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Application News

Liquid Chromatograph Nexera™ XS

Determination of Dexamethasone and its organic impurities content as per USP monograph UHPLC method

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User Benefits

- Shimadzu Nexera XS can be effectively used for assay and organic impurities test of Dexamethasone as per the USP monograph UHPLC method
- The Nexera XS easily meets with all the acceptance criteria as per the USP monograph for Dexamethasone

■ Introduction

Dexamethasone (see Fig. 1) is a glucocorticoid medication used to treat rheumatic problems, a number of skin diseases, severe allergies, asthma, chronic obstructive lung disease, croup, brain swelling, eye pain following eye surgery, and along with antibiotics in tuberculosis. It may be given by mouth, as an injection into muscle and vein, as a topical cream or ointment for the skin or as a topical ophthalmic solution to the eye. The long-term use of dexamethasone may result in thrush, bone loss, cataracts, easy bruising, or muscle weakness. Dexamethasone also has anti-inflammatory and immunosuppressant effects.

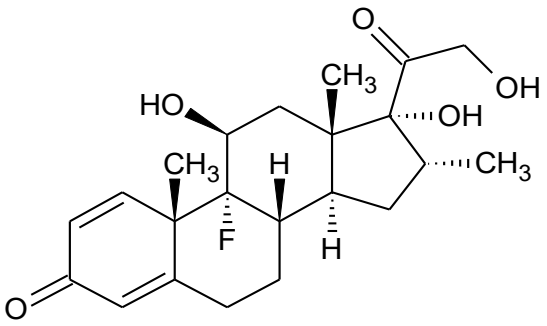

Fig. 1 Structure of Dexamethasone

The United States Pharmacopeia (USP) monograph defines an UHPLC (Ultra High-Performance Liquid Chromatography) method for high throughput fast analysis demanding a competent UHPLC system.

Here, we demonstrate the analysis for Dexamethasone as per the official monograph in USP using Shimadzu Nexera XS (see Fig. 2) fast LC system in compliance with system suitability requirements of the monograph.

Nexera series

Key features- Analytical Intelligence

- Automated support functions utilizing digital technology, such as machine-to-machine communication (M2M), Internet of things (IoT), and Artificial Intelligence (AI), that enable higher productivity and maximum reliability.

- Allows a system to monitor and diagnose itself, handle any issues during data acquisition without user input, and automatically behave as if it were operated by an expert.

- Supports the acquisition of high quality, reproducible data regardless of an operator's skill level for both routine and demanding applications.

Fig. 2 Nexera™ XS system

■ Experimental

Chromatographic conditions, mobile phase preparations, standard and sample preparations were done in accordance with the USP monograph for dexamethasone (see Table 1 for assay and Table 2 for organic impurities). System suitability parameters were also checked as per the requirements of USP monograph.

Assay

Solution A: 3.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile.

Diluent: Acetonitrile/Water = 56:44

System suitability solution: 0.3 mg/mL of USP dexamethasone RS and 20 µg/mL of USP betamethasone RS in diluent. Sonicate to dissolve as needed.

Standard solution: 0.3 mg/mL of USP dexamethasone RS in diluent. Sonicate to dissolve as needed.

Sample solution: 0.3 mg/mL of dexamethasone in diluent. Sonicate to dissolve as needed.

Table 1 LC acquisition parameters for Assay

Column	: 100mm x 2.1 mm I.D., 1.7 µm USP packing L1
Oven temperature	: 35°C
Mobile Phase A	: Solution A
Mobile phase B	: Solution B
Gradient program (B %)	: 0.0-10.0 min → 24.0 (%); 10.0-15.0 min → 24.0-55.0 (%); 15.0-16.0 min → 55.0-90.0 (%); 16.0-16.1 min → 90.0-24.0 (%); 16.1-20.0 min → 24.0 (%).
Flow Rate	: 0.4 mL/min
Total Run Time	: 20.0 min
Injection Volume	: 2.0 µL
Autosampler Temperature	: 10°C
Detector wavelength	: 240 nm

Organic impurities

Solution A: 3.4 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile.

Diluent: Acetonitrile/water = 56:44

System suitability solution: 0.3 mg/mL of USP dexamethasone RS and 20 µg/mL of USP betamethasone RS in diluent. Sonicate to dissolve as needed.

Standard solution: 4.0 µg/mL of USP dexamethasone RS, 6.0 µg/mL each of USP betamethasone RS and USP desoximetasone RS, and 12.0 µg/mL of USP dexamethasone acetate RS in diluent.

Sample solution: 4.0 mg/mL of dexamethasone in diluent. Sonicate to dissolve as needed.

Table 2 LC acquisition parameters for Organic impurities

Column	: 100 mm x 2.1 mm I.D.,; 1.7 µm USP packing L1
Oven temperature	: 35°C
Mobile Phase A	: Solution A
Mobile phase B	: Solution B
Gradient program (B %)	: 0.0-10.0 min → 24.0 (%); 10.0-15.0 min → 24.0-55.0 (%); 15.0-16.0 min → 55.0-90.0 (%); 16.0-16.1 min → 90.0-24.0 (%); 16.1-20.0 min → 24.0 (%).
Flow Rate	: 0.4 mL/min
Total Run Time	: 20.0 min
Injection Volume	: 2.0 µL
Autosampler Temperature	: 10°C
Detector wavelength	: 240 nm

■ Results for Assay

The retention time of dexamethasone in standard and sample solutions is found to be about 8.961 minutes (see Fig. 3). The resolution between betamethasone and dexamethasone in system suitability solution is found to be 1.65 (see Fig. 4). The tailing factor for peak due to dexamethasone in the standard solution is well within the system suitability criteria of NMT 2.0 (see Table 3). The % relative standard deviation (%RSD) for retention time and peak area for six replicates of standards solution complies with the acceptable criteria of RSD NMT 0.73% (see Table 3) Overlay of five replicates of standards is shown in Fig. 5.

Table 3 Dexamethasone Assay standard

Parameter	Observed	USP Criteria
%RSD Retention Time (n=6)	0.056	NMT 0.73
%RSD Area (n=5)	0.161	NMT 0.73
Tailing factor	1.795	NMT 2.0

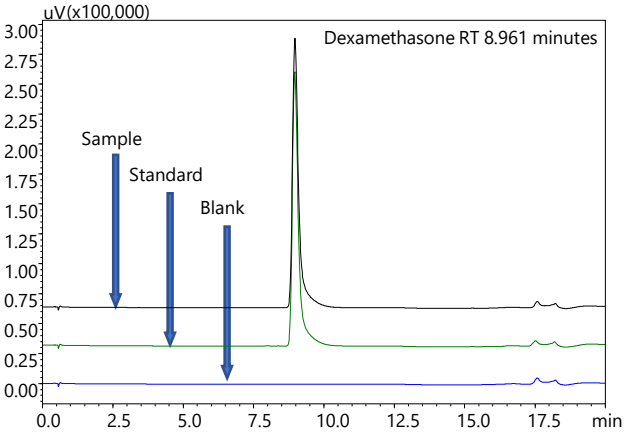

Fig. 3 Overlay of Assay sample, standard and blank

Sample analysis for Assay

The results obtained for analysis of sample using this method is found to be within the acceptance criteria of 97.0-102.0% (see Table 4).

Table 4 Content of Dexamethasone

Sample	Content (%)	USP Criteria (%)
Preparation-1	101.3	97.0-102.0
Preparation-2	100.7	

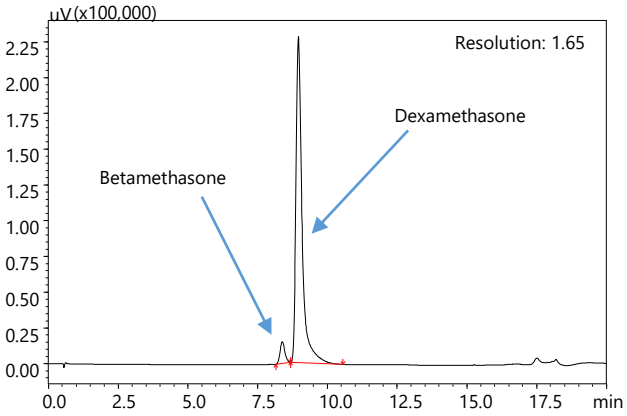

Fig. 4 System suitability solution

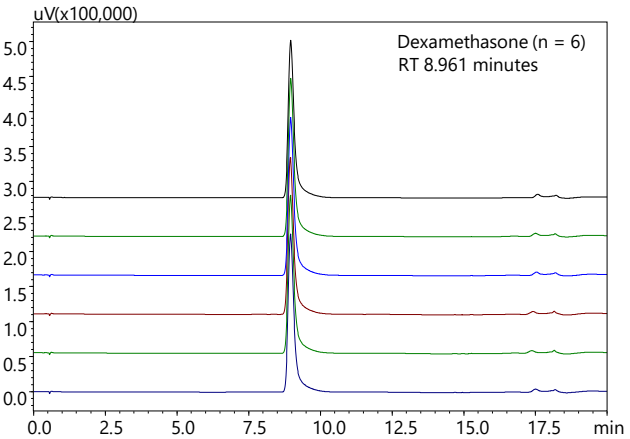

Fig. 5 Overlay chromatograms of Assay standards

■ Results for Organic impurities

The resolution between the peaks due to betamethasone and dexamethasone in the system suitability solution is found to be 1.65 which within the acceptance criteria of NLT 1.5 (see Fig. 4). The retention time of dexamethasone in

standard and sample solutions (see Fig. 6 and Fig. 7, respectively) is found to be about 8.9 minutes. The observed %RSD for area and retention time of betamethasone, dexamethasone, desoximetasone and dexamethasone acetate for replicate injections in standard solution is within the criteria of NMT 5.0 (see Table 5). The observed relative retention time for betamethasone, desoximetasone and dexamethasone acetate w.r.t. dexamethasone is found to be 0.934, 1.632 and 1.790 respectively.

Table 5 Dexamethasone organic impurities standard

Analyte	Parameter	%RSD (n=6)	USP Criteria
Dexamethasone	Retention time	0.090	NMT 5.0
	Area	0.888	NMT 5.0
Betamethasone	Retention time	0.086	NMT 5.0
	Area	0.278	NMT 5.0
Desoximetasone	Retention time	0.030	NMT 5.0
	Area	0.086	NMT 5.0
Dexamethasone acetate	Retention time	0.022	NMT 5.0
	Area	0.143	NMT 5.0

Sample analysis for Organic impurities

The percentage of betamethasone, desoximetasone, and dexamethasone acetate in the portion of dexamethasone taken is calculated using below equation.

$$\text{Result} = (r_u / r_s) \times (C_u / C_s) \times 100$$

where,

r_u = peak response of betamethasone, desoximetasone, or dexamethasone acetate from the sample solution.

r_s = peak response of the corresponding USP reference standard from the standard solution.

C_s = concentration of the corresponding USP reference Standard in the Standard solution (mg/mL).

C_u = concentration of dexamethasone in the sample solution (mg/mL).

The percentage of 16α-methylprednisone, dexamethasone 7,9-diene, and any individual unspecified impurity in the portion of dexamethasone taken is calculated using:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times (1/F) \times 100$$

where,

r_u = peak response of 16α-methylprednisone, dexamethasone 7,9-diene, or any individual unspecified impurity from the sample solution

r_s = peak response of dexamethasone from the standard solution

C_s = concentration of USP dexamethasone RS in the standard solution (mg/mL)

C_u = concentration of dexamethasone in the sample solution (mg/mL)

F = relative response factor for respective impurities.

The content of individual known impurity, any unspecified unknown impurity and total impurities were found to be within the acceptance criteria (see Table 6, Table 7 and Table 8). The typical chromatogram of sample showing overlay with blank is shown in Fig. 7.

Table 6 Dexamethasone Organic impurities sample

Label claim	Main peak area %	Total impurities %area
Sample-1	99.691	0.309
Sample-2	99.687	0.313
USP criteria	-	NMT 0.5

Table 7 Dexamethasone organic impurities USP acceptance criteria

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
16α-Methylprednisone	0.86	1.0	0.15
Betamethasone	0.94	-	0.15
Dexamethasone	1.00	-	-
Dexamethasone 7,9-diene	1.40	1.7	0.10
Desoximetasone	1.58	-	0.15
Dexamethasone acetate	1.74	-	0.30
Any individual unspecified impurity	-	1.0	0.10
Total impurities	-	-	0.5

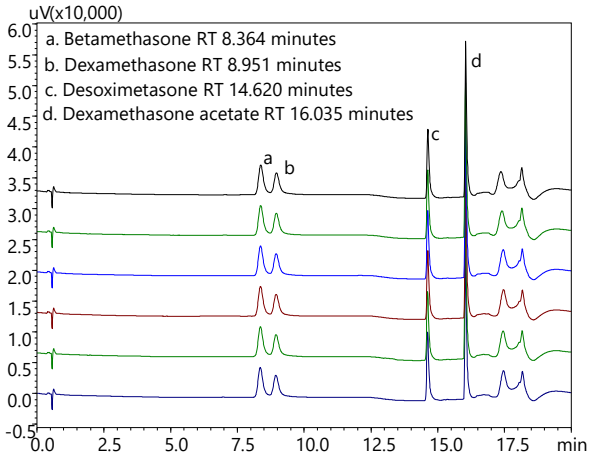

Fig. 6 Overlay chromatograms of Organic impurities standards

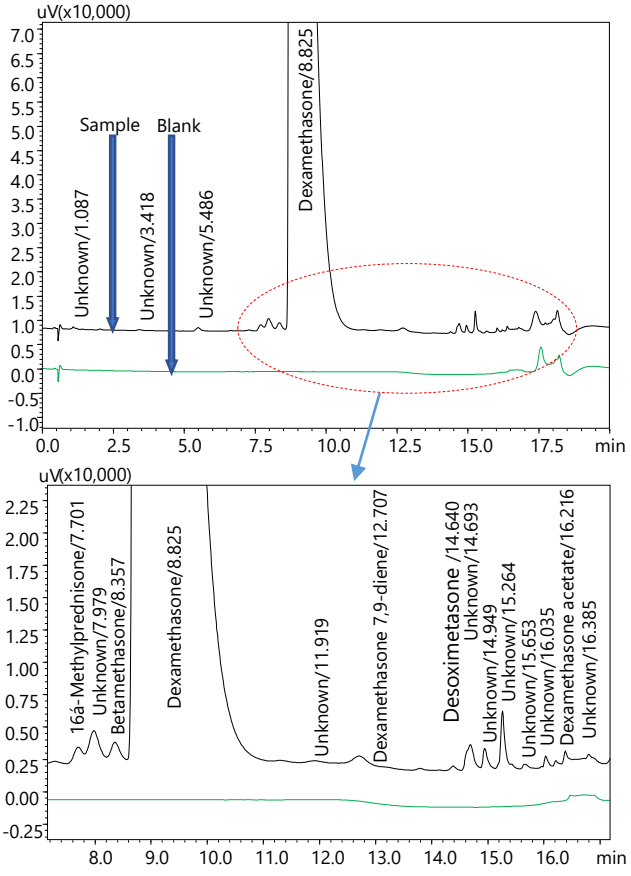

Fig. 7 Overlay of Organic impurities sample and blank

Table 8 Percent area for impurity peaks in samples

Peak#	Name	RRT	Sample-1	Sample-2
1	Unknown	0.122	0.005	0.005
2	Unknown	0.382	0.004	0.004
3	Unknown	0.614	0.018	0.017
4	16á-Methylprednisone	0.862	0.024	0.024
5	Unknown	0.893	0.054	0.054
6	Betamethasone	0.935	0.025	0.025
7	Dexamethasone	0.987	99.691	99.687
8	Unknown	1.333	0.005	0.006
9	Dexamethasone 7,9-diene	1.422	0.024	0.030
10	Dosimethasone	1.638	0.011	0.010
11	Unknown	1.644	0.025	0.025
12	Unknown	1.672	0.017	0.017
13	Unknown	1.708	0.048	0.048
14	Unknown	1.751	0.005	0.005
15	Unknown	1.794	0.008	0.008
16	Dexamethasone acetate	1.814	0.003	0.003
17	Unknown	1.833	0.007	0.007

■ Conclusion

- This study successfully demonstrated the performance of Shimadzu Nexera series UHPLC system to determine the content of dexamethasone and its organic impurities in conformity with the USP monograph acceptance criteria and system suitability requirements.
- The reproducibility i.e., the relative standard deviation of retention time and area for standard are well within the acceptance criteria of NMT 0.73% for assay test and NMT 5.0% for the organic impurities test.
- The resolution between the peaks due to betamethasone and dexamethasone is found to be well within the acceptance.
- The relative retention times for all known impurities complies with the acceptance value in the USP monograph.
- The sample shows content of dexamethasone and its organic impurities within the permissible limits of USP.

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