Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Testing and Research
Division of Pharmaceutical Analysis

Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of NDMA in Ranitidine Drug Substance and Drug Product

Background: Ranitidine HCl is a prescription and over the counter medication used to treat acid reflux. The drug is a histamine-2 receptor antagonist (acid inhibitor or H2 blocker). Some of the common H2 receptor blockers include: Ranitidine (Zantac), Nizatidine (Axid), Famotidine (Pepcid, Pepcid AC) and Cimetidine (Tagamet, Tagamet HB).

As GC based methods had been observed to elevate NDMA levels in tested materials an alternative method which prevents the degradation of ranitidine and the subsequent formation of NDMA was therefore needed. A liquid chromatography with high resolution mass spectrometer (LC-HRMS) was developed and validated to measure the levels of NDMA in ranitidine drug substance and drug product.

Conclusions:

An LC-HRMS method was developed and validated following ICH Q2(R1) for the detection and quantitation of NDMA in ranitidine drug substance and drug product. The limit of detection (LOD), limit of quantitation (LOQ) and range of the method are summarized below:

	NDMA
LOD (ng/mL)	0.32
(ppm)	0.011
LOQ (ng/mL)	1.0
(ppm)	0.033
Range (ng/mL)	1.0 - 100
(ppm)	0.033 – 3.33

LC-HRMS Method for the Determination of NDMA impurity in Ranitidine Drug Substance or Drug Product

Purpose
This method will be used to quantitate N-nitroso-*di*-methylamine (NDMA) impurity in ranitidine drug substance or drug product.

Principle
N-nitroso-*di*-methylamine (NDMA) impurity is separated from ranitidine by reverse phase chromatography and is detected by a high-resolution and high-mass accuracy (HRAM) mass spectrometer. High sensitivity detection is achieved by monitoring the accurate *m/z* value of the protonated impurity ion. Quantitation is performed by comparing the peak area of the NDMA impurity in extracted ion chromatogram of the samples to the peak area of the NDMA reference standard in an external calibration standard.

Reagents
- NDMA Reference Standard
- Formic acid, LC/MS grade (Fisher A117-50 or equivalent)
- Acetonitrile, LC/MS grade (Fisher A955-4 or equivalent)
- Methanol, LC/MS grade (Fisher A456-4 or equivalent)
- Water, LC/MS grade or equivalent

Equipment/Instrument
- HPLC or UHPLC system equipped with temperature-controlled autosampler and column compartment
- Q Exactive™ hybrid quadrupole-orbitrap mass spectrometer (Thermo-Fisher Scientific)
- HPLC column: Ace C18-AR, 3 µm 100 Å, 50 x 4.6 mm (Mac-Mod, Part No. ACE1190546)
- Analytical Balance
- Vortex Mixer
- 15 mL glass centrifuge tubes
- Wrist action shaker
- 0.22 µm PVDF syringe filters
- Centrifuge
- HPLC vials

Mobile phase preparation
- Mobile phase A (0.1% formic acid in water): mix formic acid and water at a volume ratio of 1:1000
- Mobile phase B (0.1% formic acid in acetonitrile): mix formic acid and acetonitrile at a volume ratio of 1:1000

Diluent and Blank: Methanol

NDMA Intermediate Stock Standard preparation (100 ng/mL)

Prepare a 100 ng/mL intermediate stock standard solution in methanol using commercially available NDMA reference stock standard solution.

Working Standard Preparation (2.0 ng/mL)
Transfer a 1.0 mL aliquot volume of the intermediate stock standard into a 50 mL volumetric flask and dilute to volume with methanol. Prepare fresh daily.

Drug substance sample preparation
Accurately weigh 120 mg of drug substance into a 15 mL glass centrifuge tube. Add 4.0 mL of methanol and mix the solution using a vortex mixer until dissolved.

Drug product sample preparation
Crush the appropriate number of tablet(s) to obtain a target concentration of 30 mg/mL of API in methanol, and transfer into a 15 mL glass centrifuge tube. Add the appropriate volume of methanol and mix for about a minute using a vortex mixer. Shake the sample for 40 minutes using a mechanical wrist action shaker.

After extraction, centrifuge the sample for 15 minutes at 4500 rpm. Filter the supernate using a 0.22 µm PVDF syringe filter, discard the first 1 mL and transfer the filtered sample into an hplc vial for LC/MS analysis.

Chromatographic Conditions

HPLC Column	HPLC column: Ace C18-AR, 3 µm 100 Å, 50 x 4.6 mm (Mac-Mod, Part No. ACE1190546)		
Column Temp.	30 °C		
Flow Rate	0.5 mL/min		
Mobile Phase A	0.1% formic acid in water		
Mobile Phase B	0.1% formic acid in acetonitrile		
Gradient	Time (min)	A%	B%
	0	95	5
	1.0	95	5
	3.0	80	20
	7.0	0	100
	9.0	0	100
	9.1	95	5
	14.0	95	5
Injection Volume	3 µL		
Autosampler Temp.	4 - 8 °C		
Needle Wash	80:20, Methanol:Water with 0.1% Formic Acid		

Mass spectrometer conditions

- Instrument

Q Exactive™ mass spectrometer (Thermo-Fisher)

- Ion Source Settings
  Note: Ion source parameters can be adjusted to achieve the desired sensitivity.

Sheath Gas Flow Rate	55 arbitrary units
Aux Gas Flow Rate	15 arbitrary units
Sweep Gas Flow Rate	0 units
Spray Voltage	3.5 kV
Capillary Temp.	400 °C
Aux Gas Heater Temp.	350 °C

- Scan Settings
  Note: 1) The scan start-end time should be adjusted for the user's HPLC system since the retention time of the NDMA impurity may vary between different HPLC systems, 2) The divert valve can be used to divert the eluent to waste when a scan is not performed.

NDMA Impurity	
Scan Type	PRM
Polarity	Positive
Scan Start -End (min)	0 - 3.2
m/z Isolated for PRM	75.0553
NCE	80
Isolation Window	1.5 m/z
Scan Range	50 – 95 *m/z*
Microscans	3
Resolution	35,000
AGC target	2e5
Maximum IT	100 ms

Injection Sequence

- Inject Blank (use diluent) at least once at the beginning of a sequence
- Inject Standard solution for six consecutive times before the injection of the first sample
- Inject Standard solution once every six injections of samples and at the end of a sequence.
- Example:

Order	Solution	No. of Injections
1	Blank	2
2	Standard	6
3	Blank	1
4	Sample 1	1
5	Sample 2	1
6	Sample 3	1
7	Sample 4	1

8	Sample 5	1
9	Sample 6	1
10	Standard	1
…	…	…

System Suitability

- The % RSD (n = 6) of the NDMA peak areas for the first six injections of the standard solution should be no more than 10%.
- The cumulative % RSD of the NDMA peak areas should be no more than 15%. (cumulative % RSD of the peak area is calculated by combining the initial six replicate injections of the standard solution and each subsequent bracketing standard).

Data Processing

- NDMA peak areas from the extracted ion chromatograms (EIC) with a *m/z* tolerance of 15 ppm are used for quantitation. The NDMA *m/z* value to be extracted is listed below:

NDMA Impurity	
m/z to be extracted	75.0553

- The retention time difference of the NDMA impurity in the analyzed samples should not be more than 2% of the retention time of the corresponding NDMA peak in the reference standard solution.

Calculation

Drug Substance:

$$\text{NDMA impurity (ppm)} = \frac{A_{spl}}{As} \times C_s \times \frac{1\ mg}{1\times 10^6\ ng} \times \frac{V}{W} \times 10^6$$

Where: A_{spl} = Area of the NDMA impurity peak in the sample solution
As = Average area (n = 6) of the NDMA impurity peak from the first six consecutive injections of the standard solution
C_s = Concentration of the NDMA impurity in the standard solution (ng/mL)
W = Weight of drug substance (mg)
V = Volume of the diluent in the sample solution (mL)

Drug Product:

$$\text{NDMA impurity (ppm)} = \frac{A_{spl}}{As} \times C_s \times \frac{1\ mg}{1\times 10^6\ ng} \times \frac{1}{30\ mg/mL} \times 10^6$$

Where: A_{spl} = Area of the NDMA impurity peak in the sample solution
As = Average area (n = 6) of the NDMA impurity peak from the first six consecutive injections of the standard solution

C_s = Concentration of the nitrosamine impurity in the standard solution (ng/mL)

Report

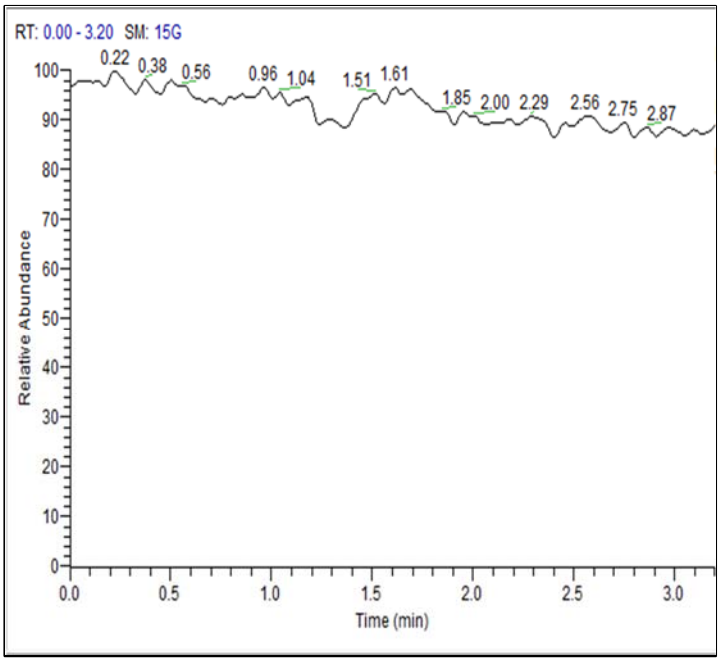
- Report the nitrosamine impurity content in ppm with three significant figures if the value is ≥ LOD
- Report 'not detected' if no nitrosamine impurity is detected or the value is < LOD

References

1. FY19-107-DPA-S, LC-MS Method for Detection of Six Nitroso Impurities in ARB Drugs.
2. FY19-159-DPA-S, Evaluation of Analytical Methods in Ranitidine - NDMA Paper.
3. FY19-059-DPQR-S, Review of Potential NDMA Formation from Ranitidine HCl (July 5, 2019)

Example Chromatograms

Methanol Blank



NDMA (2.0 ng/mL Standard)

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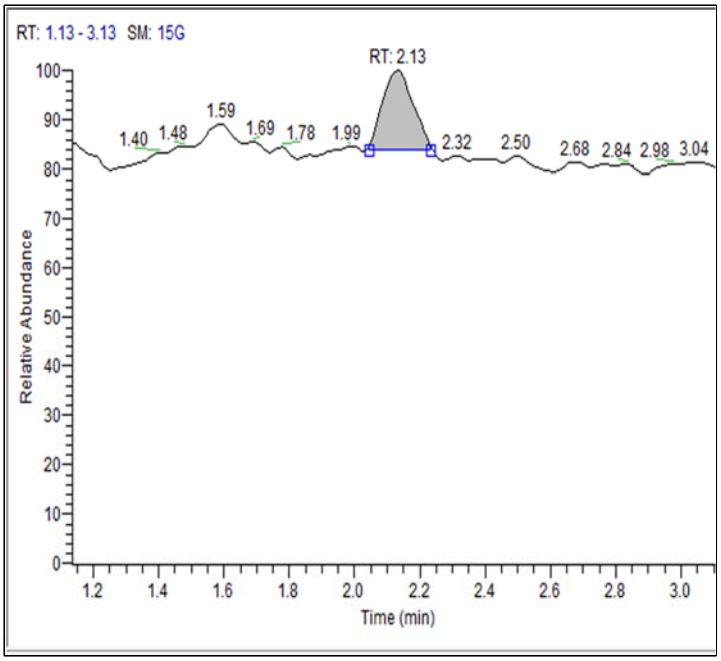
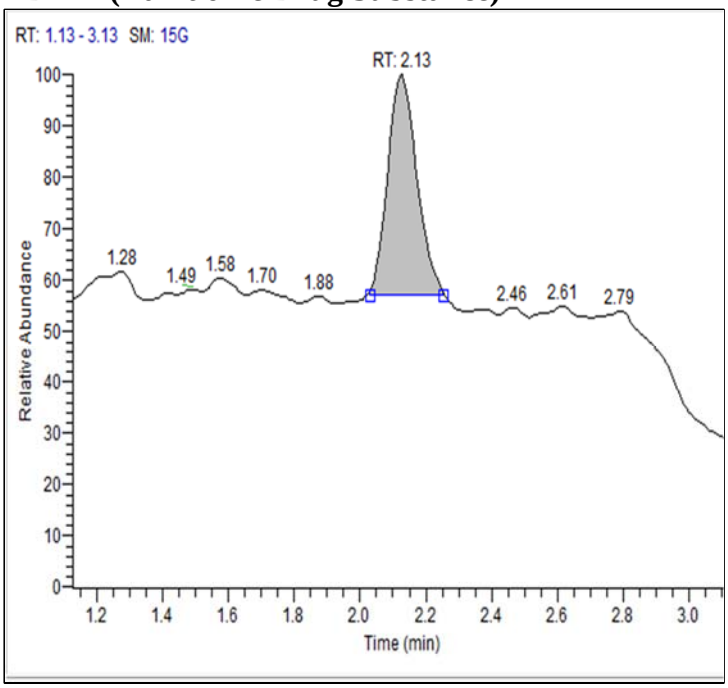


NDMA (Ranitidine Drug Substance)



NDMA (Ranitidine Drug Product)

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