

Two Chromatography Methods for Determining Carbohydrates in Coffee

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Introduction

Brewed coffee has emerged as one of the most consumed beverages in the world, and green coffee (unroasted beans) is one of the most globally traded agricultural commodities. Coffee is grown in over 70 countries, primarily in Latin America, Southeast Asia, and Africa. The top coffee importing countries are the United States, Germany, Japan, France, Italy, Spain, Canada, and the United Kingdom.

In recent years, interest in the physiology and biochemistry of green coffee beans and their role in the quality of final roasted coffee has grown. Drinks made from green coffee beans have been introduced in the market. Green and roasted coffees are tested at several stages of their production and processing. Green coffee beans are tested for bean density, brightness, acidity, pH, moisture content, and total soluble solids. Roasted coffee is tested for caffeine, chlorogenic acids, lipids, carbohydrates, total polyphenols, total proteins, and aflatoxins.

Coffee carbohydrates constitute the major part (at least 50% of the dry weight) of raw coffee beans. Carbohydrates in coffee contribute to its flavor as they undergo complex changes (react with amino acids, i.e., the Maillard reaction) during the roasting process. They act as aroma binders, foam stabilizers, and also impart viscosity to the coffee beverage. Carbohydrates are also good tracers for assessing the authenticity of soluble (instant) coffee.

Currently, the Association of Analytical Chemists (AOAC) Official Method 995.136, which is based on high-performance anion-exchange (HPAE) chromatography with pulsed amperometric detection (PAD), is used for determining the free and total carbohydrates in instant coffee.¹ This method is also used by the British Standards Institution for testing coffee and coffee products.

This study first tested the AOAC Official Method 995.13 on a Thermo Scientific Dionex ICS-3000 Ion Chromatography System. Carbohydrates in instant and green coffee were separated on a Thermo Scientific Dionex CarboPac™ PA1 column, and measured by electrochemical detection with disposable Au on polytetrafluoroethylene (PTFE) working electrodes. A few proposed modifications of the official method achieved separation of two pairs of sugars, which are otherwise difficult to resolve.

A fast method using the Dionex CarboPac SA10 column (with electrolytically generated eluent) was then tested for determining the common coffee carbohydrates. This column has been shown to achieve fast, high resolution separation of mono- and disaccharides commonly found in food samples.² The Dionex CarboPac SA10 column is composed of a wide-pore macroporous substrate coated with strong anion-exchange latex of nanobeads. The combination of the high capacity provided by the substrate and the new internal chemistry of the nanobead functionality delivers high resolution and short analysis time for the common sugars of interest in food and beverages.

The testing here demonstrates the linearity, precision, and recovery of common coffee carbohydrates in instant and green coffee. It compares the two methods and discusses their respective advantages and disadvantages. Note that the disposable working electrodes used in these methods provide short equilibration times and greater electrode-to-electrode reproducibility compared to conventional working electrodes. Both of the described methods provide good sensitivity, consistent response, and can be routinely used for sugar analysis in coffee applications. The fast method is recommended when rapid separation is desired, keeping in mind that two pairs of sugars (namely, rhamnose–galactose and fructose–ribose) are not resolved. In applications where all 11 common coffee carbohydrates need to be resolved, the AOAC Official Method 995.13 (with minor modifications) is recommended.

Experimental Details

Thermo Scientific Dionex ICS-3000 or ICS-5000 Ion Chromatography System including:

Gradient or isocratic pump, with the vacuum degas option installed

DC Detector/chromatography module

Injection loop, 10 μ L (for method based on AOAC Official Method 995.13)/injection valve with an internal 0.4 μ L injection loop (for fast method)

Dionex ICS-3000 ED Electrochemical Cell disposable gold electrode

Electrochemical detector

Conditions

Modified AOAC Official Method 995.13

Columns: Dionex CarboPac PA1 Analytical,
4 \times 250 mm,
Dionex CarboPac PA1 Guard
4 \times 50 mm

Flow Rate: 1.0 mL/min

Inj. Volume: 10 μ L (full loop)

Column Temp.: 25 $^{\circ}$ C

Detector Temp.: 30 $^{\circ}$ C

Eluent: DI water from 0–50 min,
300 mM NaOH from 50–65 min
DI water from 65–80 min
(re-equilibration)

Postcolumn

Base: 300 mM NaOH

Flow Rate for

Postcolumn Base: 0.6 mL/min

Fast Method

Columns: Dionex CarboPac SA10 Analytical,
4 \times 250 mm,
Dionex CarboPac SA10 Guard,
4 \times 50 mm

Flow Rate: 1.5 mL/min

Inj. Volume: 0.4 μ L (full loop)

Column Temp.: 45 $^{\circ}$ C

Detector Temp.: 30 $^{\circ}$ C

Eluent: 1 mM KOH

Eluent Source: EGC II KOH with CR-ATC

Both

Detection: PAD

Background: 30–70 nC

Noise: 30–60 pC

Working

Electrode: Disposable Au on PTFE

Reference

Electrode: Ag/AgCl mode

Carbohydrate Waveform

Sample Preparations

Instant coffee samples were prepared according to AOAC Method 995.13.

Green Coffee

1 g of green coffee beans was mixed with 10 mL of DI water and sonicated for 15 min. The supernatant was passed through a 0.2 μ m filter, and was further diluted with water if needed. Samples were used within 24 h. (Extracts from ground green coffee beans, obtained using this procedure, gave similar results.)

Results

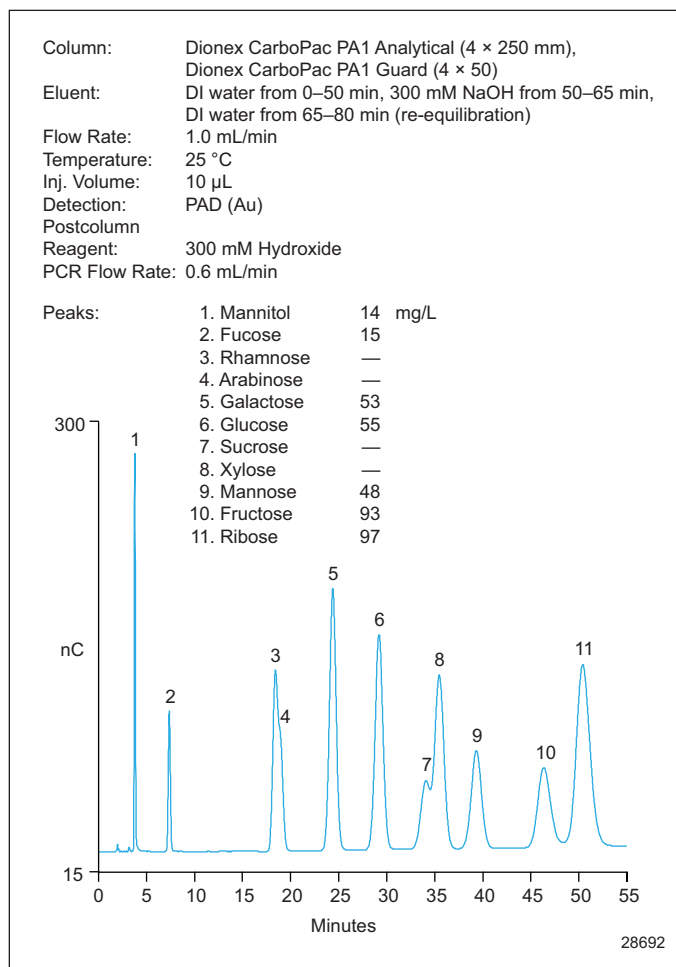
Modified AOAC Method 995.13 Separation

Figure 1 shows the separation of the carbohydrates present in a mix of standards. All the carbohydrates elute in 55 min with a total run time of 80 min (including column wash and equilibration steps). Note that the later eluting peaks are broader relative to the early eluting peaks, as expected from an isocratic method.

Rhamnose–arabinose (Figure 1, peaks 3 and 4) and sucrose–xylose (Figure 1, peaks 6 and 7) are not completely resolved. The resolution issue for these sugars has been addressed in the official method.

If the rhamnose–arabinose peaks are not resolved, the method suggests excluding rhamnose from the mixed standard solution. For the other pair, the AOAC method recommends 2 to 3 injections of the specified carbohydrates standard solution or an increase of the re-equilibration time in order to achieve a good separation of glucose, sucrose, and xylose.

FIGURE 1. Chromatogram of mixed coffee carbohydrate standards, using the AOAC official method 995.13.

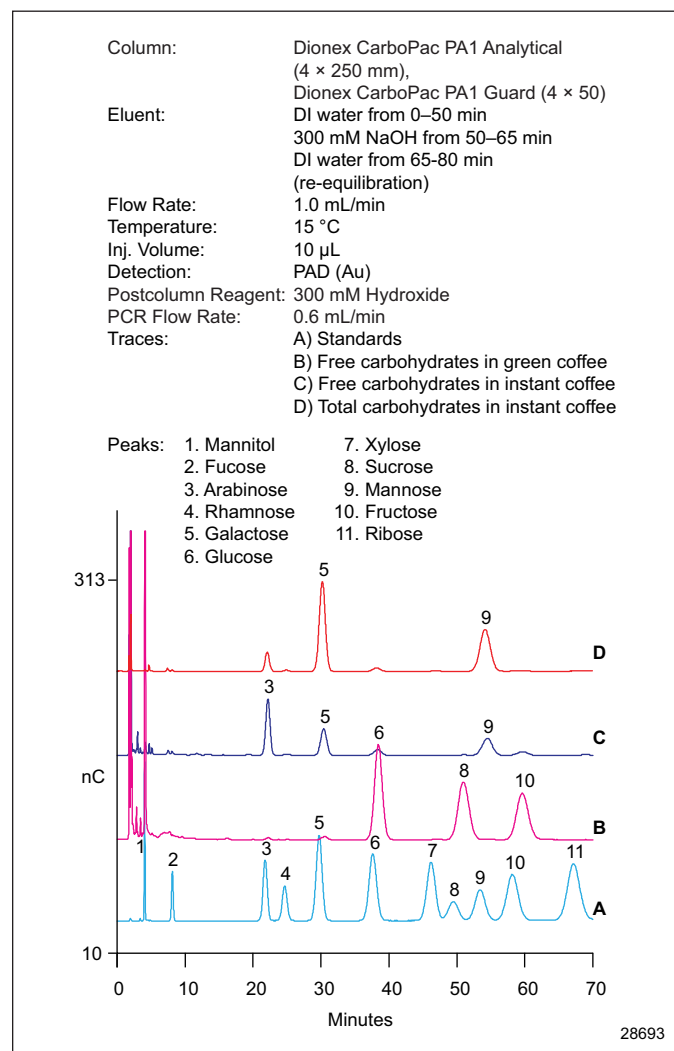


As an alternative, the column temperature may be lowered to 15 °C (referred to here as modification 1) to achieve separation of all 11 carbohydrates in the mixed standard solution. Note that the run time is increased (Figure 2), and all the sugars now elute in 70 min. In addition, arabinose elutes before rhamnose, and xylose before sucrose, compared to the elution order at 25 °C, suggesting that the interaction of these sugars with the stationary phase at low temperature (15 °C) is different than at 25 °C.

Figure 2 also shows representative chromatograms of extracts from green coffee beans (B), and extracts of free carbohydrates (C), and total carbohydrates (D) from instant coffee.

The primary carbohydrates present in extracts from green coffee beans (B) were mannitol, glucose, sucrose, and fructose. In comparison, in the instant coffee sample tested, the major free carbohydrates were arabinose, galactose, and mannose, and the minor sugars were glucose and fructose. In the extract for total carbohydrates from instant coffee, the sugars were mainly arabinose, galactose, and mannose in the sample tested. This gives an indication of how the sugars present in green coffee have changed during roasting and other heat treatment processes (e.g., extraction, spray drying).

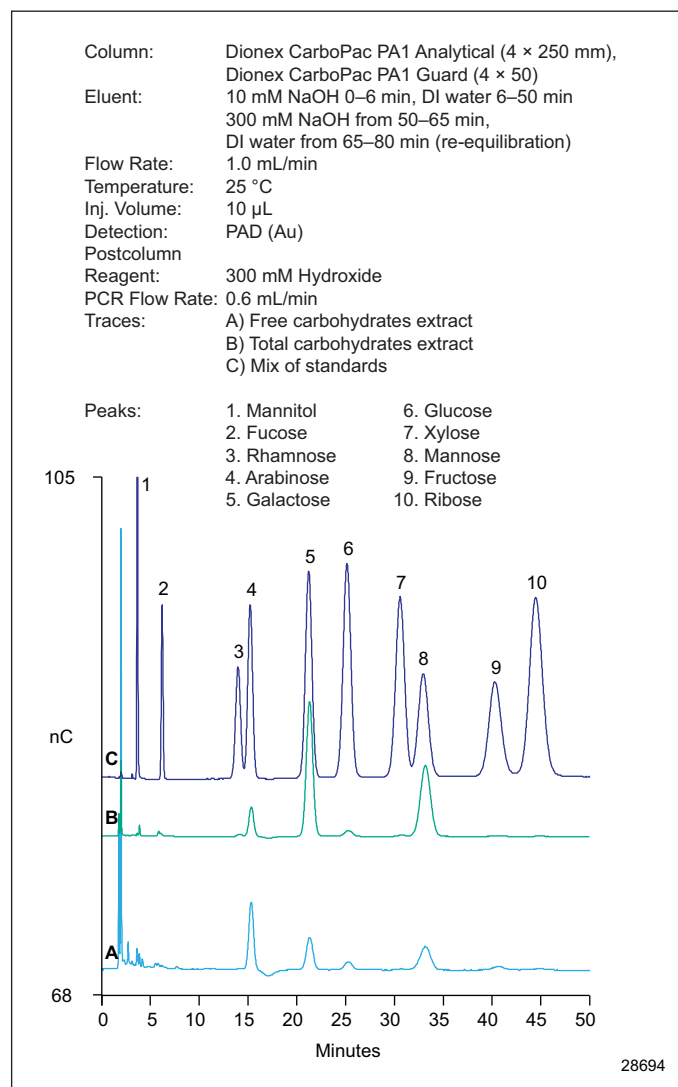
FIGURE 2. Chromatograms of mixed coffee carbohydrate standards (A), free carbohydrate in extract of green coffee beans (B), free carbohydrates in instant coffee (C), and total carbohydrates in instant coffee (D); using the modified AOAC Official Method 995.13 (T = 15 °C).



The first set of co-eluting peaks, rhamnose and arabinose, was also resolved by modifying the conditions of the mobile phase: by eluting with 10 mM hydroxide for the first 6 min, then switching to DI water (i.e., using a step change; Figures 3 and 4). Note that only the mobile phase was modified; all other chromatography conditions were the same as in AOAC Method 995.13. Because extracts from instant coffee typically do not contain sucrose (Figure 2, C and D), sucrose can be eliminated from the mix of standards that will be used when analyzing samples from instant coffee (Figure 3C).

For determining sugars in instant coffee, the suggested changes (referred to as modification 2) to the official

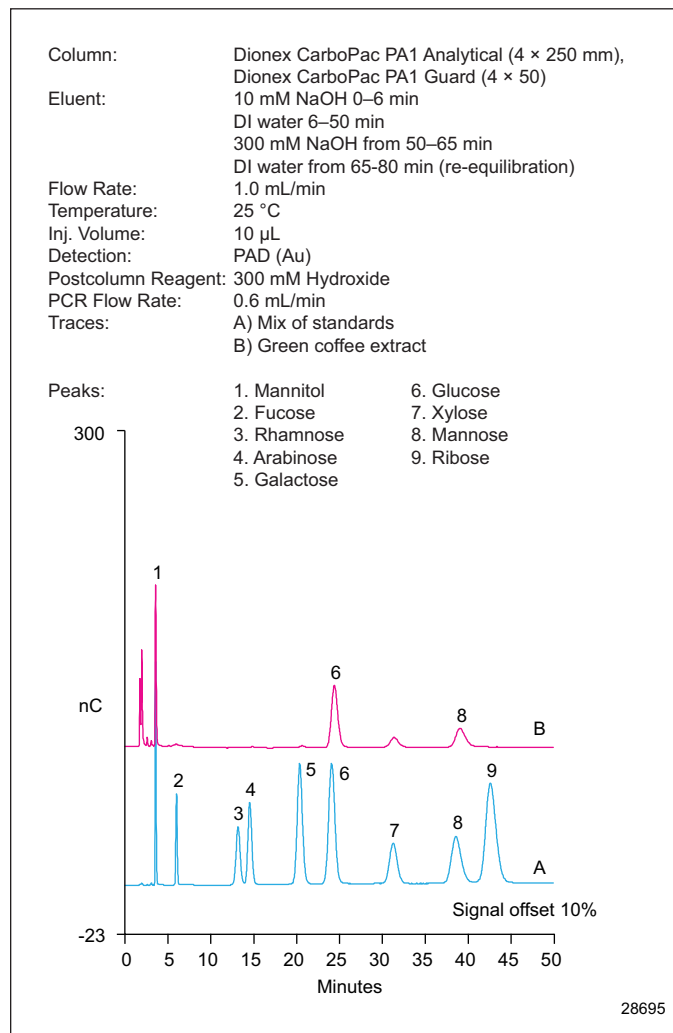
FIGURE 3. Chromatograms of free carbohydrates extract from instant coffee (A), total carbohydrates extract from instant coffee (B), and mixed carbohydrate standards (C); using the modified AOAC Official Method 995.13 (10 mM hydroxide for 6 min, and sucrose not included in mix of standards).



method include: (a) elution with 10 mM hydroxide for the first 6 min, then switch to DI water, and (b) exclude sucrose from the mix of standards. Note that flavored instant coffees are more likely to contain sucrose, so modification 1 will be more appropriate.

Typically, green coffee samples do not contain xylose and mannose (Figure 2B). When analyzing green coffee samples, exclude xylose and mannose from the mix of standards (Figure 4A). Similar to the original AOAC Official Method 995.13, all the sugars elute in 50 min. The suggested method changes (referred to as modification 3) for analyzing extracts from green coffee include: (a) elution with 10 mM NaOH for the first 6 min, followed by DI water, and (b) exclusion of xylose and mannose from the mix of standards, while maintaining all other chromatography conditions in AOAC Method 995.13.

FIGURE 4. Chromatograms of mixed coffee carbohydrate standards (A), free carbohydrates extract from green coffee beans (B); using the modified AOAC Official Method 995.13 (10 mM hydroxide for 6 min, and xylose and mannose not included in mix of standards).



Precision

The precisions for six replicate injections of a mixture of sugar standards for the AOAC Official Method 995.13 with modification 1 (i.e., with column temperature 15 °C) ranged from 0.2 to 0.68% for RT and from 4.5 to 5.4% for peak area. The precisions for the official method with proposed modifications 2 and 3 were in the range of 0.19 to 1.84% for RT, and 1.05 to 4.96% for peak area.

Accuracy

The average recovery for the sugars (using modification 2 of the Official Method) in the instant coffee samples ranged from 70 to 116%. For green coffee samples (using the official method with modification 3), the average recovery ranged from 73 to 95%. The between-day recovery precision for the coffee sugars in the spiked samples averaged 12% over three days.

Fast Method Separation

The mixture of coffee carbohydrate standards separated on a Dionex CarboPac SA10 column is shown in Figure 5. All the sugars elute within 8 min. This is significantly faster than the other methods used for analyzing common sugars in food and beverages.

However, note that two pairs of sugars co-elute under the current configuration. These are rhamnose–galactose and fructose–ribose (Figure 5, peaks 5 and 9).

FIGURE 5. Chromatogram of mixed coffee carbohydrate standards using the fast method.

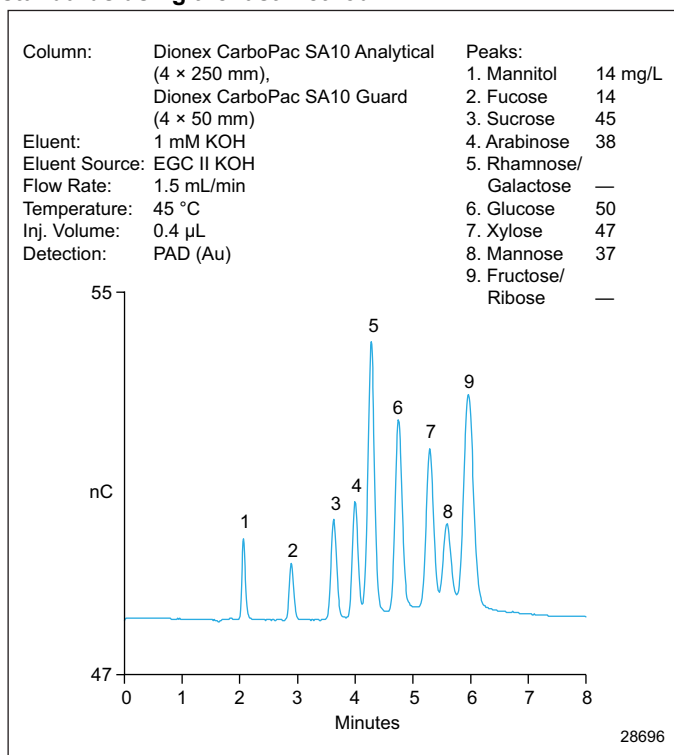
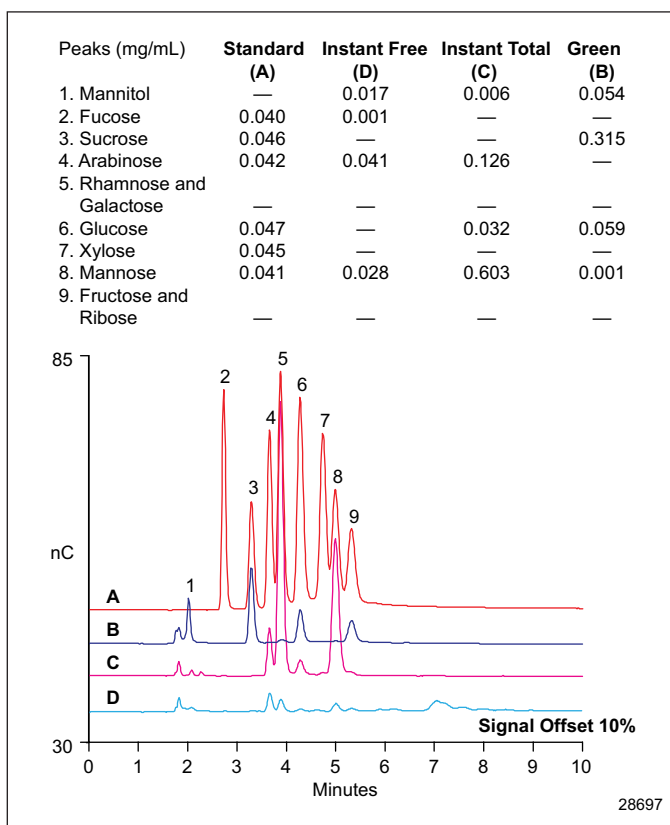


Figure 6 shows representative chromatograms for green and instant coffee. The green coffee sample has mannitol, sucrose, glucose, and fructose (assignment is based on the knowledge that green coffee samples have minimal or no ribose), whereas the instant coffee samples have arabinose, galactose (assignment is based on the knowledge that instant coffee samples have minimal or no rhamnose), glucose, and mannose.

FIGURE 6. Chromatograms of a mixture of coffee carbohydrate standards (A), free carbohydrates from green coffee beans (B), free carbohydrates (C), and total carbohydrates (D) extract from instant coffee; using the fast method. Chromatographic conditions same as listed in Figure 5.



Linearity and Precision

The linearity of the method was determined by injecting calibration standards ranging from 5 to 900 mg/L. The coefficients of determination were between 0.9942–0.9998. Precisions ranged from 0.13 to 0.21% for RT and 1.35 to 4.65% for peak area.

The two methods (the AOAC Official Method and the fast method) gave similar intra-day and between day (over three days) RT and peak area precisions.

Accuracy

Intra-day concentration RSDs for coffee extracts were in the range of 0.2 to 1.8%. The average recovery for the sugars in instant and green coffee ranged from 74 to 127%. The between-day recovery precision for the coffee sugars in the spiked samples ranged from 1.5 to 24% (average 10%) over three days.

Summary

Both methods are sensitive, accurate, reliable, and differ primarily in their total analysis time and peak resolutions for coffee carbohydrate determinations.

AOAC Official Method 995.13

- A longer run time (80 min) compared to proposed fast method
- For certain sugars that might be difficult to resolve with the official method, minor modifications suggested

A Fast Method Proposed Using the Dionex CarboPac SA10 Column

- Resolves 7 of the 11 coffee carbohydrates in 8 min (two additional peaks are co-elutions of two pairs of carbohydrates)
- Only addition of DI water for continuous operation

References

1. Methods of Test for Coffee and Coffee Products. Instant Coffee: Determination of Free and Total Carbohydrate Contents by High-Performance Anion-Exchange Chromatography. BSI Standards Development; BS 5752: Part 15, **1995**.
2. Zheng, T.; Woodruff, A.; Pohl, C.; Rao, S.; Flook, K.; Agroskin, Y. *A New Solution for Fast and Rugged Biofuel Carbohydrate Analysis*. Thermo Fisher Scientific Poster, LPN 2563-01, **2010**.

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