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# Impact of the Claristep® Filtration System on Recovery and Adsorption of Various Therapeutic Proteins at Low Sample Volumes

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## Abstract

We tested the novel filter device Claristep® for the preparation of protein samples containing one of four different target molecules, i.e. an anti-malaria vaccine candidate, aviscuminum, interferon alfa-2B or monoclonal antibody (mAb) P2G12. In comparison to the standard protocols for sample preparation prior to analysis by either reversed-phase or SEC HPLC, the Claristep® filter did not exhibit a significant difference in protein concentration if the target molecules were present with at least 1.0 g $L^{-1}$. A significantly reduced product concentration was observed in the Claristep® filtered samples compared to the standard protocol for mAb P2G12 if the concentrations were lower. The protein adsorption to the filter material was well described by a Langmuir adsorption isotherm. Besides, the Claristep® filters facilitated a rapid sample preparation with minimal volume losses.

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# Introduction

Biopharmaceutical samples often contain insoluble particles like cell debris or protein aggregates from their biotechnological production processes. These particles can damage analytical instruments, e.g. by blocking capillaries of auto-samplers of analytical devices or chromatography columns, and are typically removed by centrifugation or filtration. Whereas centrifugation can only separate based on differences in density, the latter method is often preferable because an absolute particle removal can be achieved. However, syringe filters usually have large sample dead volumes of about 100 µL, which is disadvantageous as only small sample volumes < 200 µL are typically available in early phase small-scale experiments. Also, nowadays often only < 50 µL of sample are required for state-of-the-art analytical devices like (U)HPLC consoles.

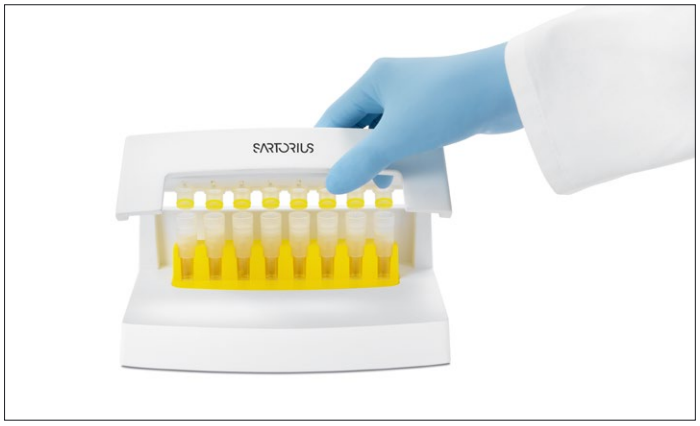

Figure 1: Claristep® Filtration System

If sample volumes are small, unspecific protein binding to the filter material can occur and either impair quantification or completely prevent sample analysis due to alterations in sample composition. In addition, large numbers of samples ~10–500 per day (depending on batch size) have to be analyzed for release testing of biopharmaceuticals, which can be time and labor-intensive using regular single-use filters. Therefore, we tested a novel multiplexed filtration device (Claristep®) in a direct comparison with regular sample preparation for an anti-HIV antibody as well as for a recombinant vaccine candidate, a potential anti-cancer toxin and interferon alfa-2B.

# Materials and Methods

In-process samples for a malaria vaccine candidate (M = 89.9 kDa) were taken at different steps during downstream processing. The concentration of the malaria vaccine candidate in-process samples ranged from 3.03 to 4.20 mg $mL^{-1}$. The matrix of in-process sample 1 was 0.075 M Imidazol, 0.020 M Tris, 0.050 M sodium chloride, pH 8.0, the matrix of in-process sample 2 was 0.020 M Imidazol, 0.25 M sodium chloride, pH 8.0, the matrix of in-process sample 3 was 0.010 M sodium dihydrogen phosphate, 0.50 M sodium chloride, pH 7.4. The samples were analyzed by reversed-phase HPLC on a Jupiter C5, 250 × 4.6 mm, 5 µm, 30 nm (Phenomenex, Torrance) column. Elution was carried out at 1.0 mL $min^{-1}$ by a linear gradient from 15 % (v/v) water/acetonitrile (95/5 v/v) to 60 % (v/v) water/acetonitrile (5/95 v/v) in the presence of 0.2 % (v/v) TFA in 25 min. Detection wavelength was 220 nm.

Therapeutic toxin aviscuminum samples were in the dimension of 0.52 to 0.96 mg $mL^{-1}$, sample buffer was 0.020 M Tris, 0.085 M sodium chloride, 0.0010 M EDTA, 0.1 % (v/v) polysorbate 80, pH 8.0. Aviscuminum (M = 57.3 kDa) samples were subjected to reversed-phase chromatography using a Luna CN, 150 × 2.0 mm, 3 µm, 10 nm (Phenomenex, Torrance) column. Samples were eluted at a column temperature of 50 °C with 0.4 mL $min^{-1}$ by a linear gradient from 17 % (v/v) water/acetonitrile (95/5 v/v) to 88 % (v/v) of water/acetonitrile (5/95 v/v) in 5 min in the presence of 0.1 % TFA (v/v). Detection was carried out at 220 nm. Interferon alfa-2B (M = 19.4 kDa) samples were analyzed according to the monograph 07/2015:1110 of the European pharmacopoeia [1] by reversed phase chromatography for related proteins. Protein concentration of interferon alfa-2B samples were 2.6 and 2.9 mg $mL^{-1}$. The matrix was 0.025 M sodium acetate, 0.315 M sodium chloride, pH 4.5. Separation of interferon alfa-2B and its related proteins was achieved on a Jupiter C18, 250 × 4.6 mm, 5 µm, 30 nm (Phenomenex, Torrance) column.

Monoclonal antibody samples were diluted to $1.0 \times 10^{-3}$, $1.0 \times 10^{-2}$, $1.0 \times 10^{-1}$ and 1.0 mg $mL^{-1}$. The sample matrix was 0.9 % (m/v) sodium chloride solution. Monoclonal antibody P2G12 (M = 150 kDa) drug substance samples were separated by size, using a Yarra SEC-X150 1.8 µm, 4.6 × 150 mm (Phenomenex, Torrance) column. The mobile phase was 0.1 M sodium dihydrogen phosphate buffer, pH 6.8. The analytes were detecteted at 220 nm.

All samples prepared by the Claristep® method (Figure 1) were prepared by filtering 60.0 µL (anti malaria vaccine, aviscuminum and interferon alfa-2B) or 100.0 µL (mAb P212) through the Claristep® filter device using 0.2 µm regenerated cellulose filters. Standard sample preparation was carried out by centrifugation at 16,000 × g for 10 min and injection of the supernatant. All samples were analyzed as triplicates on an Ultimate 3000 (U)HPLC System with integrated Pump, auto-sampler, column oven and UV|VIS-detector (ThermoScientific, Waltham).

# Results and Discussion

Up to eight samples can be filtered simultaneously by a single operation of the Claristep® device. Standard 9-mm auto-sampler vials are used as receiver containers facilitating further sample processing and analysis. The sample preparation required only 10 % of the time compared to sample preparation by traditional syringe filters. The Claristep® filters had a small dead volume of a few microliter, e.g. if 60 µL of sample was loaded, the filtrate volume was about 30 µL.

All proteins independent on their size of 19.4 to 150 kDa passed through the filter without any significant detectable losses at sample concentrations of ≥ 1.0 mg $mL^{-1}$. By HPLC analysis, there were no or only slight protein losses for Claristep® preparation compared to the standard protocol. Recoveries were ≥ 96 % for all anti-malaria vaccine-, avis-cuminum- and interferon alfa-2B samples (Figure 2). HPLC-analyses showed an average coefficient of variation of 2.2 % for the samples prepared by centrifugation whereas 2.3 % were observed for the Claristep® setup. Using a two-sided student's t-test we found that only one sample pair, i.e. in-process sample 2 for the anti-malaria vaccine candidate (Figure 2B), exhibited a significant difference between the preparation methods ($t = 3.49$, $t_{crit} = 2.78$, $p = 0.05$, $df = 4$), with the Claristep® filtration reducing the protein concentration by 2.6 %, most likely due to adsorption to the filter material.

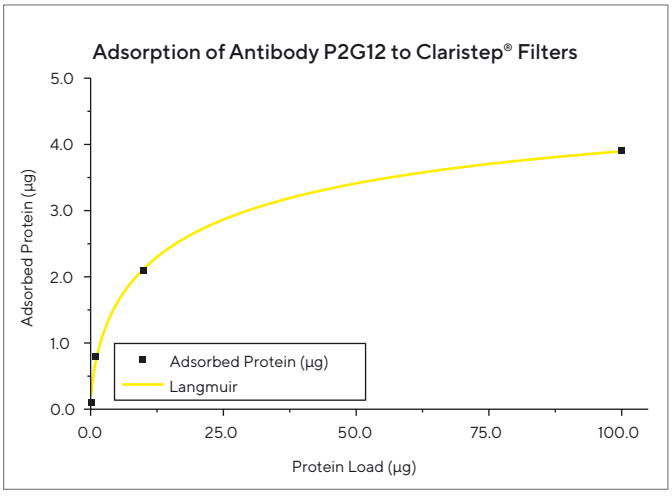


Figure 3: Langmuir fit of protein adsorption to Claristep® filter for monoclonal antibody P2G12. Adsorption of antibody P2G12 in dependency of total protein load on sample filtration by Claristep® filters.

In addition, HPLC analyses of samples of the monoclonal antibody P2G12 (Figure 2D), showed a relative increase in product loss with decreasing absolute protein concentration for the Claristep® setup compared to the standard procedure, which was significant according to a two-sided student's t-test, i.e. of 21.0 % for $1.0 \times 10^{-1}$ mg $mL^{-1}$ ($t = 15.89$,

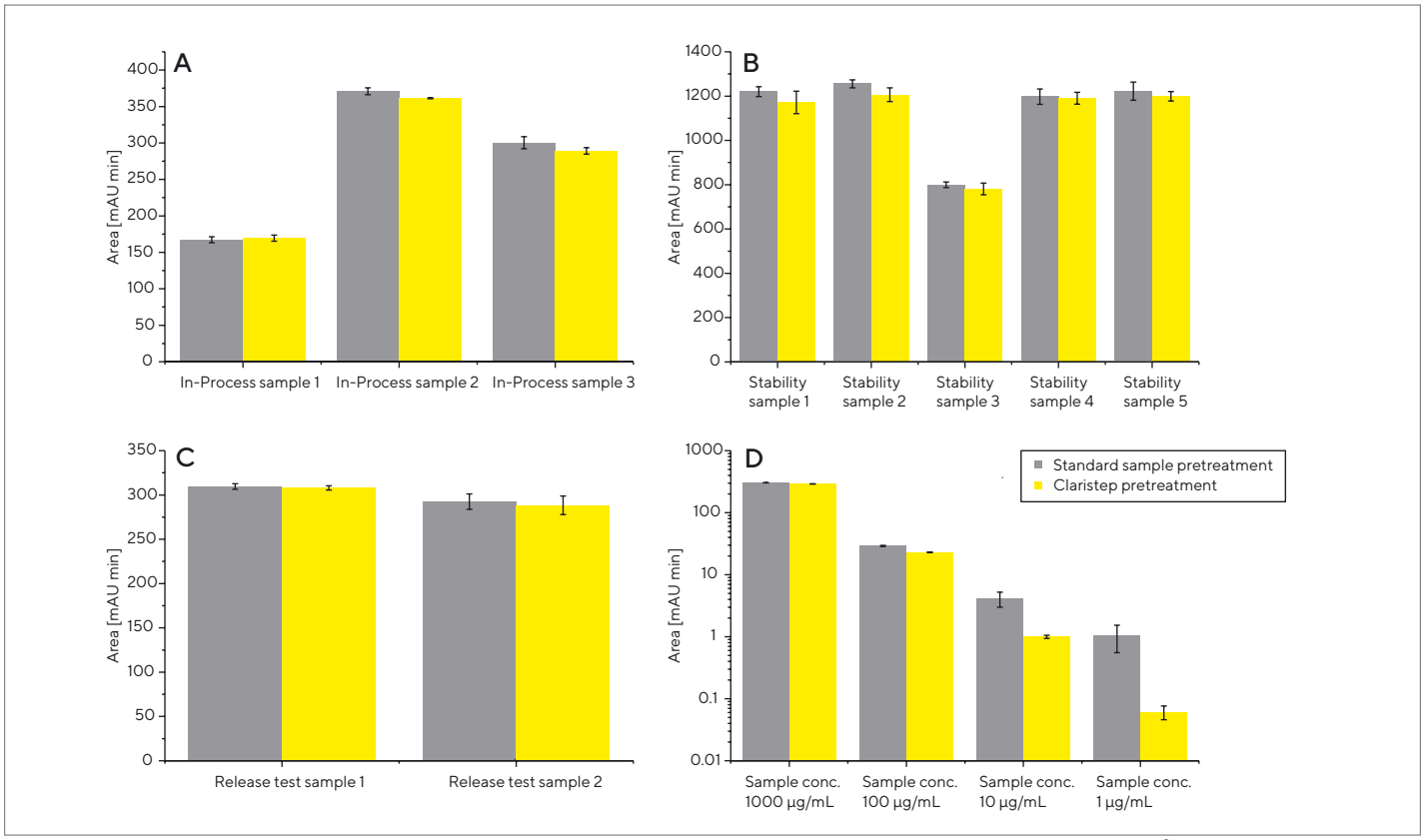


Figure 2: Recovery of four representative target proteins after standard analysis pre-treatment or preparation with Claristep®. Columns represent the integrated peak areas obtained for anti-malaria vaccine candidate (A) aviscuminum (B), interferon alfa-2B (C) and monoclonal antibody P2G12 (D) by HPLC analysis after preparation. Error bars indicate the standard deviation of three replicate measurements.

$t_{crit}$ = 2.78, p = 0.05, df = 4), 75.8 % (t = 4.82, $t_{crit}$ = 2.78, p = 0.05, df = 4) for $1.0 \times 10^{-2}$ mg $mL^{-1}$ and 94,1 % (t = 3.47, $t_{crit}$ = 2.78, p = 0.05, df = 4) for samples with an antibody concentration of $1.0 \times 10^{-3}$ mg $mL^{-1}$. The adsorption effect of P2G12 to the Claristep® filter material resembled a Langmuir adsorption isotherm (adjusted $R^2$ = 0.9949, Equation 1, Figure 3).

$$y = \frac{a \times b \times x^{1-c}}{1 + b^* \times} \qquad (1)$$

We observed a saturation of ~ 4.0 µg protein per filter. Therefore, loading small sample volumes of < 100 µL and | or samples with low protein concentrations of < 1.0 mg $mL^{-1}$ may lead to distorted results, i.e. an underestimation of protein in the sample. This can be critical in pharmaceutical analysis and individual investigations and validation should be carried out for new target proteins even though using 100 µL sample with concentration of ≥ 1.0 mg $mL^{-1}$ is likely to be a safe standard. In general, the coefficients of variation in case of the monoclonal antibody were higher than before but the Claristep® setup performed more consistently than the centrifugation method, i.e. the coefficients of variation were at least less than half of what we observed for centrifugation, e.g. 27.2 % (centrifugation) vs. 6.1 % (Claristep®) for the $1.0 \times 10^{-2}$ mg $mL^{-1}$ sample.

## Conclusion

The novel Claristep® filtration device was easy to use and facilitated a rapid sample preparation. Protein losses were negligible compared to the standard sample preparation protocol if protein concentrations were 1.0 g $L^{-1}$. If applicable, a pre-adsorption of the filter with a defined non-analyte protein (e.g. bovine serum albumin) may reduce the protein losses that we observed for mAb concentrations below 1.0 g $L^{-1}$ and can thus render the method compatible with analytical methods that typically require only low concentrations of the target molecule, e.g. surface plasmon resonance.

## References

1. *Interferon alfa-2 concentrated solution.* European Pharmacopoeia. 8.3: p. 2502-2505.

## Abbreviations

| | |
|---|---|
| df | Degrees of freedom |
| EDTA | Ethylene diamine tetraacetic acid |
| HPLC | High performance liquid chromatography |
| mAb | Monoclonal antibody |
| TFA | Trifluoroacetic acid |
| p-value | Probability value |
| t-test | Student's t-test |
| Tris | 2-Amino-2-(hydroxymethyl)propane-1,3-diol |
| (U)HPLC | (Ultra-) high performance liquid chromatography |

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