DETERMINATION OF NDMA IN METFORMIN AND RANITIDINE PRODUCTS BY LC-MS/MS

Pharmaceutical Laboratory
Applied Sciences Group, Health Sciences Authority
11 Outram Road, Singapore 169078

Disclaimer: The testing method below provides option and guidance for the users to determine NDMA in Metformin and Ranitidine products. The method should be validated by users to ensure it is fit for its intended use.

1 Scope

This document outlines the test method for the determination of *N*-Nitroso-*di*-methylamine (NDMA) in Metformin and Ranitidine products by Liquid Chromatography Hybrid Tandem Mass Spectrometry (LC-MS/MS).

2 Determination of NDMA by LC-MS/MS

2.1 Reagents and Chemicals

N-Nitroso-*di*-methylamine (NDMA)
N-Nitroso-*di*-methylamine-D6 (NDMA-D6)
Methanol (MeOH), HPLC grade
Formic acid, MS grade
Dichloromethane (DCM), analytical grade
Deionized water (DI water)
Diluent I: 20% methanol in DI water containing 10 ng/mL NDMA-D6
Diluent II: DCM containing 10 ng/mL NDMA-D6

2.2 Instruments and Apparatus

Liquid Chromatography Tandem Mass Spectrometry (QTRAP 6500+ MS/MS coupled with Agilent 1290 Infinity LC)
Centrifuge
Ultrasonic bath
Volumetric flask (Class A, 10 mL)
Membrane syringe filter (PTFE 0.2 µm)
Micropipette
2 mL vials
1.5 mL Eppendorf tube
Conical bottom centrifuge tube, Polypropylene (PP)

2.3 LC-MS/MS Method

HPLC

Column:	Phenomenex® Gemini C18 analytical column (4.6 mm x 100 mm, 3 µm) or equivalent		
Column oven temperature:	40 °C		
Injection volume:	5 µL		
Mobile phase A:	0.1% formic acid in DI water		
Mobile phase B:	Methanol		
Flow rate:	0.35 mL/min		
Gradient:	Time (min)	Mobile phase A (%)	Mobile phase B (%)
	0	80	20
	5.5	80	20
	6.0	5	95
	9.0	5	95
	9.5	80	20
	12	80	20

[Note: The flow rate or run time may be varied to obtain optimum separation.]

MS/MS

MS:	QTRAP 6500+					
Polarity:	Positive					
Ionization mode:	APCI (Atmospheric Pressure Chemical Ionization)					
MS parameter:	CUR: 20 psi; CAD: Medium; TEP: 400 °C; GSI: 40 psi					
Valve switches*:	Time (min)			Position		Remark
	0.0-3.5			A		To waste
	3.5-5.5			B		To MS
	5.5-12.0			A		To waste
MRM:	ID	Q1	Q3	DP	EP	CE
	NDMA 1	75.0	43.0	60	7	21
	NDMA 2	75.0	58.0	60	7	16
	NDMA IS	81.0	46.0	60	7	21

[*Note: Valve switches window may adjust depends on the different system to avoid excessive contamination of MS detector from API and excipients (subject to the RT of the target analyte)]

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 and dilute with methanol.
2. *Stock Internal Standard Solution* (20 mg/L): Prepared from commercially available NDMA-D6 standard and dilute with methanol.
3. *Intermediate Standard Solution* (1 mg/L): Accurately transfer 500 µL of *Stock Standard Solution* to a 10 mL volumetric flask and top up to volume using 20% methanol solution.
4. *Intermediate Internal Standard Solution* (1 mg/L): Accurately transfer 500 µL of NDMA-D6 *Stock Internal Standard Solution* to a 10 mL volumetric flask and top up to volume using methanol.
5. *Working Standard Solution*s (with 10 µg/L IS; prepared in 10 mL volumetric flask individually):

Working Standard Solution	Standards Conc.	Vol of *Mix Stock Standard solution* (1 mg/L)	Vol of *Mix Stock IS Solution* (1 mg/L)	
1	0	0	100 µL	Top up to volume (10 mL) using 80% methanol
2	0.5 µg/L	5 µL	100 µL	
3	1 µg/L	10 µL	100 µL	
4	5 µg/L	50 µL	100 µL	
5	10 µg/L	100 µL	100 µL	
6	20 µg/L	200 µL	100 µL	
7	50 µg/L	500 µL	100 µL	
8	100 µg/L	1000 µL	100 µL	

[Note: Protect all *Standard Solutions* from light.]

2.4.2 Sample Preparation

1. General sample preparation

i. Weigh 10 units of sample together and calculate the average weight of each unit.
ii. Accurately weigh an amount of powdered sample, equivalent to 500 mg of API into a 15 mL PP conical bottom centrifuge tube.
iii. Add 10 mL of Diluent I, vortex to mix well and sonicate for 10 min.
iv. Filter the supernatant into a HPLC vial through a 0.2 µm Nylon membrane filter.

[Notes]

i. Scale down the sample amount if necessary;
ii. Centrifuge may be required before the filtration if the sample solution is difficult to pass through the membrane syringe filter (e.g. Transfer about 1 mL mixture to a 1.5 mL Eppendorf tube, centrifuge the mixture at 15000 rpm for 5 min at room temperature);
iii. Protect sample solutions from light.
iv. In the situation when the amount of powder is too much for effective sample extraction, reduce the powder amount or increase the extraction solvent volume. In this case, the LOD of the method will be affected and needed to be recalculated accordingly.

2. Sample preparation for extended release Metformin drug product

i. This sample preparation is applicable to the extended release Metformin tablets or raw material that is not suitable to be extracted by the diluent directly (e.g. when diluent was added to the sample, it would become gel-like mixture and unable to do the filtration).
ii. Weigh 10 units of sample together and calculate the average weight per unit.
iii. Accurately weigh an amount of powdered sample, equivalent to 500 mg of API into a conical bottom centrifuge tube.
iv. Add 10 mL of Diluent II with a glass bulb pipette.
v. Vortex to mix well and shake the mixture with an orbital shaker at 350 rpm for 10 min.
vi. Add 10 mL of 1N HCl solution into the conical bottom centrifuge tube.
vii. Vortex to mix well and shake the mixture with an orbital shaker at 350 rpm for 10 minutes and then centrifuge the mixture for 10 minutes at 4000 rpm
viii. Carefully dip a micropipette to the bottom to withdraw 5 ml of organic layer and transfer to a 15 mL conical bottom centrifuge tube.
ix. Evaporate the solvent under nitrogen stream at room temperature to almost dry.
x. Add ~2 mL 20% methanol and sonicate to re-dissolve the residues. Transfer the mixture to a 5 mL volumetric flask. Wash the tube with 20% methanol (~1 mL, twice). Transfer the washing solution to the same volumetric flask and top up to volume using 20% methanol.
xi. Filter the supernatant into a HPLC vial through a 0.2 µm Nylon membrane filter.

[Notes]

i. Scale down the sample amount if necessary;
ii. Protect sample solutions from light.

3. Sample preparation for Ranitidine liquid products

i. This sample preparation is applicable to the Ranitidine liquid products (e.g. Injection or syrup). Less solvent was used for the sample extraction to obtain reasonable method LOD and LOQ.
ii. Accurately transfer 1 mL liquid sample into a 2 mL volumetric flask.
iii. Add 0.4 mL methanol and 20 µL *Intermediate Internal Standard Solution* (1 mg/L), top up to 2 mL with DI water, vortex to mix well and sonicate for 10 min.
iv. Filter the supernatant into a HPLC vial through a 0.2 µm Nylon membrane filter.

[Notes]
i. Scale down the sample amount if necessary;
ii. Protect sample solutions from light.

2.4.3 Spiked Sample Preparation

Spiked Sample Solution: Prepare the *Spiked Sample Solution* as described for the Sample Preparation in Section 2.4.2 with addition of NDMA reference standard at LOD level (e.g. add 5 µL of *Intermediate Standard Solution* (1 mg/L) to 10 mL *Sample Solution* to obtain the concentration of 0.5 ng/mL for 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. Select method: *NDMA_MRM_APCI_12min*;
2. Inject solvent blank (20% methanol).
3. Inject *Working Standard Solution 1-8* (Duplicate).
4. Inject solvent blank.
5. Inject *Sample Blank*.
6. Inject *Sample Solution* (Duplicate) [Note: dilute sample using diluent accordingly when the concentration of the *Sample Solution* exceeds the calibration range.].
7. Inject *Spiked Sample Solution.*
8. Inject solvent blank.
9. Flush LC-MS/MS system immediately after the analysis.

2.6 Interpretation of Results

1. For negative identification, the result is valid only if:
i. No peaks corresponding to NDMA was observed in the chromatogram obtained from the *Sample Solution.* Positive results are obtained in *Spiked Sample Solution*;

ii. Report as 'Not Detected' and indicate the LOD of the substances.

2. For positive identification, the result is valid only if:

i. The peaks corresponding to NDMA in the chromatogram of all the ion pairs

from the *Sample Solution* have close retention time (±0.3 min) to the peaks from the *Standard Solutions* chromatogram;

ii. The deviation of the ion ratios of NDMA obtained from the *Standard Solutions* and *Sample Solution* for the two MRM transitions are not more than 20%.

3. The quantification is performed using the peak area ratios of [NDMA: (75.0/43.0) / NDMA IS (81.0/46.0)] through linearity plot from *Working Standard Solutions 1,3-8*. The quantification result is valid only if:

i. The deviation of the peak area ratio of [NDMA: (75.0/43.0) / NDMA IS (81.0/46.0)] obtained from duplicated sample solution are not more than 20%;

ii. The linearity coefficient of the calibration plot is greater than 0.99;

iii. Report as 'Less than LOQ' and indicate the LOQ of the substances if the peak area ratio of [NDMA: (75.0/43.0) / NDMA IS (81.0/46.0)] is above the peak area ratio of *Standard Solution* of 0.5 ng/mL but less than the peak area ratio of *Standard Solution* of 1 ng/g.

4. LOD and LOQ

Instrument LOD	0.5 ng/mL
Instrument LOQ	1 ng/mL

	Method used for sample preparation		
	General	Extended release Metformin drug product	Ranitidine drug product in liquid form
Method LOD*	10 ng/g	10 ng/g	40 ng/g**
Method LOQ*	20 ng/g	20 ng/g	80 ng/g**

* Method LOD and LOQ are with respect to API

** The value here is based on the Ranitidine Injection, 50 mg/2mL. For other product, the method LOD and LOQ should be calculated individually.

2.7 Calculation

1. Calculation of NDMA content in sample with respect to drug products (per unit)

Content of NDMA in drug product = $[(C_{Spl} \times V_{Spl} \times Dil) / W_{Spl}] \times W_{Unit}$

Where:

Content of NDMA in drug product: ng/unit

C_{Spl} : Concentration of NDMA obtained from liner plot, ng/mL

V_{Spl} : Volume of *Sample Solution*, mL (e.g. 10 mL for General sample preparation and complicated matrix sample preparation; 2 mL for Liquid injection sample preparation)

W_{Spl} : Weight of sample used for sample preparation, g
W_{Unit} : Weight of each unit of sample, g (e.g. g/tab or g/cap etc.)
Dil : Dilution factor

2. Calculation of NDMA content in sample with respect to API

Content of NDMA with respect to API = $[(C_{Spl} \times V_{Spl} \times Dil) / W_{Spl}] \times W_{Unit} / S_{Unit}$

Where:

Content of NDMA with respect to API: ng/g
C_{Spl} : Concentration of NDMA obtained from liner plot, ng/mL
V_{Spl} : Volume of *Sample Solution*, mL (e.g. 10 mL for General sample preparation and complicated matrix sample preparation; 2 mL for Liquid injection sample preparation)
W_{Spl} : Weight of sample used for sample preparation, g
W_{Unit} : Weight of each unit of sample, g (e.g. g/tab or g/cap etc.)
S_{Unit} : Strength of the drug product per unit, g (e.g. 0.5 for 500 mg/tab)
Dil : Dilution factor

3. Calculation of method LOD and LOQ with respect to API

Method LOD (ng/g) = $(0.5 \times V_{Spl}) / A_{API}$

Method LOQ (ng/g) = $(1 \times V_{Spl}) / A_{API}$

Where:

0.5 : Instrument LOD, ng/mL
1 : Instrument LOQ, ng/mL
V_{Spl} : Volume of *Sample Solution*, mL (e.g. 10 mL for general sample preparation and extended release Metformin sample preparation; 2 mL for Liquid injection sample preparation)
A_{API} : Amount of API used for sample preparation, g (e.g. 500 mg for general sample preparation; 25 mg for Ranitidine Injection, 50 mg/2mL)