The goal of optimizing the capture step was to maximize yield. The sample load had a minor impact on both yield and aggregate content. The inclusion of NaCl in the intermediate wash buffer improved HCP removal (data not shown). The levels of leached ligand were both low and evenly distributed over the different conditions in the design space; therefore, we could not obtain a model for ligand leakage.

A sweet spot analysis at three different NaCl concentrations with the following criteria: (i) yield higher than 90%; (ii) HCP levels lower than 70 ppm; and (iii) aggregate content less than 14%, was performed with MODDE software v8. The sweet spot i.e., the spot at which all three criteria were met (represented by the red patch in Fig 11) increased with increasing NaCl concentration in the wash solution. The graphs in Figure 11 suggest that a wash solution containing at least 450 mM NaCl should be used in the capture step.

Conclusions

We have developed a high-throughput process development workflow that is based on the application of PreDictor plates for the screening phase and the use of small-scale columns such as HiScreen for the optimization phase. This new workflow was used to develop a capture step involving binding, wash, and elution conditions for the purification of a MAb. Application of this new workflow produced a capture step with high yields and low levels of host cell proteins and leached Protein A. In addition, there was a reduction in the amount of sample and time required for the process development workflow.

High-throughput screening of a large experimental space during the screening phase led to a significant reduction in the number of experiments we had to conduct in the optimization phase with column chromatography. In addition, the ability to screen a large experimental space eliminates the risk of developing a sub-optimal process in the end. The procedure described here provides an efficient and robust solution for high-throughput process development.

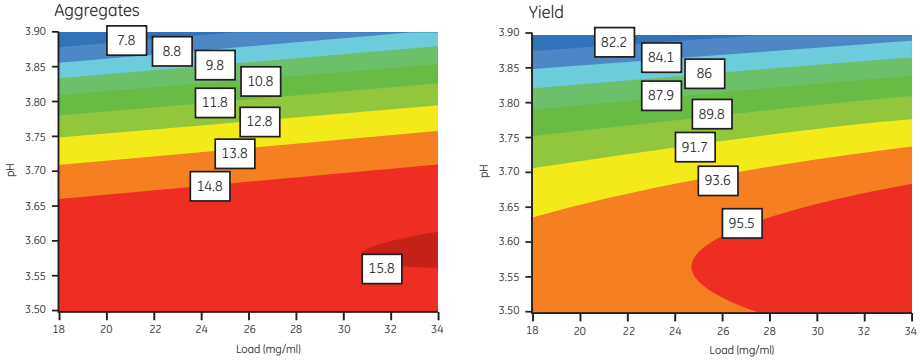


Fig 10. Response surfaces of yield and aggregates. The pH (3.5 to 3.9) at which the elution step was performed had the greatest impact on both the yield and the level of aggregate formation. A decrease in pH had a positive effect on yield but increased the aggregate content.

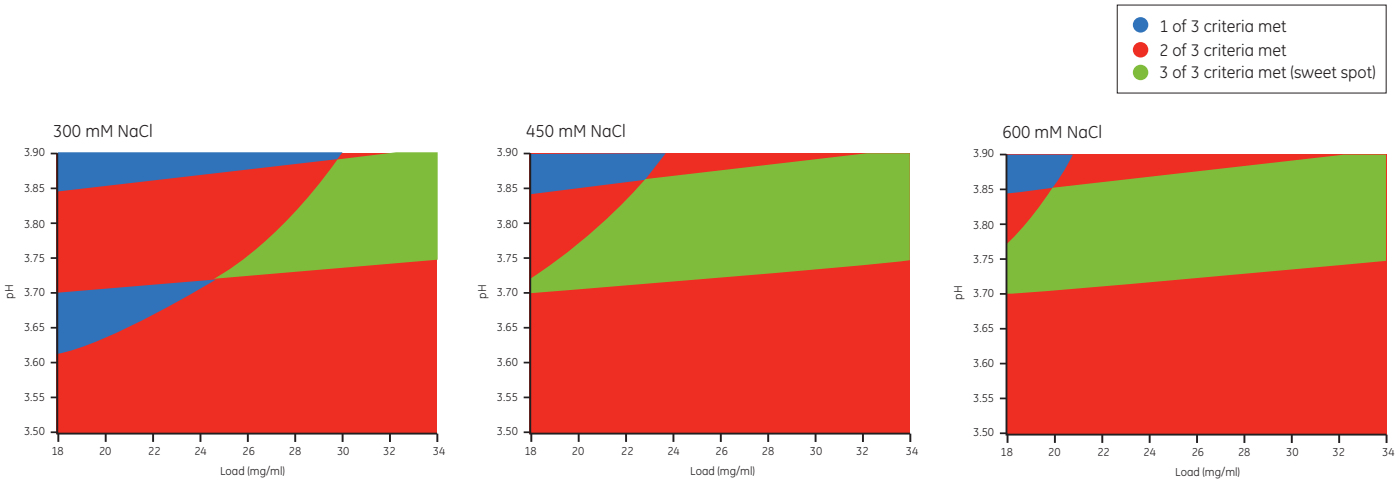


Fig 11. Sweet spot analysis of the capture step with the following criteria yield (90% to 100%), aggregates (0% to 14%) and HCP (0 to 70 ppm) at different levels of NaCl in the wash. The sweet spot is the red patch in the graph where all three criteria were met.

References

1. Instruction, PreDictor plates, 28-9258-34 AB.
2. Application note: High throughput process development with PreDictor plates, GE Healthcare, 28-9403-58, Edition AA (2006).
3. Bergander, T. *et al.* High-Throughput Process Development: Determination of Dynamic Binding Capacity Using Microtiter Filter Plates Filled with Chromatography Resin. *Biotechnol. Prog.* **24**, 632-639 (2008).

Ordering information

Product	Quantity	Code No.
PreDictor MabSelect SuRe, 6 µl	4 × 96-well filter plates	28-9258-23
PreDictor MabSelect SuRe, 20 µl	4 × 96-well filter plates	28-9258-24
HiTrap MabSelect SuRe	5 × 1 ml	11-0034-93
HiScreen MabSelect SuRe	1 × 4.7 ml	28-9269-77

Related literature

Data file: MabSelect SuRe	11-0011-65

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