

# Application News

## No.L467

### High Performance Liquid Chromatography

## Analysis of Sugars in Orange Juice and Grape Juice by Prominence-i and Differential Refractive Index Detector

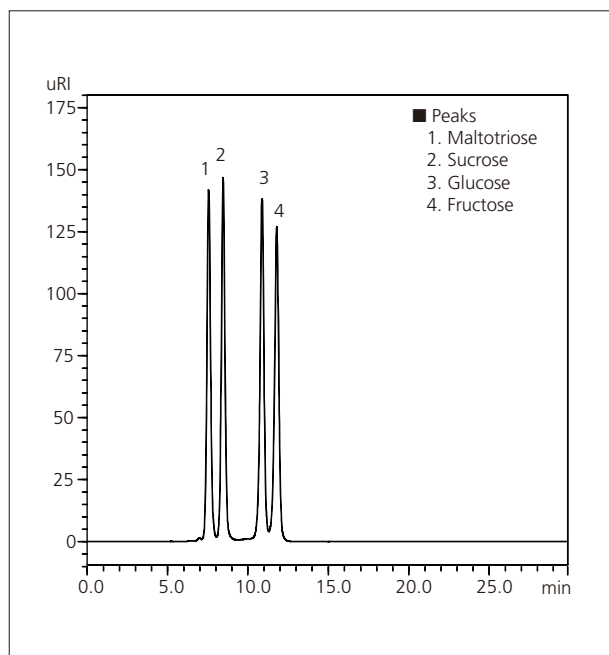
As sugars display little ultraviolet absorption, a differential refractive index detector or an evaporative light scattering detector is used for their detection.

The new Prominence-i integrated high-performance liquid chromatograph can be connected to the RID-10A differential refractive index detector. Since the column oven can accommodate a 30-cm column for use in sugar analysis (ligand exchange column), and the temperature can be controlled up to 85 °C, it therefore supports applications that require a long column and high column temperature.

Here, we introduce an example of sugar analysis in juices using the Prominence-i with the RID-10A.

### ■ Analysis of Sugar Standard Solution

Fig. 1 shows the results of analysis of a standard mixture of four sugars (maltotriose, sucrose, glucose, fructose) using a 10 µL injection (each at 20 g/L). The analytical conditions were as shown in Table 1. For the analytical column, we used the Shim-pack SCR-101N, a specialized sugar-analysis column that supports both the gel filtration and ligand exchange modes.



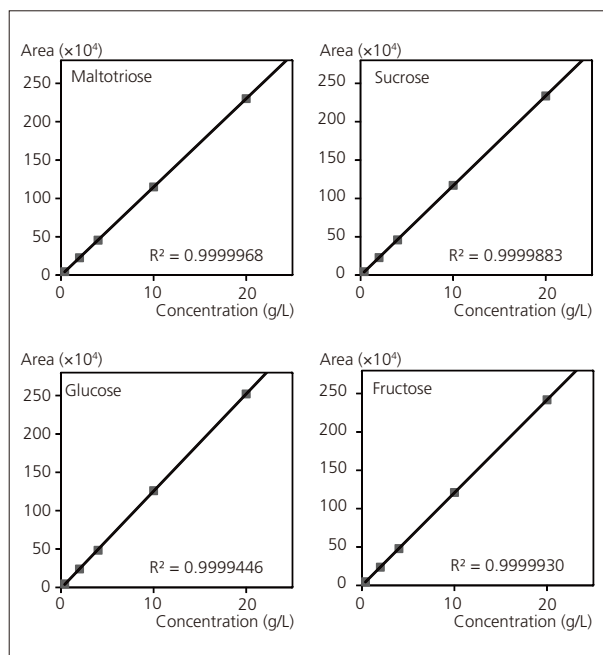
**Fig. 1 Chromatogram of a Standard Mixture of Four Sugars (20 g/L each, 10 µL injected)**

**Table 1 Analytical Conditions**

Column	: Shim-pack SCR-101N (300 mm L. × 7.9 mm I.D., 10 µm)
Mobile Phase	: Water
Flowrate	: 0.6 mL/min
Column Temp.	: 80 °C
Injection Volume	: 10 µL
Detection	: RID-10A
	Polarity +, Cell temp. 40 °C, Response 1.5 sec

### ■ Linearity

Fig. 2 shows the linearity obtained using the conditions listed in Table 1. Calibration curves were generated for the four sugars using concentrations ranging from 0.4 to 20 g/L, and the mean area value obtained from each set of the three repeat measurements. Excellent linearity was obtained, with a coefficient of determination greater than  $R^2=0.9999$  for all of the substances.



**Fig. 2 Calibration Curves of a Standard Mixture of 4 Sugars (0.4 - 20 g/L, 10 µL injected)**

### ■ Analysis of Orange Juice

Fig. 3 and 4 show the chromatograms obtained from analysis of Orange Juice A and Orange Juice B, respectively. Both Orange Juice A and B were diluted with water to obtain 10-fold dilutions, respectively, and after filtering the solutions through a 0.2 μm membrane filter, 10 μL each was injected. The analytical conditions used were the same as those shown in Table 1. Sucrose, glucose and fructose were detected in both types of orange juice. Table 2 shows the content values for the respective sugars detected in the juices.

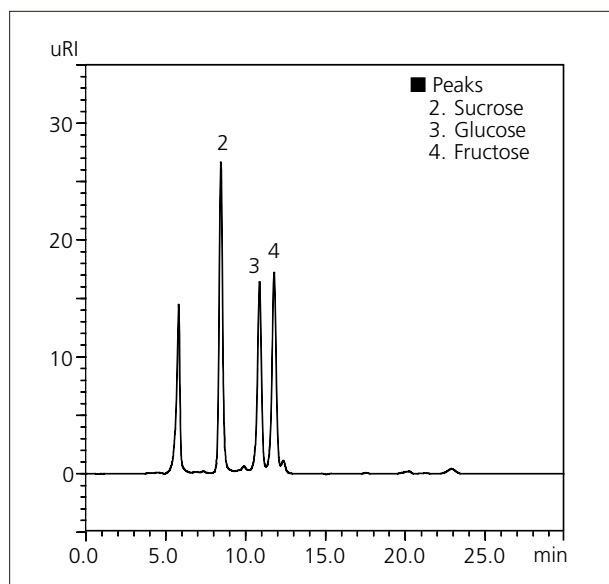


Fig. 3 Chromatogram of Orange Juice A (10 μL injected)

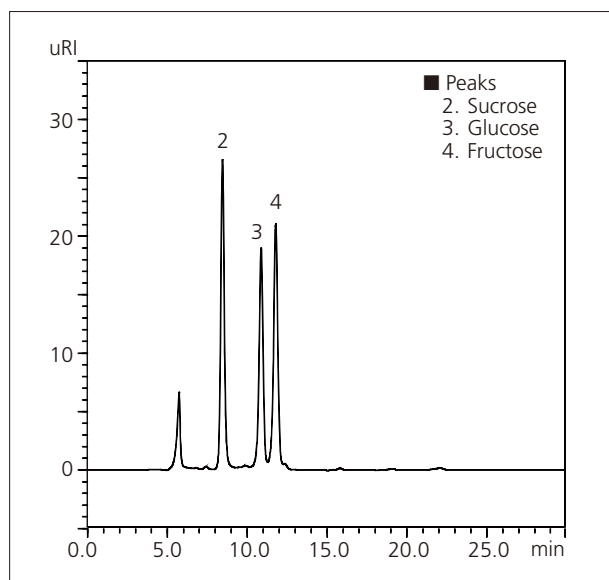


Fig. 4 Chromatogram of Orange Juice B (10 μL injected)

Table 2 Content of Each Sugar in Orange Juices

	Content (g/L)	
	Orange juice A	Orange juice B
Sucrose	36	36
Glucose	25	28
Fructose	27	34

### ■ Analysis of Grape Juice

Fig. 4 shows a chromatogram obtained from analysis of grape juice. The grape juice was diluted with water to obtain a 10-fold dilution, and after filtering the solution through a 0.2 μm membrane filter, 10 μL of the prepared sample was injected. The analytical conditions used were the same as those shown in Table 1. Glucose and fructose were detected in the grape juice. Table 3 shows the content values of the detected sugars.

Table 3 Content of Each Sugar in Grape Juice

	Content (g/L)
Glucose	50
Fructose	56

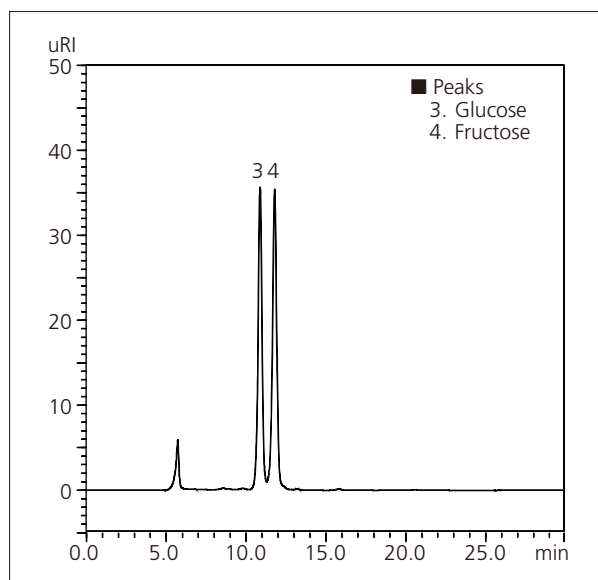


Fig. 5 Chromatogram of Grape Juice (10 μL injected)