

Determination of Monosaccharides in Acid-Hydrolyzed Corn Stover Using HPAE-PAD

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INTRODUCTION

Lignocellulosic biomass provides 1.3 billion tons of raw materials annually and is a common feedstock for fermentation systems used for biofuel production.^{1,2} Corn stover—the above-ground portion of the plant minus the kernels—accounts for a large percentage of the global biomass available for this purpose. The corn stover is acid-hydrolyzed to release a water-soluble mixture of carbohydrates, typically consisting of arabinose, glucose, galactose, mannose, xylose, fructose, and cellobiose, among other non-carbohydrate substances in 0.5 to 1.5% (w/w) sulfuric acid. The concentration of these sugars can vary with the feedstock, its pretreatment, hydrolysis, and storage conditions. Knowledge of the monosaccharide content of acid-hydrolyzed corn stover allows evaluation of the effectiveness of a new hydrolysis process and/or an estimation of the product yield (e.g., ethanol, butanol, methanol, hydrogen, etc.).

Carbohydrates lack a good chromophore and therefore require high concentrations to be detected by UV absorbance or RI, and non-carbohydrate ingredients of acid-hydrolyzed corn stover can interfere with carbohydrate determinations. Pulsed amperometric detection (PAD) has a broad linear range for monosaccharides and most other carbohydrates, and is selective for compounds that can be detected under a given set of electrochemical conditions.^{3,4}

High-performance anion-exchange chromatography (HPAE) can separate glucose, galactose, arabinose, xylose, mannose, fructose, cellobiose, and other carbohydrates. The CarboPac® PA1 anion-exchange column resolves monosaccharides from the unretained and undetected non-carbohydrate components typical of acid-hydrolyzed plant-derived materials. The hydroxide eluent concentration and column temperature determines the extent of resolution by altering the selectivity of the column. Generally, at high hydroxide eluent concentrations (e.g., 200 mM NaOH), monosaccharides elute rapidly and are poorly resolved. At lower hydroxide eluent concentrations (e.g., 0.5–50 mM), monosaccharides are resolved but elution times are longer. Minor variations in eluent concentration or column temperature can cause unique resolution of carbohydrates. The use of eluent generation (EG) ensures that high-purity hydroxide eluent with accurate concentrations is used in the application.

Here, the authors present two methods for determination of carbohydrates in an undiluted corn stover hydrolysate without prior pH neutralization. The first method rapidly estimates the total free carbohydrate content in less than 10 min, and the second method separates the monosaccharides for speciation. Data is presented that shows the control possible over column selectivity by adjusting eluent concentration and column temperature, and evaluate performance, including precision, accuracy, and linearity for both methods.

EXPERIMENTAL

Instrument

Dionex ICS-3000 ion chromatography system consisting of:

DP Dual Gradient pump with vacuum degas option and GM-4 Gradient Mixer.

Eluent Generator with EGC II KOH eluent generator cartridge (EluGen® II Hydroxide; P/N 058900).

Continuously Regenerated Anion Trap Column (CR-ATC; P/N 060477).

DC Detector/Chromatography Module equipped with temperature control, injection valve(s) with 0.2 µL (P/N 068383) or 25 µL injection loop, and Electrochemical Detector (P/N 061718) with combination pH/Ag/AgCl reference electrode (P/N 061879).

Carbohydrate PTFE Disposable Au Working Electrodes (P/N 066480, package of 6) or conventional Au Working Electrode (P/N 061749). The standard carbohydrate concentration system uses the 2 mil PTFE gaskets (included with the package of disposable electrodes, P/N 060141), or the 1 mil gaskets (P/N 045972) for the conventional electrode. The high carbohydrate concentration system uses the 15 mil PTFE gasket (P/N 057364).

AS Autosampler (with diverter valve for dual systems), and 2 mL vial tray.

EO Eluent Organizer, including pressure regulator, and four 2 L plastic bottles for each system.

Chromeleon® Chromatography Data System software.

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Chromatography Conditions

Column:	CarboPac® PA1 Analytical (4 × 250 mm, P/N 35391)
Flow Rate:	1.0 mL/min
Injection Volume:	0.2 or 25 µL
Detection:	Pulsed amperometry, gold electrode
Waveform:	Four-potential carbohydrate waveform (see Technical Note 21) ⁵
Reference Electrode:	Ag/AgCl (Ag mode)

Rapid Method

Eluent A:	200 mM NaOH (manually prepared)
Column Temperature:	30 °C
Detector Temperature:	30 °C

Table 1. Rapid Method Gradient Program			
Time (min)	% A	NaOH concentration (mM)	Comments
0.0	100	200	Inject sample, elute mono-, di-, and oligosaccharides
10.0	100	200	End run

Speciation Method

Eluent A:	DI water
Eluent B:	200 mM NaOH (manually prepared)
Column Temperature:	25 °C
Detector Temperature:	25 °C

Table 2. Speciation Method Gradient Program					
Time (min)	% A	% B	KOH Concentration (mM)	NaOH Concentration (mM)	Comments
0.0	100	0	0.5	0	Inject sample
35.0	100	0	0.5	0	Elute monosaccharides with 0.5 mM KOH
35.1	0	100	0.5	200	Step to 200 mM NaOH*
50.0	0	100	0.5	200	Elute disaccharides and oligosaccharides
50.1	100	0	0.0	0	Begin column rinse with water (eluent generator current off)
55.0	100	0	0.0	0	End column rinse with water
55.1	100	0	0.5	0	Begin column re-equilibration with KOH (eluent generator current on)
60.0	100	0	0.5	0	End run

SAMPLES AND STANDARDS

Corn stover acid hydrolysate was a generous gift from the National Bioenergy Center of the National Renewable Energy Laboratory, Golden, CO, USA. Undiluted corn stover hydrolysate was centrifuged at 16,000 × g for 10 min prior to injection. Supernatant was diluted 400-fold with water if needed. Carbohydrate standards were obtained from Sigma-Aldrich, and diluted in water to desired concentrations.

RESULTS

Rapid Method

The rapid method (Table 1) for estimation of carbohydrates is based on a strong hydroxide eluent (200 mM) that quickly elutes monosaccharides, disaccharides, and oligosaccharides. Corn stover acid hydrolysate can be analyzed > 10 min for estimation of total monosaccharide content.

Separation

Figure 1 shows overlaid chromatograms of undiluted corn stover hydrolysate (blue trace) and standards (magenta trace) using the rapid method. Panel B expands the signal axis to reveal greater detail. Arabinose (peak 1) is resolved from coeluting galactose, glucose, mannose, and xylose (peak 2). Fructose (peak 3) is also resolved, but coelutes with other unidentified carbohydrates, making quantification inaccurate. Cellobiose (peak 4) elutes between 6–7 min, but the peak also contains other carbohydrates. The combined peak area for peaks 1 and 2 is an estimate of the monosaccharide content of corn stover acid hydrolysate.

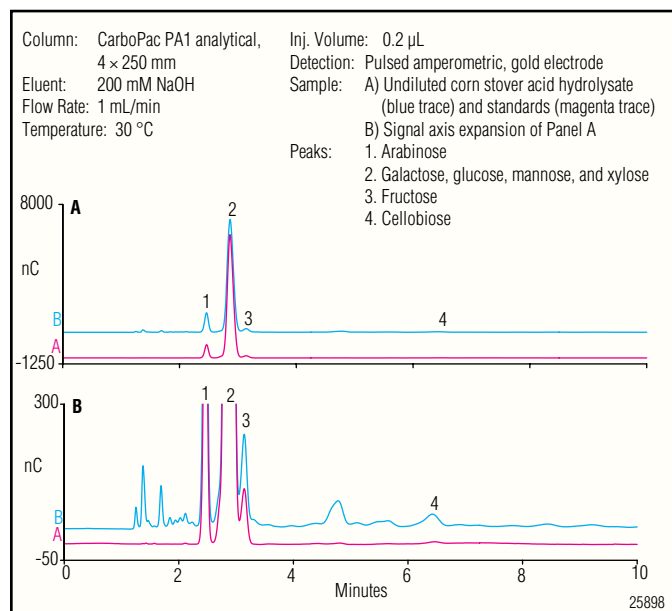


Figure 1. Rapid separation of monosaccharides in undiluted corn stover acid hydrolysate (blue trace) and standards (magenta trace) using the CarboPac PA1 with 200 mM NaOH at 30 °C.

Method Performance

Values of r^2 for the calibration ranges used in this study are presented in Table 3. This table also presents the calculated concentration of total monosaccharides (arabinose, glucose, galactose, mannose, xylose, fructose, and cellobiose) based on the measured peak areas. The estimated total monosaccharide concentration in the corn stover hydrolysate was 199 mg/mL. The concentration provided by NREL for this sample was 137 mg/mL. The spike recovery of arabinose, glucose, xylose, mannose, and galactose was 96%. We did not obtain meaningful spike recovery measures for fructose or cellobiose due to interferences from coeluting carbohydrates.

Table 4 presents monosaccharide retention time and peak area RSDs for replicate injections of undiluted corn stover hydrolysates using the rapid method. Retention time RSD was 0.2%, and peak area RSD ranged from 0.7–5%

Table 3. Rapid Method Linearity and Accuracy

Saccharide	Calibration Range		Measured Concentration in Undiluted Corn Stover		
	(mg/mL)	r^2	Mean (mg/mL)	RSD (%)	% Spike Recovery
Arabinose	1.4–14	0.9992	14	0.7	96
Glucose, Xylose, Mannose, Galactose	26–255	0.9997	176	0.4	96
Fructose	0.4–4	0.9512	98	1.8	292*
Cellobiose	0.03–0.3	0.9581	2.1	2.3	234*
Total	27–274	0.9997	199	0.4	96

*Coeluting peaks, inaccurate measurements.

Table 4. Precision of Replicate Injections of Undiluted Corn Stover Hydrolysate Using the Rapid Method

Saccharide	Undiluted Corn Stover (n=20 injections) RSDs (%)	
	Retention Time	Peak Area
Arabinose	0.22	0.69
Glucose, Xylose, Mannose, Galactose	0.17	0.79
Fructose	0.16	1.25
Cellobiose	0.16	4.78

Speciation Method

Chromatography for carbohydrate speciation of acid-hydrolyzed corn stover is based on a weak hydroxide eluent (0.5 mM) that alters the selectivity in a manner that resolves the different types of monosaccharides and disaccharides. Figure 2 shows overlaid chromatograms of carbohydrate standards using 0.5, 2.0, 25, and 50 mM KOH, produced by the EG. A step gradient to 200 mM NaOH enables a rapid elution of more strongly retained carbohydrates. Corn stover acid hydrolysate can be analyzed in < 60 min for speciation of monosaccharides.

Separation

These chromatograms show the resolutions possible by varying hydroxide eluent concentration. For example, a slight improvement in separation between rhamnose and arabinose, and between sucrose and xylose, occurs when the eluent concentration is reduced from 2 mM to 0.5 mM KOH. Under both eluent concentrations, xylose, glucose, galactose, and mannose are well resolved. When the eluent concentration is raised to 25 mM, rhamnose, arabinose, sucrose, and xylose, are fully resolved. Although not extensively presented here, column temperatures are also a powerful factor affecting column selectivity. Even a few degrees can significantly impact separations. Table 5 shows a comprehensive list of carbohydrates found in plant material, and their behavior on the CarboPac PA1 column under varying eluent concentrations. This table also demonstrates the effect of column temperature on selectivity at the 200 mM NaOH eluent concentration, comparing retention times at 25 and 30 °C.

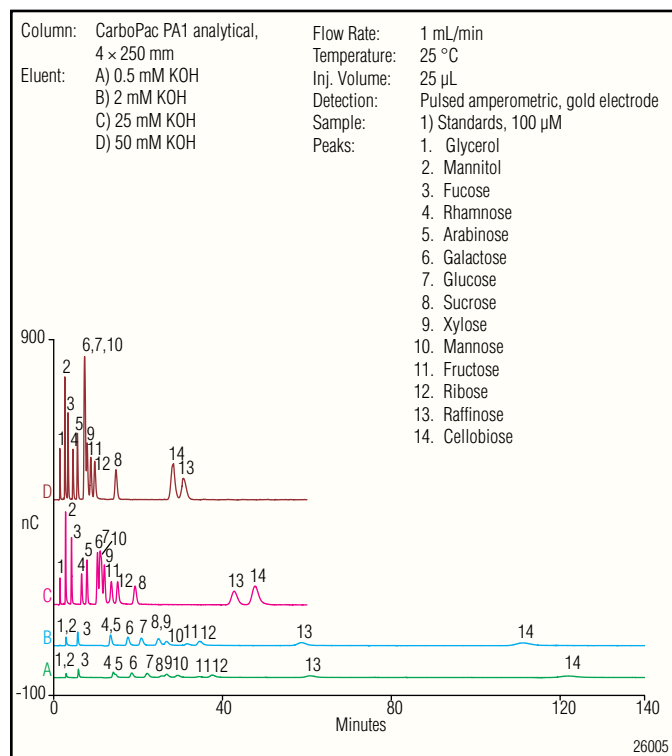


Figure 2. Carbohydrate standards separated with 0.5, 2, 25, and 50 mM KOH. Varying base concentration enables changes in column selectivity and resolution of some carbohydrates.

Table 5. Retention Times for Common Plant Carbohydrates at Different Hydroxide Concentrations

Analyte	Retention Time (min), 1.0 mL/min Flow Rate, Isocratic									
	mM KOH, 25 °C							mM NaOH		
								25 °C	30 °C	
	0.5	1	2	5	10	25	50	100	200	200
Void	1.3	1.3	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Propylene Glycol	1.5	1.4	1.4	1.4	1.4	1.4	1.3	1.4	1.4	1.3
Glycerol	1.6	3.0	3.0	1.5	1.5	1.5	1.5	1.5	1.5	1.4
myo-Inositol	1.7	1.6	1.6	1.6	1.6	1.6	1.5	1.5	1.5	1.4
Erythritol	1.8	1.7	1.7	1.6	1.6	1.6	1.6	1.6	1.6	1.5
Xylitol	2.0	1.9	1.9	1.8	1.8	1.8	1.8	1.7	1.7	1.6
Arabitol	2.2	2.1	2.1	2.1	2.1	2.1	2.0	1.9	1.8	1.7
Galactitol	2.5	2.4	2.4	2.4	2.4	2.3	2.2	2.1	2.0	1.9
Sorbitol	2.5	2.1	2.6	2.5	2.5	2.4	2.3	2.2	2.0	1.9
Ribitol	2.5	2.5	2.5	2.4	2.4	2.4	2.3	2.2	2.0	1.9
Mannitol	3.0	3.0	3.0	2.9	2.9	2.8	2.7	2.4	2.2	2.2
Trehalose	3.5	3.4	3.4	3.3	3.3	3.2	3.0	2.7	2.4	2.2
Fucose	5.8	5.8	5.8	5.4	5.0	4.2	3.4	2.6	2.0	1.9
Maltitol	9.2	9.7	9.7	8.9	8.8	7.9	6.7	5.1	3.8	3.4
2-Deoxy-D-Glucose	11.1	11.2	10.9	9.9	9.2	7.2	5.5	3.8	2.7	2.6
Oxygen Dip	12.3	12.3	12.3	12.3	12.4	12.4	12.4	12.8	n/a	n/a
Octyl-Glucopyranose	13.2	13.2	11.6	11.5	11.2	10.9	10.0	8.0	7.1	NT
Arabinose	13.9	14.0	13.6	12.0	10.7	7.8	5.7	3.7	2.6	2.5
Rhamnose	14.5	14.4	13.7	11.6	9.7	6.6	4.6	3.0	2.2	2.1
Galactosamine	15.1	15.0	14.3	12.4	10.9	7.8	5.5	3.6	2.2	2.4
Mannosamine	17.3	17.1	16.2	13.8	11.7	8.0	5.5	3.5	2.5	2.3
Galactose	18.2	18.4	17.8	15.7	14.1	10.3	7.3	4.6	3.1	2.4
Glucosamine	20.3	20.1	18.9	16.0	13.5	8.9	6.0	3.8	2.6	2.4
Glucose	21.8	21.8	21.1	18.2	15.8	11.0	7.4	4.6	3.0	2.9
Turanose 1*	23.0	21.8	20.8	17.7	15.4	10.8	7.4	4.6	3.0	NT
Thioglucose	24.5	23.7	23.5	21.8	21.8	19.1	16.2	12.0	8.1	7.4
Lyxose	24.8	24.9	23.5	19.4	16.3	10.4	6.9	4.2	2.8	2.7
Sucrose	25.2	25.8	25.5	23.5	23.0	19.2	14.9	9.8	6.0	5.4
Xylose (D-)	26.2	26.5	25.0	21.0	17.7	12.0	7.9	4.8	3.1	2.9
Mannose	29.0	28.8	27.0	22.1	18.1	11.3	7.3	4.3	2.9	2.7
N-Acetyl-Glucosamine	31.5	31.0	28.5	22.5	17.6	10.6	6.8	4.1	2.7	2.6
Fructose (D-)	33.7	33.7	32.1	26.3	21.6	13.6	8.8	5.2	3.3	3.2
Sorbose	35.2	35.7	33.6	27.0	22.1	13.8	9.0	5.3	3.4	3.2
Ribose	38.0	36.1	35.1	30.3	24.0	15.2	9.5	5.6	3.5	3.3
Melibiose	44.5	45.3	42.4	34.4	28.5	18.5	11.7	6.6	3.9	3.7
Raffinose	61.5	62.1	60.6	53.5	51.8	42.9	30.9	18.6	9.9	8.5
Gentiobiose	104	102	96.7	77.9	65.9	42.3	25.9	13.5	6.8	6.3
Cellulose (-D)	122	119	113	89.5	74.9	46.8	28.2	14.1	7.0	6.5
Turanose 2*	134	197	195	NT	NT	47.9	27.5	13.6	6.9	6.2
Maltose	141	236	220	167	143	88.8	50.9	23.3	10.1	9.2
Palatinose	> 120	> 120	125	112	88.1	48.7	28.8	14.4	7.4	6.5
Maltotriose	> 240	> 240	> 240	> 240	> 240	> 240	> 240	96.1	31.1	27.1
Maltotetrose	> 240	> 240	> 240	> 240	> 240	> 240	> 210	> 180	99.4	NT
Maltohexose	> 240	> 210	> 210	> 210	> 210	> 210	> 210	> 210	> 210	NT

*Two turanose peaks were observed; NT=not tested, n/a=not available

4 Determination of Monosaccharides in Acid-Hydrolyzed Corn Stover Using HPAE-PAD

Figure 3 shows the separation of undiluted and non-pH-neutralized corn stover acid hydrolysate and carbohydrate standards at comparable concentrations. Alcohols, glycols, and aditols eluted early, followed by monosaccharides. Many disaccharides and oligosaccharides required higher eluent strength (e.g., 200 mM NaOH) to elute rapidly. The EG system can be assisted with manually prepared eluent, up to 200 mM NaOH, without replumbing. The use of a manually prepared hydroxide-assisted EG still provides many of the benefits of eluent generation while extending its useful range to higher eluent concentrations for periodic column wash, or for elution of more highly retained carbohydrates. Without the two Dionex high-carbohydrate concentration accessories (0.2 µL injection loop and 15 mil electrochemical detector gasket), the corn stover acid hydrolysate must be diluted (e.g., 400-fold) prior to injection (Figure 4).

Method Performance

Values for r^2 are presented in Table 6 using the speciation method. This table also presents the measured concentrations of arabinose, glucose, galactose, mannose, xylose, fructose, and cellobiose. An estimate for the total concentration in the corn stover hydrolysate was 190 mg/mL. The spike recovery of these sugars ranged from 73–102%.

Table 6. Speciation Method Linearity and Accuracy					
Saccharide	Calibration Range		Measured Concentration in Undiluted Corn Stover		
	(mg/mL)	r^2	Mean (mg/mL)	RSD (%)	% Spike Recovery
Arabinose	1.4–14	0.9982	14	0.6	77
Galactose	0.6–6.3	0.9996	5.4	0.9	92
Glucose	3.8–38	0.9906	33	0.5	73
Xylose	21–210	0.9951	132	0.5	89
Mannose	0.1–1.4	0.9956	0.7	0.9	102
Fructose	0.4–4	0.9993	4.5	0.9	87
Cellobiose	0.03–0.3	0.9937	1.0	1.1	75
Total			190		

Table 7 presents the monosaccharide retention time and peak area RSDs for replicate injections of undiluted corn stover hydrolysates using the speciation method on two separate days. Retention time RSDs ranged from 0.06–0.18%, and peak area RSD ranged from 0.6–9%.

Table 7. Speciation Method Precision of Replicate Injections of Undiluted and Non-pH-Neutralized Corn Stover Acid Hydrolysate								
Saccharide	Retention Time				Peak Area			
	Day 1 (n=12)		Day 2 (n=17)		Day 1 (n=12)		Day 2 (n=17)	
	Mean (min)	RSD (%)	Mean (min)	RSD (%)	Mean (nC*min)	RSD (%)	Mean (nC*min)	RSD (%)
Arabinose	13.2	0.13	13.1	0.10	30.7	2.4	33.9	1.2
Galactose	17.3	0.12	17.3	0.12	16.7	2.8	18.8	2.5
Glucose	20.4	0.13	20.4	0.11	74.7	1.7	79.5	1.2
Sucrose	ND*	ND	ND	ND	ND	ND	ND	ND
Xylose	24.2	0.14	24.0	0.12	159.4	1.7	173.4	0.6
Mannose	27.7	0.17	27.6	0.12	2.7	3.7	3.2	7.2
Fructose	32.1	0.18	32.1	0.13	9.0	3.3	10.1	2.6
Cellobiose	38.1	0.07	38.1	0.06	2.9	9.3	3.3	7.8

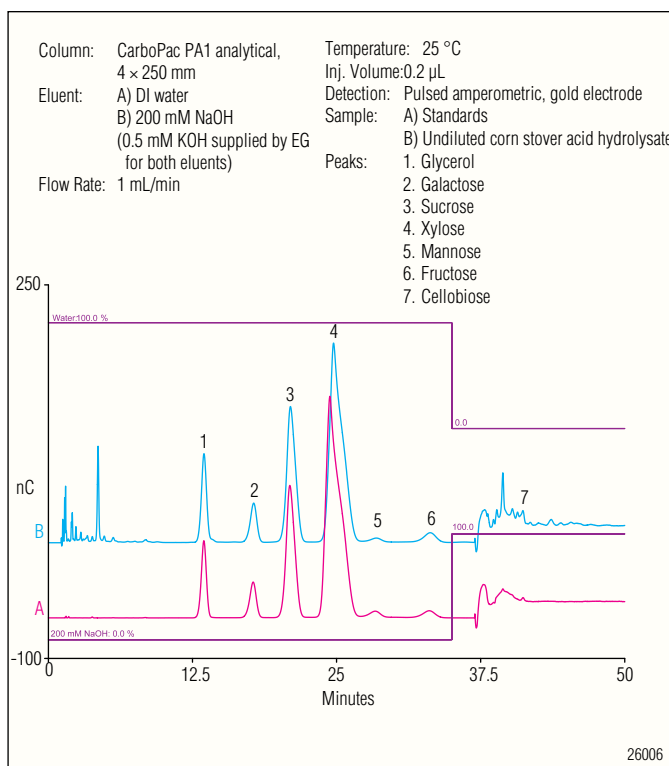


Figure 3. Carbohydrate standards (trace A) and undiluted corn stover acid hydrolysate (trace B) separated using 0.5 mM KOH with a step to 200 mM NaOH for rapid elution of cellobiose.

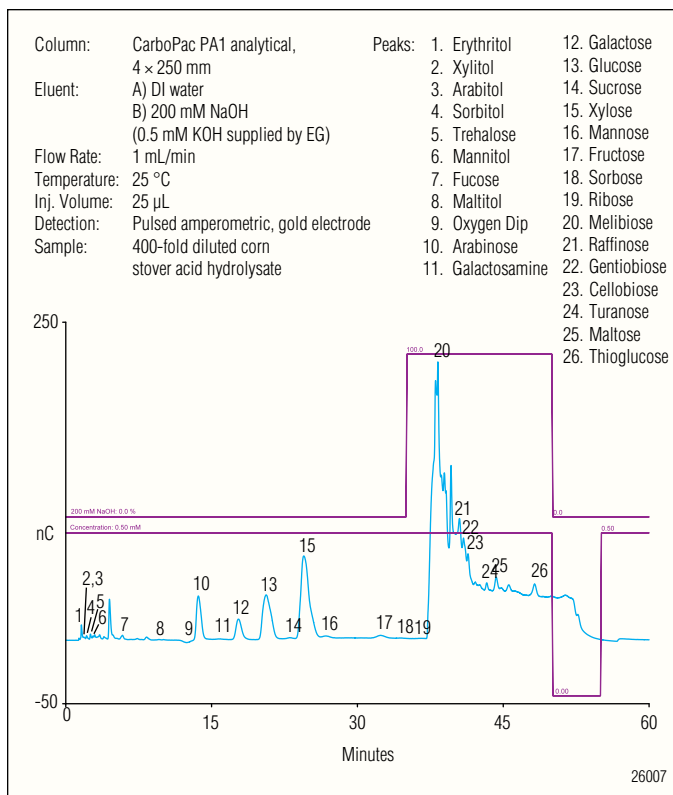


Figure 4. Separation of carbohydrates in acid-hydrolyzed corn stover diluted 400-fold in water prior to analysis using a 25 µL injection volume.

SUMMARY

- HPAE-PAD can be used for either a fast (10 min) estimation of total free monosaccharides, or a speciation method for determination of each type of carbohydrate in non-neutralized (acidic) and undiluted acid-hydrolyzed corn stover. This choice of methods allows the fermentation scientist to estimate product yield quickly, or measure carbohydrate composition for research or routine monitoring.
- Varying eluent hydroxide concentrations and column temperature enable the separation of difficult to resolve carbohydrates.
- EG enables reliable hydroxide eluent production without carbonate contamination, ensuring a more rugged application.
- HPAE-PAD, both with and without EG, is a powerful technique offering detector specificity and versatility in column selectivity for determination of carbohydrates in biocrop feedstock supporting the biofuels industry.
- For more details on the rapid method, see Dionex Application Note 225,¹ and for the speciation method, see Application Note 226.²

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