Analytical Performance of Capillary High-Performance Anion-Exchange with Pulsed Amperometric Detection

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Executive Summary

Capillary-scale high-performance anion-exchange with pulsed amperometric detection (HPAE-PAD) offers substantial benefits over conventional-scale HPAE-PAD, including improved mass detection limits, smaller sample volumes, and reduced eluent consumption. Capillary HPAE-PAD is ideally suited for analytical determination of carbohydrates in a range of matrices across multiple applications.

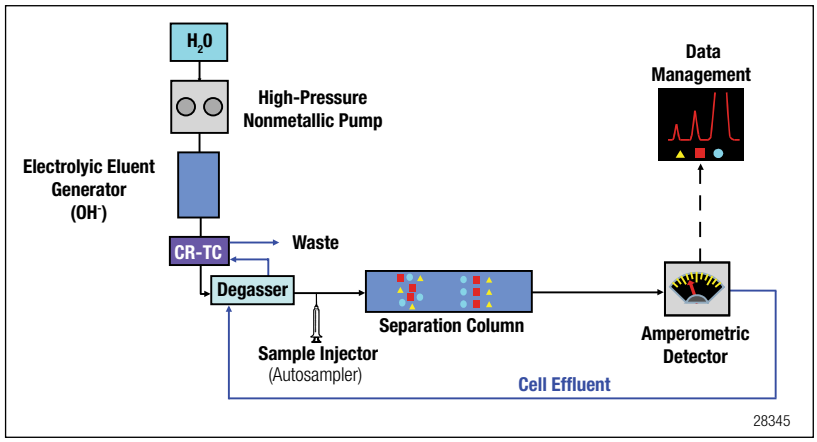

Figure 1. Block diagram of a capillary Reagent-Free™ HPAE-PAD system with electrochemical detection.

Key Words

Capillary HPAE-PAD, ICS-5000, Carbohydrates

Abstract

Capillary high-performance anion-exchange with pulsed amperometric detection (HPAE-PAD) is a technique that uses capillary columns (e.g., 0.4 mm i.d. columns) packed with ion-exchange resin and a newly designed capillary amperometric cell with a gold working electrode and a palladium hydrogen (PdH) reference electrode. The cell body is made of titanium and serves as a counter electrode. An electrolytic eluent generator optimized for operation at capillary flow rates is also included in the system.

To characterize the new technique, the authors generated analytical performance data under a number of different conditions based on eluent composition and flow rate. Separation efficiency and concentration detection limits were comparable with those achieved using analytical-scale chromatography. However, the mass detection limits were significantly improved, and the range of linearity of calibration plots was broader than what is typically achieved using analytical-scale chromatography.

Next, the authors used capillary HPAE-PAD for the analysis of carbohydrates in cell cultures, fermentation broths, protein hydrolyzates, beverages, and food samples. Because of its low flow rates (5–10 µL/min), capillary HPAE-PAD offers the convenience of extremely low eluent usage (1 L of water lasts approximately 2 months). An additional advantage is that the system requires only very small injection volumes (0.4 µL).

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Experimental

Instrument: Thermo Scientific™ Dionex™ ICS-5000 Ion Chromatography System, including:

- Autosampler with low-volume injector valve (0.40 µL)
- Dual pump (DP) with flow range from 1 to 100 µL/min
- Capillary Eluent Generator (CapEG)
- Capillary Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column
- Thermo Scientific™ Dionex™ IC Cube™ cartridge with capillary Thermo Scientific™ Dionex™ CarboPac™ PA20 column, 0.40 × 150 mm
- Capillary ED cell (130 nL dead volume) equipped with PdH reference electrode and disposable gold working electrode (1 mm diamter)

System Control and Data Processing: Thermo Scientific™ Dionex™ Chromeleon™ 6.8 Chromatography Data System software

Chromatographic Conditions: See figures for detail.

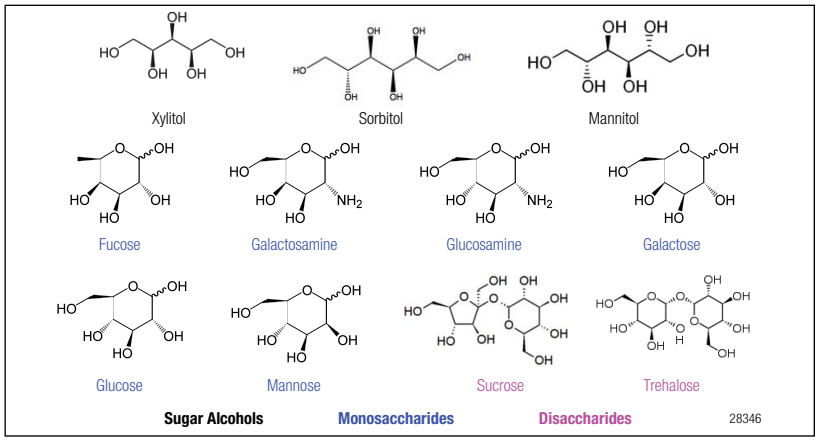


Figure 2. Selected molecular structures: sugar alcohols, monosaccharides, and disaccharides.

Table 1. Calibration performance of capillary and analytical systems.

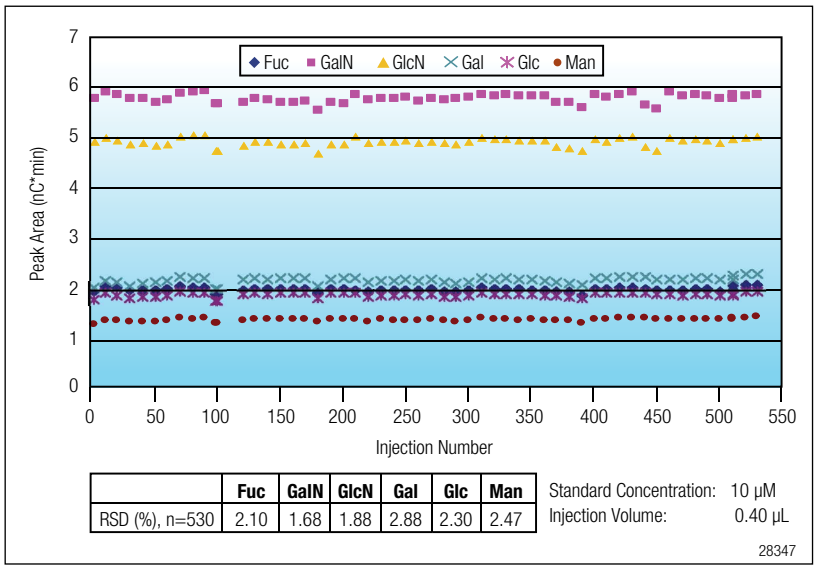
	Linear Range		Correlation Coefficients	
	Capillary	Analytical	Capillary	Analytical
Fuc	0.024–50	0.023–20	0.9958	0.9932
GalN	0.018–25	0.014–20	0.9998	0.9920
GlcN	0.029–25	0.023–20	0.9949	0.9987
Gal	0.054–25	0.029–20	0.9964	0.9932
Glc	0.056–50	0.027–50	0.9986	0.9961
Man	0.068–50	0.059–50	0.9956	0.9975

1. Reference electrode: PdH (capillary); Ag/AgCl (analytical)
2. Eluent: EG generated (capillary); manually prepared eluent (analytical)
3. Injection volume: 0.40 µL (capillary); 25 µL (analytical)
4. Analytical system conditions: flow rate 0.25 mL/min; separation column Dionex CarboPac PA20, 3 × 150 mm; eluent 10 mM NaOH

Table 2. Limits of detection using capillary and analytical systems.

	LOD (µM)		LOD (picogram)	
	Capillary	Analytical	Capillary	Analytical
Fuc	0.024	0.023	1.6	94.4
GalN	0.018	0.014	1.3	62.7
GlcN	0.029	0.023	2.1	103.0
Gal	0.054	0.029	3.9	130.9
Glc	0.056	0.027	4.0	121.6
Man	0.068	0.059	4.9	265.7

1. Reference electrode: PdH (capillary); Ag/AgCl (analytical)
2. Eluent: EG generated (capillary); manually prepared eluent (analytical)
3. Injection volume: 0.40 µL (capillary); 25 µL (analytical)
4. Analytical system conditions: flow rate 0.25 mL/min; separation column Dionex CarboPac PA20, 3 × 150 mm; eluent 10 mM NaOH



	Fuc	GalN	GlcN	Gal	Glc	Man
RSD (%), n=530	2.10	1.68	1.88	2.88	2.30	2.47

Figure 3. Response stability over two-week period: six monosaccharides, with PdH reference electrode.

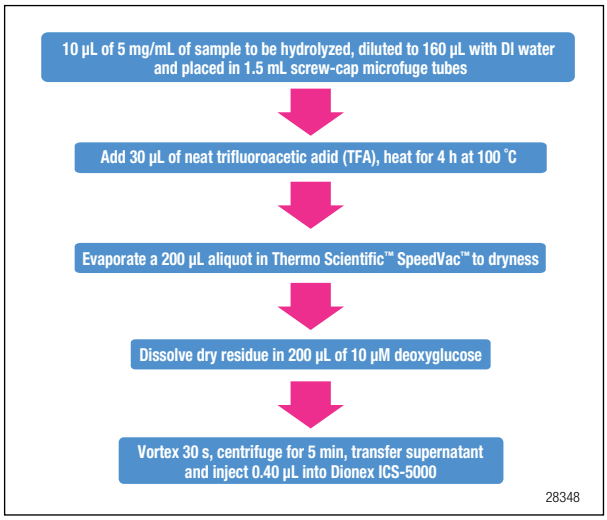


Figure 4. Hydrolysis of a monoclonal antibody.

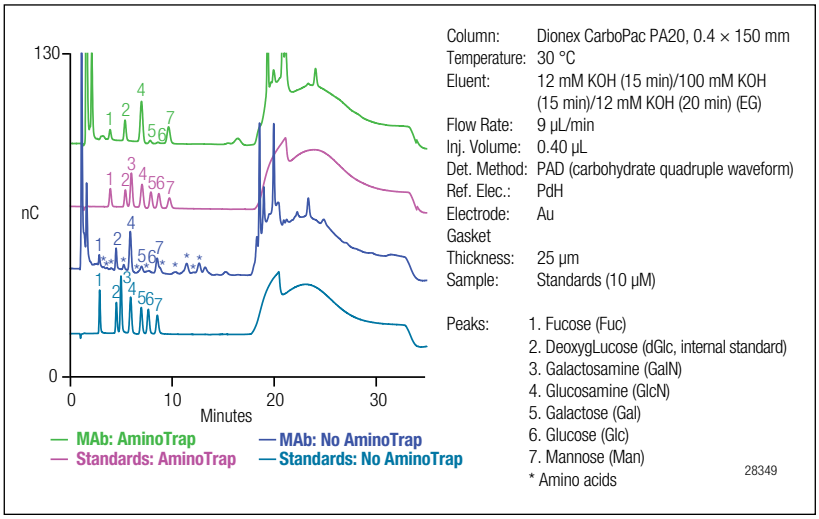


Figure 5. Separation of monoclonal antibody hydrolysate with and without Thermo Scientific™ Dionex™ AminoTrap™ column. The Dionex AminoTrap column is designed to eliminate amino acid interference. It is installed between injector and separation column.

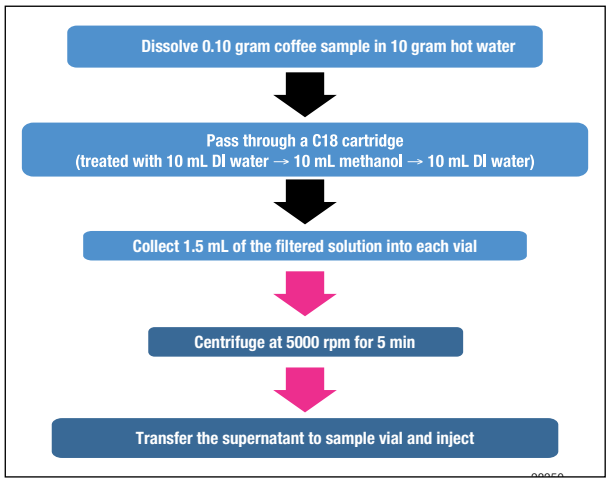


Figure 6. Preparation of a soluble coffee sample for free carbohydrate analysis.

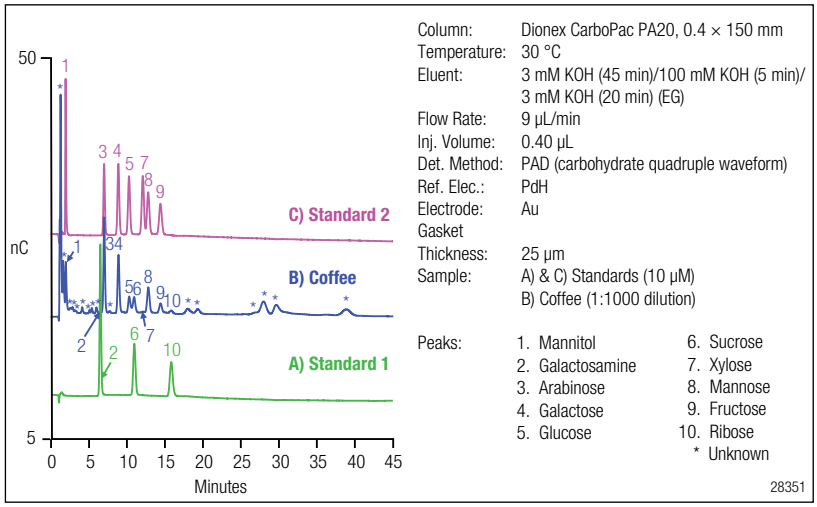


Figure 7. Separation of coffee sugars.

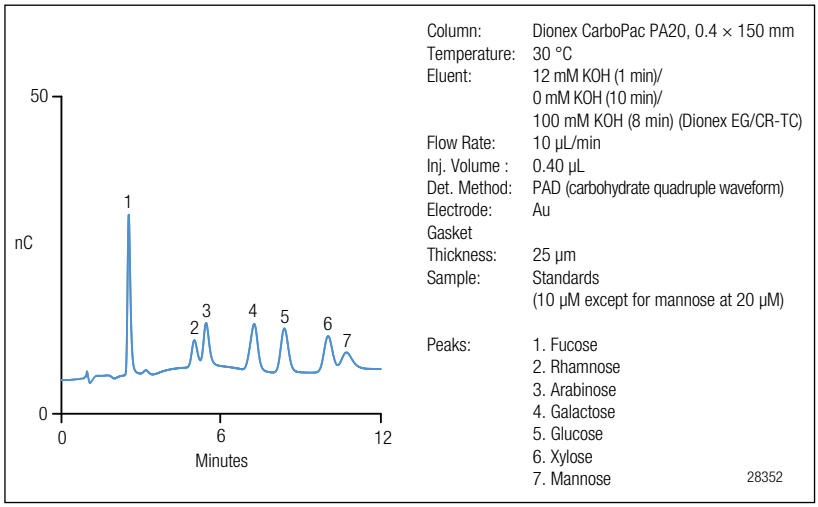
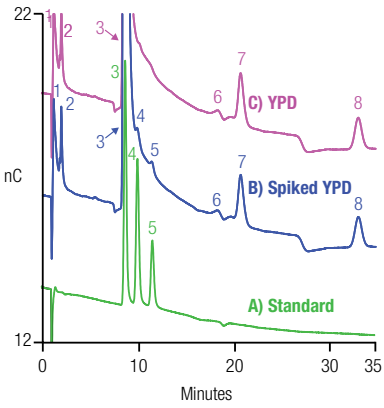


Figure 8. Separation of wood sugars. Two critical pairs—rhamnose/arabinose and xylose/mannose are separated in a single run.



Column:	Dionex CarboPac PA20, 0.4 × 150 mm
Temperature:	30 °C
Eluent:	7 mM KOH (35 min)/100 mM KOH (5 min)/ 7 mM KOH (20 min) (EG)
Flow Rate:	9 µL/min
Inj. Volume:	0.40 µL
Det. Method:	PAD (carbohydrate quadruple waveform)
Electrode:	Disposable Au
Ref.:	PdH
Gasket	
Thickness:	25 µm
Sample:	A) Standard (10 µM) B) Spiked Sample (10,000 dilution spiked with 1 µM sucrose and fructose) C) Sample (10,000 dilution)
Peaks:	1. Glycerol/Inositol 2. Trehalose 3. Glucose 4. Sucrose 5. Fructose 6–8. Unknown

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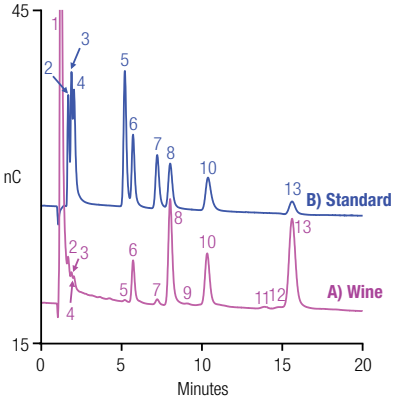
Figure 9. Separation of cell culture medium yeast extract; peptone-dextrose (YPD).



Column:	Dionex CarboPac PA20, 0.4 × 150 mm
Temperature:	30 °C
Eluent:	10 mM KOH (20 min)/100 mM (5 min)/ 10 mM KOH (20 min) (EG)
Flow Rate:	9 µL/min
Inj. Volume:	0.40 µL
Det. Method:	PAD (carbohydrate quadruple waveform)
Ref. Elec.:	PdH
Electrode:	Au
Gasket	
Thickness:	25 µm
Sample:	A) Wine sample (1:500 dilution) B) Standard (10 µM)

Peaks:		
1. Unknown	7. Galactose
2. Sorbitol	8. Glucose
3. Mannitol	9. Unknown
4. Trehalose	10. Fructose
5. Galactosamine	11–12. Unknown
6. Arabinose	13. Proline

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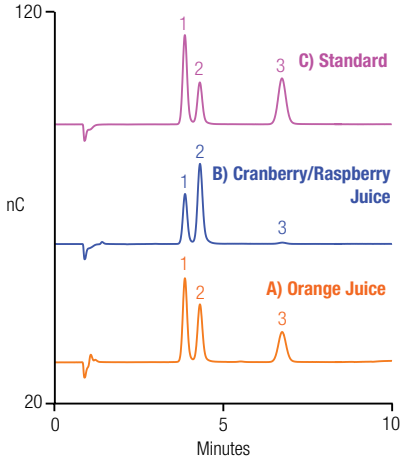
Figure 10. Separation of sugars, amino sugars, and amino acids in wine sample and standard.



Column:	Dionex CarboPac PA20, 0.4 × 150 mm
Temperature:	30 °C
Eluent:	50 mM KOH (EG)
Flow Rate:	10 µL/min
Inj. Volume:	0.40 µL
Det. Method:	PAD (carbohydrate quadruple waveform)
Ref. Elec.:	PdH
Electrode:	Au
Gasket	
Thickness:	25 µm
Samples:	A) & B) Juice samples (1:5000 dilution) C) Standard (20 µM)
Peaks:	1. Glucose 2. Fructose 3. Sucrose

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Figure 11. Separation of sugars in juice samples and standard.

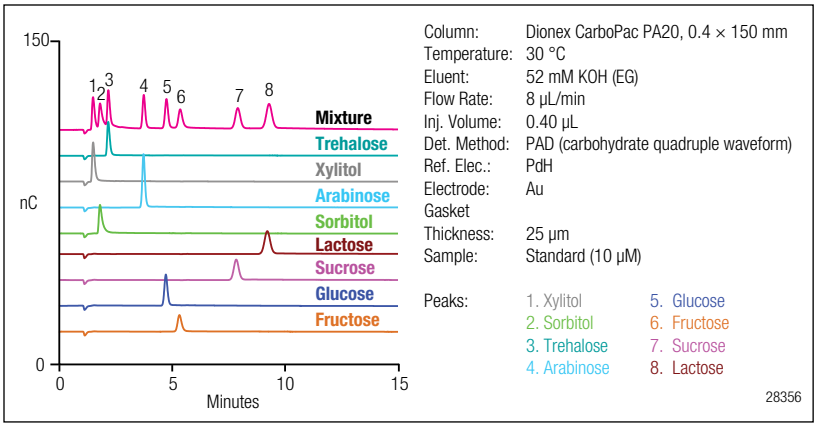

Figure 12. Separation of sugar alcohols, mono- and disaccharides found in dietary fiber.

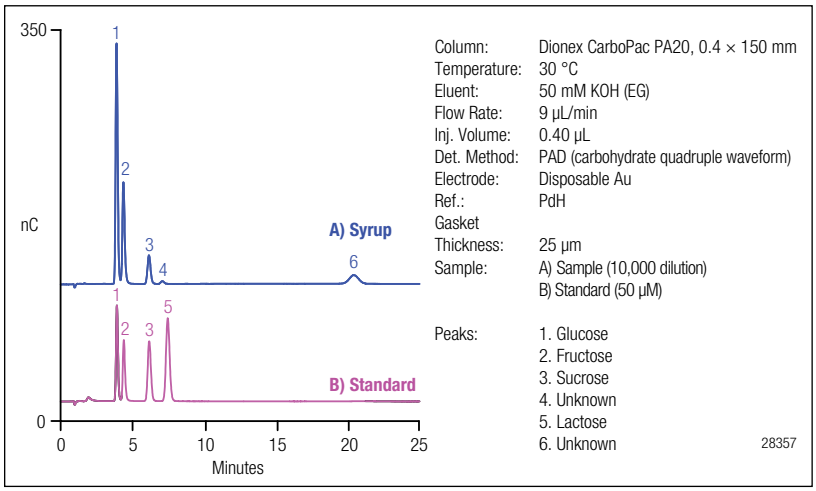

Figure 13. Separation of sugars in chocolate syrup.

Conclusion

Capillary-scale HPAE-PAD using the Dionex ICS-5000 system equipped with a capillary ED cell and PdH reference electrode enables determination of carbohydrates in a range of applications. This capillary-scale HPAE-PAD platform provides analytical performance comparable to conventional-scale HPAE-PAD using a regular ED cell and Ag/AgCl reference electrode, with the additional advantages of minimal eluent consumption (<15 mL/day), extremely small sample sizes (400 nL injection volumes), and improved mass detection limits.

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