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Determination of *N*-Nitrosodimethylamine and *N*-Nitrosodiethylamine in Medicines

1. Scope

This method is applicable to the determination of *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) in sartan, ranitidine and metformin drug substances and drug products.

2. Method

After extraction, *N*-nitrosodimethylamine and *N*-nitrosodiethylamine are 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;

Formic acid, HPLC grade;

Deionized water, resistivity ≧ 18 MΩ・cm (at 25°C);

N-Nitrosodimethylamine (NDMA), 200 μg/mL in methanol, reference standard;

N-Nitrosodiethylamine (NDEA), 5000 μg/mL in methanol, reference standard;

N-Nitrosodimethylamine-d_6 (NDMA-d_6), 1000 μg/mL in dichloromethane, isotope-labelled internal standard;

N-Nitrosodiethylamine-d_4 (NDEA-d_4), isotope-labelled internal standard.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL, 50-mL.

2.3.2. Centrifuge tube: 15 mL, PP.

2.3.3. Membrane filter: 0.22 μm, PVDF.

2.3.4. Mortar and pestle.

2.4. 50% methanol

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

2.5. Mobile phase

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2.5.1. Solvent A

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

2.5.2. Solvent B

Dilute 1 mL of formic acid with methanol to 1000 mL, and filter with a membrane filter.

2.6. Internal standard solution preparation

Accurately transfer 1 mL of NDMA-d_6 isotope-labelled internal standard to a 10-mL volumetric flask, and transfer about 1 mg of NDEA-d_4 isotope-labelled internal standard accurately weighed to a 10-mL volumetric flask. Dissolve and dilute to volume with methanol as the internal standard stock solutions. Store in a freezer and protect from light. When to use, mix appropriate volume of each internal standard stock solution, and dilute with 50% methanol to 200 ng/mL as the internal standard solution.

2.7. Standard solution preparation

Accurately transfer 50 μL of NDMA reference standard and 10 μL of NDEA reference standard to a 10-mL volumetric flask and a 50-mL volumetric flask, respectively, and dilute to volume with methanol as the standard stock solutions. Store in a freezer and protect from light. When to use, mix appropriate volume of each the standard stock solution and the internal standard solution, and dilute with 50% methanol to 2-50 ng/mL (containing 20 ng/mL of the internal standard) as the standard solutions.

2.8. Standard calibration curve establishment

Accurately inject 10 μL of the standard solutions into LC-MS/MS separately, and operate according to the following conditions. Establish the standard calibration curves of NDMA and NDEA by the ratios of the peak area of NDMA and NDEA to that of the internal standard vs. the concentrations of NDMA and NDEA.

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

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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: 10 µL.

MS parameters (1):

Ionization mode: $APCI^+$.

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)
NDMA	75 > 43*	28	21
	75 > 58	28	15
NDEA	103 > 75*	25	13
	103 > 47	25	29
NDMA-d_6 (I.S.)	81 > 46	106	25
NDEA-d_4 (I.S.)	107 > 77	48	16

*Quantitative ion.

MS parameters (2):

Ionization mode: $APCI^+$.

Capillary voltage: 4 kV.

Probe temperature: 400°C.

Ion source temperature: 130°C.

Cone gas flow: 150 L/hr.

Desolvation gas flow: 950 L/hr.

Detection mode: multiple reaction monitoring (MRM). Detected ion pair,

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cone voltage and collision energy are as follows.

Analyte	Precursor ion (*m/z*) > product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
NDMA	75 > 43*	28	12
	75 > 58	28	8
NDEA	103 > 75*	18	10
	103 > 47	18	10
NDMA-d_6 (I.S.)	81 > 46	28	10
NDEA-d_4 (I.S.)	107 > 77	8	8

*Quantitative ion.

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. 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 add 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. Drug product

Grind at least 10 tablets of the sample 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 add 50% methanol to volume. Transfer to a 15-mL centrifugal tube, and centrifuge at 2500 xg for 10 min. Filter the

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supernatant with a membrane filter, and take the filtrate as the sample solution.

2.10. Identification and quantification

Accurately inject 10 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 NDMA or NDEA based on the retention time and the relative ion intensities(note). Calculate the amount of NDMA or NDEA in the sample by the following formula:

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

Where,

C: the concentration of NDMA or NDEA in the sample solution calculated by the standard calibration curve (ng/mL)

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

M: the weight of sample (g)

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

Relative ion intensity (% of base peak)	Tolerance (%)
＞50	± 20
＞20-50	± 25
＞10-20	± 30
≦10	± 50

Remark

1. The limits of quantitation (LOQs) of NDMA and NDEA for Ranitidine are 0.1 μg/g and 0.05 μg/g, respectively, and those for other drugs are both 0.02 μg/g.
2. 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

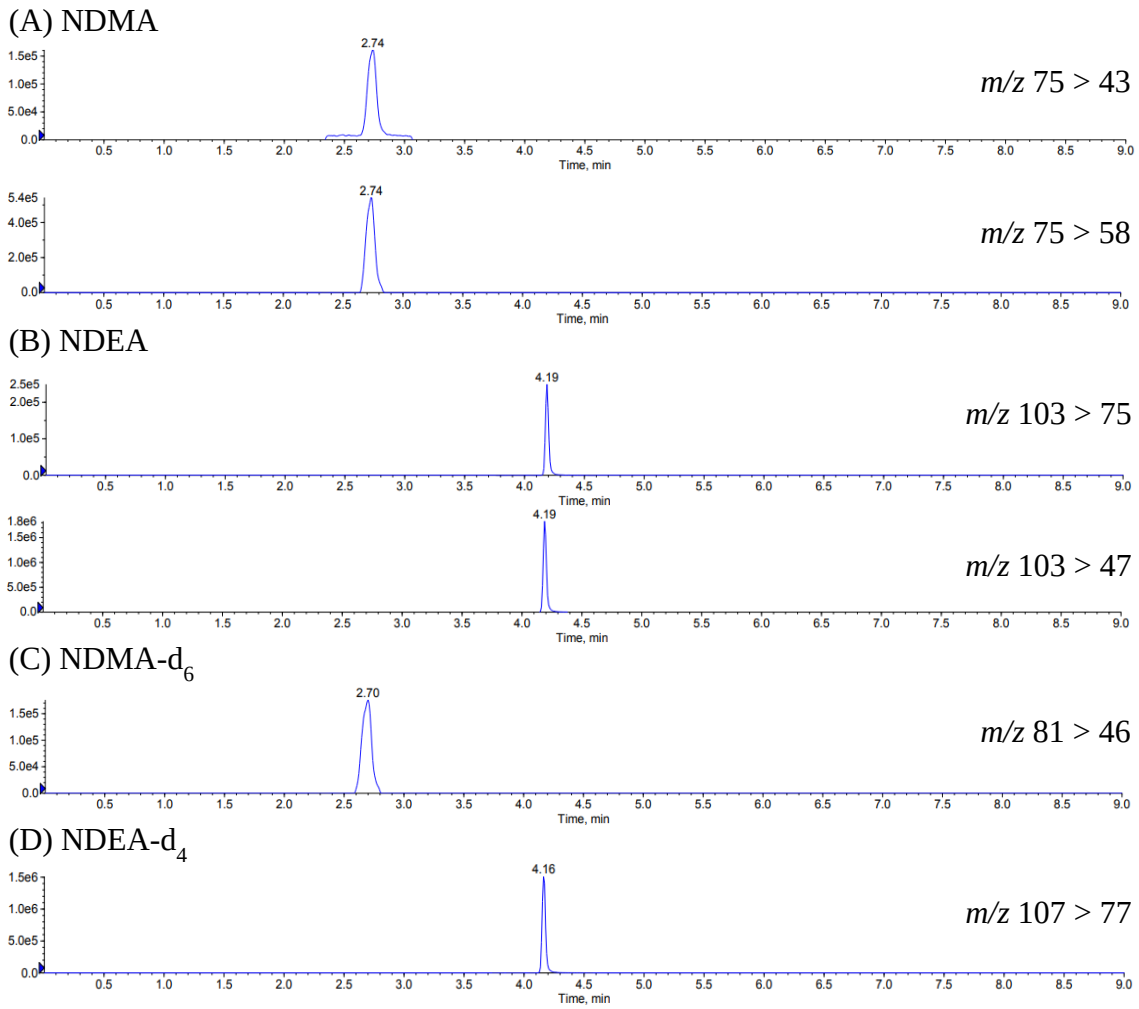

Figure. The MRM chromatograms of NDMA (A), NDEA (B), NDMA-d_6 internal standard (C) and NDEA-d_4 internal standard (D) analyzed by LC-MS/MS.