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Application News

No.L494

High Performance Liquid Chromatography

Analysis of Omeprazole by "i-Series" for USP and JP Methods

Omeprazole, a drug that effectively suppresses the excessive secretion of gastric acid, is often used for the treatment of gastric ulcer and duodenal ulcer, in addition to the treatment of reflux esophagitis. Acting as a Proton Pump Inhibitor (PPI), omeprazole is included in the WHO Model List of Essential Medicine, and considered an important component of basic medical care.
This Application News introduces an example of analysis of omeprazole in accordance with the Japanese Pharmacopoeia (JP) and the United States Pharmacopeia (USP). Also presented here is an example of analysis that can be completed in a significantly shorter time than that described in the USP General Chapter 621 Chromatography.
The Nexera-i integrated UHPLC was used for the analysis by the procedure described in the USP. The Nexera-i supports the use of analytical conditions specified for both HPLC and UHPLC. In the case of compliance (HPLC conditions) with the Japanese Pharmacopoeia, we conducted analysis using the Prominence-i integrated HPLC.

The USP Method - Original Method

The analytical conditions specified in the USP monograph are shown in Table 1. The results of analysis of the system suitability test solution (0.1 mg/mL, acetonitrile-boric acid solution) specified in the omeprazole test method are shown in the upper chromatogram of Fig. 1. The results obtained sufficiently satisfy the threshold required with respect to both tailing factor and relative standard deviation (n = 6) specified in the monograph (Table 4).

Table 1 Analytical Conditions (USP Original Method)

System	: Nexera-i
Column	: Shim-pack GIST C8 (150 mmL. × 4.6 mm I.D., 5 µm)
Mobile Phase	: Acetonitrile/Phosphate (Na) Buffer (pH 7.6) = 1/3 (v/v)
Flowrate	: 0.80 mL/min
Column Temp.	: 40 ˚C
Injection Volume	: 20 µL
Detection	: UV 280 nm (Cell temp. 40 ˚C)

Table 2 Selection of Column for Speed Enhancement

	Column Size	L/dp	Ratio
USP Original Method	150 mmL. × 4.6 mm I.D., 5 µm	30000	1 (100 %)
USP Fast Method	50 mmL. × 3.0 mm I.D., 2 µm	25000	0.83 (-17 %)

Table 3 Analytical Conditions (USP Fast Method)

System	: Nexera-i
Column	: Shim-pack GIST C8 (50 mmL. × 3.0 mm I.D., 2 µm)
Mobile Phase	: Acetonitrile/Phosphate (Na) Buffer (pH 7.6) = 1/3 (v/v)
Flowrate	: 0.85 mL/min
Column Temp.	: 40 ˚C
Injection Volume	: 8 µL
Detection	: UV 280 nm (Cell temp. 40 ˚C)

Speed Enhancement for USP Method

The permissible ranges within which the analytical conditions may be modified are specified in the USP General Chapters: <621> Chromatography. Changing these analytical conditions within range makes it possible to shorten the analysis time. For details regarding changes that can be used to allow fast USP-compliant analysis, please refer to Application News L464.
Shortening analysis time can be accomplished in two ways, 1) by shortening the column, and 2) by increasing the flowrate (linear velocity). To preserve the resolution of the column, the column length and particle size may be modified as long as the ratio of L (column length) to dp (column particle size) remains in the specified range (permissible range: -25 % to +50 %). We selected a column size of 50 mmL. × 3.0 mm I.D., and 2 µm particle size. For further details, please see Table 2. The flowrate, proportional to the column cross-sectional area, and inversely proportional to the particle diameter (see text for permissible limits), was determined as 0.85 mL/min.
The instrument used for the analysis was the Nexera-i high-speed integrated UHPLC, suitable for multi-sample processing. The Nexera-i permits analysis using both HPLC and UHPLC conditions, without requiring changes to plumbing or flow cell type. This flexibility can allow legacy HPLC methods to be quickly transferred to UHPLC speed and performance.
Table 3 shows the analytical conditions using the higher speed analysis, and the chromatogram obtained from analysis of the system suitability test solution is shown in the lower part of Fig. 1. The analysis time was reduced more than 80 percent compared to that using the analytical conditions of Table 1 (Fig. 1 upper).
The results of the system suitability test are shown in Table 4. Clearly, the threshold value has been satisfied even using high-speed analysis conditions.

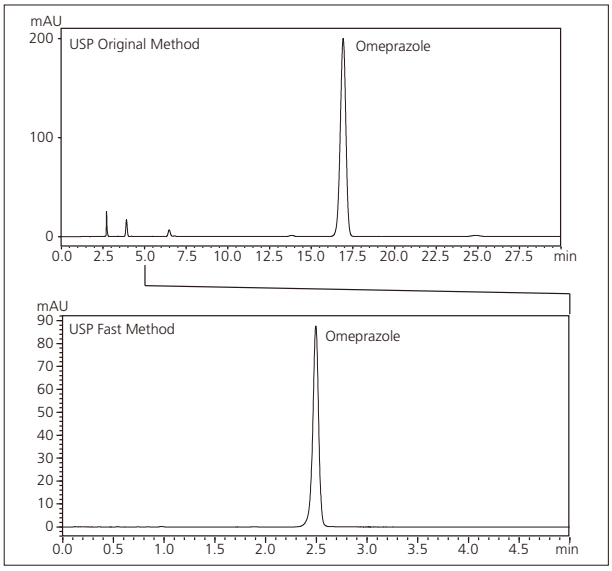

Fig. 1 Chromatograms Conforming to USP Method (Upper: USP Original Method, Lower: USP Fast Method)

Table 4 Results of System Suitability Test Using USP Method (Original Method and Fast Method)

System Suitability Requirements		Analytical Conditions			
		USP Original Method (Table 1)		USP Fast Method (Table 3)	
		Results	Judgments	Results	Judgments
USP Tailing Factor for Omeprazole	≦ 1.5	0.94	PASS	0.89	PASS
Relative Standard Deviation for Omeprazole (n = 6)	≦ 1.0 %	Rt 0.097 %	PASS	Rt 0.081 %	PASS
		Area 0.022 %	PASS	Area 0.121 %	PASS

Analysis According to Japanese Pharmacopeia

The analytical conditions specified in the 16th Edition of the Japanese Pharmacopeia are shown in Table 5. For the instrument, the integrated HPLC Prominence-i was used. The system suitability test specified in the Japanese Pharmacopeia includes three items, "Test for required detectability", "System performance", and "System repeatability". The respective chromatograms are shown in Figs. 2-4.

Regarding the test for required detectability, both the system suitability test solution (5 mg/L, prepared using mobile phase) and this solution diluted five-to-one with mobile phase are measured, and their peak areas compared. The peak area of the omeprazole in the five-to-one diluted solution was compared to the results obtained using the system suitability solution, and was determined to be approximately 20 % (within permissible range of 15-25 %).

For evaluation of system performance, omeprazole and 1,2-dinitrobenzene are dissolved in sodium borate-ethanol solution (at 100 mg/L and 250 mg/L, respectively). The solution is analyzed, and the resolution of omeprazole and 1,2-dinitrobenzene is verified. The results indicated a resolution of about 24 (permissible range is 10 or greater).

For evaluation of system repeatability, six repeat analyses of the system suitability test solution were conducted, and the peak area relative standard deviation was checked. A relative standard deviation of 0.2 % was obtained (permissible range is 2.0 % or less).

These results are summarized in Table 6.

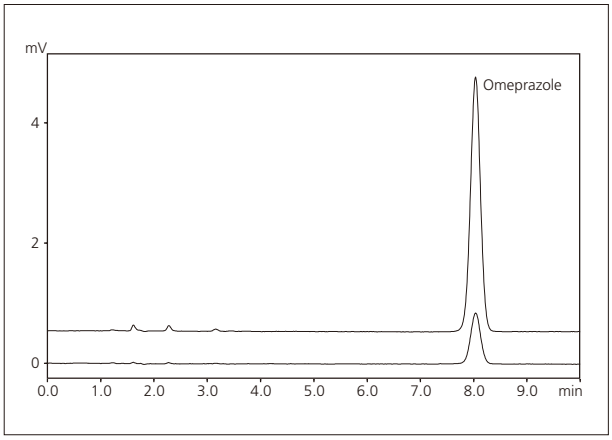

Fig. 2 Chromatogram According to JP Method – Test for Required Detectability (Upper: 5 mg/L, Lower: 1 mg/L)

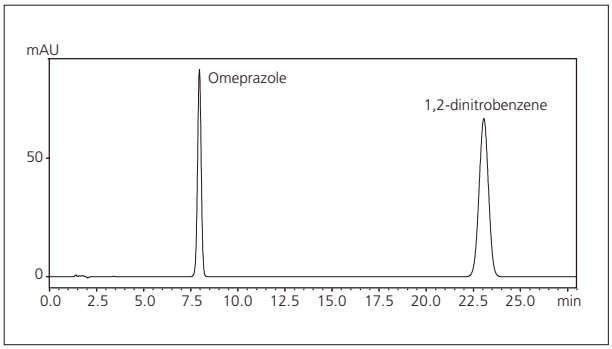

Fig. 3 Chromatogram According to JP Method – System Performance

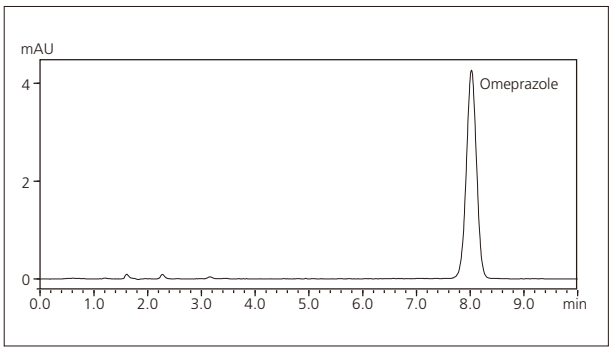

Fig. 4 Chromatogram According to JP Method – System Repeatability

Table 5 Analytical Conditions (JP Method)

System	: Prominece-i
Column	: Shim-pack GIST C8 (150 mmL. × 4.6 mm I.D., 5 µm)
Mobile Phase	: Phosphate (Na) Buffer (pH 7.6) / Acetonitrile = 29/11 (v/v)
Flowrate	: 1.3 mL/min
Column Temp.	: 25 ˚C
Injection Volume	: 10 µL
Detection	: UV 280 nm (Cell temp. 40 ˚C)

Table 6 Results of System Suitability Test (JP Method)

System Suitability Requirements		Results	Judgments
Test for Required Detectability	Area 15 to 25 %	19.7 %	PASS
System Performance	Resolution ≧ 10	23.6	PASS
System Repeatability	%RSD Area ≦ 2.0 %	0.202 %	PASS

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