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A Fast Method for Sugar Analysis of Instant Coffee Samples

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ABSTRACT

Purpose: To demonstrate a fast method for analyzing sugars in instant coffee using High-Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD) and a High Concentration Carbohydrate Analysis kit

Methods: Instant coffee sugars are ionized in a strong base, separated by High-Performance Anion-Exchange (HPAE) chromatography, and detected by Pulsed Amperometric Detection (PAD). Analysis is facilitated by the Thermo Scientific™ Dionex™ Integrion™ HPIC™ system.

Results: Using this method, carbohydrates present in soluble, as well as total, carbohydrate extracts of instant coffee were quantified in less than six minutes. The results for the method linearity, precision, and robustness are presented.

INTRODUCTION

Coffee is the most widely consumed hot beverage worldwide. World consumption of coffee has steadily increased in past 50 years, growing from 57.9 million bags in 1964 to 142 million bags in 2012.[1] The process of bringing the harvested coffee fruits to consumers as a beverage involves a series of steps. Greater control of each step of this process improves the ability to produce a high quality coffee. The carbohydrates are an important constituent of the coffee beans, forming about 50% of the green coffee bean. These carbohydrates undergo complex changes during the roasting process and can affect the final taste and aroma properties of the coffee. Carbohydrate content is used for detecting coffee adulteration[2]. Because carbohydrates represent a high percentage of the coffee bean's mass, their impact on viscosity plays an important role in soluble coffee processing.[3]

Thermo Scientific Application Note 280[4] (AN280) described a fast ion chromatography (IC) method for analysis of carbohydrates in coffee using the Thermo Scientific™ Dionex™ CarboPac™ SA10 column with electrolytically generated eluent. The current study updates the fast method described in AN280 with a Dionex CarboPac SA10-4 µm column. Due to smaller particle size, this column provides more efficient peaks and better resolution, making the sample analysis easier and more reliable. Moreover, the system used for the mono- and disaccharide analysis is also updated. The new system combines flexibility and ease-of-use with high sensitivity and selectivity, bringing a new level of convenience and cost-effectiveness to simple sugar analysis.

MATERIALS AND METHODS

Isolation of Carbohydrates

Soluble coffee was used without grinding or homogenization. The free and total carbohydrate extracts were prepared using the methods described in AOAC Method 995.13.[5]

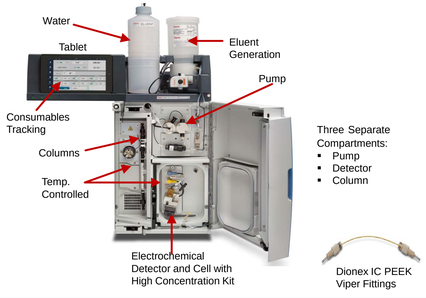
Chromatography

See chromatograms for conditions.

Instrument

Dionex Integrion HPIC system, RFIC model (Figure 1) configured for electrochemical detection, including eluent generation and Thermo Scientific™ Dionex™ IC PEEK Viper™ fittings (Figure 1, bottom left). The flow diagram is shown in Figure 2.

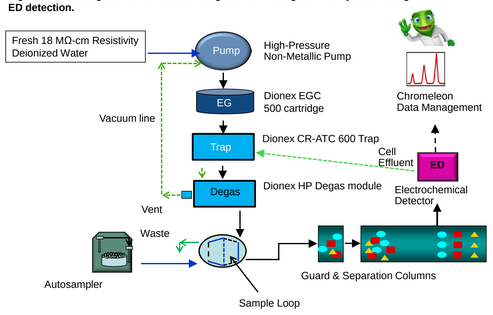
Figure 1. Dionex Integrion HPIC System configured for electrochemical detection.



Data Analysis

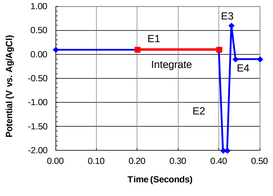
Thermo Scientific™ Chromeleon™ Chromatography Data System software, version 7.2 SR4.

Figure 2. Flow diagram for the Dionex Integrion HPIC Reagent-Free system configured for ED detection.



In PAD using the four-potential waveform, the disposable working electrode is pulsed through the different potentials at set times, completing two cycles within one second (Figure 3). This waveform is optimized to provide a clean, stable gold layer in preparation for detection of the next eluting peak.

Figure 3. Four-potential carbohydrate waveform.



Time (s)	Potential (V)	Integration
0.00	+0.10	
0.20	+0.10	Start
0.40	+0.10	End
0.41	-2.0	
0.42	-2.0	
0.43	0.60	
0.44	-0.10	
0.50	-0.10	

1. Detection potential (E1)
2. Duration time of E1 (t1)
3. Integration period (Integrate)
4. Reductive cleaning potential (E2)
5. Duration time of E2 (t2)
6. Oxidative cleaning potential (E3
7. Duration time of E3 (t3)
8. Pre-detection (oxide reduction) potential (E4)
9. Duration time of E4 (t4)

RESULTS

Separation

Figure 4 shows a standard sample containing nine common sugars separated using a Dionex CarboPac SA10-4µm column using the proposed method. The nine sugars are well resolved within six min. Using this method, two sugar pairs, namely rhamnose/galactose and fructose/ ribose, coelute. Rhamnose and ribose were not added to the standard mix. The separation conditions are chosen based on the knowledge that rhamnose and ribose are not present in the coffee samples studied. But for samples that potentially contain coeluting sugars, the longer modified AOAC Method 995.13[5], described in AN280[4], should be used. Figure 5 shows representative chromatograms for sugars present in free (red line) and total carbohydrate (blue line) extracts for a commercial instant coffee sample, sample A , prepared as described in the AOAC method[5]. Free carbohydrate coffee extract for the instant coffee samples used in this study mainly contains four sugars—arabinose, galactose, mannose, and fructose. Whereas, the total sugar extract mainly contains arabinose, galactose, glucose, and mannose. Both extracts contain other sugars at lower concentrations.

Figure 4. Analysis of nine common carbohydrate sugars.

Columns: Dionex CarboPac SA10-4µm, 4 × 250 mm
Dionex CarboPac SA10 Guard 4 × 50 mm
Temperature: 40 °C
Flow Rate: 1.5 mL/min
Eluent: 1 mM KOH
Eluent Source: EGC 500 KOH Cartridge with Thermo Scientific™ Dionex™ CR-ATC 600
Injection Volume: 0.4 µL (push full)
Detection: PAD, disposable Au electrode with high concentration kit

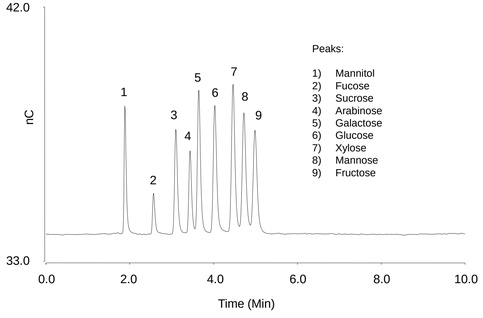
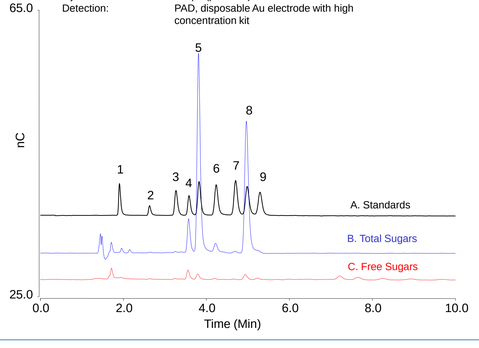


Figure 5. Free and total carbohydrate sugars present in an instant coffee sample A.

Columns: Dionex CarboPac SA10-4µm, 4 × 250 mm
Dionex CarboPac SA10 Guard 4 × 50 mm
Temperature: 40 °C
Flow Rate: 1.5 mL/min
Eluent: 1 mM KOH
Eluent Source: EGC 500 KOH Cartridge with Thermo Scientific™ Dionex™ CR-ATC 600
Injection Volume: 0.4 µL (push full)
Detection: PAD, disposable Au electrode with high concentration kit



Linearity and Precision

The linearity of the method was studied using nine concentration levels each of the nine sugars used in this study. For all sugars except fucose and arabinose the concentration levels were between 3 and 800 mg/L. The calibration data showed that the responses were linear for all the concentration levels studied, with coefficients of determination ≥0.997 (Table 1). To determine method precision, seven replicate injections of a standard sample containing 100 mg/L sugars (except fucose and arabinose, which were at 50 and 25 mg/L, respectively) were used. The RSD values for retention time as well as peak area were < 2% indicating excellent injection to injection precision.

Table 1. Method linearity and precision.

Coffee Sugar	Range (mg/L)	R-squared	RT RSD	Peak Area RSD
Mannitol	3.125–800	0.999	0.16	1.86
Fucose	0.7–200	0.999	0.18	1.49
Sucrose	3.125–800	0.999	0.09	0.60
Arabinose	1.56–400	0.999	0.09	0.58
Galactose	3.125–800	0.999	0.00	0.84
Glucose	3.125–800	0.999	0.00	1.28
Xylose	3.125–800	0.997	0.07	1.18
Mannose	3.125–800	0.998	0.06	0.83
Fructose	3.125–800	0.999	0.06	2.05

Accuracy

Recovery studies were performed to assess method accuracy. Both free and total carbohydrate extracts were prepared in triplicate (n=3). As shown in Figure 6 and 7, recoveries for all (except mannose in both carbohydrates extracts) sugars in free as well as total carbohydrate extracts of sample A were 80–120%, well within the 70–130% limit prescribed by the U.S. FDA in the ORA laboratory manual.[6] Sample B showed similar trends (not shown).

Figure 6. Recovery studies on the free carbohydrate sugars present in instant coffee sample A.

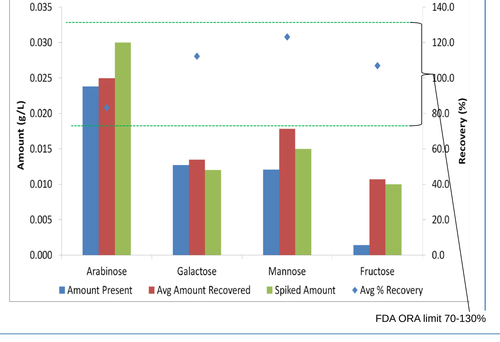
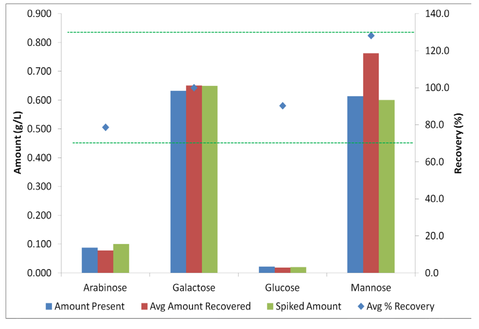


Figure 7. Recovery studies on the total carbohydrate sugars present in instant coffee sample A.



CONCLUSIONS

- The smaller particle size of the Dionex CarboPac SA10-4µm column allows for higher resolution separation of all nine sugars within 6 min.
- The method demonstrated excellent precision and accuracy, making it an ideal candidate for fast coffee sugar analysis of both free and total carbohydrate extracts.
- The method proposed here offers increased reliability and sensitivity due to electrolytic eluent generation as well as the ease-of-use features of the Dionex Integrion HPIC system.

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