APPLICATION NOTE

Improved Method for Determination of Biofuel Sugars by HPAE-PAD

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Keywords
Lignocellulosic biomass, Dionex Integrion HPIC system, Fermentable sugars, Biomass hydrolysis, Electrochemical detection, Biomass-to-biofuel conversion

Goal

To develop a fast ion chromatography (IC) method for the determination of sugars in biofuel samples using a Thermo Scientific™ Dionex™ CarboPac™ SA10-4µm column with electrolytically generated eluent and a high pressure IC system with electrochemical detection.

Introduction

Significant reduction in the supplies of underground fossil fuels and increased production of greenhouse gasses has led to a worldwide focus on alternative paths for energy generation from renewable sources, such as lignocellulosic biomass[1-2]. The breakdown of cellulose and hemicellulose contained in lignocellulosic biomass feedstocks produces five- and six-carbon sugars that can be fermented into biofuels such as ethanol[3] and biodiesel[4].

The efficiency of biomass hydrolysis into fermentable sugars is used to assess the overall efficiency of biomass-to-biofuel conversion, and is directly related to target biofuel yield and process economics.[5] The determination of carbohydrates in hydrolysates derived from lignocellulosic biomass is a crucial step in biofuel production. However, development of robust analytical methods remains a challenge. Current methods suffer from several drawbacks, such as low throughput, poor analyte resolution, and lack of applicability to a wider set of biomass samples[6].

High performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) can be used to determine sugars from biomass hydrolysate samples. HPAE-PAD has been shown to deliver fast determinations of carbohydrates in biomass hydrolysate samples using the Thermo Scientific™ Dionex™ CarboPac™ SA10-4µm column.[7] Determination of carbohydrates in acid-hydrolyzed corn stover samples at 10-fold dilution was shown in Thermo Scientific Application Note 1089 (AN1089).[8] The method uses electrolytically generated hydroxide eluent and a 62 mil gasket in the electrochemical flow cell to resolve and detect the eight

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common lignocellulosic biomass derived fermentable sugars, including xylose, sucrose, arabinose, galactose, glucose, mannose, fructose, and cellobiose.

The current work updates the system used for the carbohydrate analysis in AN1089.[8] The new system combines flexibility and ease-of-use with high sensitivity and selectivity, bringing a higher level of convenience and cost effectiveness to simple sugar analysis. The improved Thermo Scientific™ Dionex™ IC PEEK Viper™ fitting reduces the dead volume in the flow path and enhances chromatographic performance. The method proposed here separates eight common carbohydrate sugars in less than eight minutes. The shorter run time allows for shorter sample turnaround times and reduced eluent consumption, thereby improving the overall process economics. Using this method, carbohydrates present in 10 individual biofuel samples were quantified.

Conditions

Column:	Thermo Scientific Dionex CarboPac SA10-4µm, 4 × 250 mm (P/N 088233), CarboPac SA10-4µm Guard 4 × 50 mm (P/N 088234)
Column Temp:	45 °C
Compartment Temp:	30 °C
Flow Rate:	1.5 mL/min
Eluent:	1 mM KOH
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge (P/N 075778) with Dionex CR ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
Electrochemical Cell Gasket:	62 mil
Reference Electrode:	pH-Ag/AgCl
Sampler Tray Temp:	Ambient
Injection Volume:	0.4 µL (Push_Full mode)

Samples

The biomass hydrolysate samples tested in this study were derived from corn stover, switchgrass, and/or energy cane. These biomass samples were pretreated either individually or as mixtures (mixed feedstocks) with any one of the reagents: acid, alkali, or ionic liquids. The pretreatments were followed by enzymatic hydrolysis with Cellic® Ctec2 and Cellic Htec2 (Novozymes, CA) to yield near-complete hydrolysis of biomass oligosaccharides.

All biomass hydrolysate samples were diluted 10-fold with DI water before use.

Note: Lignocellulosic-biomass-derived samples have complex matrices. Highly retained contaminants in these samples will occupy a portion of the anion exchange sites, limiting the number of sites available for retention of analyte anions, especially after continued use. This may result in loss of column capacity and shortened retention times. If such column contamination is suspected then the Dionex CarboPac SA10-4µm column can be readily cleaned using 100 mM KOH or NaOH. Refer to Appendix A "CarboPac SA10-4µm Column Care" in the column manual (Document# 065579-02) for column washing procedure and additional information on column care.

Carbohydrate Waveform

Time (s)	Potential (V)	Integration
0.00	+0.10	
0.20	+0.10	Begin
0.40	+0.10	End
0.41	-2.0	
0.42	-2.0	
0.43	+0.6	
0.44	-0.1	
0.50	-0.1	

Equipment

- A Thermo Scientific™ Dionex™ Integrion™ HPIC™ system was used in this work. The Dionex Integrion HPIC system is an integrated ion chromatograph that includes:
  - Dionex Integrion HPIC system pump
  - Detector compartment temperature control
  - Electrochemical detector (P/N 22153-62035) and cell (P/N 072044)
  - pH-Ag/AgCl reference electrode (P/N 061879)
  - Carbohydrate disposable Au working Electrode, pack of 6 (two 2.0 mil gaskets included) (P/N 066480)
  - AS-AP autosampler (P/N 074925) with cooling tray option (recommended) and 1.5/0.3 mL vial tray (P/N 074936)
- Sterile assembled microcentrifuge tubes with screw cap, 1.5 mL (Sarstedt P/N 72.692.005)
- Nalgene Rapid-Flow 0.2 µm filter units, 1000 mL, nylon membrane, 90 mm diameter (Thermo Scientific P/N 164-0020)
- Consumables
  - Part numbers are shown in Table 1

Table 1. Consumables for the Dionex Integrion HPIC system.

Product name	Description of High Pressure Device	Part Number
Thermo Scientific™ Dionex™ IC PEEK Viper™ fitting tubing assembly kits	Dionex IC PEEK Viper fitting tubing assembly kit for the Dionex Integrion HPIC system: Includes one each of P/Ns: 088815-088821	088798
Dionex IC PEEK Viper fitting tubing assemblies	Guard to separator column: 0.007 × 4.0 in (102 mm)	088815
	Valve to guard column: 0.007 × 5.5 in (140 mm)	088816
	EGC Out to CR-TC Eluent In: 0.007 × 6.5 in (165 mm)	088817
	Separator to ED Cell In: 0.007 × 7.0 in (178 mm), ED	088819
	CR-TC Out to Degasser In: 0.007 × 9.5 in (241 mm)	088821
Thermo Scientific™ Dionex™ AS-AP Autosampler vials	Package of 100, polystyrene vials, caps, septa, 0.3 mL	055428
4-Port Injection Valve pod	Install in place of 6-port valve pod. The 4-port pod has an internal sample loop of 0.4 µL.	074699
Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator cartridge	Eluent generator cartridge when using 4 µm particle columns	075778
Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column	Continuously regenerated trap column used with Dionex EGC KOH 500 cartridge	088662
Dionex HP EG Degasser	Degasser module	075522
Electrochemical Detector (ED)	Without cell, with shipping container	22153-62035
Electrochemical cell	Includes knob and, support block	072044
pH-Ag/AgCl reference electrode	Reference electrode	061879
Au on PTFE electrodes	Working electrode, package of six	066480
High concentration carbohydrate analysis kit	Includes 62 mil gasket and modified spacer block	085324
62 mil gasket	If purchased separately, package of two	075499
pH Buffer, pH 7	Reference electrode pH calibration standard	SB115-500*
pH Buffer, pH 10	Reference electrode pH calibration standard	SB107-500*

* Fisher Scientific P/N

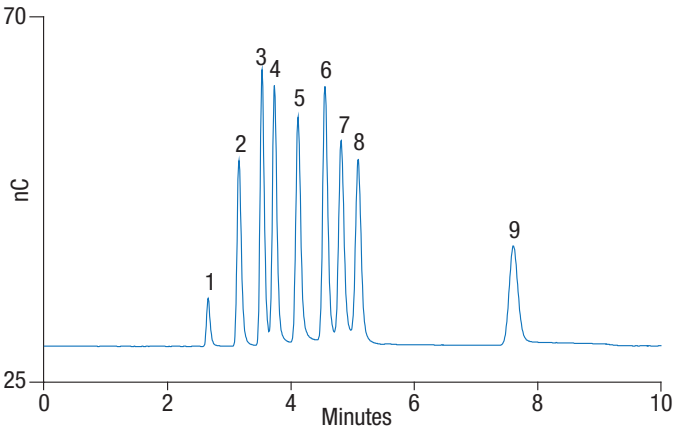
Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better
- L(–)-Fucose (Sigma, P/N F2543)
- D-Galactose (Sigma, P/N G0625)
- D(+)-Mannose (Sigma P/N M6020)
- D-Fructose (Sigma P/N F25X43)
- D-Xylose (Aldrich P/N X-10705)
- Sucrose (Sigma P/N 84097)
- D-Glucose (P/N 1910-01)
- D(-)-Arabinose (Sigma P/N A3131)
- D(+)-Cellobiose (Sigma P/N C7252)

Results

Separation

Figure 1 shows a representative chromatogram obtained using a standard mix containing eight common biofuel sugars at 0.6 g/L each, analyzed on Dionex CarboPac SA10-4µm column. Fucose was used as an internal standard. All of the peaks were well resolved and the separation was completed within 8 min.



Column:	Dionex CarboPac SA10-4µm Analytical, 4 × 250 mm	Peaks:	1. Fucose
Eluent:	1 mM KOH		2. Sucrose
Flow Rate:	1.5 mL/min		3. Arabinose
Inj. Volume:	0.4 µL		4. Galactose
Temp:	45 °C		5. Glucose
Detection:	PAD (Au)		6. Xylose
Gasket:	62 mil		7. Mannose
			8. Fructose
			9. Cellobiose

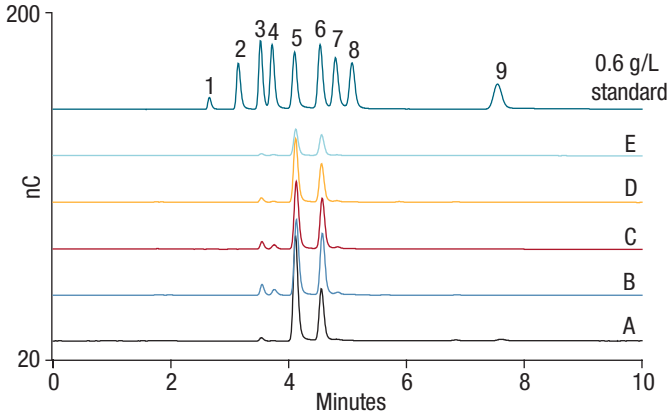
Figure 1. Separation of biofuel sugars on the Dionex CarboPac SA10-4µm column using proposed method (0.6 g/L standard).

Linearity and Precision

Using this method, calibration curves were generated for the eight biofuel sugars. The results are included in Table 2. The method is linear for more than two orders of magnitude from 0.005 to 2 g/L for all eight carbohydrates. This wide dynamic range is an important feature required of any method designed to measure sugars present in biomass hydrolysates, as it is ideal for determination of the sugars that are typically present in divergent ratios. The retention time and peak area relative standard deviations (RSD) are less than 1.26% across all sugars, indicating excellent method precision.

Accuracy

To determine method accuracy, 10 field biomass hydrolysate samples were procured. The samples were collected at various stages of biomass processing and conversion of lignocellulosic biomass to ethanol. Method accuracy was evaluated by first quantifying the sugars present in the biomass hydrolysate samples. From this set, five samples were randomly selected for recovery studies. Representative chromatograms for the selected five biofuel samples are shown in Figure 2. The major sugars present in these samples are glucose and xylose.



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Detection:	PAD (Au)		6. Xylose
Gasket:	62 mil		7. Mannose
			8. Fructose
			9. Cellobiose

Figure 2. Representative chromatograms of five lignocellulosic biomass based biofuel samples A-E.

All of the biofuel sugars that are present above the lowest calibration standard were spiked with 50–150% of the original amount found in the biofuel sample. The original amounts present, spiked amounts, and percent spike recoveries are included in Table 2. For all four samples studied here, excellent recoveries were obtained for all spiked biomass sugars.

Robustness

Due to the complex matrices that are characteristic of biomass samples, it is critical to ensure minimal matrix effect on retention time and peak area stability during a long sequence run. The method robustness was studied by injecting the standard sugar mix at frequent intervals during a 200 injection sequence run of the biofuel samples. The results included in Tables 3 and 4 show excellent retention time and peak area reproducibility respectively across the four injections of standard sugar mix located at different positions in the sequence.

Table 2. Calibration (at 11 levels between 0.006 to 2 g/L of each of the biofuel sugars) and precision data (0.6 g/L, n=7) for eight common biofuel sugars.

Parameter	Sucrose	Arabinose	Galactose	Glucose	Xylose	Mannose	Fructose	Cellobiose
Coe. Of Determination	0.996	0.995	0.999	0.993	0.992	0.992	0.997	0.999
RT RSD (%)	0.12	0.08	0.08	0.10	0.06	0.10	0.05	0.04
Area RSD (%)	0.28	0.47	0.51	0.62	0.66	0.69	1.26	0.28

Table 3. Biofuel sugar recovery studies (n=7).

Sample Name	Sugar	Avg. Amount (g/L)	Spike (g/L)	Recovery (%)	RSD (%)
A	Arabinose	0.02	0.02	100	0.1
	Glucose	1.17	0.8	106	0.1
	Xylose	0.5	0.5	114	0.0
	Cellobiose	0.03	0.04	98.7	0.0
B	Arabinose	0.01	0.10	94.1	0.0
	Galactose	0.07	0.05	97.6	0.0
	Glucose	1.07	0.8	89.4	0.0
	Xylose	0.8	0.5	95.6	0.0
C	Arabinose	0.09	0.10	91.2	0.0
	Galactose	0.05	0.05	129	0.1
	Glucose	1.03	0.8	93.8	0.0
	Xylose	0.7	0.5	104	0.0
D	Arabinose	0.04	0.02	88.3	0.3
	Galactose	0.00	0.01	117	0.1
	Glucose	0.72	0.8	97.7	0.0
	Xylose	0.4	0.5	111	0.0
E	Arabinose	0.03	0.02	95.3	0.1
	Galactose	0.01	0.02	99.1	0.0
	Glucose	0.55	0.8	100	0.0
	Xylose	0.4	0.5	108	0.0

Table 4. Retention time robustness.

Inj. No.	Retention Time (min)								
	Fucose	Sucrose	Arabinose	Galactose	Glucose	Xylose	Mannose	Fructose	Cellobiose
1	2.66	3.16	3.53	3.74	4.13	4.56	4.83	5.10	7.63
75	2.68	3.18	3.55	3.75	4.13	4.58	4.83	5.12	7.63
100	2.66	3.16	3.53	3.73	4.12	4.56	4.82	5.10	7.60
200	2.66	3.15	3.53	3.73	4.10	4.53	4.79	5.08	7.55

Table 5. Peak area robustness.

Inj. No.	Area (nC*min)								
	Fucose	Sucrose	Arabinose	Galactose	Glucose	Xylose	Mannose	Fructose	Cellobiose
1	0.46	2.09	2.91	3.08	3.10	3.48	2.99	2.78	2.20
75	0.44	2.03	2.85	3.03	3.01	3.35	2.88	2.74	2.16
100	0.44	2.05	2.88	3.05	3.04	3.37	2.93	2.75	2.20
200	0.44	2.03	2.84	3.03	3.02	3.33	2.91	2.72	2.21

Conclusion

An improved method for quantification of biomass sugars that can be converted to biofuels is presented here. The method is able to separate all eight key biomass sugars within 8 min. The response data for all biomass sugars studied here showed excellent correlation with concentration. The method provides excellent peak area reproducibility, as well as retention time precision within the concentration levels studied. Spike recovery studies performed to demonstrate method accuracy showed good recovery from all of the spiked samples, suggesting that the method is accurate. Finally, the method is robust with retention time and peak area remaining stable when subjected to over 200 biofuel sample injections. In summary, the method proposed here is a convenient, precise, and robust way to quantify biomass sugars in complex hydrolysate samples and will improve the reliability of determining biomass-to-biofuel efficiency calculations.

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