Published, Dec 30, 2022
RA01I008.001

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

1. Scope

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

2. Method

After extraction, *N*-nitroso salbutamol 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: XSelect HSS T3, 3.5 μm, 3 mm i.d. × 15 cm, or an equivalent product.

2.1.2. Ultrasonicator.

2.1.3. 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 salbutamol, 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, and filter with a membrane filter.

2.4.2. Solvent B

Dilute 1 mL of formic acid and 200 mL of acetonitrile with methanol to 1000 mL, and mix well.

2.5. Standard solution preparation

Transfer appropriate amount of *N*-nitroso salbutamol reference standard

accurately weighed into a 10-mL volumetric flask, dissolve and dilute to volume with methanol to 1000 μg/mL as the standard stock solutions. Store at -20℃ and protect from light. Upon use, mix appropriate volume of the standard stock solution and dilute with deionized water to 0.025-100 ng/mL as the standard solution.

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 salbutamol by the peak area of *N*-nitroso salbutamol vs. the concentrations of *N*-nitroso salbutamol.

LC-MS/MS operating conditions(note):

Column: XSelect HSS T3, 3.5 μm, 3 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 → 1.0	95 → 95	5 → 5
1.0 → 5.0	95 → 50	5 → 50
5.0 → 6.5	50 → 50	50 → 50
6.5 → 7.5	50 → 35	50 → 65
7.5 → 8.5	35 → 35	65 → 65
8.5 → 9.5	35 → 0	65 → 100
9.5 → 12.0	0 → 0	100 → 100
12.0 → 12.1	0 → 95	100 → 5
12.1 → 15.0	95 → 95	5 → 5

Flow rate: 0.6 mL/min.

Inject volume: 10 μL.

Ion spray voltage: 4.5 kV.

Ionization mode: ESI^-.

Ion source temperature: 450°C.

Nebulizer gas, Gas 1: 50 psi.

Heated gas, Gas 2: 60 psi.

Curtain gas: 25 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 salbutamol	267 > 151* 267 > 204	18	33 34

* Quantitative ion pair

Note: 1. All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

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

Time (min)	Position
0.0 → 5.0	Waste
5.0 → 9.0	MS
9.0 → 15.0	Waste

2.7. Sample solution preparation

Transfer about 0.01 g of sample accurately weighed to a 10-mL volumetric flask, and add 8 mL of deionized water. Mix well, sonicate for 10 min, and dilute with deionized water to volume. Transfer to a 15-mL 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 salbutamol based on the retention time and the relative ion intensities(note). Calculate the amount of *N*-nitroso salbutamol in the sample by the following formula:

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

Where,

C: the concentration of *N*-nitroso salbutamol 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 the follows.

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

Remark

1. Limit of quantification (LOQ) for *N*-nitroso salbutamol is 0.025 μg/g.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. Wu, J., Ding, C., Ge, Q., Li, Z., Zhou, Z. and Zhi, X. 2011. Simultaneous determination of ipratropium and salbutamol in rat plasma by LC-MS/MS and its application to a pharmacokinetic study J. Chromatogr. B 879: 3475-3483.
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

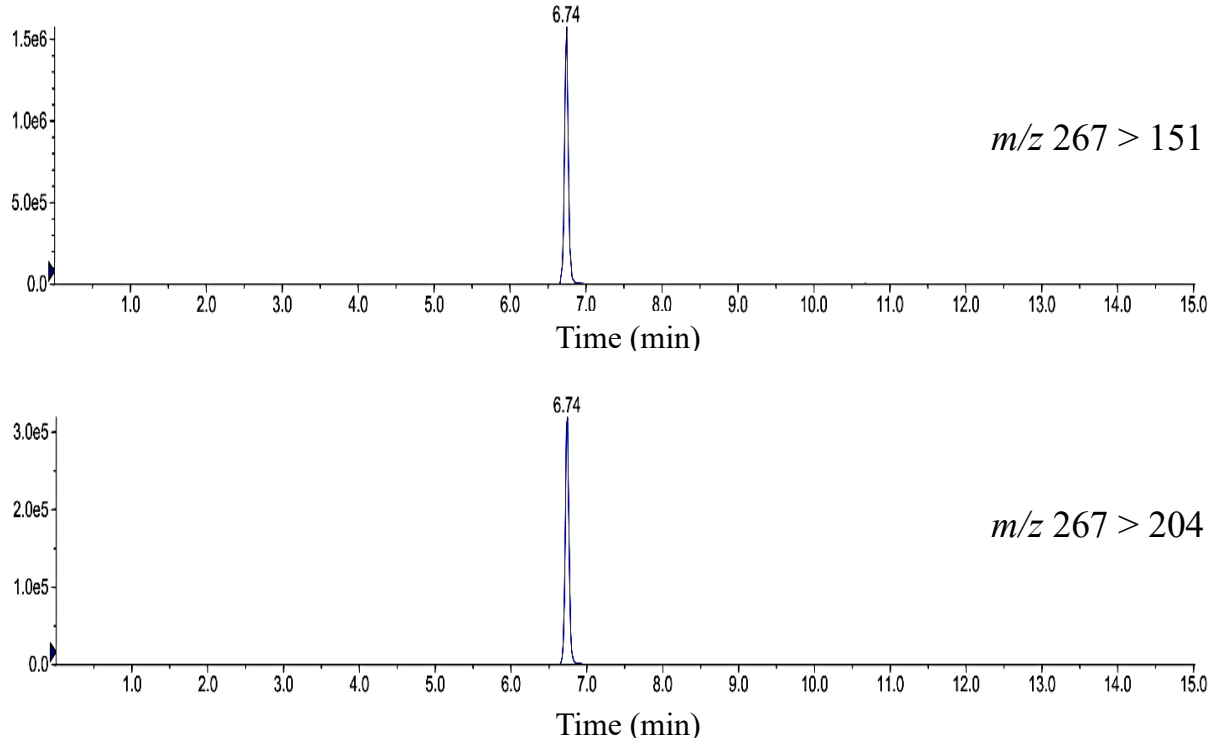

Figure. The MRM chromatograms of *N*-nitroso salbutamol standard analyzed by LC-MS/MS.