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Determination of *N*-Nitroso-*N*-Methyl-4-Aminobutyric Acid in Sartan Drug Substances and Drug Products

1. Scope

This method is applicable to the determination of *N*-nitroso-*N*-methyl-4-aminobutyric acid (NMBA) in sartan drug substances including candesartan, irbesartan, losartan, olmesartan, telmisartan and valsartan, and their products.

2. Method

After extraction, NMBA is determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

2.1. Equipments

2.1.1. Liquid chromatograph/tandem mass spectrometer

2.1.1.1. Ion source: atmospheric pressure chemical ionization, APCI.

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 ≧ 2500 ×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-*N*-methyl-4-aminobutyric acid (NMBA), reference standard;

N-Nitroso-*N*-methyl-*4*-aminobutyric acid-d_3 (NMBA-d_3), isotope-labelled internal standard.

2.3. Apparatus

2.3.1. Volumetric flask: 10-mL and 20-mL.

2.3.2. Centrifuge tube: 15-mL, PP.

2.3.3. Membrane filter: 0.22-μm, PVDF.

2.4. 50% methanol

Mix methanol and deionized water at the ratio of 1:1 (v/v).

2.5. Mobile phase

2.5.1. Solvent A

Dilute 2 mL of formic acid with deionized water to 1000 mL, and filter

with a membrane filter.

2.5.2. Solvent B

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

2.6. Internal standard solution preparation

Transfer 10 mg of NMBA-d_3 isotope-labelled internal standard accurately weighed to a 20-mL volumetric flask, dissolve and dilute to volume with methanol as the internal standard stock solution, and then store in a freezer and protect from light. When to use, dilute appropriate volume of the internal standard stock solution with 50% methanol to 500 ng/mL as the internal standard solution.

2.7. Standard solution preparation

Transfer 10 mg of NMBA reference standard accurately weighed to a 20-mL volumetric flask, dissolve and dilute to volume with methanol as the standard stock solution, and then store in a freezer and protect from light. When to use, mix appropriate volume of the standard stock solution and the internal standard solution, and dilute with 50% methanol to 5-100 ng/mL (containing 50 ng/mL of the internal standard) as the standard solutions.

2.8. Standard calibration curve establishment

Accurately inject 3 μL of the standard solutions into LC-MS/MS separately, and operate according to the following conditions. Establish the standard calibration curves of NMBA by the ratio of the peak area of NMBA to that of the internal standard vs. the concentration of NMBA.

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)	Solvent A (%)	Solvent B (%)
0.0 → 1.0	95 → 95	5 → 5
1.0 → 5.0	95 → 0	5 → 100
5.0 → 6.5	0 → 0	100 → 100
6.5 → 6.6	0 → 95	100 → 5

6.6 → 9.0	95 → 95	5 → 5

Flow rate: 0.6 mL/min.

Injection volume: 3 μL.

Ion source: $APCI^+$.

Nebulizer current: 5 μA.

Curtain gas: 25 psi.

Gas 1: 50 psi.

Collision gas: medium.

Temperature: 400°C.

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

Analyte	Precursor ion (*m/z*) > product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)
NMBA	147 > 117*	50	9
	147 > 87	50	17
NMBA-d_3 (I.S.)	150 > 120	36	9

*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	Detector
5.0 → 9.0	Waste

2.9. Sample solution preparation

2.9.1. Sartan drug substance (active pharmaceutical ingredient)

Transfer about 1 g of sample accurately weighed to a 10-mL volumetric flask, and add 1 mL of the internal standard solution and 7 mL of 50% methanol. Sonicate for 30 min, and dilute with 50% methanol to volume. Transfer to a 15-mL centrifugal tube, and centrifuge at 2500 xg for 10 min. Filter the supernatant with a membrane filter, and take the filtrate as the sample solution.

2.9.2. Sartan drug product

Accurately weigh at least 10 tablets of the sample, and calculate the average weight per tablet. Then, grind them to powder. Transfer about 1 g of the powdered sample accurately weighed to a 10-mL volumetric flask, and add 1 mL of the internal standard solution and 7 mL of 50% methanol. Sonicate for 30 min, and dilute with 50% methanol to volume. Transfer to a 15-mL centrifugal tube, and centrifuge at 2500 xg for 10 min. Filter the supernatant with a membrane filter, and take the filtrate as the sample solution.

2.10. Identification and quantification

Accurately inject 3 µL of the sample solution and the standard solutions into LC-MS/MS separately, and operate according to the conditions in section 2.8. Identify NMBA based on the retention time and the relative ion intensities(note). Calculate the amount of NMBA in the sample by the following formula:

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

Where,

C: the concentration of NMBA 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 for 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. The limit of quantification (LOQ) for NMBA is 0.05 µg/g.
2. If the result is showed as the amount of NMBA per tablet (µg/Tab.), it is

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calculated by multiplying the amount of NMBA in the sample (μg/g) by the average weight per tablet (g/Tab.).

3. Further validation shall be done when interference compounds appear in samples.

Reference

Ripollés, C., Pitarch, E., Sancho, J. V., López, F. J. and Hernández, F. 2011. Determination of eight nitrosamines in water at the ng L^{-1} levels by liquid chromatography coupled to atmospheric pressure chemical ionization tandem mass spectrometry. Anal. Chim. Acta 702: 62-71.

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Reference chromatogram

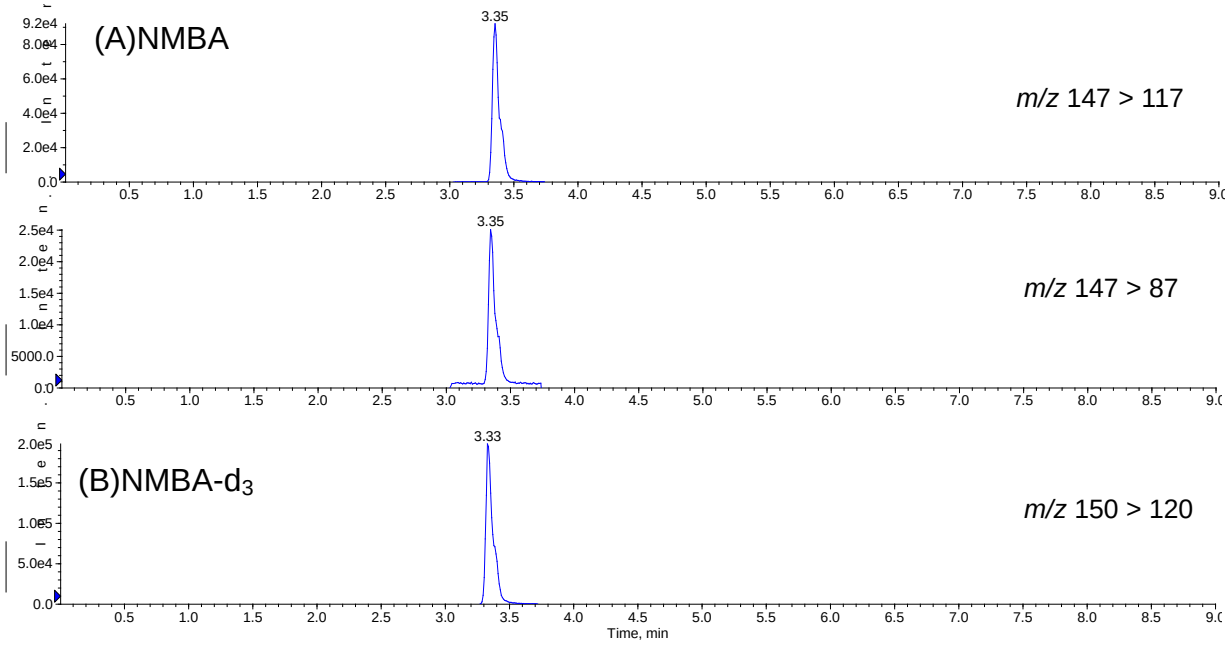

Figure. The MRM chromatograms of NMBA (A) and NMBA-d_3 internal standard (B) analyzed by LC-MS/MS.