Application News
No. L532
High Performance Liquid Chromatography

Analysis of Purified Glucose and Glucose Hydrate in Accordance with the Japanese Pharmacopoeia

Glucose is known as the most abundant sugar in the natural world, and is also used as a pharmaceutical product to take energy parenterally.

Test methods for purified glucose and glucose hydrates (hereinafter referred to glucose) were added to Supplement I to the Japanese Pharmacopoeia, 17th Edition, which was published in December 2017 [1]. In this test method, HPLC method using a refractive index detector is adopted for the purity test and the assay. This article introduces an example of analysis of glucose based on the Japanese Pharmacopoeia.

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System Suitability Test

Three types of test solutions shall be analyzed in the system suitability test. Table 1 shows the compositions of each test solution.

Fig. 1 shows the chromatograms of the system suitability solution (top), standard solution (2) (middle), and standard solution (bottom). Table 2 shows the analytical conditions. In this analysis, maltotriose, maltose, glucose, and fructose were eluted in that order.

Table 3 shows the retention time of glucose and the relative retention time of each compound. Table 4 shows the criteria and results of the system suitability test. System suitability was satisfied for all items.

Table 1 Compositions of Test Solutions

Name	Components	Conc.
System Suitability Solution	Maltose	100 mg/L
	Maltotriose	100 mg/L
	Fructose	100 mg/L
Standard Solution (2)	Glucose	15 mg/L
Standard Solution	Glucose	30 g/L

Table 2 Analytical Conditions

System	: Prominence™-i
Column	: GL Science InertSphere Sugar-2 (300 mm L. × 7.8 mm I.D., 9 µm)
Mobile Phase	: Water
Flow Rate	: 0.3 mL/min
Column Temp.	: 85 °C
Injection Volume	: 20 µL
Detection	: Refractive Index Detector (RID-20A)
Cell Temp.	: 60 °C

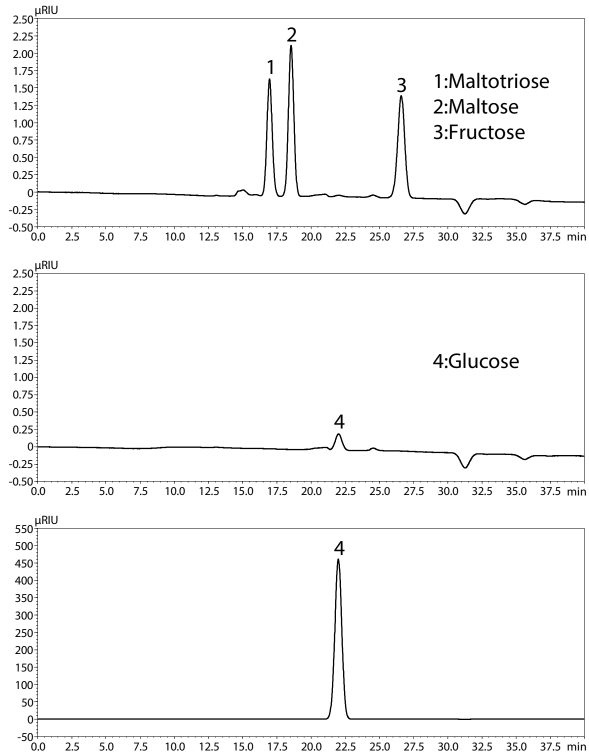

Fig. 1 Chromatograms of Four Sugar Components Top: System Suitability Solution, Middle: Standard Solution (2), Bottom: Standard Solution

Table 3 Retention Times of Compounds

Items	Compound	Reference (Approx.)	Result
Retention Time (min)	Glucose	21	22.0
Relative Retention Time	Glucose	1	1
	Maltotriose	0.7	0.77
	Maltose/Isomaltose	0.8	0.84
	Fructose	1.3	1.21

Table 4 Results of System Suitability Test

Test items	Criteria	Result	Judgement
Resolution between Maltotriose and Maltose (System Suitability Solution)	≥ 1.3	2.1	Passed
Relative Standard Deviation of Peak Area (N=6) (Standard Solution)	≤ 1.0%	0.25%	Passed

Linearity of Calibration Curve

Fig. 2 shows the calibration curve for the glucose analyzed under the conditions in Table 1. The calibration curve was prepared in the concentration range between 10 and 50 g/L. As a result, good linearity with a contribution ratio (R^2) of 0.9999 or more was obtained.

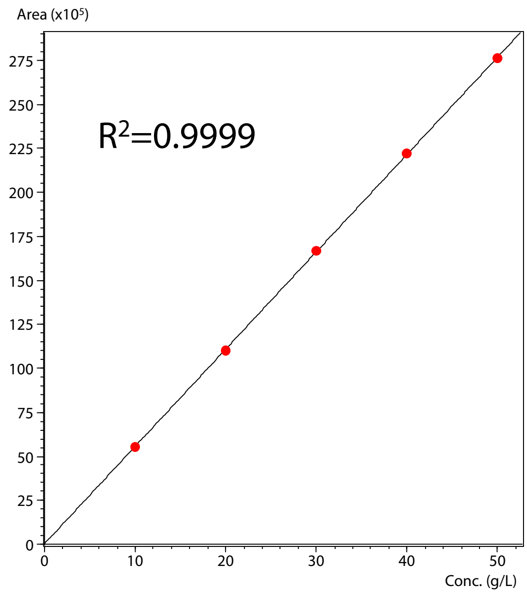

Fig. 2 Linearity of Calibration Curve

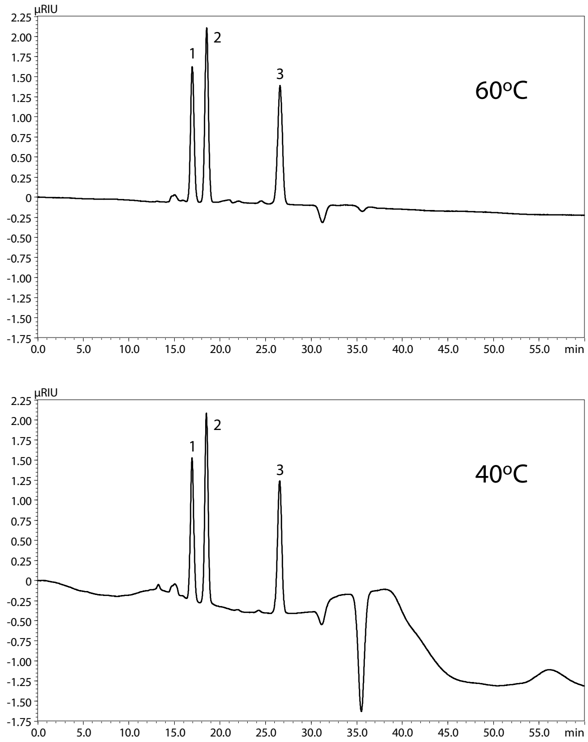

Fig. 3 Contribution of Cell Temperature on Baseline

Contribution of Cell Temperature

In general, refractive index detectors are extremely susceptible to the temperature, and particularly, temperature change causes baseline drift. RID-20A, refractive index detector of Shimadzu, features an optical system with dual temperature control to maintain a constant temperature in the detector cell. Baseline drift is dramatically reduced in some cases, depending on the setting temperature of the detector cell.

Fig. 3 shows the chromatograms of the system suitability solution when the cell temperature was set to 60 °C (top) and to 40 °C (bottom). The baseline of the chromatogram with the 40 °C setting is extremely unstable, suggesting that it was not possible to maintain a constant temperature in the cell due to the large temperature difference between the column and the cell. Thus, when using a differential refractive index detector, it is important to minimize the temperature difference between the column and cell.

Reference

(1) Japanese Pharmacopoeia, 17th Edition, Supplement I (Notification No. 348 of the Ministry of Health, Labour and Welfare, December 1, 2017.

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