

Rapid and Sensitive Determination of Biofuel Sugars by Ion Chromatography

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Key Words

Corn Stover, Electrochemical Detection, High Concentration Samples, Disposable Electrodes, Sugars in Biomass, Dionex CarboPac SA10

Introduction

The uncertainty associated with the supply of petroleum and the general shift in public opinion toward environmentally friendly lifestyle choices have increased interest and research in feasible alternative fuels. Biofuels have emerged as an attractive alternative to fossil fuels; however, the development of robust analytical methods remains a challenge.

A common feedstock for biofuel alcohol production is corn stover. It consists of the leaves and stalks of maize plants left after harvesting. Corn stover processing involves dilute acid treatment followed by enzymatic reactions to convert the sugars to fuel alcohol. Acid hydrolysis releases the water-soluble mixture of carbohydrates, usually consisting of arabinose, glucose, galactose, mannose, xylose, fructose, and cellobiose. It is critical to determine carbohydrates during biofuel alcohol production to ensure process yield and product quality. The carbohydrates in biomass samples are often quantified by high-performance liquid chromatography (HPLC) using refractive index detection.¹

The refractive-index-based method is not specific, and could have interferences from other compounds in the complex biomass matrices. A Thermo Scientific Dionex CarboPac PA1 column can separate biomass carbohydrates in under 10 min, but only with group separation of mono-, di-, and trisaccharides.² To resolve individual carbohydrates requires separations lasting over an hour.³

Goal

The goal of this work was to develop a rapid (<8 min) and robust method with minimal sample pretreatment using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) to determine the sugars of interest in the highly concentrated samples encountered in biofuel processing.

Equipment

- Thermo Scientific Dionex ICS-3000 or Dionex ICS-5000 system including:
 - SP Single or DP Dual Pump, Gradient or Isocratic, with the Vacuum Degaser option
 - EG Eluent Generator Module (P/N 061714)
 - DC Detector/Chromatography Compartment
 - High-Pressure Injection Valve, 4-port (P/N 074699)
 - ED Electrochemical Detector (PN 072042)
 - Gold on PTFE Disposable Electrode (6 pack, P/N 066480)
 - pH, Ag/AgCl Reference Electrode (P/N 061879)
 - PTFE gasket, 15 mil (P/N 057364)
 - AS-AP Autosampler
- Thermo Scientific Dionex Chromeleon Chromatography Data System (CDS) software
- EO Eluent Organizer with two 2 L Plastic Bottles (P/N 072057)
- Vial Kit, 0.3 mL Polypropylene with Caps and Septa, 100 Each (P/N 055428)
- Thermo Scientific Nalgene 250 mL HDPE Narrow-Mouth Bottle (P/N 2002-0008)

- Nalgene™ 1000 mL HDPE Narrow-Mouth Bottle (P/N 2002-0032)
- Nalgene 250 mL 0.2 µm nylon filter units (P/N 153-0020)
- Nalgene 1000 mL 0.2 µm nylon filter units (P/N 154-0020)

Reagents and Standards

Reagents

Deionized water, Type I reagent grade, 18 MΩ-cm resistivity or better filtered through a 0.2 µm filter immediately before use

Standards

- Fucose (Sigma P/N F2252)
- D(+)-Galactose (Sigma-Aldrich P/N G-0625)
- D(+)-Mannose (Sigma-Aldrich P/N M-6020)
- D(-)-Fructose (J.T. Baker P/N M556-05)
- D-Xylose (Sigma-Aldrich P/N X107-5)
- Sucrose (Sigma P/N S-9378)
- D-Glucose, monohydrate (J.T. Baker P/N 1910-01)
- D(-)-Arabinose (Sigma-Aldrich P/N A-3131)

Conditions

Method

Columns:	Dionex CarboPac™ SA10 Analytical, 4 × 250 mm (P/N 074641)
	Dionex CarboPac SA10 Guard, 4 × 50 mm (P/N 074902)
Eluent:	1 mM KOH
Eluent Source:	Dionex EGC II KOH with Dionex CR-ATC
Flow Rate:	1.5 mL/min
Inj. Volume:	0.4 µL (full loop)
Temperature:	45 °C
Cell Temp.:	30 °C
Backpressure:	2500 psi
Detection:	PAD
Background:	30–70 nC
Working Electrode:	Gold Carbohydrate Disposable
Reference Electrode:	
Mode:	Ag/AgCl mode
Noise:	30–60 pC

Carbohydrate Waveform

Time (s)	Potential (V)	Integration
0.00	+0.1	
0.20	+0.1	Begin
0.40	+0.1	End
0.41	-2.0	
0.42	-2.0	
0.43	+0.6	
0.44	-0.1	
0.50	-0.1	

Reference electrode in Ag/AgCl mode

Preparation of Solutions and Reagents

Eluent Solution

1 mM Potassium Hydroxide

Generate the potassium hydroxide (KOH) eluent on line by pumping high-quality degassed, deionized (DI) water through the Dionex EGC II KOH cartridge. Chromeleon™ software tracks the amount of KOH used and calculates the remaining cartridge lifetime. Although eluents can be prepared manually, the use of eluent generation is strongly recommended because it ensures the high-purity hydroxide eluent and accurate concentrations required for this application.

Consistent preparation of a 1 mM hydroxide eluent or a 10 mM hydroxide eluent (if proportioning is used) is difficult due to variable carbonate contamination. The impact of carbonate contamination is significant when using low concentration hydroxide eluents. If eluents are prepared manually (not recommended), use NaOH instead of KOH and prepare according to the general instructions for hydroxide eluents in Dionex (now part of Thermo Scientific) Technical Note 71.⁴ For this application, electrolytic eluent generation delivers superior performance.

Stock Standard Solutions

Dissolve solid standards in DI water to prepare a 200 mg/mL stock solution for each carbohydrate (Table 1). Maintain the stock solution at -20 °C until needed.

Working Standard Solutions

Prepare working standards in DI water by diluting the stock solutions. Store working standards at 4 °C. Make all dilutions gravimetrically to ensure high accuracy.

Sample Preparation

Corn Stover and Wood Hydrolysates

Centrifuge corn stover and wood hydrolysate samples at 16,000 g for 10 min to ensure elimination of particulates, and inject at dilutions of 1/100, 1/150, and 1/20 with DI water for analysis. Acid-hydrolyzed corn stover was donated by the National Renewable Energy Laboratory in Boulder, Colorado.

Precautions

Treated biomass samples have high concentrations of sugars such as xylose, glucose, and galactose, so carry-over may be observed. A syringe flush of 500 µL DI water reduces the carryover and is recommended between samples. A column wash at 100 mM KOH for 20 min is recommended if retention time shifting is observed. The application of 100 mM KOH changes the system equilibrium; re-equilibration at 1 mM for 30 min is recommended to achieve high retention time precision.⁵ Replace the reference electrode every six months and the disposable working electrode every four weeks.

Analyte	Range (mg/mL)	Coeff. of Determin. (r ²)	Concentration Used for Precision Injections (mg/mL)	RT (min)	Retention Time Precision (RSD) ^a	Peak Area (nC*min)	Peak Area Precision (RSD) ^a
Fucose	0.4–2	0.9918	0.5	2.8	<0.01	3.53	2.42
Sucrose	0.4–2	0.9884	0.6	3.5	0.12	2.85	2.32
Arabinose	0.4–2	0.9937	0.5	3.9	0.11	3.49	2.21
Galactose	0.4–2	0.9887	0.5	4.1	0.08	5.03	2.65
Glucose	0.4–2	0.9894	0.5	4.5	<0.01	5.40	2.38
Xylose	0.4–2	0.9923	0.5	5.1	0.08	4.78	2.31
Mannose	0.4–2	0.9916	0.5	5.3	<0.01	3.71	2.26
Fructose	0.4–2	0.9981	0.5	5.7	0.06	2.46	1.67

^aRelative standard deviation, n = 6

Results and Discussion

Figure 1A shows the separation of the sugars in a standard mix; fucose, sucrose, arabinose, galactose, glucose, xylose, mannose, and fructose are easily resolved in 8 min. The resolution of these sugars in a short run time makes this method suitable for on-line monitoring of biofuel samples.

Figure 1B shows the sugars in acid-hydrolyzed corn stover (sample dilution 100-fold). The major sugars present in this acid-hydrolyzed corn stover sample are arabinose, galactose, glucose, and xylose. The high concentration of xylose indicates that the corn stover sample is rich in hemicellulose. This method can be used for measuring xylose in biomass samples, which is typically used for monitoring efficiency of biomass pretreatment and fermentation processes.

Linear Range

Biomass samples typically have a sugar concentration in the range of 100–200 mg/mL, and sugars are typically analyzed after a 100- or 150-fold dilution. The linearity of the method was determined by injecting calibration standards in triplicate covering the expected range of the sugars of interest in the samples (0.4–2 mg/mL) shown in Table 1. The coefficients of determination obtained from the calibration curves were between 0.9984–0.9989, using least squares regression. For analyzing samples with lower sugar concentrations or for sugars that are present at low concentrations, an appropriate calibration range must be selected.

Precision

Peak area and retention time (RT) precisions were determined for six replicate injections of a mixture of sugar standards. The concentration used for precision injections was 0.5 mg/mL for each of the biofuel sugars (Table 1). The RT precisions ranged from <0.01–0.12%. The peak area precisions were in the range 1.7–2.7%. The high RT precisions are attributed to consistent generation of high-purity KOH using the eluent generator.

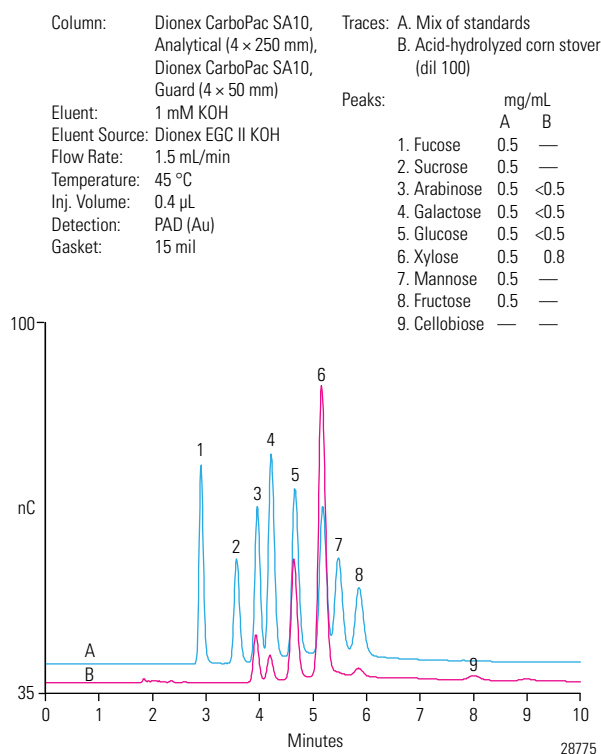


Figure 1. Separation of biofuel sugars (A) and acid-hydrolyzed (diluted 100-fold) corn stover sample (B) on the Dionex CarboPac SA10 column

Intraday (three injections) and between-day precisions of biofuel sugars in acid-hydrolyzed corn stover were evaluated over three days. The RT and peak area precisions are summarized in Table 2. The between-day RT precisions ranged from 0.9–1.5% and peak area precisions were 6–8%. The intraday RT precisions were in the range 0.09–0.28%, and peak area precisions were in the range 0.4–5.0% (data not shown). These precisions demonstrate that this method can be used for complex biomass matrices such as acid-hydrolyzed corn stover.

Accuracy

Method accuracy was evaluated by measuring recoveries in spiked corn stover samples (Table 3). Samples were spiked with analytes at a level that was 50–100% of the amount determined in the original sample. Recoveries were calculated from the difference in response between the spiked and unspiked samples. Intraday concentration precision for corn stover was 3%. The average recovery for the sugars ranged from 69–112%. The between-day recovery precision for the eight biofuel sugars in the spiked samples ranged from 1–10% over three days.

Injection Loop (2.5 μ L) and Postcolumn Addition of Base

The method described here can also be used with the 2.5 μ L injection loop with a 15 mil gasket. The linear range of detection for the biofuel sugars for a 2.5 μ L injection is 0.001–0.1 mg/mL (coefficients of determination ranging from 0.9853–0.9990), and samples must be diluted accordingly to fall within the calibrated range.

To achieve a wider linear range, this method can also be used with postcolumn addition of more concentrated hydroxide (100/200 mM) to the eluent stream.

Postcolumn addition can be made through a mixing tee using the second pump of the DP.6

The linear range of detection for the biofuel sugars with postcolumn addition of base (with a 2.5 μ L injection and 15 mil gasket) is 0.05–1.0 mg/mL, with coefficients of determination ranging from 0.9972–0.9999. Figure 2 shows a chromatogram of the mix of carbohydrate standards with postcolumn addition of base (200 mM hydroxide). Note that this configuration requires additional hardware (i.e., a mixing tee and reaction coil) and reagent (postcolumn base).

Table 2. Between-day (n = 3 days) RT and peak area precisions (triplicate injections of corn stover acid hydrolysate^a)

Analyte	RT (min)	RT Precision (RSD)	Peak Area (nC*min)	Peak Area Precision (RSD)
Arabinose	3.91	1.23	0.54	7.95
Galactose	4.17	1.34	0.38	7.60
Glucose	4.62	1.36	1.84	7.99
Xylose	5.14	1.41	4.88	6.78

^aDilution factor 150

Table 3. Biofuel sugar recoveries in corn stover acid hydrolysate (n = 3 days)

Analyte	Amount Added (mg/mL)	Amount Detected (mg/mL)	Recovery (%)	Recovery (RSD)
Fucose	1.01	0.81	80.5	6.1
Sucrose	0.87	0.61	69.6	2.3
Arabinose	0.90	0.87	96.7	1.5
Galactose	1.01	0.98	98.0	10.1
Glucose	1.00	0.98	99.2	6.6
Xylose	0.59	1.33	112.7	5.2
Mannose	0.99	0.86	87.3	3.2
Fructose	1.02	1.08	108.5	1.0

Column: Dionex CarboPac SA10, Analytical (4 \times 250 mm), Dionex CarboPac SA10, Guard (4 \times 50 mm)
 Eluent: 1 mM KOH
 Eluent Source: Dionex EGC II KOH
 Flow Rate: 1.5 mL/min
 Temperature: 45 $^{\circ}$ C
 Inj. Volume: 2.5 μ L
 Detection: PAD (Au)
 Gasket: 15 mil
 Postcolumn Base: 200 mM Sodium hydroxide
 Postcolumn Flow Rate: 0.4 mL/min

Peaks: 1. Fucose 0.5 mg/mL
 2. Sucrose 1.0
 3. Arabinose 1.0
 4. Galactose 1.0
 5. Glucose 1.0
 6. Xylose 1.0
 7. Mannose 1.0
 8. Fructose 1.0

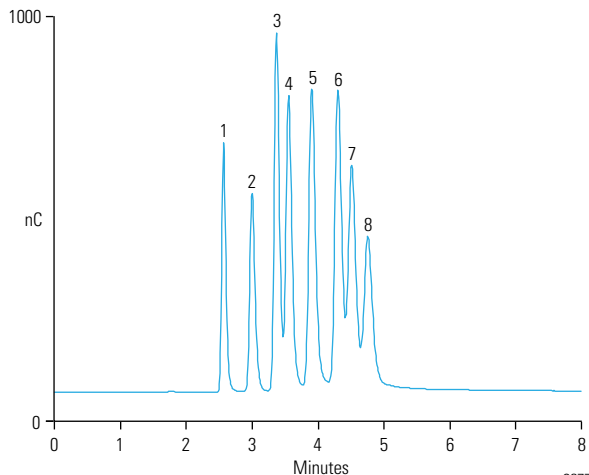


Figure 2. Separation of biofuel sugars on the Dionex CarboPac SA10 column; postcolumn base: 200 mM NaOH

Conclusion

This work describes a HPAE-PAD method for the accurate determination of common sugars in biomass such as acid-hydrolyzed corn stover. The method uses the Dionex CarboPac SA10 column with electrolytically generated hydroxide eluent, enabling fast analysis of the biofuel sugars. The method is shown to have a linear range suited for handling high concentration samples with minimal sample treatment, high precisions, and acceptable recoveries. The disposable gold working electrode provides consistently high detector response, assuring greater instrument-to-instrument and lab-to-lab reproducibility. This configuration needs only the addition of DI water for continuous operation. In summary, the described HPAE-PAD method using the Dionex CarboPac SA10 column is accurate and reliable, and can be adopted for on-line monitoring of sugar levels in biomass applications.

Suppliers

VWR, 1310 Goshen Parkway, West Chester, PA 19380, U.S.A., Tel: 800-932-5000.

Sigma-Aldrich Chemical Co., P.O. Box 2060, Milwaukee, WI 53201, U.S.A., Tel: 800-558-9160.

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