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Fast 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 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 ion chromatography (HPIC™) system with electrochemical detection.

Introduction

Lignocellulosic biomass is a key renewable source for alternative energy generation.[1–2] As new sources of biomass are explored, approaches to maximize energy generation are becoming important for biofuel producers. Monitoring the release of 5- and 6-carbon sugars, which can be fermented into biofuels like ethanol[3] and biodiesel[4], is required for evaluating the overall efficiency of biomass-to-biofuel conversion and is directly related to target biofuel yield and process economics.[5] Hence, the determination of carbohydrates in hydrolysates derived from lignocellulosic biomass is a crucial step in biofuel production, although development of robust analytical methods still remains a challenge. Currently used methods suffer from several drawbacks such as low throughput, poor analyte resolution, and non-applicability to a wider set of biomass samples.[6]

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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 Dionex CarboPac SA10-4µm column.[7] The Dionex CarboPac SA10-4µm column uses smaller resin particles for more efficient separations resulting in more accurate peak integration and more reliable results. 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 in AN1089 uses electrolytically generated hydroxide eluent and a 62 mil gasket in the electrochemical flow cell to resolve and detect the eight common lignocellulosic biomass-derived fermentable sugars, including xylose, sucrose, arabinose, galactose, glucose, mannose, fructose, and cellobiose. The use of a 62 mil gasket with the Dionex CarboPac SA10 column was recently shown to extend the linear range of carbohydrate determinations.[9] This allows concentrated samples like biomass hydrolysates to be analyzed without significant dilution, leading to improved method accuracy and convenience.

The system used for the carbohydrate analysis in AN1089 was recently updated in AN1161.[10] The new system used in AN1161 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™ fittings reduce the dead volume in the flow path and enhance chromatographic performance.

This work updates the column used in AN1161 with a shorter 4 × 150 mm format column. The combination of the smaller resin particle size of the Dionex CarboPac SA10-4µm column and the shorter column used here results in separation of eight common sugars in less than six minutes. This is significant time savings as compared to the eight-minute runtime achieved in AN1089. The shorter run time allows for faster sample turnaround times and reduced eluent consumption, thereby improving the overall process economics. Results for method linearity, accuracy, and robustness are presented here. Moreover, using this method, carbohydrates present in 10 individual field biofuel samples were quantified.

Experimental

Conditions

Column:	Dionex CarboPac SA10-4µm, 4 × 150 mm (P/N 088652) Dionex CarboPac SA10-4µm Guard 4 × 30 mm (P/N 088654)		
Column Temperature:	45 °C		
Compartment Temperature:	30 °C		
Flow Rate:	1.5 mL/min		
Eluent:	1 mM KOH		
Eluent Source:	Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778) with Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)		
Working Electrode:	Gold disposable on PTFE (P/N 066480)		
Electrochemical Cell Gasket:	62 mil		
Reference Electrode:	pH-Ag/AgCl		
Sampler Tray Temperature:	Ambient		
Injection Volume:	0.4 µL (Push_Full mode)		
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	

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 20-fold with deionized (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 therefore shortened retention times. If such column contamination is suspected, 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 the recommended column washing procedure and additional information on column care.

Equipment

A Thermo Scientific™ Dionex™ Integrion™ HPIC™ system (P/N 22513) with RFIC option, was used in this work. The Dionex Integrion HPIC system is an integrated ion chromatograph that includes:

- SP single pump module
- 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)

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 F2543)
- D-Xylose (Aldrich® P/N X-10705)
- Sucrose (Sigma P/N 84097)
- D-Glucose (J. T. Baker® P/N 1910-01)
- D(-)-Arabinose (Sigma P/N A3131)
- D(+)-Cellobiose (Sigma P/N C7252)

Table 1. Consumables for the Dionex Integrion HPIC System.

Product	Description	Part Number
Dionex IC PEEK Viper fitting tubing assembly kits	Dionex IC Viper fitting tubing assembly kit for the Integrion HPIC: 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
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
Dionex EGC 500 KOH eluent generator cartridge	Eluent generator cartridge when using 4 µm particle columns	075778
Dionex CR-ATC 600 electrolytic 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	Fisher Scientific P/N SB115-500
pH Buffer, pH 10	Reference electrode pH calibration standard	Fisher Scientific P/N SB107-500

Consumables

Part numbers are shown in Table 1.

Results

Separation

A representative chromatogram of a standard mix containing eight of the common biofuel sugars at 0.6 g/L concentration each obtained using a Dionex CarboPac SA10-4µm 4 × 150 mm column is shown in Figure 1. Fucose was used as an internal standard. All the peaks were well resolved and the separation was completed within six minutes. The smaller particle size and shorter column format result in a faster run time.

Linearity

A sufficiently wide dynamic range is required of any method designed to measure sugars present in biomass hydrolysates. This is because the sugars are typically present in divergent ratios in these samples. Using this method, peak area calibration curves more than two orders of magnitude wide, between 0.005 and 2 g/L were generated for all eight sugars. This calibration range is suitable for handling samples without the need for significant dilution, which in turn will avoid dilution errors. The results from linear curve fitting of the data are included in Table 2.

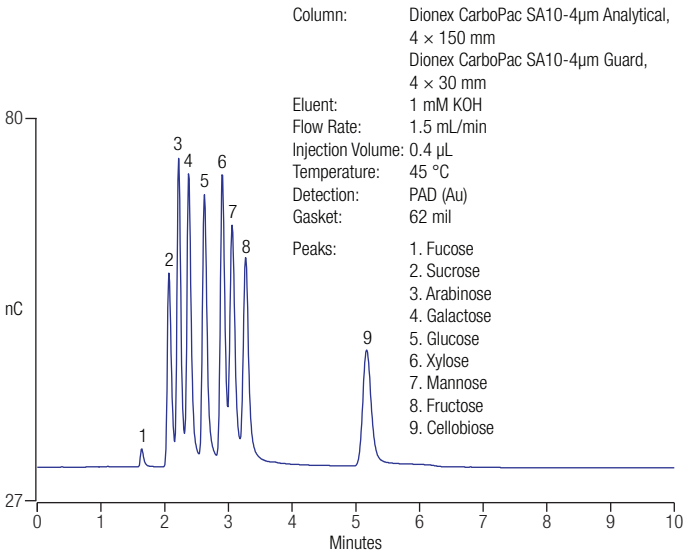


Figure 1. Separation of biofuel sugars on the Dionex CarboPac SA10-4µm 4 × 150 mm column using proposed method (0.6 g/L standard).

Table 2. Calibration (at 11 levels between 0.006 and 2 g/L) data (n=3) for each of the eight common biofuel sugars.

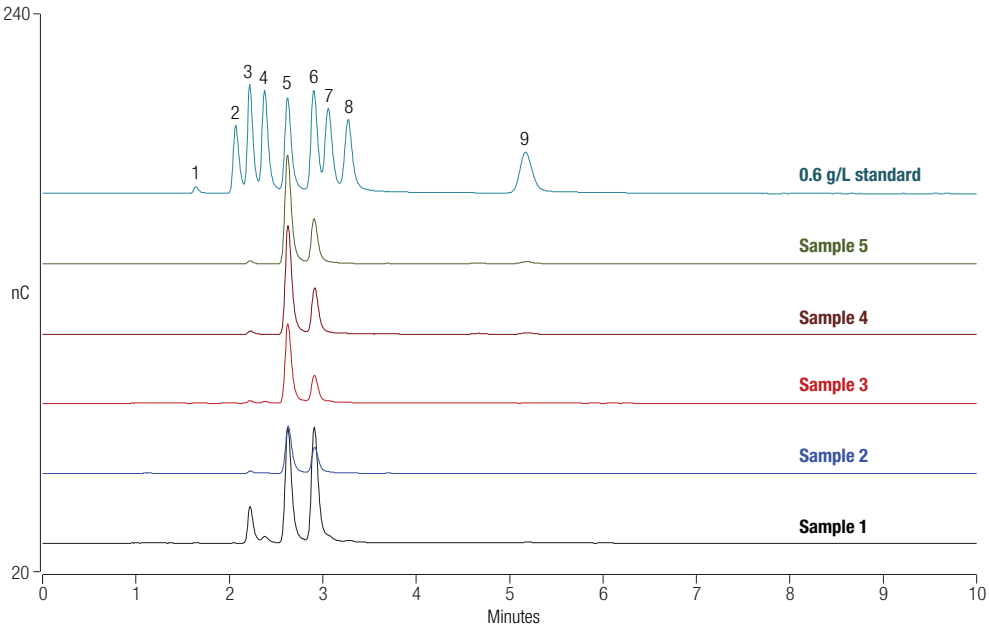
Peak Name	Retention Time (min)	Resolution	Concentration Range (g/L)	Coefficient of Determination
Sucrose	2.13	1.4	0.006–2	1.000
Arabinose	2.28	1.4	0.006–06	1.000
Galactose	2.45	2.0	0.006–06	1.000
Glucose	2.71	2.1	0.006–1	0.999
Xylose	3.00	1.2	0.006–06	0.999
Mannose	3.17	1.5	0.006–06	0.999
Fructose	3.40	10	0.006–1	0.999
Cellobiose	5.48	-	0.006–2	0.999

Accuracy

Method accuracy was evaluated by first quantifying the sugars present in biomass hydrolysate samples. To determine method accuracy, ten field biomass hydrolysate samples were procured. The samples were collected at various stages of biomass processing and conversion of lignocellulosic biomass to ethanol. From this set, five samples were randomly selected for recovery studies to determine method accuracy. Representative chromatograms for the selected five biofuel samples are shown in Figure 2. The major sugars present in these samples are glucose and xylose. All the biofuel sugars that are present above the lowest calibration standard were spiked with 50–150% of the original amount found in the sample. The original amounts present, spiked amounts, and percent spike recoveries are included in Table 3. For all four samples studied here, excellent recoveries were obtained for all spiked biomass sugars.

Robustness

Biomass-derived samples have complex matrices, which may cause loss of column capacity with prolonged use. As a result, it is critical to ensure minimal matrix effect on retention time and peak area stability during a long sequence run. At the same time, it is important to demonstrate that slight variations in method parameters during routine use do not affect the chromatographic performance in a significant way. Here, the method robustness was studied in two ways.



Column:	Dionex CarboPac SA10-4µm Analytical, 4 × 150 mm Dionex CarboPac SA10-4µm Guard, 4 × 30 mm
Eluent:	1 mM KOH
Flow Rate:	1.5 mL/min
Injection Volume:	0.4 µL
Temperature:	45 °C
Detection:	PAD (Au)
Gasket:	62 mil
Peaks:	1. Fucose 2. Sucrose 3. Arabinose 4. Galactose 5. Glucose 6. Xylose 7. Mannose 8. Fructose 9. Cellobiose

Figure 2. Representative chromatograms of five lignocellulosic biomass based biofuel samples 1–5.

Table 3. Recovery studies performed on five different biofuel samples (n=7).

Sample	Sugar	Average Amount (g/L)	Theoretical Spiked Amount (g/L)	Average Recovery (%)	RSD
Sample 1	Arabinose	0.04	0.02	95.8	0.91
	Galactose	0.02	0.01	106	0.68
	Glucose	0.43	0.25	89.2	0.71
	Xylose	0.41	0.32	85.4	0.56
	Mannose	0.02	0.02	110	0.07
	Fructose	0.01	0.02	79.3	0.25
Sample 2	Arabinose	0.01	0.02	98.8	0.02
	Galactose	0.00	0.01	96.3	0.09
	Glucose	0.27	0.25	99.6	0.01
	Xylose	0.15	0.32	96.4	0.01
	Mannose	0.01	0.02	115	0.11
	Fructose	0.01	0.02	74.2	0.07
Sample 3	Arabinose	0.01	0.02	99.2	0.38
	Galactose	0.01	0.01	103	0.35
	Glucose	0.46	0.25	89.1	0.78
	Xylose	0.17	0.32	95.8	0.21
	Mannose	0.01	0.02	108	0.16
	Fructose	0.02	0.02	78.6	0.37
Sample 4	Arabinose	0.02	0.02	92.6	0.08
	Glucose	0.64	0.25	84.2	0.06
	Xylose	0.27	0.32	93.0	0.03
	Fructose	0.02	0.02	77.5	0.09
Sample 5	Arabinose	0.02	0.02	90.5	0.05
	Galactose	0.00	0.01	103	0.07
	Glucose	0.64	0.25	86.7	0.01
	Xylose	0.28	0.32	89.3	0.02

First, variation in method performance was monitored over a 180-injection sequence run of the biofuel samples. Changes in retention time and peak area were measured over all the samples by injecting a standard containing 0.6 g/L of all eight biofuel sugars (Table 4) at frequent intervals. This resulted in 21 standard injections during the total sequence run time of ~33 h. Figures 3A and 3B show retention time and peak area trending of all the 21 standard injections. Maximum retention time and peak area change values observed were 0.8% and 1.8%, both for xylose. Hence, there was no significant trending of either peak area or retention time during this experiment. This result indicates that the method can withstand sample matrix effects in a typical sequence run.

Table 4. Method robustness to biomass sample matrix effects (n=21).

Biofuel Sugar	Retention Time (min)	Retention Time RSD	Average Peak Area (nC·min)	Peak Area RSD
Fucose	1.66	0.36	0.47	2.38
Sucrose	2.12	0.42	2.01	2.09
Arabinose	2.27	0.35	3.12	1.34
Galactose	2.44	0.41	3.33	0.92
Glucose	2.69	0.45	3.41	1.00
Xylose	2.99	0.44	3.56	2.35
Mannose	3.15	0.48	3.30	2.26
Fructose	3.38	0.59	3.26	2.10
Cellobiose	5.44	0.68	2.51	1.01

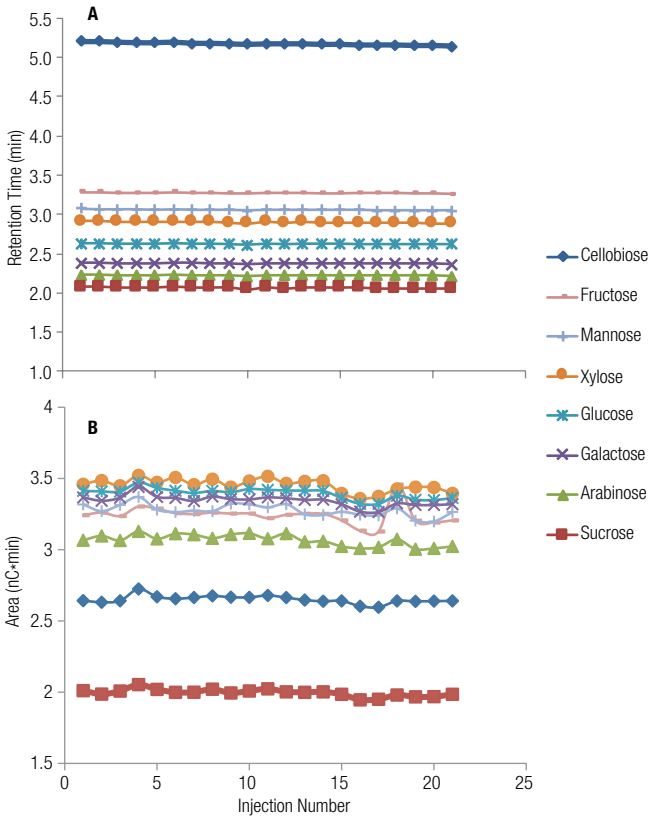


Figure 3. Retention time stability to sample matrix effects (A), and peak area stability to sample matrix effects (B).

Next, the effect of variations in eluent, column temperature, flow rate, and eluent concentration on key chromatographic parameters was studied. A few key method parameters were intentionally varied ±10% and changes in retention time, peak resolution, and peak asymmetry were studied on two different columns.

The results included in Tables 5 and 6 show minimal changes in retention time and peak resolution. The peak asymmetries for all eight sugars tested here also showed minimal change with the ±10% method variations on both columns (not shown). The largest impact on the individual retention times are observed, as expected, with changing the eluent flow rate.

Table 5. Robustness studies on column 1 using 0.6 g/L standard (n=3).

Parameter	RT % Difference									Resolution % Difference							
	Fuc	Suc	Ara	Gal	Glu	Xyl	Man	Fru	Cel	Fuc	Suc	Ara	Gal	Glu	Xyl	Man	Fru
-10% Temp	-1.3	-4.6	-2.5	-2.7	-4.2	-4.7	-6.0	-3.7	-6.6	-20.2	22.5	-3.1	-12.3	-0.1	-21.7	27.8	-6.3
+10% Temp	1.0	3.7	2.2	2.3	3.6	3.8	5.0	3.1	6.0	13.0	-19.4	0.7	11.0	-4.2	15.3	-32.4	5.5
-10% Flow	-11.1	-11.1	-11.0	-11.1	-11.1	-11.1	-11.0	-11.2	-11.0	1.1	2.1	-2.7	-1.0	-0.6	1.3	-3.4	-5.1
+10% Flow	9.1	9.1	9.1	9.1	9.1	8.7	8.8	9.0	9.1	-0.8	0.5	-0.7	0.2	-2.0	2.4	2.7	2.0
-10% Eluent Conc.	0.0	-0.1	-0.1	-0.1	-0.2	-0.4	-0.4	-0.3	-0.2	0.2	0.9	0.2	-0.8	-1.6	0.5	0.2	0.5
+10% Eluent Conc.	0.0	-0.1	0.2	0.0	0.0	0.0	0.2	0.3	0.4	-0.8	5.9	-3.6	-0.3	-0.3	2.9	0.4	-0.2

Table 6. Robustness studies on column 2 using 0.6 g/L standard (n=3).

Parameter	RT % Difference									Resolution % Difference							
	Fuc	Suc	Ara	Gal	Glu	Xyl	Man	Fru	Cel	Fuc	Suc	Ara	Gal	Glu	Xyl	Man	Fru
-10% Temp	-1.1	-4.6	-2.6	-2.6	-4.3	-4.7	-5.9	-3.7	-6.8	-22.6	20.8	-1.9	-13.4	0.9	-20.9	25.7	-7.5
+10% Temp	1.9	4.1	2.4	2.6	3.9	4.3	5.5	3.6	6.3	10.8	-23.9	1.3	11.2	-2.5	14.6	-33.5	4.3
-10% Flow	-10.5	-11.0	-11.0	-10.9	-11.0	-10.9	-10.9	-11.1	-11.0	-1.7	-0.5	0.6	-2.9	-0.9	-0.8	-4.7	-2.9
+10% Flow	9.4	9.1	9.1	9.2	9.0	8.9	9.1	9.1	9.1	-0.9	1.0	0.9	-0.8	0.3	3.5	1.1	1.4
-10% Eluent Conc.	0.0	0.0	-0.1	-0.1	-0.3	-0.3	-0.3	-0.3	-0.3	0.5	-0.7	0.4	-2.0	0.1	0.3	-0.8	-0.5
+10% Eluent Conc.	0.5	0.0	0.0	0.3	0.0	0.3	0.3	0.3	0.3	-2.4	0.0	4.7	-3.1	2.4	-0.8	-1.3	-1.1

Conclusion

This application note presents an improved method for quantification of biomass sugars that can be converted to biofuels. The method can separate all eight key biomass sugars within six minutes. The response data for all biomass sugars studied here show excellent correlation with concentration. The method provides excellent peak area as well as retention time precision within the concentration levels studied. Spike recovery studies performed to demonstrate method accuracy showed good recovery from all the spiked samples, implying that the method is accurate. Finally, the method is robust with retention time and peak area remaining stable over 180 injections of the biofuel samples. In summary, the method proposed here is a convenient, precise, and robust method to quantify biomass sugars in complex hydrolysate samples and will improve the reliability of biomass-to-biofuel efficiency calculations.

References

1. Hill, J.; Nelson, E.; Tilman, D.; Polasky, S.; Tiffany, D. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *PNAS.* **2006,** *103(30),* 11206–11201.
2. Ragauskas, A.J.; et al. The path forward for biofuels and biomaterials. *Science.* **2006,** *311,* 484–489.
3. Goldemberg, J. Ethanol for a sustainable energy future. *Science,* **2007,** *315,* 808–810.
4. Alonso, D. M.; et al. Catalytic conversion of biomass to biofuels. *Green Chem.* **2010,** *12,* 1493–1513.
5. Wyman C.E. Ethanol from lignocellulosic biomass: Technology, economics and opportunity. *Bioresour. Technol.* **1994,** *50,* 3–16.
6. Sluiter, J.B.; et al. Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. *J. Agric. Food Chem.,* **2010,** *58(16),* 9043–9053.
7. Thermo Scientific Application Update 192 (AU192). [Online] Carbohydrate Determination Biofuel Samples. https://appslab.thermofisher.com/App/2277/au192-carbohydrate-determination-biofuel-samples (accessed Jul. 22, 2016).
8. Thermo Scientific Application Note 1089 (AN1089). Determination of Carbohydrates in Acid Hydrolysates of Wood. [Online] https://tools.thermofisher.com/content/sfs/brochures/AN-1089-Carbohydrates-Acid-Hydrolysates-Wood-AN70941-EN.pdf (accessed Jul. 22, 2016).
9. Thermo Scientific Technical Note 186 (TN186). The effect of working electrode gasket thickness on the sensitivity and linearity of carbohydrate response by pulsed amperometric detection. [Online] http://tools.thermofisher.com/content/sfs/brochures/TN-186-IC-PAD-Gasket-Thickness-Carbohydrate-Response-TN72178-EN.pdf (accessed Dec. 28, 2016).
10. Thermo Scientific Application Note 1161 (AN1161). Improved method for determination of biofuel sugars by HPAE-PAD [Online] http://tools.thermofisher.com/content/sfs/brochures/AN-1161-IC-HPAE-PAD-Biofuel-Sugars-AN72165-EN.pdf (accessed Jan. 5, 2017).

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