

Introduction

High throughput process development has been used as a tool for selecting suitable adsorbents for downstream processes and for the optimisation of process conditions for large scale chromatography. This approach has reduced the volume of feed required for process development and the time taken to determine optimum process conditions which in turn has reduced the cost of drug development. More recently these techniques have been used for new applications such as supporting process development, manufacturing and quality control.

Batch release tests must be cost effective and rapid and therefore are not generally performed using complex feedstocks. In this poster we describe a novel use for high throughput process development techniques to support manufacture and quality assurance of an affinity adsorbent, Mimetic Blue® SA HL P6XL, developed and manufactured exclusively by ProMetic BioSciences Ltd (PBL).

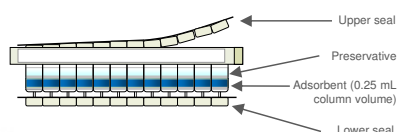
PuraPlate™ (96-column block format, 0.25 mL column volume) was used in high throughput mode to demonstrate performance consistency between different batches of Mimetic Blue® SA HL P6XL adsorbent using a complex feed (human source plasma). Due to the nature of both the PuraPlate™ and the beaded agarose base matrix, the test was completed under gravity flow. The captured albumin was eluted using a buffer containing 30 mM sodium octanoate. Process samples were collected and analysed using SDS-PAGE, size exclusion chromatography and Nephelometry.

The PuraPlate™ high throughput investigation showed comparable binding, recovery and purification for all eight batches of Mimetic Blue® SA HL P6XL adsorbent. This work demonstrates that high throughput techniques can be used to monitor batch to batch consistency of chromatography adsorbents using complex feedstocks.

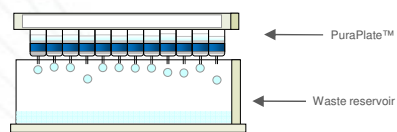
PuraPlate™ Operation



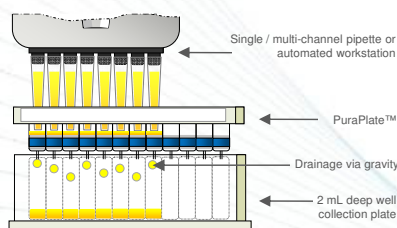
1. Removal of seals from PuraPlate™ (96 x 0.25 mL CV)



2. Drainage of PuraPlate™ in waste reservoir



3. Sample application (e.g. load, wash, elution, CIP)



PuraPlate™ Applications

High Throughput Screening	Testing a range of adsorbents using a single set of generic chromatography conditions for lead candidate identification
Process Optimisation	Investigating a range of different load, wash and elution applications, varying strategies such as pH, conductivity and additives
Adsorbent Recycling & Stability	Investigating numerous solvents, detergents and denaturants to identify optimum Clean in Place (CIP) strategies
Batch Consistency	Evaluating multiple batches of a single adsorbent for performance consistency

High Throughput Investigation – Adsorbent Batch Consistency

Eight manufactured batches of Mimetic Blue® SA HL P6XL were evaluated in PuraPlate™ format using generic conditions selected to reveal any difference in purification performance.

Elution samples were analysed by SDS-PAGE and size exclusion chromatography to show HSA recovery profiles including the presence of any residual plasma proteins (product impurity profile).

Non-bound and elution samples were tested by Nephelometry for HSA binding and recovery capacities.

TABLE 1

Experimental conditions for the capture and purification of human serum albumin (HSA) from human source plasma with eight manufactured batches of Mimetic Blue® SA HL P6XL adsorbent in PuraPlate™ format.

Platform	PuraPlate™
Column parameters	0.25 mL column volume (0.8 cm diameter, 0.5 cm bed height)
Adsorbent	Mimetic Blue® SA HL P6XL
Equilibration buffer	50 mM sodium phosphate, pH 7.5 – 3 x 1 mL
Load	Human source plasma – 1 x 0.25 mL
Wash buffer	50 mM sodium phosphate, pH 7.5 – 2 x 0.75 mL
Elution buffer	20 mM sodium phosphate, 150 mM NaCl, 30 mM sodium octanoate, pH 6.0 – 2 x 0.75 mL
Clean in Place (CIP)	0.5 M NaOH – 2 x 0.75 mL

FIGURE 1

Non-reduced SDS-PAGE of elution samples from Mimetic Blue® SA HL P6XL (batches 1 to 8) PuraPlate™ investigation using human source plasma.

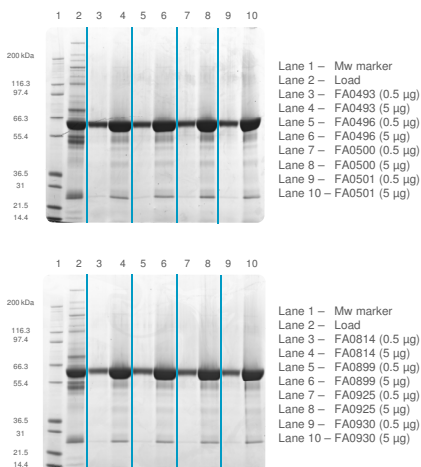


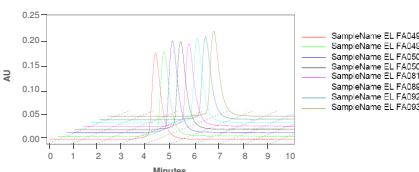
TABLE 2

Mimetic Blue® SA HL P6XL % purity results, as determined by densitometry.

Batch	Purity (%)
1	92
2	92
3	90
4	90
5	93
6	94
7	93
8	94

FIGURE 2

Overlaid elution profiles for all eight Mimetic Blue® SA HL P6XL adsorbent batches, as performed by size exclusion chromatography.



High purity values comparable to densitometry results with peak areas within 7% variation of the mean.

TABLE 3

Mimetic Blue® SA HL P6XL (batches 1 to 8) HSA binding capacity and recovery results, as determined by Nephelometry.

Batch	Binding Capacity (g/L of adsorbent)	Recovery (g/L of adsorbent)	Recovery (%) from Bound
FA0493	28	27	98
FA0496	24	27	≥100
FA0500	28	30	≥100
FA0501	26	26	100
FA0814	26	25	98
FA0899	24	27	≥100
FA0925	25	27	≥100
FA0930	26	28	≥100

Conclusions

- The PuraPlate™ high throughput technique investigated demonstrated a high level of consistency between different batches of Mimetic Blue® SA HL P6XL. This supports the use of cost effective and rapid high throughput techniques, such as PuraPlate™, to monitor batch to batch consistency of adsorbents using complex feedstocks to provide more significant performance related information and additional quality assurance data.
- Mimetic Blue® SA HL P6XL in this experiment provided HSA binding capacities and recoveries of ~26 g/L of adsorbent with >90% purity. Capacities in excess of 30 g/L of adsorbent are achievable under optimised conditions [1] & [2].
- SDS-PAGE shows comparable elution profiles for all eight batches of adsorbent tested (<5% coefficient of variance [CV]).
- Size exclusion chromatography gave comparable peak shapes and retention times as well as high purity values for all eight batches of adsorbent tested.
- HSA recoveries from bound were all within 5% CV and ~100% recovery was obtained for the majority of batches tested.

References

- Mimetic Blue® Affinity Adsorbents brochure (Issue: 060510), ProMetic BioSciences Ltd.
- An improved performance adsorbent for the downstream processing of serum albumin and related fusion proteins, IBC's BioProcess International™ Conference & Exhibition Poster, October 12-16, 2009, ProMetic BioSciences Ltd.

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