

Summary

A miniaturized column system in standard microplate format, harbouring exchangeable arrays with up to 96 individual MediaScout® RoboColumns, is adapted for automated operation in a commercial liquid handling workstation (TECAN Freedom Evo®). The combined robotic system allows to perform automated high throughput small scale bio-chromatographic separations by running up to eight individual columns simultaneously.

Application examples include protein separations by step gradient elution after binding the samples to cation or anion exchangers, elution after binding to affinity chromatography media by changed isocratic conditions, as well as de-salting under isocratic conditions. These applications are successfully implemented for parameter elucidation and optimization in process development of therapeutic protein production and in-process monitoring of fermentation broth for mAB-production.

In particular sample preparation for mass spectrometry analysis in antibody screening using common gravity induced de-salting columns is a time-consuming procedure. The combined use of automated desalting of mAB-samples, after a Protein A purification step, and the dedicated mass spectrometer enables to speed up the process significantly with the capacity to run and analyze hundreds samples in parallel per day.

System configuration

Media Scout® ready-to-use RoboColumns are available pre-packed with any available chromatographic media to defined compression, in order to replicate conditions in larger columns. Up to 96 of them may be fixed onto a 96 array compatible base plate, in any desired order (Fig. 2). Utilizing injection moulding technique, the RoboColumn is developed with fixed bed height, made from polypropylene.

The RoboColumn inlet is equipped with a patented needle adapter seal, utilizing an appropriate sized o-ring located at the top of the column (Fig. 1).

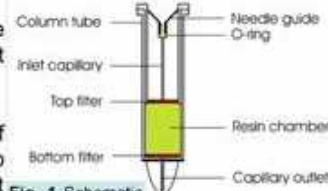


Fig. 1 Schematic drawing of a MediaScout® RoboColumn.

Therefore the eight channel liquid delivery system (LiHa) of the robotic workstation (Fig. 4) can be reversibly connected to the columns, in order to allow uptake and loading of different volumes of samples and buffer solutions in the individual steps of the separation procedure.

Liquid flow in the columns is driven by positive liquid displacement, rather than by air pressure or gravity, thus mimicking the situation in columns individually connected to a one channel stand-alone chromatography system.

To integrate the 96 column array onto the platform of the robotic workstation and to operate it conform to chromatographic requirements, the height adjustable Te-Chrom plate holder (Fig. 2) was developed.

This construction enables the optional use of appropriate elution plates of different sizes.

For shown experiments, a 96 RoboColumn array was packed with Sephadex™ G-25 Superfine SEC media (GE Healthcare).

After a Protein A purification step mAB-samples were loaded onto the 96 RoboColumn array and eluted into a standard deep well plate.

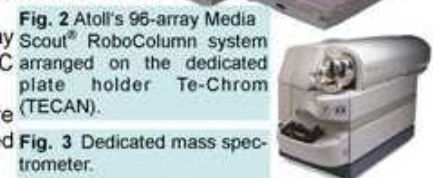


Fig. 2 Atoll's 96-array Media Scout® RoboColumn system arranged on the dedicated plate holder Te-Chrom (TECAN).

The fractional desalted mAB-samples of different batches were transmitted to the auto sampler of a dedicated mass spectrometer (Fig. 3) in order to cross-check the mAB-structure against a standard pattern, e.g. grade of glycosylation, amino acid sequence of heavy and light chain.

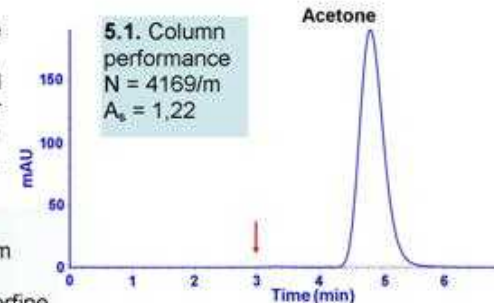


Fig. 4 TECAN Freedom EVO® liquid handling workstation.

Method development

Before the experimental setup could be adapted to the automated robotic system, a comparable LC-column (Fig. 6), packed with the desired media, was checked for packing performance using a common HPLC-System and continuous monitoring.

Fig. 5
 Column: MediaScout® MiniChrom
 Dimensions: 5 x 25 mm (CV 0.5 ml)
 Packing media: Sephadex™ G-25 Superfine
 Flow rate: 0.5 ml/min (150 cm/h)
 Detection: OD 280 nm
 Sample injection: 3 min



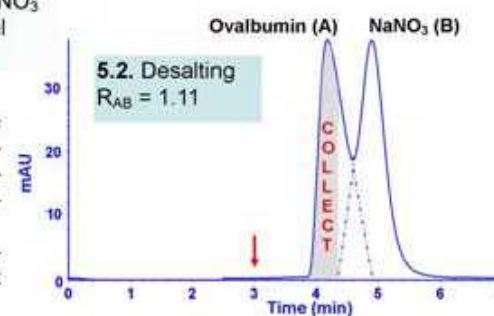
5.1. Column performance
 $N = 4169/m$
 $A_s = 1,22$



Fig. 6 MediaScout® MiniChrom 5-25.

Experiments:
 5.1. Sample: 20 µl of 1% Acetone
 5.2. Sample: 20 µl of Ovalbumin, NaNO₃
 c(Ovalbumin) = 1 mg/ml
 c(NaNO₃) = 10 mg/ml

In order to determine the elution volume respectively the fraction range of the protein sample, isocratic elution of an ovalbumin / sodium nitrate-mixture was performed. Due to experimental setup a total desalting of the protein sample ($R \geq 1.50$) is not required.



5.2. Desalting
 $R_{AB} = 1.11$

Proof of concept

7.1. MS after non continuous chromatography.
 Liquid flow induced by gravity.

Column: illustra NAP™-5 (GE Healthcare)
 Dimensions: 9.5 x 28.7 mm (CV 2.0 ml)
 Packing media: Sephadex™ G-25 DNA grade
 Flow rate: n.a. (gravity)
 Process time: **50 min/8 samples**

Fig. 7 Mass spectra of reduced mAB after fractional desalting using different operational modes show a perfect match.

Sample: 90 µg reduced mAB in 60 µl 20% C₂H₅N/ 1% HCOOH
 c(mAB) = 1,50 µg/µl

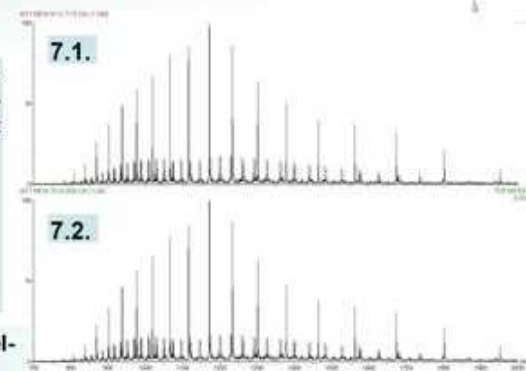


Fig. 8 Schematic drawing of illustra NAP™-5 column.

7.2. MS after true continuous, parallel-chromatography.
 Liquid flow driven by positive liquid displacement and controlled through dispensing speed of the robotic system.

Column: MediaScout® RoboColumn
 Dimensions: 5 x 30 mm (CV 0.6 ml)
 Packing media: Sephadex™ G-25 Superfine
 Flow rate: 0.5 ml/min (150 cm/h)
 Process time: **5 min/8 samples**

Fig. 9 Row of MediaScout® RoboColumns.

Optimization

Fig. 10 Mass spectra of reduced mAB after fractional desalting varying sample concentration. Quality of mass spectra is not affected by decreasing sample concentration.

Column: MediaScout® RoboColumn
 Dimensions: 5 x 30 mm (CV 0.6 ml)
 Packing media: Sephadex™ G-25 Superfine
 Flow rate: 0.5 ml/min (150 cm/h)

Sample: Reduced mAB in 60 µl 20% C₂H₅N/ 1% HCOOH

10.1. c(mAB) = 1.5 µg/µl
 m(mAB) = 90 µg
 10.2. c(mAB) = 1.3 µg/µl
 m(mAB) = 78 µg
 10.3. c(mAB) = 1.0 µg/µl
 m(mAB) = 60 µg
 10.4. c(mAB) = 0.6 µg/µl
 m(mAB) = 36 µg
 10.5. c(mAB) = 0.3 µg/µl
 m(mAB) = 18 µg

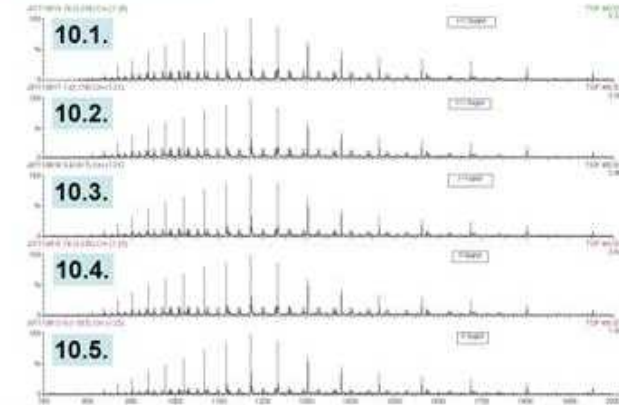


Fig. 11 Row of MediaScout® RoboColumns 5-30 with fixed stainless steel tips inserted.



Conclusions

- Atoll's 96 MediaScout® RoboColumn array was successfully adapted on TECAN's liquid handling workstation Freedom Evo® for chromatographic applications.
- The transfer from a common one channel stand-alone chromatography system into a fully automated, parallel chromatography system was successfully completed.
- Time consuming manually desalting of protein samples (mAB) using common gravity induced columns, was effectively replaced by automated HTS-chromatography using robotically operated MediaScout® RoboColumns. Process time per sample was reduced about 10 times.
- The consumption of sample was optimized to a minimum without any affect on experimental data.
- **Costs per experiment could be significantly reduced due to saving process time, API, process-relevant products and solvents.**

Acknowledgments

TECAN (Switzerland and Germany) is gratefully acknowledged for generously supporting this work by providing Freedom EVO® hard- and software, as well as for helpful practical training and numerous advices.