Determination of Carbohydrates in Fruit Juice Using Capillary High-Performance Anion-Exchange Chromatography

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High-performance anion-exchange chromatography (HPAE) coupled with pulsed amperometric detection (PAD) is a well-established technique to identify and quantify carbohydrates in food and beverage samples. This technique is important for quality control, nutritional labeling, authenticity testing, and production process monitoring because it provides key metrics of product quality and related properties, contamination, or adulteration. HPAE-PAD allows direct quantification of nonderivatized carbohydrates with minimal sample preparation and also resolves most carbohydrates from sugar alcohols and organic acids, while not detecting sodium chloride commonly present in fruit juices.[1]

With HPAE, carbohydrates are ionized in the strong base eluent (typically sodium or potassium hydroxide) and separated by anion-exchange chromatography. Thermo Scientific™ Dionex™ CarboPac™ columns are optimized for carbohydrate separations. The Dionex CarboPac resin is packed in a polymeric column body that is inert to hydroxide eluents, thereby reducing column and electrode fouling from metal contamination. Using the carbohydrate-optimized waveform, PAD is sensitive and specific for carbohydrates by detecting mostly compounds containing hydroxyl functional groups. As a result, its sensitivity for carbohydrates is greater than for other analyte classes by several orders of magnitude.

Figure 1 shows the determination of carbohydrates in fruit juices by HPAE-PAD using a capillary Dionex CarboPac PA20 column and PAD (Table 1). Scaling down from analytical to a capillary scale system provides many benefits for carbohydrate analysis. One of the most significant benefits is the use of eluent generation (EG) to produce ultrapure carbonate-free potassium hydroxide eluent. The eluent (electrolytically generated on-line from deionized water) provides excellent reproducibility for carbohydrate analysis. This reduction in scale also allows generation of 200 mM potassium hydroxide compared to the limit of 100 mM potassium hydroxide available on a conventional analytical platform. Thus, capillary HPAE-PAD provides greater application flexibility to elute

Column:	Dionex CarboPac PA 20, 0.4 × 150 mm
Temperature:	30 °C
Eluent:	50 mM potassium hydroxide (EG)
Flow Rate:	10 µL/min
Inj. Volume:	0.40 µL
Detection:	PAD, 4–potential carbohydrate, Au
Ref. Electrode:	PdH
Gasket Thickness:	25 µm
Samples:	Juice samples (5000× dilution) Standard (20 µM)
Peaks:	1. Glucose 2. Fructose 3. Sucrose

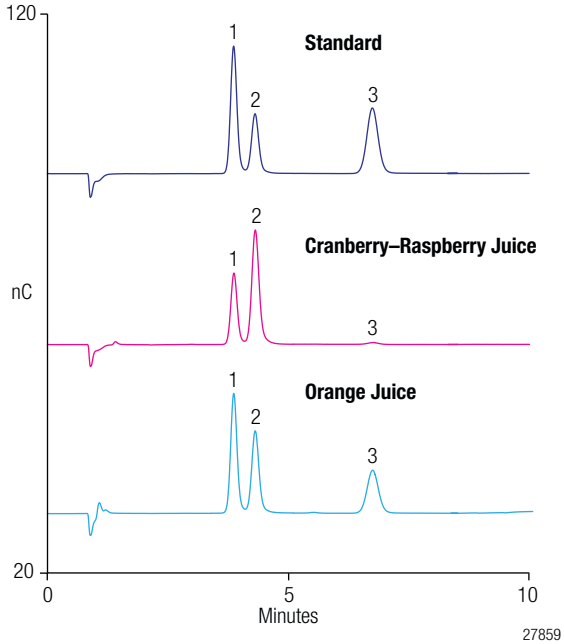

Figure 1. Analysis of juices for carbohydrates by capillary HPAE-PAD.

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strongly retained sugars and to implement routine on-line column washes without requiring manually prepared strong hydroxide wash solutions. Using capillary eluent generation, the pump delivers deionized water isocratically, eliminating the need for a pump with proportionating capability.

In capillary HPAE-PAD, the system is also always on and ready for analysis, simplifying the overall workflow. No eluent preparation is required; no startup or equilibration time is needed. Also, because the system is always on, the stability of the PAD detector is further improved, thus requiring less frequent calibrations. Even under continuous operation, the EG cartridge will last for 18 months, which provides an overall lower cost of ownership.

Capillary HPAE-PAD and Reagent-Free™ IC (RFIC™) technology further expands the capabilities and simplifies the determination of carbohydrates using HPAE with PAD.

Table 1. Carbohydrate waveform*.

Time (sec)	Potential (V)	Gain Region	Integration	Ramp
0.00	+0.95	Off	Off	Ramp
0.20	+0.95	On	On (Start)	Ramp
0.40	+0.95	Off	Off (End)	Ramp
0.41	–1.15	Off	Off	Ramp
0.42	–1.15	Off	Off	Ramp
0.43	+1.45	Off	Off	Ramp
0.44	+0.75	Off	Off	Ramp
0.50	+0.75	Off	Off	Ramp

*This waveform is used with the PdH reference electrode.

Equipment and Conditions

- Thermo Scientific Dionex ICS-5000 capillary IC system including:
  - DP Dual Pump, capillary with degas option
  - DC Detector/Chromatography module equipped with Thermo Scientific Dionex IC Cube™ module
  - ED Electrochemical Detector, capillary
  - EG Eluent Generator
  - AS Autosampler
  - An electrochemical cell, capillary, with PdH reference electrode, disposable Au working electrode
  - Thermo Scientific Dionex Chromeleon™ Chromatography Data System software

All experimental conditions are listed in Figure 1.

Sample Preparation

Filter and dilute samples 5000× prior to analysis.

Conclusion

Glucose, fructose, and sucrose in fruit juices are well resolved using a Dionex CarboPac PA20 column in capillary format and an electrolytically generated potassium hydroxide eluent. Capillary RFIC systems expand the application of IC to carbohydrate analysis for the food and beverage industries by bringing enhanced mass sensitivity, ease-of-use, and reproducibility to routine determination of carbohydrates.

References

1. De Vries, J.W.; Nelson, A.L. *Food Technol.*, **1994,** (July) 76–77.

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