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

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SUMMARY

The determination of carbohydrates in food and other matrices is always a topic of high concern. The exact determination of sugar content is not only essential when thinking of foods targeted to diabetics. Such sensitive analytical methods are also interesting to manufacturers of sugar based pharmaceutical products or for research projects in the field of carbohydrates. Here, we compare the HPLC analysis with electrochemical detection (ECD) versus refractive index detection (RID) for the determination of glucose, sucrose, and arabinose.

INTRODUCTION

Many different methods for sugar analysis are available, using different columns and detection facilities. Due to the missing chromophore, carbohydrates are often analysed with refractive index detection. Refractive index detectors (RID) are universal detectors applicable for a large variety of analytical tasks. They are often chosen for their robustness and easy handling. Compared to other detectors they are in many cases more cost effective. Whenever the critical factor of analysis is sensitivity, it depends on the limit values that should be reached if a RID is applicable. Compared to UV detection for example, the RID is characterized by lower sensitivity. Alternatives for detecting substances without a chromophore are light scattering detection (ELSD) or electrochemical detection (ECD). The following application faces the differences of electrochemical detection and refractive index detection on the exemplary separation of glucose, sucrose, and arabinose. The focus of determination was set to the calculation of the limit of detection (LOD).

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SAMPLE PREPARATION

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 method ran isocratically at a flow rate of 0.6 mL/min with 0.015 M sodium hydroxide as mobile phase. The mobile phase was prepared in plastic flasks using a 50 % (w/w) carbonate free NaOH stock solution. After diluting with water to 0.015 M, 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 eluent.

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 1)**.

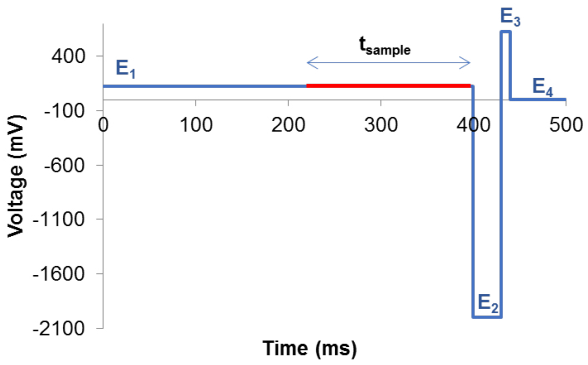


Fig. 1 4-step PAD potential waveform for the detection of monosaccharides and other carbohydrates, the sample detection occurs during the highlighted time period t_{sample}

RESULTS

For electrochemical detection, the use of sodium hydroxide as mobile phase is necessary. That is why the separation was performed on an Eurokat sodium (Eurokat Na) column. Due to the sodium ligands, this column is suitable to tolerate the basic conditions. A 0.015 M sodium hydroxide solution was used as mobile phase for the RID and ECD measurements. The detailed method parameters are described in the materials & methods section. Eurokat Na columns are usually operated at a temperature of 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 to minimize the temperature gradient, the column temperature was set to 60 °C. **Fig 2** (ECD) shows the measurement of a mixed sugar standard at a concentration of 10 µg/mL for each component. **Fig 3** (RID) shows the chromatogram of the same mixture at a concentration of 1 mg/mL. All analytes were baseline separated. Based on these measurements the LOD (S/N=3) was determined. The calculated results and single values for signal and noise are summarized in **Tab 1**. The achieved performances reveal significant disparities in sensitivity. Whereas the LOD amounts for the RID measurements lie in a range from 7–10 µg/mL, the ECD results are ranged between 1–8 ng/mL. For glucose and arabinose, the sensitivity of the ECD is about 5000 times higher compared to the one of the RID. The sensitivity for sucrose is about 1000 times higher.

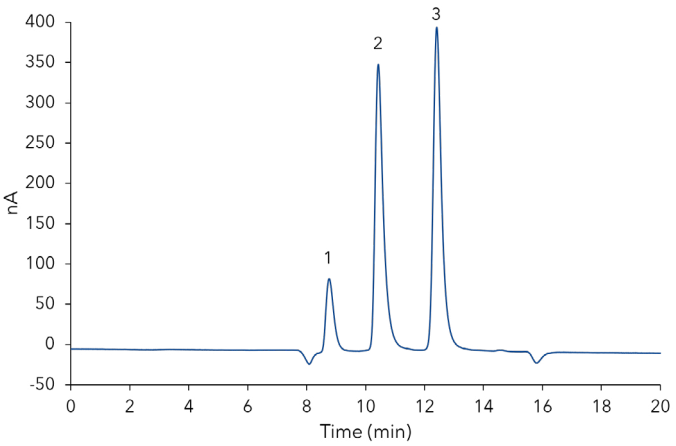


Fig. 2 ECD chromatogram of mixed sugar standard at 10 µg/mL, 1) sucrose 2) glucose 3) arabinose

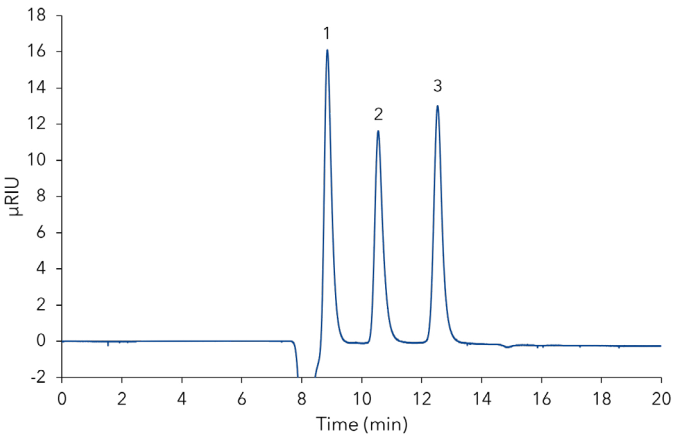


Fig. 3 RID chromatogram of mixed sugar standard at 1 mg/mL, 1) sucrose 2) glucose 3) arabinose

Tab. 1 Calculated LOD values for ECD and RID measurements of sucrose, glucose, and arabinose

	ECD			RID		
	Signal (nA)	**Noise (nA)**	**LOD (S/N=3) (ng/mL)**	**Signal (µRIU)**	**Noise (µRIU)**	**LOD (S/N=3) (µg/mL)**
Sucrose	97.3950	0.2509	7.7283	16.4670	0.0403	7.3420
Glucose	355.9940	0.2509	2.1144	11.7480	0.0403	10.2911
Arabinose	401.8500	0.2509	1.8731	13.2020	0.0403	9.1577

CONCLUSION

Both, ECD and RID, have their benefits. Concerning the operation, the RID is much easier to handle. Also, the common Eurokat column temperatures of 60 °C to 85 °C are no problem and the baseline stabilizes quickly, assuming the flow cell is flushed sufficiently. The SenCell, as the name implies, is not only sensitive during measurement but also to changes in the method settings. It is for example necessary to stabilize the cell after every change in the waveform settings. Furthermore, high temperature gradients from column to cell are debilitating, causing a noisy and drifting baseline. But regarding responsivity, the ECD is one step ahead. Recapitulated, both detectors are very suitable for the analysis of carbohydrates. Dependent on the presupposed limits of detection and quantification the right detector must be chosen.

MATERIALS AND METHODS

Tab. 2 Method parameters

Column temperature	60 °C
Injection volume	20 µL
Injection mode	Partial Loop
Detection 1	RID
Data rate	20 Hz (for RID only)
Detection 2	ECD

Tab. 3 Pump parameters

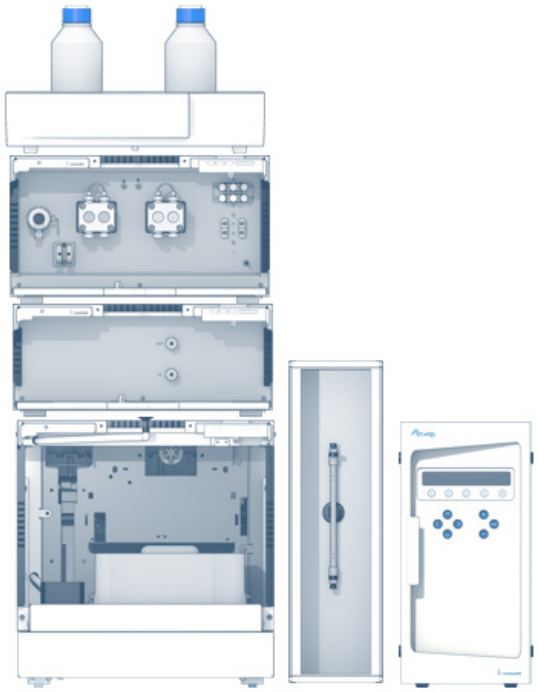
Eluent (A)	0.015 M sodium hydroxide
Gradient	isocratic
Flow rate	0.6 mL/min
Run time	20 min

Tab. 4 ECD settings (pulsed mode)

E1	0.10 V	t1	0.40 s
E2	-2.00 V	t2	0.02 s
E3	0.60 V	t3	0.01 s
E4	-0.10 V	t4	0.20 s
Cell temperature	40 °C	ts	200 ms
Range	200 - 500 nA		
Polarity	+		
Compensation	On		
Spacer position	2		
Filter	Off		

Tab. 5 System configuration

Instrument	Description	Article No.
Pump	AZURA P 6.1L, HPG	APH35EA
Autosampler	AZURA AS 6.1L	AAA00AA
Detector 1	AZURA RID 2.1L	ADD31
Detector 2	AZURA ECD 2.1	A1651
Flow cell (detector 2)	SenCell - HyREF (Pd/H2) Reference electrode/Au Working electrode	A1652-3
Thermostat	AZURA CT 2.1	A05852
Column	Eurokat Na, 300 x 8 mm ID	30GX210EKN
Column	Eurokat Na, 30 x 8 mm ID	03GX210EKN
Software	ClarityChrom 8.1 – workstation, autosampler control included	A1670
Software	ClarityChrom 8.1 – System Suitability Extension (SST)	A1677



RELATED KNAUER APPLICATIONS

VEV0083 – Sensitive and selective analysis of wood sugars and uronic acids for biofuel research with electrochemical detection

VFD0160 – Determination of sugars in different matrices

VFD0161 – Determination of sugars in honey using HILIC separation and RI detection

