Fast process development of a single-step purification using ÄKTA avant systems

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CY13371-12May20-AN

Fast process development of a single-step purification using ÄKTA™ avant systems

This study demonstrates a rational strategy for rapid process development of a single-step purification. Anion exchange chromatography was used to develop a capture step for purifying recombinant S-transaminase from *E. coli*. The aim was to achieve sufficient purity for immobilizing the enzyme onto a chromatographic support. First, initial screening of chromatography media and process conditions was performed using prefilled PreDictor™ 96-well filter plates and Assist software. Next, the Design of Experiments (DoE) functionality, incorporated in UNICORN™ 6 control software, was used with ÄKTA avant 25 system to perform a DoE optimization of loading and elution conditions. DoE was also used to confirm that the optimized process conditions were robust. After optimization, scale-up was performed in two steps (40-fold and 400-fold) with HiScale™ and AxiChrom™ columns using ÄKTA avant 150 system.

Introduction

Left- and right-handed enantiomers of a molecule often have different properties and biological activities. When preparing pharmaceuticals that are racemic, it is important to know the effects of the two different enantiomers. In the best scenario one of the enantiomers represents the positive effect while the other is inactive, but in the worst case the other form could be harmful. An increasingly important technology for selective enantiomer production is the use of enzymatic reactions.

(S)-transaminase (S-TA) is an enzyme used for asymmetric synthesis of chiral amines via transfer of an amino group (Fig 1). S-TA, a thermostable mutant originating from *Arthrobacter citreus*, has a broad substrate range and excellent stereoselectivity of an enantiomeric excess over 99% (1). In this study, a single anion exchange chromatography (AIEX) step was developed for the purpose of immobilizing the purified protein onto a solid support for use as a biocatalyst. Biocatalysts are an important industrial application for intermediate suppliers that require new, cost-effective methods for producing pure enantiomers that can later be used as raw material by pharma companies.

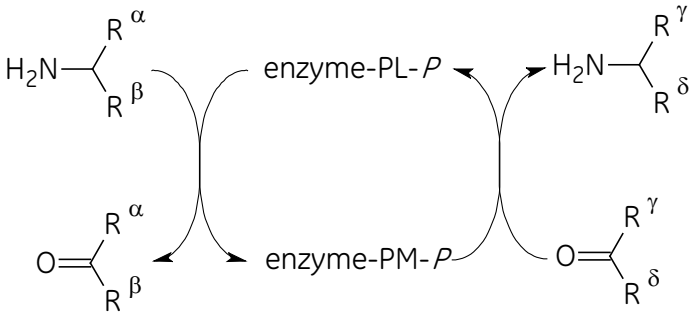


Fig 1. Transaminases catalyze asymmetric synthesis of chiral amines by transferring an amino group from an amino donor to an amino acceptor via the cofactor PLP (pyridoxal 5'-phosphate). PLP carries the amine group between the donor and acceptor molecule through the reversible conversion of PLP to PMP (pyridoxamine 5'-phosphate). Illustration modified from D. Koszelewski *et al* (1).

Figure 2 shows the process development strategy used in this study. Initial screening was performed using PreDictor AIEX screening plates to test four different chromatography media (resins). ÄKTA avant 25 liquid chromatography system was used for optimization of loading and elution conditions using prepacked HiTrap™ columns and DoE in UNICORN 6. DoE was also used to test robustness of the optimized conditions using HiScreen™ prepacked columns. Using ÄKTA avant 150 system, HiScale and AxiChrom columns were used for scaling up 40- and 400-fold, respectively.

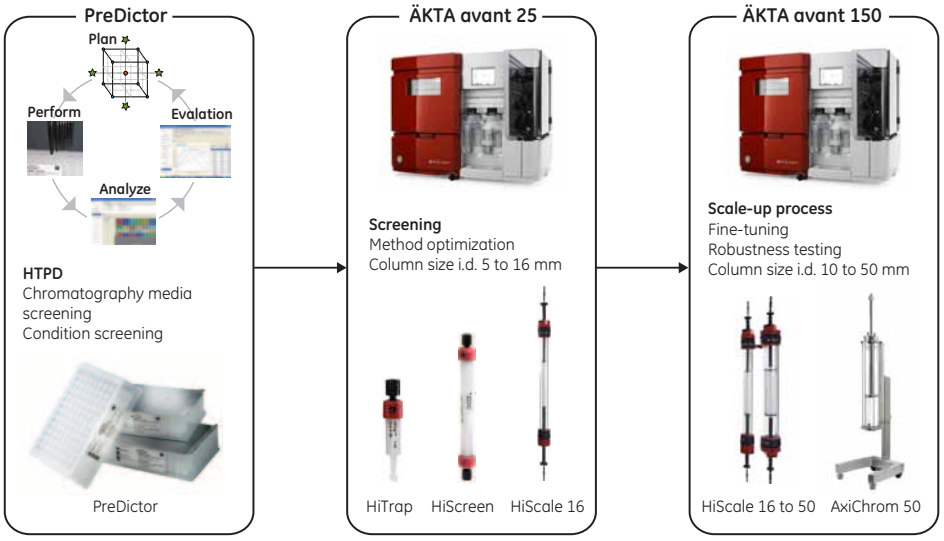


Fig 2. Process development workflow. High throughput process development (HTPD) using prefilled PreDictor 96-well filter plates for chromatography media and condition screening, followed by optimization of process conditions using ÄKTA avant 25 and UNICORN 6, and then fine-tuning and scale-up using ÄKTA avant 150 system.

Materials and methods

Sample preparation

A variant of the S-TA enzyme (M_r 53 054; pI 5.69), originating from *Arthrobacter citreus*, was overexpressed in recombinant *E. coli*. Spray-dried *E. coli* cells (2.5 g; Cambrex AB, Karlskoga, Sweden) were suspended for about 1 h at 4ºC in 25 ml buffer, pH 7.65 (50 mM sodium phosphate buffer, 0.5 mM pyridoxal-5'-phosphate [PLP; Sigma], 5% v/v glycerol, and 10 mM Triton X-100). After suspending the cells, homogenization was performed four times using EmulsiFlex-C3 High Pressure Homogenizer (Avestin, Inc.) at a pressure of ~20 000 psi and then clarified by centrifugation at 20 000 rpm for 30 min (JA-25.50 Beckman Rotor).

Analysis

The identity of the target protein was confirmed by SDS-PAGE analysis. SDS-PAGE was run on an ExcelGel™ SDS Gradient 8-18 with ExcelGel SDS Buffer Strips (anode and cathode) using a Multiphor™ II electrophoresis system. SDS sample application strips were used for sample loading, enabling up to 52 samples to be run on the same gel. After staining with Deep Purple™ Total Protein Stain, the gel was scanned using an Ettan™ DIGE Imager and analyzed using ImageQuant™ TL software. The identity of S-TA was confirmed by mass spectrometry (Ettan MALDI-ToF Pro system) of the strongest band on the SDS-PAGE gel.

Total protein concentration was measured at 562 nm (Pierce™ BCA Protein Assay Kit). A standard curve was prepared using 100 mM sodium phosphate buffer with 0.5 mM PLP, and BSA was used as the standard.

Chromatography media screening using HTPD

Initial screening of media and loading conditions was performed using PreDictor 96-well filter plates (PreDictor AIEX Screening Plate, 20 µl) prefilled with four different anion exchange (AIEX) media: Capto™ Q, Capto DEAE, Q Sepharose™ Fast Flow, and Capto adhere.

The experimental design, generated using Assist software, varied pH and conductivity for the four types of media tested (Fig 3).

Samples were pH adjusted with 100 mM sodium phosphate buffer. The plate was equilibrated three times for 1 min with 200 µl buffer on a microplate shaker at 1100 rpm. Sample (100 µl) was mixed with 100 µl buffer, loaded on the plate, incubated for 60 min, and then washed (3 × 200 µl). The plates were incubated for 20 min with elution buffer consisting of 100 mM sodium phosphate buffer with the corresponding pH and 0.5 M NaCl. All solutions were removed using vacuum suction (0.5 bar). All buffers were prepared using a Tecan Freedom EVO™ robot (Tecan, Inc). Fractions were stored for analysis with BCA protein assay and SDS-PAGE. Response data from the analysis of eluted samples were evaluated using Assist software.

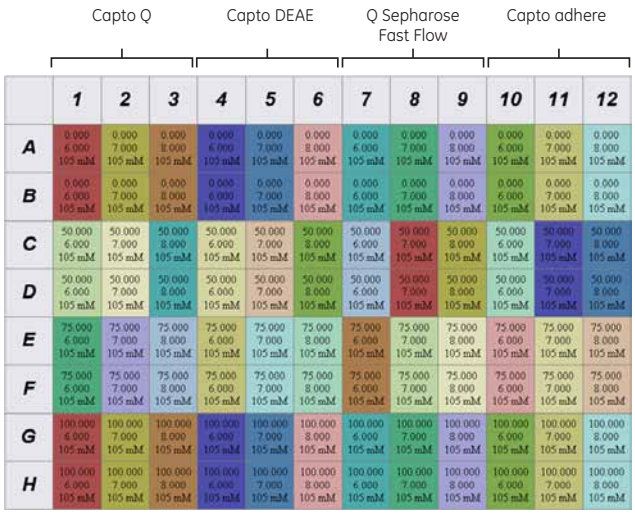


Fig 3. AIEX PreDictor plate experimental design. For the four different types of media, pH (6, 7, and 8) and conductivity (0 mM, 50 mM, 75 mM, and 100 mM NaCl) were varied.

Optimization of chromatography conditions using ÄKTA avant 25 system

Capto DEAE was found to be the optimal chromatography media during the media screening, and HiTrap Capto DEAE 1 ml columns were used with ÄKTA avant 25 to test optimal loading and elution conditions. The optimization was performed using DoE integrated in UNICORN software.
To optimize loading conditions, the Rechtschaffner experimental design was used allowing 13 different combinations of the tested parameters: loading pH, sample load volume, and flow rate (see Table 2 for run scheme). The Rechtschaffner experimental design uses a minimum number of experiments while allowing a maximum amount of information to be obtained. The column was equilibrated with 5 CV (column volumes) of 100 mM sodium phosphate buffer with 0.5 mM PLP at pH 6, 6.5, and 7. The sample was loaded at a concentration of ~4.0 mg/ml in different volumes, 1.5, 8.25, and 15 ml at the different pHs. The flow rate was varied between 0.5, 2.25, and 4 ml/min. The column was washed with the same buffer for 7 CV. A step elution using 100 mM sodium phosphate buffer, 0.5 mM PLP, and 1 M NaCl for 15 CV was used for elution. Two milliliter fractions were collected.

Elution conditions were optimized using the conditions obtained from the loading optimization. Elution pH and conductivity was varied according to a CCC (central composite circumscribed) experimental design allowing 11 different combinations of the parameters to be tested (Table 1). HiTrap Capto DEAE 1 ml column was equilibrated with 7 CV of 100 mM sodium phosphate, 0.5 mM PLP, pH 6 and then 10 ml sample was added at a concentration of ~4.0 mg/ml at the corresponding pH. The 11 different step elutions were performed varying pH (6 to 7) and NaCl concentration (200 mM to 1 M), and 1 ml fractions were collected.

Table 1. Run scheme for elution optimization on HiTrap Capto DEAE using CCC design

Run no.	Elution conductivity (mM NaCl)	Elution pH
1	200	6.5
2	600	6.5
3	900	6.8
4	600	6.5
5	600	6.5
6	1000	6.5
7	900	6.2
8	300	6.8
9	300	6.2
10	600	6.0
11	600	7.0

Robustness test using DoE

Two HiScreen Capto DEAE columns, coupled in series for a 20 cm bed height, were used on an ÄKTA avant 150 system to test the robustness of the optimized purification step. A fractional factorial experimental design generated by DoE in UNICORN 6 was used, allowing five different combinations of the controlled parameters to be tested: the target elution conductivity (NaCl concentration; i.e., the gradient slope), and the sample load volume (see Table 4). The columns were equilibrated with 5 CV of 100 mM sodium phosphate buffer, 0.5 mM PLP, pH 6 at 3.5 ml/min. The sample was loaded at a concentration of ~4.0 mg/ml in different volumes (14.5, 16, and 19 ml). The columns were washed with the same buffer for 7 CV. A linear gradient using 100 mM sodium phosphate buffer with 0.5 mM PLP and 1 M NaCl was used for 15 CV for elution at pH 6, and 30 ml fractions were collected.

Gradient elution study

S-TA was purified using ÄKTA avant 150 and HiScale 16/20 columns packed with Capto DEAE to a bed height of 20 cm. Purification of S-TA was performed using either step or gradient elution, and the eluted fractions were analyzed by gel filtration (size exclusion chromatography) to determine whether monomer and/or active dimer were present in each peak fraction. In the gel filtration analysis, the eluted peak fractions were run on prepacked Superdex™ 200 10/300 GL columns. Superdex columns were equilibrated with 2 CV of 100 mM sodium phosphate buffer including 0.5 mM PLP and 60 mM NaCl, after which 100 µl sample was applied followed by 1.5 CV of buffer. The flow rate was set at 0.5 ml/min using ÄKTA avant 25 system, and HMW Calibration Kit was used as a standard. By using gradient elution, the active dimer could be separated from the monomer.

Scale-up using ÄKTA avant 150 system

An initial scale-up (40-fold) was performed using ÄKTA avant 150 system and a HiScale 16/20 column packed with Capto DEAE to a bed height of 20 cm. The buffers used were 100 mM sodium phosphate, 0.5 mM PLP, pH 6. The column was equilibrated with 5 CV, and then 103 ml sample was loaded (corresponding to 67 ml of undiluted lysate). The column was washed with 7 CV of start buffer followed by gradient elution using 100 mM sodium phosphate buffer including 1 M NaCl. The flow rate was 20 ml/min, and 20 ml fractions were collected.

ÄKTA avant 150 system and an AxiChrom 50/300 column packed with Capto DEAE media to a bed height of 20 cm were used for further scale-up (400-fold). The gradient elution method was simliar to that used for HiScale. Lysate (800 ml; corresponding to 625 ml undiluted lysate) was loaded onto the column. The flow rate was 150 ml/min, and 700 ml fractions were collected.

Results and discussion

Screening of Capto AIEX media using HTPD

Chromatography media and binding conditions were screened using PreDictor AIEX screening plates containing four different types of media. Purity and recovery were analyzed. Due to the complex nature of the sample, an overall purity of ~32% was obtained for the four different media types when varying the parameters salt and pH (Fig 4). However, the amount of recovered target protein was not the highest in the samples that gave the highest purity.

The media screening analysis revealed two candidates—Capto DEAE and Capto adhere. Capto adhere is a strong anion exchanger and also enables multimodal interactions, whereas Capto DEAE is a weak anion exchanger. The results indicated an effect of load volume (data not shown), where the percentage of S-TA in the eluate increased with higher load. This effect was more prominent for Capto adhere, which did not work well with a smaller sample load. Analyzing the amount of S-TA obtained when varying loading pH and conductivity revealed that a higher amount of target protein was obtained using Capto DEAE (Fig 5). Elution of the target protein from Capto adhere required 2 M NaCl at pH 3, but even at those conditions, the recovery was lower than for Capto DEAE (data not shown). Thus, Capto DEAE was chosen for further optimization. The results also showed that low pH and lower conductivity favored a higher yield.

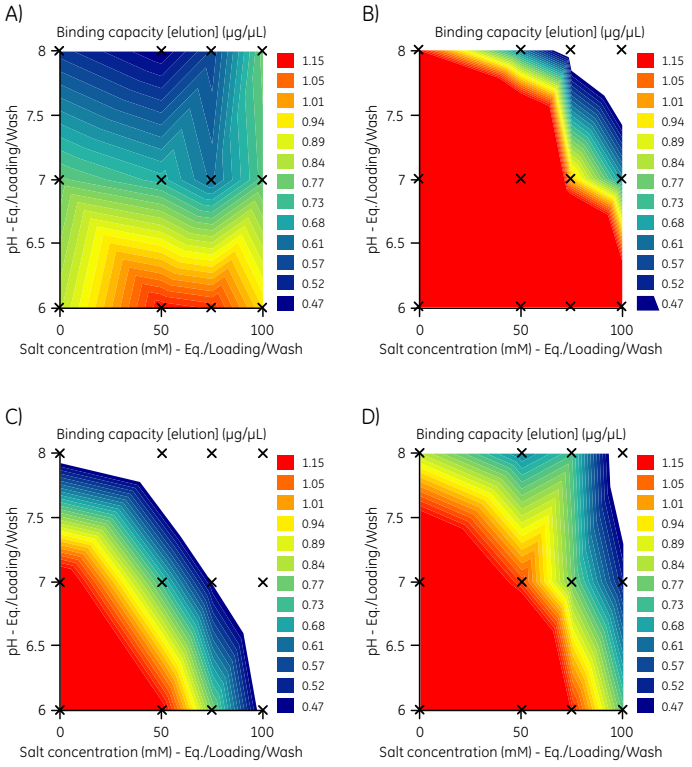


Fig 5. Surface plots generated using Assist software show the optimal region of the chosen parameters for (A) Capto Q, (B) Capto DEAE, (C) Q Sepharose Fast Flow, and (D) Capto adhere. Results indicate that the highest amount of target protein was obtained with Capto DEAE. Protein concentration is given in µg per µl.

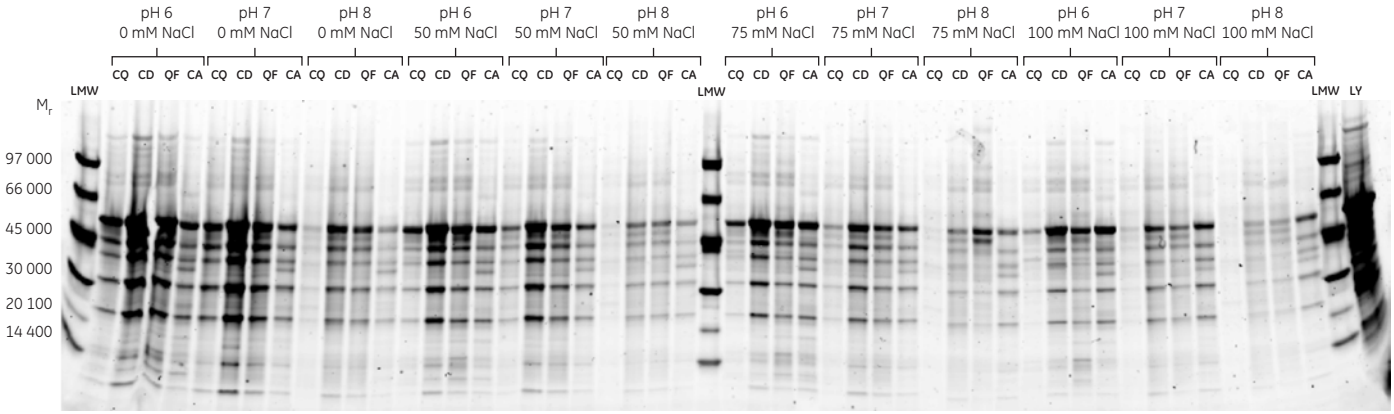


Fig 4. SDS-PAGE analysis of the fractions from the HTPD chromatography experiments. The pH was varied from 6 to 8, and the conductivity from 0 to 100 mM NaCl. The target protein, S-TA, has a relative molecular mass (M_r) of 53 054. The target protein band with the highest intensity was obtained using Capto DEAE for all conditions used. CQ=Capto Q, CD=Capto DEAE, QF=Q Sepharose Fast Flow, CA=Capto adhere, LY=Lysate, and LMW=low molecular weight marker (LMW-SDS Marker Kit). The molecular mass of the intact protein monomer was also confirmed by MALDI-ToF (data not shown).

Optimizing loading conditions

Table 2 shows the run scheme and results for the DoE optimization of loading conditions using HiTrap Capto DEAE 1 ml columns. The study revealed that at increased load more protein was recovered; however, more protein was also lost in the flowthrough and the data indicate the nonlinear relationship is probably due to displacement effects. This was also confirmed by the SDS-PAGE analysis (Fig 6) and BCA protein assay analysis (Table 2).

The robustness study results indicate that an even higher load would have been beneficial. The overall purity increased to about 40% to 50%, most likely sufficient for the purpose of immobilizing the protein onto a solid support.

Table 2. Experimental conditions and results from the loading study

Run no.	Loading pH	Flow rate (ml/min)	Sample load* (ml)	Purity (%)	Target protein in peak (mg)	Target protein in flow-through fraction (mg)
1	6	0.5	15	51	6.8	3.3
2	6.5	2.25	8.25	45	3.1	1.2
3	7	0.5	1.5	37	0.5	0.1
4	7	0.5	15	49	7.4	3.0
5	7	4	1.5	41	0.5	0.2
6	6.5	2.25	8.25	48	3.2	1.3
7	6.5	2.25	15	51	6.4	3.2
8	7	2.25	8.25	40	2.5	2.7
9	6	0.5	1.5	42	0.7	0.2
10	6	4	15	47	6.6	4.7
11	6.5	4	8.25	38	2.8	2.6
12	6	4	1.5	54	0.9	0.2
13	6.5	2.25	8.25	44	3.3	2.5

*~4.0 mg/ml

The previous HTPD chromatography experiments also indicated that more target protein was recovered with higher sample load volume (data not shown), and statistical analysis of the loading study showed similar results (Fig 7). The results were analyzed in UNICORN and demonstrated that load volume was the most significant factor, whereas flow and pH had little influence (using a confidence interval of 0.90).

The summary of fit plot shown in Figure 7 indicated a good model. The load volume contributed largely to the measured variation which could be the reason that the other variables were not significant. In this case, one outlier was detected. However, using the Rechtschaffner design does not allow excluding runs without also decreasing the support for the response surface modeling (the model that includes the interaction and curvature terms). The large contribution

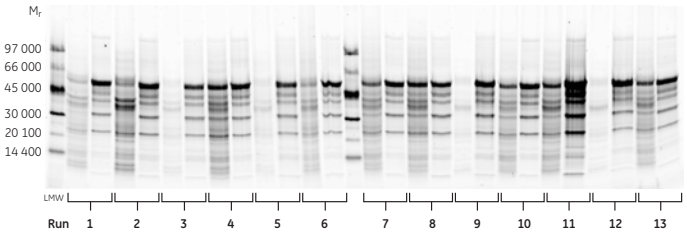


Fig 6. SDS-PAGE analysis of samples from the loading study. Flowthrough and peaks loaded according to the run scheme in Table 2. LMW=low molecular weight marker.

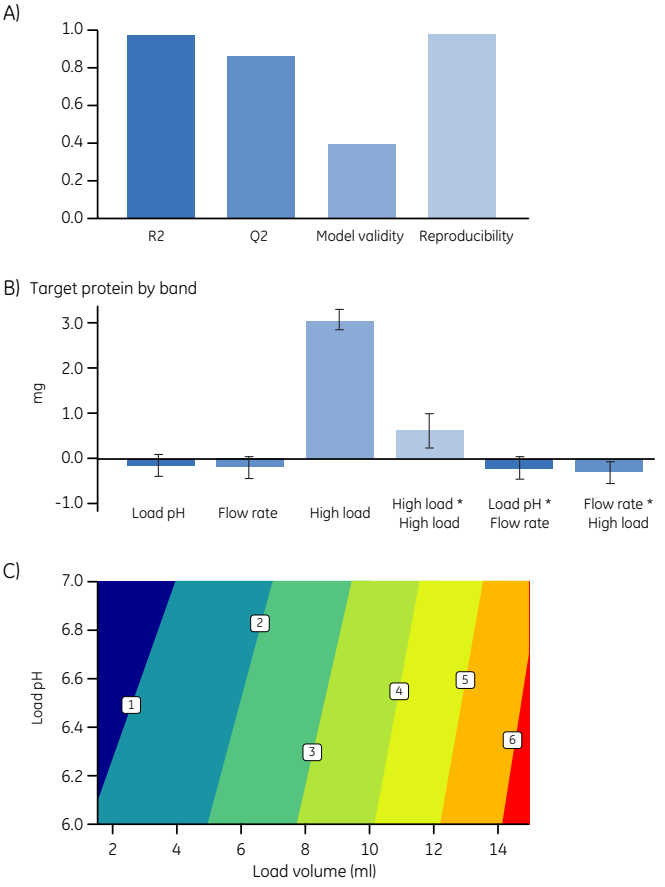


Fig 7. Statistical analysis of the results obtained in the loading optimization study using DoE in UNICORN. The model is based on the total mass of S-TA eluted from the column in milligrams. (A) The summary of fit plot indicates a good model. (B) The coefficient plot shows that the load is the most significant factor, while flow and pH have little influence on the result. (C) The surface plot shows an increasing amount of S-TA eluted as more sample is loaded onto the column. This plot also indicates that high flow rates could be used at low pH, giving amounts of recovered target protein comparable to lower flow rates (data not shown).

from load volume could indicate that a repetition of the design is necessary using a tighter interval in load volume and/or a broader range of the other controlled parameters.

Using load volume as the one single parameter in the model resulted in an extremely low replicate variation, as compared to the residual variation, which gave a low model validity (lack-of-fit value, p=0.088). However, the flow rate and pH were kept in the model since the residual variation was reasonable and because the chromatographic results indicated a slight pH and flow dependency (Fig 7).

Optimizing elution conditions

Table 3 shows the results for the elution optimization study using prepacked HiTrap Capto DEAE 1 ml columns. The chromatograms for the different runs showed that more target protein is obtained at low pH, 6 to 6.5, and at salt concentrations of 500 to 700 mM NaCl (Fig 8). The SDS-PAGE analysis showed similar purity as in the loading study (~45%; Fig 9).

Statistical analysis of the elution study correlates with the results, showing that pH below 6.5 and salt concentration between 500 and 700 mM affects the amount of protein retrieved (Fig 10C). The summary of fit plot indicated a good model (Fig 10A). Two significant coefficients are included in the model, and although the linear term for salt concentration was not significant, the quadratic term was, and therefore the linear term was kept in the model (Fig 10B).

Column:	HiTrap Capto DEAE 1 ml
Sample:	10 ml S-TA (~4.0 mg/ml)
Start buffer:	100 mM sodium phosphate, 0.5 mM PLP, pH 6
Elution buffer:	100 mM sodium phosphate buffer, 200 to 1000 mM NaCl, 15 CV
Gradient:	25 CV, 0 to 100% step elution
Flow rate:	4 ml/min
System:	ÄKTA avant 25
Detection:	UV absorbance 280 nm

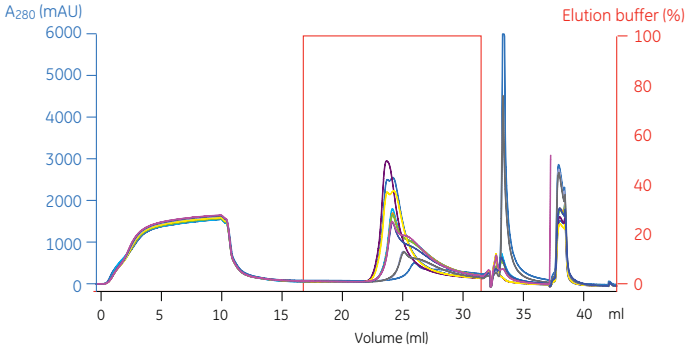


Fig 8. Elution optimization study. Absorbance trace at 280 nm for the different chromatographic runs. Sample was loaded for each run and step elution was performed according to the DoE experimental design.

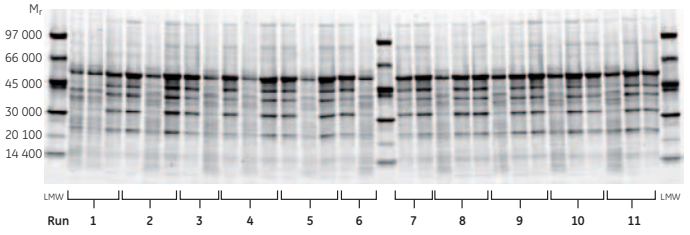


Fig 9. SDS-PAGE analysis of the elution study. Run order and conditions are according to Table 3. LMW=low molecular weight marker.

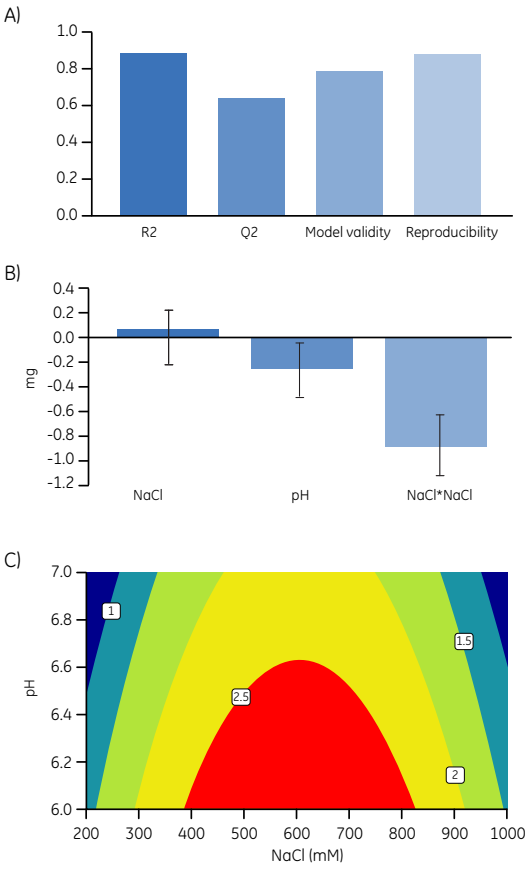


Fig 10. Statistical analysis of elution study. Total amount of S-TA (mg) eluted from the column was used to generate a statistical DoE model in UNICORN. (A) The summary of fit plot revealed a significant model. (B) Two significant coefficients in the model showed that both the salt quadratic term (NaCl*NaCl) and pH were significant. (C) The contour plot showed that the optimal elution area was below pH 6.4 and at a salt concentration between 500 and 700 mM.

Table 3. Experimental conditions and results for elution study

Run no.	Elution salt conc. (mM NaCl)	Elution pH	Amount target protein in peak (mg)
1	200	6.5	0.8
2	600	6.5	2.7
3	900	6.8	1.2
4	600	6.5	2.5
5	600	6.5	3.0
6	1000	6.5	1.4
7	900	6.2	2.0
8	300	6.8	1.8
9	300	6.2	1.8
10	600	6.0	2.9
11	600	7.0	2.1

Robustness test of optimized conditions

Two HiScreen Capto DEAE columns connected in series, giving a bed height of 20 cm, were used to perform a robustness test on the optimized process conditions using DoE. Figure 11 displays the SDS-PAGE analysis of the different runs from the robustness test. The different runs correspond to a ~10% change in the loading salt concentration and loading volume. The SDS-PAGE analysis and BCA protein assay showed no significant change in the obtained purity or amount of target protein (calculated as ~45% to 50% and 62 to 92 mg target protein for the different runs; Table 4). With a ~10% change in both loading salt concentration and loading volume, the statistical analysis displayed no significant model for neither purity nor the recovered amount of target protein (Fig 12). Thus, the process was deemed to be robust for the tested conditions.

Table 4. Experimental conditions and results for the DoE robustness test using the fractional factorial experimental design

Run no.	Loading conductivity (mM NaCl)	Loading volume (ml)	Purity (%)	Target protein (mg)
1	1.1	14.5	49	68
2	0.9	14.5	48	92
3	0.9	19	46	84
4	1	16.8	50	68
5	1.1	19	48	69
6	1	16.8	50	62
7	1	16.8	49	70

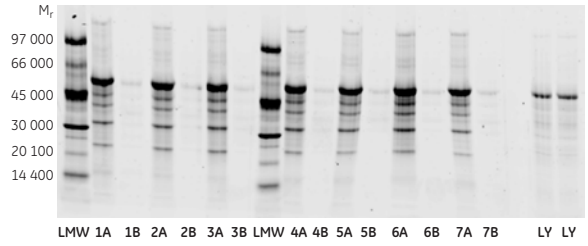


Fig 11. SDS-PAGE analysis of the robustness study. Lane number corresponds to the run order in Table 4. For each lane, A=eluted peak 1 and B=eluted peak 2. LY=Lysate; LMW=low molecular weight marker.

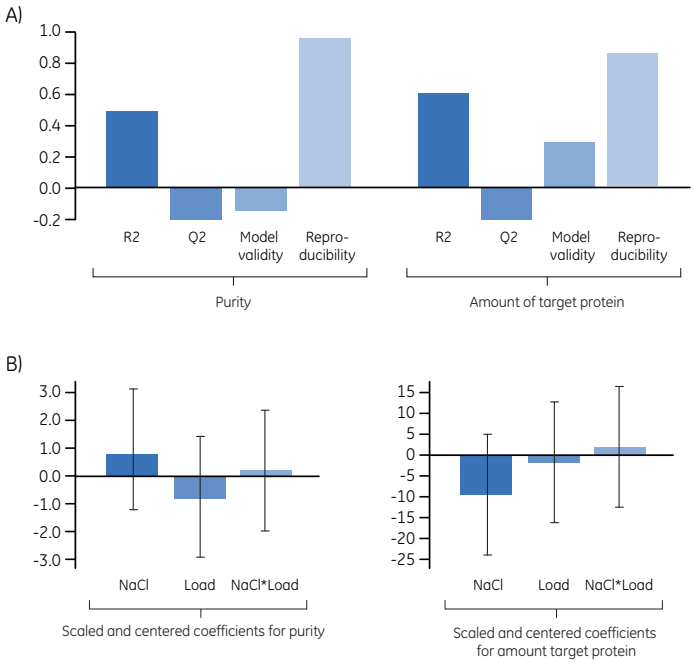


Fig 12. Statistical analysis of the robustness study. Purity and total amount of S-TA (mg) eluted from the column was analyzed using MLR (multiple linear regression). The summary of fit plot (upper) shows no significant model and no significant terms were obtained for the models generated (lower). The tested conditions were therefore deemed to be robust.

Gradient elution study

A gradient elution study was performed since our attempts to separate the active dimer from the monomer were not successful using step elution. Purification of S-TA using either step or gradient elution were run in order to compare the methods and determine whether monomer and/or dimer were present in the eluted peaks. ÄKTA avant 150 and HiScale 16/20 columns packed with Capto DEAE to a bed height of 20 cm were used for the study.

Figure 13 shows the SDS-PAGE analysis of the peak obtained from the step elution, and the two major peaks obtained from the gradient elution. The eluted peak fractions were analyzed using gel filtration (size exclusion chromatography) on prepacked Superdex 200 10/300 GL columns.

The gel filtration runs (Fig 14) indicated an approximate relative molecular weight (M_r) of 158 000 for the S-TA dimer which is an overestimation of the molecular weight, probably due to the non-globular behavior of the S-TA dimer accelerating its retention as compared to a globular protein passing through the gel filtration column. The results were also verified by the SDS-PAGE analysis showing that S-TA is the largest dominating protein and no other protein in the sample could generate such a large peak in the gel filtration.

A comparison of the gel filtrated samples and the HMW marker is shown in Figure 14. The results indicate that the active form of the enzyme, the dimer, is present in the first eluted peak (E1) from the gradient elution but not the second peak. The second peak (E2) contains smaller proteins, while the peak from the step elution contains a mixture of monomer and dimer. Gradient elution was therefore the preferred method, since the active dimer could be separated from the nonactive monomer. Hence, scaling up was performed using gradient elution.

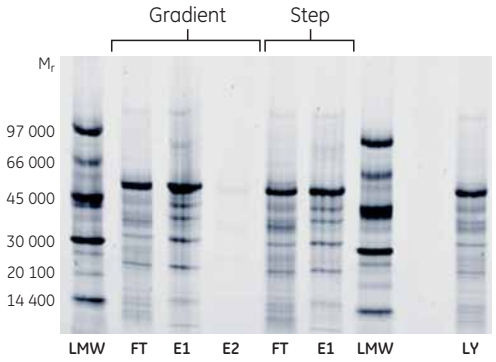


Fig 13. SDS-PAGE analysis of purification runs using either gradient or step elution. FT=Flowthrough, E1=eluted peak 1, E2=eluted peak 2, and LMW=low molecular weight marker. E1 corresponds to S-TA and an increase in intensity can be seen between the lysate (LY) and purified material.

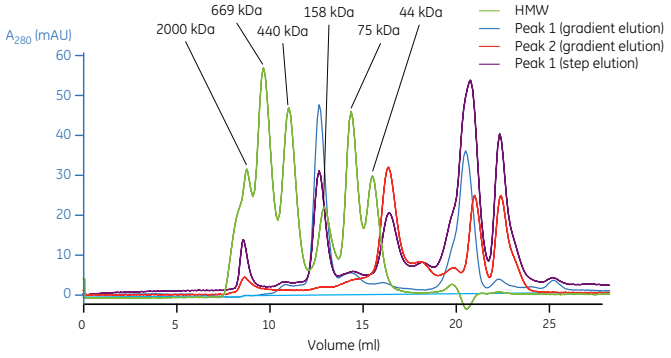
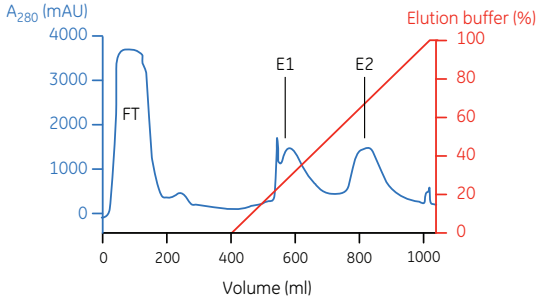


Fig 14. A comparison of the peaks obtained from the gel filtration analysis and HMW markers (HMW Marker Kit; green line), indicating that the S-TA dimer (the active form of the enzyme) is present in eluted peak 1 of the gradient elution run (E1; blue line). Eluted peak 2 from the gradient run (E2; red line) contains no dimer, and the only peak from the step elution (purple line) indicates a mixture of monomer and dimer.

Seamless scale-up using ÄKTA avant 150 system

For initial scaling up, HiScale 16/20 columns were packed with Capto DEAE to a bed height of 20 cm (Fig 15). The figure also shows a further scale-up to AxiChrom 50. The 40-fold scale-up using HiScale columns showed similar results to the 400-fold scale-up with AxiChrom. The SDS-PAGE analysis showed a purity of around 45% for both scale-up studies (Fig 16). AxiChrom 50 column was packed with Capto DEAE using ÄKTA avant 150 and the Intelligent Packing functionality in UNICORN.

Column:	HiScale 16/20
Media:	Capto DEAE
Sample:	103 ml S-TA (~4.0 mg/ml)
Start buffer:	100 mM sodium phosphate, 0.5 mM PLP, pH 6
Elution buffer:	100 mM sodium phosphate buffer, 200 to 1000 mM NaCl, 15 CV
Gradient:	25 CV, 0 to 100% linear gradient
Flow rate:	20 ml/min
System:	ÄKTA avant 150
Detection:	UV absorbance 280 nm



Column:	AxiChrom 50/300
Media:	Capto DEAE
Sample:	800 ml S-TA (~4.0 mg/ml)
Start buffer:	100 mM sodium phosphate, 0.5 mM PLP, pH 6
Elution buffer:	100 mM sodium phosphate buffer, 200 to 1000 mM NaCl, 15 CV
Gradient:	25 CV, 0 to 100% linear gradient
Flow rate:	150 ml/min
System:	ÄKTA avant 150
Detection:	UV absorbance 280 nm

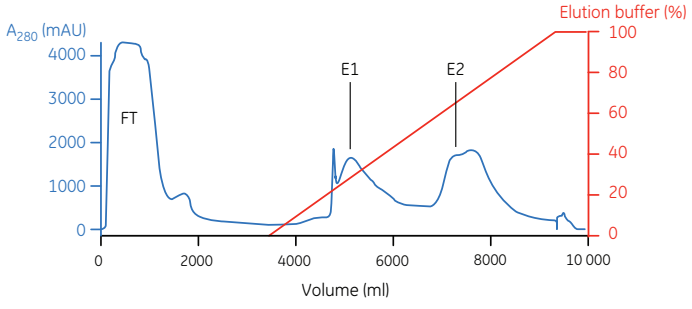


Fig 15. Chromatograms from the scale-up studies with a gradient elution using 1 M NaCl. Two major peaks eluted (E1 and E2), the first one corresponding to the target protein. FT=Flowthrough.

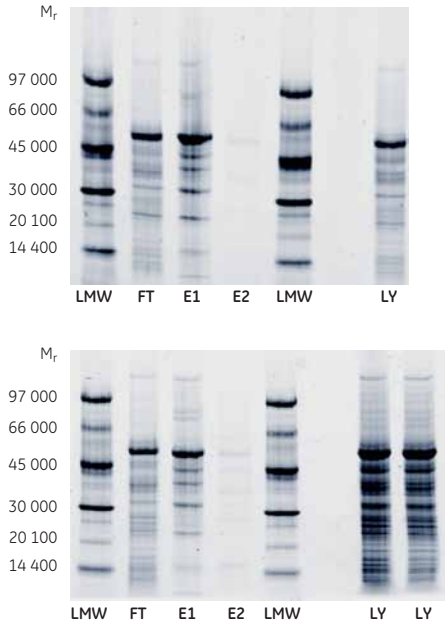


Fig 16. SDS-PAGE analysis of the 40-fold (upper) and 400-fold (lower) scale-up using HiScale and AxiChrom columns, respectively. FT=Flowthrough, E1=eluted peak 1, E2=eluted peak 2, LY=lysate, and LMW=low molecular weight marker.

Summary and conclusions

In this study, a single anion exchange chromatography capture step was developed for the purification of S-TA. Due to the complex nature of the sample, a purity of 40% to 50% was achieved. However, preliminary results suggest that immobilization of S-TA on NHS-activated Sepharose Fast Flow shows a higher specific activity for the immobilized purified enzyme compared to immobilized lysed material. A second purification step may be required if a higher purity is needed for a more effective biocatalytic reaction.

A higher load indicated a higher yield probably due to displacement effects on the column. Gradient elution was a better choice compared to step elution because of the capability to separate the active dimer from the non-active monomer and other contaminants.

Table 5. Summary of the different scale-up steps

Column	System	Scale-up factor	Feed load (ml)	Target protein (mg)
HiTrap	ÄKTA avant 25	1	1.5-15	0.5-7.5
HiScreen × 2	ÄKTA avant 150	9.4	14.5-19	60
HiScale 16/20	ÄKTA avant 150	40	67	160
AxiChrom 50/300	ÄKTA avant 150	400	625	1891

This study demonstrates a rapid strategy for process development and scale-up of a recombinant protein purification (Table 5). PreDictor 96-well filter plates, together with Assist software, provided quick and efficient media screening and optimization. For optimization of loading and elution conditions, DoE integrated in UNICORN 6 was used with HiTrap prepacked columns. DoE was also used later, with prepacked HiScreen columns, to confirm the robustness of the optimized process conditions. Finally, smooth scale-up was achieved using the same BioProcess™ media in larger HiScale and AxiChrom columns. Methods used in ÄKTA avant 25 were easily rescaled to larger columns in ÄKTA avant 150 using UNICORN 6.1. ÄKTA avant systems and dedicated column formats reduced process time and facilitated seamless scale-up.

References

1. Koszelewski, D. *et al.* ω–Transaminases for the synthesis of non-racemic α-chiral primary amines. *Trends Biotechnol.* **28**, 324-332 (2010).

Acknowledgements

We thank Cambrex AB (Karlskoga, Sweden) for kindly providing us with the sample used in this study.

Ordering information

Product	Code no.
Systems	
ÄKTA avant 25	28-9308-42
ÄKTA avant 150	28-9763-37
Software	
UNICORN 6.1 local or remote workstation license with DVD	28-9806-84
UNICORN 6.1 local or remote workstation license without DVD	28-9835-31
UNICORN 6.1 DVD package	28-9806-81
ImageQuant TL Software	28-9380-94
Plates and columns	
PreDictor AIEX Screening Plate, 20 µl	28-9432-89
HiTrap Capto DEAE 5 × 1 ml Columns	28-9165-37
HiScreen Capto DEAE 1 × 4.7 ml Column	28-9269-82
Superdex 200 10/300 GL Column	17-5175-01
HiScale 16/20 Column	28-9644-41
AxiChrom 50/300 Column	28-9018-31
Electrophoresis and imaging	
Multiphor II System	18-1018-06
ExcelGel SDS Gradient 8-18	80-1255-53
ExcelGel SDS Buffer Strips (anode and cathode)	17-1342-01
IEF/SDS Application Strips, 52 samples, 5-20 µl	18-1002-26
Deep Purple Total Protein Stain	RPN6305
LMW-SDS Marker Kit	17-0446-01
HMW-SDS Marker Kit	17-0615-01
Ettan DIGE Imager	63-0056-42

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First published Sept. 2010

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28-9827-80 AA 09/2010