Application Note AN-P-086

Quality assurance of instant coffee

Free and total carbohydrate determination with IC-PAD according to AOAC 995.13 and ISO 11292

Coffee is an extremely popular beverage with a significant economic importance. Quality assurance, including tracing adulterants in coffee, is therefore an established process and a requirement for consumers. Carbohydrates which make up to 50% of raw coffee beans function as flavor, viscosity, and aroma agents [1]. They also serve as authenticity tracers, because unadulterated soluble coffee is exclusively made from pure roasted coffee [2,3]. There are clear specification criteria for the quality assessment by ISO 24114 and AFCASOLE (e.g., a limit of <2.46% total glucose and <0.45% total xylose expressed as mass fractions of total carbohydrates) [3]. AOAC 995.13 and ISO 11292 give analytical requirements for instant coffee quality tests regarding analysis of free and total carbohydrates. Ion chromatography (IC) allows precise quantification of the mandatory analytes arabinose, fructose, galactose, glucose, mannose, sucrose, mannitol, and xylose according to AOAC and ISO. The presented IC method is extremely sensitive and overcomes a very common challenge of possible analyte coelutions, such as with rhamnose.

SAMPLE AND SAMPLE PREPARATION

Instant coffee powders (≈ 300 mg per 100 mL) of two instant coffee brands (Jacobs coffee GOLD and a customer sample) were prepared as described in AOAC and ISO to determine the amount of free carbohydrates (arabinose, fructose, galactose, glucose, mannose, sucrose, and mannitol) and total carbohydrates (arabinose, galactose, glucose, mannose, xylose, and mannitol) after acid hydrolyzation.

For the determination of free carbohydrates, coffee powders were dissolved in 100 mL ultrapure water (UPW) and then filtered (0.25 µm). For total carbohydrate analysis, coffee powders were hydrolyzed in HCl (0.1 mol/L) at 100 °C (150 minutes), diluted to 100 mL with UPW, and filtered with an Ag^+-H^+-cartridge combination. A final dilution (10 to 50-fold) is recommended with UPW.

EXPERIMENTAL

The carbohydrates specified above for dissolved (free) and total carbohydrate analysis were baseline separated on a Metrosep Carb 2 column with a binary high-pressure gradient combined with a flow gradient (940 Professional IC Vario ONE/HPG configuration) (Figure 1). Amperometric detection was performed after PCR with 300 mmol/L NaOH to improve the detection sensitivity of the method.

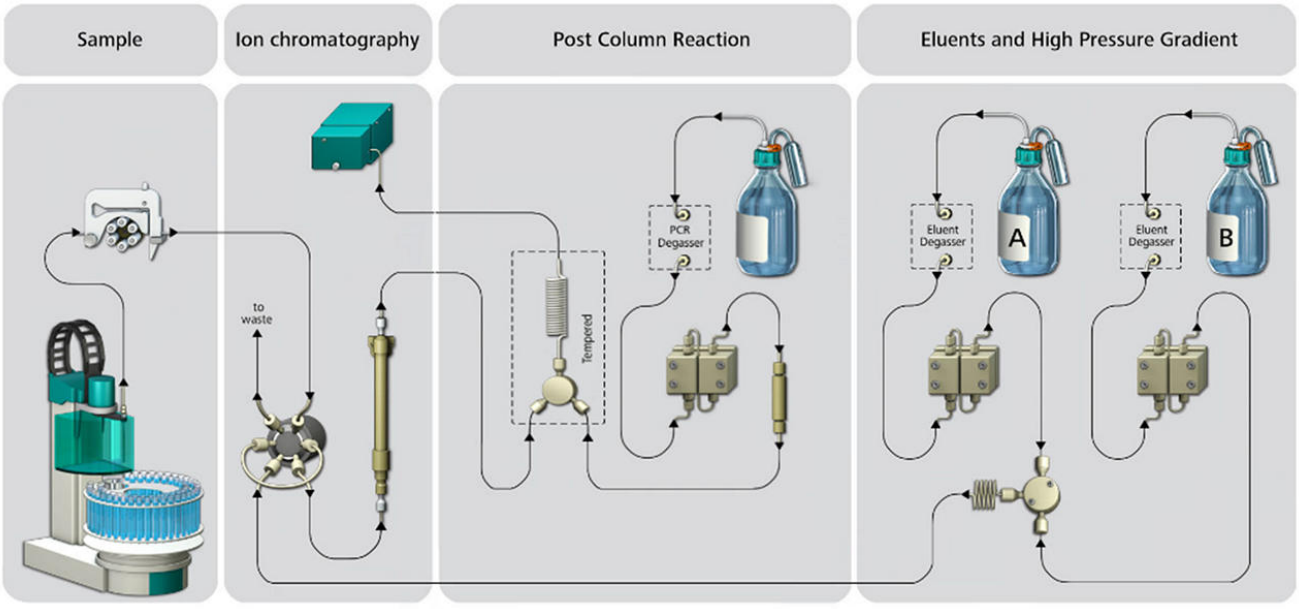


Figure 1. Schematic showing the sample flow path from sample introduction with an 858 Professional Sample Processor, to the 940 Professional IC with column (Metrosep Carb 2 - 250/4.0), amperometric detector (Wall-Jet cell with Au and Pd electrodes), and the high pressure gradient pumps for eluent A (UPW) and B (200 mmol/L NaOH and 1 mmol/L NaAc). To increase sensitivity, 300 mmol/L NaOH is added as a PCR (Post Column Reaction) solution. Chromatography for anions is often referred as HPAEC (high performance anion exchange chromatography) but is simplified here to the generic term of IC.

RESULTS

For the two analyzed instant coffee samples, the free carbohydrate content (results not shown) after dissolution in UPW ranged from 0.2 to 27 g/kg. The mass fractions show unique patterns for both samples. In the Jacobs brand, arabinose and mannose dominate (≈ 35 mass%), while the largest peaks for the customer-provided instant coffee brand corresponded to glucose (≈ 20 mass%) and fructose (almost 40 mass%).

Total carbohydrate content after acid hydrolysis is especially crucial for quality control and purity assessment (Table 1). ISO 24114 set limitations for total glucose and xylose of 2.32 and 0.42%, respectively. The total carbohydrate content of both tested samples show a distinct distribution (Table 1 and Figure 2). Both brands contain similar fractions of galactose and arabinose. Glucose, mannose, and xylose contents differ in a broader range.

Looking more closely at the quality criteria, the purity of Jacobs coffee GOLD can be approved as unadulterated product. The customer-provided brand indicates adulteration and would fail a respective control.

Table 1. Carbohydrate concentrations (g/kg) determined by IC-PCR- PAD after acid hydrolysis in two instant coffee samples (Jacobs coffee GOLD and a customer sample). The total carbohydrate content is expressed as the individual mass fractions (M%) of mannitol, arabinose, galactose, glucose, mannose, and xylose (ISO 11292). Additionally, quantification of rhamnose, fructose, ribose and sucrose is possible (Figure 2). Purity indicators are given by the limits for total glucose (<2.32%) and total xylose (<0.42%) (ISO 24114:2011).

	Jacobs (g/kg) [M%]	Customer (g/kg) [M%]
Mannitol	ND	9 [2.6%]
Arabinose	28.3 [6.5%]	36 [10.2%]
Galactose	190.0 [43.9%]	197.8 [56.2%]
Glucose	6.3 [1.5%]	34.2 [9.7%]
Mannose	207.1 [47.8%]	68.5 [19.4%]
Xylose	1.2 [0.3%]	6.7 [1.9%]
Total carbohydrate content	436.9 [100%]	352.2 [100%]

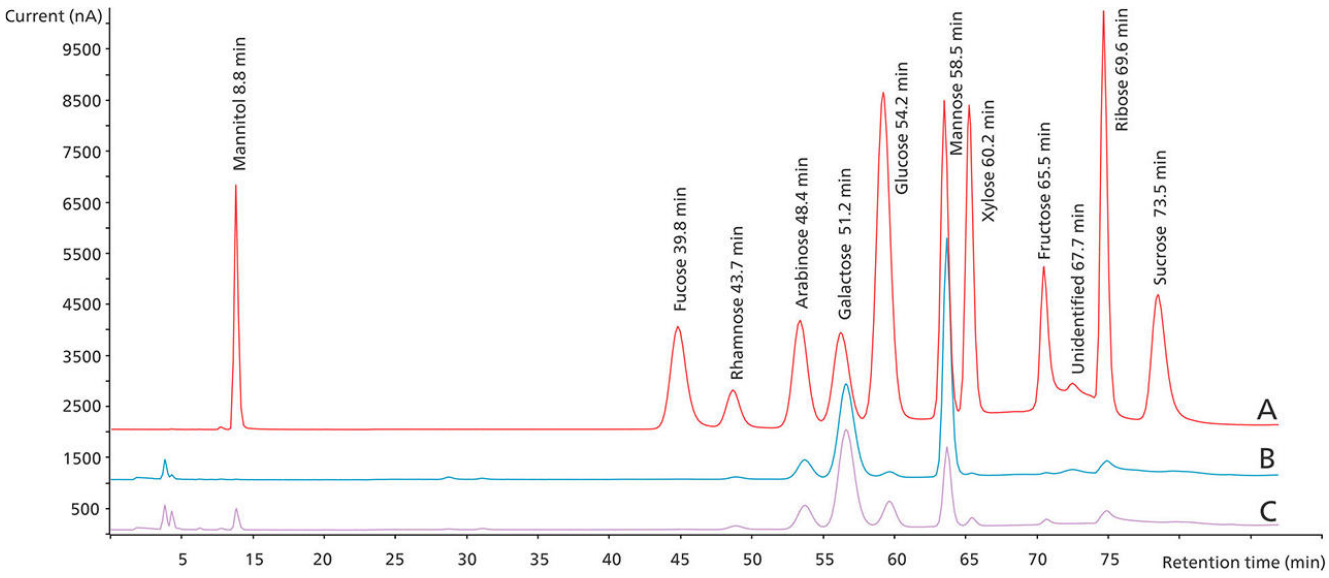


Figure 2. Chromatogram overlay of (A) a 5 mg/kg mixed carbohydrate standard and the diluted (1:10) samples of (B) Jacobs coffee and (C) customer instant coffee after acid hydrolysis. The separation and detection were performed according to the setup listed in Figure 1. For better performance, quantification of fructose and ribose should be performed using peak height.

CONCLUSION

With the presented method the requirements of AOAC 995.13 and ISO 11292 for the determination of dissolved and total carbohydrates in instant coffee are fulfilled. An excellent separation of the required carbohydrates can be achieved by combination of a binary high-pressure gradient and a flow gradient on a Metrosep Carb 2 column. An additional benefit of this method eliminates peak overlap between rhamnose and arabinose, an overall constraint of the ISO-method. Overall, the precise quantification of all required carbohydrates plus fucose and ribose can be performed.

Automation and Inline Sample Preparation are additional improvements to increase the sample throughput and save laboratory time and money.

IC with amperometric detection is a robust, highly specific and precise valuable addition for analytical laboratories performing carbohydrate analysis.

REFERENCES

1. Arya, M.; Rao, L. J. M. An Impression of Coffee Carbohydrates. Crit Rev Food Sci Nutr 2007, 47 (1), 51–67. https://doi.org/10.1080/10408390600550315.
2. Girard, P.; Stöber, P.; Blanc, M.; Prodolliet, J. Carbohydrate Specification Limits for the Authenticity Assessment of Soluble (Instant) Coffee: Statistical Approach. *J AOAC Int* 2006, 89 (4), 999–1003.
3. AFCASOLE (Association of European Producers of Soluble Coffee) Statement on the Authenticity of Soluble Coffees of 6 July 1995; as Confirmed by the ECF (European Coffee Federation, Legal Successor of AFCASOLE) in January 2007.

CONTACT

Metrohm ANZ
56 Buffalo Road
2111 Gladesville
info@metrohm.com.au

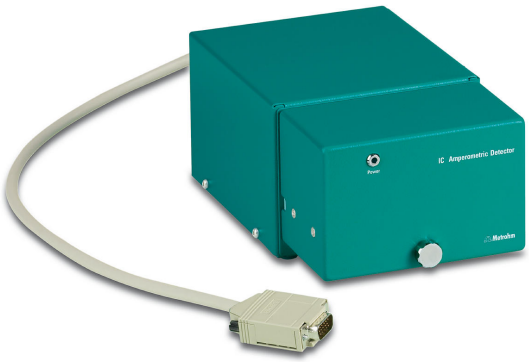
CONFIGURATION

940 Professional IC Vario ONE/HPG

The 940 Professional IC Vario ONE/HPG is the intelligent IC instrument **without suppression** with **binary high-pressure gradient**. It can be extended with the 942 Extension Modules to up to a quaternary gradient system. The instrument can be used with any separation and detection methods.

Typical areas of application:

- Carbohydrate analysis with pulsed amperometric detection (PAD) after gradient elution
- Gradient applications with UV/VIS detection with or without post-column derivatization

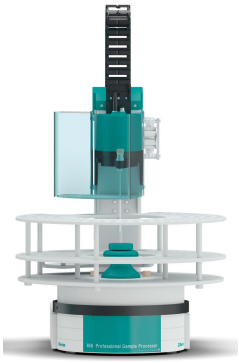


IC Amperometric Detector

Compact and intelligent amperometric detector for intelligent IC instruments. Outstanding selectivity due to the four measuring modes: DC, PAD, flexIPAD and CV, as well as the excellent signal/noise ratio and the very fast stabilization of the measuring signal guarantee the highest in measurement precision.

Metrosep Carb 2 - 250/4.0

The Metrosep Carb 2 - 250/4.0 IC column is particularly suitable for the determination of carbohydrates using alkaline eluents and pulsed amperometric detection. The high-capacity anion exchanger column is based on a styrene-divinylbenzene copolymer. It is stable in the range of pH = 0 - 14 and separates monosaccharides and disaccharides. It is also suitable for the analysis of sugar alcohols, anhydrous sugars, amino sugars, etc. The 250 mm version of the Metrosep Carb 2 separation column is optimized for complex separations.



858 Professional Sample Processor – Pump

The 858 Professional Sample Processor – Pump processes samples from 500 µL to 500 mL. The sample transfer takes place either with the installed bidirectional two-channel peristaltic pump or with an 800 Dosino.

942 Extension Module Vario HPG

The 942 Extension Module Vario HPG is an extension module for the Professional IC Vario line of instruments. It permits the addition of a further eluent in a Professional IC Vario high-pressure gradient system.

Typical applications

- High-pressure gradient systems with up to four eluents

IC equipment Wall-Jet cell: Carb (Au, Pd)

Equipment comprised of Wall-Jet cell with additional accessories. For carbohydrate analysis with a gold working electrode and a Pd reference electrode.