Application News

No.L489

High Performance Liquid Chromatography

Analysis of Mannitol Using RID-20A Differential Index Detector

Mannitol possesses characteristics that include low moisture absorption and low reactivity, and is therefore used as an excipient for pharmaceuticals. USP methods for D-mannitol (hereafter, "mannitol") were amended in 2014 to specify the use of refractive index detection with a 7.8 mm × 300 mm L19 column.
The RID-20A differential refractive index detector features an optical system with dual temperature control to absorb the impact of subtle changes in temperature, thereby permitting chromatograms to be generated with a stable baseline. Here, we introduce an example of mannitol analysis using the RID-20A.

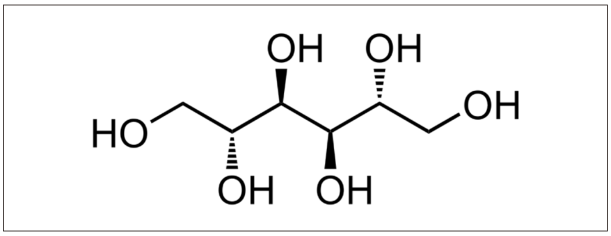

Fig. 1 Structure of D-Mannitol

System Suitability

Fig. 1 shows the structural formula of mannitol. The test method for mannitol specifies that two types of system suitability test standard solutions be analyzed. The upper data of Fig. 2 shows the system suitability test results for Standard Solution 1, consisting of isomalt and maltitol standards (each 1 g/L), and the lower data of Fig. 2 shows the results using Standard Solution 2, consisting of mannitol and sorbitol standards (each 25 g/L). Table 1 shows the analytical conditions.
Table 2 shows the reference values for the system suitability test, in addition to the analysis results. The results confirm that system suitability is satisfied with respect to all the criteria.

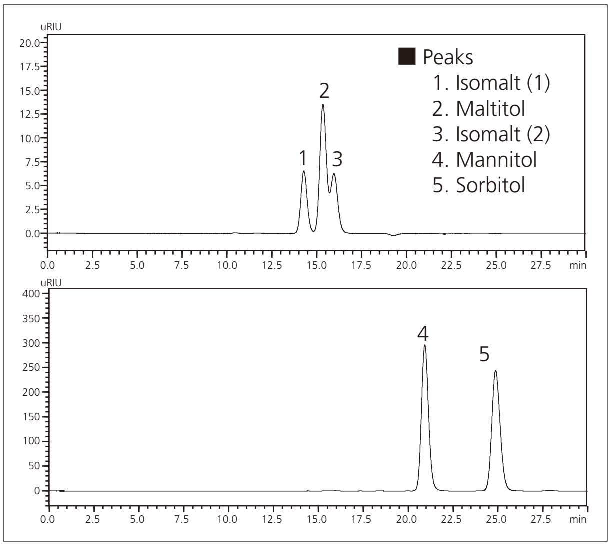

Fig. 2 Chromatograms of a Standard Mixture of Four Sugar Alcohols
Upper: System Suitability Solution 1 (Isomalt, Maltitol)
Lower: System Suitability Solution 2 (Mannitol, Sorbitol)

Table 1 Analytical Conditions

System	: Prominence
Column	: Shim-pack SCR-101C (300 mm L. × 7.9 mm I.D., 10 µm)
Mobile Phase	: Water
Flowrate	: 0.5 mL/min
Column Temp.	: 85 °C
Injection Volume	: 20 µL
Detection	: RID-20A

Table 2 Results of System Suitability Test

System Suitability Assessment Item	Target Substance	Reference Value	Analysis Result
Retention Time	Mannitol	Approximately 20 minutes	20.9 minutes
Relative Retention Time with Respect to Mannitol	Isomalt (1)	Approximately 0.6	0.68
	Maltitol	Approximately 0.69	0.73
	Isomalt (2)	Approximately 0.73	0.76
	Sorbitol	Approximately 0.12	1.2
Resolution	Mannitol and sorbitol	Greater than 2.0	5.0
Relative Standard Deviation of Peak Area Value	Mannitol	Less than 1.0 %	0.03 %

Linearity

Fig. 3 shows the calibration curve of mannitol analyzed using the conditions of Table 1. The correlation coefficient over the concentration range from 1 to 100 g/L is $R^2 = 0.999$, demonstrating excellent linearity.

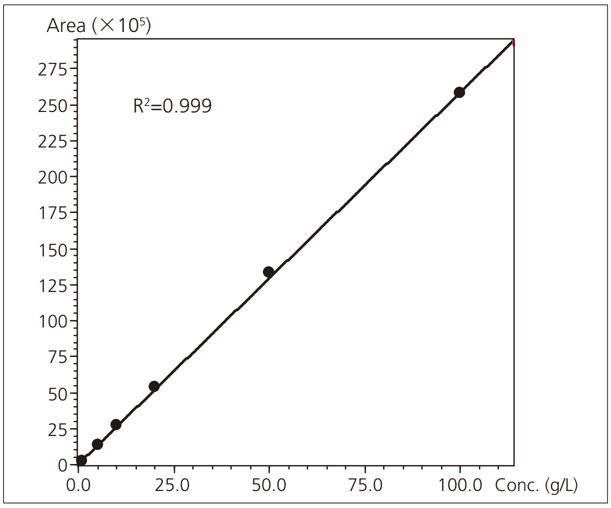

Fig. 3 Linearity of Calibration Curve

Effect of Column Temperature

Fig. 4 shows the chromatograms obtained in analysis of the four sugar alcohol standard samples using the two column oven temperatures of 85 °C and 60 °C. The upper pair of chromatograms are those of isomalt and maltitol, and the lower pair, of mannitol and sorbitol. When the column temperature is lowered to 60 °C, the retention time of mannitol becomes 23 minutes, at which point the system suitability requirement is no longer satisfied.

When analysis is conducted at a high column temperature like 85 °C, it is important that the temperature be maintained uniformly over the entire length of the column. The CTO-20AC column oven used in this analysis is equipped with forced air circulation, thereby permitting stable analysis even at high temperatures.

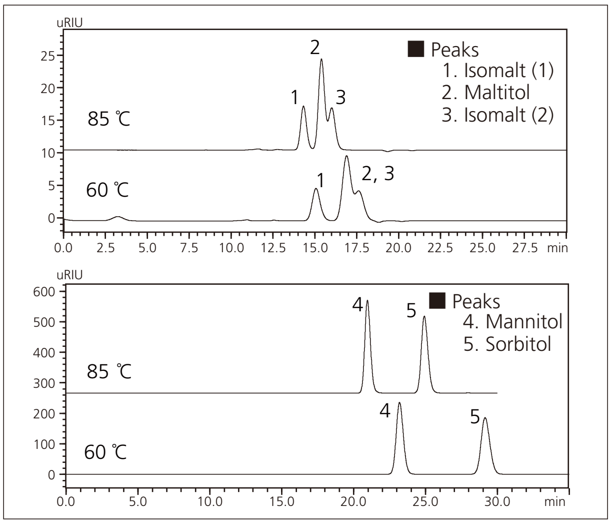

Fig. 4 Chromatograms of a Standard Mixture of Four Sugar Alcohols (85 °C, 60 °C)

Analysis of a Pharmaceutical Excipient

Fig. 5 shows an example of analysis of a pharmaceutical excipient, consisting mainly of mannitol, using a 20 µL injection of 50 g/L sample solution. The chromatogram of the excipient is shown in the upper portion of the figure, while an expanded view of the chromatogram is shown in the lower portion. Trace levels of other substances, including sorbitol and isomalt, were also detected in the excipient.

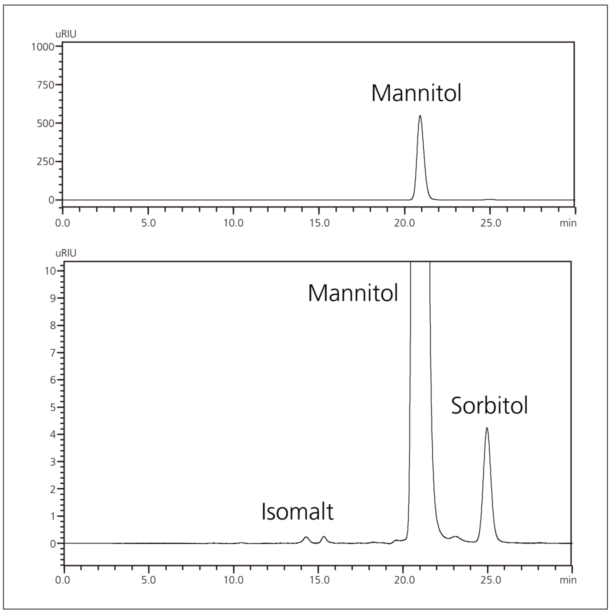

Fig. 5 Chromatogram of Pharmaceutical Excipient
Upper: Chromatogram
Lower: Expanded Chromatogram

Reference

1) United States Pharmacopeia, Second Supplement to USP 37–NF 32

The samples used to produce this application were kindly provided by Nihon Generic Co., Ltd.

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