Science Together

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Start up, shut down - Screening of pressure sensitive columns with the 8 port 2-position valve

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SUMMARY

Using valves in FPLC is most often necessary due to manual injection, column switching or fractionation. But also common analytical HPLC applications can be improved by adding valves. Widespread analytical application areas are for example column screenings to find the suitable column. A large variety of valves is available, but not all are suitable for the screening of pressure sensitive columns. This note describes an application of a KNAUER 8 port 2-position valve in a screening process with Eurokat columns.

INTRODUCTION

The KNAUER Eurokat polymer columns are based on a sulfonated cross-linked styrenedivinylbenzene copolymer and available in several ionic forms (H, Ca, Pb and Ag). This cation exchanger is characterized by an outstanding density of functional groups, making it the ideal choice for ion exclusion, size exclusion and ligand exchange chromatography. Due to the degree of cross-linkage of the polymer, the Eurokat columns are pressure sensitive. They are operated at an elevated temperature ranging from about 60 °C to 85 °C. To prevent a sudden pressure increase it is also necessary to raise the flow rate slowly and gradually until the desired value is reached. Due to the permanent flow required for Eurokat columns, the use of a multiposition valve for screening purposes is not applicable. However, as it allows simultaneous flow on two columns, the KNAUER 8 port 2-position valve is a good alternative to allow screening with sensitive Eurokat columns..

Additional Information

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RESULTS

Single standards for identification and a mixed standard of six carbohydrates (arabinose, mannitol, cellobiose, xylose, glucose, xylitol) and glycerine were measured on two different Eurokat columns. The columns were connected to the KNAUER 8 port 2-position valve. Thereby it is possible to maintain a permanent and constant flow rate on both columns and the time-consuming step of changing the column, heating it up and slowly increase the flow rate is not necessary. **Fig. 1** shows the connection of the columns and other devices to the valve. In position 1 the Eurokat Na column is in the injector flow path. When position 2 is selected the analysis is carried out on the Eurokat Ca column. Instead of configuring two separate pumps an AZURA HPG pump was altered, so that the columns could be operated each with one pump head.

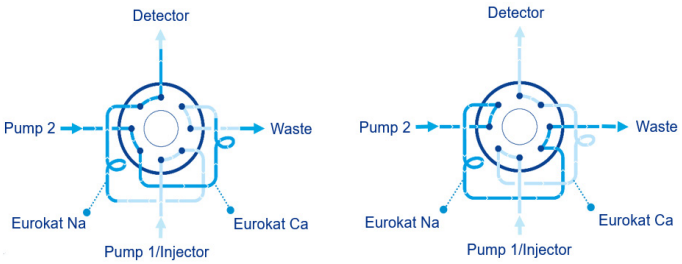


Fig. 1 Switching of 8 port 2-position valve. Position 1 – left, position 2 - right.

On the Eurokat Na column not all substances were separated properly. Mannitol and glucose are eluting at the same time as well as glycerine and arabinose. **Fig. 2** shows the measurement of the mixed standard on the sodium phase.

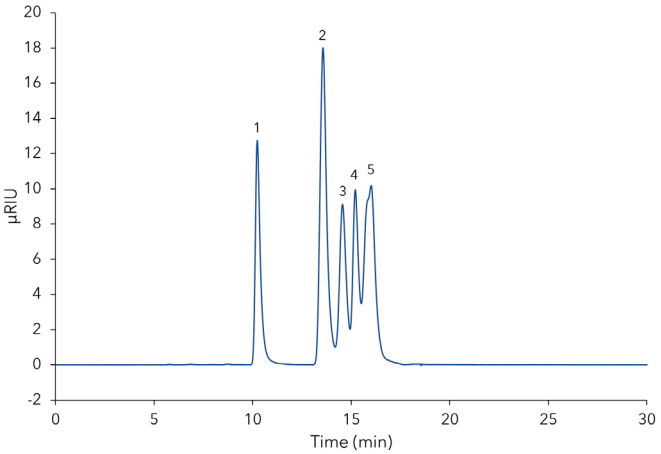


Fig. 2 Mixed standard on Eurokat Na. 1 - Cellobiose, 2 - Mannitol/Glucose, 3 - Xylose, 4 - Xylitol, 5 - Glycerin/Arabinose.

Using the additional valve, it is now possible to directly inject the sample onto the second column without the need of any changes. On the Eurokat Ca column the resolution of the substances could be improved. The elution order is different from the measurement on the sodium phase. Only glucose and xylose were not baseline separated. **Fig. 3** shows the chromatogram of the mixed standard on the calcium phase.

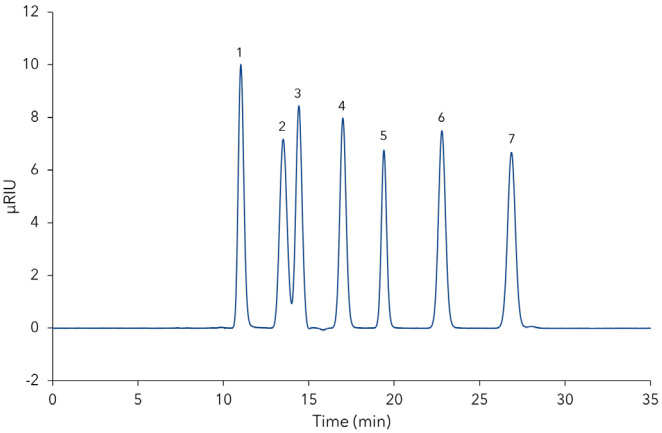


Fig. 3 Mixed standard on Eurokat Ca. 1 - Cellobiose, 2 - Glucose, 3 - Xylose, 4 - Arabinose, 5 - Glycerin, 6 - Mannitol, 7 - Xylitol.

SAMPLE PREPARATIONS

All standards were dissolved in water.

CONCLUSION

Depending on the valve position one column is in the flow path of the injector and detector, whereas the second column is also ready for measurement. The standards or samples can be analysed on the first column and afterwards directly on the second one because the flow rate is kept constant for both. Concerning the complex handling of Eurokat columns, it is not necessary to change the column manually, heat it up again and slowly rise the flow rate. For screening tasks with pressure sensitive columns or column materials the use of the KNAUER 8 port 2-position valve is advisable to save time that otherwise is spend on changing and equilibrating the second column. Besides screening challenges there are more applications the 8 port 2-position valve can be used for, e.g. comprehensive or "heart-cut" 2D-LC.

MATERIALS AND METHODS

Tab. 1 Method parameters

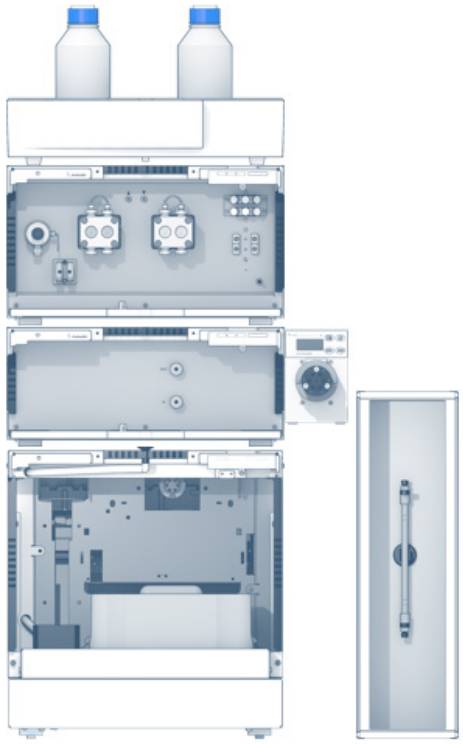
Column temperature	75 °C
Injection volume	20 µL
Injection mode	Partial loop
Detection	RID
Data rate	20 Hz
Time constant	0.05 s

Tab. 2 Pump parameters

Eluent (A)	Water
Flow rate	0.5 mL/min
Gradient	isocratic

Tab. 3 System configuration

Instrument	Description	Article No.
Pump 1	AZURA P6.1L, HPG	APH35EA
Autosampler	AZURA AS 6.1L	AAA00AA
Detector	AZURA RID 2.1L	ADC01
Thermostat	AZURA CT 2.1	A05852
Valve drive	AZURA VU 4.1	AWA01
Valve	High-pressure injection valve, 8 Port	AVC38AC
Column 1	Eurokat Na, 300 x 8 mm ID	30GX210EKN
Column 2	Eurokat Ca, 300 x 8 mm ID	30GX360EKN
Software	ClarityChrom 8.1 – workstation, autosampler control included	A1670



RELATED KNAUER APPLICATIONS

VFD0150 – Xylitol Extraction via HPLC Purification from Fermented Biomass

VFD0160 – Determination of sugars and natural sugar substitutes in different matrices

VFD0148J – Determination of mannose and mannooligosaccharides with an improved RI detector

