



High throughput chromatographic separations at small scale in a liquid handling workstation

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1. Small scale separations of biomolecules by chromatography

Chromatography is still one of the most powerful separation methods for the isolation of biomolecules from complex biological sources.

Common methods

- IEX, HIC, IMAC, MMC, AF, SEC (including de-salting)

Performed at small scale for analytical purposes, i.e.,

- Sample preparation (e.g. for subsequent MS)
- Resin screening (e.g. to select useful separation media)
- Parameter screening (in scale-down process optimization)
- Process validation



1. Small scale separations of biomolecules by chromatography

Some advantages of small scale bio-chromatography

- Low consumption of valuable samples
- Reduced amount of chemicals (reagents, buffers, etc.)
- Small resin volumes
- Reduced waste
- Larger number of samples per time
- Reduced expenses per sample



2. Demand for HTP-techniques in small scale separation.

Where numerous samples are to be processed in a short time, e.g.

- Cell culture screening for selection of mAbs
- In-process monitoring in biopharmaceutical production
- 2D-parameter optimization in process chromatography development (i.e. pH versus ionic strength in IEC)
- Medical diagnostic applications of bio-chromatography

3. Brief history of small chromatography columns for bioseparation

Inner dimensions of commercial pre-packed columns

<u>Trade name</u>	<u>Length × Inner Diameter</u>	<u>Column Volume</u>	<u>vendor</u>
	(mm × mm)	(ml)	
HiTrap™	25.0 × 7.0	1.0	GEH
AcroSep™	14.8 × 9.4	1.0	Pall
ToyoScreen	6.4 × 31.0	1.0	Tosoh Bioscience
Fractogel® EMD			
Scout Column	19.4 × 8.1	1.0	Merck KGaA
Econo-Pac®	36.0 × 5.9	1.0	BioRad
MediaScout®			
MiniChrom	50.0 × 5.0	1.0	Atoll
	20.0 × 8.0	1.0	Atoll
	25.0 × 5.0	0.5	Atoll
	10.0 × 5.0	0.2	Atoll
	5.0 × 5.0	0.1	Atoll



4. Approaches towards automated multiple small scale separations

4.1 Batch adsorption, performed in standard 96-well filter plates

Advantages

- Simple operations, multiple sample processing in a relative short time, abundant equipment (shakers, centrifuges, vacuum manifolds)
- Up to 4 x 96 samples in parallel

Disadvantages

- One theoretical plate per separation step, incomplete separation (pore volume retains previous fraction)
- Time consume for equilibration (shaking) and elution (limited membrane permeability)
- Vacuum operation often inadequate for protein separation



4. Approaches towards automated multiple small scale separations

4.2 Spin columns

Advantages

- Simple operations, multiple sample processing in a relatively short time, abundant equipment (centrifuges)
- Unlimited number of samples (up to about 24 samples in parallel)

Disadvantages

- Asymmetric flow distribution, relative to column length axis (induced by inhomogeneity of centrifugal field)

4. Approaches towards automated multiple small scale separations

4.3 Mini column arrays, operated by positive liquid displacement

Advantages

- True chromatography mode (symmetric flow distribution relative to column length axis)
- 10-100 theoretical plates (depends on resin type and column geometry)
- Simple operations, multiple sample processing in a short time
- Unlimited number of samples (yet up to 8 samples simultaneously)
- Manual method development possible (pipet columns)

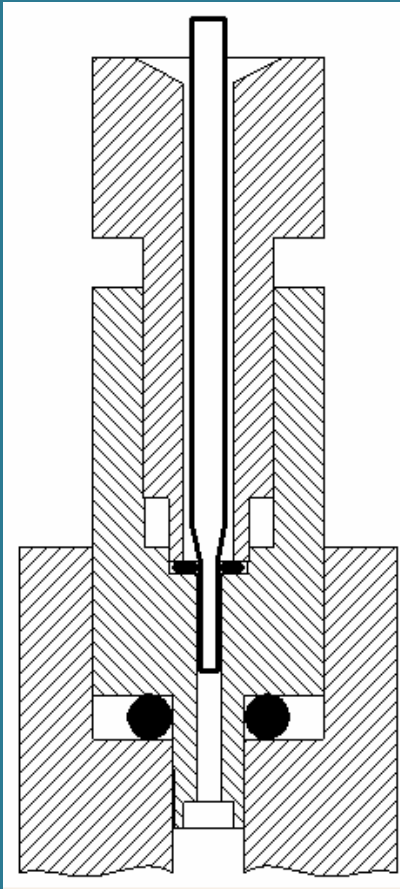
Disadvantages

- Gravity flow not feasible, due to resin compression
- Multiple controlled liquid delivery systems required
- Efforts for method implementation



5. Development of Versatile RoboColumn 96-Array

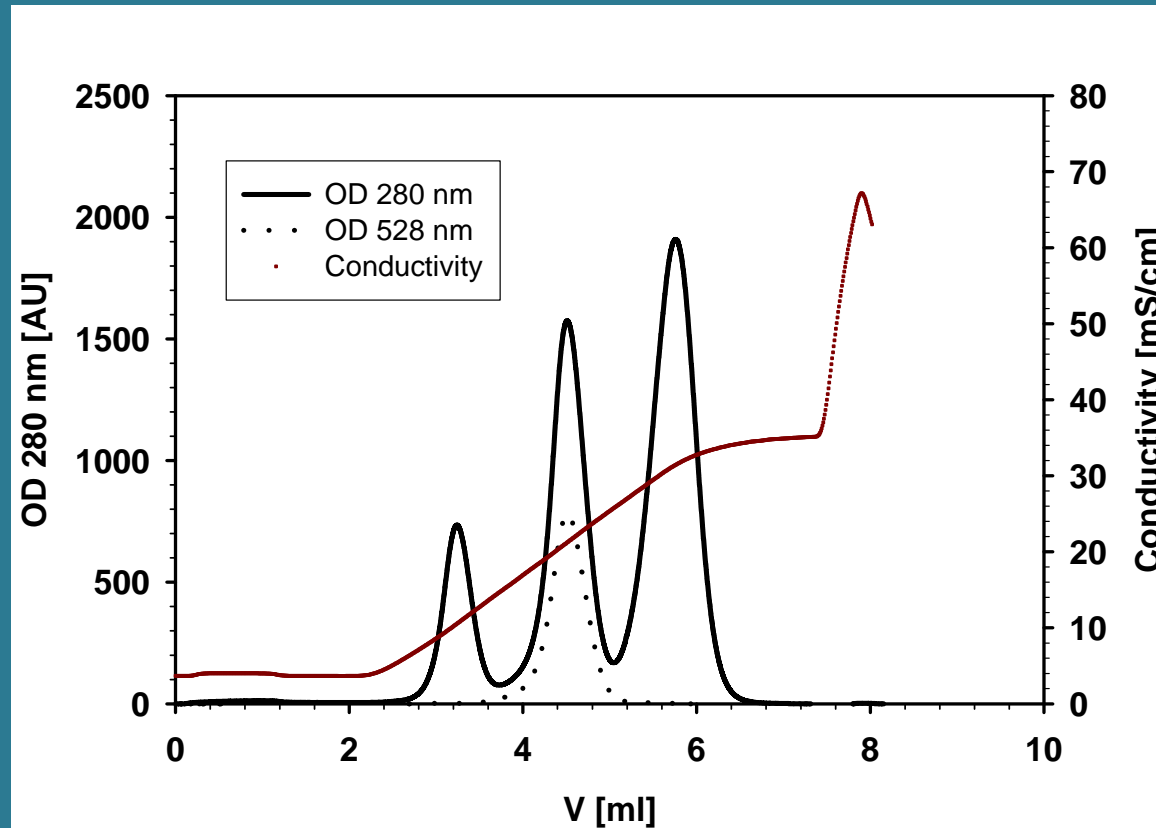
5.1 Column adapter for repeated connection to fixed metal tips and prototype



Taken from Tim Schroeder, Diploma Thesis, Research Center Jülich, Germany, 2005

5. Development of Versatile RoboColumn 96-Array

5.2 Separation of Proteins (RNase A, cytochrome c, lysozyme) on **prototype** small column with top fixed tip connector (gradient elution, continuous monitoring)



Column Dimensions (L × ID): 22 × 2.4 (mm × mm)

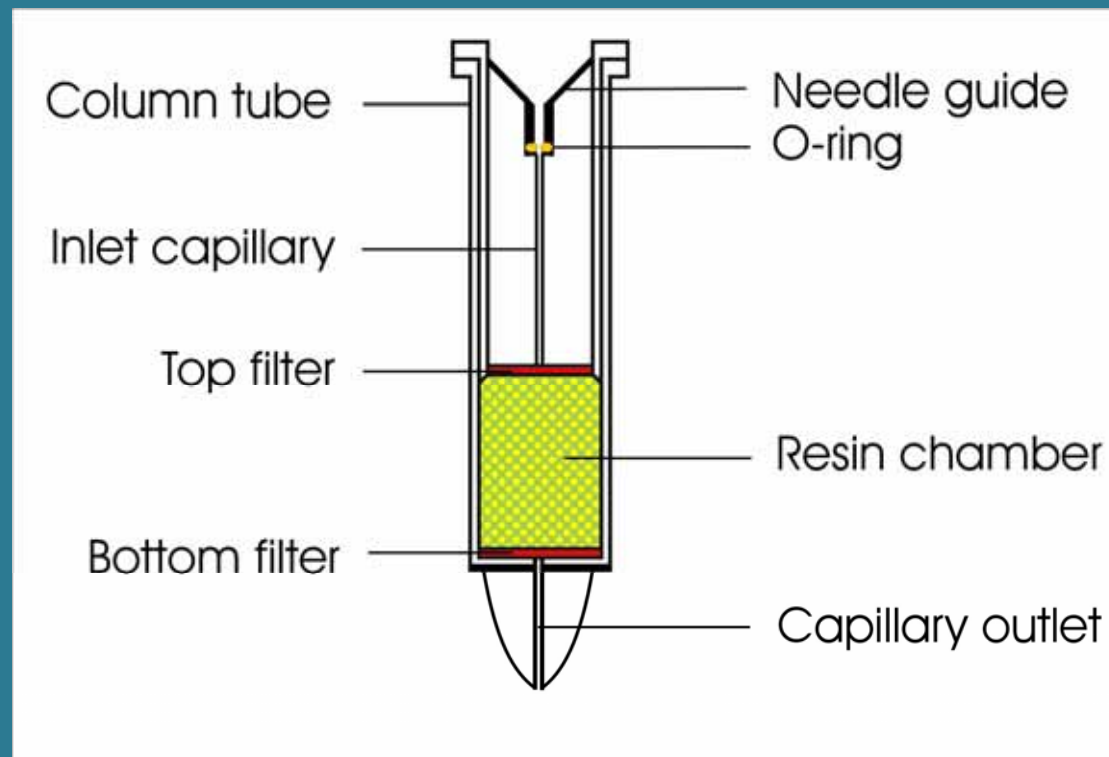
Column Volume: 0.1 ml

Packing Media: SP Sepharose XL

Taken from Tim Schroeder,
Diploma Thesis, Research
Center Jülich, Germany, 2005

5. Development of Versatile RoboColumn 96-Array

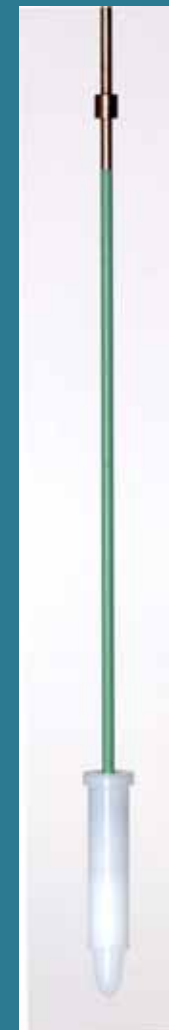
5.3 Commercial version of low volume RoboColumn with top fixed tip connector and capillary outlet



Column Dimensions (L × ID): 10 x 5 (mm × mm)

Column Volume: 0.2 ml

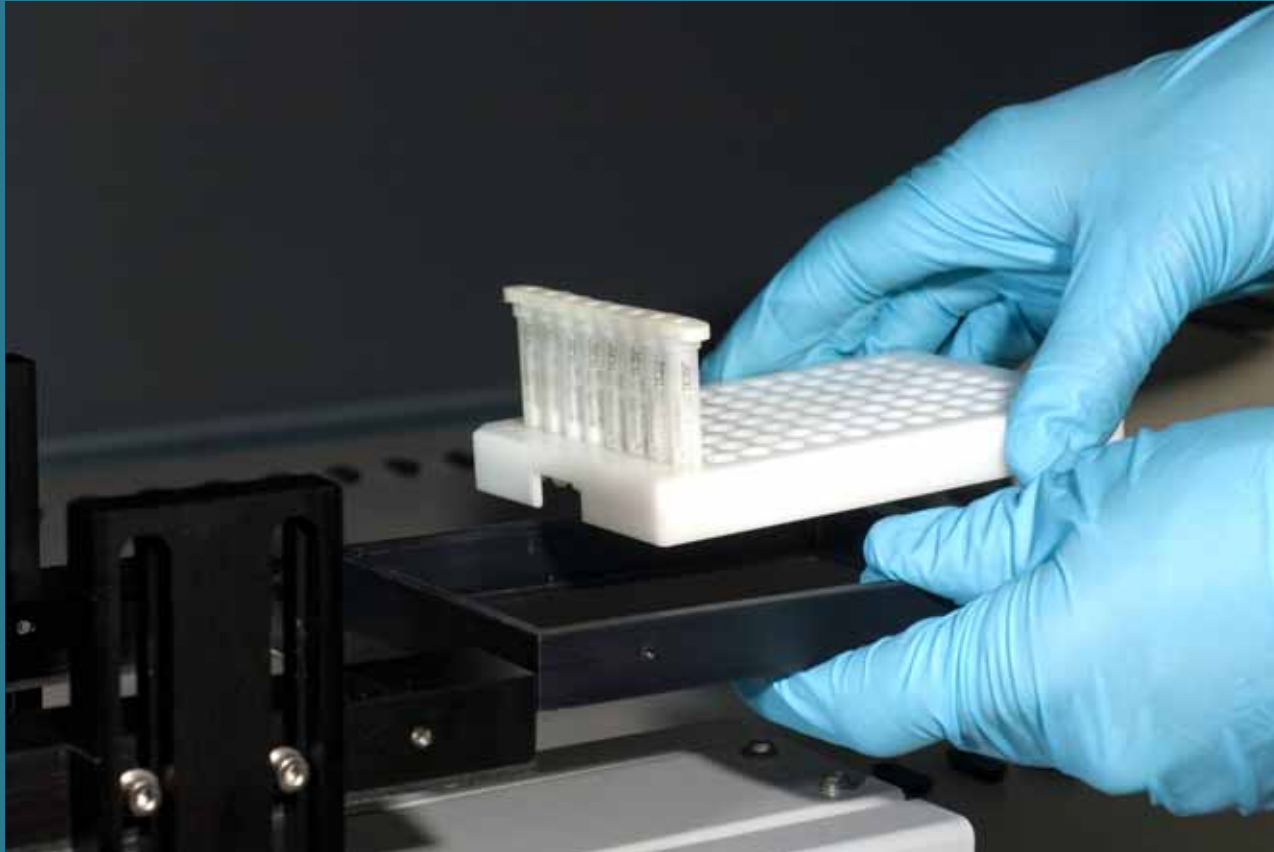
Packing Media: any desired





5. Development of Versatile RoboColumn 96-Array

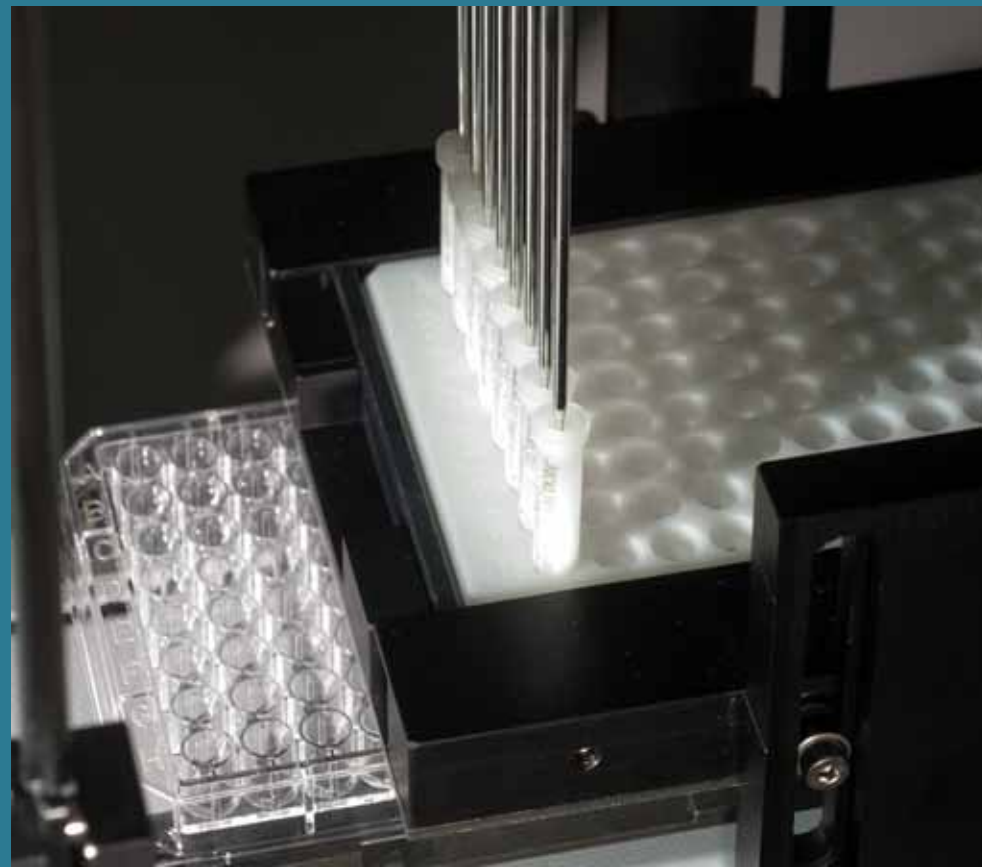
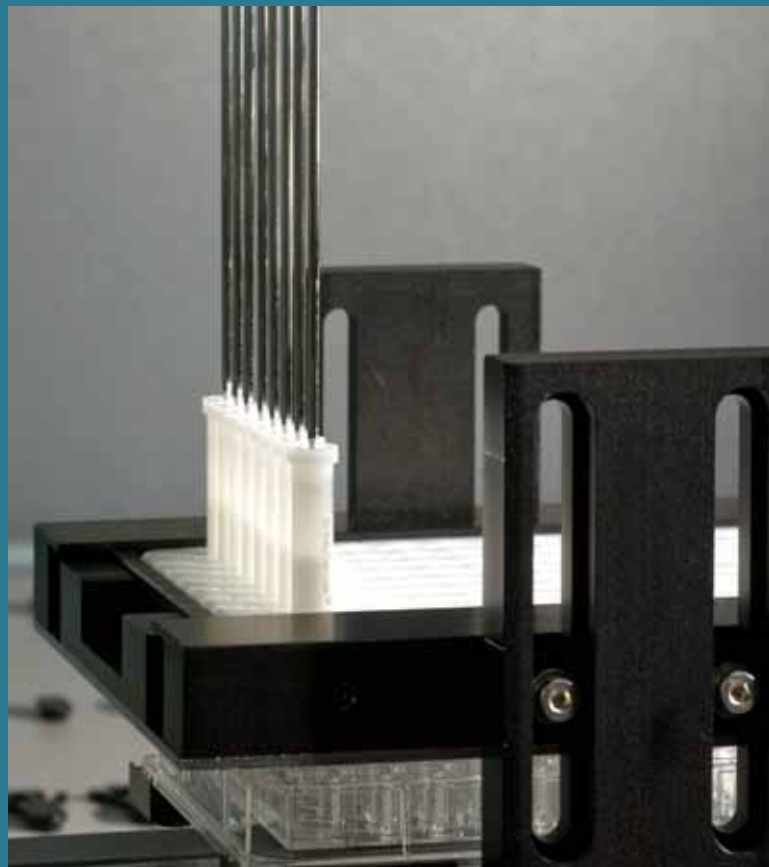
5.4 RoboColumns mounted on 96-well microplate compatible base plate



Insertion of column array into dedicated plate holder of a liquid handling workstation

5. Development of Versatile RoboColumn 96-Array

5.5 Eight RoboColumns reversibly connected to eight independent liquid delivery systems via inserted fixed metal tips



Fractions are collected into standard 96-well microtiter plates on a controlled transport system of a Te-Stack module

6. Proof of Concept for RoboColumn Arrays

6.1 System Configuration



**Tecan Freedom Evo Pipetting Robot Configured As
Chromatography Workstation**



6. Proof of Concept for RoboColumn Arrays

6.1 System Configuration

- **Freedom EVO (TECAN)**

 - Liquid handling arm (LiHa)
 - Robotic manipulator (RoMa)
 - Te-Stack™ with plate holder
 - MTP-reader (infinite M200)

- **MediaScout® RoboColumns (Atoll)**

 - packed with desired
chromatography media



6. Proof of Concept for RoboColumn Arrays

6.2 Automated Separation of Cytochrome c from Vitamin B₁₂ on a Cation Exchanger

Step 1 Column equilibration

Step 2 Sample application

Step 3 Elution of Vitamin B₁₂

Step 4 Elution of Cytochrome c

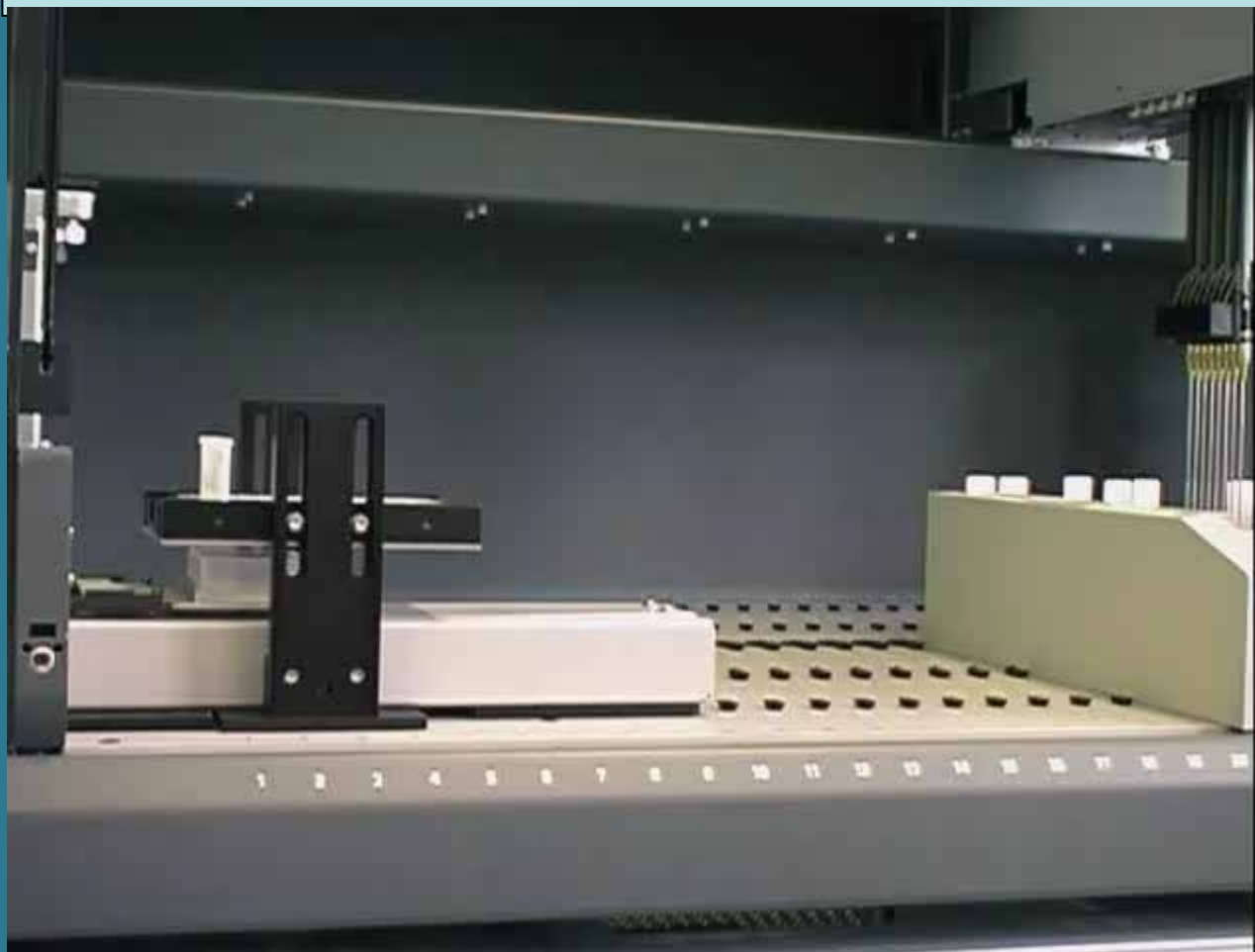
Step 5 Evaluation of microplate



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6.2 Automated Separation of Cytochrome c from Vitamin B₁₂ on a Cation Exchanger

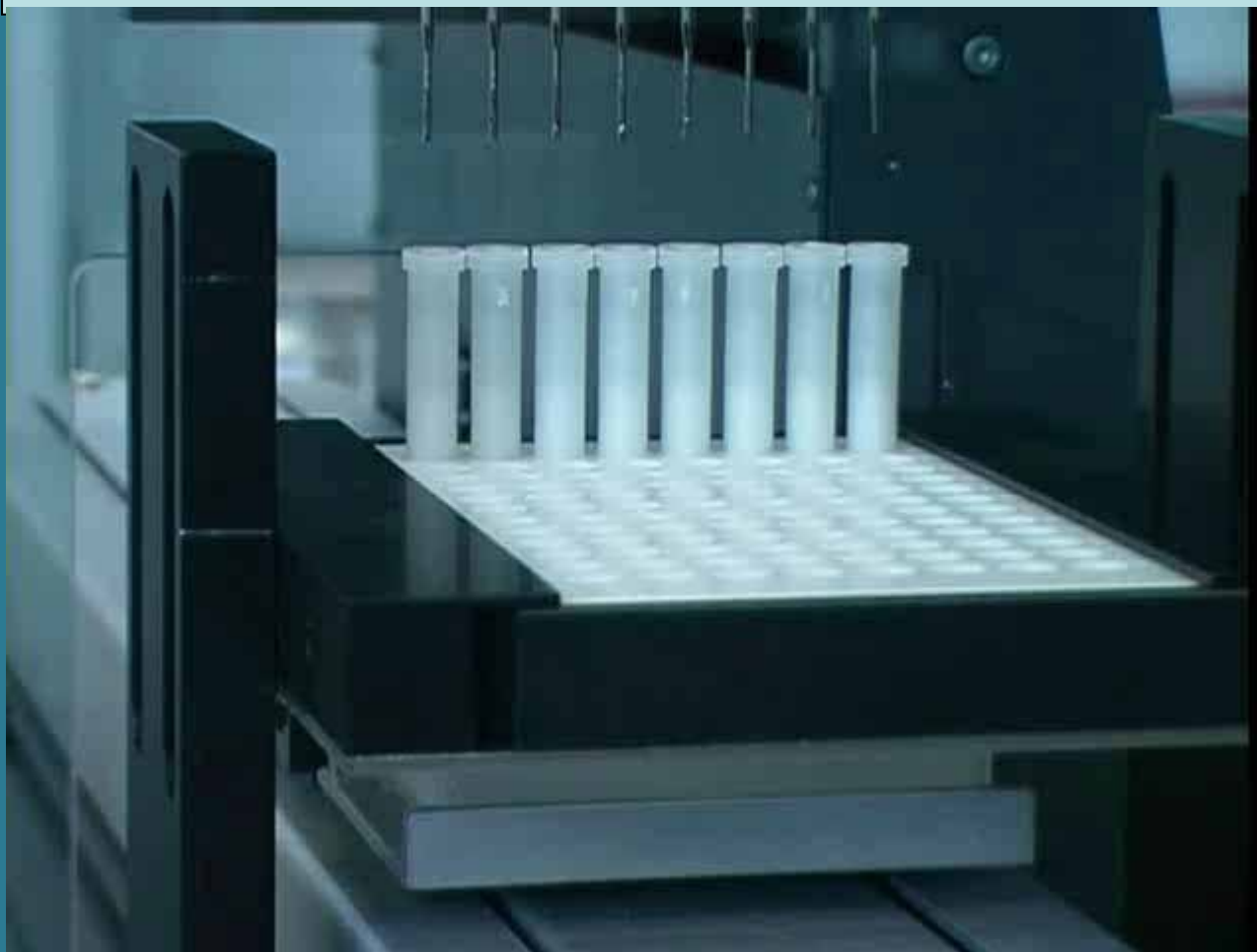
Step 1 Column equilibration



6. Proof of Concept for RoboColumn Arrays

6.2 Automated Separation of Cytochrome c from Vitamin B₁₂ on a Cation Exchanger

Step 2 Sample application



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6.2 Automated Separation of Cytochrome c from Vitamin B₁₂ on a Cation Exchanger

Step 3 Elution of Vitamin B₁₂

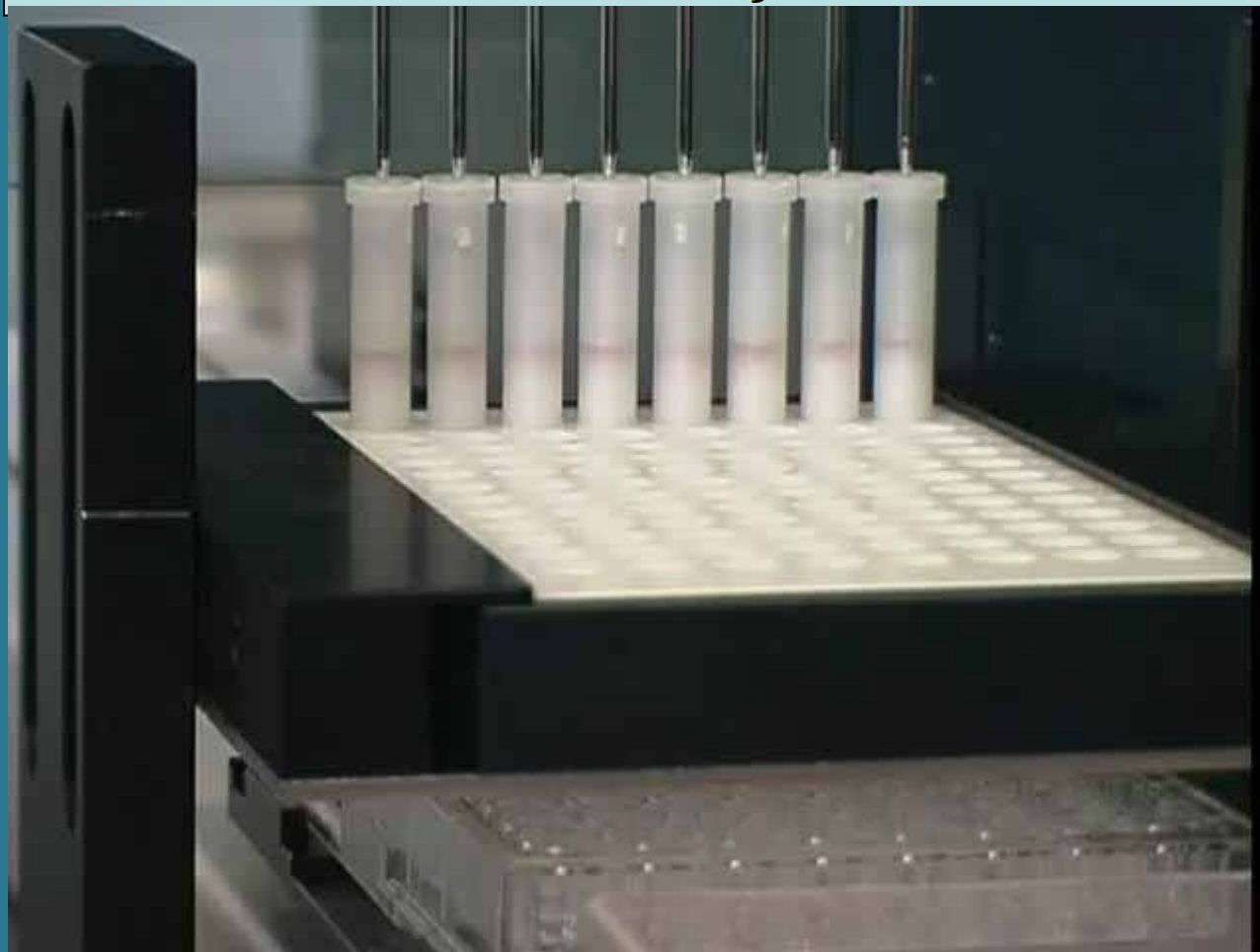




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Step 4 Elution of Cytochrome c





6. Proof of Concept for RoboColumn Arrays

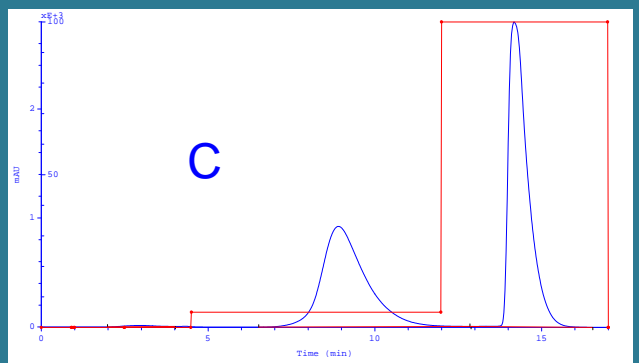
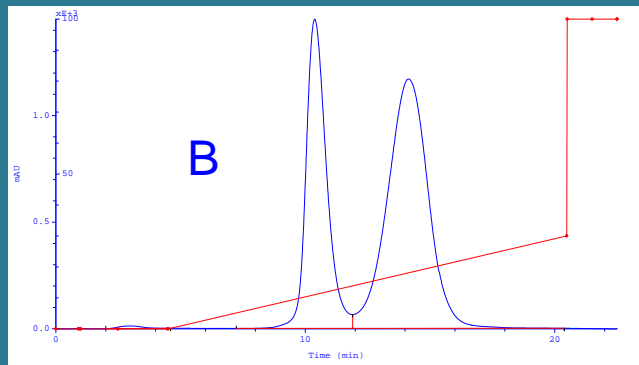
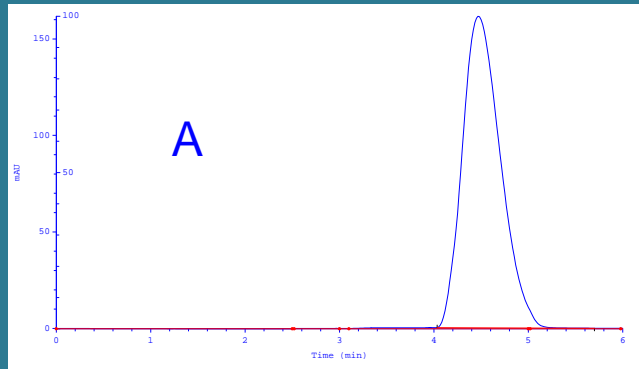
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Step 5 Evaluation of microplate



6. Proof of Concept for RoboColumn Arrays

6.3 Verification of Small Column Performance (MiniChrom 5-10)



Column 5 x 10 mm (c.v. 0.2 ml)

Packing media Toyopearl SP-650S

Flow rate 0.5 ml/min (150 cm/h)

Detection OD 280 nm

Sample (A) 20 μ l 1% acetone

$$A_s = 1.3$$

$$N = 65 \text{ (6500/m)}$$

Sample (B, C) lysozyme, cytochrome c (each 1 mg/ml)

Gradient (B) 0 – 1 M NaCl in 0.02 M Na-phosphate buffer, pH 7.0 within 30 c.v.

(C) 0.1 M NaCl (step 1)

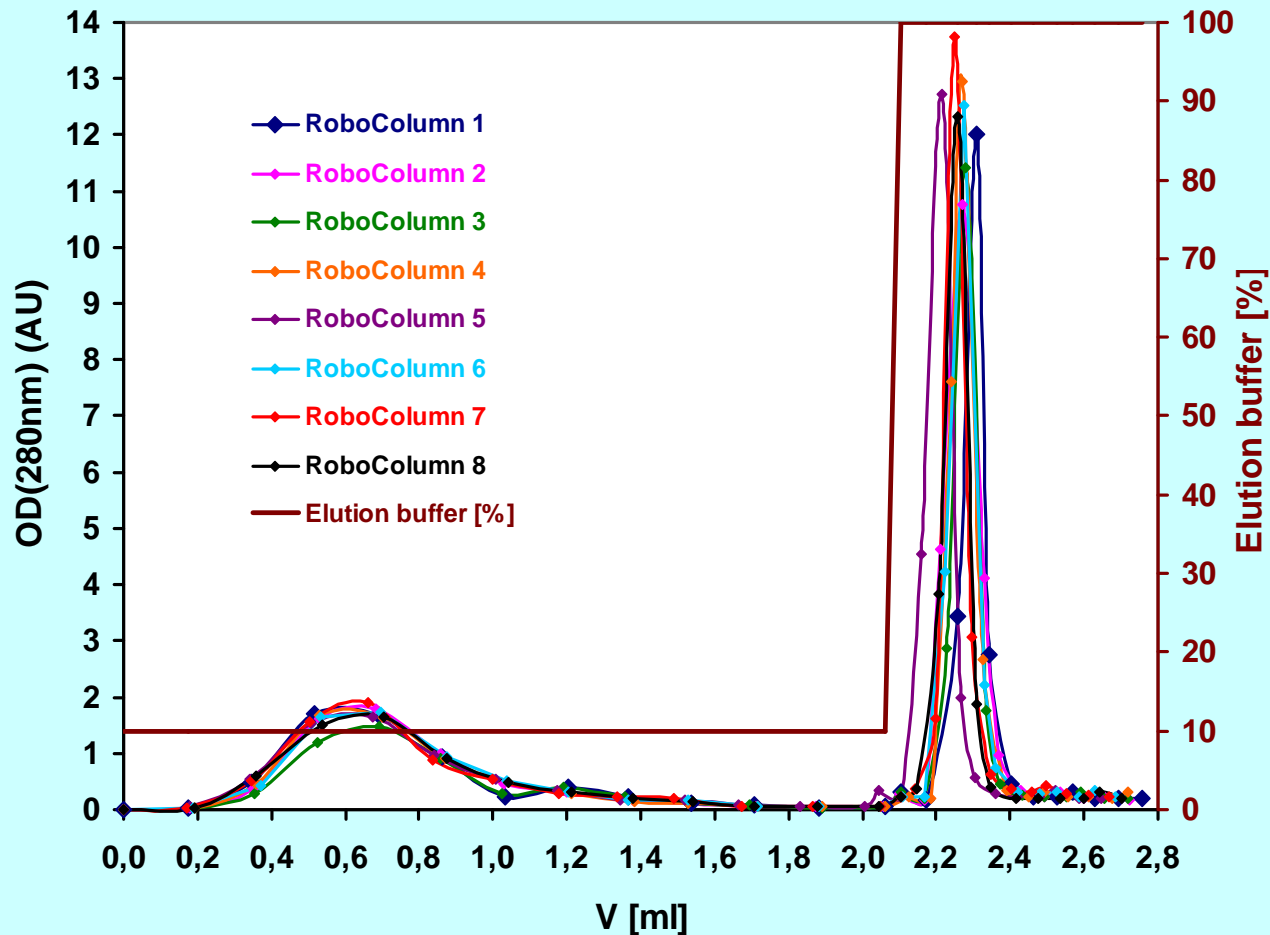
1.0 M NaCl (step 2)

same buffer as in (B)

6. Proof of Concept for RoboColumn Arrays

6.4 Simultaneous Automated Separation of Eight Protein Samples

Step gradient elution of cytochrome c and lysozyme on an eight-row of MediaScout® RoboColumns, packed with Toyopearl® SP-650S



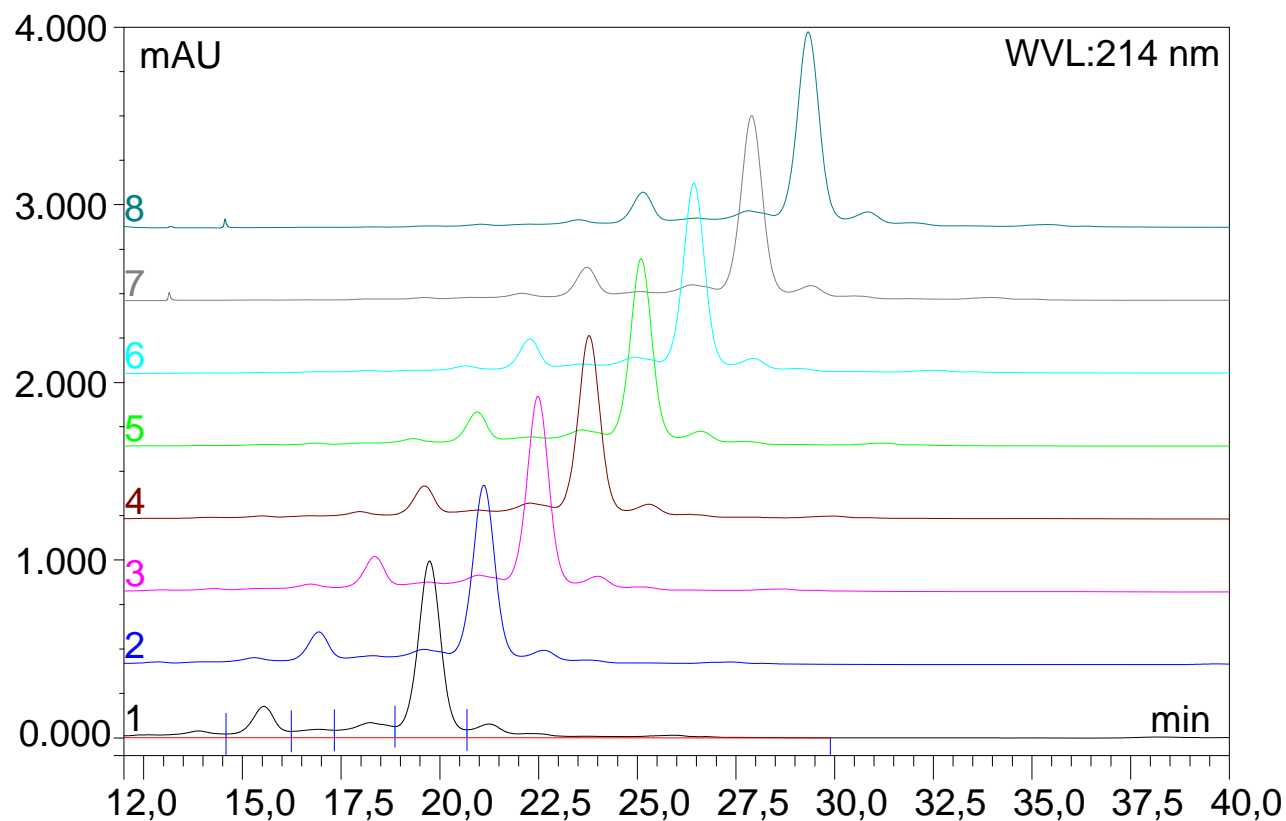
Example application of HTP chromatographic separations: Automated in-process monitoring of

Procedure: **mAb production**

- Samples were taken from fermentation broth
- Loaded onto RoboColumns packed with ProA capture resin
- Washed
- Eluted into 96-well plate
- Neutralised
- Analysed by HPLC-CIEC

7. Example applications of HTP Chromatographic separations

In-process monitoring by CIEX of mAb samples from fermentation broth, after rapid purification on RoboColumns, packed with ProSep[®] vA Ultra





8. Summary and Conclusions

- RoboColumns were operated **simultaneously** by the liquid handler of a modified commercial robotic workstation (Tecan Freedom EVO), utilizing fixed metal tips and positive liquid displacement
- Miniaturized RoboColumns (0.2 ml c.v.) were used successfully for small scale separation of proteins
- The chromatographic performance of RoboColumns in small scale protein separations is comparable with columns individually connected to a chromatography system
- RoboColumns can be packed with a variety of process relevant chromatography media
- RoboColumns allow automated HTP processing of multiple samples, e.g. for in-process monitoring applications



9. Acknowledgments

Tecan (Switzerland and Germany) is gratefully acknowledged for generously supporting this work by providing Freedom EVO hardware and software, as well as for helpful practical training and numerous advices!

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