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Method of Test for *N*-Nitroso Paroxetine in Paroxetine Drug Substance

1. Scope

This method is applicable to the determination of *N*-nitroso paroxetine in paroxetine drug substances.

2. Method

After extraction, *N*-nitroso paroxetine 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: Symmetry C18, 3.5 μm, 4.6 mm i.d. × 15 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;
Ammonium formate, reagent grade;
Deionized water, resistivity ≥ 18 MΩ · cm at (25°C);
N-nitroso paroxetine, reference standard;
N-nitroso paroxetine-d_5 isotope-labeled internal 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:

Accurately weigh 0.63 g of ammonium formate and transfer into a 1 L volumetric flask and dilute to volume with deionized water. Filter with a membrane filter.

2.4.2. Solvent B: Acetonitrile

2.5. Internal standard solution preparation

Transfer about 5 mg of *N*-nitroso paroxetine-d_4 internal reference standard accurately weighed into a 10 mL volumetric flask, dissolve and dilute to volume with methanol as the internal standard stock solution. Store in a refrigerator. Upon use, dilute the internal standard stock solution with methanol to 5 ng/mL as the internal standard solution.

2.6. Standard solution preparation

Transfer about 5 mg of *N*-nitroso paroxetine reference standard accurately weighed into a 10 mL volumetric flask, dissolve and dilute to volume with methanol as the standard stock solution. Store in a refrigerator. Upon use, mix appropriate volume of the standard stock solution and the internal standard solution, and dilute with methanol to 0.3-20 ng/mL (containing 0.5 ng/mL internal standard) as the standard solutions.

2.7. Standard calibration curve establishment

Accurately inject 5 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 paroxetine by the ratios of the peak area of *N*-nitroso paroxetine to that of the internal standard vs. the concentrations of *N*-nitroso paroxetine.

LC-MS/MS operating conditions(note):

Column: Symmetry C18, 3.5 μm, 4.6 mm i.d. × 15 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 → 2.0	35 → 35	65 → 65
2.0 → 7.0	35 → 5	65 → 95
7.0 → 9.0	5 → 5	95 → 95
9.0 → 9.1	5 → 35	95 → 65
9.1 → 12.0	35 → 35	65 → 65

Flow rate: 0.8 mL/min.

Inject volume: 5 μL.

Ion spray voltage: 5.5 kV.

Ionization mode: ESI^+.

Ion source temperature: 500°C.

Nebulizer gas, Gas 1: 50 psi.
Heated gas, Gas 2: 60 psi.
Curtain gas: 35 psi.
Collision gas: high.
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 paroxetine	359 > 192*	50	23
	359 > 329	50	11
N-nitroso paroxetine-d_4 (I.S.)	363 > 196	45	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 → 4.6	Waste
4.6 → 5.6	MS
5.6 → 12.0	Waste

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

2.8. Sample solution preparation

Transfer about 0.1 g of sample accurately weighed to a centrifuge tube, and add 1 mL of the internal standard solution and 9 mL of methanol. Mix well, sonicate for 5 min and centrifuge at 3000 ×g for 5 min. Filter the supernatant with a membrane filter, and take the filtrate as the sample solution.

2.9. Identification and quantification

Accurately inject 5 μL of sample solution and standard solution into LC-MS/MS separately, and operate according to the conditions in section 2.7. Identify *N*-nitroso paroxetine based on the retention time and the relative ion intensities(note). Calculate the amount of *N*-nitroso paroxetine in the sample by the following formula:

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

Where,

C: the concentration of *N*-nitroso paroxetine 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

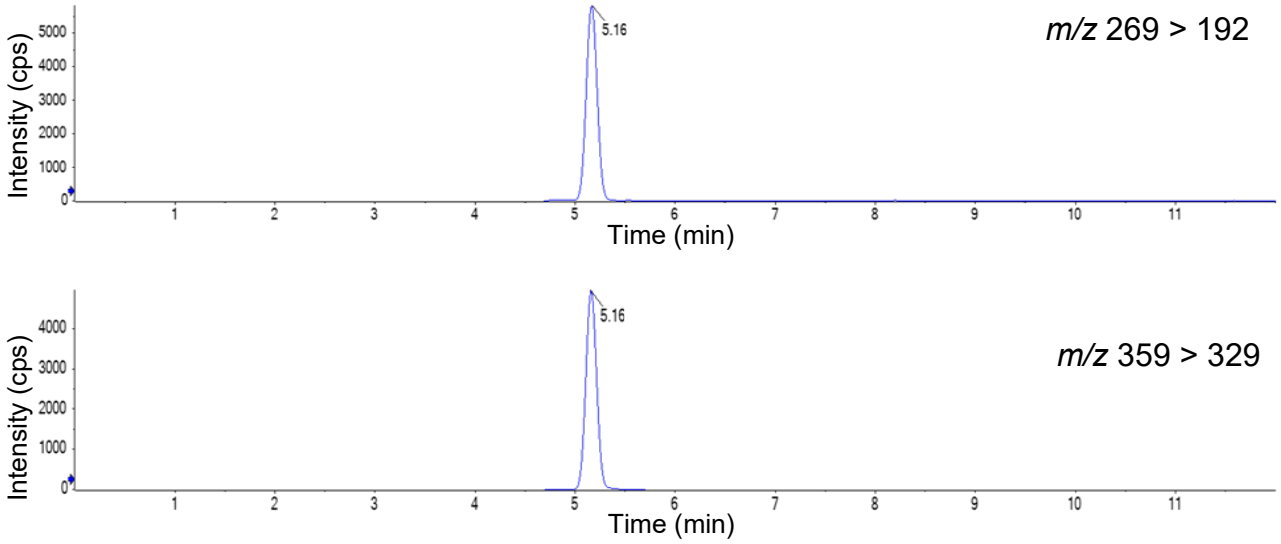
1. Limit of quantification (LOQ) for *N*-nitroso paroxetine is 0.03 μg/g.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. Wang, M., Zhou, W., Zhang, Q. and Huang, M. 2013. Development and validation of a LC-MS/MS method. Adv. Mater. Res. 722: 255-259.
2. Jhee, O. H., Seo, H. K., Lee, M. H., Jeon, Y. C., Shaw, L. M., Lee, S. H., Hur, Y., Kim, K. H., Lee, H. S., Lee, S. E. and Kang, J. S. 2007. Determination of paroxetine in plasma by liquid chromatography coupled to tandem mass spectrometry for pharmacokinetic and bioequivalence studies. Arzneim-Forsch. 57: 455-461.

Reference chromatogram

(A) *N*-nitroso paroxetine



(B) *N*-nitroso paroxetine-d_4 (I.S.)

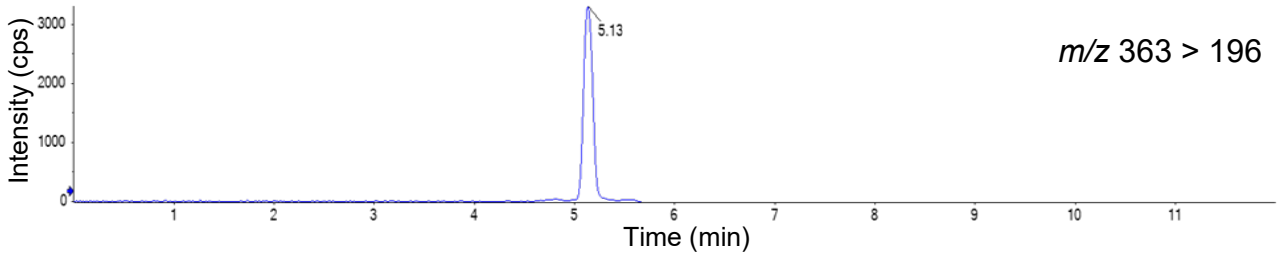


Figure. The MRM chromatograms of *N*-nitroso paroxetine standard (A) and *N*-nitroso paroxetine-d_4 internal standard (B) analyzed by LC-MS/MS.