Increasing sensitivity of carbohydrate analysis by switching from refractive index to electrochemical detection

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SUMMARY

Nowadays sugar substitutes are used in many products, not only for diabetic purposes but to make products more attractive for customers. Furthermore, people are interested in a healthier lifestyle which includes consuming less sugar. Therefore, quality control of sugar and sugar substitutes in food and beverages is compulsory to assure the correct labelling of products and composition of ingredients. Using KNAUER Eurokat columns in combination with an electrochemical detector expands the application area of carbohydrate analysis.

INTRODUCTION

Sweet taste is favoured by human beings. People instinctively desire the pleasure of sweetness, which resulted in a preference for sweet foods and beverages[1]. Since sugar is rich in calories, a lot of people are switching to light products containing sugar substitutes. These products contain less calories and are often obtained from natural crude materials, e.g. wood fibres of the birch. This application focuses on the determination of commonly used sugars and natural sugar substitutes. Sucralose (E 955) is a high-intensity sweetener, about 600 times higher than saccharose.

Mannitol (E 421) and sorbitol (E 420) have about half the intensity of saccharose, while xylitol (E 967) has a quite equal intensity as commonly used sugar[2]. In KNAUER application **VFD0160** the separation of saccharose, sucralose, glucose, fructose, mannitol, xylitol and sorbitol was performed and detection was carried out via the measurement of the refractive index using the AZURA RID 2.1L. The following application reproduces the measurements using electrochemical detection with post column sodium hydroxide addition to enhance the sensitivity.

Additional Information

RESULTS

For electrochemical detection of carbohydrates, the use of sodium hydroxide is necessary, either as mobile phase or applied via post column addition. Eurokat polymer columns are usually operated at a temperature of 60 - 75 °C. However, the measuring cell of the ECD can be operated in a temperature range from 10 – 40 °C. The great temperature difference from column to ECD cell caused a drifting baseline. To stabilize the baseline and minimize the temperature gradient, the column temperature was set to 60 °C. A 5-point calibration in a range from 1.00 to 10.00 µg/mL for the seven compounds was recorded. The calibration curves showed a very good linearity with R2 > 0.9996 for all analytes. **Fig. 1** shows the mixed standard at a concentration of 8.00 µg/mL.
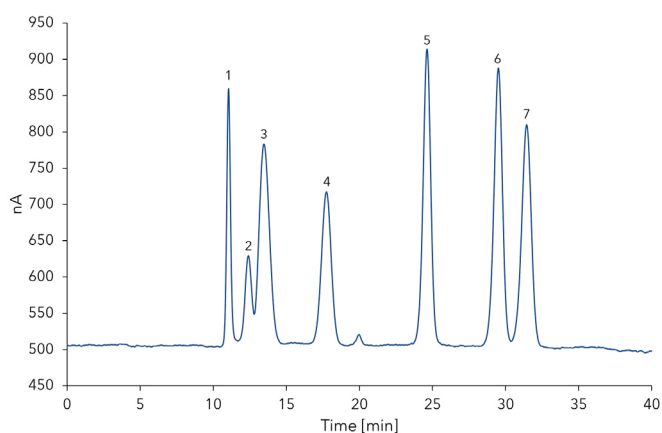
Tab. 1 Calculated LOD and LOQ values

Substance	LOD (S/N=3) [µg/mL]	LOQ (S/N=10) [µg/mL]
Saccharose	0.07	0.25
Sucralose	0.23	0.77
Glucose	0.10	0.32
Fructose	0.13	0.44
Mannitol	0.07	0.22
Xylitol	0.07	0.23
Sorbitol	0.09	0.29

Referring to application note VFD0160 connatural samples of chewing gum and toothpaste were extracted. **Fig. 2** and **Fig. 3** show an overlay of the mixed standard and the extracted samples.
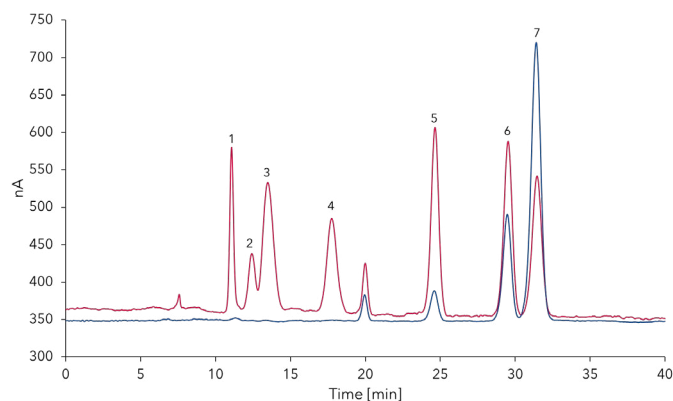

Fig. 1 Mixed standard at a concentration of 8.00 µg/mL. 1 - saccharose, 2 - sucralose, 3 - glucose, 4 - fructose, 5 - mannitol, 6 - xylitol, 7 - sorbitol.

Based on the measurement of the lowest calibration point 1.00 µg/mL, the limit of detection (LOD) and limit of quantification (LOQ) was calculated. For LOD a signal-to-noise ratio (S/N) of S/N=3 was taken as basis and S/N=10 for LOQ. The following **Tab. 1** summarizes the calculated values for each compound.

Fig. 2 Overlay of mixed standard (red) and extracted chewing gum (blue). 1 - saccharose, 2 - sucralose, 3 - glucose, 4 - fructose, 5 - mannitol, 6 - xylitol, 7 - sorbitol.
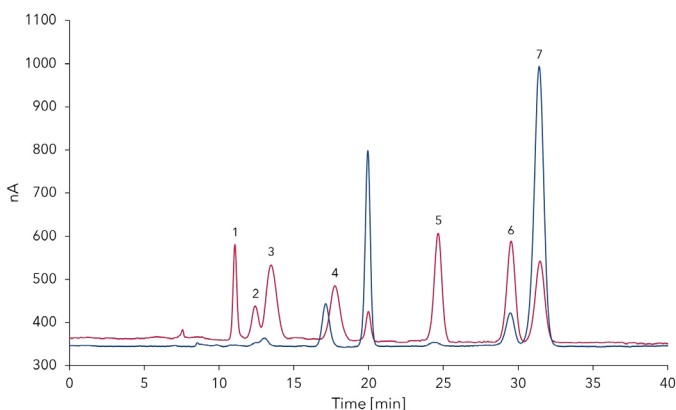

Fig. 3 Overlay of mixed standard (red) and extracted toothpaste (blue). 1 - saccharose, 2 - sucralose, 3 - glucose, 4 - fructose, 5 - mannitol, 6 - xylitol, 7 - sorbitol.

RESULTS

The amount of sugar and sugar substitutes in the samples was calculated in g/100g. **Tab. 2** shows the results of sample measurement. The chewing gum contains high amounts of xylitol and sorbitol. Furthermore, mannitol was detected but none of the other sugars. This goes along with the available product information. The extracted toothpaste sample also contains xylitol and sorbitol. Again, this correlates to the provided product data. Unfortunately, no detailed information about the exact amounts of sugars and sugar substitutes in the analysed samples is available.

Tab. 2 Results of sample measurements (n.d. = not detected)

Peak	Compound	Chewing gum in g/100g	Toothpaste in g/100g
1	Saccharose	n.d.	n.d.
2	Sucralose	n.d.	n.d.
3	Glucose	n.d.	n.d.
4	Fructose	n.d.	n.d.
5	Mannitol	3.42	0.49
6	Xylitol	11.98	3.00
7	Sorbitol	37.68	28.65

SAMPLE PREPARATIONS

Before running the system, it was passivated with 20% nitric acid at a flow rate of 1mL/min for about 30 minutes. Afterwards it was flushed with water until the pH was neutral. The mobile phase for post column addition was prepared in plastic flasks using a 50% (w/w) carbonate free NaOH stock solution. After diluting with water to 200 mM, the sodium hydroxide eluent was transferred to a plastic bottle. The eluent was degassed using ultra sonication and additionally vacuum filtrated. To keep the eluent carbon dioxide free, a filter was installed on top of the bottle. All prepared standards were dissolved in deionized water.

For ECD detection a SenCell high sensitivity electrochemical flow cell with a gold working electrode was used and operated at 40 °C. The column temperature was set to 60 °C. The ECD was operated in pulsed mode using a 4-step PAD potential waveform **(Fig 4)**.
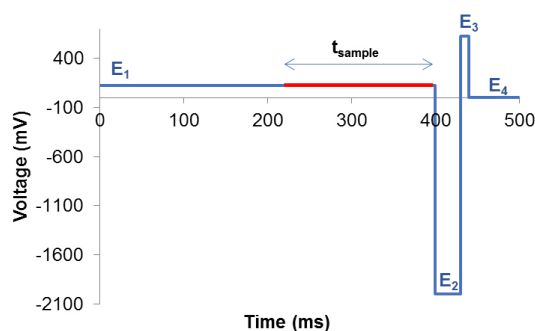

Fig. 4 4-step PAD potential waveform for the detection of monosaccharides and other carbohydrates, the sample detection occurs during the highlighted time period tsample

5 g of the chewing gum or toothpaste sample were weighed into a conical flask. Then 50 mL of deionized water were added. The flask was heated under stirring to about 50 °C for approximately 20 minutes. After cooling to room temperature, the extract was filtered through a 0.45 µm syringe filter. After dilution 1:2000 with water, 20 µL of the sample were injected.

CONCLUSION

The calibration determined in KNAUER application note VFD0160 using refractive index detection lies in a range from 0.25 mg/mL up to 2.00 mg/mL. Using the electrochemical detector ECD 2.1 it was possible to calibrate a range which is about 250 times lower. That makes it possible to detect even residues of carbohydrates in food, beverages and other products. Hence, the applied method is suitable for quality control of sugar free or "light" labelled products for example, where very low concentrations of carbohydrates must be determined. A striking benefit over already published methods using electrochemical detection is the use of the same column as with the refractive index detector. Therewith, a very wide range of concentrations on the same, very long-lasting column can be analysed.

MATERIALS AND METHODS

Tab. 3 Instrument setup

Column temperature	60 °C
Injection volume	20 µL
Injection mode	Partial loop
Detection	ECD

Tab. 4 Pump parameter

Eluent (A)	Water
Flow rate	0.5 mL/min
Post column eluent (B)	200 mM sodium hydroxide
Post column flow rate	0.8 mL/min
Gradient	Isocratic

Tab. 5 ECD settings (PAD)

E1	0.10 V	t1	0.04 s
E2	-2.00 V	t2	0.02 s
E3	0.60 V	t3	0.01 s
E4	-0.10 V	t4	0.02 s
Cell temperature	40 °C	ts	200 ms
Range	1 µA		
Polarity	+		
Compensation	On		
AST position	2		
Filter	0.02 Hz		

Tab. 6 System configuration

Instrument	Description	Article No.
Pump	AZURA P 6.1L, HPG	APH35EA
Autosampler	AZURA AS 6.1L	AAA00AA
Detector	AZURA ECD 2.1	A1651
Flow cell	SenCell - HyREF (Pd/H2) Reference electrode/Au Working electrode	A1652-3
Thermostat	AZURA CT 2.1	A05852
Column	Eurokat Ca, 300 x 8 mm ID	30GX360EKN
Software	ClarityChrom 8.1 – Workstation, autosampler control included	A1670
Software	ClarityChrom 8.1 – System Suitability Extension (SST)	A1677
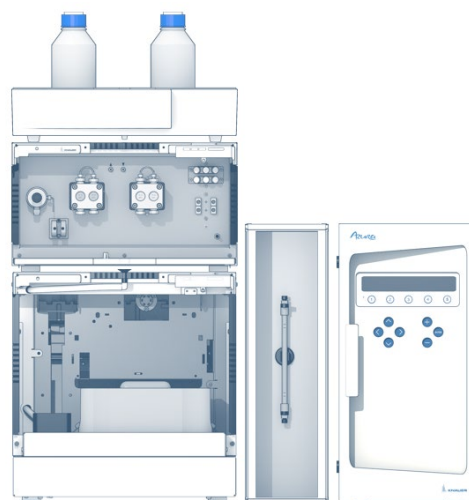
REFERENCES

[1] Zygler, A., Wasik, A., Kot-Wasik, A., Namieśnik, J. Determination of nine high-intensity sweeteners in various foods by high-performance liquid chromatography with mass spectrometric detection. Anal Bioanal Chem 400(7):2159-2172 (2011).

[2] Kuhner, P. Kennzeichnung von Zusatzstoffen. Bundeszentrum für Ernährung https://www.bzfe.de/inhalt/kennzeichnung-von-zusatzstoffen-1881.html (February 6, 2020).

RELATED KNAUER APPLICATIONS

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

VFD0183 – Sensitivity boost - comparison of electrochemical and refractive index detection for sugar analysis