
	Title	Revision No.	Date	Document No.
	DETERMINATION OF NDMA IN RANITIDINE PRODUCTS BY LC-MS/MS	Ver-001	12 Sep 2019	PHARM QNDMA_RAN_LCMSMS

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

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

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1 Scope

This document outlines the test method for the determination of N-nitroso-dimethylamine (NDMA) in 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, HPLC grade (MeOH)
Formic acid, MS grade
Deionized water (DI water)
Diluent: MeOH / DI water (80/20)

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:	0.1% formic acid in Methanol		
Flow rate:	0.35 mL/min		
Gradient:	Time (min)	Mobile phase A (%)	Mobile phase B (%)
	0	80	20
	1	80	20
	12	5	95
	16	5	95
	16.1	80	20
	20	80	20

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

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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; CXP: 11					
Valve switches*:	Time (min)		Positon		Remark	
	0.0-3.5		A		To waste	
	3.5-5.3		B		To MS	
	5.3-20		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 be adjusted depending on the different system to avoid excessive contamination of MS detector from API and excipients (subject to the RTs of the target analytes)]


2.4 Standard, Sample, Sample Blank and Spiked Sample Preparation

2.4.1 Standard Preparation

1. Stock Standard Solution (20 mg/L): Prepared from commercially available NDMA standard (solid or liquid form) in 10 mL volumetric flask, top up to volume with MeOH.
2. Stock Internal Standard Solution (20 mg/L): Prepared 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 Diluent.
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 with MeOH.
5. Working Standard Solutions (with 10 µg/L IS; prepared in 10 mL Volumetric flask individually):

Working Standard Solution	Standards Conc.	Vol of Intermediate Stock Standard solution (1 mg/L)	Vol of Intermediate Stock IS Solution (1 mg/L)	Top up to volume (10 mL) with Diluent
1	0	0	100 µL	
2	5 µg/L	50 µL	100 µL	
3	10 µg/L	100 µL	100 µL	
4	20 µg/L	200 µL	100 µL	
5	50 µg/L	500 µL	100 µL	
6	100 µg/L	1000 µL	100 µL	

[Note: Protect all Standard Solutions from light.]

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2.4.2 Sample Preparation

1. Weigh 10 tablets together and calculate the average mass of one tablet.
2. Accurately weigh an amount of powdered sample, corresponding to 500 mg of the API into a 15 mL PP conical centrifuge tube.
3. Add 100 μ L Intermediate Stock IS Solution (1 mg/L) and 10 mL of diluent, vortex to mix well and sonicate for 10 min [Note: Scale down the sample amount if necessary].
4. Transfer about 1 mL mixture to a 1.5 mL Eppendorf tube, centrifuge the mixture at 15000 rpm for 5 min at room temperature.
5. Filter the supernatant into a HPLC vial through a 0.2 μ m PTFE Membrane filter.
[Note: Protect sample solutions from light. In the situation when the amount of powder is too much for effective sample extraction, please 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.]

2.4.3 Spiked Sample Preparation


1. Accurately weigh an amount of powdered sample, corresponding to 500 mg of API into a 15 mL PP Conical bottom centrifuge tube.
2. Transfer 45 μ L Intermediate Stock Standard solution and 100 μ L Intermediate Stock Internal Standard Solution into the tube.
3. Repeat step 3-5 as in [Section 2.4.2](#) to obtain Spike Sample Solution [4.5 ng/mL in Spiked Sample Solution, corresponding to 0.09 μ g/g in sample, LOD of 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_RAN_MRM_APCI;
2. Inject solvent blank (Diluent).
3. Inject Standard Solutions.
4. Inject solvent blank.
5. Inject Sample Blank.
6. Inject Sample Solution (Duplicate) [Note: dilute sample with 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.

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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 NDMA.

The LOD for the NDMA is listed as below:

	NDMA
Instrument LOD	4.5 ng/mL
Method LOD	0.09 µg/g

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

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 corresponding peaks from the Standard Solutions chromatogram;
 - ii. The deviation of the ion ratios of NDMA obtained from the Standard Solution (working standard solution 2, 5 µg/L) and Sample Solution for the two MRM transitions are not more than 20%.
3. The quantification is performed using the peak area ratios from ion pair of NDMA: (75.0/43.0)/NDMA IS through linearity 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;
 - iii. Report as 'Less than 0.3 µg/g' if the analysis result of NDMA is above 0.09 µg/g but less than 0.3 µg/g;

3 References

1. Determination of N-nitrosodimethylamine in Valsatan Active Pharmaceutical Ingredient and the Related Medicinal Products, Taiwan Food and Drug Administration (TFDA), OMCL TW_TFDA-B
2. Determination of NDMA by LC/UV in Valsartan Active Substances and Finished Products, French National Agency for Medicines and Health Products Safety Laboratory Controls Division Ref. 18A0399-01