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Everything solved - Carbohydrate content in instant coffee with PAD

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SUMMARY

DIN ISO 11292:1995 regulates the determination and analysis of carbohydrates in instant coffee using anion exchange chromatography combined with pulsed amperometric detection (PAD). Here an adapted method is provided, using a polymer-based cation-exchange column in the Pb^{2+} ionic form and PAD.

INTRODUCTION

Instant coffee is very popular in many areas of the world. It is made from dried coffee extract either using spray-drying or freeze-drying[1]. As carbohydrates are main ingredients of coffee beans, one quality measure for instant coffee is its free and total sugar content. This analysis is regulated by ISO 11292:1995[2] or AOAC method 995.13. The carbohydrates can act as aroma binders and foam stabilizers. They can also influence the viscosity of the drink and are a good tracer for assessing the authenticity[3]. The free content is determined just after dilution of the powder. While the total sugar content requires a hydrolysis step prior to the determination. According to ISO 11292:1995 the contents of the following carbohydrates are of interest: arabinose, fructose, galactose, glucose, mannose, sucrose, mannitol and xylose. Furthermore, the determination of total glucose and total xylose content is an indicator to evaluate the authenticity of instant coffee products. The specification limit of an indicator carbohydrate is the maximum permitted concentration, above which a soluble coffee is considered as adulterated. The limit is defined as the sum of the maximum content and the expanded uncertainty[4].

Additional Information

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RESULTS

A five-point calibration for the eight carbohydrates in a range from 0.35 µg/mL to 5.00 µg/mL was prepared. All calibration curves showed good linearity with a correlation coefficient of $R^2 > 0.9999$. **Fig. 1** exemplarily displays the chromatogram of a mixed standard at a concentration of β=5.00 µg/mL.

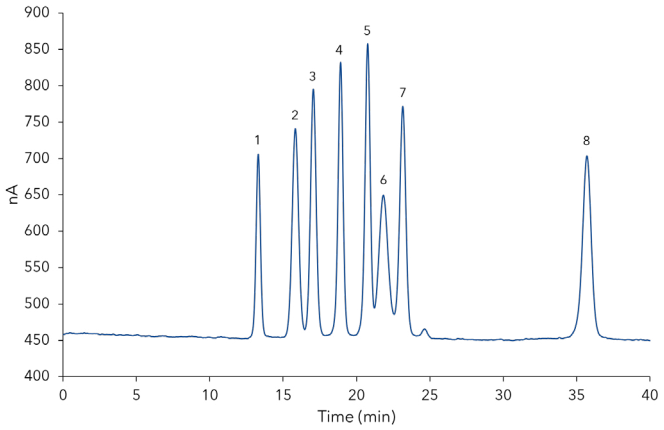

Fig. 1 Chromatogram of mixed standard with β=5.00 µg/mL. 1 - saccharose, 2 - glucose, 3 - xylose, 4 - galactose, 5 - arabinose, 6 - mannose, 7 - fructose, 8 - mannitol.

The dry matter of two instant coffee samples was determined according to DIN 10764 4:2007 03[5] by heating to 95 °C under atmospheric pressure. For both samples a mass loss of w=2.79 g/100 g was calculated. Furthermore, the repeatability of the method was determined with multiple measurements (n=5). The relative standard deviation for peak area and retention time was calculated. **Tab. 1** displays the results for repeatability.

Tab. 1 Repeatability (n=5) for peak area and retention time

Peak	Compound	% RSD Retention time	% RSD Area
1	Saccharose	0.04	0.43
2	Glucose	0.04	1.19
3	Xylose	0.00	0.88
4	Galactose	0.02	0.59
5	Arabinose	0.00	0.81
6	Mannose	0.04	1.07
7	Fructose	0.03	1.76
8	Mannitol	0.01	0.97

The two different instant coffee samples were prepared following the described sample preparation for free and total hydrocarbon content. **Fig. 2** shows the measurement of free carbohydrate content for one of the samples.

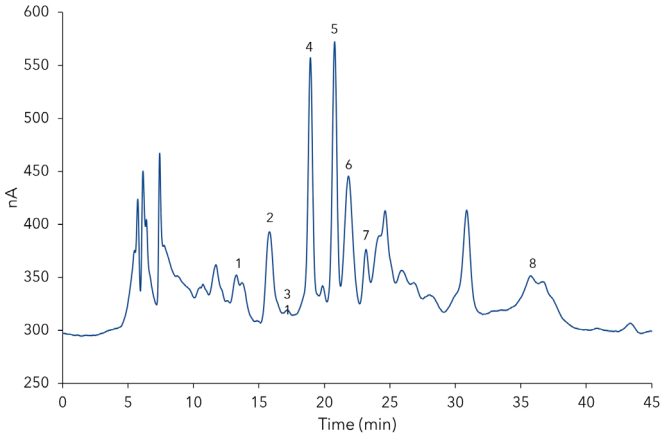

Fig. 2 Chromatogram of free hydrocarbon content. 1 - saccharose, 2 - glucose, 3 - xylose, 4 - galactose, 5 - arabinose, 6 - mannose, 7 - fructose, 8 - mannitol.

Fig. 3 shows the determination of the total content of the same sample after hydrolysis.

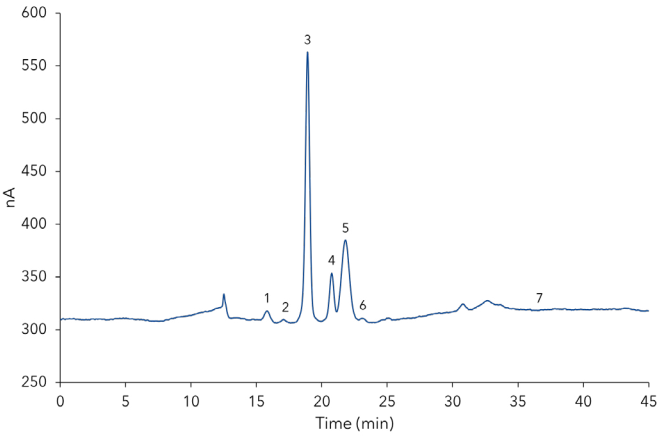

Fig. 3 Chromatogram of hydrolyzed coffee sample. 1 - glucose, 2 - xylose, 3 - galactose, 4 - arabinose, 5 - mannose, 6 - fructose, 7 - mannitol.

The following **Tab. 2** summarizes the measured amounts for the free and total hydrocarbon content in the instant

RESULTS

coffee samples. The mass loss was considered for calculation. In order to avoid incorrect declarations that adulterated products are 100% pure soluble coffee, the ISO 24114:2011-04 regulates the criteria for the authenticity of instant coffee products[4]. Therefore, the total content of two indicator carbohydrates, xylose and glucose, is considered. The specification limit for total glucose content is 2.46% and 0.45% for total xylose content. Both measured samples show similar amounts for the free and total carbohydrate content. The values for the two indicator carbohydrates are within the specification limit of ISO 24114:2011-04. So, the measured samples could be labelled as pure soluble coffee.

Tab. 2 Free and total carbohydrate content in samples

Peak	Compound	Sample 1 free [g/100g]	Sample 1 total [g/100g]	Sample 2 free [g/100g]	Sample 2 total [g/100g]
1	Saccharose	0.26	-	0.09	-
2	Glucose	0.58	1.00	0.54	1.29
3	Xylose	0.01	0.18	0.01	0.23
4	Galactose	0.98	23.33	0.95	17.71
5	Arabinose	1.23	3.97	0.96	3.16
6	Mannose	0.57	13.52	0.96	16.47
7	Fructose	0.18	0.48	0.16	0.40
8	Mannitol	-	0.26	0.05	0.13
Total		**3.80**	**42.74**	**3.71**	**39.39**

SAMPLE PREPARATIONS

When using a system that is not bioinert, it needs to be passivated with 20% nitric acid at a flow rate of 1mL/min for about 30 minutes. Afterwards flush with deionized water until the pH value is neutral. The 300 mM sodium hydroxide (NaOH) solution for post column addition was prepared in plastic flasks using a 50% (w/w) carbonate free NaOH stock solution. After dilution the NaOH 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 calibration standards were dissolved in deionized water. The ECD was operated in pulsed mode using a 4-step PAD potential waveform **(Fig. 4)**.

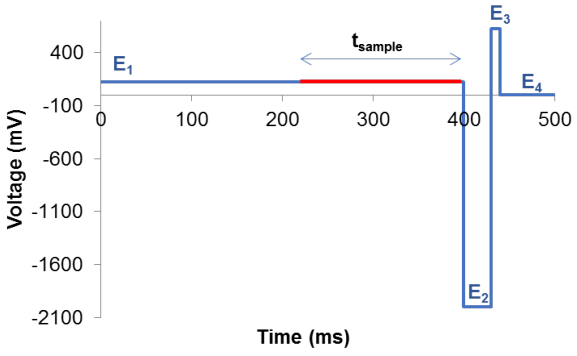

Fig. 4 4-step PAD potential waveform for the detection of monosaccharides and other carbohydrates. The sample detection occurs during the highlighted time period t_{sample}.

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SAMPLE PREPARATIONS

Free carbohydrate content: Approximately 300 mg of instant coffee sample were weighed into a 100 mL volumetric flask and 70 mL deionized water were added. The flask was swirled until the sample was dissolved completely and filled up to the mark with water. 5 – 10 mL of this solution were filtered through a disposable C18 cartridge, the first millilitres were discarded. Here, Macherey Nagel Chromabond C18 cartridges (REF 730003) were used. The filtrate was then again filtered through a 0.2 µm syringe filter and diluted with water in a ratio of 1:10. 20 µL of the prepared sample were injected.

Total carbohydrate content: Approximately 300 mg of instant coffee sample are weighed into a 100 mL volumetric flask and 50 mL of 1.0 M hydrochloric acid were added. The flask was swirled slightly and afterwards placed in a boiling water bath for 150 minutes. Every 30 minutes the solution was swirled by hand. After cooling the sample down to room temperature, the flask was filled up to mark with deionized water. The sample solution was filtered through a folded filter. 3 mL of this filtrate were filtered through a disposable cartridge in silver form and the first millilitre was discarded. Here, Macherey Nagel Chromafix PS-Ag^+ cartridges (REF 731865) were used. At last, the sample was filtered through a 0.2 µm syringe filter and diluted with water in a ratio of 1:10. 20 µL of the prepared sample were injected.

Determination of dry matter: The dry matter of the soluble coffee products was determined according to DIN 10764 4:2007 03[5] by heating under atmospheric pressure.

CONCLUSION

The provided adapted method using a polymer-based cation-exchange column in the Pb^{2+} ionic form and pulsed amperometric detection is suitable for the analysis of carbohydrates in instant coffee and to confirm authenticity of soluble coffee products. Using NaOH post column addition makes it possible to apply polymer columns and therewith, a very wide range of concentrations on the same, very long-lasting column can be analysed.

MATERIALS AND METHODS

Tab. 3 Method parameters

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

Tab. 4 Pump parameters

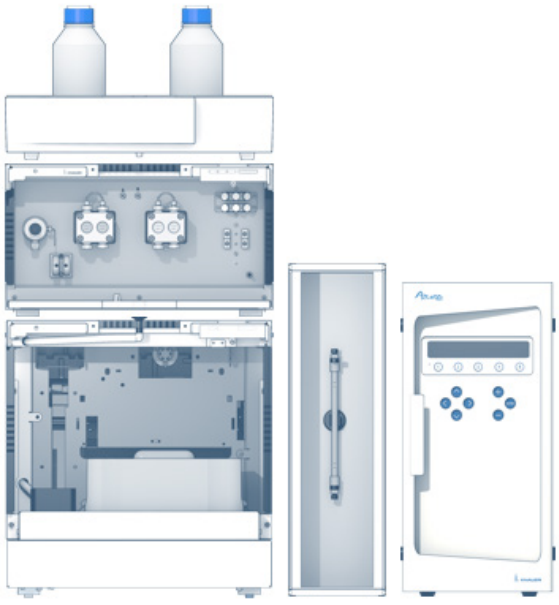
Eluent	water
Gradient	isocratic
Flow rate	0.5 mL/min
Post column eluent	300 mM sodium hydroxide
Gradient	isocratic
Flow rate	0.8 mL/min
Run time	45 min

Tab. 5 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	1 µA		
Polarity	+		
Compensation	On		
AST	2		
Filter	0.02 Hz		

Tab. 6 System configuration

Instrument	Description	Article No.
Pump	AZURA P6.1L, HPG	APH35EA
Autosampler	AZURA AS6.1L	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	Agilent Metacarb 87P, 300 x 7.8 mm ID	
Software	ClarityChrom 8.1 – workstation, autosampler control included	A1670
Software	ClarityChrom 8.1 – System Suitability Extension (SST)	A1677



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[1] Bjarnadottir, A. Instant coffee: Good or bad? https://www.healthline.com/nutrition/instant-coffee-good-or-bad#what-it-is (December 11, 2019).

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[3] Antec Scientific. Carbohydrate analysis in instant coffee according to adapted ISO method 11292:1995. https://antecscientific.com/downloads/notes/food/220_006_01%20-%20Carbohydrate%20Analysis%20in%20Instant%20Coffee.pdf (December 11, 2019).

[4] ISO 24114:2011-04 Instant coffee – Criteria for authenticity. https://www.iso.org/obp/ui/#iso:std:iso:24114:ed-1:v1:en (December 11, 2019).

[5] DIN 10764-4:2007-03 Analysis of coffee and coffee products – Determination of loss in mass of soluble coffee – Part 4: Method for soluble coffee and soluble coffee products by heating under atmospheric pressure (routine method) (2007).

RELATED KNAUER APPLICATIONS

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

VFD0186 – Increasing sensitivity of carbohydrate analysis by switching from refractive index to electrochemical detection