Automated Parallel Chromatographic Separations in Process Development

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Summary

Development and optimization of chromatographic separation conditions is normally a time consuming task in the large scale production of proteins such as monoclonal antibodies.
In this study a 96 well formatted MediaScout® RoboColumn® array was adapted for automated operation in a modified commercial liquid handling workstation and used for the development of a purification strategy for a recombinant, secretionary enzyme by screening different cation exchange chromatography media according to optimize yield and purity (Fig. 1).
The quality of the purified enzyme was checked by using cutting-edge high throughput LabChip-SDS Electrophoreses (Caliper).
The combined approach of Atoll's MediaScout® chromatography tools and Tecan's liquid handling workstation Freedom EVO® enables to speed up the development of a purification process by nearly one order of magnitude.

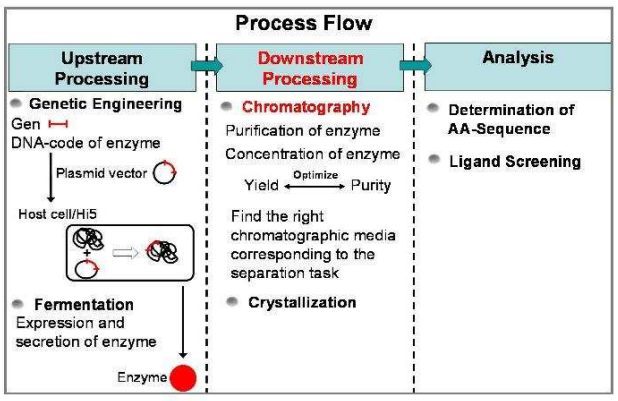

FIGURE 1

API development. Process flow.

Experimental Setup

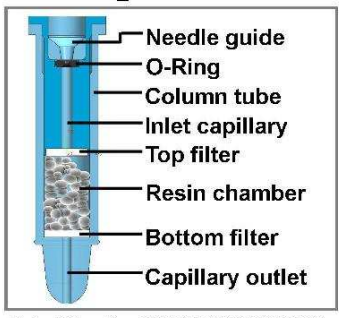

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FIGURE 2

MediaScout® RoboColumn®.

FIGURE 3

Liquid handling workstation Tecan Freedom EVO. Modified for use with RoboColumn®.

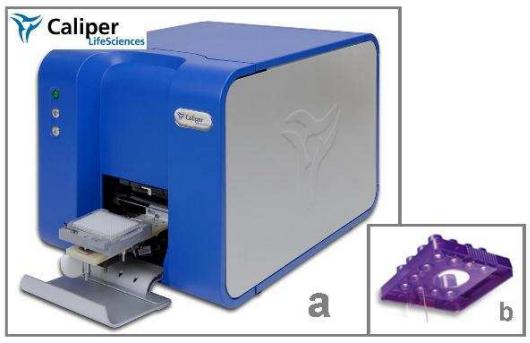


FIGURE 4

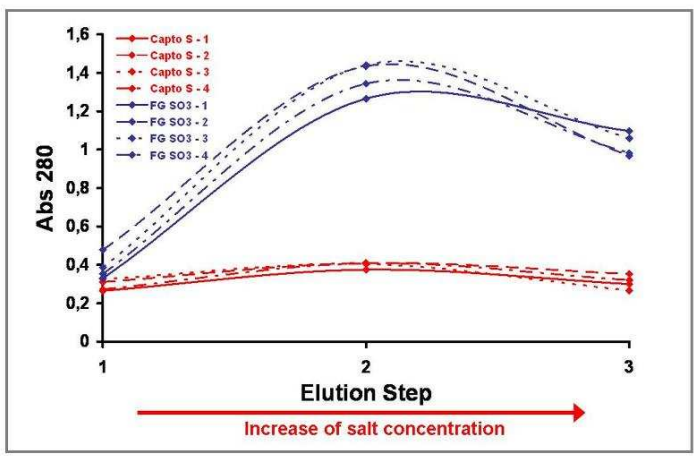
a LabChip GXII System.
b Microfluidic chip.

Purification

FIGURE 5

Atomated small scale purification of enzyme using RoboColumn®. Elution profile.

- Fractogel® EMD SO_3^- shows a higher overall protein binding capacity than Capto™ S.
- Elution profile does not include data about the purity of the desired enzyme. ⟶ SDS Electrophoreses



Column	RoboColumn®
V_{Column}	200 µl
Media 1	Fractogel® EMD SO_3^-
Media 2	Capto™ S
V_{Sample}	6.0 ml
Flow Rate	150 cm/h
Binding buffer	10 mM MOPS, pH 6.8 50 mM NaCl
Elution buffer 1	10 mM MOPS, pH 6.8 150 mM NaCl
Elution buffer 2	10 mM MOPS, pH 6.8 400 mM NaCl
Elution buffer 3	10 mM MOPS, pH 6.8 800 mM NaCl

SDS Electrophoreses

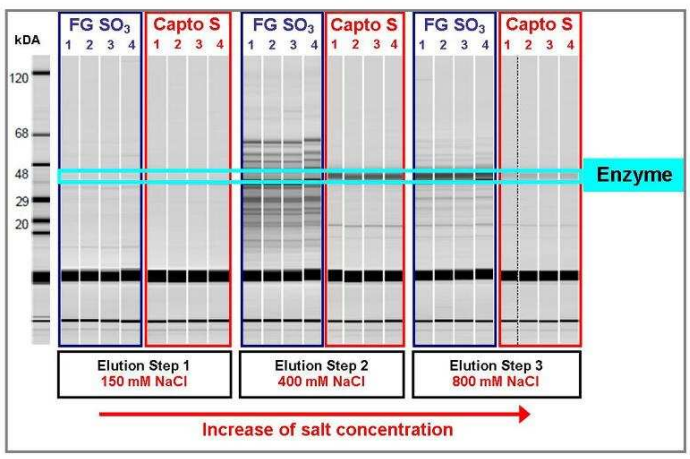
FIGURE 6

SDS Electrophoreses after purification of enzyme using LabChip technology.

- Capto™ S shows a more specific binding behavior and therefore higher purity for the desired enzyme.

Media	Fractogel® SO_3	Capto™ S
Yield	++	+
Purity	--	++
∑	0	+++

- Fractogel® EMD SO_3^- is binding a lot of undesired proteins (impurities) as well.



Conclusions

- Atoll's 96 MediaScout® RoboColumn® array was successfully adapted on TECAN's liquid handling workstation Freedom Evo® for chromatographic applications.
- Small scale automated high throughput separations using bio-chromatography were sucessfully applied for screening of cation exchange chromatography media in process develoment.
- The desired enzyme was sucessfully concentrated and purified using MediaScout® RoboColumn® packed with Capto™ S.
- Using the combined approach of Atoll's MediaScout® RoboColumn®, Tecan's liquid handling workstation Freedom EVO and Caliper's LabChip technology enables to reduce costs per experiment significantly due to saving process time, API, process-relevant products and solvents.