Platform purification of a domain antibody

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Platform Purification of a Domain Antibody

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Introduction

Monoclonal antibodies (MAbs) are very successful for treatment of several different cancers and tumors. However, the low tissue penetration has led to the development of smaller sized biopharmaceuticals (Ab fragments) such as Fabs, single chain Fv (scFv) and domain antibodies (Dabs).

These molecules lack the Fc part of the antibody making a platform purification approach using Protein A impossible. However, with the introduction of the Protein L based affinity chromatography media (Capto™ L) new possibilities are introduced for capture of Dabs. By using high performance multimodal media for polishing, remaining contaminants are removed to trace levels. In this study a three-step process platform was developed for purification of Dab (Fig 2).

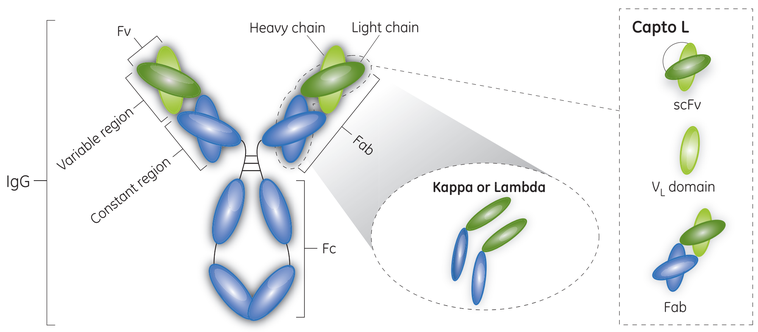

Fig 1. Different kinds of Mab fragments.

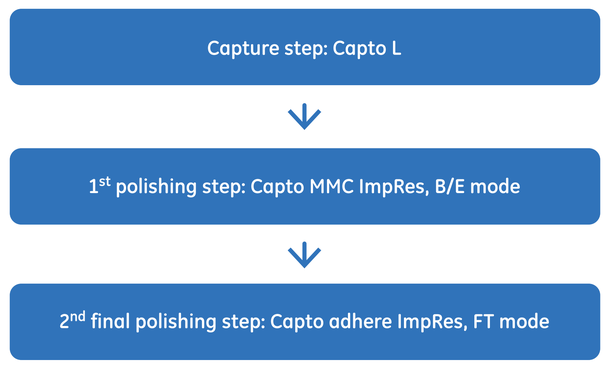

Fig 2. Summary of the platform purification approach for a Dab.

Capture step using Capto L

Based on DoE results, a wash step where high amounts of NaCl (> 500 mM) is added, gives significant reduction of *E. coli* proteins (ECP) in the following elution step.

The capture step was performed accordingly, using Capto L.

The resulting chromatogram is found in Fig 3. The product was eluted without NaCl since a wash without salt was included in the method. This had previously shown to be crucial for good recovery.

Running conditions

Sample:	Dab expressed in *E. coli*. Mw: 12.9 kDa, theoretical pI: 9.2. Heat treated supernanat loaded.
Sample volume:	2.7 L, 837 mg (0.31 g/L)
Column:	Capto L packed in XK 26/20 column, VT: 59.5 ml
Flow rate:	12.5 ml/min (residence time of 4 min)
Binding buffer:	20 mM sodium citrate, 800 mM NaCl, pH 5.0
Wash buffer 1:	20 mM sodium citrate, 800 mM NaCl, pH 5.0 (8 CV, Column Volumes)
Wash buffer 2:	20 mM sodium citrate, pH 5.0 (1.5 CV)
Elution buffer:	20 mM sodium citrate, pH 2.8 (5 CV)

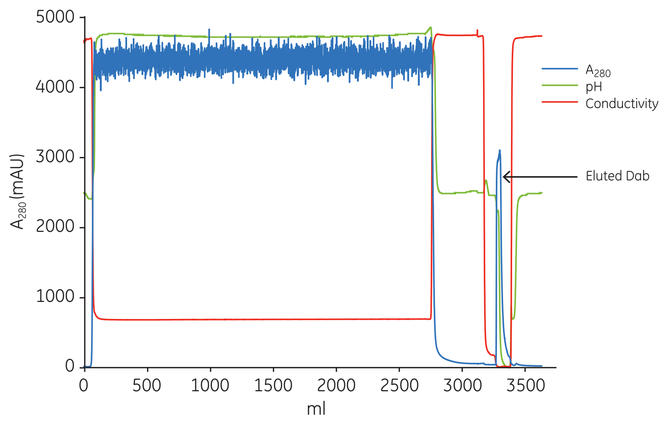

Fig 3. Chromatogram from the capture step using Capto L.

1st Polishing step using Capto MMC ImpRes

The use of a Monte Carlo simulation showed a robust area for yield and ECP content from pH 5.3-5.6 and a load of 18-20 g/l (Fig 4). This step was very effective in reducing an unknown yellow/brown colored impurity. This impurity could be followed in the chromatography at 400 nm (green trace in Fig 5).

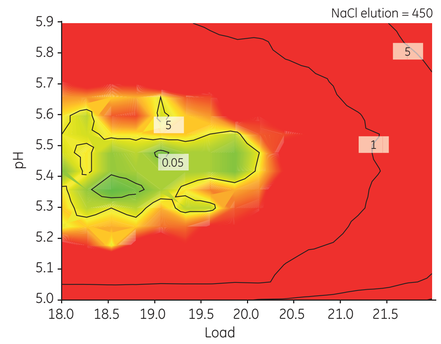

Fig 4. Monte Carlo simulation of yield and ECP content in the Capto MMC ImpRes step. The green surface shows the area where yield > 83% and an ECP content < 75 ppm could be achieved in a robust manner. The risk of failure limit was 0.1%. 100 000 experiments were performed in silico.

Running conditions

Sample:	Dab purified by Capto L and pH adjusted to 5.5 by the addition of 0.2 M NaOH
Sample volume:	27.4 ml, 324 mg (11.8 g/L)
Column:	Capto MMC ImpRes packed in HiScale™ 16/100 column, V_T: 17.5 ml
Flow rate:	4.4 ml/min (residence time of 4 min)
Starting buffer:	20 mM sodium citrate, pH 5.5
Wash buffer:	20 mM sodium citrate, 100 mM NaCl, pH 5.5 (3 CV, isocratic)
Elution buffer:	20 mM sodium citrate, 500 mM NaCl, pH 5.5 (7 CV, step)
CIP:	500 mM NaOH

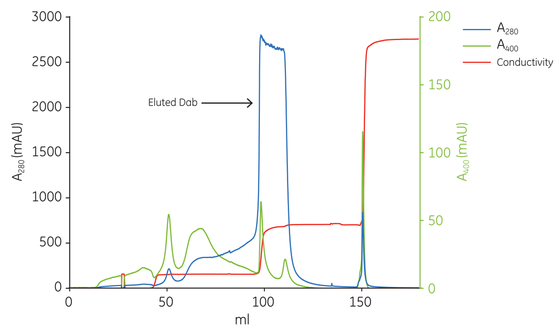

Fig 5. Chromatogram from the Capto MMC ImpRes first polishing step.

2nd polishing step using Capto adhere ImpRes

For the second and final polishing step Capto adhere ImpRes was chosen and optimized in a flowthough mode, Fig 6.

Running conditions

Sample:	Dab elution pool from Capto MMC ImpRes step, buffer exchanged on HiPrep™ 26/10 Desalting column to starting buffer.
Sample volume:	40 ml, 244 mg (6.1 g/L)
Columns:	3 × HiTrap™ Capto adhere ImpRes 1 ml connected in series. V_T: 3 ml
Flow rate:	1.5 ml/min (residence time of 2 min)
Starting buffer:	25 mM Tris-HCl, pH 8.5
Wash buffer:	25 mM Tris-HCl, pH 8.5 (7 CV)
Strip solution:	500 mM HAc (8 CV)
CIP:	1 M NaOH (3 CV)

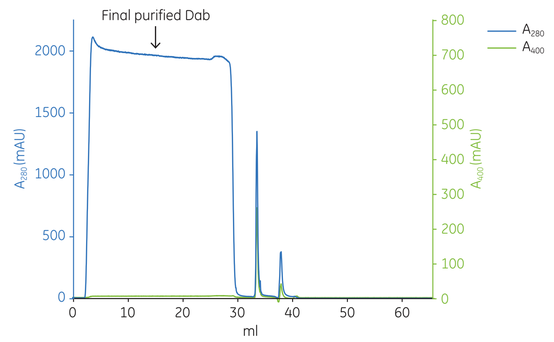

Fig 6. Chromatogram from the Capto adhere ImpRes polishing step.

Study and analyses performed

DoE

Response surface modeling (RSM) study with a Central composite circumscribed (CCC) design (star distance = 1.2) using the DoE software MODDE 9.1 (Umetrics, Sweden).

E. coli proteins (ECP) assay

Gyrolab system with anti-ECP antibodies (Cygnus Technologies), see Table 1.

Endotoxin analysis

Endosafe PTS cartridge from Charles Rivers Laboratories, see Table 1.

SDS-PAGE

PhastGel™ Homogeneous 20 (GE Healthcare) and stained with Coomassie™. SDS-PAGE analysis showed only one band for the final purified Dab (data not shown).

LC-MS

Zorbax® 300SB-C8 column (Agilent Technologies). LC-MS analysis showed a major peak of the correct molecular weight (data not shown).

Conclusions

- A three-step process platform for purification of a Dab has been developed and verified with a final total yield of 81%
- In the capture step high NaCl content with either high (> 8) (data not shown) or low (< 6) pH effectively reduces ECP
- The first polishing step efficiently removes a yellow/brown colored impurity, visible at 400 nm
- Final Dab product shows high purity according to all performed analyses

Table 1. Summary table of yield and purity analysis of a Dab platform purification

Step	Step yield, %	Endotoxin, EU/ml	Endotoxin, EU/mg	ECP, ng/ml	ECP, ppm	Protein L, ppm
Dab start	100	*	>2 000 000*	*	>200 000*	N.A.
Capture step: Capto L	99.6	18	1.53	1846	159	< LOQ†
1st Polishing step: Capto MMC ImpRes	86.4	1.8	0.20	54	9.0	< LOQ
2nd Polishing step: Capto adhere ImpRes	93.9	< 0.5 (< LOQ)	< 0.09 (< LOQ)	30	5.5	< LOQ
Total yield:	**80.8**					

* *Not analyzed, data based on previous experience.*
† *Limit of quantitation.*

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