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Fast HPLC Analysis for Fermentation Ethanol Processes

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Abstract

The production of ethanol from a renewable resource such as corn utilizes a fermentation process with enzymes and yeast to convert starches and sugars to ethanol. To improve the productivity of the fermentation process, certain stress factors affecting the activity of the yeast are carefully managed throughout the process. These include monitoring the relative concentrations of glucose, ethanol, lactic acid and acetic acid. This information is then used to optimize fermentation conditions. HPLC analysis can easily provide information about the critical components of fermentation and is widely used throughout the U.S. in producer labs. Typical HPLC run times are 20 to 30 minutes. This application note describes the use of a Waters Breeze HPLC System in obtaining the necessary information in 10 minutes. With HPLC run times reduced by more than 50%, plant operators can determine component concentrations more quickly, resulting in superior fermentation process control. Consequently, plant managers have the potential to increase ethanol plant productivity and reduce loss due to fermentation failure.

Introduction

Legislation and public interest for alternative fuels call for increasing the use of ethanol/gasoline blends in the transportation industry. While some regions of the world are driving to limit fossil fuel consumption in non-transportation related applications or reduce greenhouse gas emissions, others are focused on reducing their energy dependency on imported oil[1]. The United States produced over 3.9 billion gallons of ethanol in 2005, about a 120% increase from 2001. There are more than 100 ethanol biorefineries in 19 states across the country and more than an additional 110 worldwide. In the U.S. alone, the biore-fineries have the capacity to produce more than 4.7 billion gallons of ethanol a year.[2] The production of ethanol from a renewable resource such as corn utilizes a fermentation process with enzymes and yeast to convert starches and sugars to ethanol. To improve the productivity of the fermentation process, certain stress factors affecting the activity of the yeast are carefully managed throughout the process. These include monitoring the relative concentrations of glucose, ethanol, lactic acid, and acetic acid.[3]

This information is then used to optimize fermentation conditions. HPLC analysis can easily provide information about the critical components and is widely used throughout the U.S. in producer labs. Typical HPLC run times are 20 to 30 minutes.[4]

This note describes using a Waters Breeze HPLC System to obtain the necessary information in 10 minutes. With HPLC run times reduced by more than 50%, plant operators can determine component concentra-tions more quickly, resulting in superior fermentation process control. Consequently, plant managers have the

potential to increase ethanol plant productivity and reduce loss due to fermentation failure.

Experimental

LC conditions

Breeze system	1515 HPLC pump[5], 717 plus autosampler, external column heating module, 2414 RI detector, Breeze software.
Column:	7.8 x 150 mm IC-Pak 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 seconds
Sampling rate:	5 pts/second
RI detection temp:	30 °C

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 (Milwaukee, WI). The Ethanol Industry Standard was purchased from Midland Scientific (Omaha, NE). Dextrin is a mixture of polysaccharides containing a small amount of low molecular weight polysaccharides6. Chromatography indicated 92.1% polysaccharide with more than 3 glucose units, 2.7% maltotriose (3 glucose units), 1.7% maltose (2 glucose units), 2.6% glucose, and 0.9% unidentified oligosaccharide7. This data was used in to calculate the standard concentrations.

Preparation of Standard Solutions

The stock standard mixture solution was prepared by weighing a certain amount of each component into a 25 ml volumetric flask and then add-ing de-ionized water (Millipore Milli-Q) to the mark. The stock standard solution was then diluted to make a series of standard solutions. For example, the original stock solution was diluted to 5%, 10%, 30%, 50%, and 70% of the original concentration. All standard solutions and samples were filtered using GHP Acrodisc, 0.45 µm, 25 mm diam-eter syringe filters (WAT200514).

Mobile Phase

Dilute sulfuric acid (0.50 mM) was prepared by a two-step dilution. First, about 1600 ml de-ionized water were added to a 2000 ml volumetric flask. Then, using a 10 ml pipette (calibrated in 1/10), 5.5 ml 96.0% sulfuric acid was transferred into the 2000 ml volumet-ric flask. The flask was filled to the 2000 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

Ethanol fermentation broth contains mostly sugars and alcohols. The major components of interest are dextrin, maltotriose, maltose, glu-cose, lactic acid, glycerol, acetic acid, and ethanol. The fast separation shown in Figure 1 was optimized for these components on a Waters system by changing a combination of column dimensions, column temperature, and concentration of the mobile phase and flow rate (see Experimental). The peaks were identified separately by obtaining chromatograms of the individual components under the same condi-tions. The calibration curves were generated automatically in Breeze software from the chromatograms of a series of standard mixtures at several concentrations. Table 1 summarizes calibration information.

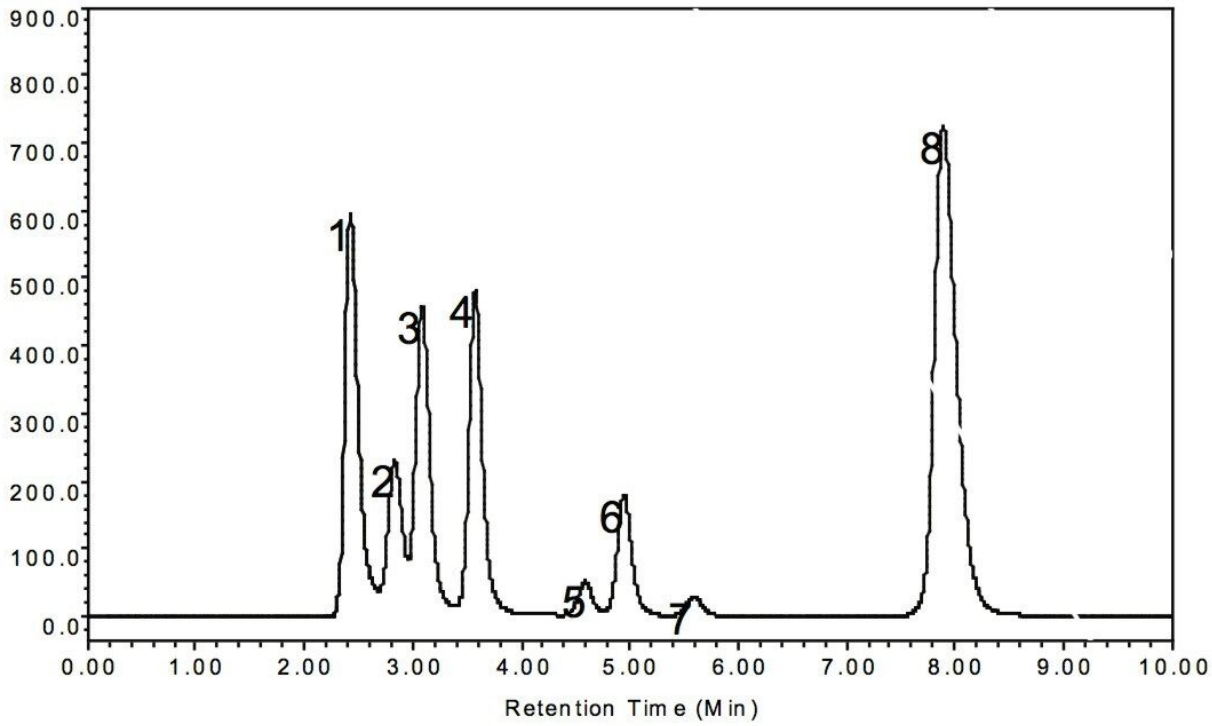

Figure 1. HPLC separation of 8 major fermenting mash components: 1. dextrin, 2. maltotriose, 3. maltose, 4. glucose, 5. lactic acid, 6. glycerol, 7. acetic acid, and 8. ethanol.

The relationship between peak area and the concentration was linear over the entire concentration range examined. (Peak height vs. con-centration was also linear over the same range). Figure 2 is a typical calibration curve.

To test this fast HPLC methodology, six injections of a commercial Industrial Fuel Ethanol Standard were quantified and the results com-pared with the label values from the vendor. The results are well within an acceptable +/-10% (Table 2).

Compound	Retention Time (min)	Concentration Range (g/100ml)	R^2	Slope ($x10^5$)	Intercept ($x10^3$)
Dextrin	2.43	0.169 - 3.65	0.9992	17.4	13.4
Maltotriose	2.83	0.0519 -1.12	0.9998	21.1	0.03
Maltose	3.08	0.118 - 2.55	0.9999	22.5	-5.58
Dextrose	3.57	0.111 - 2.39	0.9999	23.1	-8.92
Lactic Acid	4.59	0.0169 -0.363	0.9999	16.4	-2.14
Glycerol	4.95	0.0608 -1.31	0.9999	17.0	-7.49
Acetic Acid	5.59	0.0173 -0.372	0.9999	12.1	-8.31
Ethanol	7.89	0.697 - 5.00	0.9999	8.79	64.1

Peak Area = (standard concentration × slope) + intercept

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

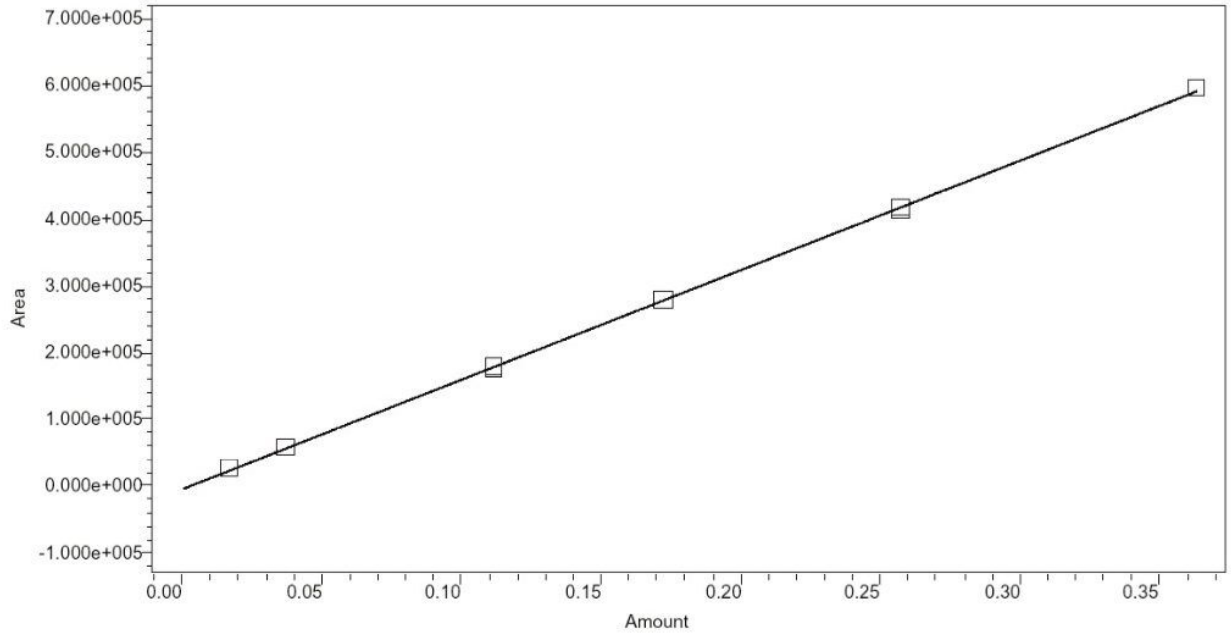

Figure 2. Lactic acid calibration curve, R2 = 0.9999.

Component	Vendor's Value (g/100ml)	Analysis Results[1] (g/100ml)	Relative error (%)
Dextrin	3.25	3.076 ±0.017	-5.35
Maltotriose	1.00	1.073 ±0.003	7.30
Maltose	2.00	1.976 ±0.009	-1.20
Glucose	2.00	1.909 ±0.007	-4.55
Lactic Acid	0.30	0.305 ±0.001	1.67
Glycerol	1.00	1.046 ±0.004	4.60
Acetic Acid	0.30	0.289 ±0.002	-3.67
Ethanol	12.00	12.14 ±0.04	1.17

1: Results of 6 measurements. The standard deviation is also shown as the measurement uncertainty.

Table 2. Commercial industrial fuel ethanol standard comparison.

Acid peak retention times are affected by the pH (the sulfuric acid concentration) of the mobile phase. Another test of this fast HPLC methodology included examining the effect of small changes in the mobile phase concentration on peak retention times. Experiments were run with mobile phases ranging from 90% (0.452 mM sulfuric acid) to 103% (0.515 mM sulfuric acid) of the recommended 0.50 mM sulfuric acid mobile phase concentration. At least 20 injections were made and results calculated for each mobile phase concentration. The Breeze report in Figure 3 is an example with 4 different mobile phase concentrations showing mean, standard deviation and %RSD results for retention time components 1-8. Figure 4 summarizes the retention time data for the mobile phase concentrations examined and indicates minimal variation.

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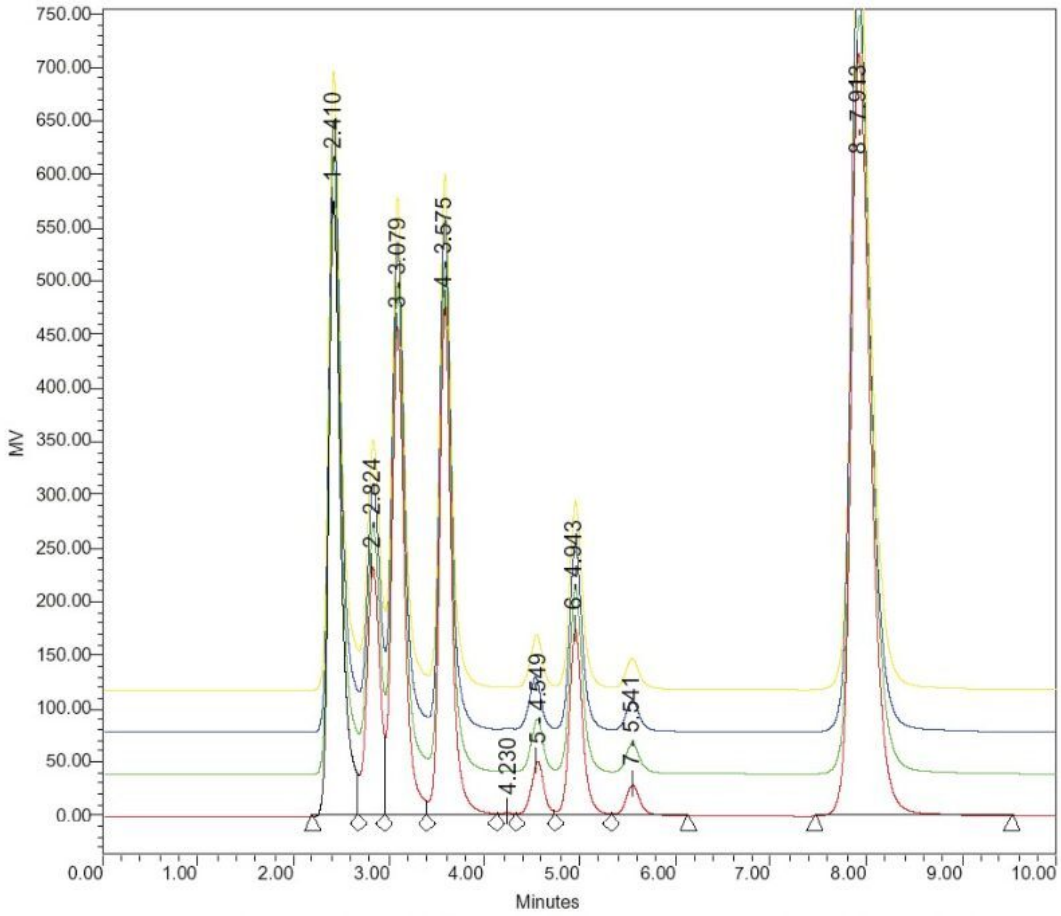

Component Summary For Retention Time

	Sample Name	Inj.	Channel	Vial	1	2	3	4	5	6	7	8
1	0452mM	1	410	42	2.413	2.825	3.080	3.575	4.526	4.943	5.537	7.907
2	0515mM	1	410	42	2.410	2.824	3.079	3.575	4.549	4.943	5.541	7.913
3	0479mM	1	410	42	2.411	2.825	3.080	3.575	4.537	4.943	5.537	7.912
4	0497mM	1	410	42	2.410	2.824	3.080	3.575	4.544	4.944	5.538	7.916
Mean					2.411	2.824	3.080	3.575	4.539	4.943	5.538	7.912
Std. Dev.					0.001	0.001	0.000	0.000	0.010	0.000	0.002	0.003
% RSD					0.1	0.0	0.0	0.0	0.2	0.0	0.0	0.0

Figure 3. Breeze report.

Table 3 summarizes the concentration determined for the commercial industrial fuel ethanol standard and overall, the largest peak retention time shift is within ±0.7% of the mean retention time and the component concentration results are within ±8.7% of the reference values.

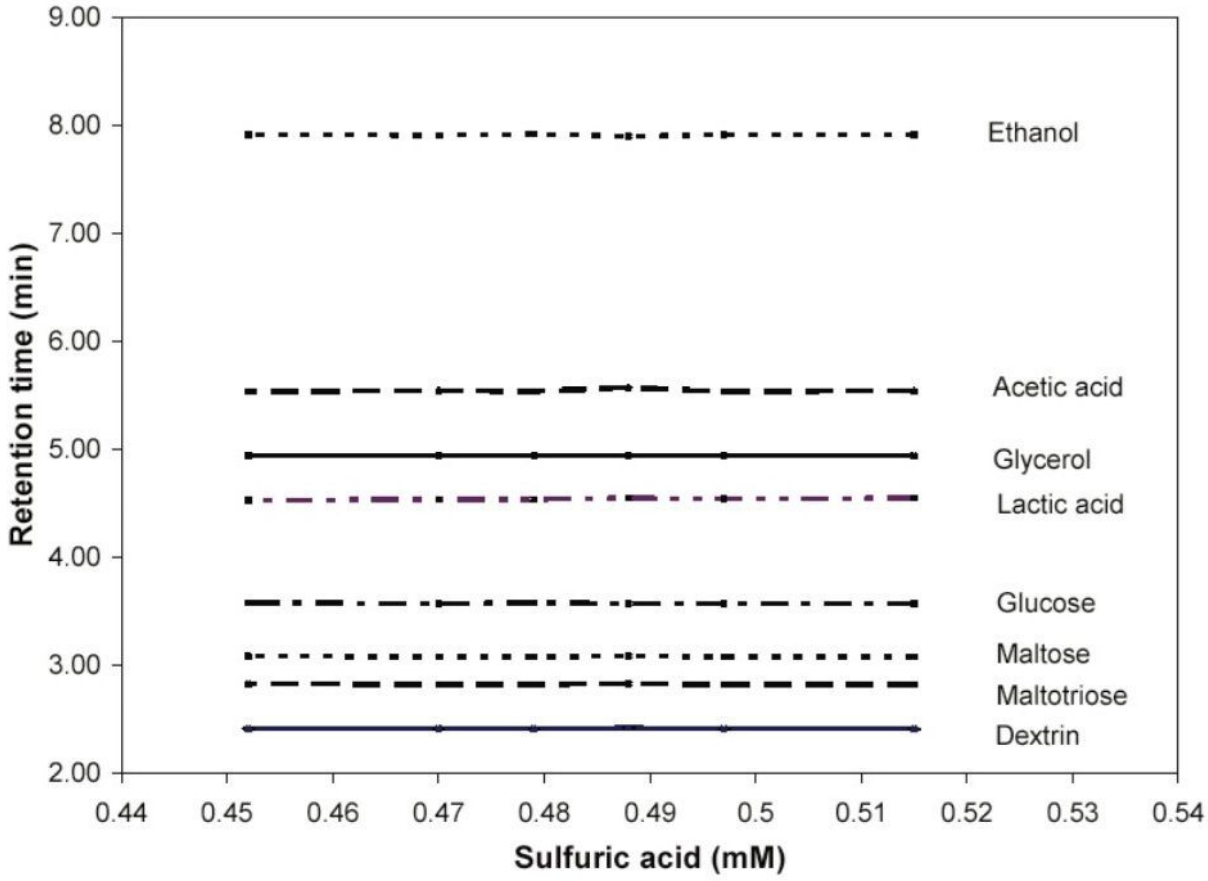

Figure 4. Retention time reproducibility summary.

Component	Concentrations (in g/100ml) measured in different sulfuric acid concentrations[1]					
	0.452mM[2]	0.470mM[3]	0.479mM[2]	0.488mM[2]	0.497mM[4]	0.515mM[3]
Dextrin	2.999 ± 0.020	3.008 ± 0.013	3.018 ± 0.026	2.983 ± 0.026	3.034 ± 0.013	2.995 ± 0.023
Maltotriose	1.064 ± 0.008	1.068 ± 0.006	1.066 ± 0.008	1.061 ± 0.008	1.074 ± 0.005	1.064 ± 0.009
Maltose	1.937 ± 0.012	1.950 ± 0.009	1.942 ± 0.016	1.948 ± 0.014	1.958 ± 0.009	1.932 ± 0.014
Glucose	1.870 ± 0.012	1.885 ± 0.009	1.875 ± 0.015	1.883 ± 0.013	1.892 ± 0.008	1.865 ± 0.015
Lactic Acid	0.306 ± 0.002	0.307 ± 0.002	0.304 ± 0.002	0.304 ± 0.002	0.304 ± 0.001	0.301 ± 0.002
Glycerol	1.017 ± 0.006	1.026 ± 0.005	1.022 ± 0.008	1.024 ± 0.007	1.030 ± 0.005	1.016 ± 0.008
Acetic Acid	0.275 ± 0.002	0.280 ± 0.003	0.274 ± 0.002	0.280 ± 0.002	0.276 ± 0.002	0.274 ± 0.003
Ethanol	11.930±0.072	12.025±0.057	12.016±0.083	12.005±0.088	12.065±0.057	11.935±0.097

1: The standard deviation is shown as uncertainty. 2: Number of measurement is 30. 3: Number of measurement is 20. 4: Number of measurement is 21.

Table 3. Repeatability using several mobile phase concentrations.

Conclusion

A 10 minute HPLC analysis of eight major fuel ethanol fermentation components can be reliably performed on a Waters Breeze HPLC system. The resolution in a typical commercial industrial fuel ethanol standard provides precise, accurate quantitative results. Small changes in mobile phase composition that are larger than expected during mobile phase preparation still provide results within acceptable limits.

In addition to providing quality data, the Waters methodology that utilizes an IC-Pak Ion Exclusion column requires less than 50% of the time of current HPLC analyses. Faster analysis times may translate to superior process control, increased productivity, and reduced product loss for the ethanol plant.

References

1. 1. Hileman, B. Energy for a Sustainable Future, C&EN. 84:7, 70-75, 2006.
2. Renewable Fuels Association Home Page: www.ethanolrfa.org.
3. Kohl, S. “Ethanol 101-5: Managing Stress Factors”, Scott Kohl, *Ethanol Today*, 1:36-37, 2004.
4. Optimizing Ethanol Production with High Performance Liquid Chromatography, Waters Application Note no. 720000455EN.

5. Data were acquired using a 1525 Binary pump because the system was available. Since this method uses isocratic elution, a 1515 HPLC pump is suitable for this application.
6. Personal communication with vendor; product contains approximately 10% low molecular weight fraction.
7. 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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1515 Isocratic HPLC Pump <https://www.waters.com/534311>

2414 Refractive Index (RI) Detector <https://www.waters.com/514425>

Available for purchase online:

Ion Exclusion, 7 µm, 7.8 x 150 mm Column <
https://www.waters.com/waters/partDetail.htm?partNumber=WAT010295>

Shodex SH-G, 7 µm, 6 x 50 mm Guard Column <
https://www.waters.com/waters/partDetail.htm?partNumber=WAT034243>

Acrodisc, Syringe Filter <https://www.waters.com/waters/partDetail.htm?partNumber=WAT200514>

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