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Application News

High Performance Liquid Chromatograph

Analysis of Saccharides Using Integrated HPLC and Size Exclusion-Ligand Exchange (Na Type) Column

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User Benefits

- Saccharides contained in fruit juices can be analyzed simultaneously.
- There is no need to prepare the mobile phase since analysis can be done using only water as mobile phase.
- The integrated HPLC system allows for the installation of a refractive index detector without changing the footprint.

■ Introduction

Saccharides are the main component of sweetness in food and an important source of energy. However, excessive saccharide intake has been reported to increase the risk of lifestyle-related diseases such as obesity and dental caries[1)]. Monitoring the amount of saccharide in food plays an important role in quality control and nutritional management.

Saccharides have almost no ultraviolet absorption, so they are detected using differential refractive index detector, evaporative light scattering detector, a mass spectrometer, etc. Our integrated HPLC can be connected with these detectors. High column oven temperature such as around 80 ˚C required in size exclusion - ligand exchange mode is available because upper limit of controllable temperature is 90 ˚C. In this article, saccharide analysis in fruit juice using an integrated HPLC and differential refractive index detector is introduced.

■ Analyses of standard solutions

Shim-pack™ SUR-Na column was employed for this investigation. This column provides combined separation modes of size exclusion and sodium-type ligand exchange. Easy operation for HPLC analysis without preparing complicated mobile phase can be performed because of employing only water as the mobile phase. Fig. 1 shows the appearance of integrated HPLC employed for this study. Table 1 shows the analytical conditions.

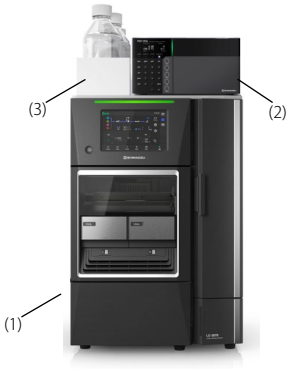

Fig. 1 Appearance of integrated HPLC
(1)LC-2070 (2)RID-20A (3)Reservoir tray

Table 1 Analytical conditions

System	: LC-2070A
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 temperature	: 80 ℃
Flow rate	: 0.6 mL/min
Injection volume	: 10 µL
Vial	: TORAST Vial *3
Detection	: Refractive index detector (RID-20A) Polarity +, Cell temp. 40 ℃, Response 1.5 sec

*1 P/N：S228-59529-01, *2 P/N：S228-59529-02, *3 P/N：GLCTV-901

Fig. 2 shows the chromatogram of a mixed standard solution of four saccharides (maltotriose, sucrose, glucose, and fructose).

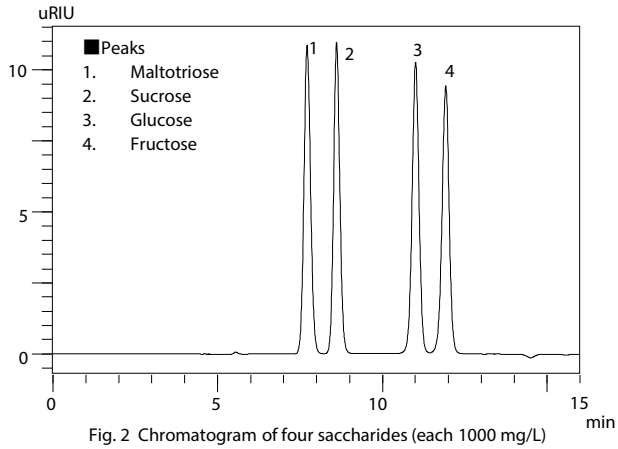

Fig. 2 Chromatogram of four saccharides (each 1000 mg/L)

■ Calibration curves

Table 2 shows the coefficients of determination of respective calibration curves for saccharides created from the analysis results of the mixed standard solution. Calibration curves were created within a concentration range of 20 to 2000 mg/L. All the calibration curves showed good linearity with the coefficients of determination $r^2 = 0.9999$ or higher.

Table 2 Coefficients of determination of calibration curves

Compound	Linearity(r^2)
Maltotriose	0.9999
Sucrose	0.9999
Glucose	0.9999
Fructose	0.9999

■ Analytical method robustness

Table 3 shows the relative standard deviations (RSDs) of retention time and peak area obtained by six times consecutive analyses of each 50 mg/L of mixed standard solution. Satisfactory results of less than 0.02% for retention time RSDs and less than 0.6% for peak area RSDs were obtained for all compounds.

Table 3 %RSD for six times consecutive analyses (Each 50 mg/L)

Compound	Retention time(%RSD)	Peak area(%RSD)
Maltotriose	0.02	0.35
Sucrose	0.02	0.45
Glucose	0.02	0.35
Fructose	0.02	0.52

■ Analyses of various types of fruit juice

Figs. 3, 4, 5, 6, and 7 show the chromatograms for orange juice, mandarin juice, grape juice, pineapple juice, and grapefruit juice respectively. Each juice was diluted 50 times with water, filtered through a 0.2 µm membrane filter*4, then subjected to HPLC analysis. Table 4 shows the saccharide contents of each juice.

*4 Pall Acrodisc 13 mm with 0.2 um PVDF P/N：4455T

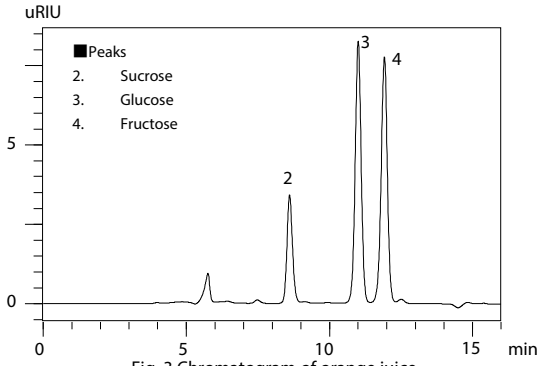

Fig. 3 Chromatogram of orange juice

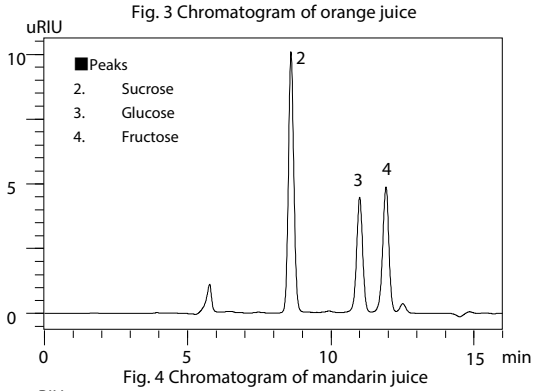

Fig. 4 Chromatogram of mandarin juice

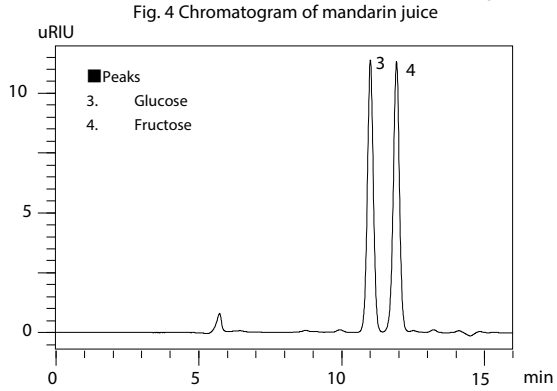

Fig. 5 Chromatogram of grape juice

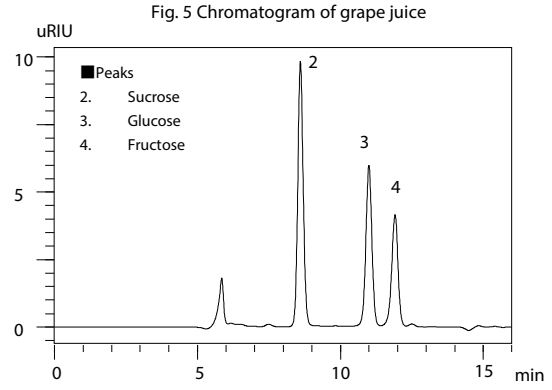

Fig. 6 Chromatogram of pineapple juice

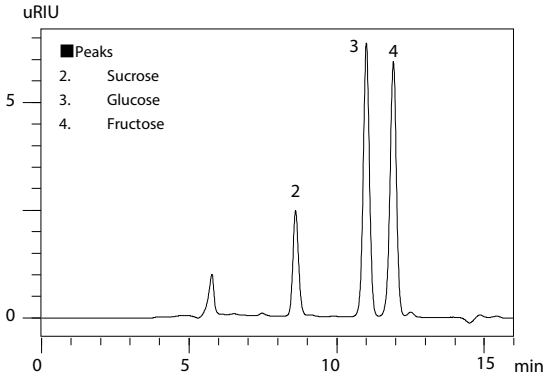

Fig. 7 Chromatogram of grapefruit juice

Table 4 Saccharide contents in each fruit juice

Juice	Sucrose	Glucose	Fructose
Orange	1.6×10^4	4.0×10^4	4.2×10^4
Mandarin	4.6×10^4	2.2×10^4	2.6×10^4
Grape	N.D.	5.6×10^4	6.0×10^4
Pineapple	4.5×10^4	2.9×10^4	2.3×10^4
Grapefruit	1.1×10^4	3.1×10^4	3.2×10^4

N.D.=Not Detected

500 mg/L of maltotriose, sucrose, glucose, and fructose were spiked to each juice and evaluated "spike and recovery". Table 5 shows the results of the spike and recovery test. We got satisfactory results with a recovery rate of 86-100% for all compounds.

Table 5 Spike and recovery rate from each fruit juice

Juice	Maltotriose	Sucrose	Glucose	Fructose
Orange	100	93	91	90
Mandarin	99	87	95	94
Grape	99	100	88	87
Pineapple	99	86	92	92
Grapefruit	98	93	92	91

■ Conclusion

Simultaneous analyses of saccharides in various types of juice were performed. The saccharides were detected using integrated HPLC connected to differential refractive index detector. It was possible to separate two monosaccharides of glucose and fructose by using Shim-pack SUR-Na having combined separation mode of size exclusion and ligand exchange as the separation column. Satisfactory results were obtained for the linearity of the calibration curves, the analytical method resulted very robust, showing excellent reproducibility and recovery rates.

<References>

1) World Health Organization (2015)
Guideline：Sugars intake for adults and children
〈http://apps.who.int/iris/bitstream/10665/149782/1/9789241549028_eng.pdf〉

<Related Applications>

1. Analysis of saccharides using integrated HPLC and size exclusion-ligand exchange (Ca type) column
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01-00931-EN First Edition: Sep. 2025

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