Application Note AN-P-086

速溶咖啡的质量保证

Free and total carbohydrate determination with IC-PAD according to AOAC 996.04 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 996.04 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.

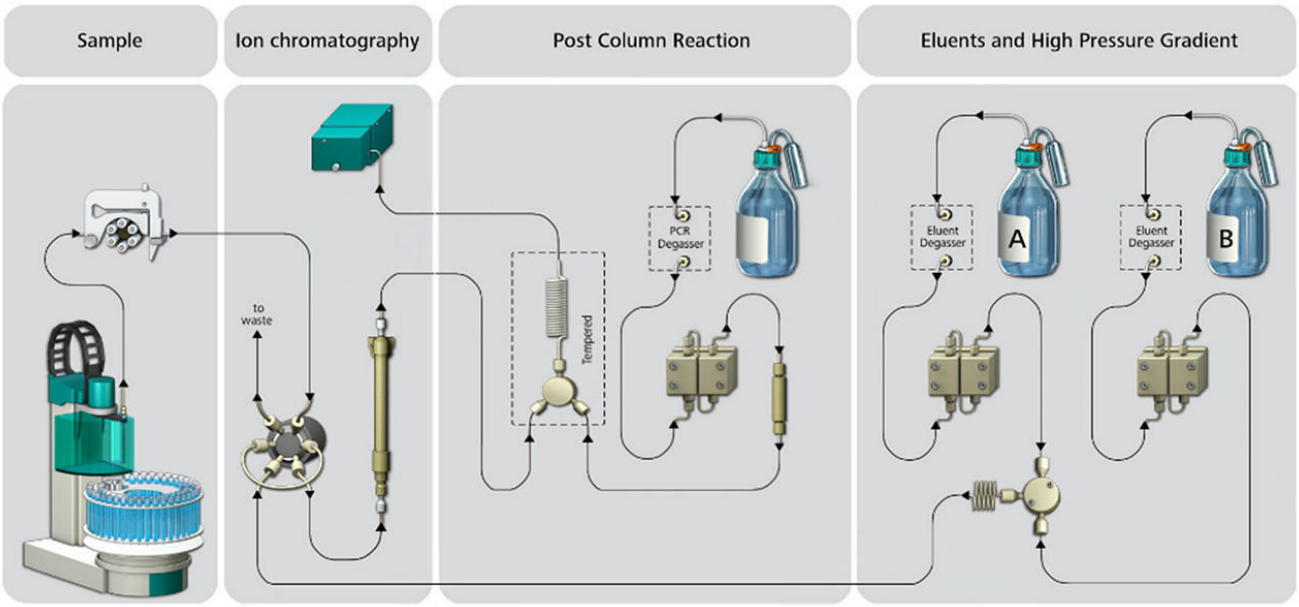

Figure 1. Schematic showing the sample flow path from sample introduction with an 858 Professional Sample Processor, to the 940 Professional IC with column (Metrosep Carb 2 - 250/4.0), amperometric detector (Wall-Jet cell with Au and Pd electrodes), and the high pressure gradient pumps for eluent A (UPW) 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%]

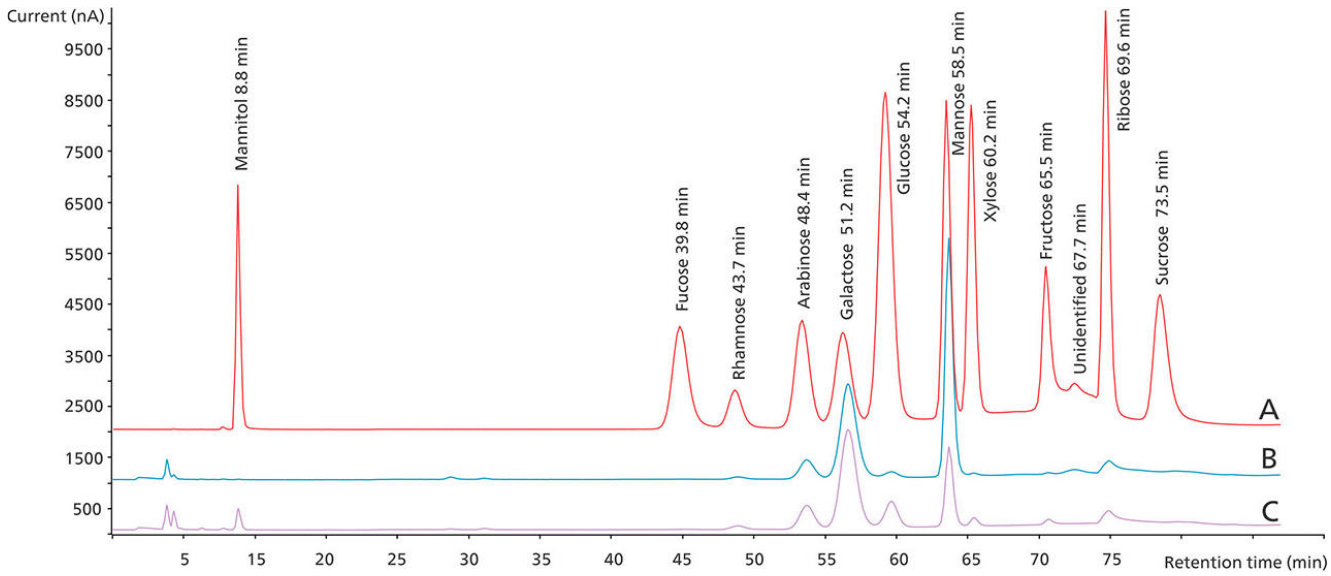

Figure 2. Chromatogram overlay of (A) a 5 mg/kg mixed carbohydrate standard and the diluted (1:10) samples of (B) Jacobs coffee and (C) customer instant coffee after acid hydrolysis. The separation and detection were performed according to the setup listed in Figure 1. For better performance, quantification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

CONCLUSION

With the presented method the requirements of AOAC 996.04 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. Araya and Rao (2007), Crit Rev Food Sci Nutr. 47(1), 51–67.
2. Girard et al. (2006), J AOAC Int. 89(4), 99–1003.
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.

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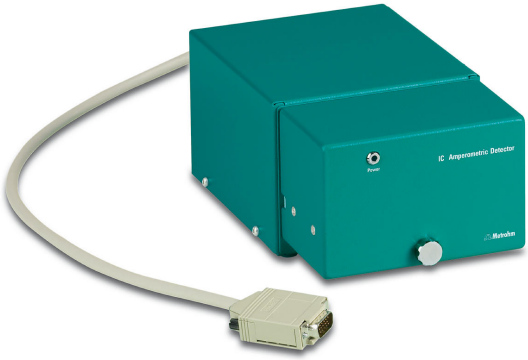
CONFIGURATION

940 Professional IC Vario ONE/HPG

940 Professional IC Vario ONE/HPG 是智能型 IC 仪器,**无抑制**,带**二元高压梯度**。 可使用 942 Extension Modul 将其扩展至四元梯度系统。 该仪器可使用各种分离和检测方法。

典型的应用范围:

- 梯度淋洗后使用脉冲安培检测法(PAD)分析碳水化合物
- 使用 UV/VIS 检测的梯度应用,带或不带柱后衍生反应

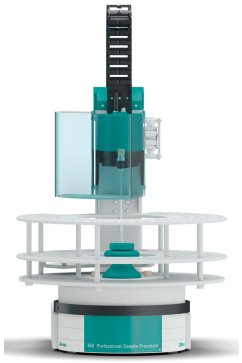


IC Amperometric Detector

用于智能型离子色谱仪器的紧凑智能型安培检测器。多种可选的不同测量模式:DC、PAD、flexIPAD 和 CV,以及卓越的信号/干扰比例关系和极快进入测量准备就绪状态,这一切确保测量的高度精确性。

Metrosep Carb 2 - 250/4.0

Metrosep Carb 2 - 250/4.0 离子色谱柱特别适用于使用碱性淋洗液测定碳水化合物和脉冲安培检测。该高容量阴离子交换柱基于苯乙烯/二乙烯基苯聚合物。它在 pH = 0 - 14 的范围内较稳定,可分离单糖和双糖。此外还可用于分析糖醇、脱水糖和低聚糖等。250 mm 款型的 Metrosep Carb 2 分离柱极为适用于复杂的分离要求。



858 Professional Sample Processor – Pump

858 Professional Sample Processor – Pump 可处理体积在 500 µL 至 500 mL 之间的样品。进行样品转移时,既可以使用内置的双向双通道蠕动泵、也可通过 800 Dosino 来进行。

942 Extension Module Vario HPG

942 Extension Module Vario HPG 是 Professional IC Vario 仪器系列的扩展模块。 它可向 Professional IC Vario **高压梯度系统**中添加第二种淋洗液。

典型应用

- 高压梯度系统,多达四种淋洗液

CarbAuPd

装备由喷壁式流通池组成,带有附件。用于碳水化合物分析,带一个金工作电极和一个钯参比电极。