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Application News

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High Performance Liquid Chromatography

Method Transfer and High-Speed Analysis of Impurities of Drug by Prominence-i

In February 2014, the Japanese Pharmacopoeia - Sixteenth Edition, Supplement Ⅱ was newly issued. Bepotastine besilate[1)], newly listed among the official monographs (chemicals, etc.), is a selective histamine H1 antagonist which is prescribed for treatment of itching of the eyes, often associated with allergic conjunctivitis.
Here, using the new Prominence-i integrated high-performance liquid chromatograph, we conducted analysis of substances chemically related to bepotastine besilate in accordance with the Japanese Pharmacopoeia, while at the same time comparing the analysis results between those using the Prominence-i (with the standard configuration or with the delay volume-compatible system kit) and those obtained using both the conventional integrated LC-2010 model as well as a third-party company's LC system. In addition, we introduce an example of method transfer from that used with Shimadzu's conventional system or that of a third-party LC system, respectively. At the same time, we introduce an example of high-speed analysis using the Prominence-i (with Low-volume tubing kit installed) in which the analysis time is shortened to about one-fourth the time, while conforming to the Japanese Pharmacopoeia default of conditions.

Method Transfer for Analysis of Bepotastine Besilate Standard

Purity testing for substances related to bepotastine besilate was conducted in accordance with the Japanese Pharmacopoeia (JP). Table 1 shows the analytical conditions used for analysis of a standard solution of bepotastine besilate (4 mg/L). Fig. 1 shows the chromatograms obtained using a third-party LC system (upper) and the Prominence-i (lower), respectively.
Similarly, Fig. 2 shows the chromatograms obtained using the LC-2010 (upper) and the Prominence-i with the delay volume-compatible system kit option (lower), respectively. It is clear from Fig. 1 and 2 that there is separation compatibility among the third-party LC system and the LC-2010 and Prominence-i.
System suitability testing was conducted in accordance with the Japanese Pharmacopoeia (Purity (2) Related substances – System suitability). Table 2 on the following page shows the results of the system suitability test, which clearly indicates that all of the conditions specified in the Japanese Pharmacopoeia are satisfied.

Table 1 Analytical Conditions

Column	: Shim-pack VP-C8 (150 mm L × 4.6 mm I.D., 5 µm)
Flowrate	: 1.0 mL/min
Mobile Phase	: A) 50 mmol/L Phosphate (Potassium) Solution (pH 3.0) B) Acetonitrile Containing 5.7 mmol/L 1-pentanesulfonate monohydrate B Conc. 30 %
Column Temp.	: 40 °C
Injection Volume	: 20 µL
Detection	: LC-2030C 3D at 220 nm
Flow Cell	: Conventional Cell for Integrated

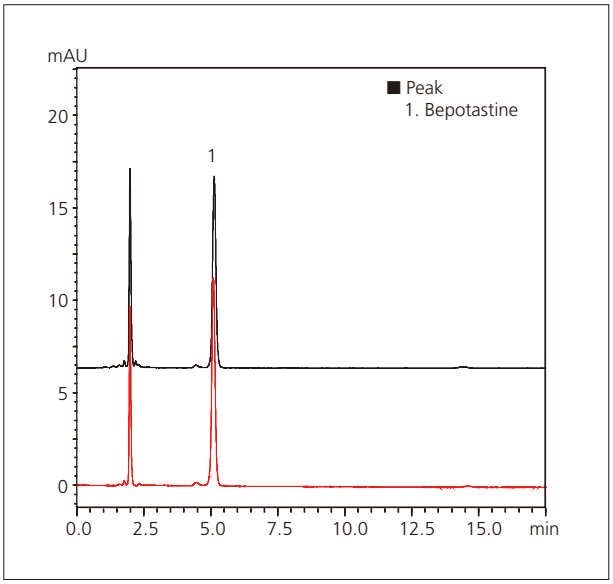

Fig. 1 Chromatograms of Bepotastine Besilate
Upper: Third-Party LC System
Lower: Prominence-i

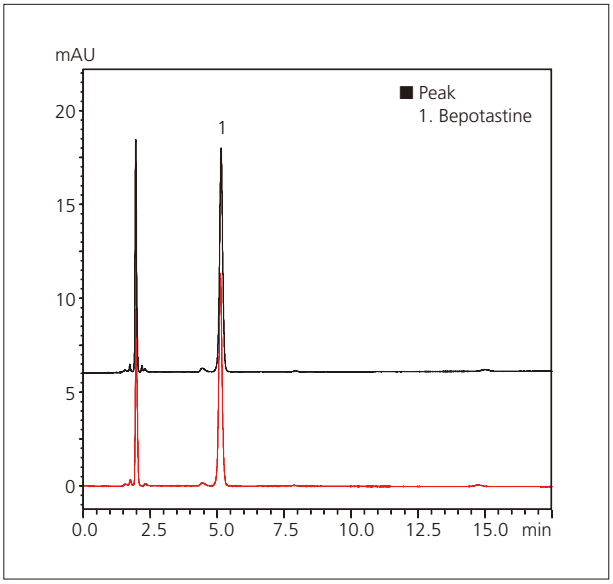

Fig. 2 Chromatograms of Bepotastine Besilate
Upper: LC-2010
Lower: Prominence-I (with Delay Volume-Compatible System Kit)

Table 2 Results of System Suitability Test Specified in Japanese Pharmacopeia (JP)

Tubing Kit		System Suitability Items			Reference Value	Results	Judgment
Conventional (lower chromatogram of Fig. 1)	None (standard plumbing)	Detection check	Peak area	20-fold dilution of Std. solution	3.5 ≦ X ≦ 6.5	5.0	PASS
		System performance	Number of theoretical plates	Bepotastine	≧ 3000	8300	PASS
			Symmetry coefficient	Bepotastine	0.8 ≦ Y ≦ 1.5	1.01	PASS
		System repeatability	Relative standard deviation	Bepotastine peak area	≦ 2.0	0.16	PASS
LC-2010-Compatible (lower chromatogram of Fig. 2)	Delay volume-compatible system kit	Detection check	Peak area	20-fold dilution of Std. solution	3.5 ≦ X ≦ 6.5	5.2	PASS
		System performance	Number of theoretical plates	Bepotastine	≧ 3000	8500	PASS
			Symmetry coefficient	Bepotastine	0.8 ≦ Y ≦ 1.5	1.01	PASS
		System repeatability	Relative standard deviation	Bepotastine peak area	≦ 2.0	0.13	PASS
High-Speed Analysis (Fig. 3)	Low-volume tubing kit	Detection check	Peak area	20-fold dilution of Std. solution	3.5 ≦ X ≦ 6.5	4.8	PASS
		System performance	Number of theoretical plates	Bepotastine	≧ 3000	9400	PASS
			Symmetry coefficient	Bepotastine	0.8 ≦ Y ≦ 1.5	1.22	PASS
		System repeatability	Relative standard deviation	Bepotastine peak area	≦ 2.0	0.27	PASS

High-Speed Analysis of Impurities of Bepotastine Besilate

Using an ultra-high-speed analytical column, substances chemically related to bepotastine besilate were investigated. During high-speed analysis, the optional "Low-volume tubing kit" was used.

The chromatogram in the lower segment of Fig. 3 was obtained from measurement of a standard solution of bepotastine besilate (4 mg/L) using the analytical conditions of Table 3. The data in the upper segment of Fig. 3 is the same as that shown in the lower segment of Fig. 1. Referring to the chromatogram in the lower segment of Fig. 3, the peak corresponding to the principal component bepotastine is clearly detected at 1.2 minutes. In addition, both the analysis time and mobile phase consumption were kept to about one-fourth their respective previous values while maintaining separation. As for sensitivity, compared to that using conventional analysis, as shown in Fig. 1, improvement of about 8.5 fold was achieved.

Table 2 shows the system suitability test results. Even with the faster analysis, all of the conditions specified in the Japanese Pharmacopoeia were clearly satisfied.

Table 3 Analytical Conditions -High Speed Analysis-

Column	: Shim-pack XR-C8 (75 mm L × 3.0 mm I.D., 2.2 µm)
Flowrate	: 1.0 mL/min
Mobile Phase	: A) 50 mmol/L Phosphate (Potassium) Solution (pH 3.0) B) Acetonitrile Containing 5.7 mmol/L 1-pentanesulfonate monohydrate B Conc. 30 %
Column Temp.	: 40 °C
Injection Volume	: 1 µL
Detection	: LC-2030C 3D at 220 nm
Flow Cell	: High-speed High-sensitivity Cell

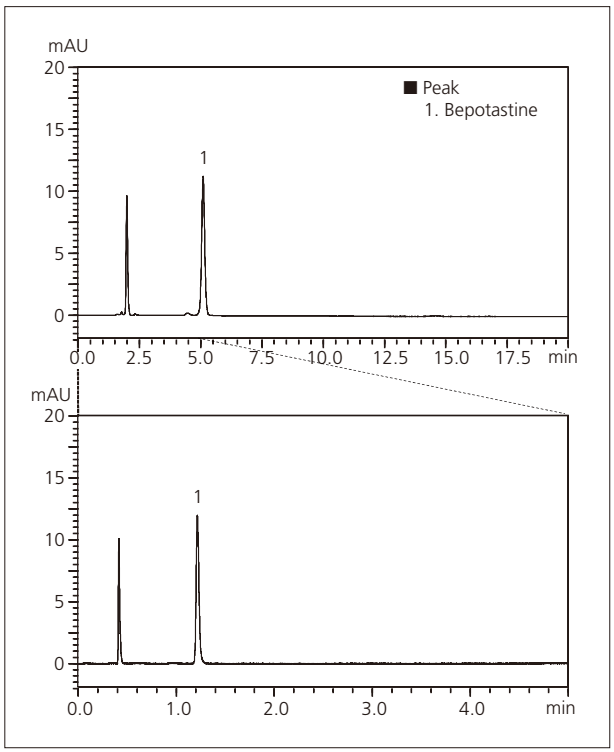

Fig. 3 Chromatograms of Bepotastine Besilate – High-Speed Analysis
Upper: Conventional Analysis
Lower: High-Speed Analysis (with Low-volume Tubing Kit)

[References]
1) Japanese Pharmacopoeia - Sixteenth Edition, Supplement II
Official Monographs (Chemicals, etc.) - Bepotastine Besilate

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