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응용 자료

Fast Analysis for Fermentation Ethanol with the Breeze 2 HPLC System

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Abstract

This application note describes a fast HPLC analysis method that was developed on the Waters Breeze 2 HPLC System to analyze samples within 10 minutes.

Introduction

Recent legislation and public interest in alternative fuels have resulted in an increased use of ethanol/gasoline blends in the transportation industry. In some regions of the world, there is a drive to limit fossil fuel consumption for other applications or reduce greenhouse gas emissions. In other regions, there are initiatives to reduce energy dependency on imported oil.[1] The United States produced over 6.5 billion gallons of ethanol in 2007, about a 67% increase from 2005. There are 139 ethanol biorefineries in 24 states across the U.S., which have the capacity to produce about 7.9 billion gallons of ethanol per year.[2]

The production of ethanol from a renewable resource, such as corn, utilizes a fermentation process where enzymes and yeast are used to convert starches and sugars to ethanol. To improve the productivity of the fermentation process, certain stress factors affecting yeast activity are carefully analyzed throughout the process. These include monitoring the relative concentrations of glucose, ethanol, lactic acid, and acetic acid.[3]

Monitoring results are then used to optimize fermentation conditions in order to increase productivity. HPLC analysis can provide information about critical components easily, and is widely used throughout the U.S. in producer labs. Typical HPLC runtimes are 20 to 30 minutes.[4] As ethanol production levels increase, there is a need for more rapid monitoring of the fermentation process.

This application note describes a fast HPLC analysis method that was developed on the Waters Breeze 2 HPLC System to analyze samples within 10 minutes.[5]

Experimental

The Breeze 2 HPLC System is the next generation in a series of systems designed for routine chromatographic applications. The Breeze 2 HPLC System used in this method is comprised of the Waters 1525 Binary HPLC Pump[6], 2707 Autosampler, external column heating module, 2414 Refractive Index (RI) Detector, and Breeze 2 Software.

LC conditions:

LC system:	Breeze 2 HPLC
Column:	IC-Pak 7.8 x 150 mm Ion Exclusion Pre-column 6.0 x 50 mm, SH-1011P
Column temp:	75 °C
Flow rate:	1.0 mL/min
Mobile phase:	0.5 mM sulfuric acid
Injection volume:	5 µl
RI sensitivity:	32
RI time constant:	0.2
Sampling rate:	5 pts
RI detection temp:	30 °C

Acquisition and processing methods

Data were acquired using the Breeze 2 Software. From data acquisition to real-time monitoring and total results management, the intuitive Breeze 2 Software interface requires minimal training, is easy to setup, learn, and operate.

Materials

Dextrin (Type I: from corn), maltotriose, maltose (monohydrate Grade I), glucose monohydrate, L(+)-lactic acid (SigmaUltra, 98%), glycerol, acetic acid (96.0%) sulfuric acid (ACS reagent), and ethyl alcohol were purchased from Sigma-Aldrich. Dextrin is a mixture of polysaccharides containing a small amount of low molecular weight polysaccharides.[7]

Chromatography indicated dextrin contains 92.1% polysaccharide (more than 3 glucose units), 2.7%

maltotriose (3 glucose units), 1.7% maltose (2 glucose units), 2.6% glucose, and 0.9% unidentified oligosaccharide.[8] This data was used in to calculate the standard concentrations.

Preparation of standard solutions

The stock standard mixture solution was prepared by adding each component into a 25 mL volumetric flask and dissolving into de-ionized water (Millipore Milli-Q). The stock standard solution was then diluted to 5%, 10%, 30%, and 70% of the original concentration. All standard solutions and samples were filtered using GHP Acrodisc, 4.5 $x10^{-4}$ mm diameter syringe filters (WAT200514).

Mobile phase

Dilute sulfuric acid (0.50 mM) was prepared by a two-step serial dilution. First, about 160 mL de-ionized water was added to a 200 mL volumetric flask. Then, using a 2 mL pipette (calibrated in 1/100), 0.553 mL 96.4% sulfuric acid was transferred into the 200 mL volumetric flask. The flask was filled to the 200 mL mark with de-ionized water to make a 50 mM sulfuric acid stock solution. Next, 10.0 mL of the 50 mM solution were transferred to a 1000 mL volumetric flask. Diluting this with de-ionized water to the 1000 mL mark made the 0.50 mM sulfuric acid mobile phase.

Results and Discussion

The major components of interest in the ethanol fermentation broth are dextrin, maltotriose, maltose, glucose, lactic acid, glycerol, acetic acid, and ethanol. The fast separation shown in Figure 1 was obtained on the Breeze 2 HPLC System.

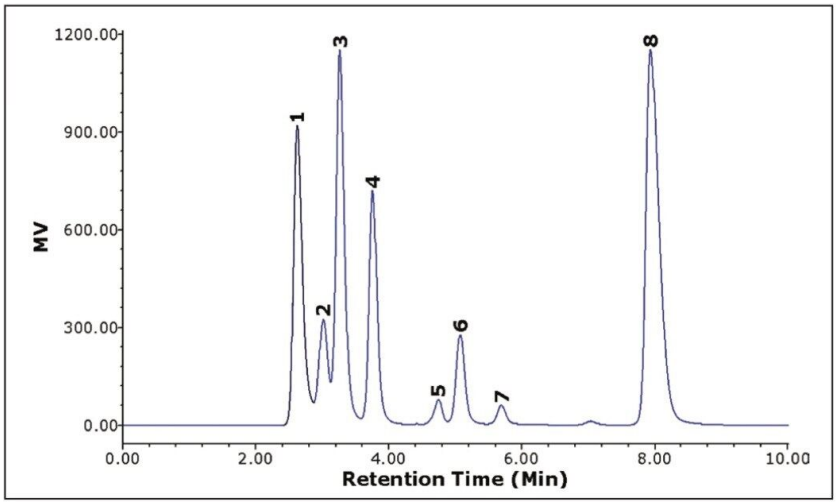

Figure 1. HPLC separation of eight major fermenting mash components: 1) dextrin, 2) maltotriose, 3) maltose, 4) glucose, 5) lactic acid, 6) glycerol, 7) acetic acid, and 8) ethanol.

The calibration curves were generated automatically in Breeze 2 Software from the chromatograms of a series of standard mixtures at several concentrations. Table 1 summarizes the calibration results.

Compound	Retention time (min)	Concentration range (g/100 mL)	R^2
Dextrin	2.63	0.22 – 4.36	>0.9998
Maltotriose	3.06	0.06 – 1.14	>0.9998
Maltose	3.27	0.20 – 4.04	>0.9999
Dextrose	3.76	0.11 – 2.21	>0.9999
Lactic Acid	4.70	0.02 – 0.36	>0.9999
Glycerol	5.08	0.06 – 1.28	>0.9999
Acetic Acid	5.70	0.02 – 0.46	>0.9998
Ethanol	8.05	0.76 – 15.18	>0.9999

Table 1. The calibration curves between the peak area and the concentration.

The relationship between peak area and the concentration was linear over the entire concentration range examined. Figure 2 is a screen capture of a typical calibration curve for dextrin.

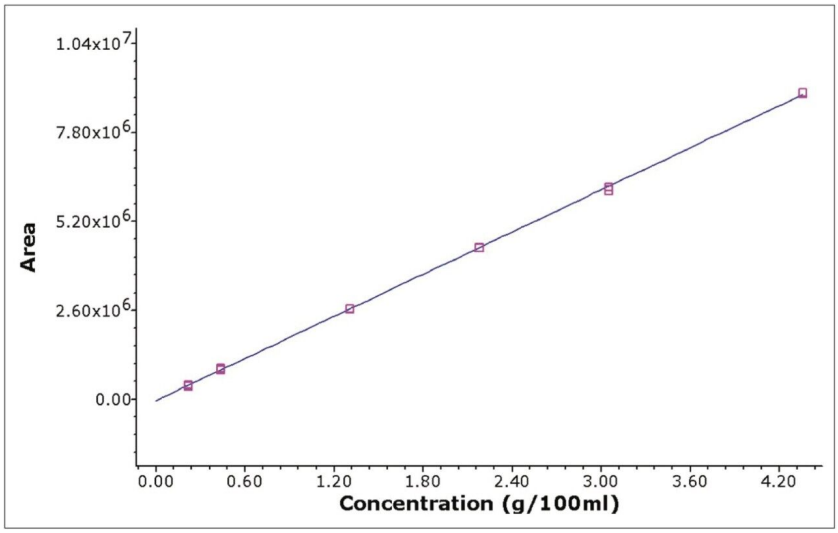

Figure 2. Dextrin calibration curve, R^2 = 0.9998.

To test the accuracy and the reproducibility of this fast HPLC analytical method, 10 injections of a homemade fuel ethanol sample were quantified and the results were compared with the concentration value obtained from sample preparation. The results were precise and well below the acceptable +/-10% limit , as shown in Table 2. The peak retention times of these injections are also included in Table 2. Excellent peak retention time reproducibility was obtained.

Component	Concentration (g/100 mL)	Measured results (g/100 mL)	Concentration results % RSD	Relative error (%)	Retention time % RS
Dextrin	2.18	2.15	3.7	-1.4	0.2
Maltotriose	0.569	0.563	3.7	-1.1	0.2
Maltose	2.02	1.99	3.4	-1.5	0.2
Dextrose	1.10	1.09	3.0	-0.9	0.2
Lactic acid	0.181	0.181	2.3	0	0.1
Glycerol	0.638	0.635	2.4	-0.5	0.1
Acetic acid	0.231	0.229	2.1	-0.9	0.1
Ethanol	7.59	7.52	1.6	-0.9	0.1

Table 2. Homemade fuel ethanol sample measurement and peak retention time reproducibility (n=10).

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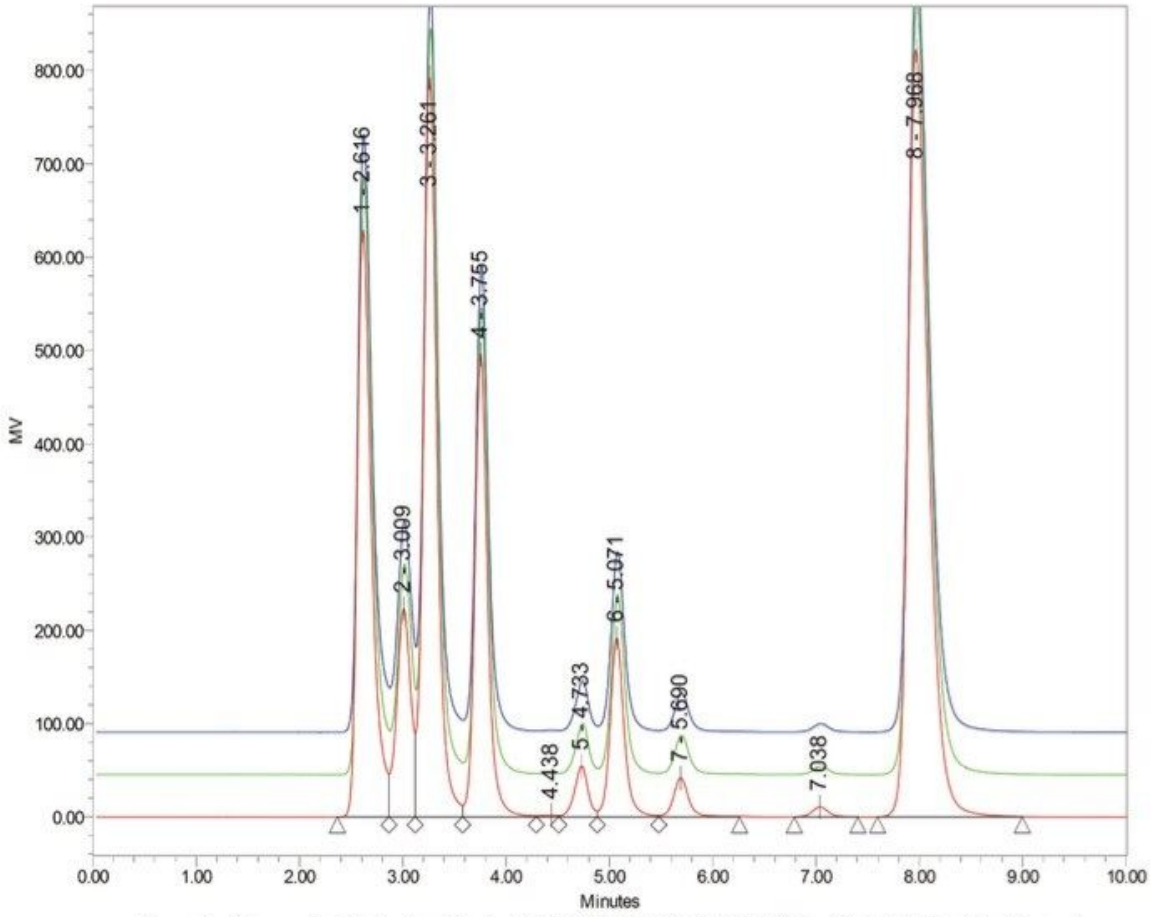
Project Name Ethanol Fast Nov 08

Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System



Sample Name: 5; Date Acquired: 11/19/2008 8:11:48 PM EST; Vial: 1:C,6; Injection: 1

Sample Name: 5; Date Acquired: 11/19/2008 8:22:05 PM EST; Vial: 1:C,6; Injection: 2

Sample Name: 5; Date Acquired: 11/19/2008 8:32:23 PM EST; Vial: 1:C,6; Injection: 3

Peak Summary with Statistics

Peak Name: 1

	Sample Name	Vial	Inj.	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	Amount	Units
1	5	1:C,6	1	1	2.616	6107987	17.92	628963	3.051	g/100ml
2	5	1:C,6	2	1	2.623	6187556	18.01	639170	3.051	g/100ml
3	5	1:C,6	3	1	2.622	6201652	18.02	640748	3.051	g/100ml
Mean					2.620	6165731.592		636293.664	3.051	
Std. Dev.					0.004	50502.748		6397.19	0.00	

Report Method: Peak Summary Report

Figure 3. An example of a peak summary report generated by Breeze 2 Software.

Conclusion

A 10 minute HPLC analysis of eight major fuel ethanol fermentation components were reliably performed on the Breeze 2 HPLC System. The resolution in this fast analysis for fuel ethanol broth samples provides fast and accurate quantitation. Faster analysis times may translate to superior process control, increased productivity, and reduced product loss for the ethanol producers.

The breeze 2 HPLC also provides ethernet control and sample temperature control. These new features will help to ensure consistent analytical results for QA/QC plant laboratories.

References

1. Hileman, B. Energy for a Sustainable Future. C&EN, 84: 7, 70-75, 2006.
2. Website: Renewable Fuels Association, “Changing the climate ethanol industry outlook 2008”. http://www.ethanolrfa.org.
3. Kohl, S. Ethanol 101-5: Managing Stress Factors, Ethanol Today, January 2004, 36-37.
4. Optimizing Ethanol Production with High Performance Liquid Chromatography, Waters Application Note No. 720000455EN.
5. Fast HPLC Analysis for Fermentation Ethanol Processes, Waters Application Note No. 720001896EN.
6. 1515 HPLC pump can also be used for this application.
7. Personal communication with vendor; product contains approximately 10% low molecular weight fraction.
8. Composition of the dextrin, maltotriose, maltose, and an unidentified impurity were estimated by the peak areas of each component. No adjustments were made for differences in RI response factors.

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2414 Refractive Index (RI) Detector for Modular LC Systems <https://www.waters.com/134721830>

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