DETERMINATION OF N-NITROSODIMETHYLAMINE (NDMA) IN METFORMIN PRODUCTS BY HRAM-GCMS

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1 Scope

This document outlines the test method for the determination of NDMA in metformin products by Exactive GC Orbitrap Mass Spectrometer (HRAM-GCMS).

2 Determination of NDMA in Metformin Products by HRAM-GCMS

2.1 Reagents and Chemicals

N-Nitrosodimethylamine (NDMA)
N-Nitrosodimethylamine-D6 (NDMA-D6)
Methanol (MeOH), HPLC grade
Dichloromethane (DCM), AR grade
1N Hydrochloride acid (1N HCl)

2.2 Instruments and Apparatus

Thermo Scientific Exactive GC Orbitrap Mass Spectrometer equipped with a TRACE 1310 Gas Chromatograph and a TRIPLUS RSH Auto-Sampler
Orbital Shaker
Centrifuge
Volumetric flask (Class A)
Glass bulb pipette
Membrane filter (PTFE 0.2 µm)
Micropipette
Conical bottom centrifuge tube, polypropylene (PP)
2 mL vials
1.5 mL Eppendorf tube
Glass tube with cap

2.3 GCMS parameters

GC Conditions [1]	
Inlet temperature	250 °C
Transfer line temperature	250 °C
Column	HP – INNOWAX 30m x 0.25mm x 0.25µm
Injection type	Splitless with surge at 84.7 kPa for 0.5 min
Injection volume	2 µL

Flowrate	1 mL/min of helium at constant flow mode
Oven programme	40 °C for 0.5min→200 °C at 20 °C /min→250 °C at 60 °C/min and hold for 3min
Runtime	12.33 min
MS Parameters	
Polarity	Positive
EI energy	-30ev
Solvent delay	4 min
Full scan	Resolution, 60,000; AGC, target $1e^6$; Maximum IT auto; scan range, 30 to 400 *m/z*.
Targeted-SIM	Resolution, 30,000; AGC, target $5e^5$; Maximum IT auto; Isolation window, 1.0 *m/z*.

Nitrosamine compounds in inclusion list:

Nitrosamine	Accurate Mass
NDMA	74.0475
NDMA-D6	80.0851

2.4 Standard, Sample, Sample Blank and Spiked Sample Preparation

2.4.1 Standard Preparation

1. *Stock Standard Solution* (20 mg/L): Prepare from commercially available standards (solid or liquid form) in 10 mL volumetric flask, top up to volume with MeOH.
2. *Stock Internal Standard Solution* (20 mg/L): Prepare from commercially available NDMA-D6 standard and dilute with MeOH.
3. *Intermediate Stock Standard Solution* (1 mg/L): Accurately transfer 500 µL of Stock Standard Solution to a 10 mL volumetric flask and top up to volume with MeOH.
4. *Intermediate Stock Standard Solution* (0.1 mg/L): Pipet 1 mL of *Intermediate Stock Standard Solution* (1 mg/L) to a 10 mL volumetric flask and top up to volume MeOH.

5. *Intermediate Stock Internal Standard Solution* (1 mg/L): Accurately transfer 500 µL of *Stock Internal Standard Solution* to a 10 mL volumetric flask and top up to volume with MeOH.
6. *Working Standard Solutions* (with 10 µg/L IS; prepared in 10 mL volumetric flask individually) as shown in Table 1.

Table 1

Working Standard Solution	Concentration (µg/L)	Volume of *Intermediate Stock Standard Solution 1* (1 mg/L), µL	Volume of *Intermediate Stock IS Solution* (1 mg/L), µL	Top up to volume with DCM
1	0	0	100	
2	1	10	100	
3	5	50	100	
4	10	100	100	
5	20	200	100	
6	50	500	100	
7	100	1000	100	

2.4.2 Sample Preparation

1. Weigh 10 tablets together and calculate the average mass per tablet.
2. Accurately weigh an amount of powdered sample, corresponding to 500 mg of the active pharmaceutical ingredient (API) into a conical bottom centrifuge tube. [Scale down the sample amount proportionally if sample is insufficient].
3. Add 10 mL of 1N HCl into the conical bottom centrifuge tube.
4. Vortex to mix well and shake the mixture with an orbital shaker at 350 rpm for 10 minutes.
5. Add 100 µL of *Intermediate Stock IS Solution* (1 mg/L).
6. Add 10 mL of DCM with a glass bulb pipette.
7. Vortex to mix well and shake the mixture for 10 min.
8. Centrifuge the mixture for 10 minutes at 4000 rpm.
9. Carefully, dip a micropipette tip to the bottom to withdraw *ca.* 1-2 ml of organic layer and transfer to a syringed fitted with membrane filter.
10. Collect the clear filtered solution in a HPLC vial for analysis.

Note: If the organic layer is not clear due to suspension, transfer a portion of organic solution *ca.* 1 mL to an Eppendorf vial and centrifuge it at 15000 rpm for 5 min.

2.4.3 Spiked Sample Preparation

1. Accurately weigh an amount of powdered sample, corresponding to 500 mg of API into a conical bottom centrifuge tube.
2. Add 10 mL of 1N HCl into the conical bottom centrifuge tube.
3. Vortex to mix well and shake the mixture with an orbital shaker for 10 minutes.
4. Add 100 µL of *Intermediate Stock IS Solution* (1 mg/L) and 50 µL of *Intermediate Stock Standard Solution* (0.1 mg/L).
5. Repeat step 6-10 as described in Section 2.4.2 to obtain *Spiked Sample Solution* (10 ng/mL NDMA).

2.4.4 Sample Blank Preparation

Sample Blank Solution: Prepare the Sample Blank as described for the Sample Preparation in Section 2.4.2 but without the sample addition.

2.5 Test Procedure

1. Ensure the mass calibration results are valid prior to analysis. [Note: The validity of calibration result is 7 days.]
2. Select method: Lab Method_Nitrosamine analysis by EX GC
3. Inject DCM.
4. Inject *Working Standard Solutions 1-7* duplicate.
5. Inject DCM.
6. Inject *Sample Blank Solution*.
7. Inject *Sample Solution* duplicate [Note: Dilute sample with proper adjustment of *Intermediate Stock IS Solution* when the concentration of the *Sample Solution* exceeds the calibration range.].
8. Inject *Spiked Sample Solution.*
9. Inject DCM.

2.6 Interpretation of Results

1. The positive identification, the result is valid only if:
   i. The peak corresponding to NDMA in the chromatogram from the *Sample Solution* have close retention time (± 2.5%) to the peak from the *Working Standard Solution 4* (10 µg/L);
   ii. The mass error of the peak corresponding to NDMA (Full Scan Mode) obtained from the *Standard Solution* and *Sample Solution* must be within the ±5 ppm mass error to respective theoretical value.
2. The negative identification, the result is valid only if:

i. No peak corresponding to NDMA observed in the chromatogram obtained from the Sample Solution. Positive results are observed in the *Spiked Sample Solution*.

ii. Report as 'Not Detected' and indicate the LOD accordingly.

Scan type	Full Scan
Instrument LOD	0.5 ng/mL
Method LOD	10 ng/g

[Note: LOD is based on 500 mg of API in the drug substance.]

3. The quantification is performed using peak area ratios of NDMA/NDMA-D6 through linearity calibration plot from *Standard Solutions*. The quantification result is valid only if:
   i. The deviation of the peak area ratios for NDMA obtained from duplicated sample solution are not more than 20%;
   ii. The linearity coefficient of the calibration plot is greater than 0.99;
   Report as 'Less than 20 ng/g' if the analysis result of NDMA is above 0.5 ng/mL but less than 1.0 ng/mL.

3 Reference

1. Official United States Food and Drug Administration (USFDA) documents, FY19-006-DPA-S, Combined Direct Injection N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) Impurity Assay by GC/MS

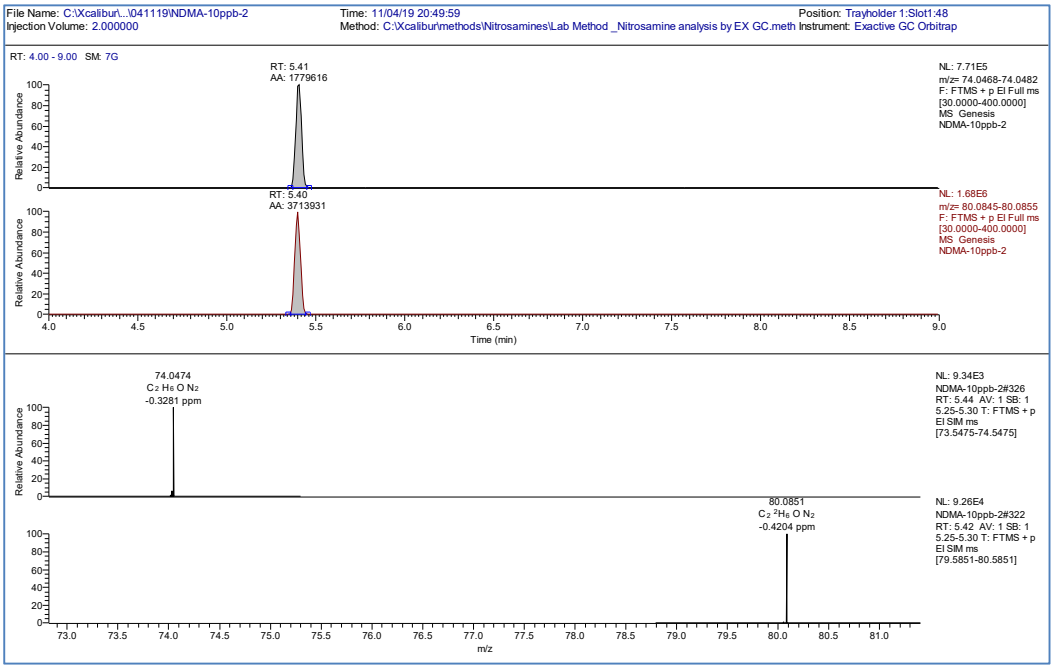

Fig1. Typical chromatograms and mass spectra of NDMA and NDMA-D6