Science Together

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Sugar screening using Eurokat columns

Karyna Oliynyk, Svea Stephan, Yannick Krauke, Kate Monks; applications@knauer.net
KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin;
www.knauer.net

SUMMARY

In biorefineries, bacteria and yeast are used for the fermentation of C_5 and C_6 sugars into bio-ethanol. The by-products from bio-ethanol production are also of value and can be used in other applications. To determine the most suitable column for the analysis of fermentation samples, sugar and sugar alcohol standards were screened using ion exchange Eurokat columns. Measurements using these columns enable the identification and quantification of fermentation broth components, as well as the determination of any impurities that may be present. Screenings using Eurokat Ca, Na, Pb and H columns provided high detection and separation performance for sugars and sugar alcohols. This performance demonstrated that Eurokat columns are suitable for the separation of biorefinery products, as well as for other applications.

INTRODUCTION

Eurokat columns separate substances via a combination of size exclusion and ligand exchange mechanisms. The column packing material is composed of sulfonated cross-linked styrene-divinylbenzene. These polymers are extremely stable in aqueous media over the entire pH-range, resulting in the longer lifetime of Eurokat columns when compared to conventional silica-based columns. By modification of the column with Ca-, Na-, Pb- and H-Ions different column-sample interactions can be selected for, allowing for the selection of different separation dynamics.

Due to their high functional group density, Eurokat columns are recommended for ion exclusion, size exclusion and ligand exchange chromatography. Eurokat H is suitable for the determination of organic acids, including complex mixtures, carbohydrates, alcohols and sugar alcohols. Eurokat Ca and Pb work optimally for the determination of carbohydrates with a degree of polymerization (DP) < 4 and are not suitable for higher carbohydrates. Eurokat Na is suitable for the analysis of oligosaccharides with a DP of up to 6.[1] To acquire an overview of the separation specifications of these columns and to enable a comparison of their screening results, sixteen sugar and sugar alcohol standards were screened using four ion exchange columns.

Additional Information

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SAMPLE PREPARATION

All standards were dissolved in distilled water (concentration 10 mg/ml) and filtered with Sartorius RC 0.45 µm syringe filters. The stock solutions were diluted to 2 mg/ml before injection.

RESULTS

Sixteen sugars and sugar alcohols were screened using four differently modified columns: Eurokat Ca, Na, Pb and H. Eurokat Ca demonstrated the best separation of regarded samples out of all the columns. Different substance classes eluted from this column as groups of different retention times t_R: oligosaccharides had the shortest retention (t_R < 13 min), monosaccharides were found in the middle of the peak distribution (13 < t_R < 20 min) and sugar alcohols were retained the longest (t_R > 20 min). All measured standards are listed in eluting order in **Tab. 2**.

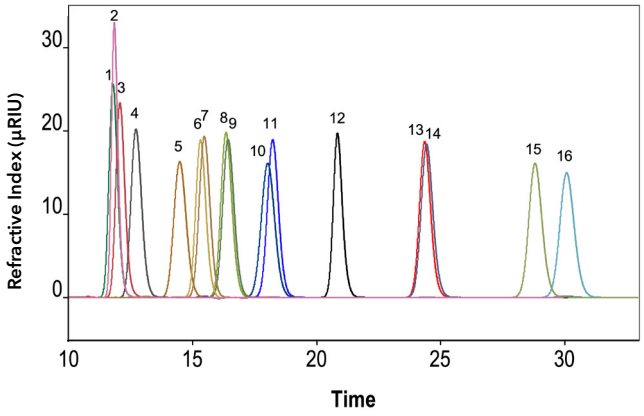

Fig. 1 Measurement of sugar standards on the Eurokat Ca column. Flow rate 0.5 ml/min, temperature 75 °C, sample concentration 2 mg/ml, injection volume 20 µl. Components are listed in **Tab. 2**

Eurokat Na is suitable for the detection of oligosaccharides and their separation from other substances, however it is not suitable for the separation of mixtures of oligosaccharides.

The chromatogram for Eurokat Na showed an inconsistent peak distribution. Oligosaccharides **(1–4 in Fig. 2)**, as well as monosaccharides and sugar alcohols **(8–13 in Fig. 2)**, showed similar retention times, meaning their separation was not possible. A mixture consisting of an oligosaccharide with a monosaccharide or a sugar alcohol could however be separated using this column. Eurokat Na allowed for the separation of mixtures, e.g. arabinose and fructose, that cannot be separated using a Eurokat Ca column **(Fig. 6)**. Retention times of all the measured standards are listed in **Tab. 3**.

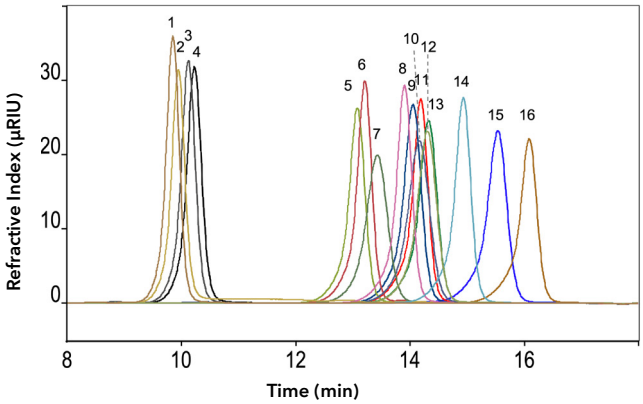

Fig. 2 Measurement of sugar standards on the Eurokat Na column. Flow rate 0.5 ml/min, temperature 75 °C, sample concentration 2 mg/ml, injection volume 20 µl. Components are listed in **Tab. 3**

Measurements using the Eurokat Pb column showed the highest retention times of all 4 columns. Similarly to the Eurokat Ca-column, oligosaccharides eluted from Eurokat Pb at low retention times (t_R < 18 min), followed by monosaccharides (18 < t_R < 24 min) and then sugar alcohols at high retention times (t_R > 24 min) **(Fig. 3)**.

The elution range for the sugar alcohols was found to exceed 25 minutes. Eurokat Pb allowed not only the separation of different substance types but also the separation of sugar alcohols from one-another.

A list of all the standards measured using this column and their retention times are shown in **Tab. 4**. Although a Pb modified column can be used in analysis stages they cannot be used in purification applications in the food industry, because of lead´s neurotoxicity.[2]

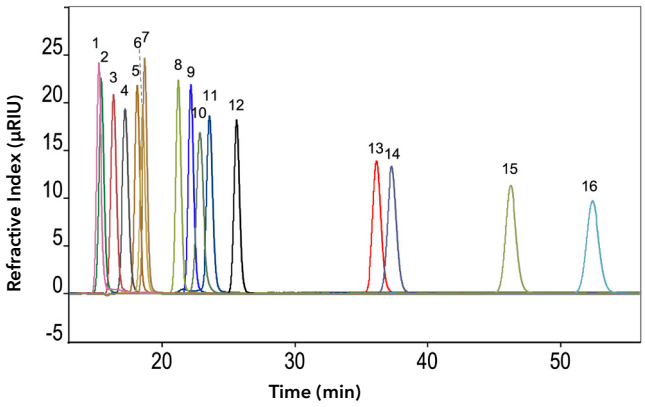

Fig. 3 Measurement of sugar standards on the Eurokat Pb column. Flow rate 0.5 ml/min, temperature 75 °C, sample concentration 2 mg/ml, injection volume 20 µl. Components are listed in Tab. 4

Eurokat H showed a separation of lower resolution than Eurokat Ca, however some specific substances could be effectively separated using this column, e.g. rhamnose and glucose (Fig. 8). Sucrose interacts with H-Ions on the column´s surface, resulting in its breakdown into two respective monomers with merging peaks (Fig. 4). This problem does not occur to any other oligosaccharides tested in this experiment. The three other disaccharides measured in this experiment eluted from the column at low retention times (< 8 min). All measured standards with their retention times are listed in Tab. 5.

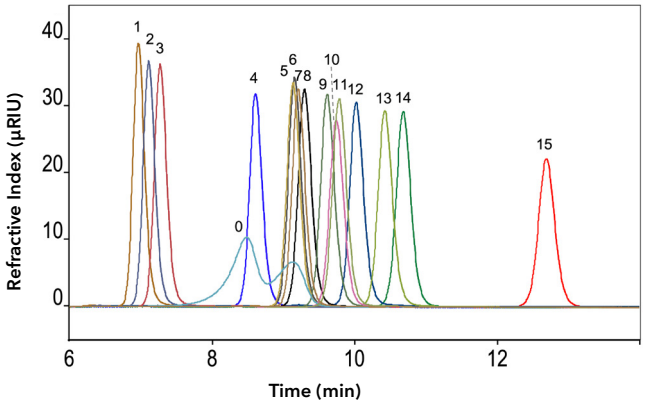

Fig. 4 Measurement of sugar standards on the Eurokat H column. Flow rate 0.7 ml/min, temperature 60 °C, sample concentration 2 mg/ml, injection volume 20 µl. Components are listed in Tab. 5

In Fig. 5–8 the separation of different substance pairs using Eurokat Ca and the other Eurokat models were compared to highlight any differences in the separation properties of the columns.

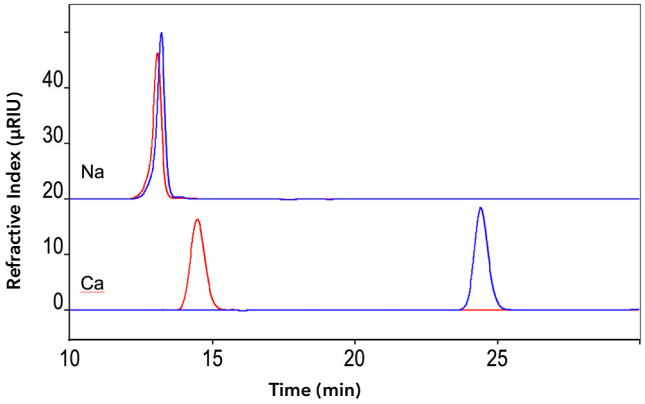

Fig. 5 Glucose (red) and mannitol (blue) measured with Eurokat Na (above) and Eurokat Ca (below) columns. Flow rate 0.5 ml/min, temperature 75 °C, sample concentration 2 mg/ml, injection volume 20 µl.

In Fig. 5 Eurokat Ca showed a clear baseline separation of glucose and mannitol (Resolution = 10.59), whilst the peaks of the same components eluted from Eurokat Na almost simultaneously (Resolution = 0.26). On the contrary, Fig. 6 shows that arabinose and fructose can be better separated using Eurokat Na (Resolution = 1.94) when compared to Eurokat Ca (Resolution = 0.21).

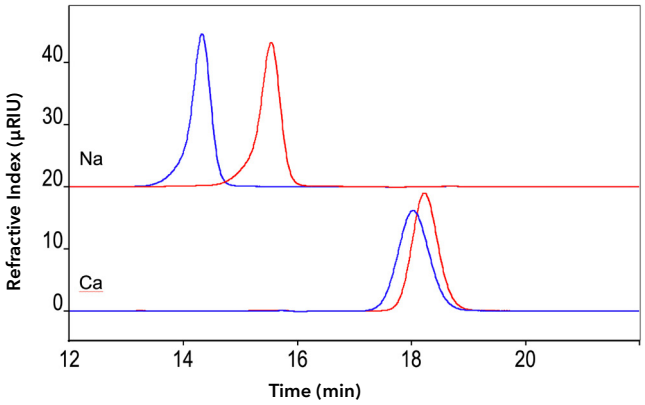

Fig. 6 Arabinose (red) and fructose (blue) measured with Eurokat Na (above) and Eurokat Ca (below) columns. Flow rate 0.5 ml/min, temperature 75 °C, sample concentration 2 mg/ml, injection volume 20 µl

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In most cases the Eurokat Ca is suitable for baseline or close-to-baseline separation. However, there are some sugar mixtures where other column modifications are required in order to achieve satisfactory separation. An example for each column (Eurokat Pb and H) is shown in **Fig. 7 and 8**.

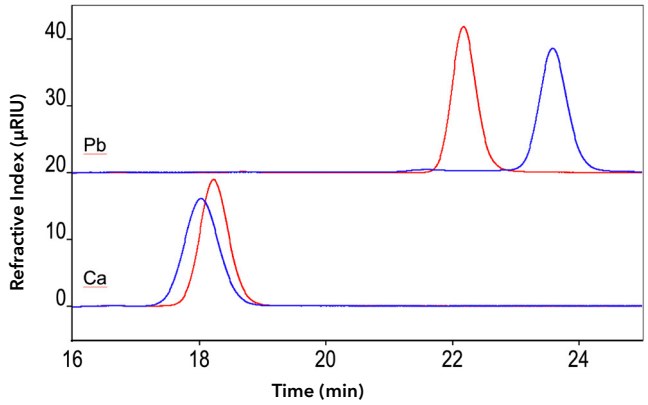


Fig. 7 Arabinose (red) and fructose (blue) measured with Eurokat Pb (above) and Eurokat Ca (below) columns. Flow rate 0.5 ml/min, temperature 75 °C, sample concentration 2 mg/ml, injection volume 20 µl.

Fig. 7 shows that the separation of arabinose and fructose is significantly improved when using Eurokat Pb (Resolution = 1.80) than when using Eurokat Ca (Resolution = 0.21).

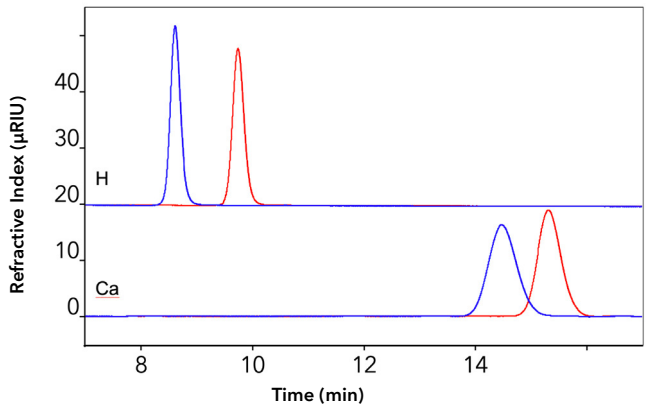


Fig. 8 Rhamnose (red) and glucose (blue) measured with Eurokat H (above) and Eurokat Ca (below) columns. Ca: flow rate 0.5 ml/min, temperature 75 °C; H: flow rate 0.7 ml/min, temperature 60 °C; sample concentration 2 mg/ml, injection volume 20 µl.

Although rhamnose and glucose were separable using both columns, a baseline separation was only possible using Eurokat H (Resolution = 3.11). The resolution using Eurokat Ca was 0.94.

CONCLUSION

Sixteen sugar and sugar alcohol standards were screened using the Eurokat Ca, Na, Pb and H columns. Comparison of the elution profiles of the standards demonstrated **(Fig. 1–4)**, that the Ca- and Pb- modified columns provided the best sample distribution for this substance range.

To determine the most suitable column for a separation, it is useful to know (or have at least a presumption of) the sample composition. As shown in **Fig. 1–4** oligosaccharides elute at low, monosaccharides at mid-range and sugar alcohols at high retention times.

When using Eurokat Na and H columns monosaccharides and sugar alcohols eluted in the same time range. Each column has its own elution range, so it is not possible to define general retention time limits for every type of substance in each of the columns. The choice of which column to use for a particular experiment should be based on the composition of the sample. Depending on the sample composition, sometimes an analysis that uses two different Eurokat columns may be necessary.

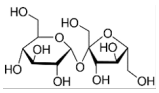
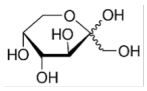
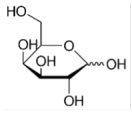
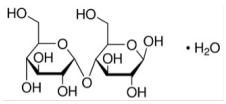
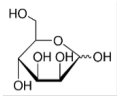
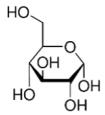
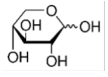
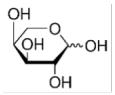
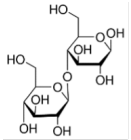
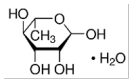
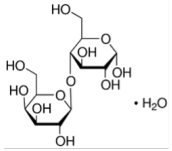
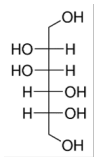
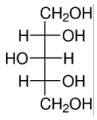
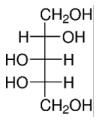
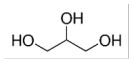
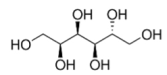
A high peak resolution (Res) results in better separation. A one hundred percent separation is achievable at Res = 2.[3] Retention times for all of the measured standards when using each of the referenced columns are listed in the **Tab. 2–5**.

If Eurokat columns are applied for purification tasks it is important to consider that the use of lead in the food sector is not allowed because of its toxicity, therefore the Eurokat Pb column is not suitable for purification purposes in the food industry.

MATERIALS AND METHODS

The analysis of sugars requires an isocratic HPLC system equipped with a degasser, autosampler, column oven and a refractive index detector. Other configurations are also available. Please contact KNAUER to configure a system that fits your needs. To protect the column from sample impurities installation of a pre-column packed with the same material as the main column is always recommended.

Tab. 1 Measured analytes

Analyte	CAS#	Chemical formula	Chemical structure
Sucrose	57-50-1	$C_{12}H_{22}O_{11}$	
D-(-)-Fructose	57-48-7	$C_6H_{12}O_6$	
D-(+)-Galactose	59-23-4	$C_6H_{12}O_6$	
D-(+)-Maltose monohydrate	6363-53-7	$C_{12}H_{22}O_{11} \cdot H_2O$	
D-(+)-Mannose	3458-28-4	$C_6H_{12}O_6$	
D-(+)-Glucose	14431-43-7	$C_6H_{12}O_6$	
D-(+)-Xylose	58-86-6	$C_5H_{10}O_5$	
L-(+)-Arabinose	5328-37-0	$C_5H_{10}O_5$	
D-(+)-Cellobiose	528-50-7	$C_{12}H_{22}O_{11}$	
L-Rhamnose monohydrate	10030-85-0	$C_6H_{12}O_5 \cdot H_2O$	
D-Lactose monohydrate	64044-51-5	$C_{12}H_{22}O_{11}$	
D-Mannitol	69-65-8	$C_6H_{14}O_6$	
Xylitol	87-99-0	$C_5H_{12}O_5$	
L-(-)-Arabitol	7643-75-6	$C_5H_{12}O_5$	
Glycerol	56-81-5	$C_3H_8O_3$	
D-Sorbitol	50-70-4	$C_6H_{14}O_6$	

Tab. 2 List of retention times of all standards with Eurokat Ca column

No.	Sugar	Retention time (min)
1	D-(+)-Cellobiose	11.79
2	Sucrose	11.84
3	D-(+)-Maltose monohydrate	12.07
4	D-Lactose monohydrate	12.72
5	D-(+)-Glucose	14.48
6	L-Rhamnose monohydrate	15.32
7	D-(+)-Xylose	15.47
8	D-(+)-Galactose	16.35
9	D-(+)-Mannose	16.43
10	D-(-)-Fructose	18.03
11	D-(+)-Arabinose	18.23
12	Glycerol	20.84
13	L-(-)-Arabitol	24.36
14	D-Mannitol	24.44
15	Xylitol	28.81
16	D-Sorbitol	30.09

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Tab. 3 List of retention times of all standards with Eurokat Na column

No.	Sugar	Retention time (min)
1	D-(+)-Cellobiose	9.85
2	Sucrose	9.95
3	D-(+)-Maltose monohydrate	10.12
4	D-Lactose monohydrate	10.23
5	D-(+)-Glucose	13.08
6	D-Mannitol	13.22
7	L-Rhamnose monohydrate	13.43
8	D-Sorbitol	13.91
9	D-(+)-Galactose	14.06
10	D-(+)-Mannose	14.16
11	L-(-)-Arabitol	14.19
12	D-(+)-Xylose	14.31
13	D-(-)-Fructose	14.33
14	Xylitol	14.94
15	D-(+)-Arabinose	15.54
16	Glycerol	16.09

Tab. 4 List of retention times of all standards with Eurokat Pb column

No.	Sugar	Retention time (min)
1	Sucrose	15.23
2	D-(+)-Cellobiose	15.43
3	D-(+)-Maltose monohydrate	16.34
4	D-Lactose monohydrate	17.24
5	D-(+)-Glucose	18.12
6	L-Rhamnose monohydrate	18.53
7	D-(+)-Xylose	18.71
8	D-(+)-Galactose	21.22
9	D-(+)-Arabinose	22.18
10	D-(+)-Mannose	22.84
11	D-(-)-Fructose	23.59
12	Glycerol	25.62
13	L-(-)-Arabitol	36.18
14	D-Mannitol	37.31
15	Xylitol	46.27
16	D-Sorbitol	52.42

Tab. 5 List of retention times of all standards with Eurokat H column

No.	Sugar	Retention time (min)
1	D-(+)-Cellobiose	6.97
2	D-(+)-Maltose monohydrate	7.11
3	D-Lactose monohydrate	7.27
4	D-(+)-Glucose	8.61
5	D-(+)-Mannose	9.14
6	D-(+)-Galactose	9.16
7	D-(+)-Xylose	9.21
8	D-(-)-Fructose	9.30
9	D-Mannitol	9.62
10	L-Rhamnose monohydrate	9.75
11	D-Sorbitol	9.79
12	D-(+)-Arabinose	10.02
13	L-(-)-Arabitol	10.43
14	Xylitol	10.68
15	Glycerol	12.69
0	Sucrose	—

Tab. 6 Method parameters

	Value Ca, Na and Pb columns	Value H column
Column temperature	75 °C	60 °C
Injection volume	20 µL	20 µL
Injection mode	Partial Loop	Partial Loop
Detection	RI	RI
Data rate	10 Hz	10 Hz

Tab. 7 Pump program

	Value Ca, Na and Pb columns	Value H column
Eluent (A)	water	0.01 N – H_2SO_4 in water
Flow rate	0.5 ml/min	0.7 ml/min
Gradient	isocratic	isocratic

Configuration of the system used in this application is listed in **Tab. 8**. Method parameters and pump program for each column are shown in **Tab. 6–7**.

Tab. 8 System configuration

Instrument	Description	Article No.
Pump 1	AZURA P6.1L LPG	APH34EA
Autosampler	AZURA AS 6.1L	AAA50AA
Detector	AZURA RID 2.1L	ADD31
Thermosthat	AZURA CT 2.1	ATC00
Column	Eurokat Ca, 10 µm, Column 300 x 8 mm	30GX360EKN
	Eurokat Na, 10 µm, Column 300 x 8 mm	30GX210EKN
	Eurokat Pb, 10 µm, Column 300 x 8 mm	30GX350EKN
	Eurokat H, 10 µm, Column 300 x 8 mm	30GX340EKN
	Eurokat Ca, 10 µm, Column 30 x 8 mm	03GX360EKN
	Eurokat Na, 10 µm, Column 30 x 8 mm	03GX210EKN
	Eurokat Pb, 10 µm, Column 30 x 8 mm	03GX350EKN
	Eurokat H, 10 µm, Column 30 x 8 mm	03GX340EKN
Software	ClarityChrom (version 8.2.2.102)	A1670

Fig. 9 Instrument composition

Information about the minimum configuration of the Sugar Screening System: **A46014**

REFERENCES

[1] Column Care and Regeneration – Eurokat
https://www.knauer.net/Dokumente/columns/lc_columns/guides/Column%20Care%20and%20Use_Eurokat_2018.pdf

[2] EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on Lead in Food. EFSA Journal 2010; 8(4):1570. [151 pp.]. doi:10.2903/j.efsa.2010.1570
Chemical structures image source:
https://www.sigmaaldrich.com/

[3] Gaby Aced, Hermann J. Möckel, Liquidchromatographie: Apparative, theoretische und methodische Grundlagen der HPLC, Weinheim; New York; Basel; Cambridge: VCH, 1991, p. 29–30

RELATED KNAUER APPLICATIONS

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VFD0148J Determination of mannose and mannooligosaccharides with an improved RI detector

VFD0149J Determination of xylitol in microbial fermentation broth

VFD0160 Determination of sugars and natural sugar substitutes in different matrices

VFD0187 Eurokat column coupling