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Application News

No. L584

High Performance Liquid Chromatography

JP- and EP-Compliant Analysis of Impurities of COVID-19 Drug Dexamethasone

In July 2020, Japan's Ministry of Health, Labour and Welfare (MHLW) approved the anti-inflammatory drug dexamethasone as a drug for treatment of the infectious disease COVID-19 caused by the novel coronavirus [1]. Dexamethasone is the second officially-approved COVID-19 drug in Japan.

Dexamethasone is a steroid drug that is used with a variety of diseases, including severe infections and interstitial pneumonia, and is already a widely-used drug. It has been reported that administration of dexamethasone also reduced the death rates of COVID-19 patients that required mechanical ventilators or oxygen inhalation in large-scale clinical research in the United Kingdom.

Procurements of drug substances and pharmaceutical additives from overseas and shipments of manufactured drugs to other countries are increasing, but the standards and test methods for dexamethasone differ depending on the applicable pharmacopoeia. Therefore, testing conforming to the test method recognized in the destination country is necessary when shipping to other countries.

This article introduces analyses based on the test requirements for impurities of dexamethasone in the Japanese Pharmacopoeia (JP) and the European Pharmacopoeia (EP).

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JP-Compliant Analysis of Impurities of Dexamethasone

Dexamethasone was analyzed according to the item of Purity (2) Related compounds of the JP [2]. Fig. 1 shows the chromatogram of the standard solution (5.94 mg/L) [2], and Table 1 shows the analytical conditions. Shim-pack™ VP-phenyl was used for the analytical column because phenylsilanized silica gel is specified as the packing material in the JP. The flow rate was set to 1.15 mL/min because it is specified that the flow rate should be adjusted so that the retention time of dexamethasone is approximately 13 minutes in the JP.

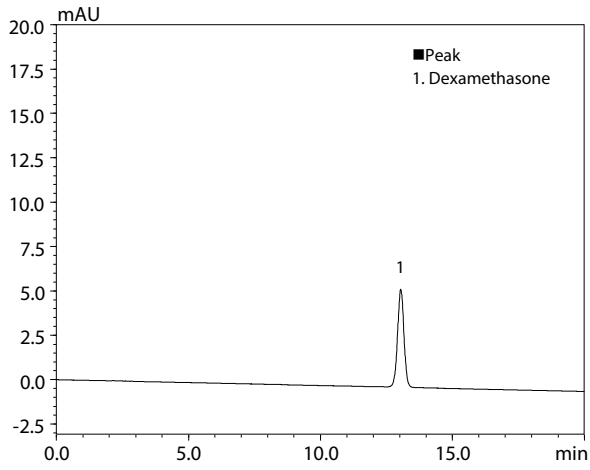

Fig. 1 Chromatogram of Standard Solution (5.94 mg/L) (Japanese Pharmacopoeia)

Table 1 Analytical Conditions (Japanese Pharmacopoeia)

System	:	Nexera™ lite
Column	:	Shim-pack VP-phenyl *1 (250×4.6 mm I.D., 5 µm)
Flow rate	:	1.15 mL/min
Mobile phase	:	(ammonium) formate buffer (pH 3.6) */ acetonitrile=67:33 * 1.32 g/L of ammonium formate in water. Adjust with formic acid to a pH of 3.6.
Column temp.	:	25 °C
Injection volume	:	10 µL
Vial	:	TORAST-H Glass Vial, 1.5mL Amber Glass with Cap & Septa (Shimadzu GLC) *2
Detection	:	UV 254 nm
Sample	:	Dexamethasone standard solution (5.94 mg/L)

*1: P/N 228-59928-92, *2: P/N 370-04300-03

EP-Compliant Analysis of impurities of Dexamethasone

Next, Dexamethasone was analyzed according to the item of Related compounds of the EP [3]. Fig. 2 shows the chromatogram of reference solution (a) [3], and Table 2 shows the analytical conditions. The lower part of Fig. 2 shows an enlarged chromatogram for retention times from 10 min to 30 min.

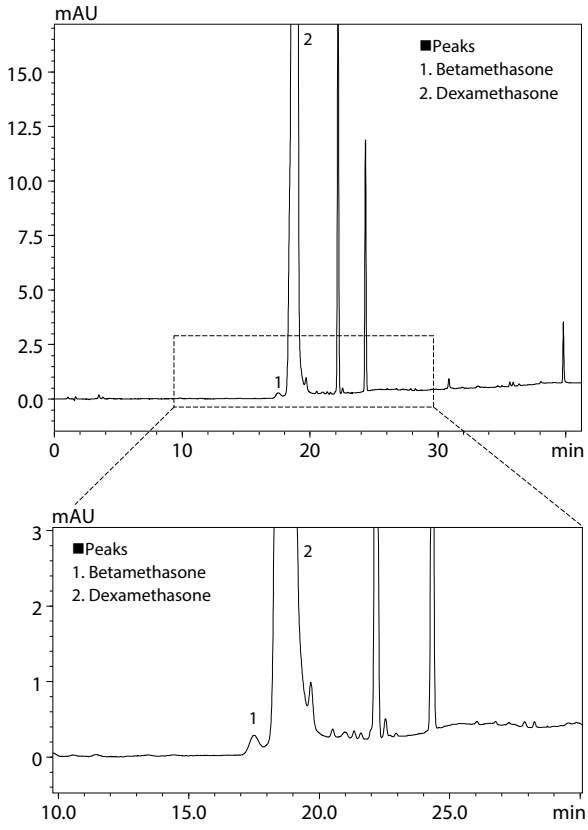

Fig. 2 Chromatogram of Reference Solution (a) (European Pharmacopoeia)

Shim-pack Scepter™ C18 was used for the analytical column because the EP specifies endcapped octadecylsilanized silica gel (ODS).

Although the isocratic elution is specified in the JP test method described above, whereas the gradient elution in the EP method.

Table 2 Analytical Conditions (European Pharmacopoeia)

System	:	Nexera lite
Column	:	Shim-pack Scepter C18 *3 (150×4.6 mm I.D., 5 µm)
Flow rate	:	1.2 mL/min
Mobile phase	:	A) 25% acetonitrile * B) acetonitrile * mix 250 mL of acetonitrile with 700 mL of water and allow to equilibrate; dilute to 1000 mL with water and mix again
Time program	:	0%B (0-15 min)→100%B (40 min)→ 0%B (40.01- 55 min)
Column temp.	:	45 °C
Injection volume	:	20 µL
Vial	:	TORAST-H Glass Vial, 1.5 mL Amber Glass with Cap & Septa (Shimadzu GLC)
Detection	:	UV 254 nm
Sample	:	Dexamethasone reference solution (a)

*3: P/N 227-31020-05

JP- and EP-Compliant System Suitability Tests

System suitability tests were conducted based on the JP and EP. In the JP, tests are carried out for the number of theoretical plates, symmetry factor, and system repeatability using a standard solution having dexamethasone as its main compound. However, in the EP, the test is conducted for the peak-to-valley ratio of dexamethasone to betamethasone, an impurity that is eluted immediately before dexamethasone. It may be noted that the value of this peak-to-valley ratio is calculated automatically on the Shimadzu LabSolutions™ workstation in the same manner as the number of theoretical plates, symmetry factor, and repeatability.

Table 3 shows the test results. Fig. 3 shows a comparison of the chromatograms in 6 repeated tests, as specified in the JP. Although the test items and standard values are different depending on the pharmacopoeia, judgments of "Passed" were obtained for all items in the test methods provided in both of the JP and the EP.

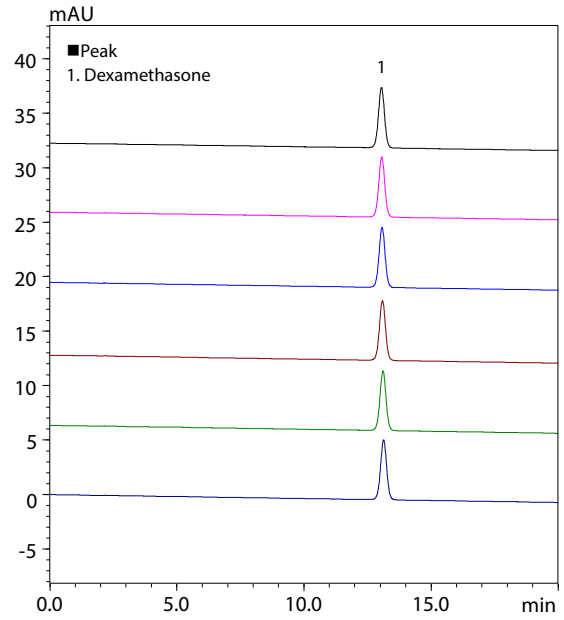

Fig. 3 Comparison of Chromatograms in 6 Repeated Tests with Standard Solution (5.94 mg/L) (Japanese Pharmacopoeia)

Conclusion

As described in this article, analyses of dexamethasone were carried out based on the "Related compounds" item in the Japanese Pharmacopoeia and the European Pharmacopoeia. A Shimadzu Nexera lite system was used in both analyses.

A comparison of the liquid chromatographic conditions specified in the Japanese and European pharmacopoeias showed that, except for the detection conditions, the column stationary phase and other test conditions differed greatly. Although the items required in the two system suitability tests also differ depending on the pharmacopoeia, judgments of "Passed" were obtained for all items in the test methods provided in both of the JP and the EP.

Table 3 Results of System Suitability Tests

		System suitability requirements		Results	Judgements
JP	Confirmation of detection	dexamethasone	8-12%	9.7	PASSED
	Number of theoretical plate	dexamethasone	≥5000	14125	PASSED
	Symmetry factor	dexamethasone	≤1.5	1.0	PASSED
	%RSD	dexamethasone	≤1.0	0.034	PASSED
EP	Peak-to-valley ratio	betamethasone and dexamethasone	≥2.0	4.2	PASSED

<References>
(1) Ministry of Health, Labour and Welfare, Novel Coronavirus Response Headquarters, "Clinical Management of Patients with COVID-19 – A guide for front-line healthcare workers Ver. 2.2," July 17, 2020
(2) Japanese Pharmacopoeia 17th Edition, "Dexamethasone"
(3) European Pharmacopoeia 8.8, 01/2014: 0388, "Dexamethasone"

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