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Application News

High Performance Liquid Chromatograph Nexera™ lite

Fermentation Monitoring of Yeast Using Size Exclusion-ligand Exchange (Na-type) Column

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User Benefits

- Oligosaccharides, monosaccharides, and sugar alcohols of less than trisaccharide can be separated.
- There is no need to prepare complicated mobile phase since just water can be used as mobile phase for HPLC analysis.
- The variations of saccharides and ethanol in the fermentation process caused by microbes can be monitored.

■ Introduction

Quantitative determinations of saccharides are performed in various fields, including food products. Especially in the production of alcoholic beverages and biofuels, where ethanol is produced from saccharides through microbial fermentation, it is important to perform quantitative analysis and monitoring of saccharides accurately for process design and quality control purposes.

Shim-pack™ SUR-Na ligand exchange chromatography column provides the combination of two separation modes of size exclusion and sodium-type ligand exchange to analyze saccharides with high separation performance. There is no need for mobile phase preparation since only water is used as the mobile phase, resulting in easy execution of saccharide analysis.

■ Analysis of standard mixture

Fig. 1 shows the flow path diagram of this system setup. Shim-pack SUR-Na requires water as the mobile phase to separate saccharides, it allows to employ the simple system configuration of the Nexera lite isocratic setup connected with RID-20A differential refractive index detector.

Table 1 shows the analytical conditions. A retention index was created by analyzing twenty standard saccharides, involving oligosaccharides, monosaccharides, and sugar alcohols of less than trisaccharide.

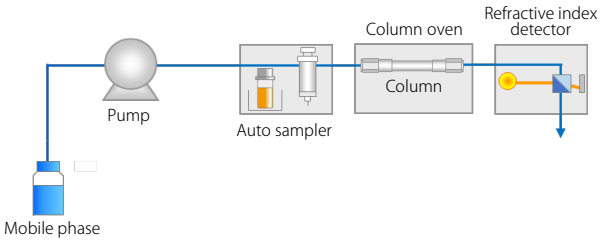


Fig. 1 Flow path diagram

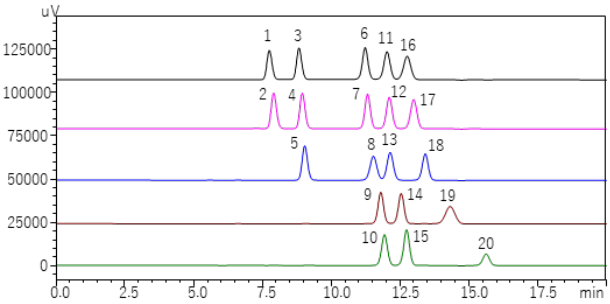
Table 1 Analytical conditions

System	:	Nexera lite
Column	:	Shim-pack SUR-Na *1 (250 mm × 7.8 mm I.D., 8 µm)
Guard Column	:	Shim-pack SUR-Na(G) *2 (50 mm × 7.8 mm I.D., 8 µm)
Mobile phase	:	Water
Column temp.	:	80 ℃
Flow rate	:	0.6 mL/min (Single column)
		0.5 mL/min (Double columns)
Injection vol.	:	20 µL (Retention index)
		2 µL (Standards, Samples)
Detection	:	Refractive index (RID-20A)

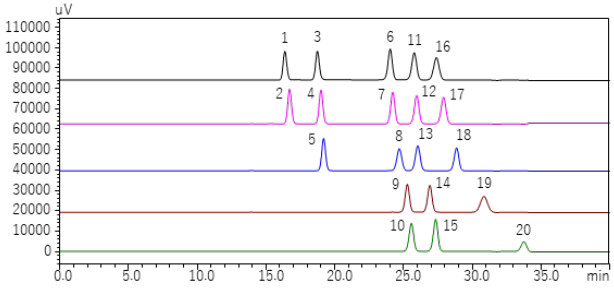
*1 P/N：S228-59529-01
*2 P/N：S228-59529-02

■ Retention index

The chromatogram for a single column is shown in Fig. 2(a), the chromatogram for two connected columns in Fig. 2(b), and the retention index in Table 2.



(a) Chromatograms using a single column setup



(b) Chromatograms using two columns connected in series

1. Raffinose
2. Maltotriose
3. Sucrose
4. Maltose
5. Lactose
6. Glucose
7. Mannitol
8. Rhamnose
9. Sorbitol
10. Galactose
11. Mannose
12. Fructose
13. Xylose
14. Xylitol
15. Inositol
16. Fucose
17. Arabinose
18. Glycerol
19. Ribose
20. Ethanol

(1 g/L, each)

Fig. 2 Chromatograms of twenty saccharides

Table 2 Retention index for twenty saccharides

	name	Single column (min)	Double columns (min)
1	Raffinose	7.71	16.40
2	Maltotriose	7.87	16.74
3	Sucrose	8.78	18.77
4	Maltose	8.90	19.03
5	Lactose	8.99	19.22
6	Glucose	11.18	24.06
7	Mannitol	11.27	24.25
8	Rhamnose	11.48	24.73
9	Sorbitol	11.75	25.31
10	Galactose	11.88	25.61
11	Mannose	11.97	25.82
12	Fructose	12.06	26.00
13	Xylose	12.09	26.07
14	Xylitol	12.50	26.95
15	Inositol	12.69	27.37
16	Fucose	12.70	27.43
17	Arabinose	12.95	27.95
18	Glycerol	13.37	28.90
19	Ribose	14.29	30.89
20	Ethanol	15.60	33.79

■ Calibration curves

Calibration curves were created for four of the twenty saccharides (sucrose, glucose, fructose, and ethanol) to determine the concentrations in samples. Calibration curves for respective saccharides are shown in Fig. 3. Good linearities were obtained with coefficients of determination of 0.999999 or more for all.

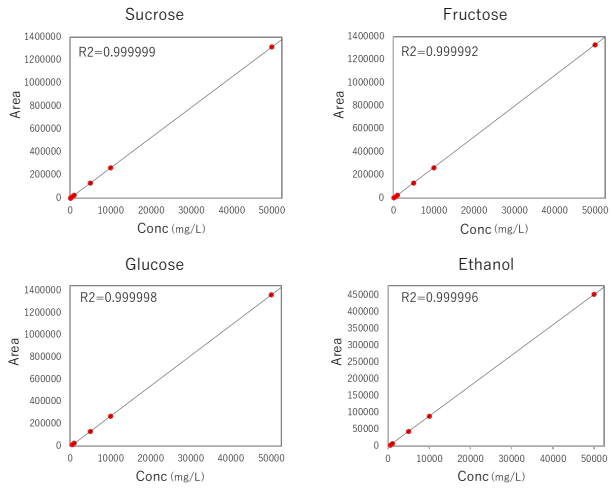


Fig. 3 Calibration curves

■ Analysis of yeast fermentation cultures

Glucose and fructose, which are monosaccharides, can be separated on Shim-pack SUR-Na by utilizing its two separation modes of size exclusion and ligand exchange.

Here, yeast fermentation culture in molasses was analyzed under the conditions of "single column" and "sample" in Table 1. Fig. 4 shows the chromatograms of four samples in different fermentation times.

Based on the quantitative determination results of sucrose, glucose, fructose, and ethanol, the fermentation process in which sucrose (disaccharide) was decomposed into monosaccharides glucose and fructose (both monosaccharides) then finally ethanol was yielded along with the fermentation time. Fig. 5 shows the results of plotting the concentration changes of each component from the quantitative results.

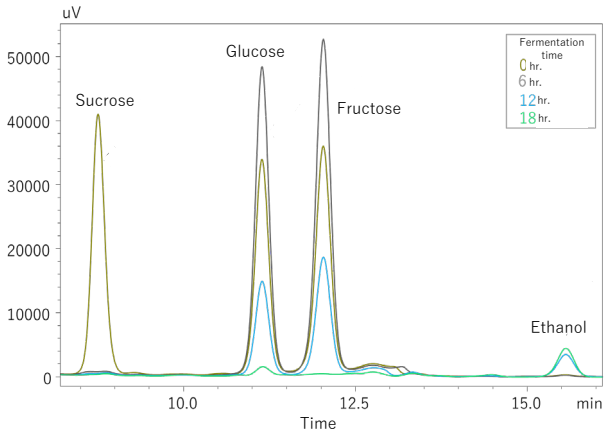


Fig. 4 Chromatogram of yeast fermentation culture

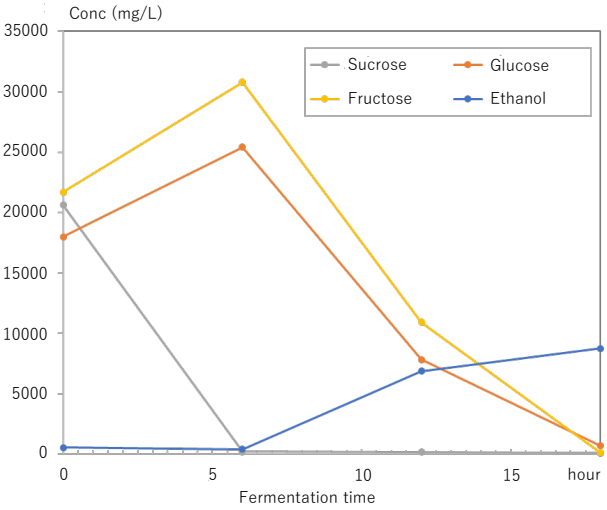


Fig. 5 Variations of concentration for respective saccharides

■ Conclusion

In this article, the retention index and the calibration curves for saccharides and sugar alcohols were created, and as an application, quantitative monitoring of fermentation process was performed based on HPLC analyses of the yeast fermentation culture. Since Shim-pack SUR-Na can easily analyze a variety of saccharides, it is expected to be used in a wide range of application fields, including the energy and food industries.

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