

Science Together



AZURA® SMB systems

Chromatography for continuous separations



**CHROMATOGRAPHIC
SPECIALTIES INC.**



1-800-267-8103 • chromspec.com • tech@chromspec.com



Why better choose SMB instead of batch chromatography

SMB chromatography is a HPLC technique for the separation of binary mixtures with high productivity and purity.



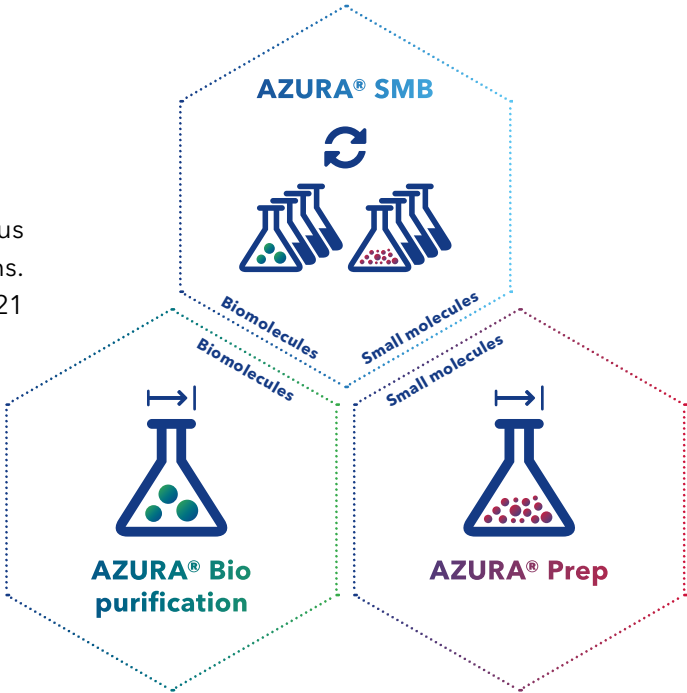
Get higher productivity and purity than with comparable batch systems – even with a smaller system.

Save up to 90 % of the solvent and reduce the solid phase costs up to 80 %

Gain nearly undiluted product and minimize concentration efforts.

AZURA® purification systems

KNAUER offers system solutions for continuous separation tasks as well as for batch separations. Visit us online or take a look at page 20 and 21 for more information.



Introducing SMB chromatography

Simulated moving bed chromatography (SMBC) is increasingly applied as a separation technique in the pharmaceutical industry, production of fine chemicals and in the field of bioengineering. SMB is a method in process chromatography that enables substance mixtures to be continuously separated and extracted in two fractions.

By repeated use of the SMB process each partial fraction can be separated into a further fraction – down to binary substance mixtures. Typically, the SMB process is set up in advance for a two component mixture. Following this, both substances can be immediately extracted in pure form.

What is the difference between batch LC and SMBC?

Batch chromatography (single-column)	SMB chromatography (multi-column)
Unlimited number of fractions	Two fractions, no waste
Recovery typically below 80 %	Recovery up to 100 %
EITHER high purity OR high yield	High purity AND high yield
Isocratic or gradient	Isocratic
High solvent consumption	Can be as low as 10 % of batch consumption
Very diluted product	Product concentration comparable with input concentration (feed)

Limited to binary mixtures?

The SMB process is ideally suited for two-component separations (Fig. 1a). For the task of separating and collecting multiple fractions, classical batch LC might be the better option (Fig. 1b).

SMB chromatography can also be used for the separation of more than two peaks (multi-component mixtures). Therefore it is possible to “split the chromatogram” at a certain point (Fig. 1 c).

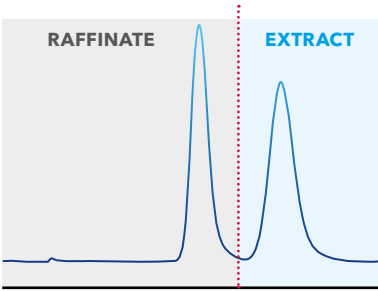


Fig. 1a: Basic binary mixture for a SMB separation.

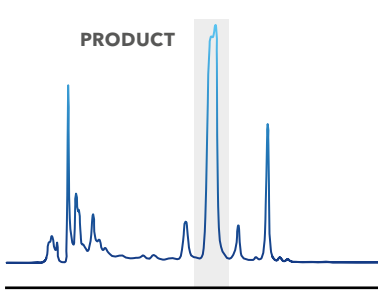


Fig. 1b: Typical multi-component mixture for classical batch chromatography.

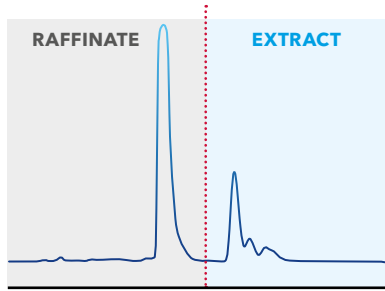


Fig. 1c: Multi-component mixture, can be separated in two different fractions with SMB.

PurityChrom® MCC
Intuitive and highly functional control software

Flexibility

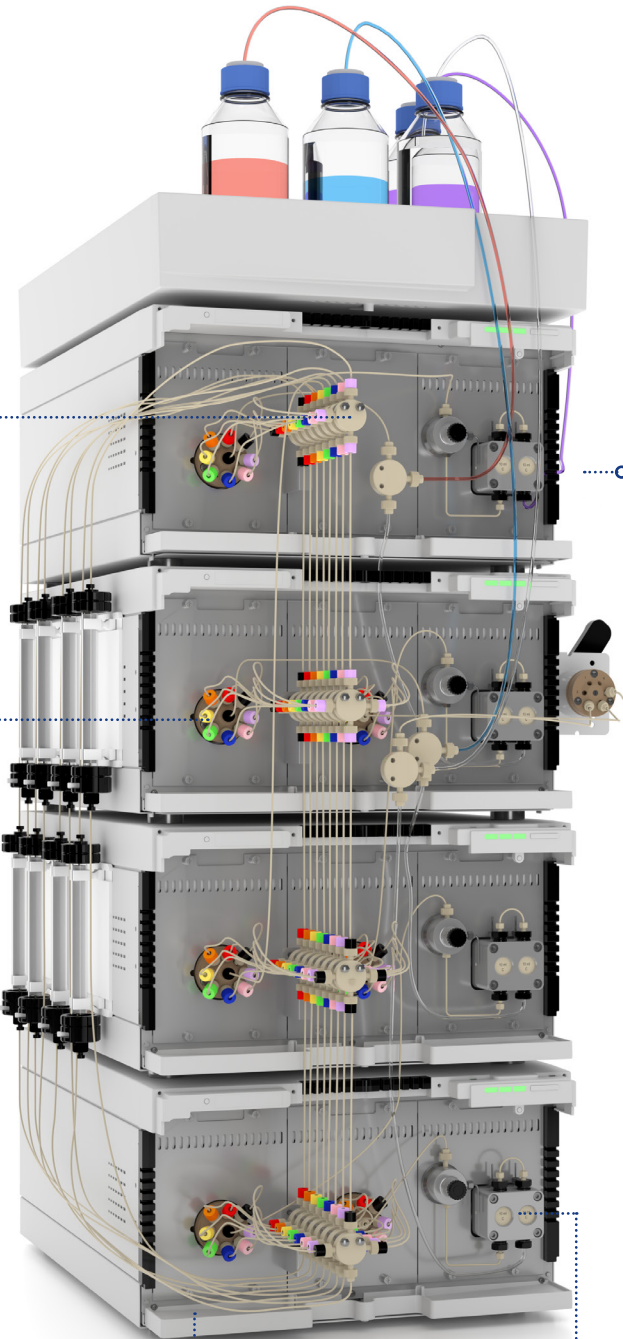
Up to 8 columns at max. 130 bar

Multi-position valve

Standard valves for flexible zone definition and low maintenance costs

Temperature control

Columns can be heated or cooled (requires additional equipment).



Gram scale

Continuous flow rates up to 30 ml/min and columns up to 30 mm ID allow to increase your throughput up to several hundred grams.

Small footprint

The AZURA® SMB systems require little space on the lab bench.

Biocompatible

Biocompatible version available. Perfect solution for the continuous purification of small molecules.

AZURA® SMB Lab

This SMB system is optimized for separation tasks on a scale of several hundred grams. The standard configuration consists of four AZURA® assistants ASM 2.2L with seven multi-position valves and four AZURA® pumps P 4.1S as well as our user-friendly software PurityChrom® MCC including required IT hardware. Depending on the special

requirements of every separation the SMB system can be freely configured via valve switch (e.g. closed-loop, open-loop, 3-zone) and is upgradable with detectors and flow meter. See table for available configurations of the AZURA® SMB Lab. Individual configuration is available on request.

Available configurations for AZURA® SMB Lab

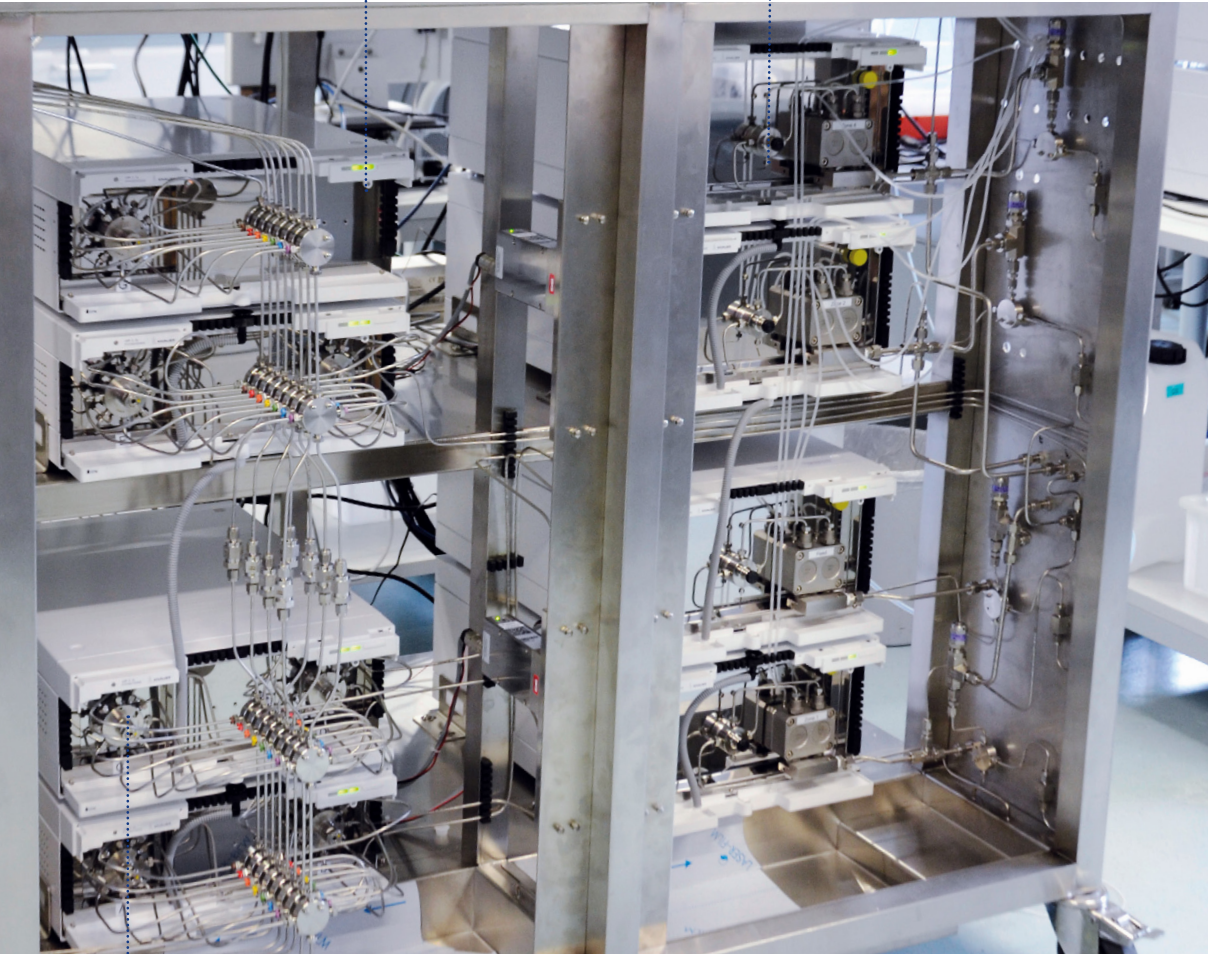
Max. continuous flow rate	Max. flow rate*	Number of columns	Max. pressure*	Art. No.	Description
30 ml/min	50 ml/min	8	130 bar	A29101	Stainless steel
		8	130 bar	A29100	Biocompatible (PAEK, ceramic)

* The maximum operating parameters for flow and pressure depend on the specific columns and customer application.

PurityChrom® MCC
Intuitive and highly functional control software

Wide field of applications
Up to 8 columns at max. 100 bar

Kilogram scale
Continuous flow rates up to 250 ml/min allow very high throughput on a kilogram scale



Multi-position valve
Standard valves for flexible zone definition and low maintenance requirements

Temperature control
Columns can be heated or cooled (requires additional equipment)

AZURA® SMB Pilot

The AZURA® SMB Pilot is designed for the separation of binary mixtures on a hundred gram to kilogram scale and is typically used with columns up to 50 mm ID. Its special emphasis is put on the continuous operation mode and highest productivity.

The SMB standard configuration consists of four AZURA® pumps P 2.1L and seven 8-port multi-position valves integrated into four AZURA® assistants ASM 2.2L. Our user-friendly software PurityChrom® MCC and the required IT hardware are also included. We offer several variations of the standard system configuration.



Available configurations for AZURA® SMB Pilot

Max. continuous flow rate	Max. flow rate*	Number of columns	Max. pressure*	Art. No.	Description
250 ml/min	500 ml/min	8	100 bar	A29201*	Stainless steel

* The maximum operating parameters for flow and pressure depend on the specific columns and customer application.

Upgrade kits for AZURA® SMB systems

Heating and column organisation

Save space and time with our SMB oven or multi-column stands



A29902

A29903

A29901

Description	Art. No.
Oven for AZURA® SMB Lab and AZURA® SMB Pilot systems (8 KNAUER columns with up to 250 × 50 mm inner dimension)	A29903
Column holder for 8 SMB columns with up to 250 × 50 mm	A29901
Column holder for 8 SMB columns with 8, 16 mm ID and up to 250 mm length or 20 mm ID and 150 mm length	A29902

Batch-upgrade

Add a batch system into your SMB

Upgrade your system and perform simple separation tasks or estimate your separation parameters directly without buying an additional batch LC system. The Batch-Upgrade kits include a detector and an injection valve.

Flow rate	Material	Art. No.
up to 100 ml/min	Stainless steel	A29601
	Biocompatible	A29600
up to 500 ml/min	Stainless steel	A29603
	Biocompatible	A29602

Installation

Standard installation and familiarization - including system installation, instruction to system and software as well as general considerations for SMB operation.

	Art. No.
Europe	A0000SMBEU
International	A0000SMBIN

System and process control

High-accuracy mass flow meters for highest process stability

Number of mini-CORI-FLOW™	Position	Monitors	Monitoring level	Field of application
1	Column	Process stability	+	Process evaluation; standard systems
2	Column; feed pump	Process stability; feed flow	++	Process evaluation; demanding separations; expensive feed; low feed flow
4	At every pump	Pump flow	++++	Production process; complete control over system stability

Description	Art. No.
One mini CORI-FLOW™ M13 for AZURA® SMB Lab, incl. accessories	A29800
Four mini CORI-FLOW™ M13 for AZURA® SMB Lab, incl. accessories	A29801
One mini CORI-FLOW™ M14 for AZURA® SMB Pilot, incl. accessories	A29802
Four mini CORI-FLOW™ M14 for AZURA® SMB Pilot, incl. accessories	A29803
One mini CORI-FLOW™ M13 Hastelloy for AZURA® SMB Lab, incl. accessories	A29805

Columns for SMB processes

Every SMB set comes with a special test certificate

KNAUER offers purpose-built SMB sets. Additionally to the quality test of every single column, SMB sets are tested for reproducibility to ensure the appropriateness for SMB processes.



For a stable SMB process, a set of identical and robust HPLC columns is essential as in every of the four zones the separation has to be identical to reach a continuous process. It is recommended to order one additional column in a SMB set (needed columns + 1) to have one column with exactly the specifications of the SMB set for determination of the parameters needed for process layout. The particle size of the separation material should be 15 µm or larger to ensure a robust purification process.

While the stationary phase always depends on the application, the recommended column dimensions are mainly dependent on the amount of sample that must be purified.

Typical parameters for preparative columns

Column dimension	Loadability* compared to analytical 250 x 4 mm column	Recommended flow rate [ml/min]
250 x 4 mm	1 x (analytical reference)	1
250 x 8 mm	4 x	4
250 x 16 mm	16 x	16
250 x 20 mm	25 x	25
250 x 30 mm	56 x	55
250 x 50 mm	156 x	150



* The mass and volume loadabilities always depend on the specific sample and application as well as on the stationary phase filled in the HPLC column. The chart only gives a first clue and can be higher or lower than the given numbers. Scale-up factor (SF) calculation: $SF = ID^2(\text{preparative})/ID^2(\text{analytical})$

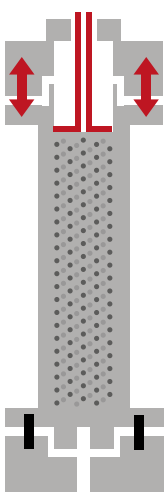
High flexibility through variable column hardware types

When to use which column hardware solution?

KNAUER offers stationary phases for nearly any application in normal- or reversed phase mode as well as for special purification tasks in ion exclusion and ligand exchange mode for example. Nearly all KNAUER phases are also available in larger particle sizes for easy upscaling to SMB processes.

dAX preparative column hardware

- **Dynamic axial compression** for an extended lifetime
- No formation of void volume at the column inlet possible



Special demand for **extended column life time**

dAX Hardware
Extremely high life time
Excellent Reproducibility
More cost-intensive

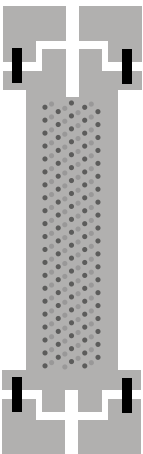
Refill possible



An overview of all KNAUER columns can be found on www.knauer.net/columns

Standard preparative column hardware

- **Flanged or standard threaded**
- Without axial compression



Standard flanged Hardware

- Simple
- cost-effective
- versatile

All classical **prep applications**

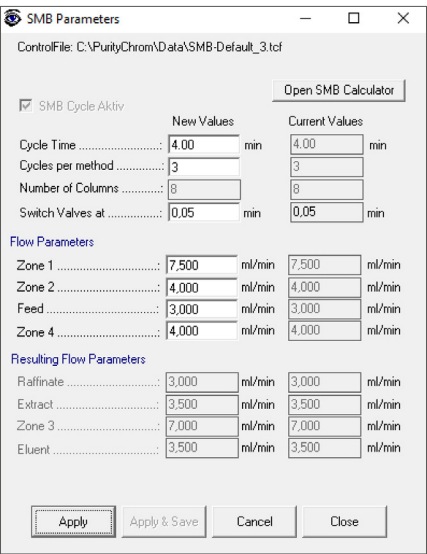
No refill option

PurityChrom® MCC

Software for multi-column chromatography (MCC)

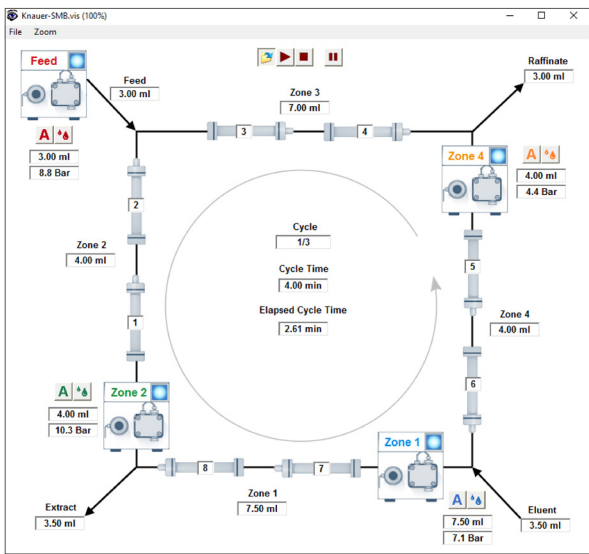
SMB Parameter Wizard

Insert your calculated adsorption parameters. The SMB Parameter Wizard will calculate the operation point. The parameters can easily be transferred to the SMB method file.



System visualization

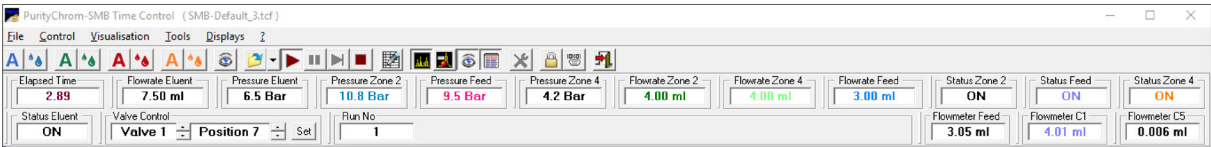
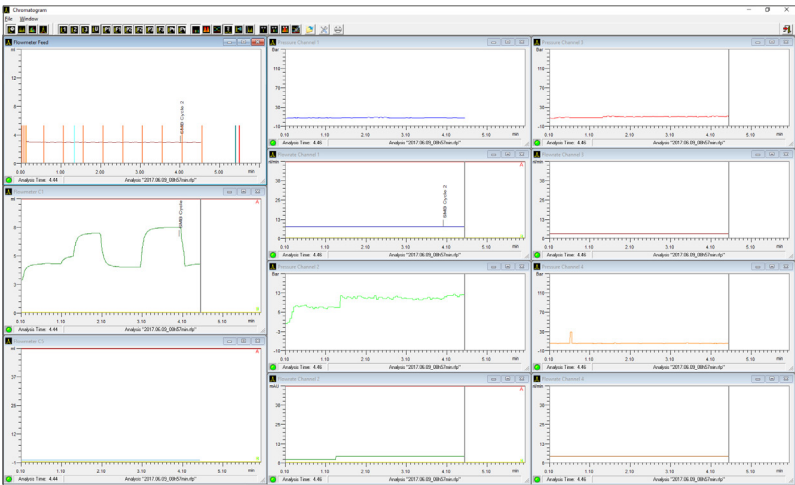
The system visualization is everything you need to control the SMB system. The visualization can be freely configured. Keep track of all the information.



Software for multi-column chromatography (MCC)

System monitor

Monitor as many channels as necessary at once and keep full control of your SMB process all the time.



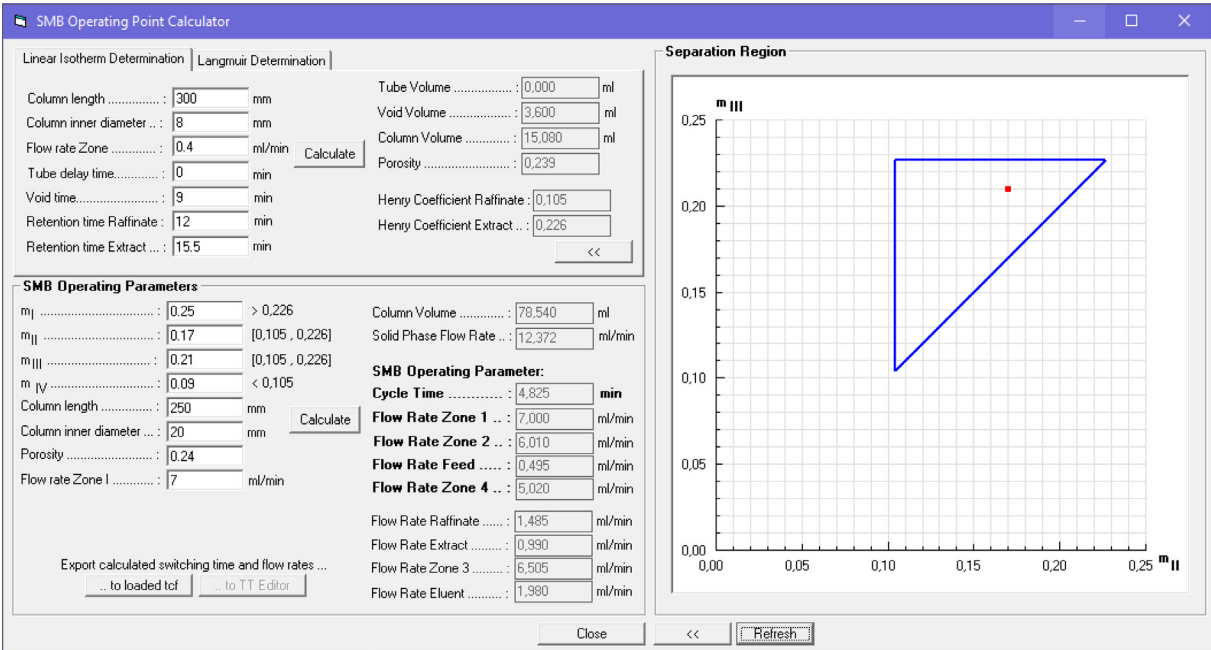
Security options

The user management ensures the integrity of your separation methods. In addition every change made through the method is protocolled by the software and saved in the result files. Our PurityChrom® software is 21 CFR part 11 compliant.

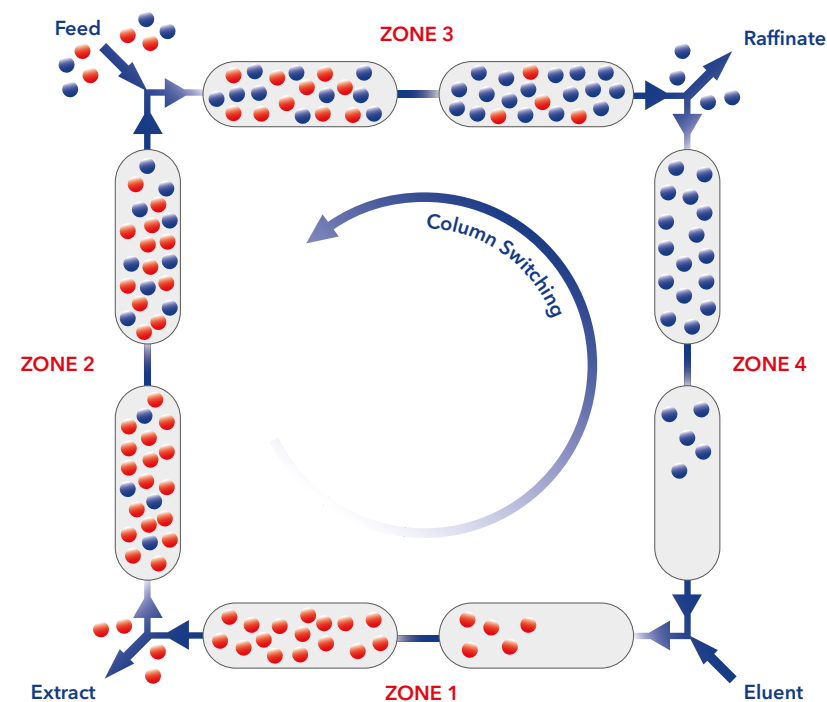
SMB-Default_3.tcf		
Time [min]	Function	Parameter
0.00	Feed	100.0 , 0.0 , 0.0 , 0.0 3.00 ml/min Constant Flow
0.00	Zone 4	100.0 , 0.0 , 0.0 , 0.0 4.00 ml/min Constant Flow
0.00	Pressure Pumps	Maximum Pressure Pump 1 = 150.0 Bar
0.05	Valve Position	All Valves = Next Position
0.10	Start Chromatogram	Channel 1,2,3,P1,P2,F2,P3,F3,P4,F4 (500 ms)
0.55	Valve Position	All Valves = Next Position
1.05	Valve Position	All Valves = Next Position
1.33	Zone 2	100.0 , 0.0 , 0.0 , 0.0 4.00 ml/min Constant Flow
1.55	Valve Position	All Valves = Next Position
2.05	Valve Position	All Valves = Next Position
2.55	Valve Position	All Valves = Next Position
3.05	Valve Position	All Valves = Next Position
3.55	Valve Position	All Valves = Next Position
4.40	Stop Chromatogram	All started Channels
4.50	Stop all	

SMB Operation Point Calculator

Adsorption isotherms can be entered into an integrated starting point calculator. The generated values can be checked via a visual feedback very easily. The parameters will be transferred directly into the SMB Parameter Wizard.



The SMB principle



SMB process scheme

The SMB process enables the separation of binary mixtures by means of a simulated countercurrent between the solid and liquid phases. This is accomplished with a series of chromatography columns arranged in a ring. An eluent flow circulates through this ring. Two inlets (for feed and eluent) and two outlets (extract/red and raffinate/blue) define four separation zones. By continuously feeding sample and synchronously switching the columns against the eluent flow direction, a countercurrent is achieved between the solid and liquid phases, leading to high purity of both target fractions. The movement of the solid phase is realized by simultaneously switching seven multi-position valves (AZURA® SMB) or one central multi-position valve (former KNAUER SMB, CSEP®).

The bulk of the eluent is continuously circulated in the system, making it necessary to replace only that small amount which is removed in the extract and raffinate, thereby enabling savings of up to 90% of the eluent in comparison to a batch process. Due to the simulated countercurrent, the stationary phase is significantly better utilized with the SMB technique as compared to the batch process technique. The number of theoretical plates might be also less important, making it possible to use cost-effective larger particle size for the stationary phases.



AZURA® SMB Lab manifold, stainless steel version.

Where can a SMB separation be used?

Range of applications	Separation and extraction ...
Pharmaceutical chemistry	Chiral compound (cis-trans phytol, steroids, peptides, antibiotics, etc.)
Food chemistry	Fatty acids, carbohydrate mixtures (sucrose/molasses or fructose/glucose, etc.)
Biochemistry	Phenylalanine, fermentation/cell culture products (citric acid, sugars, antibodies, enzymes, etc.)
Petrochemistry	C8-Hydrocarbon (xylene/toluene, etc.)

How AZURA® SMB works

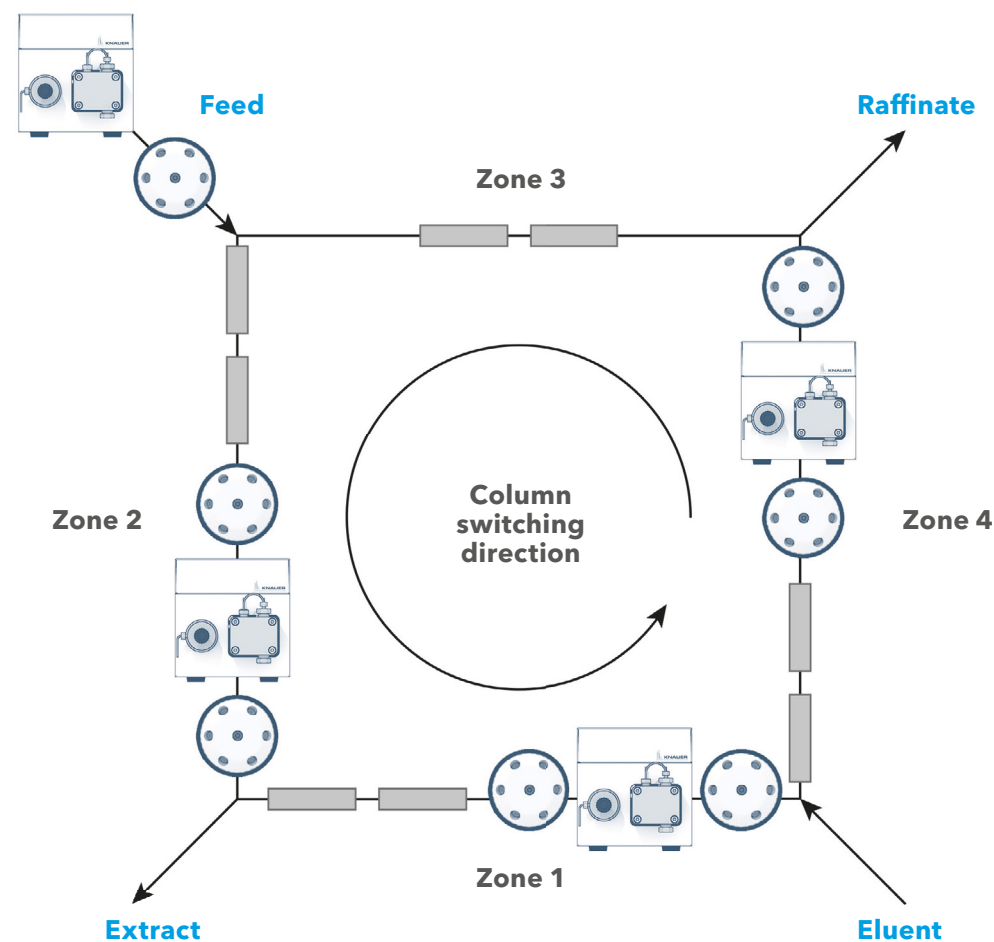
System configuration

The standard AZURA® SMB systems consists of four pumps and seven multi-position valves.

The devices are arranged as follows:

- Three pumps (Extract, Raffinate, Eluent) are placed inside the SMB cycle.
- The feed pump is placed outside the SMB cycle.
- Four valves are placed at the pump outlets.
- Three valves are placed at the pump inlets. The feed pump inlet is not connected to a multi-position valve.

Due to this configuration the SMB system can be used very flexible for many different separation modes. Additionally to the process stability AZURA® SMB systems are outperforming every other SMB system on the market regarding material and configuration flexibility as well usable pressure range.



Schematic AZURA® SMB design

Operation modes

Classical SMB chromatography

The standard configuration is designed to run classical SMB separations. This mode is perfect for the separation of binary mixtures, like sugars or e.g. pharmaceutical racemic mixture. The sep-

aration of a multi-component mixture into two different fractions is possible, too. This is typically used as one pre-purification step for very demanding separations.

Different zone configurations

In standard configuration every zone consists of two columns (2:2:2:2). To optimize the process, it might be useful to change this distribution. In case of a very effective regeneration of the solid and liquid phase in zone 1 and 4, the number of columns in these zones can be reduced. With a 1:3:3:1 configuration, a much higher productivity can be achieved. This system configuration can easily be adjusted via our software PurityChrom® MCC. The hardware does not have to be modified.

Open-/Closed-Loop

Every AZURA® SMB system can be switched between a Open- and a Closed-Loop mode. The Closed-Loop is the common SMB mode. In Open-Loop mode the SMB cycle is opened between the last column of zone 4 and the zone 1 pump. When to choose this mode?

- One impurity with very low retention time is in the sample (and eluent costs are low enough)
- To start/clean the system or change the eluent

Birch xylose converted to xylitol as sweetener



Application

Purification of xylitol by HPLC methods from fermented biomass

Process development

To optimize SMB parameters can be a very time consuming task, especially if a purity and a yield of more than 99 % with a maximum productivity

Optimization of the analytical method

The analytical HPLC method has to be transferred to an isocratic method (Fig. 2). The stationary phase must be adapted to the later SMB process requirements too. In this case, the material was changed from 10 µm to 25-56 µm. Additionally the column dimensions were changed from 300 × 8 mm to 150 × 20 mm (Fig. 3)

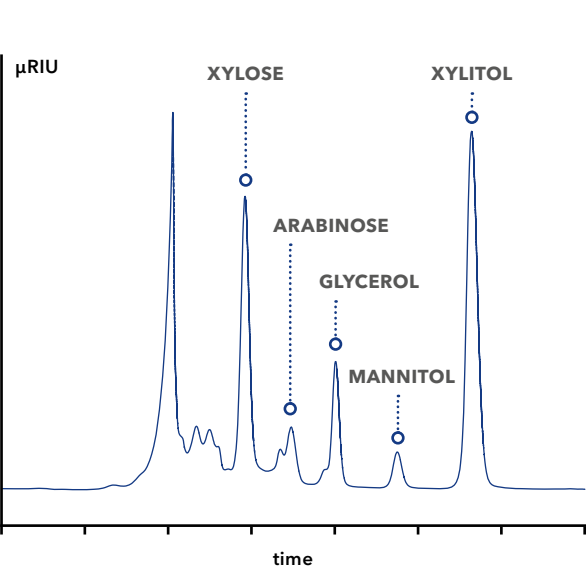


Fig.2: Analytical chromatogram of fermentation mash with identified sugar/sugar alcohols; 20 µL 1:2 dilution; Eurokat Ca 300 × 8 mm, 10 µm particle, 0.5 ml/min, 75°C, H₂O_{dd} isocratic

is needed. In this application, xylitol was purified from fermentation mash of a fed-batch process.

Transfer into preparative scale

The next step is an overloading study based on the analytical method by using a column with the same dimensions of the prospective SMB process. Volume and mass overload are evaluated and adsorption isotherms are determined based on the retention times (Fig. 3). The received parameters are transferred into the appropriate SMB scale. Figure 4 shows where the chromatogram is split based on the calculated SMB parameter.

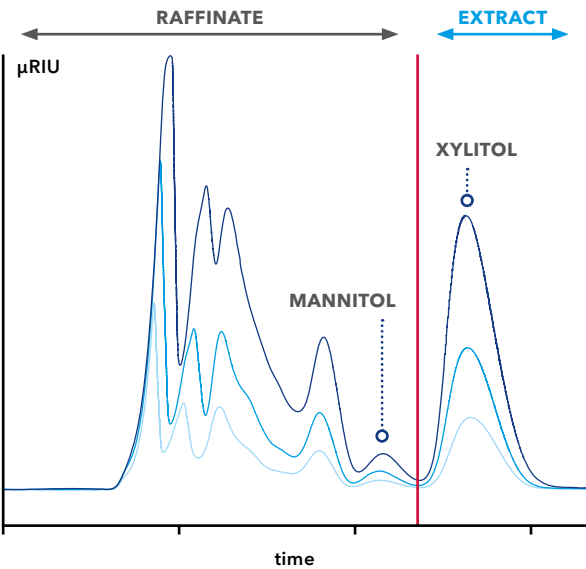


Fig.3: Semi-preparative chromatogram of fermentation mash; injection: light blue - 0.5 ml, blue - 1.0 ml, dark blue 2.0 ml; Eurokat Ca 150 × 20 mm, 25-56 µm particle, 4 ml/min 60°C, H₂O_{dd} isocratic

Evaluation of the SMB parameter

The adsorption isotherms (linear or Langmuir) can be easily inserted into the parameter wizard of our SMB software Purity-Chrom® MCC. The starting parameter will be calculated and transferred into the method automatically (Fig. 4).

Due to a contamination in the mixtures with nearly no interaction with the material, the SMB separation was done in an Open-Loop mode, so without solvent recycling.

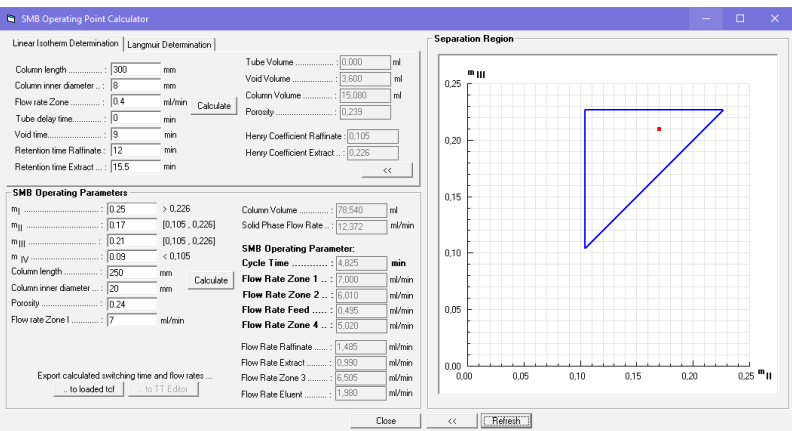


Fig.4: Parameter wizard in PurityChrom® MCC

Separation verification

Figure 5 shows an overlay of raffinate / extract and waste fraction of the 6th SMB cycle revealing a successful separation of the fractions, with 100 % purity and recovery of xylitol.

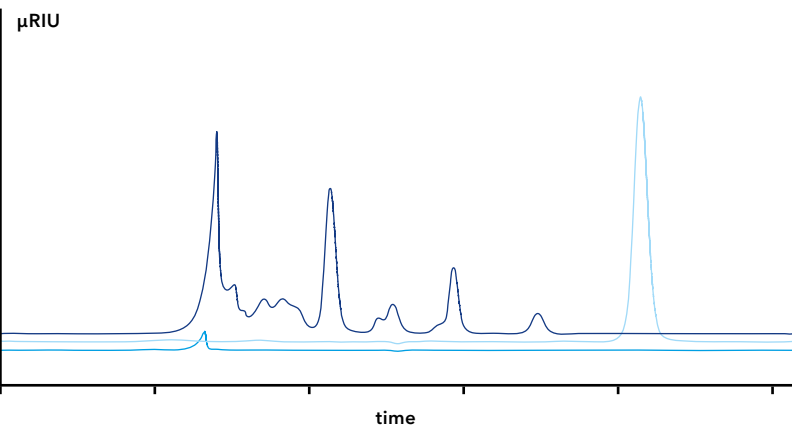
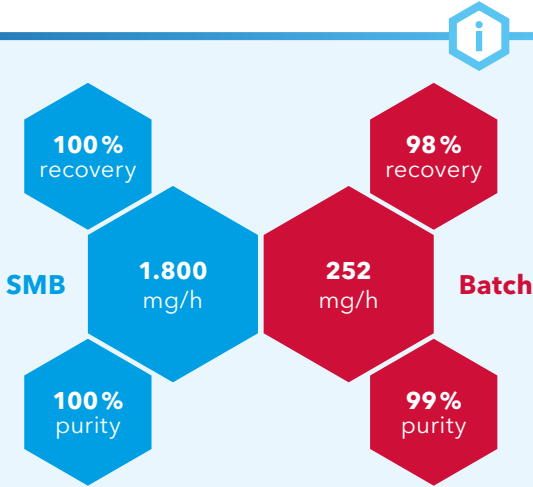


Fig.5: Overlaid analytical chromatograms of raffinate (blue); extract / xylitol (light blue); waste (blue); 20 µL 1:2 dilution; Eurokat Ca 300 × 8 mm, 10 µm particle, 0.5 ml/min, 75°C, H₂O_{dd} isocratic

"Acknowledgement: This project has received funding from the European Union's Seventh Framework Program for research, technological development and demonstration under grant agreement no FP7-KB-BE-2013-7-613802."

SMB versus Batch

The comparable batch separation (same conditions as seen in Fig. 3, dark blue chromatogram) offers nearly the same purity and recovery rate, but a significantly lower productivity of 252 mg/h. The throughput of the SMB process is with 1,8 g/h greater by the factor of seven than that of the batch process.



AZURA® batch chromatography

Whenever separation tasks are changing frequently, a classical batch LC system can be the better choice. KNAUER AZURA® Prep and AZURA® Bio purification systems are as flexible and versatile as possible.

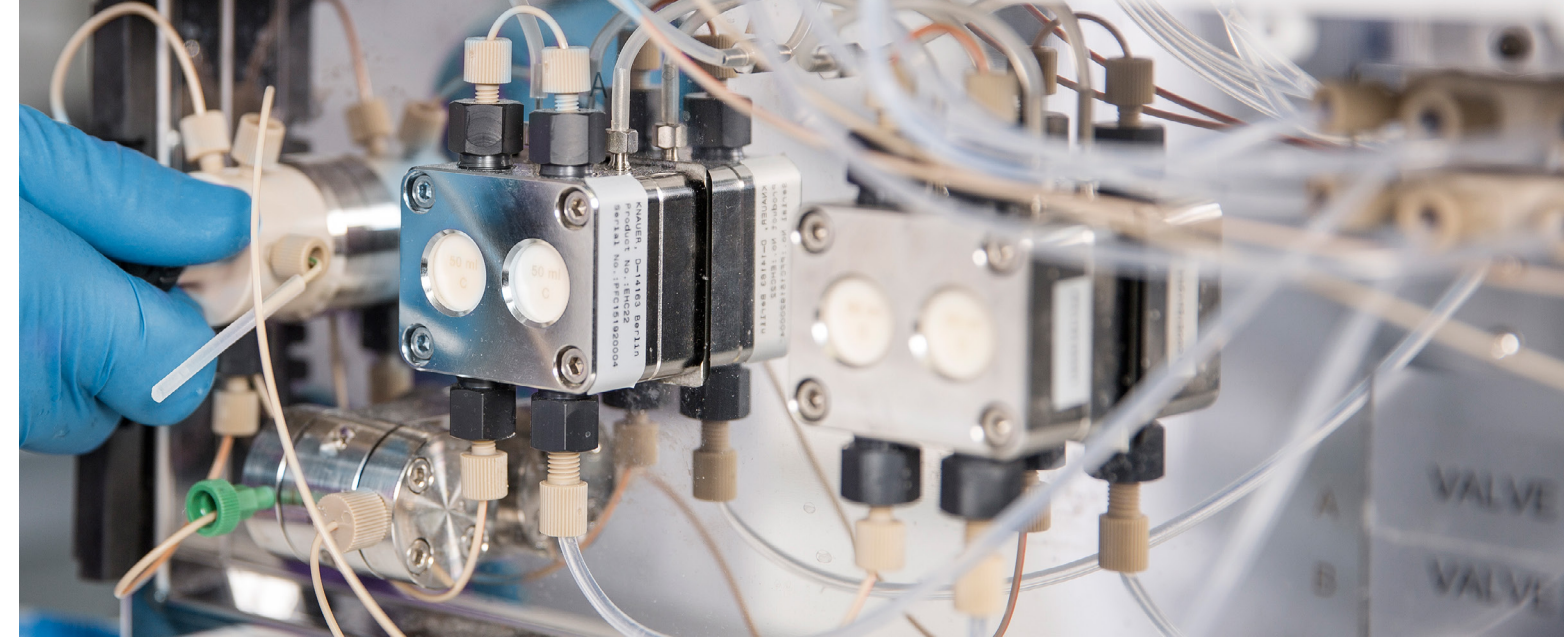
AZURA® Prep

AZURA® Prep was designed for flexibility and to comfortably handle large sample volumes. Easy to operate and maintain the AZURA® Prep systems are perfectly suited for the purification of

your products such as synthesis stages or active ingredients. The systems can be optimally adapted to the scale you need.



- Configure your system from injection to collection
- Choose between high or low pressure gradient systems from 50 to 1 000 ml/min
- User-friendly and powerful software PurityChrom®

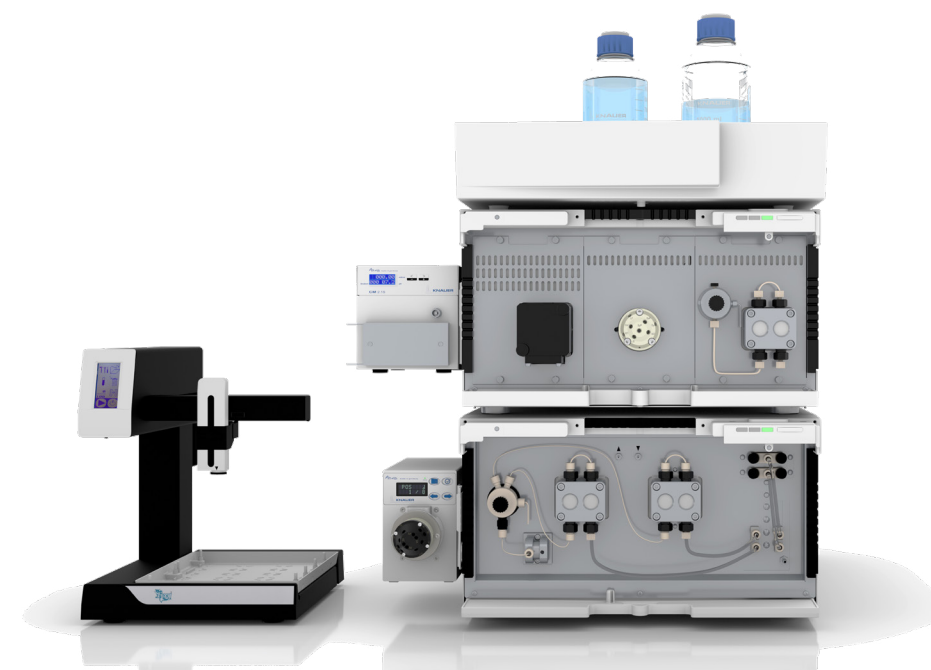


AZURA® Bio purification

Complete solution for FPLC on a minimum footprint: AZURA® Bio systems combine flexibility and reliability. The biocompatible AZURA® Bio is the perfect choice for your protein purification task. Design your AZURA® Bio system to your needs. Multiple functionalities such as column

switching, buffer and sample selection as well as fraction collection enable the user to automate their separation.

From simple to complex, from lab to pilot scale: Design your AZURA® FPLC system according to your purification task.



- Flexible and modular design
- Easy upscaling - up to 1 000 ml/min
- Powerful FPLC software PurityChrom®
- Variety of great detectors to make your sample visible

KNAUER customizable MCC systems

Special multi-column chromatography solutions

„KNAUER is the only company that could build a system according to our specifications“

Our focus is on the development of optimization and control strategies for multi-column processes. We needed a flexible system that is able to perform various multi-column processes on the highest level of technology. The hardware and the software must be flexible such that model-based optimization and control schemes can be tested on example processes. KNAUER offered the complete package for us: planning, designing, developing and manufacturing. The installation, instruction and support were very good. It was a pleasure working with KNAUER.



Prof. Dr. Ing. Sebastian Engell,
Head of Process Dynamics and
Operations
Technical University Dortmund

System components

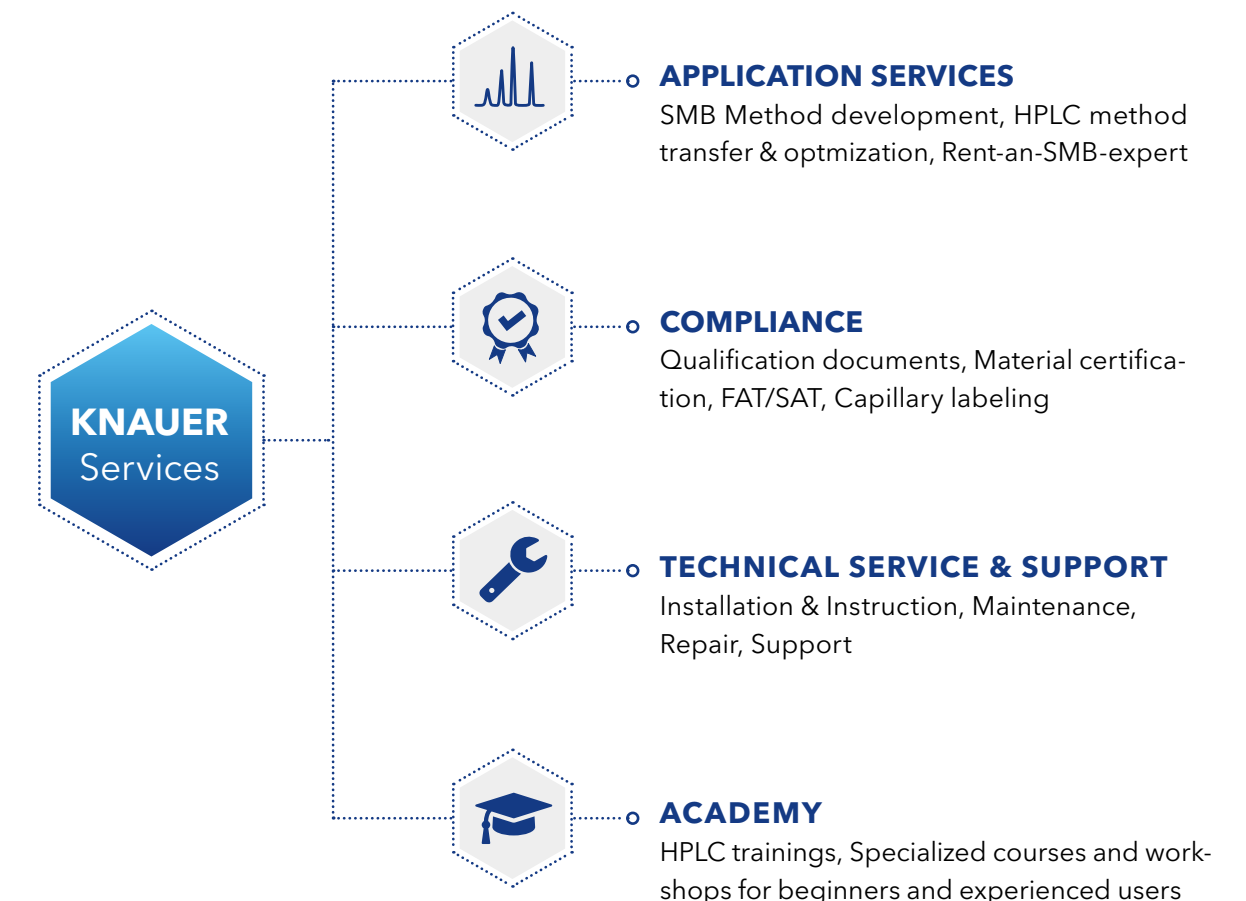
- 6 AZURA® Pumps P 6.1L HPG
- 2 AZURA® Pumps P 2.1S
- 2 AZURA® UV Detector UVD 2.1S
- 2 AZURA® Valve Drive V 2.1S
- 1 AZURA® CM 2.1S
- 24 AZURA® Valve Drive V 2.1S with multi-position valves
- Controlled via PCS by HiTec Zang

KNAUER builds up customized multi-column systems to your needs. We will support you by choosing the right devices, materials and control options.

Contact us: sales@knauer.net



KNAUER Services



Contact us

All standard user instructions, helpful video tutorials, and a structured section of frequently asked questions is freely accessible on our web page www.knauer.net. If you need further support, our friendly Support team is happy to help you via e-mail, phone or Team Viewer. They will work with you personally until all issues are resolved.

Phone: +49 30 809727-111 (workdays 9-17h CET)
 Email: support@knauer.net

Analytical
HPLC

Multi-Column
Chromatography,
SMB

Preparative
HPLC

FPLC

Osmometry

Dosing,
Metering,
Pumping

Detection

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