

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



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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 $\mu\text{L}/\text{min}$), 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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Instrument: 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 $\mu\text{L}/\text{min}$
- Capillary Eluent Generator (Cap EG)
- Continuously Regenerated–Capillary Anion Trap Column (CR-ATC)
- IC Cube™ cartridge with capillary CarboPac® PA 20 column (0.40 \times 150 mm)
- Capillary ED cell (130 nL dead volume) equipped with palladium hydrogen reference electrode and disposable gold working electrode (1 mm diameter)

System Control and Data Processing: Dionex Chromeleon® 6.8
Chromatography Data System Software

Chromatographic Conditions: see figures for detail.

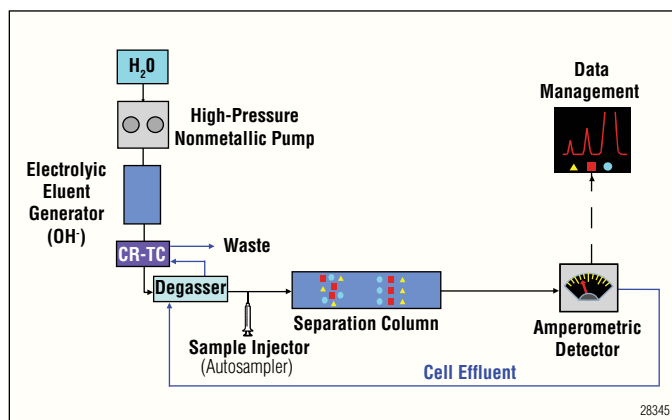


Figure 1. Block diagram of a Capillary Reagent-Free™ HPAE-PAD system with electrochemical detection.

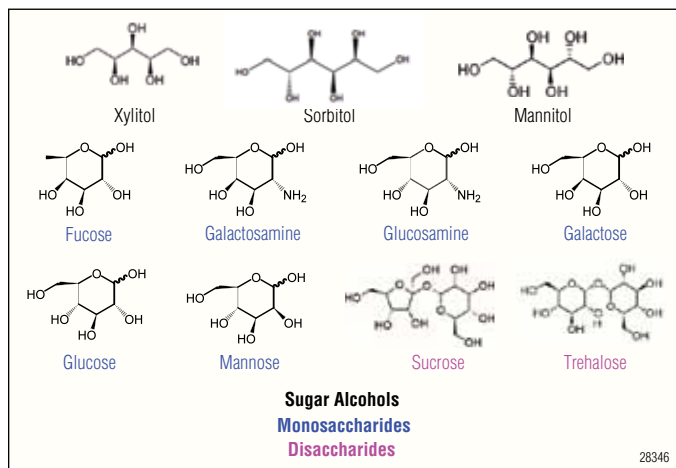


Figure 2. Selected molecular structures: sugar alcohols, monosaccharides, and disaccharides.

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

- Reference electrode: PdH (capillary); Ag/AgCl (analytical)
- Eluent: EG generated (capillary); manually prepared eluent (analytical)
- Injection volume: 0.4 μ L (capillary); 25 μ L (analytical)
- Analytical system conditions: flow rate 0.25 mL/min; separation column CarboPac PA20, 3 \times 150 mm; eluent 10 mM NaOH

Table 2. Limits of Detection (LOD) Using Capillary and Analytical Systems				
	LOD (μ M)		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

- Reference electrode: PdH (capillary); Ag/AgCl (analytical)
- Eluent: EG generated (capillary); manually prepared eluent (analytical)
- Injection volume: 0.4 μ L (capillary); 25 μ L (analytical)
- Analytical system conditions: flow rate 0.25 mL/min; separation column CarboPac PA20, 3 \times 150 mm; eluent 10 mM NaOH

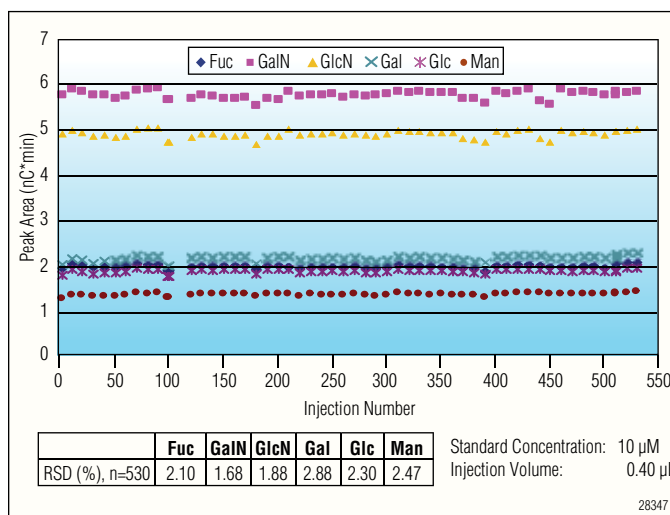


Figure 3. Response stability over two week period: six monosaccharides, with PdH reference electrode.

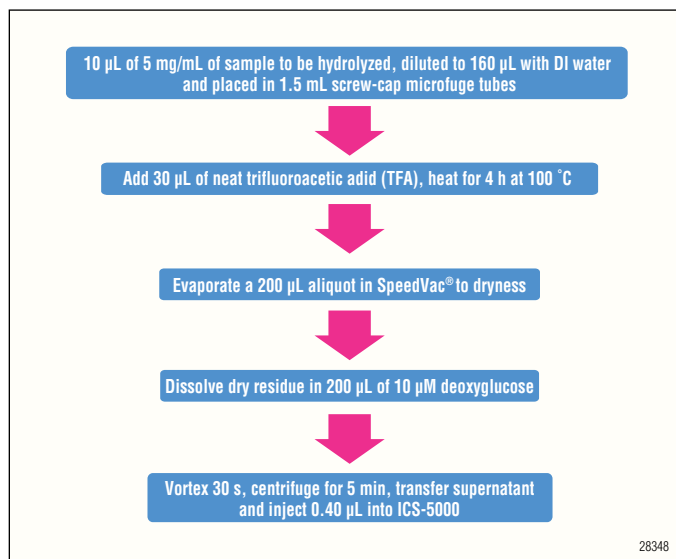


Figure 4. Hydrolysis of a monoclonal antibody.

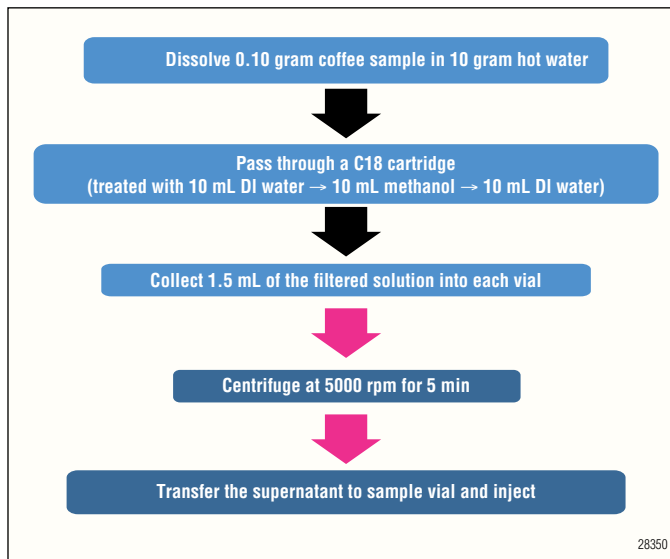


Figure 6. Preparation of a soluble coffee sample for free carbohydrate analysis.

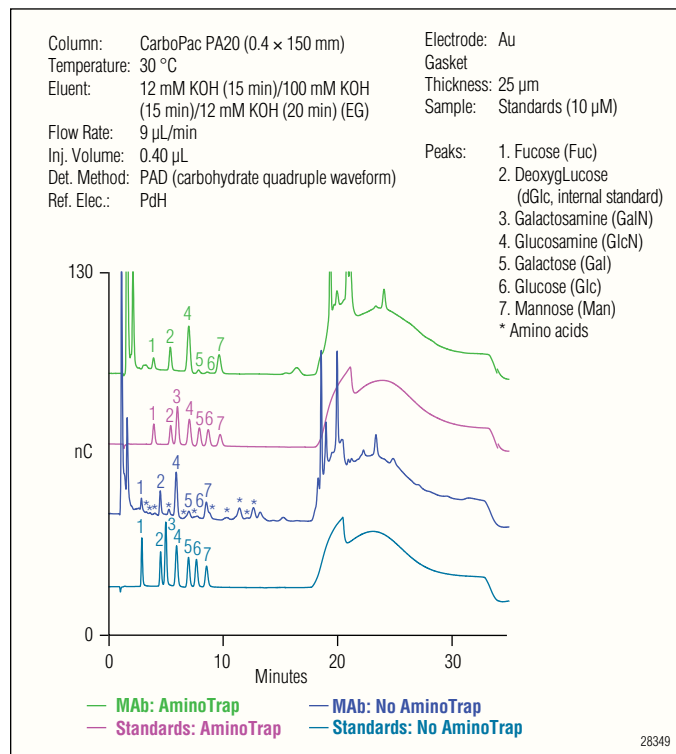


Figure 5. Separation of monoclonal antibody hydrolysate with and without AminoTrap™ column. The AminoTrap column is designed to eliminate amino acid interference. It is installed between injector and separation column.

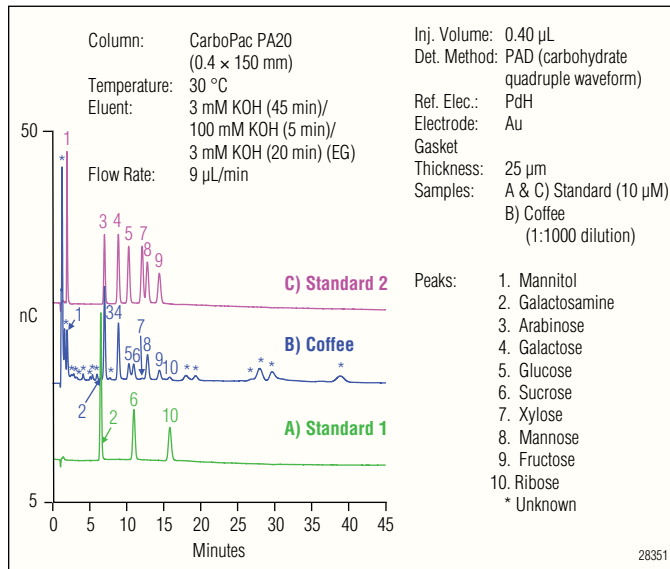


Figure 7. Separation of coffee sugars.

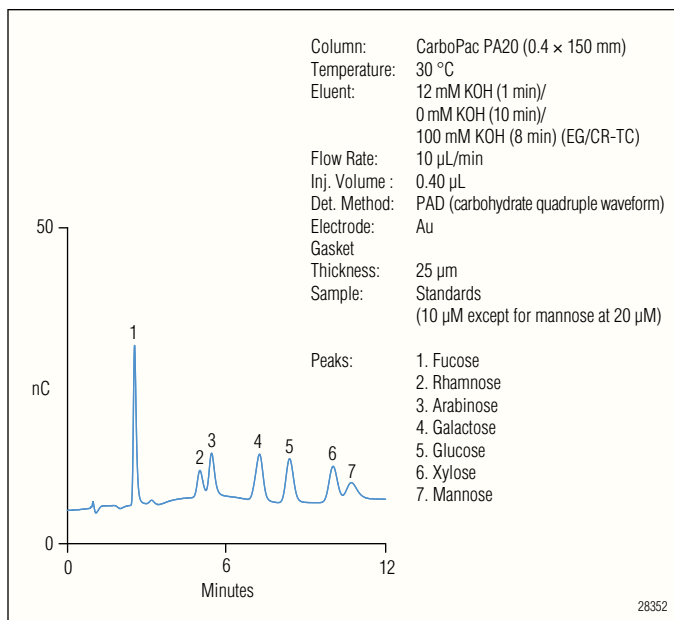


Figure 8. Separation of wood sugars. Two critical pairs—rhamnose/arabinose and xylose/mannose are separated in a single run.

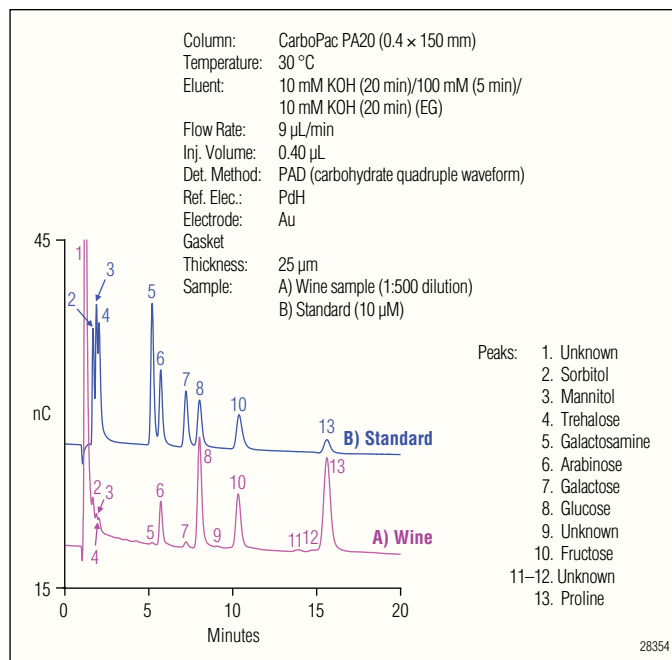


Figure 10. Separation of sugars, amino sugars, and amino acids in wine sample and standard.

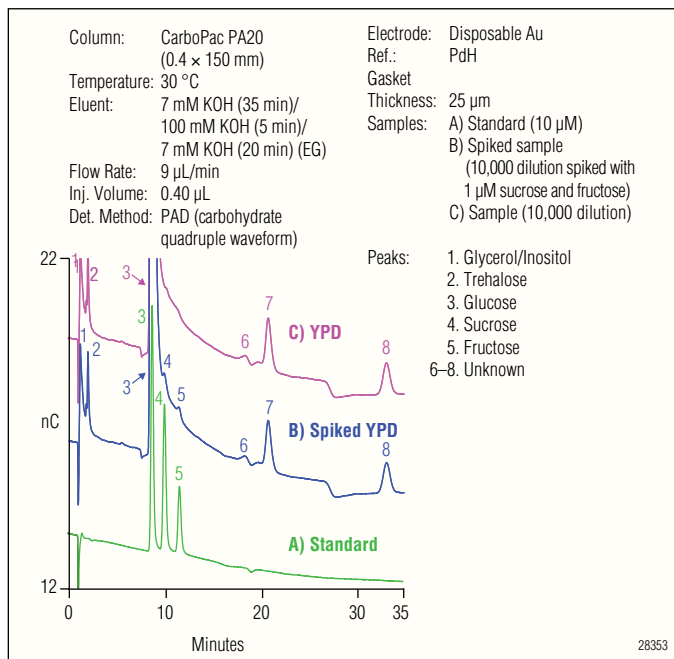


Figure 9. Separation of cell culture medium yeast extract; peptone-dextrose (YPD).

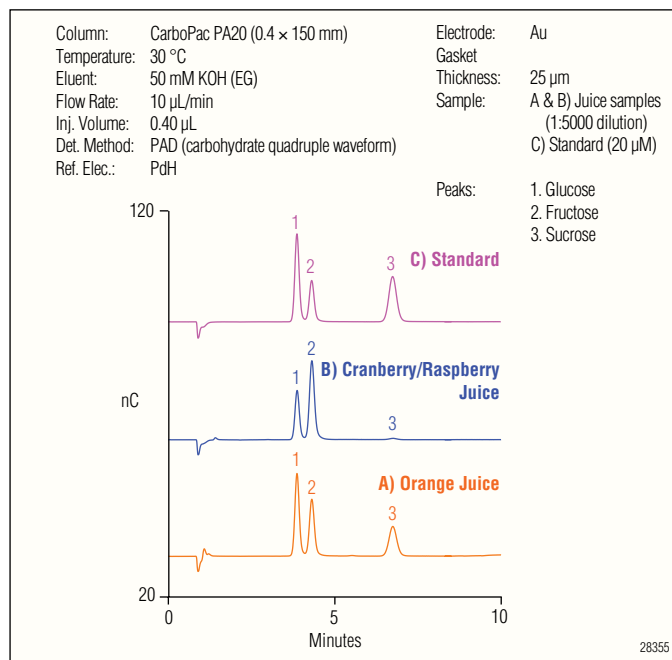


Figure 11. Separation of sugars in juice samples and standard.

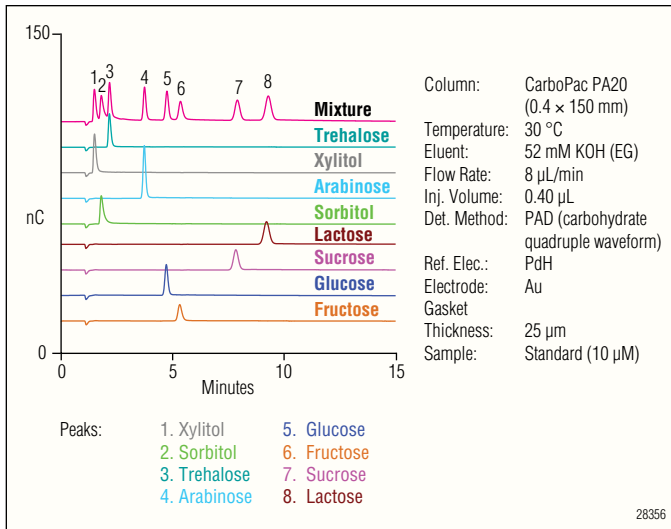


Figure 12. Separation of sugar alcohols, mono- and disaccharides found in dietary fiber.

CONCLUSION

The advantages of using capillary HPAE-PAD with newly designed capillary ED cell and PdH reference electrode include:

- Minimal eluent consumption (< 15 mL/day)
- Extremely small sample size (0.40 µL injection volume)
- Improved mass limit of detection

Capillary scale HPAE-PAD provides analytical performance comparable to conventional-scale HPAE-PAD using regular ED cell and Ag/AgCl reference electrode. A wide range of applications for carbohydrate analysis by capillary HPAE-PAD are successfully demonstrated here.

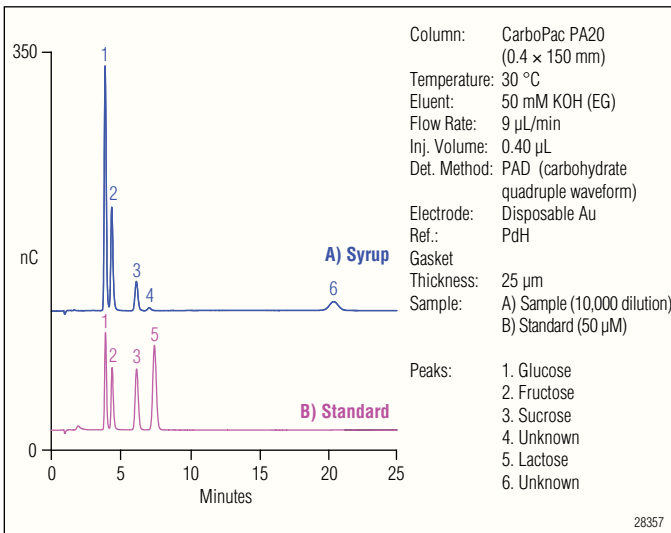


Figure 13. Separation of sugars in chocolate syrup.

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