Quality aspects of the microtiter plate workflow in the screening of chromatographic conditions

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Quality aspects of the microtiter plate workflow in the screening of chromatographic conditions

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Introduction

As an alternative to performing process development in columns, batch uptake in a 96-well microtiter format can be used to evaluate chromatographic process conditions. Batch uptake experiments correlate well with column chromatography (Figure 1).

With the development of PreDictor™ plates, i.e. 96-well filter plates filled with chromatography media (Figure 2), the batch uptake approach is now available on the market. One important quality aspect during development of the plates was to secure reproducibility with respect to chromatography media volume between wells within a plate as well as between plates. Plate lots with good quality provides a powerful instrument for high throughput process development.

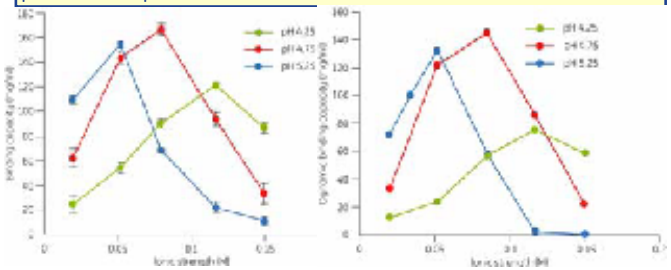

Figure 1. Batch uptake in PreDictor plates (to the left) correlates well with corresponding column experiment (to the right). Conalbumin binding capacity on Capto™ S was evaluated at various ionic strengths and different pHs.

Figure 2. PreDictor plates for condition screening, available with 7 chromatography media. Three different plates with different volumes of media are available for each medium. The choice of medium volume depends on what phase ratio liquid/solid is needed for the specific application.

Functional testing of reproducibility

The chromatography media volume variability was investigated by incubating the media with an excess of pure protein for 1 hr. The experiments were designed to have an unbound protein conc of >1mg/ml in the liquid phase after incubation, *i.e.* using high initial protein conc for the larger media volumes. Blank runs with no chromatography medium present were performed in parallel to all tests. The binding capacity in each well was determined by mass balance calculations, based on UV detection.

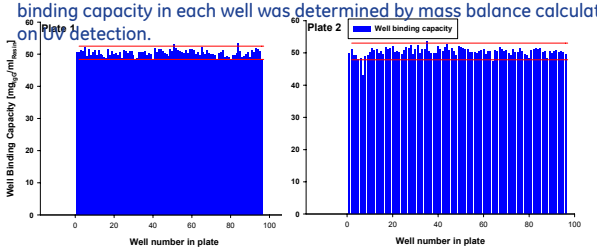

Figure 3. Evaluation of the hIgG-binding capacity on two different PreDictor MabSelect SuRe™ 6µl plates. Red lines denote 95% confidence limits for each plate. Each blue bar denotes the binding capacity for a specific well. The Relative Standard Deviation (RSD) was 1-1.2% in each plate.

The production reproducibility of the PreDictor products was studied by testing the binding capacity for 113 plates from continuous production of PreDictor plates. All media volumes for each chromatography media were tested. Each plate gave 96 data points and in total 10 848 wells were tested. High reproducibility within plates was obtained in all cases providing RSDs in the order of 1-5% (Figure 3).

Increasing robustness of experiments

Even though the throughput may be maximized by performing singular measurements, replication of the experiments is generally recommended. This increases the robustness of the batch uptake experiment by reducing variability and decreasing the risk of re-runs for specific conditions. The estimated variability within and between plates were used to calculate 95% confidence intervals for triplicate well measurements (See Figures 4-5).

The triplicate uncertainty in binding capacity was in all cases below ±7%. The uncertainty decreases at larger media volumes for all chromatography media. However, measuring the binding capacity with larger chromatography media volumes may require unrealistically high initial protein concentrations or excessive sample volumes.

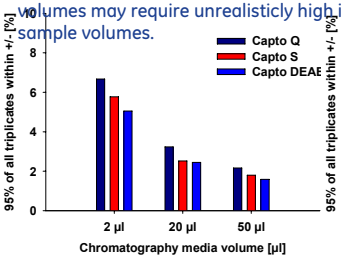

Fig 4. Reproducibility from PreDictor plates filled with the ion exchangers Capto Q, Capto S and Capto DEAE tested with different proteins.

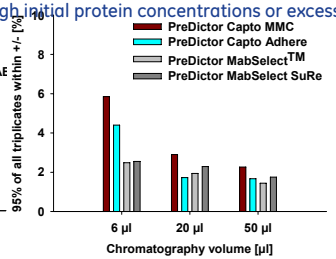

Fig 5. Reproducibility for PreDictor Capto MMC, PreDictor Capto Adhere and PreDictor MabSelect™ and PreDictor MabSelect SuRe, tested with polyclonal IgG and BSA.

Method variability vs. chromatography media volume variability

The variability from the blank runs was used as an estimate of the "non-chromatography media volume"-related error sources (e.g. factors such as pipetting, plate handling and detection).
UV detection was chosen for this study, as it is well suited for the amounts of protein used. In this case, the contribution from media volume variability was similar to the contribution from plate handling and detection (Figure 6).

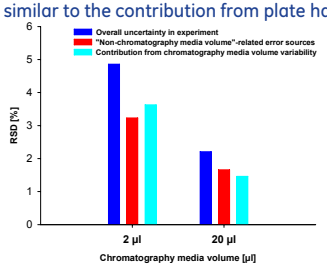

Figure 6. Overall RSD, and variability contributions from method handling and chromatography media volume.

The influence of chromatography media volume variability on the total end uncertainty of an experiment may be even smaller when less precise detection methods are used (Figure 7).

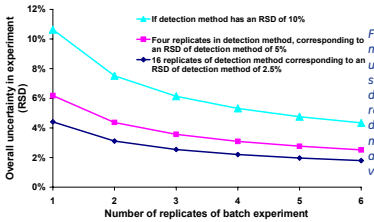

Figure 7. The effect of replicating a detection method with an RSD of 10% on the overall uncertainty in the experiment. Different curves show the effect of changing the number of detection replicates. Along the x-axis, the effect of replication of the batch experiment is shown. A detection RSD of 2.5% corresponds roughly to UV measurements in microtiter plates. Calculations are based upon 2µl chromatography media volume.

Assay replication should be considered if less precise detection methods are used. However, as stated earlier, true experiment replication is still recommended to ensure robust and efficient process development in microtiter plates.

Conclusions

- The reproducibility of chromatography media volume in PreDictor plates was good (RSD of 1.5-5% in binding capacity between wells).
- Replication of experiments should be performed to maintain the robustness of the high throughput approach in microtiter plates.
- The uncertainty in detection methods was of more concern than the chromatography media volume variability. The chromatography media volume variability was on the same level as error sources from UV detection and liquid handling.
- The quality of PreDictor microtiter plate products enables the use of batch uptake technique as a powerful tool for HTPD workflows.

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