Rapid Analysis of Sugars, Organic Acids, and Alcohols

Introduction

Bioethanol production process requires frequent rapid analyses of sugars, organic acids, and alcohols to monitor the proper operations of biomass fermentation steps. There are seven main target analytes: two maltooligosaccharides, maltotriose and maltose, its constituent sugar, glucose, and the final products, ethanol, lactic acid, acetic acid, and glycerol.

In this work, we used a Shodex™ SUGAR™ SH1011 8C for the analysis of those seven target components. Shodex™ SUGAR™ SH1011 8C is a 10-cm rapid analysis column filled with rigid styrene-divinylbenzene copolymer base material. The strong cation exchange gel works to provide a mixed mode of size exclusion and ion exclusion chromatography, which is suitable for simultaneous analysis of various sugars and organic acids. The analysis time of Shodex™ SUGAR™ SH1011 8C is one thirds of our regular 30-cm analysis column, Shodex™ SUGAR™ SH1011. Thus, it is well suited for rapid analyses of sugars and organic acids.

Column Specifications and Usable Conditions

1. Column specifications

Column Size (mm) I.D. x Length	Particle Size (μm)	Theoretical Plate Number (TPN/column)	Exclusion Limit (Pullulan)	Shipping Solvent
8.0 x 100	6	≥ 5,000	1,000	Water

Base Material
: Spherical porous particles of styrene divinylbenzene copolymer modified with sulfo group

Column Housing
: SUS 316

2. Usable conditions

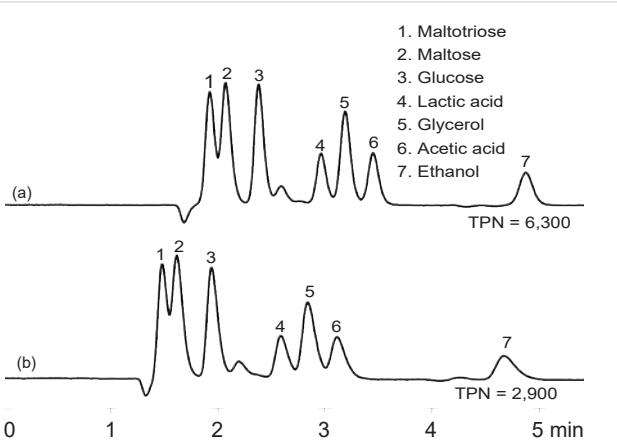
Flow Rate (mL/min)		Maximum Pressure (MPa)
Recommended	Maximum	
0.5 - 1.0	1.5	1.5

Special Features

1. Rapid analysis

Figure 1 shows an example of rapid analysis. Seven components were analyzed at 1.0 mL/min flow rate.

We achieved an analysis time under 6 minutes per sample with using an aqueous solution of sulfuric acid eluent and 65 °C column temperature. Then, we compared Shodex™ SUGAR™ SH1011 8C with other manufacturer's 10-cm length ion exclusion column by adjusting their line velocities. The theoretical plate number (TPN) of SH1011 8C for ethanol was over two times higher than that of other manufacturer's column.



Sample : 0.1 % each, 5 μL
Column : (a) Shodex SUGAR SH1011 8C (8.0 mm I.D. x 100 mm, 6 μm)
(b) Ion exclusion column from other manufacturer (7.8 mm I.D. x 100 mm, 9 μm)
Eluent : 1 mM H_2SO_4 aq.
Flow rate : (a) 1.0 mL/min, (b) 0.95 mL/min
Instrument : Shimadzu Prominance-i
Detector : RI
Column temp.: 65 °C

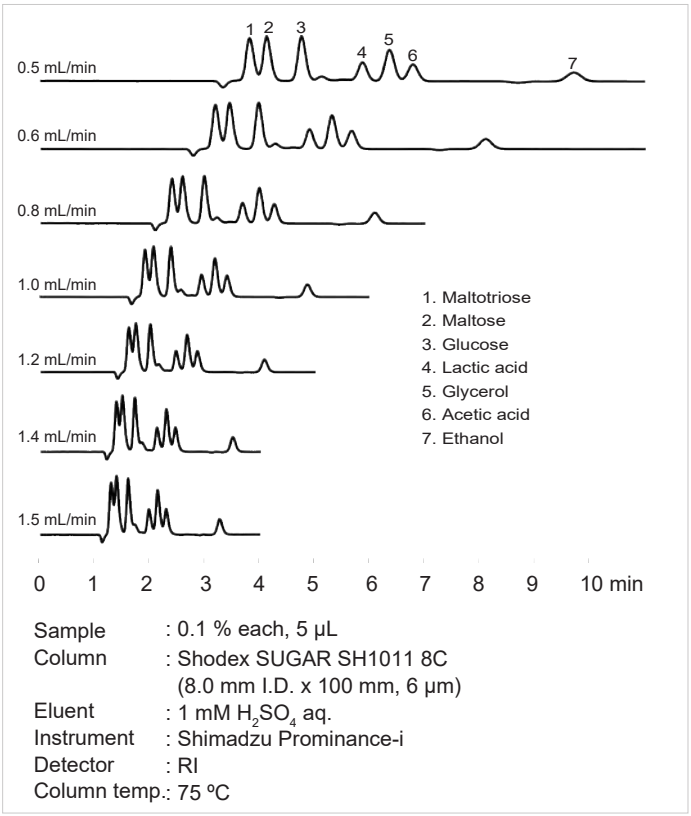
Fig. 1. Rapid analysis of sugars, organic acids, and alcohols

2. Effects of eluent flow rate

Rapid analyses of sugars, organic acids, and alcohols are required to monitor proper biomass fermentation process during the bioethanol production as they provide the decision factors to move on to the next production steps.

The effects of flow rate was studied in the flow rate range between 0.5 and 1.5 mL/min (Fig. 2). A rapid analysis within four minutes per sample was achieved under the maximum flow rate condition of Shodex™ SUGAR™ SH1011 8C (1.5 mL/min).

Figure 3 shows TPNs of ethanol obtained at each flow rate. Only a slight TPN decrease was observed at 1.5 mL/min flow rate.



Sample : 0.1 % each, 5 µL
Column : Shodex SUGAR SH1011 8C (8.0 mm I.D. x 100 mm, 6 µm)
Eluent : 1 mM H_2SO_4 aq.
Instrument : Shimadzu Prominance-i
Detector : RI
Column temp.: 75 ºC

Fig. 2. Effects of flow rate

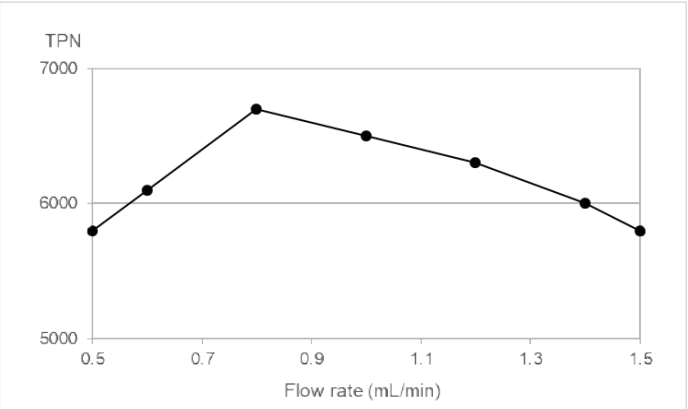
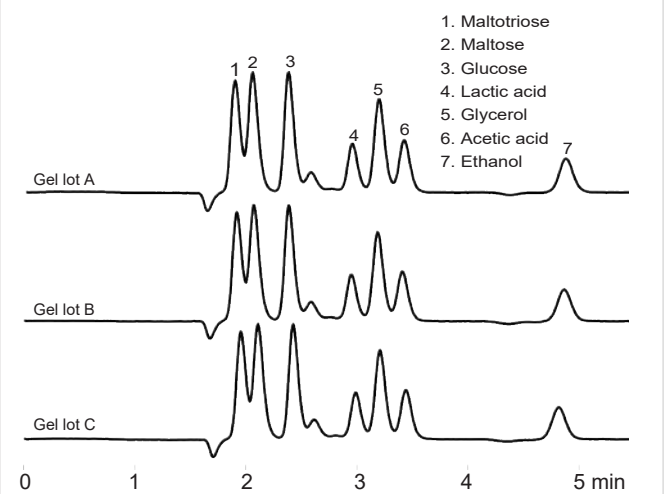


Fig. 3. TPNs of ethanol at different flow rate

3. Gel-to-gel lot reproducibility

The separation patterns and retention times of columns prepared from three different gel-lot base materials were compared (Fig. 4). The results demonstrated good reproducibility among the three gel lot columns.

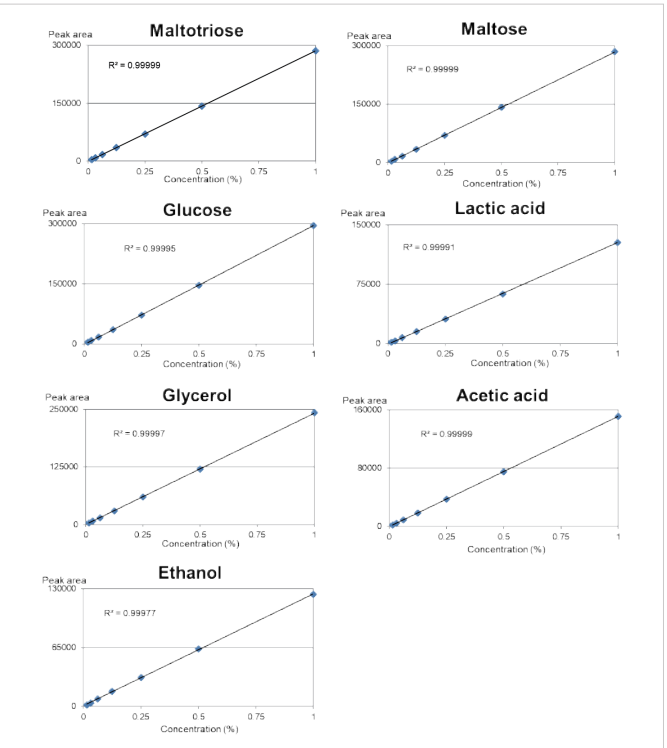


Refer to Fig.2 for the analytical condition used,
Flow rate: 1.0 mL/min

Fig. 4. Chromatograms of columns prepared from three different gel-lot base materials

4. Calibration curve

Good linearities were obtained for the calibration curves in the concentration range 0.02 to 1 % (Fig. 5). The coefficient of determination (R^2) was over 0.999 for all target analytes.

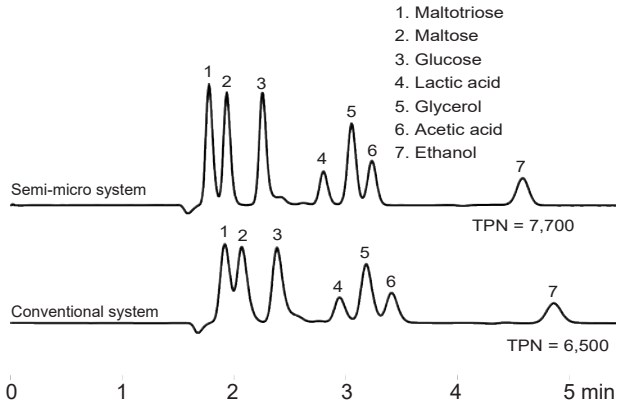


Refer to Fig. 2. for the analytical condition used,
Flow rate: 1.0 mL/min

Fig. 5. Calibration curves of the target analytesd

5. Comparisons of conventional and semi-micro HPLC systems

Shodex™ SUGAR™ SH1011 8C can be used with a conventional HPLC system. However, separations of oligosaccharides are improved when using a semi-micro HPLC system. Figure 6 shows the comparisons of chromatograms obtained by conventional and semi-micro HPLC systems. It shows that the peak shapes of all analytes were sharper when using a semi-micro system. Also, the separation between maltotriose and maltose was improved. The TPN of ethanol obtained was 1.2 times higher with the semi-micro system compared to that of conventional system.

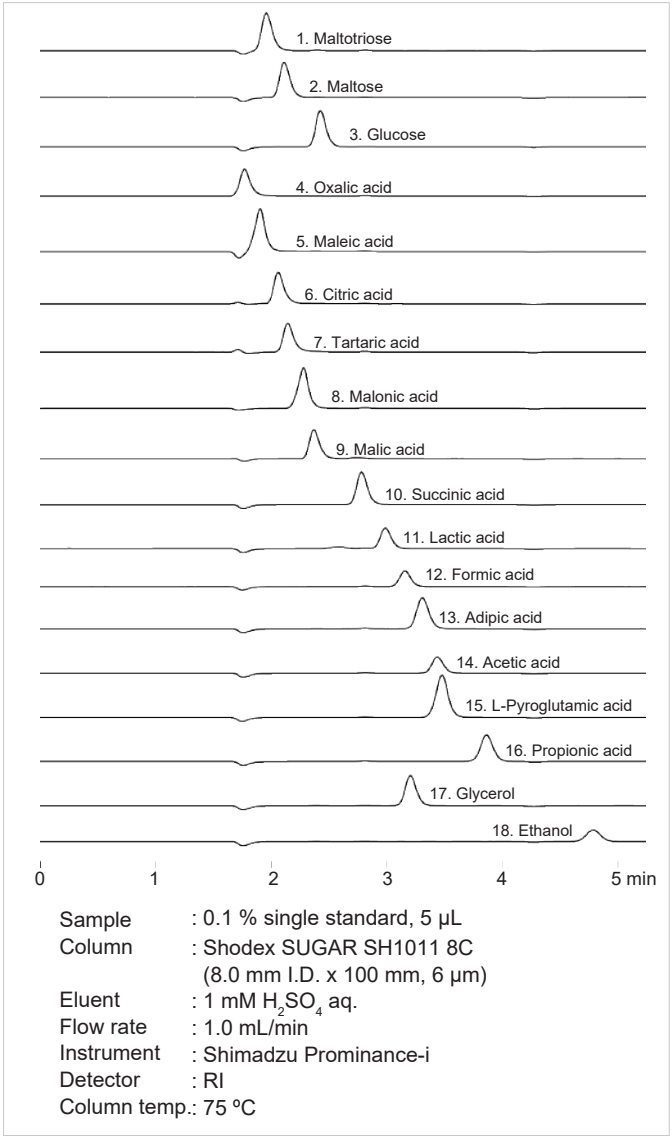


Sample : 0.1 % each, 5 µL
Column : Shodex SUGAR SH1011 8C (8.0 mm I.D. x 100 mm, 6 µm)
Eluent : 1 mM H_2SO_4 aq.
Flow rate : 1.0 mL/min
Instrument + Detector : Semi-micro; Shimadzu Nexera X2 + RI (small cell) Conventional; Shimadzu Prominance-i + RI
Column temp.: 75 ºC

Fig. 6. Comparisons of conventional and semi-micro HPLC systems

6. Retention of sugars, organic acids, and alcohols

Figure 7 shows chromatograms of 3 sugars, 13 organic acids, and 2 alcohols. Table 1 summarizes the retention times of each analyte at two sulfuric acid concentrations. Concentration of sulfuric acid in eluent does not affect the retention of sugars and alcohols much, but does affect the retention of organic acids slightly. Therefore, concentration of sulfuric acid should be adjusted depends on the type of organic acids present in the sample.



Sample : 0.1 % single standard, 5 µL
Column : Shodex SUGAR SH1011 8C (8.0 mm I.D. x 100 mm, 6 µm)
Eluent : 1 mM H_2SO_4 aq.
Flow rate : 1.0 mL/min
Instrument : Shimadzu Prominance-i
Detector : RI
Column temp.: 75 ºC

Fig. 7. Chromatograms of various sugars, organic acids, and alcohols

Table 1. Effects of sulfuric acid concentration on retention time (Rt)

(i) Sugars

Sample	Rt (min) Sulfuric acid conc.		Rt ratio (%) (b) / (a)
	(a) 1 mM	(b) 5 mM	
Maltotriose	1.96	1.96	100.15
Maltose	2.11	2.12	100.19
Glucose	2.43	2.43	100.12

(ii) Organic acids

Sample	Rt (min) Sulfuric acid conc.		Rt ratio (%) (b) / (a)
	(a) 1 mM	(b) 5 mM	
Oxylic acid	1.77	1.85	104.47
Maleic acid	1.91	2.15	112.65
Citric acid	2.06	2.19	106.02
Tartaric acid	2.14	2.29	106.86
Malonic acid	2.28	2.50	109.69
Malic acid	2.37	2.47	104.30
Succinic acid	2.78	2.81	101.11
Lactic acid	2.99	3.06	102.28
Formic acid	3.16	3.25	102.88
Adipic acid	3.31	3.33	100.63
Acetic acid	3.44	3.45	100.32
L-Pyroglutamic acid	3.48	3.76	107.90
Propionic acid	3.87	3.88	100.23

(iii) Alcohols

Sample	Rt (min) Sulfuric acid conc.		Rt ratio (%) (b) / (a)
	(a) 1 mM	(b) 5 mM	
Glycerol	3.21	3.21	100.06
Ethanol	4.79	4.79	100.04

Conclusions

The Shodex™ SUGAR™ SH1011 8C, a 10-cm length column of our regular SH1011 analytical column, is feasible analyzing seven components which are often required to be monitored during the bioethanol production. By using 1.0 mL/min flow rate, an analysis is completed within 6 minutes. This rapid analysis should contribute to reduce operation time, and consequently to reduce the cost and improve the productivities, especially in the areas of components' concentration control during the production and QC step which requires frequent multiple sample analysis.

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