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Method of Test for *N*-Nitroso Duloxetine in Duloxetine Drug Substance and Drug Products

1. Scope

This method is applicable to the determination of *N*-nitroso duloxetine in duloxetine drug substance and drug products.

2. Method

After extraction, *N*-nitroso duloxetine is determined by liquid chromatography/ tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer

2.1.1.1. Ion source: electrospray ionization, ESI.

2.1.1.2. Column: Kinetex Phenyl-Hexyl, 2.6 μm, 4.6 mm i.d. × 5 cm, or an equivalent product.

2.1.2. Ultrasonicator.

2.1.3. Vortex mixer.

2.1.4. Centrifuge: centrifugal force ≥ 3000 ×g.

2.2. Chemicals

Methanol, HPLC grade;
Acetonitrile, HPLC grade;
Formic acid, HPLC grade;
Deionized water, resistivity ≥ 18 MΩ · cm at (25°C);
N-nitroso duloxetine, reference standard.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL, amber flask.

2.3.2. Centrifuge tube: 15 mL, PP.

2.3.3. Membrane filter: 0.22 μm, PVDF.

2.4. Mobile phase

2.4.1. Solvent A:

Dilute 1 mL of formic acid with deionized water to 1000 mL. Filter with a membrane filter.

2.4.2. Solvent B:

Dilute 1 mL of formic acid with acetonitrile to 1000 mL. Filter with a membrane filter.

2.5. Standard solution preparation

Transfer about 5 mg of *N*-nitroso duloxetine reference standard accurately weighed into a 10 mL volumetric flask, dissolve and dilute to volume with methanol as the standard stock solution, and then store in a refrigerator. Upon use, dilute with methanol to 1-50 ng/mL as the standard solutions.

2.6. Standard calibration curve establishment

Accurately inject 10 μL of the standard solution into LC-MS/MS separately, and operate according to the following conditions. Establish the standard calibration curve of *N*-nitroso duloxetine by the peak area of *N*-nitroso duloxetine vs. the concentrations of *N*-nitroso duloxetine.

LC-MS/MS operating conditions(note):

Column: Kinetex Phenyl-Hexyl, 2.6 μm, 4.6 mm i.d. × 5 cm.

Column temperature: 40°C.

Mobile phase: a gradient program of solvent A and solvent B is as follows.

Time (min)	A (%)	B (%)
0.0 → 1.5	50 → 50	50 → 50
1.5 → 5.0	50 → 35	50 → 65
5.0 → 5.1	35 → 0	65 → 100
5.1 → 6.0	0 → 0	100 → 100
6.0 → 6.1	0 → 50	100 → 50
6.1 → 10.0	50 → 50	50 → 50

Flow rate: 0.6 mL/min.

Inject volume: 10 μL.

Ion spray voltage: 5.5 kV.

Ionization mode: ESI^+.

Ion source temperature: 500°C.

Nebulizer gas, Gas 1: 40 psi.

Heated gas, Gas 2: 40 psi.

Curtain gas: 40 psi.

Collision gas: Low.

Detection mode: multiple reaction monitoring (MRM). Selected ion pair, declustering potential and collision energy are as follows.

Analyte	Ion pair Precursor ion (*m/z*) > Product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)
N-nitroso duloxetine	327 > 183*	50	9
	327 > 123	30	24

* Quantitative ion pair

Note: 1. If a divert valve is available, the direction of the mobile phase can be diverted as follows.

Time (min)	Position
0.0 → 3.2	Waste
3.2 → 5.2	MS
5.2 → 10.0	Waste

2. All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.7. Sample solution preparation

2.7.1. Drug substance

Transfer about 0.1 g of sample accurately weighed to a 10 mL volumetric flask, and add 8 mL of methanol. Mix well, sonicate for 10 min, and dilute with methanol to volume. Transfer to a centrifugal tube, and centrifuge at 3000 ×g for 5 min. Filter the supernatant with a membrane filter, and take the filtrate as the sample solution.

2.7.2. Drug product

Accurately weigh at least 10 capsules. Remove the contents of the capsules and calculate the average weight of the contents per capsule. Mix the contents of the capsules, and transfer about 0.1 g of the sample accurately weighed to a 10 mL volumetric flask, and add 8 mL of methanol. Mix well, sonicate for 10 min, and dilute with methanol to volume. Transfer to a centrifugal tube, and centrifuge at 3000 ×g for 5 min. Filter the supernatant with a membrane filter, and take the filtrate as the sample solution.

2.8. Identification and quantification

Accurately inject 10 μL of sample solution and standard solution into LC-MS/MS separately, and operate according to the conditions in section 2.6. Identify *N*-nitroso duloxetine based on the retention time and the relative

ion intensities(note). Calculate the amount of *N*-nitroso duloxetine in the sample by the following formula:

$$\text{The amount of } N\text{-nitroso duloxetine in the sample } (\mu g/g) = \frac{C \times V}{M} \times 10^{-3}$$

Where,

C: the concentration of *N*-nitroso duloxetine in the sample solution calculated by the standard calibration curve (ng/mL)

V: the final make-up volume of the sample (mL)

M: the weight of the sample (g)

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤ 100%). Maximum permitted tolerances of relative ion intensities by LC-MS/MS are as follows.

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20-50	± 25
> 10-20	± 30
≤ 10	± 50

Remark

1. Limit of quantification (LOQ) for *N*-nitroso duloxetine is 0.1 μg/g.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. Swissmedic OMCL. 2024. Determination of *N*-nitroso duloxetine in duloxetine preparations with LC-MS. [https://www.edqm.eu/en/d/1937124?p_l_back_url=%2Fen%2Fsearch-edqm%3Fq%3DEuropean%2BPharmacopoeia%2BPh.Eur.%2BGeneral%2BMethod%2B2.2.40%253F%26category%3D185147%26tag%3Dpaediatric%2Bformulary%2B%2528paedform%2529%26delta%3D60%26start%3D24]
2. Fukuda, S., Nakase, Y., Imagaki, K., Kondo, K., Taniguchi, T. and Uchikawa, O. 2024. Simple and practical method for the quantitative high-sensitivity analysis of *N*-nitroso duloxetine in duloxetine drug products utilizing LC-MS/MS. ACS Omega. 9:13440-13446.

Reference chromatogram

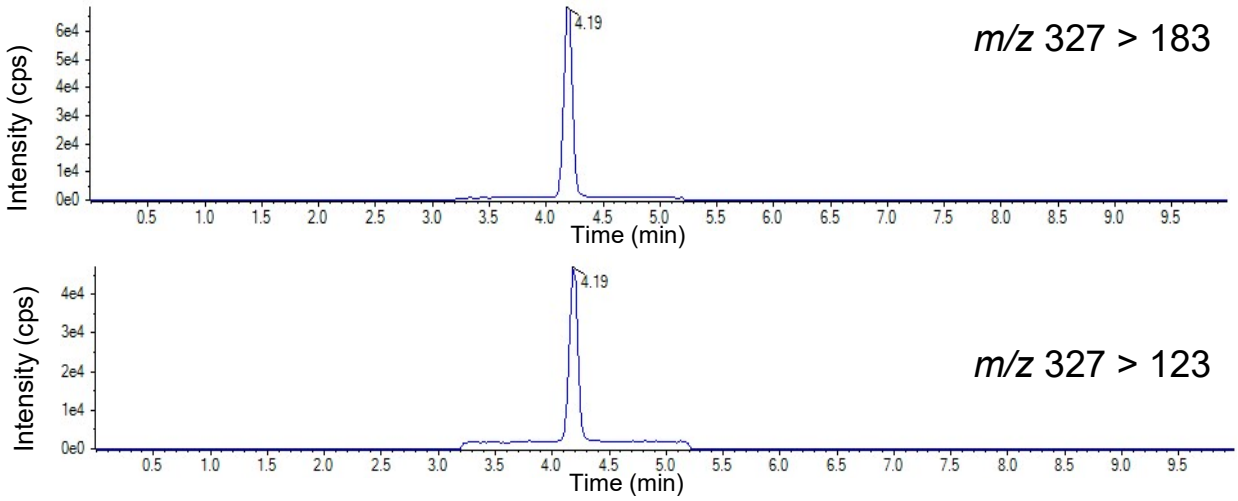

Figure. The MRM chromatograms of *N*-nitroso duloxetine standard analyzed by LC-MS/MS.