Published, Nov 2, 2023
RA01I009.001

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

1. Scope

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

2. Method

After extraction, *N*-nitroso propranolol 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 Biphenyl, 2.6 μm, 3.0 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;
Formic acid, HPLC grade;
Ammonium formate, reagent grade;
Deionized water, resistivity ≥ 18 MΩ · cm at (25°C);
N-nitroso propranolol, reference standard;
N-nitroso propranolol-d_7 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 0.063 g of ammonium formate and transfer into a 1 L volumetric flask. Add 1 mL of formic acid and dilute to volume with deionized water. Filter with a membrane filter.

2.4.2. Solvent B

Dilute 200 mL of methanol with acetonitrile to 1000 mL, and mix well. Filter with a membrane filter.

2.5. Internal standard solution preparation

Transfer about 5 mg of *N*-nitroso propranolol-d_7 internal reference standard accurately weighed into a 10 mL volumetric flask, dissolve and dilute to volume with methanol as the internal standard stock solution. 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 10 mg of *N*-nitroso propranolol reference standard accurately weighed into a 10 mL volumetric flask, dissolve and dilute to volume with methanol to as the standard stock solution. Upon use, mix appropriate volume of the standard stock solution and the internal standard solution, and dilute with methanol to 0.025~10 ng/mL (containing 0.5 ng/mL internal standard) as the standard solutions.

2.7. 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 propranolol by the ratios of the peak area of *N*-nitroso propranolol to that of the internal standard vs. the concentrations of *N*-nitroso propranolol.

LC-MS/MS operating conditions(note):

Column: Kinetex Biphenyl, 2.6 μm, 3.0 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 → 3.0	65 → 65	35 → 35
3.0 → 5.0	65 → 45	35 → 55
5.0 → 6.0	45 → 25	55 → 75
6.0 → 8.0	25 → 25	75 → 75
8.0 → 8.1	25 → 5	75 → 95
8.1 → 11.0	5 → 5	95 → 95
11.0 → 11.1	5 → 65	95 → 35
11.1 → 15.0	65 → 65	35 → 35

Flow rate: 0.4 mL/min.
Inject volume: 10 μL.
Ion spray voltage: 5.5 kV.
Ionization mode: ESI^+.
Ion source temperature: 550°C.
Nebulizer gas, Gas 1: 40 psi.
Heated gas, Gas 2: 60 psi.
Curtain gas: 50 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 propranolol	289 > 259*	40	8
	289 > 72	30	20
N-nitroso propranolol-d_7	296 > 152	30	10

* 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 → 6.5	Waste
6.5 → 8.1	MS
8.1 → 15.0	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 0.05 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 10 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 10 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 propranolol based on the retention time and the relative ion intensities(note). Calculate the amount of *N*-nitroso propranolol in the sample by the following formula:

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

Where,

C: the concentration of *N*-nitroso propranolol 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 quantitative ions divided by peak areas of qualitative 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 propranolol is 0.005 µg/g.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. Sluggett, G. W., Zelesky, T., Hetrick, E. M., Babayan, Y. and Baertschi, S. W. 2018. Artifactual degradation of secondary amine-containing drugs during accelerated stability testing when saturated sodium nitrite solutions are used for humidity control. J. Pharm. Biomed. Anal. 149: 206-213.
2. Chang, S. H., Chang, C. C., Wang, L. J., Chen, W. C., Fan, S. Y., Zang, C. Z., Hsu, Y. H., Lin, M. C., Tseng, S. H. and Wang, D. Y. 2020. A multi-analyte LC-MS/MS method for screening and quantification of nitrosamines

in sartans. J. Food Drug Anal. 28: 98-107.

Reference chromatogram

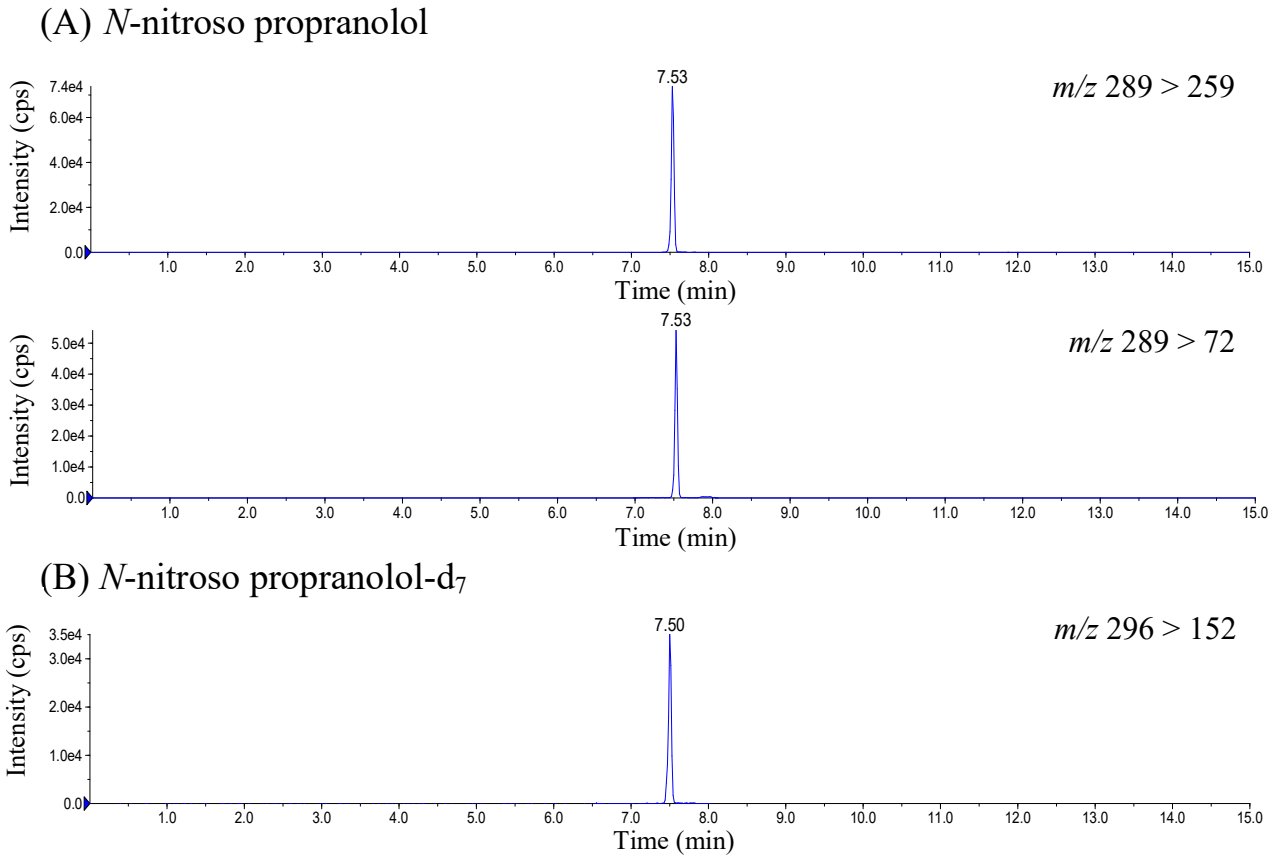

Figure. The MRM chromatograms of *N*-nitroso propranolol standard (A) and *N*-nitroso propranolol-d_7 internal standard (B) analyzed by LC-MS/MS.