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

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SUMMARY

Monosaccharides belong to the most abundant group of biomolecules in nature. They play a crucial role in metabolism, structural biology, and storage of energy. Thus, the analysis of these special type of carbohydrates is of great interest for the food industry but also for a broad range of life and material sciences. The presence of hydroxyl groups enables a specific and highly sensitive analysis using pulsed amperometric detection (PAD) with the DECADE Elite electrochemical detector as part of the dedicated AZURA® High Performance Anion Exchange Chromatography (HPAEC) system.

INTRODUCTION

The sources for the different kinds of monosaccharides can vary between food samples like honey [1] or fruits, to scientific applications like glycopeptides or they can be products of fermentation processes like the here analysed wood monosaccharides. The mixture of the seven hemicellulosic sugars fucose, rhamnose, arabinose, galactose, glucose, xylose and mannose mixed with the two uronic acids galacturonic acid and glucuronic acid, extracted from wood by heat or chemical pretreatment, are of special interest in the research for new biofuels. They are considered to be more sustainable and are expected to become a competitive commercial alternative to fuel made from corn and other food sources [2]. Carbohydrates are week acids with pKa values between 12 and 14. Consequently, they can be completely or partially ionized under basic conditions with pH >12. Due to these harsh conditions, only polymeric anion exchange columns are suitable for the monosaccharide analysis. The retention time with AZURA HPAEC is inversely correlated with pKa value and increases significantly with molecular weight of the monosaccharide.

Additional Information

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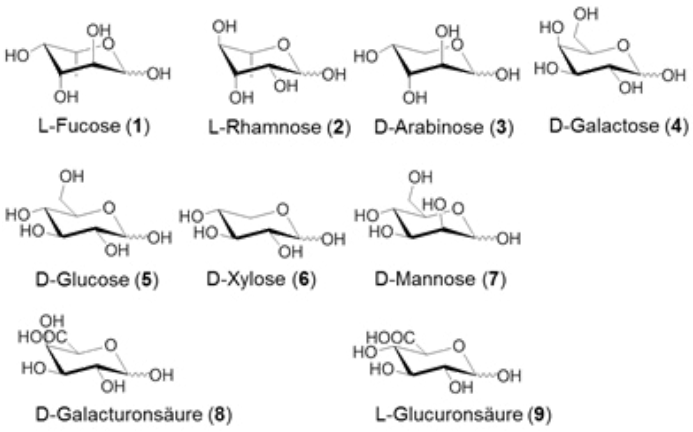
RESULTS

Using an analyte concentration of 0.1 mg/mL for the standard mixture of the nine wood monosaccharides and acids, all components could be baseline-separated (R_s > 1.5) **(Fig 1)**. The separation of the analyte peaks increases with decreasing sample concentration. The two monosaccharides xylose (6) and mannose (7) could not be baseline-separated with concentrations higher than 0.1 mg/mL. The signal to noise (S/N) ratio for each analyte was calculated from empiric data **(Tab 1)**. Noise values were determined for this concentration from two different baseline areas. For the monosaccharide sugars 1-7 the averaged noise was determined with 0.001 µA and for the uronic acids 8-9 a value of 0.1 µA was determined. Concentration curves of all analytes from 0.0125 to 0.25 mg/mL are depicted in **Fig 2**.



Pyranose structure of the seven wood monosaccharides and the two uronic acids

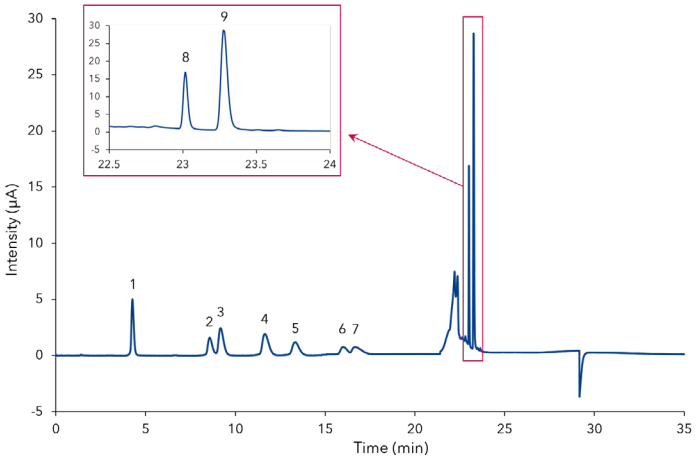


Fig. 1 Chromatogram of a standard mixture containing 0.1 mg/mL fucose (1), rhamnose (2), arabinose (3), galactose (4), glucose (5), xylose (6), mannose (7), galacturonic acid (8) and glucuronic acid (9). And a zoom into the peaks for the uronic acids

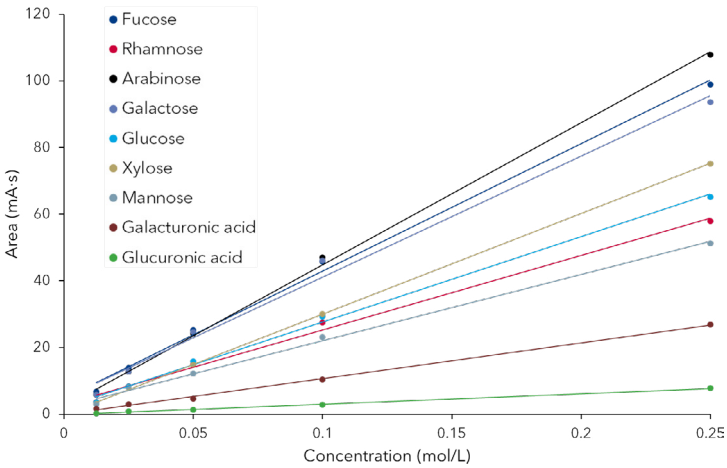


Fig. 2 Concentration curves of the described sugars and uronic acids in a concentration range between 0.0125 mg/mL to 0.25 mg/mL

Analyte	S/N
L-fucose	10000
L-rhamnose	3000
L-arabinose	4800
D-galactose	3800
D-glucose	2400
D-xylose	1600
D-mannose	1600
D-galacturonic acid	338
D-glucuronic acid	574

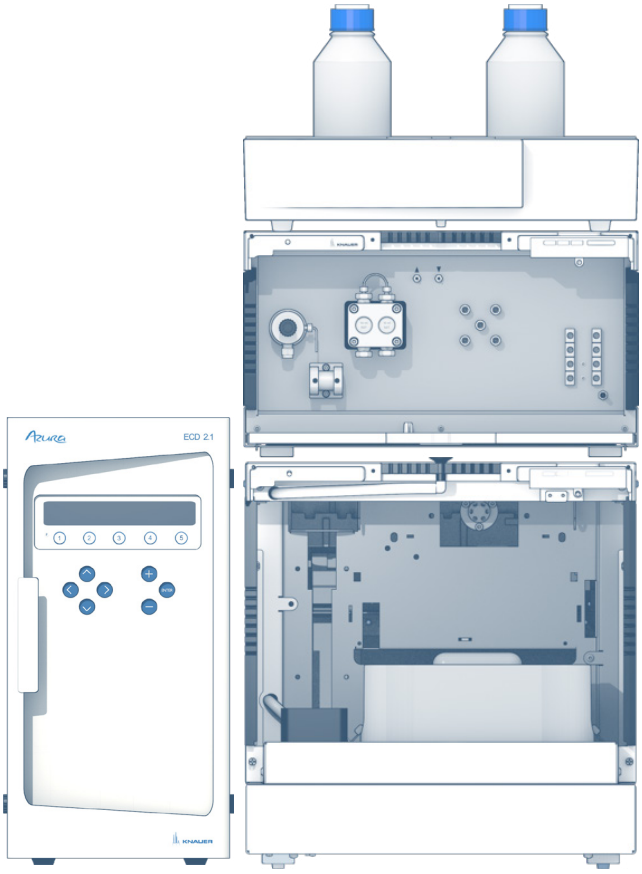
Tab. 1 Empiric determined S/N ratios for a 10 µL injection

MATERIALS AND METHODS

An AZURA glass- and metal-free High Performance Anion Exchange Chromatography (HPAEC) system was used. It was comprised of an AZURA P 6.1L LPG pump, an AZURA AS 6.1L autosampler and a DECADE Elite electrochemical detector which was also used for column tempering. The analysis was based on a step-gradient with different concentrations of NaOH solution **(Tab A2 & A3**, additional material**)**. While working with low concentrations of NaOH, carbonate ions, present in the mobile phase, can bind to the column material and thereby decrease separation efficiency. Hence, a column regeneration with higher concentrations of NaOH is recommendable for each run. Furthermore, during eluent preparation the contamination with carbonate ions should be minimized by using carbonate-free 50 % w/w NaOH solution (commercially available) and an ultrasonic degassing step before the introduction into the system. Eluents should be completely refreshed daily. With respect to the high sensitivity of the DECADE Elite detector and the etching property of the NaOH, only plastic eluent bottles, plastic eluent filters and metal-free system compartments should be used to prevent the detection of unexpected ions, silicates or borates. For detection an Antec electrochemical SenCell with Au working electrode, HyREF (Pd/H2) reference electrode and stainless steel auxiliary electrode was used with a 4-step potential waveform **(Fig A1**, additional material**)**.

CONCLUSION

High Performance Anion Exchange Chromatography (HPAEC) with pulsed amperometric detection (PAD) using the AZURA HPAEC-PAD dedicated system and applying the developed method is a highly sensitive setup for the analysis of sugar monosaccharides and other carbohydrates. The mixture of seven monosaccharides and two uronic acids could be baseline-separated with very high S/N ratios. An easy to perform method using different concentrations of NaOH allows a fast and reproducible analysis even in low concentrations. Besides the research for biofuels, the investigated sugars are components in numerous processes in nature and food applications. Thus, the current application is suitable for several issues where carbohydrates need to be specifically separated and analyzed.



REFERENCES

[1] H. Schlicke, K. Monks, KNAUER AppNote VFD0161, 2017
[2] M. J. González-Muñoz, R. Alvarez, V. Santos, J. C. Parajó, Wood Science and Technology, 2012, 46, 1–3, 271–285.

ADDITIONAL MATERIALS AND METHODS

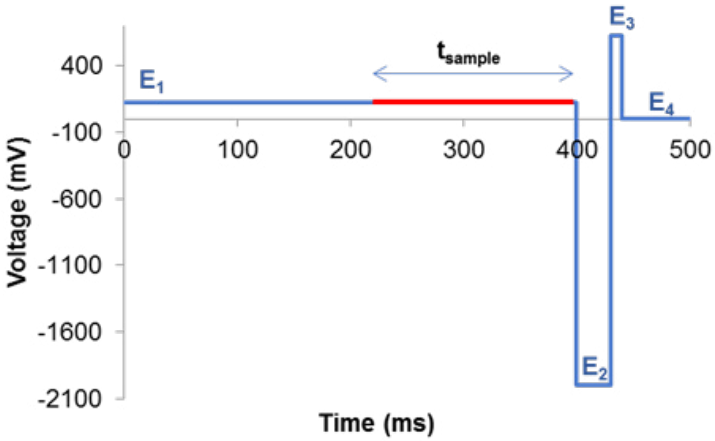


Fig. A1 4-step PAD potential waveform for the detection of mono-saccharides and other carbohydrates. The sample detec-tion occurs during the highlighted time period tsample.

Tab. A1 Method parameters

Eluent A	Water		
Eluent B	200 mM NaOH		
Eluent C	700 mM NaOH		
Flow rate	0.4 mL/min	Pressure	220 bar
Run temperature	40°C	Run time	35 min
Injection volume	10 µL	Injection mode	Full loop
Detection wavelength	ECD (40°C)	Data rate	2 Hz
		Time constant	0.2 sec

Tab. A2 Gradient method description

Time (min)	A (%)	B (%)	C (%)	Flow (mL/min)
0.00	100	0	0	0.4
11.00	100	0	0	0.4
11.02	98.8	1.2	0	0.4
19.98	98.8	1.2	0	0.4
20.00	30	0	70	0.4
25.00	30	0	70	0.4
25.50	100	0	0	0.4
35.00	100	0	0	0.4

Tab. A3 System configuration

Instrument	Description	Article No.
Pump	AZURA® P6.1L, LPG 10 mL bio inert	APH39EA
Autosampler	Autosampler AS 6.1L bio inert	AAA20AA
Detector	Electrochemical Detector DECADE Elite SCC with SenCell Au HyREF	A07545 A07546-3
Column	Dionex™ CarboPac™PA20 250x4mm	B08154-1
Precolumn	CarboPac™PA20 30x3mm	B081517
Software	ClarityChrom 7.4.2	A1670

RELATED KNAUER APPLICATIONS

VFD0062J – Fat soluble Vitamins by HPLC with electrochemical detection

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

