Faster Real-time Response to Bacterial Infection of Bioethanol Fermentation using a Short Rezex™ ROA Column

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Today, many ethanol producers are looking for ways to reduce cost and improve operational efficiency. A quick and easy way to achieve this is by using the shorter Rezex ROA 150 x 7.8 mm column as an alternative to a longer 300 x 7.8 mm column. The shorter column will not only increase throughput by 50 %, but also reduce cost by utilizing less solvent for each HPLC run.

Introduction

Political priorities as well as economic interests have fueled a dramatic growth in the biofuel industry, due much in part to research funding and tax incentives. For 2010 the world's ethanol production is estimated to be over 16 billion gallons. This number is expected to increase reaching an estimated yearly production of 20 billion gallons by 2012.[1]

The current standard analytical procedure for monitoring ethanol production uses an ion-exclusion column, such as the Phenomenex® Rezex ROA, 300 x 7.8 mm. This technology utilizes several different separation modes (gel filtration, ion-exchange, and reversed phase) to separate all of the fermentation components (sugars, organic acids, and alcohols) in one chromatographic separation.[2]

Today, many ethanol producers are adding fermenters to expand their production capacity. In order to continue using existing HPLC equipment for the increased monitoring, increased analytical throughput is needed from available instrumentation. In an effort to improve operational efficiency, several plants have begun to use the shorter Rezex ROA 150 x 7.8 mm column as an alternative to the Rezex ROA column 300 x 7.8 mm column. The shorter column increases throughput by reducing the analysis time by 50 % (from 24 to 12-minute run time). In this article, the advantages and disadvantages of using a shorter column will be discussed.

Materials and Methods

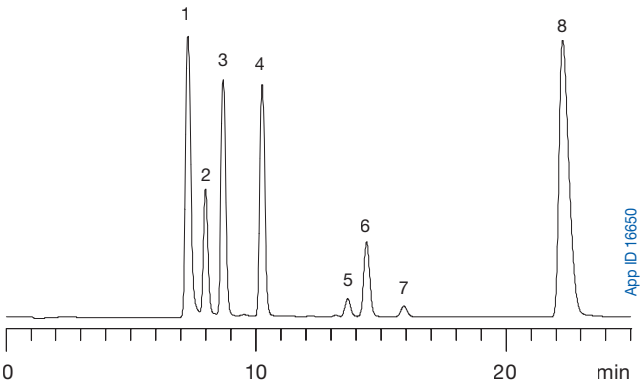
Crude samples of various bioethanol fermentation timepoints, generously provided from ICM, Inc (Colwich, KS, USA), were filtered using a 0.20 µm Phenex™-RC syringe tip filter (Phenomenex, Torrance, CA, USA). The ethanol HPLC testing standard was obtained from Midland Scientific (Omaha, NE, USA). Filtered aliquots of 10 µL were injected on a HPLC operating at a flow rate of 0.6 mL/min. The HPLC column was heated to 65 °C. SecurityGuard™ cartridges were regularly changed every 100 runs or whenever static backpressure increased 10 % above initial backpressure values to extend column lifetime. 50 % methanol was used in the autosampler needle wash to avoid bacterial contamination.

All analyses were run on a Shimadzu® LC-20AT LC system (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a SIL-10AF autosampler, degasser, and a RID-10A RI detector; data was collected using CLASS-VP™ Version 7 Software. Two dimensions of Rezex ROA columns (Phenomenex Inc., Torrance, CA, USA) were used: 150 x 7.8 mm and 300 x 7.8 mm. Aqueous mobile phase (0.005 N Sulfuric acid in water) was purchased from CHATA™ Biosystems (Ft. Collins, CO, USA).

Results and Discussion

Figure 1 shows the HPLC run of a fermentation standard using the Rezex ROA 300 x 7.8 mm column. Baseline resolution is achieved for all peaks in about 24 minutes. Runs of two bioethanol fermentation samples at the 18 and 39 hour timepoints were done using the 300 x 7.8 mm column (**Figure 2**). These runs show that early in the fermentation process, the sugar peaks are the most abundant components in the fermentation mixture. Later in the production, as sugars are converted into ethanol, the sugar peaks are depleted as the concentration of ethanol, glycerol, and organic acids increases.

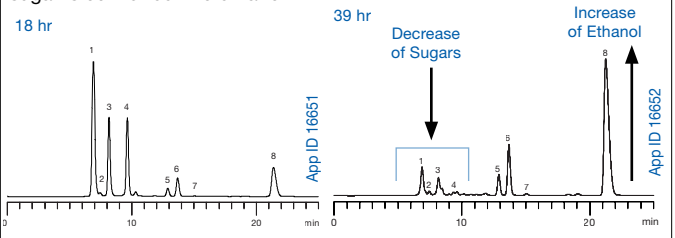
Figure 1.
A bioethanol fermentation standard was run on a 300 x 7.8 mm Rezex ROA column. Note the excellent separation of all the components.



Column: Rezex ROA-Organic acid
Dimensions: 300 x 7.8 mm
Part No.: 00H-0138-K0
Mobile Phase: 0.005 N Sulfuric acid
Flow Rate: 0.6 mL/min
Temperature: 60 °C
Detection: RI @ 40 °C
Instrument: Shimadzu® Prominence™ LC-20A System
Sample: 1. Dp4+
2. Dp3
3. Maltose
4. Glucose
5. Lactic acid
6. Glycerol
7. Acetic acid
8. Ethanol

APPLICATIONS

Figure 2.
Different fermentation timepoints run on a Rezex 300 x 7.8 mm ROA column. Note the depletion in the early sugar peaks as fermentation continues. The ethanol peak increases throughout the fermentation as sugar is converted into ethanol.



Conditions for both runs:
Column: Rezex ROA-Organic acid
Dimensions: 300 x 7.8 mm
Part No.: 00H-0138-K0
Mobile Phase: 0.005 N Sulfuric acid
Flow Rate: 0.6 mL/min
Temperature: 60 °C
Detection: RI @ 40 °C
Instrument: Shimadzu® Prominence™ LC-20A System
Sample: 1. Dp4+
2. Dp3
3. Maltose
4. Glucose
5. Lactic acid
6. Glycerol
7. Acetic acid
8. Ethanol

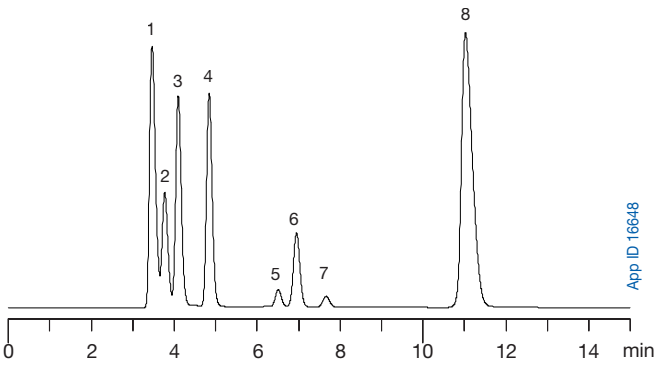
As some producers have increased the scale of their ethanol production and are now typically operating several fermentors in parallel, there is a desire to monitor these additional fermentors with existing analytical instrumentation. Monitoring multiple fermentors in parallel requires reducing the analysis time for fermentation monitoring. Quick analysis of the production process would not only increase throughput, but also help prevent the loss of an entire fermentation batch due to a bacterial infection. Lactic and acetic acid are byproducts produced by contaminated fermentation broth. These acids can inhibit or kill the yeast that converts the sugar to ethanol. Because these acids may potentially have detrimental effects on the fermentation process, producers closely monitor their levels throughout the entire production process. Regularly monitoring the process by HPLC allows operators to assess if microbial contamination is affecting the fermentation process and if remediation steps such as antibiotic addition are necessary to maximize ethanol yield.[3]

One method for reducing HPLC run time is reducing the length of the Rezex column used. The fermentation standard is run on a Rezex ROA 150 x 7.8 mm, half the length of the typical 300 mm column used (**Figure 3**). As expected, the run time using a shorter column is significantly reduced from 24 to 12 minutes. Compared to the 300 mm column, the resolution of the early eluting sugar peaks is reduced. However, baseline resolution is still achieved for the late eluting organic acids (lactic acid and acetic acid), glycerol, and alcohol peaks. The Rezex ROA 150 x 7.8 mm column will increase sample throughput 2-fold, producing faster results that allow for more real-time responses. In addition, the shorter column length utilizes less solvent. With most producers operating 24 hours a day, this will significantly help lower solvent usages and production costs.

Choosing the optimal Rezex ROA column length is dependent on whether it is critical to have an accurate quantitation of the Dp4+ and Dp3 peaks. Since the separation of sugars is based primarily on a gel filtration mechanism, there is a limitation on how much the column length can be shortened and still maintain resolution of key sugar peaks. The early eluting peaks (Dp4+, Dp3, maltose, and glucose) represent the different degrees of polymerization of the various sugars present in the sample. Monitoring of these peaks during the early timepoints of the fermentation run gives operators a good indication as to the progression of the various amylases used to break down starches to simple sugars, and dictate when yeast is added to the fermentor to start generating ethanol.

Monitoring the later eluting peaks (lactic acid, glycerol, acetic acid, and ethanol) indicates the fermentation endpoint as well as when bacterial contamination is severe enough to warrant addition of an antibiotic to limit bacterial byproducts that may inhibit ethanol production. This is still achieved with reduced analysis time when using the shorter Rezex ROA 150 x 7.8 mm column **(Figures 3-4)**.

Figure 3.
The bioethanol fermentation standard was run on a 150 x 7.8 mm Rezex ROA column. Note the limited resolution of the early eluting sugar peaks in the standard. In early fermentation monitoring such peaks may not be resolved.



Column: Rezex ROA-Organic acid
Dimensions: 150 x 7.8 mm
Part No.: 00F-0138-K0
Mobile Phase: 0.005 N Sulfuric acid
Flow Rate: 0.6 mL/min
Temperature: 60 °C
Detection: RI @ 40 °C
Instrument: Shimadzu® Prominence™ LC-20A System
Sample: 1. Dp4+
2. Dp3
3. Maltose
4. Glucose
5. Lactic acid
6. Glycerol
7. Acetic acid
8. Ethanol

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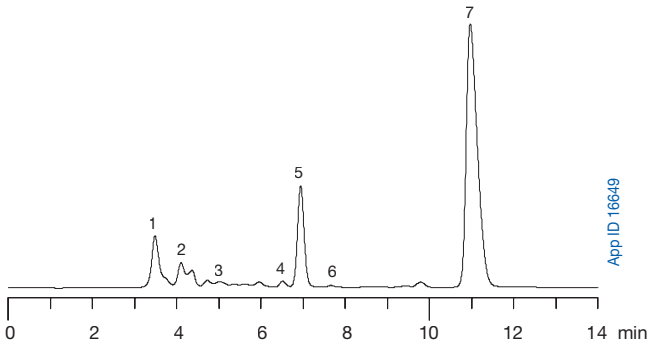
Figure 4.
A late fermentation timepoint run on a 150 x 7.8 mm Rezex ROA column. Note that the low level of remaining sugar peaks allow for some quantitation. The late eluting ethanol and organic acid peaks are well resolved on the shorter column with reduced run time.



Column: Rezex ROA-Organic acid
Dimensions: 150 x 7.8 mm
Part No.: 00F-0138-K0
Mobile Phase: 0.005 N Sulfuric acid
Flow Rate: 0.6 mL/min
Temperature: 60 °C
Detection: RI @ 40 °C
Instrument: Shimadzu® Prominence™ LC-20A System

Sample: 1. Dp4+
2. Maltose
3. Glucose
4. Lactic acid
5. Glycerol
6. Acetic acid
7. Ethanol

Conclusions

The standard HPLC column used for fermentation monitoring of bioethanol production is a Rezex ROA 300 x 7.8 mm column. This column is ideal for monitoring sugars, organic acids, and alcohols generated during bioethanol production all in one run. However, with the increase of bioethanol production, many producers are now using the shorter Rezex ROA 150 x 7.8 mm column to reduce analysis time increase productivity and to allow for more real-time responses of bacterial infection.

References

1. Unknown. *US Ethanol Production.* Retrieved October 28, 2010 from http://www.ethanol.org/index.php?id=37&parentid=8
2. G.C. Huber; G.K. Bonn. *HPLC of Carbohydrates with Cation-and Anion-Exchange Silica and Resin-Based Stationary Phases in Carbohydrate Analysis.* Chromatography Library, Vol. 58, 1995, 147-180
3. J. Mott. *Using the HPLC System in a Fuel Grade Ethanol Production Laboratory*. Shimadzu Scientific Corporation Application Note, 2006

Ordering Information

Rezex Columns					Guards		SecurityGuard™ Cartridges (mm)
							4 x 3.0
Description	Part No.	Cross Linkage	Ionic Form	Size (mm)	Part No.	Size (mm)	/10 pk
RCM-Monosaccharide	00H-0130-K0	8 %	Calcium	300 x 7.8	03B-0130-K0	50 x 7.8	AJ0-4493
RHM-Monosaccharide	00H-0132-K0	8 %	Hydrogen	300 x 7.8	03B-0132-K0	50 x 7.8	AJ0-4490
RAM-Carbohydrate	00H-0131-K0	8 %	Silver	300 x 7.8	—	—	AJ0-4491
RSO-Oligosaccharide	00P-0133-N0	4 %	Silver	200 x 10.0	03R-0133-N0	60 x 10.0	—
RNO-Oligosaccharide	00P-0137-N0	4 %	Sodium	200 x 10.0	03R-0137-N0	60 x 10.0	—
RPM-Monosaccharide	00H-0135-K0	8 %	Lead	300 x 7.8	03B-0135-K0	50 x 7.8	AJ0-4492
(for USP procedure)	00D-0135-K0	8 %	Lead	100 x 7.8	03B-0135-K0	50 x 7.8	AJ0-4492
RNM-Carbohydrate	00H-0136-K0	8 %	Sodium	300 x 7.8	03B-0136-K0	50 x 7.8	—
ROA-Organic Acid	00H-0138-K0	8 %	Hydrogen	300 x 7.8	03B-0138-K0	50 x 7.8	AJ0-4490
ROA-Organic Acid	00F-0138-K0	8 %	Hydrogen	150 x 7.8	03B-0138-K0	50 x 7.8	AJ0-4490
ROA-Organic Acid	00G-0138-E0	8 %	Hydrogen	250 x 4.6	—	—	AJ0-4490
RKP-Potassium	00H-3252-K0	8 %	Potassium	300 x 7.8	—	—	—
RFQ-Fast Acid	00D-0223-K0	8 %	Hydrogen	100 x 7.8	03B-0223-K0	50 x 7.8	AJ0-4490
							for ID: 3.2-8.0 mm

SecurityGuard Analytical Cartridges require universal holder Part No.: KJ0-4282

4 mm Diameter
for ≤ 2 mL sample volumes

15 mm Diameter
for 2 - 10 mL sample volumes

26 mm Diameter
for 10 - 100 mL sample volumes

Phenex™ Syringe Filters
Filter your samples for more consistent, reproducible results and increased column lifetime.

Membrane Type/Size	Part No.	Unit	Part No.	Unit	Part No.	Unit
0.20 µm (non-sterile)						
Phenex-RC	AF0-3203-12	100/ pk	AF0-2203-12	100/ pk	AF0-8203-12	100/ pk
(Regenerated Cellulose)	AF0-3203-52	500/ pk	AF0-2203-52	500/ pk	AF0-8203-52	500/ pk

guarantee

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