

Methods for Determining Sugars and Hydroxymethylfurfural in Biomass

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ABSTRACT

Common biomass sugars and hydroxymethylfurfural (HMF) are routinely analyzed during biofuel processing. Sugars are monitored for optimizing processes to maximize the yield of ethanol. HMF, a byproduct of processing, is quantified because it can have an inhibitory effect on ethanol yield. Additionally, there is a growing interest in HMF as a platform compound that can be used to synthesize several compounds (solvents, fuels, etc.) currently derived from crude oil.

This presentation describes high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) methods for the determination of (i) sugars and (ii) HMF in acid-hydrolyzed biomass using electrolytically generated hydroxide eluent, separation on strong anion-exchange columns, and electrochemical detection with disposable gold working electrodes.

Biomass samples often have a sugar concentration over 100 g/L, and the common sugars are fucose, sucrose, arabinose, galactose, glucose, xylose, mannose, and fructose. The method for sugar analysis described here has a short run time (8 min) with resolution of all the sugars, linear range of 0.5–2 g/L, retention time (RT) precisions of <0.01–0.12%, peak area precisions of 1.7–2.7%, and acceptable recoveries (70–112%). This method is capable of handling the high concentration biomass samples with minimal sample preparation (i.e., the strong acid hydrolysate just needs to be diluted, and the column is not affected by the amount of sulfate in the diluted sample).

The proposed method for HMF has a broad linear range (0.1–1000 µg/mL), low detection limit (0.04 µg/mL), high precision, and good recovery (112%). These methods require no eluent preparation, and due to short analysis time, good sensitivity, and consistent response, can be used for routine sugar and HMF analysis.

INTRODUCTION

Biomass Sugars

The uncertainty associated with the supply of petroleum and the general shifts in public opinion toward environmentally-friendly lifestyle choices have increased interest in and research on feasible alternative fuels. Biofuels have emerged as an attractive alternative to fossil fuel. Biofuels have significantly less carbon output and contain fewer toxins, making them a safer and cleaner alternative. Currently, the largest producers of biofuel are Brazil, USA, France, and Germany.

A common feedstock for bioethanol production is corn stover, which 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 ethanol. It is critical to analyze and quantitate the sugars during bioethanol production. Often the sugars in biomass samples are quantified by HPLC using refractive index detection.¹

In the HPAE-PAD method, carbohydrates are detected by measuring the electrical current generated by their oxidation at the surface of a gold electrode. The products of this oxidation reaction also poison the surface of the electrode and must be cleaned between measurements. This occurs automatically, twice a second, during the analysis.

HPAE-PAD is extremely selective and specific for carbohydrates because pulsed amperometry detects only those compounds that contain functional groups that are oxidizable at the detection voltage employed.

The proposed HPAE-PAD method using a CarboPac® SA10 column has a short run time of 8 min and resolves all of the eight common biofuel sugars.² In comparison, other columns (e.g., CarboPac PA1)³ can also elute biomass carbohydrates rapidly (in less than 10 min), resolving monosaccharides from disaccharides and trisaccharides. However, the analysis time for resolving the individual monosaccharides is typically long (>2 h).

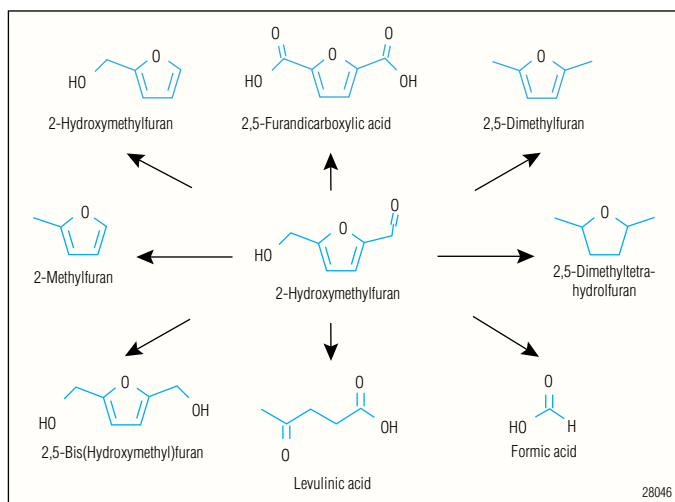


Figure 1. HMF as precursor for a range of commercial chemicals.

The CarboPac SA10 column is composed of a wide-pore macroporous substrate coated with a strong anion-exchange layer of latex nanobeads.² The highly porous structure results in increased surface area, providing higher loading capacity. The nanobead anion-exchange layer that coats the entire surface is functionalized with an alkanol quaternary ammonium group. This layer has a controlled thickness, which results in excellent mass-transfer characteristics and, consequently, high-efficiency peaks. The combination of the high capacity provided by the substrate and the new internal chemistry of the nanobead functionality delivers high resolution and short analysis time for the common sugars of interest in biofuel applications.

Hydroxymethylfurfural

The interest in HMF analysis is two-fold. Although HMF is a biomass platform chemical and can be used to synthesize a number of compounds that are currently derived from crude oil (Figure 1),^{4,5} it is also known to inhibit fermentation processes during bioethanol production.⁶ Thus, it is important to measure HMF in matrices such as treated cellulosic biomass.

Spectrophotometric and HPLC methods are available for HMF determination. One commonly used method is based on spectral absorbance at 284 nm.^{7,8} This direct absorbance measurement could have interferences from other compounds present in the complex matrices. In the HPLC method, HMF is separated on a reversed-phase column with water and methanol as the mobile phase, and then detected by UV absorbance.⁹

This work describes an HPAE-PAD-based method for the determination of HMF in acid-treated biomass (corn stover, wood hydrolysate).¹⁰ The CarboPac PA1 column used for this method is a high-capacity rugged column suitable for determining monosaccharide and disaccharides, and has a high resolution for HMF in a wide variety of matrices.

EXPERIMENTAL DETAILS

Equipment

Dionex ICS-3000 or ICS-5000 Ion Chromatography System including:

- DP Dual Pump with the vacuum degas option installed
- DC Detector/Chromatography Module
- Electrochemical Detector
- Carbohydrate PTFE Disposable Au Working Electrodes
- Ag/AgCl Reference Electrode
- PTFE Gasket: 15 mil (for sugar analysis), 3 mil (for HMF analysis)
- Autosampler
- Chromeleon® Chromatography Data System Software

Conditions

Biomass Sugars

Columns:	CarboPac SA10 Analytical (4 × 250 mm) and Guard (4 × 50 mm)
Flow Rate:	1.5 mL/min
Injection Volume:	0.4 µL (full loop)
Column Temperature:	45 °C
Detector Temperature:	30 °C
Back Pressure:	2500 psi
Eluent:	1 mM KOH
Eluent Source:	EGC II KOH with Continuously-Regenerated Anion Trap Column (CR-ATC)

HMF

Columns:	CarboPac PA1 Analytical (4 × 250 mm) Guard (4 × 50 mm)
Flow Rate:	1.0 mL/min
Injection Volume:	10 µL (full loop)
Temperature:	30 °C
Backpressure:	2400 psi
Eluent:	50 mM KOH
Eluent Source:	EGC II KOH with CR-ATC

Both Methods

Detection:	Pulsed Amperometry
Background:	30–70 nC
Working Electrode:	Carbohydrate PTFE Disposable Au Working Electrodes
Reference Electrode Mode:	Ag/AgCl mode
Noise:	30–40 pC

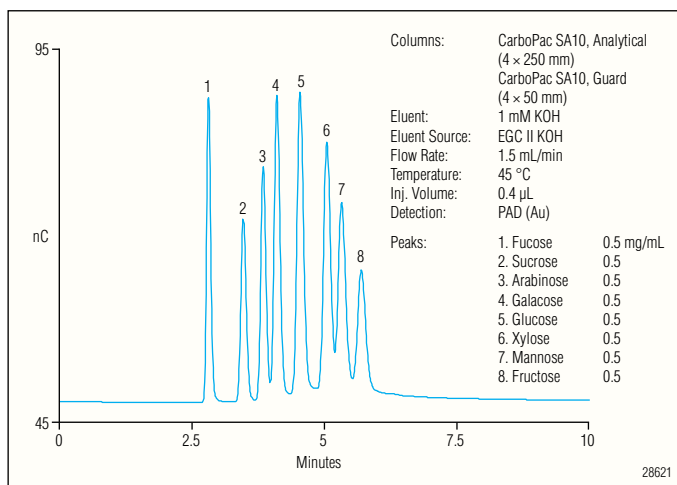


Figure 2. Separation of biofuel sugars on the CarboPac SA10 column.

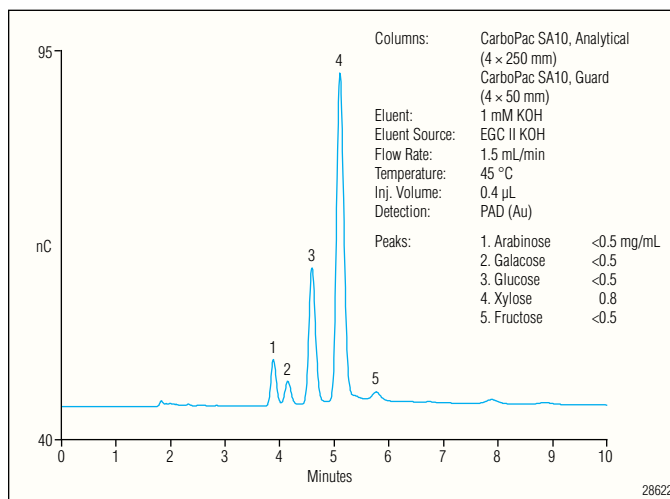


Figure 3. Separation of sugars in an acid-hydrolyzed corn stover sample diluted 100-fold.

RESULTS

Biomass Sugars

Figure 2 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 suited for online monitoring of biofuel samples. Note that if only a fast estimation of total monosaccharide concentration is needed, a CarboPac PA1 anion-exchange column (using a 200 mM hydroxide eluent) can be used.³

Figure 3 shows the sugars in an acid-hydrolyzed corn stover sample dilution 100-fold (acid-hydrolyzed corn stover was donated by the National Renewable Energy Laboratory, Boulder, CO). The sugars present in this acid-hydrolyzed corn stover sample are arabinose, galactose, glucose, and xylose. The high concentration of xylose indicates that corn stover is rich in hemi-cellulose, and xylose concentration can be monitored to optimize the acid hydrolysis of biomass.

Table 1. Linear Range and Precisions for Biofuel Sugars

Analyte	Range (mg/mL)	Coeff. of Determination (R ²)	Conc. Used for Precision Injections (mg/mL)	RT (min)	RT 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

Linear Range

Biomass samples typically have sugar concentration in the range of 150–200 mg/mL, and sugars are analyzed typically after a 100- or 150-fold dilution. The linearity of the method was determined by injecting calibration standards in triplicate covering the expected range (0.4–2 mg/mL) of the sugars of interest in the samples (Table 1). For analyzing samples with lower sugar concentrations, an appropriate calibration range needs to be selected.

Precision

The peak area and RT precisions were determined for six replicate injections of a mixture of sugar standards. The high RT precisions are attributed to consistent generation of high-purity KOH using the eluent generator.

Intraday and between-day precisions of biofuel sugars in acid-hydrolyzed corn stover were evaluated over three days (Table 2). The intraday RT precisions were in the range of 0.09–0.28%, and peak area precisions were in the range of 0.4–5.0%. The between-day RT precisions ranged from 0.9 to 1.5% and peak area precisions were 7.5%. The aforementioned precisions suggest that this method can be used for complex biomass matrices such as acid-hydrolyzed corn stover.

Table 2. Between-Day (n = 3) RT and Peak Area Precisions (Triplicate Injections of Corn Stover 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

Accuracy

The accuracy of the method 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 RSD for corn stover was 3%. The average recovery for the sugars ranged from 69 to 112%. The between-day recovery precision for the eight biofuel sugars in the spiked samples ranged from 1 to 10% over three days.

Table 3. Biofuel Sugar Recoveries in Corn Stover 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

Injection Loop and Postcolumn Addition of Base

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

To achieve a wider linear range, this method can also be used with a postcolumn addition of more concentrated hydroxide (100 or 200 mM) to the eluent stream. A postcolumn addition can be made through a mixing tee using the second pump of the DP.¹¹ The linear range

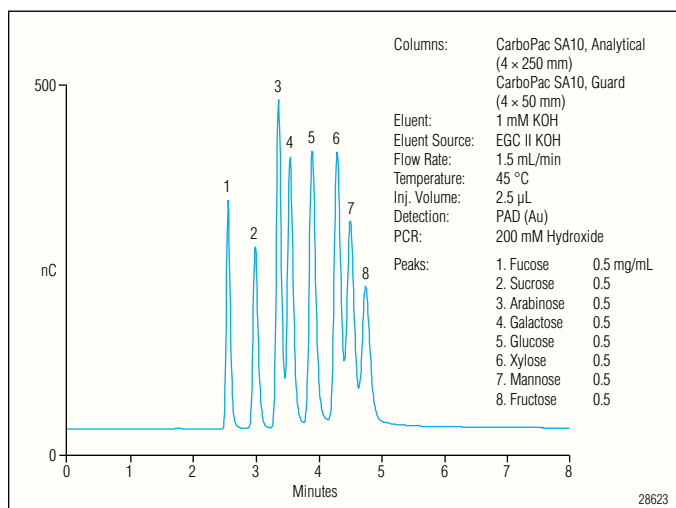


Figure 4. Separation of biofuel sugars on the CarboPac SA10 column with postcolumn reagent (PCR).

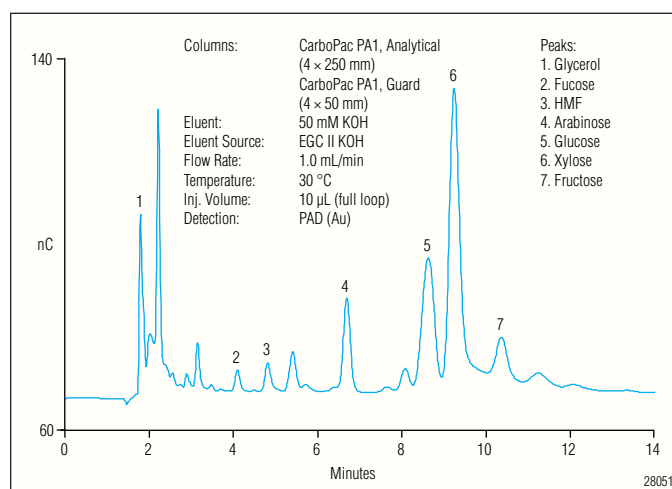


Figure 6. HMF in wood hydrolysate.

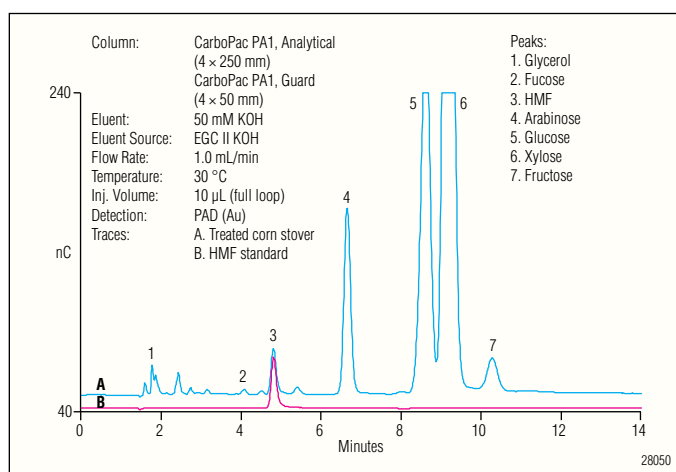


Figure 5. HMF in acid-hydrolyzed corn stover.

of detection for the biofuel sugars with a postcolumn addition of base (with a 2.5 μL injection) is 0.05–1.0 mg/mL, with coefficient of determination ranging from 0.9972 to 0.9999. Figure 4 presents a chromatogram of the mix of carbohydrate standards with a postcolumn addition of base (200 mM hydroxide). Note that this configuration requires additional hardware (i.e., the mixing tee and reaction coil) and reagent (i.e., the postcolumn base).

Hydroxymethylfurfural

Figures 5 and 6 show chromatograms of acid-hydrolyzed corn stover and wood hydrolysate. HMF was detected without interference from the other sugars in both matrices. The total run time for these samples was 15 min and provided high sample throughput, suggesting that this method could be used for online monitoring of HMF during biomass processing.

Linear Range, Limit of Quantitation, Limit of Detection

To determine linearity of the method, calibration standards were injected in triplicate covering the expected range of HMF in biomass samples (Table 4).

The limit of detection (LOD) was determined by measuring the peak-to-peak noise in a representative one-minute segment of the baseline where no peaks elute, followed by analyzing a standard at a concentration expected to provide a chromatogram with a signal-to-noise (S/N) ratio of three. Similarly, the lower limit of quantitation (LOQ) was determined by injecting a standard at a concentration that resulted in a S/N ratio of 10. Typical baseline noise for this method was 20–40 pC. The LOD and LOQ for this method were 0.04 $\mu\text{g/mL}$ and 0.10 $\mu\text{g/mL}$, respectively.

Table 4. Linear Range and Precisions for HMF Standard

Analyte	Sample Range ($\mu\text{g/mL}$)	Coeff. of Determination (R^2)	RT (min)	Concentration Used for Precision Injections ($\mu\text{g/mL}$)	RT Precision (RSD)	Peak Area ($\text{nC}\cdot\text{min}$)	Peak Area Precision (RSD)
HMF	(0.5–1000)	0.9965	4.85	5.0	0.10	4.91	0.14

Table 5. Intraday and Between-Day Precisions for Corn Stover Samples

Sample	Amount (µg/mL)	RT Precision (RSD)		Peak Area Precision (RSD)	
		Intraday	Between-Day	Intraday	Between-Day
Corn Stover	4.0	0.11	0.67	0.33	3.13

Table 6. Recoveries of HMF in Corn Stover Samples

Analyte	Sample	Amount Found (µg/mL)	Amount Added (µg/mL)	Average Recovery (n = 3 days)
HMF	Corn Stover	10.0	5.3	112.9

Precision

The peak area and RT precisions were determined for seven replicate injections of a HMF standard (Table 4). Similar to the biomass sugar analysis method, the high RT precisions are due to consistent generation of high-purity hydroxide using the eluent generator.

Intraday and between-day precisions for HMF in corn stover were evaluated over three consecutive days (Table 5). The RT precisions were in the range of 0.06–0.67%, and peak area precisions were in the range of 0.33–3.13%. The high precisions suggest that this method can be used for complex matrices.

Accuracy

The accuracy of the method was verified by determining recoveries of HMF in corn stover samples over three consecutive days. The treated corn stover sample (at 1000-fold dilution) had 4 µg/mL HMF and was spiked with 5.3 µg/mL HMF. Recoveries were calculated from the difference in response between the spiked and unspiked samples. Intraday concentration RSD was 1.7% and the average recovery of HMF in corn stover was 112% (Table 6).

SUMMARY

- Fast, accurate, reliable methods for the analysis of biomass sugars and HMF were developed.
- Methods have a linear range suited for handling high-concentration samples with minimal sample treatment, high precisions, and acceptable recoveries.
- Disposable gold working electrode provides consistently high detector response, assuring greater instrument-to-instrument and lab-to-lab reproducibility.
- This method can be used for online monitoring of sugar and HMF levels in biomass applications.
- This chromatography system only requires the addition of deionized water for continuous operation.

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