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Method of Test for *N*-nitrosodimethylamine in Metformin Drug Substance and Drug Products

1. Scope

This method is applicable to the determination of *N*-nitrosodimethylamine (NDMA) in metformin drug substance and drug products.

2. Method

After extraction, NDMA 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: 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 ≥ 4000 ×g.

2.2. Chemicals

Methanol, HPLC grade;

Formic acid, HPLC grade;

Deionized water, resistivity ≥ 18 MΩ · cm at (25°C);

N-nitrosodimethylamine (NDMA), 5.0 mg/mL in methanol, reference standard;

N-nitrosodimethylamine-d_6 (NDMA-d_6), 1 mg/mL in methanol, 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. 25% methanol

Mix methanol and deionized water at the ratio of 1:3 (v/v).

2.5. Mobile phase

2.5.1. Solvent A:

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

2.5.2. Solvent B:

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

2.6. Internal standard solution preparation

Transfer 0.5 mL of NDMA-d_6 internal reference standard into a 10 mL volumetric flask, dilute to volume with methanol as the internal standard stock solution. Store under freezing in the dark. Upon use, dilute the internal standard stock solution with methanol to 200 ng/mL as the internal standard solution.

2.7. Standard solution preparation

Transfer 0.1 mL of NDMA reference standard into a 10 mL volumetric flask, dilute to volume with methanol as the standard stock solution. Store under freezing in the dark. Upon use, mix appropriate volume of standard stock solution and the internal standard solution, and dilute with 25% methanol to 0.5-25 ng/mL (containing 10 ng/mL internal standard) as the standard solutions.

2.8. 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 NDMA by the ratios of the peak area of NDMA to that of the internal standard vs. the concentrations of NDMA.

2.9. LC-MS/MS operating conditions(note):

Column: XSelect HSS T3, 3.5 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 → 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.

Inject volume: 5 μL.

Ionization mode: $APCI^+$.

Corona voltage: 3.0 kV.

Probe temperature: 300°C.

Ion source temperature: 130°C.

Cone gas flow: 300 L/hr.

Desolvation gas flow: 1200 L/hr.

Detection mode: multiple reaction monitoring (MRM). Selected ion pair, cone voltage and collision energy are as follows.

Analyte	Ion pair Precursor ion (*m/z*) > Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
NDMA	75 > 58*	30	10
	75 > 43	60	11
NDMA-d_6 (I.S.)	81 > 46	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 → 2.4	Waste
2.4 → 5.0	MS
5.0 → 9.0	Waste

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

2.10. Sample solution preparation

2.10.1. Drug substance

Transfer about 1 g of sample accurately weighed to a centrifuge tube, and add 0.5 mL of the internal standard solution and 2 mL of methanol. Mix well, sonicate for 10 min, and centrifuge at 4000 ×g for 10 min. Transfer 500 μL of the supernatant to another centrifuge tube, and add 1.5 mL of deionized water. Mix well, sonicate for 10 min, and centrifuge at 4000 ×g for 10 min. Filter the supernatant with a membrane filter, and take the filtrate as the sample solution.

2.10.2. Drug product

Grind at least 10 tablets of the sample to powder. Transfer about 1 g of the powered sample accurately weighed to a centrifuge tube, and

add 0.5 mL of the internal standard solution and 2 mL of methanol. Mix well, sonicate for 10 min, and centrifuge at 4000 ×g for 10 min. Transfer 500 μL of the supernatant to another centrifuge tube, and add 1.5 mL of deionized water. Mix well, sonicate for 10 min, and centrifuge at 4000 ×g for 10 min. Filter the supernatant with a membrane filter, and take the filtrate as the sample solution.

2.11. 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 2.9. Identify NDMA based on the retention time and the relative ion intensities(note). Calculate the amount of NDMA in the sample by the following formula:

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

Where,

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

V: the volume of solvent for sample preparation (2.5 mL)

D: the dilution factor (4)

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

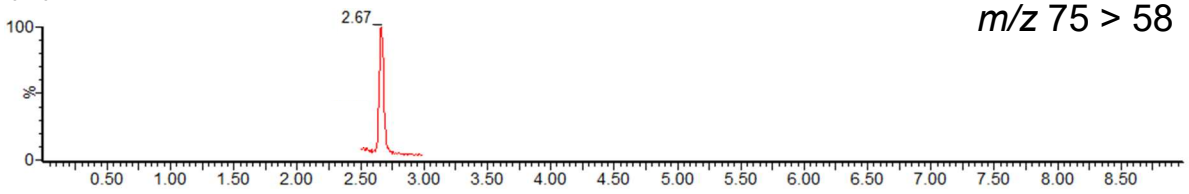
1. Limit of quantification (LOQ) for NDMA is 0.005 μg/g.
2. Further validation should be performed when interference compounds appear in samples.

Reference

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: 292-301.

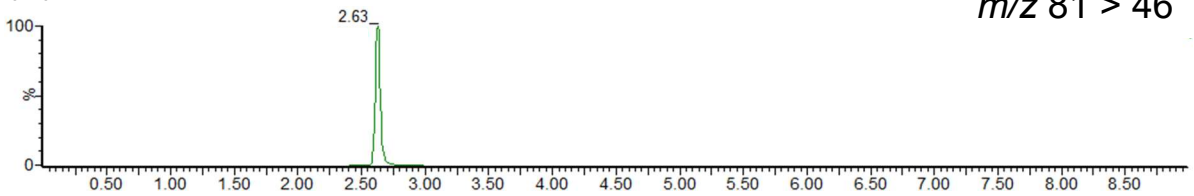
Reference chromatogram

(A) NDMA



m/z 75 > 58

(B) NDMA-d_6



m/z 81 > 46

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