U.S. FOOD & DRUG ADMINISTRATION

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Method for the Determination of NDMA in Ranitidine Drug Substance and Solid Dosage Drug Product

Background: Ranitidine is a prescription and over-the-counter drug 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).

Ranitidine medicine was suspected of being contaminated with N-nitroso-di-methylamine (NDMA), a probable human carcinogen, following notification in June 2019. Accordingly, a liquid chromatography-high resolution mass spectrometry (LC-HRMS) method was developed and validated by the Agency to determine the level of NDMA in ranitidine drug products and drug substances.

This method is a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of NDMA in ranitidine drug substance and drug product. This LC-MS method based on a QQQ platform may be used as an alternative or confirmatory method for the liquid chromatography high resolution mass spectrometry (LC-HRMS) method detailed in FY19-177-DPA-S. The QQQ platform is more widely available than the LC-HRMS platform previously shared by the agency.

Conclusions:

An LC-MS/MS 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)	0.3
(ppm)	0.01
LOQ (ng/mL)	1.0
(ppm)	0.033
Range (ng/mL)	1.0 - 100
(ppm)	0.033 – 3.33

LC-MS/MS Method for the Determination of NDMA impurity in Ranitidine Drug Substance or Solid Dosage Drug Product

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

Principle
N-nitroso-*di*-methylamine (NDMA) impurity is separated from ranitidine API by reverse phase chromatography and is detected by multiple reaction monitoring (MRM) on a triple quadrupole mass spectrometer. Quantitation is performed by comparing the NDMA quantifier ion peak area in the samples to that in external calibration standards.

Reagents
- NDMA Reference Standard
- Formic acid, LC/MS grade (Fisher A117-50 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
- Agilent 6420 Triple Quad LC/MS system with APCI source or equivalent
- HPLC column: ACE Excel C18-AR, 3 µm, 50 x 4.6 mm (MAC-MOD Analytical Inc. Part No. EXL1190546U)
- 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 methanol): mix formic acid and methanol at a volume ratio of 1:1000

Diluent and Blank: Water

NDMA Intermediate Stock Standard preparation (100 ng/mL)
Prepare a 100 ng/mL intermediate stock standard solution in water using commercially available NDMA reference stock standard solution.

Working Standard Solution Preparation (1.0 ng/mL)
Transfer a 1.0 mL aliquot volume of the intermediate stock standard into a 100 mL volumetric flask and dilute to volume with water. 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 water 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 water, and transfer into a 15 mL glass centrifuge tube. Add the appropriate volume of water 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 analysis.

Chromatographic Conditions

HPLC Column	ACE Excel C18-AR, 3 µm, 50 x 4.6 mm (MAC-MOD Analytical Inc. Part No. EXL1190546U)		
Column Temp.	30 °C		
Flow Rate	0.6 mL/min		
Mobile Phase A	0.1% formic acid in water		
Mobile Phase B	0.1% formic acid in methanol		
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	10 µL		
Autosampler Temp.	4 - 8 °C		

Mass spectrometer conditions

- Instrument
  Agilent 6420 Triple Quad LC/MS system with APCI source or equivalent.

- Ion Source Settings

Ion Source	APCI
Gas Temp. (°C)	325
Vaporizer (°C)	350
Gas flow (L/min)	5
Nebulizer (psi)	40
Capillary (V)	4000
Corona Current (μA)	5

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

- Scan Settings

Polarity: positive ion;
Scan type: MRM;
Scan Time: 1 – 3.0 min; Delta EMV(+): 200

	Precursor ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage
Quantifier	75.1	unit	43.1	unit	200	90	15	5
Qualifier	75.1	unit	58.1	unit	200	90	10	5

  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.

Injection Sequence

- Inject Blank (use diluent) at least once at the beginning of a sequence
- Inject Working Standard Solution for six consecutive times
- Inject Working 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	Working Standard Solution	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	Working Standard Solution	1
…	…	…

System Suitability

- The % RSD (n = 6) of the NDMA quantifier ion (m/z 75.1 → m/z 43.1) peak areas for the first six injections of Working Standard Solution should be no more than 10%.
- The cumulative % RSD of the NDMA quantifier ion (m/z 75.1 → m/z 43.1) peak areas for Working Standard Solution should be no more than 10%. (cumulative % RSD of the peak area is calculated by combining the initial six replicate injections of the standard solution and each subsequent bracketing standard).

Identification of NDMA

- 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 Working 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 quantifier ion peak (m/z 75.1 → m/z 43.1) in the sample solution
As = Average area (n = 6) of the NDMA quantifier ion peak (m/z 75.1 → m/z 43.1) from the first six consecutive injections of the standard solution
C_s = Concentration of the NDMA in the standard solution (ng/mL)
W = Weight of drug substance (mg)
V = Volume of the diluent in the sample solution (mL)

Drug Product:

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

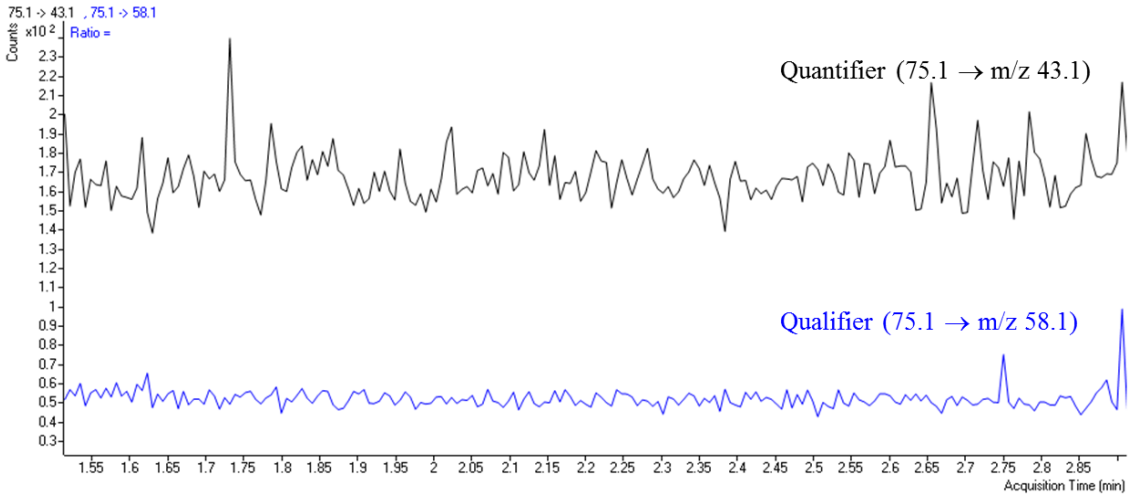
Where: A_{spl} = Area of the NDMA quantifier ion peak (m/z 75.1 → m/z 43.1) in the sample solution
As = Average area (n = 6) of the NDMA quantifier ion peak (m/z 75.1 → m/z 43.1) from the first six consecutive injections of the standard solution
C_s = Concentration of the NDMA in the standard solution (ng/mL)

Report

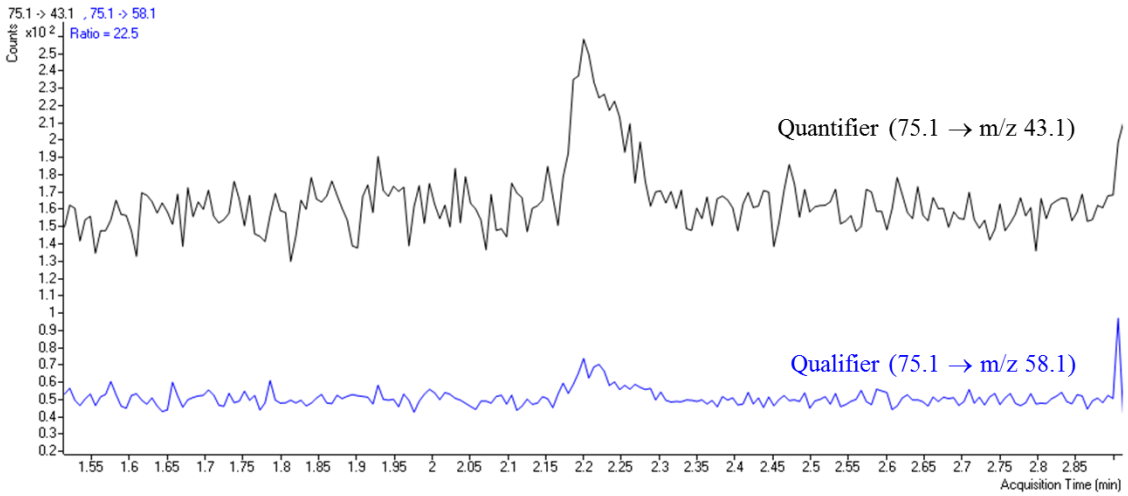
- Report the NDMA impurity content in ppm with three significant figures if the value is ≥ LOD
- Report 'not detected' if no NDMA impurity is detected or the value is < LOD

Example Chromatograms

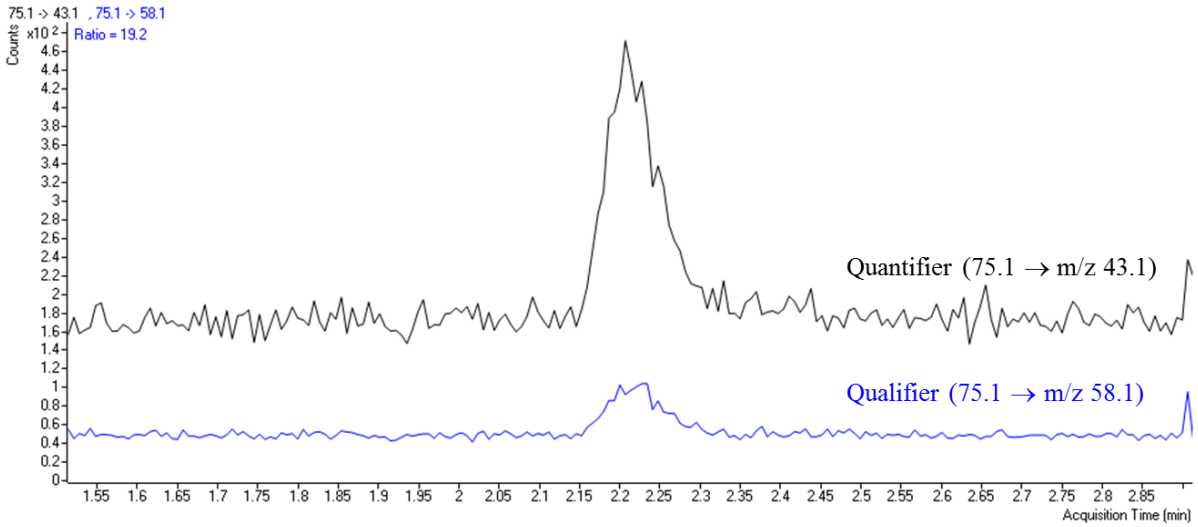
Water Blank



NDMA (0.3 ng/mL Standard)



NDMA (1.0 ng/mL Standard)



Overlapped MRM and UV chromatograms of Ranitidine Drug Product