



# Novel stability indicating LC-MS method for N-Nitroso dimethyl amine genotoxic impurity quantification in ranitidine drug substance and drug product

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## ABSTRACT

Nitrosamine impurities are potential carcinogens, which are forming from the synthesis of drug substance as byproduct and also forming in presence of NaNO<sub>2</sub>/HNO<sub>3</sub> and secondary amines (e.g., dimethyl amines, diethyl amine etc.), which should be controlled in the medication of the human beings. Hence, robust and sensitive analytical method is required to control the nitroso amine impurities in drugs. The object of this method is to quantify the N-Nitrosodimethylamine (NDMA) impurity at 0.01 ppm level in ranitidine drug substance (form-1 and form-2) and drug product (tablets and capsules) of different geography. The source of NDMA impurity is also from the dimethyl amine as a key starting material using the ranitidine synthetic process. NDMA is forming when dimethyl amine is reacting with nitrous acid. The optimized LC method conditions were ACE C18-AR 3 μm, 150 × 4.6 mm column, mobile phase A as 0.1% formic acid in water, mobile phase B as 100% methanol, 0.8 ml/minute flow with gradient (time/%mobile phase B): 0/3, 3/3, 15/15, 15.1/100, 17/100, 17.1/3, 22/3, column temperature: 40°C, injection volume 50 μl, and total run time as 22 minutes. The final response achieved with multiple reaction monitoring (MRM) type: MRM [Q1 Mass (Da):75 Q3:58.2 Time (msecond)] in atmospheric chemical ionization positive mode. The optimized method was validated against the International Council for Harmonisation Q2 (R1) and United States of Pharmacopeia general chapter for compendial method validations 1225. This method can detect up to 0.01 ppm and quantify up to 0.03 ppm, the method shows linearity from 0.03 to 20 ppm.

## INTRODUCTION

Ranitidine drug is for the gastrointestinal diseases, its IUPAC name is N-(2-[(5-[(Dimethylamino)methyl]furan-2-yl)methylthio]ethyl)-N'-methyl-2-nitroethene-1,1-diamine (Mitchard *et al.*, 1987). Ranitidine drug exists in the ranitidine HCl salt for formulation, the dosage forms of ranitidine were tablets and capsules, their strengths were 75, 150, and 300 mg. Hence, the maximum daily dosage for ranitidine HCl is 300 mg/day;

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the N-Nitrosodimethylamine (NDMA) limit will be 0.32 ppm: Ranitidine blocks the histamine H<sub>2</sub> receptor, it can decrease the acid secretions in the stomach, which in turn control the formation of ulcers in stomach. It is also treat for heartburn, erosive esophagitis, and Zollinger Ellison syndrome (<https://www.rxlist.com/zantac-side-effects-drug-center.htm>). The ranitidine HCl exists as two polymorphs as reported so far, the melting point of form I is 134°C–140°C and form II is 140°C–144°C (Wu *et al.*, 2000). The synthetic route of ranitidine HCl involves the dimethyl amine, which is a key starting material for the ranitidine compound Figure 1 and also it is precursor for NDMA impurity formation during the synthesis (Choi and Valentine, 2002; [https://www.who.int/medicines/publications/drugalerts/InformationNoteNitrosamine-impurities\\_Nov2019](https://www.who.int/medicines/publications/drugalerts/InformationNoteNitrosamine-impurities_Nov2019); Krasner *et al.*, 2013; Liu *et al.*, 2014; Mitch and Schreiber, 2008; Mitch and Sedlak, 2004; Sharma *et al.*, 2011).

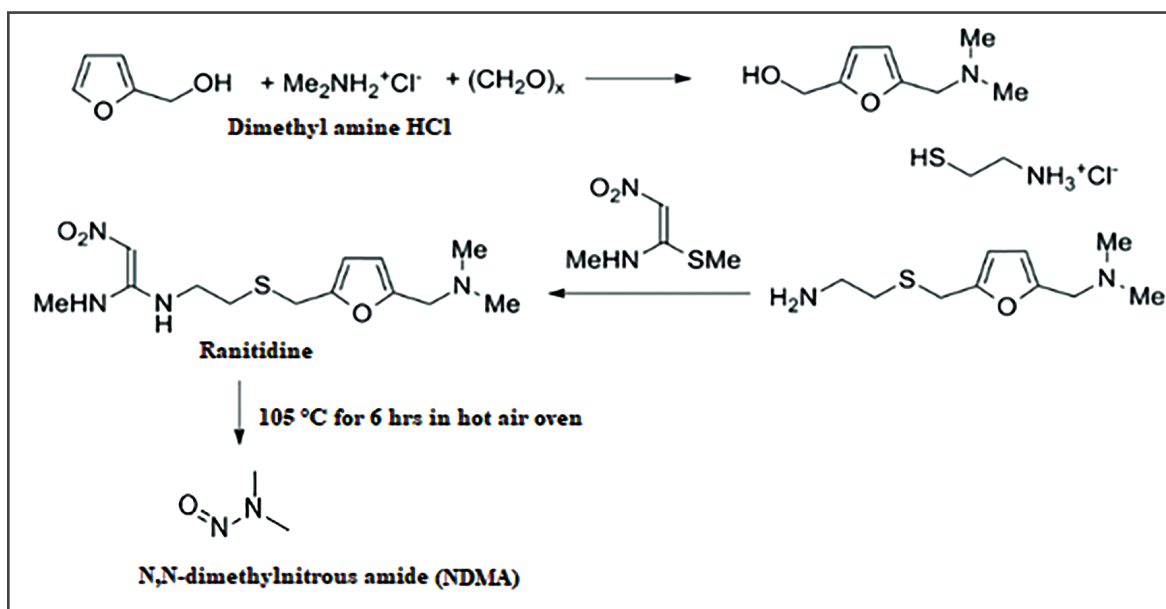


Figure 1. Synthetic scheme of ranitidine.

NDMA is potential carcinogen, which is forming from synthesis of drug substance as byproduct and also forming from the presence of  $\text{NaNO}_2/\text{HNO}_3$  and secondary amines (e.g. dimethyl amines, diethyl amine etc.), which should be controlled in the medication of the human beings. Hence, robust and sensitive analytical method should require controlling the nitroso amine impurities in drugs (<http://www.edqm.eu/sites/default/files/omcl-method-determination-ndma-valsartan-cvuseptember2018.pdf>, 2018; [https://www.agilent.com/cs/library/posters/public/Agilent\\_ASMS\\_2019\\_ThP762\\_Poster.pdf](https://www.agilent.com/cs/library/posters/public/Agilent_ASMS_2019_ThP762_Poster.pdf), 2019; Lim *et al.*, 2020; Shaikh *et al.*, 2020; USFDA, 2020a, 2020b). Many chromatography and mass spectrometric methods are available to quantify the NDMA in drug substance and drug product (Health Canada, 2020; Singapore Health Sciences Authority, 2019; The General European OMCL Network, 2020a, 2020b; USFDA, 2018, 2020c, 2020d, 2020e; US Environmental Protection Agency, 2004). But no analytical method have fully validated such specificity, robustness, ruggedness and also for degradation/stress study for NDMA production from ranitidine HCl as drug substance (form-I and form-II) and drug product. The author has proposed stress study for ranitidine drug substance (form-I and form-II), drug product (tablets and capsules), and it was shown the NDMA determination in each stress conditions. The full length validation has been performed for NDMA in ranitidine drug substance (form-I and form-II), ranitidine drug product (tablets and capsules) along with different strengths.

## MATERIALS AND METHODS

### Materials

The NDMA, N-Nitrosodiethylamine (NDEA), N-Nitroso-N-methyl-4-aminobutyric acid/N-Nitrosodibutylamine (NMBA), N-Nitrosodibutylamine (NDBA) N-Nitrosodiisopropylamine (NDIPA), and N-Nitrosoisopropylethylamine standards were

purchased from PS3 Laboratories LLP, Hyderabad, India. Millipore Milli Q purification system was used from Bangalore, India. Analytical reagent grade, methanol, sodium hydroxide, hydrochloric acid, and hydrogen peroxide were purchased from Rankem (Mumbai, India). Millipore Milli Q purification system purchased from Bangalore, India

### Equipment

Waters I-class flow through needle ultra-performance liquid chromatography (UPLC) connected with AB-Sciex 4500 mass and the data evaluation monitor with analyst 1.6.3 software.

### Method

#### UPLC method conditions

Chromatographic separation achieved from mobile phase A: 0.1% formic acid in water, mobile phase B: 100% methanol, column: ACE  $\text{C}_{18}$ -AR  $3\ \mu\text{m}$ ,  $150 \times 4.6\ \text{mm}$ , gradient (time/% mobile phase B): 0/3, 3/3, 15/15, 15.1/100, 17/100, 17.1/3, 22/3, flow: 0.8 ml/minute, injection volume:  $50\ \mu\text{l}$ , run time: 22 minutes, column temperature:  $40\text{ }^\circ\text{C}$ , detection: photo diode array (205–300), diluent: water, retention time: about 6.8 minutes.

#### Mass conditions

Mass response achieved by scan type of multiple reaction monitoring (MRM) pair [Q1 Mass (Da):75 Q3:58.2, dwell time in mseconds]: 200, polarity: positive atmospheric chemical ionization (APCI), duration: 22.00 minutes, curtain gas: 30 l, nebulizer Current (NC): 2 V, temperature:  $350\text{ }^\circ\text{C}$ , ion source gas: 55, charged aerosol detector: medium, declustering potential (DP): 50 V, entrance potential (EP): 10 V, collision energy (CE): 18, and collision cell exit potential (CXP): 9. The mass diversion with time and position were (time in minute/position of mass or

**Table 1.** System suitability injection sequence.

S. No	Solution	No of injections
1	Blank	Minimum 1
2	Linearity solutions	Each concentration-1
3	Test sample	1

waste) 2.3 minute/A to mass, 8.0 minute/B to waste, 15.0 minute/B to waste, 15.1 minute/B to mass, 20.0 minute/B to mass.

#### Preparation of standard and sample solutions

**Preparation of NDMA stock-1 solution.** Weigh about 50.0 mg of NDMA standard into a 50 ml volumetric flask containing 10 ml of methanol, dissolve by cyclomix, make up to the mark with methanol and cyclomix.

**Preparation of stock-2 solution.** Transfer 0.5 ml of stock-1 into a 50 ml volumetric flask containing 10 ml of water, make up to the mark with water and cyclomix.

**Preparation of calibration curve solutions.** Prepare linearity solutions ranging from 0.03 to 20 ppm (i.e., 0.03, 1.0, 5.0, 10.0, and 20.0 ppm) with respect to analyte concentration.

**Preparation of test sample solution for drug substance.** Weigh about 75 mg of Ranitidine HCl test sample and transfer into 5 ml volumetric flask, dissolve by water and sonication, make up to the mark with water and cyclomix.

**Preparation of test sample solution for drug product (tablet).** Crush the appropriate number of tablets to obtain target concentration of 15 mg/ml of drug substance in water and transfer into volumetric flask (50 ml), add appropriate volume of water and mix for about a minute by using vortex mixture. Shake the sample for 40 minutes using rotatory shaker.

After extraction centrifuge, the sample for 15 minutes at 4,000 rpm, filter the supernatant by using a 0.22 µm syringe

filter, discard first 1 ml, and transfer the filtered sample into a vial for liquid chromatography-mass spectrometry (LC-MS) analysis.

**Preparation of test sample solution for drug product (capsule).** Take the appropriate quantity of capsule powder to obtain target concentration of 15 mg/ml of API in water and transfer into volumetric flask (50 ml), add appropriate volume of diluent and mix for about a minute by using vortex mixture. Shake the sample for 40 minutes using the rotatory shaker.

After extraction centrifuge the sample for 15 minutes at 4,000 rpm, filter the supernatant by using a 0.22 µm syringe filter, discard first 1 ml, and transfer the filtered sample into a vial for LC-MS analysis.

**Procedure.** Equilibrate the LC-MS system with the mobile phase. Inject the prepared solutions into the LC-MS system as given in Table 1 and the typical standard chromatogram was shown in Figure 2.

**Data evaluation.** Integrate the NDMA peak only in standard and test sample.

**System suitability evaluation.** The correlation coefficient of the linearity solution should not be less than 0.99.

**Result reporting.** I. If the area of NDMA in test sample is observed less than the area of 1 ppm linearity solution then calculate the content by using three point linearity standards, i.e., 0.03, 1 and 5 ppm.

II. If the area of NDMA in test sample is observed more than the area of 1 ppm linearity solution then calculate the content by using five point linearity standards, i.e., 0.03, 1, 5, 10, and 20 ppm.

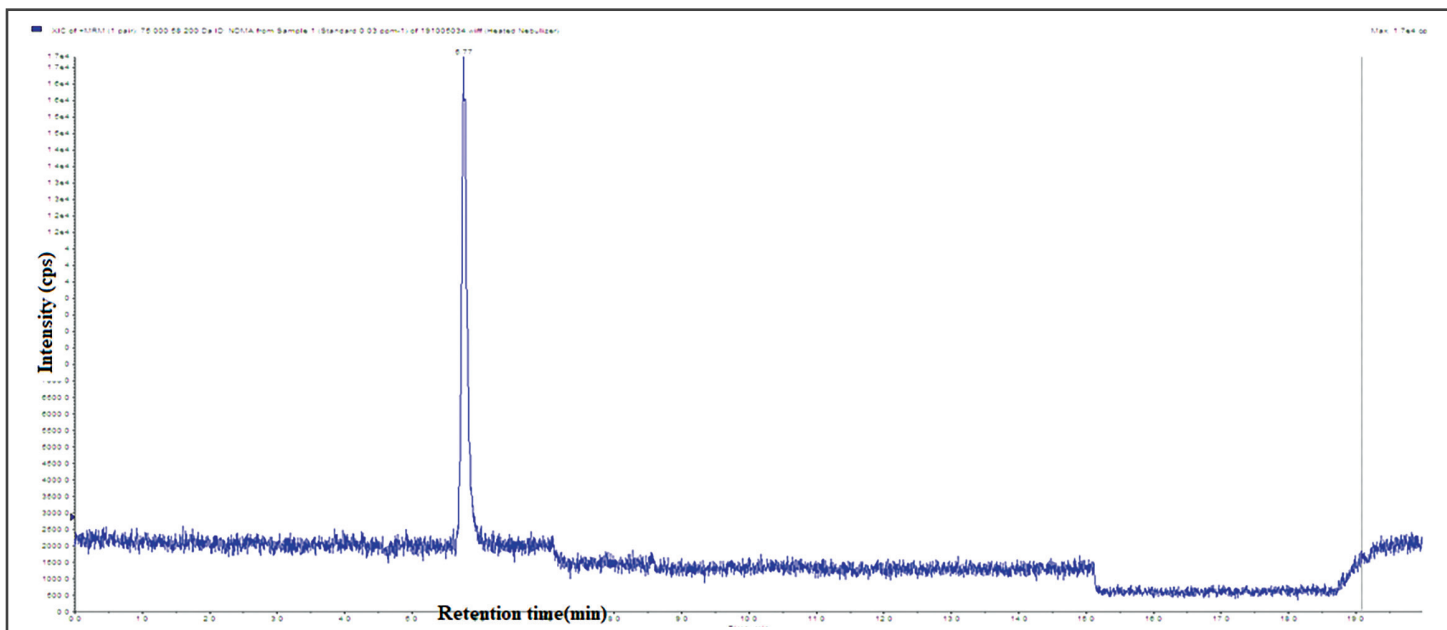
**For drug substance.** Calculate the content of NDMA using the straight line equation  $Y = mX + C$  (linearity) for each test solution and report the value of NDMA content.

$$\text{Content of NDMA ( ppm)} = (Y - C / m) \times (75/Wt)$$

where,

$Y$  = Area of test sample

$C$  = Intercept



**Figure 2.** System suitability total ion chromatogram of NDMA.

$m$  = Slope

Wt = Weight of test sample taken in mg.

**Drug product (tablets).** Calculate the content of NDMA using the straight line equation  $Y = mX + C$  (linearity) for each test solution and report the value of NDMA content.

Content of NDMA ( ppm) =  $(Y - C / m) \times$  ( Weight equivalent of tablets /Wt taken)

where,

$Y$  = Area of test sample

$C$  = Intercept

$m$  = Slope

Wt = Weight of test sample taken in mg.

**Drug product (capsules).** Calculate the content of NDMA using the straight line equation  $Y = mX + C$  (linearity) for each test solution and report the value of NDMA content.

Content of NDMA ( ppm) =  $(Y - C / m) \times$  ( Weight equivalent of capsules /Wt taken)

where,

$Y$  = Area of test sample

$C$  = Intercept

$m$  = Slope

Wt = Weight of test sample taken in mg

## RESULTS AND DISCUSSION

### LC-MS method development

The objective of the method development includes the selection of different stationary phase columns for separations of NDMA impurity from the ranitidine drug substance as well as excipients and low level detection of NDMA in presence of ranitidine drug substance and drug product. The following trials have been conducted to achieve defined objective. The objective of development trial 1 was to solubilize the ranitidine drug substance as well as the drug product. The ranitidine was freely soluble in water, and also freely soluble in methanol due to HCl salt. This was very suitable for the LC separation of NDMA in presence of ranitidine HCl. The tablets and capsules were not soluble in presence of separate water and methanol but drug substance was soluble. Hence, the water was selected for diluent, the solvent study has been screened to understand the NDMA peak retention in  $C_{18}$  column, only water and hexa fluoro isopropanol can have best retention in  $C_{18}$  column. The objective of trial 2 was to select the stationary phase to separate the NDMA along with other nitroso impurities in presence of drug substances and drug product (ranitidine HCl along with excipients). The stationary phase from polar to non-polar column was screened for the defined separation; the NDMA was separated from remaining in ACE 18 column. The objective of the trial 3 was to fix the sample concentration to achieve the 0.01 ppm limit of quantitation (LOQ) with more than 3 S/N. The 0.01 ppm response was observed with 20, 30, 40, and 50 mg ml<sup>-1</sup> sample concentration, but the % recovery was not within the regulatory requirement [as per United States of Pharmacopeia (USP), the % recovery should be 70–130], finally the chosen 15 mg ml<sup>-1</sup> where % recovery was achieved. The objective of the trial 4 was to select the mobile phase and gradient. Best separation was achieved by ACE 18 column with mobile phase A as 0.01% formic acid water and mobile phase B as acetonitrile

and water ratio as 50:50% v/v with gradient of Time/% mobile phase B as 0/8, 5/8, 8/20, 10/100, 15/100, 15.1/8, and 20/8.

### Mass parameters optimization

The objective of the mass optimization was to establish the DP, EP, CE, and CXP for the NDMA as well as the source parameters, initially the electro spray ionization source was chosen for mass checking, the NDMA response was poor and many adducts were observed and then change to APCI source. The protonated mass number of NDMA was. 75.4 m/z. The observed DP, EP for NDMA was 75.4 and 50, respectively. The product ions of precursor were 58.0 at 8 CE and 19 CXP. The source parameters were set as curtain gas as 30, NC as 2, GS1 (nebulizing gas) as 55. The observed MRM pair was 75 → 58.2.

### LC-MS method optimization

The objective of the method optimization was to demonstrate the limit of detection LOD and LOQ and check the recovery at LOQ in drug substance (form I and form II) as well as the drug product (tablets and capsules). The mobile phase B was changed from the mixer of water and acetonitrile to 100% methanol for separating the tablets having peaks and the chosen injection volume for this study was 50 µl to achieve the 0.01 ppm. The diverter parameters for mass and sample procedure was set for better recovery of NDMA in presence of excipients and degradation peaks. The finalized method evaluated procedure was followed as per Procedure Section.

### Method validation

The final developed and optimized method has been validated against the International Council for Harmonisation (ICH) Q2 (R1) and USP general chapter for compendial method validation <1225> (USP General Chapter <1225>). The method validation was performed by considering the specificity, precision, linearity, robustness, and ruggedness. The validation results were accepted by major regulatory bodies in the world.

#### Specificity

The specificity of developed method was proved by injecting NDEA, NMBA, NDIPA, NDBA and NIEPA along with NDMA. The other nitroso impurities were not co-eluting with the NDMA impurity. The stress study/degradation study has been performed to prove the method specificity.

#### Forced degradation study sample preparation

Taken 1.5 g equivalent powder of ranitidine in 100 ml volumetric flask containing 50 ml of diluent, sonicated for 20 minutes with intermediate shaking by maintaining temperature below 25°C. After sonication, added degradation solutions as mentioned below for exposure. Added neutralizing solution and made up to the mark with diluent (100 ml). Centrifuged the sample at 4,000 rpm for 10 minutes. The thermal degradation sample was injected in system after attaining to room temperature (below 25°C).

#### Degradation solutions

Acid stress—5 ml of 1 N HCl at 60°C for 5 hours.

Peroxide stress—5 ml of 1% H<sub>2</sub>O<sub>2</sub> on bench top for 2 hours.

**Table 2.** Degradation study of ranitidine.

Stress condition	NDMA content in ppm
Drug substance (form I)	
As such sample analysis	0.0
Peroxide degradation	0.0
Acid degradation	0.1
Base degradation	0.0
Water degradation	0.0
Thermal degradation	10.7
Drug substance (form II)	
As such sample analysis	17.8
Peroxide degradation	18.9
Acid degradation	16.1
Base degradation	13.0
Water degradation	15.5
Thermal degradation	111.0
Drug product (tablets)	
As such sample analysis	6.2
Peroxide degradation	6.8
Acid degradation	7.4
Base degradation	6.6
Water degradation	6.6
Thermal degradation	27.4

Water stress—5 ml of water at 60°C for 5 hours.

Thermal sample—Samples were exposed at 105°C for 6 hours.

Base stress—5 ml of 1 N NaOH at 60°C for 5 hours.

The NDMA formation was observed only in the thermal samples of drug substance and drug product (forms I, form II,

capsules, and tablets). The specificity results were captured in the Table 2.

#### Precision

Method precision has been performed by injecting six different measurements same drug substance as well as drug product with NDMA spiking 10 ppm in drug substance and 0.2 ppm in the drug product. The obtained results were less than 3% relative standard deviation (RSD). The typical method precision total ion chromatogram (TIC) and UV chromatogram of drug substance were captured in Figure 3.

#### Accuracy/recovery

Accuracy has been performed by three measurements of same drug substance as well as drug product with NDMA spiking 10 ppm in drug substance and 0.2 ppm in the drug product, the accuracy has been calculated against the test sample. The obtained results were less than 72.3%–98.6%. The typical accuracy TIC and UV chromatogram of spiked at 0.2 ppm of tablet and capsule were shown in Figures 4 and 5.

#### Linearity

Linearity is the range of the analyte that can be detected in the test sample. Linearity of the developed method has been done from 0.03 to 20 ppm. The different geographical samples are falls in the range of the linearity of the method. The correlation coefficient for the linearity was 0.9998. The calibration graph was captured in b.

#### LOD and limit of quantification

LOD is lowest amount of analyte present in the sample, it can be detected. LOQ is the lowest amount of analyte present in the sample, it can be quantified and it should be accurate and precise at that level. The LOQ and LOQ has been established by using the S/N method. The S/N ratios of LOD and LOQ met the ICH guidelines. The LOD and LOQ ion chromatogram

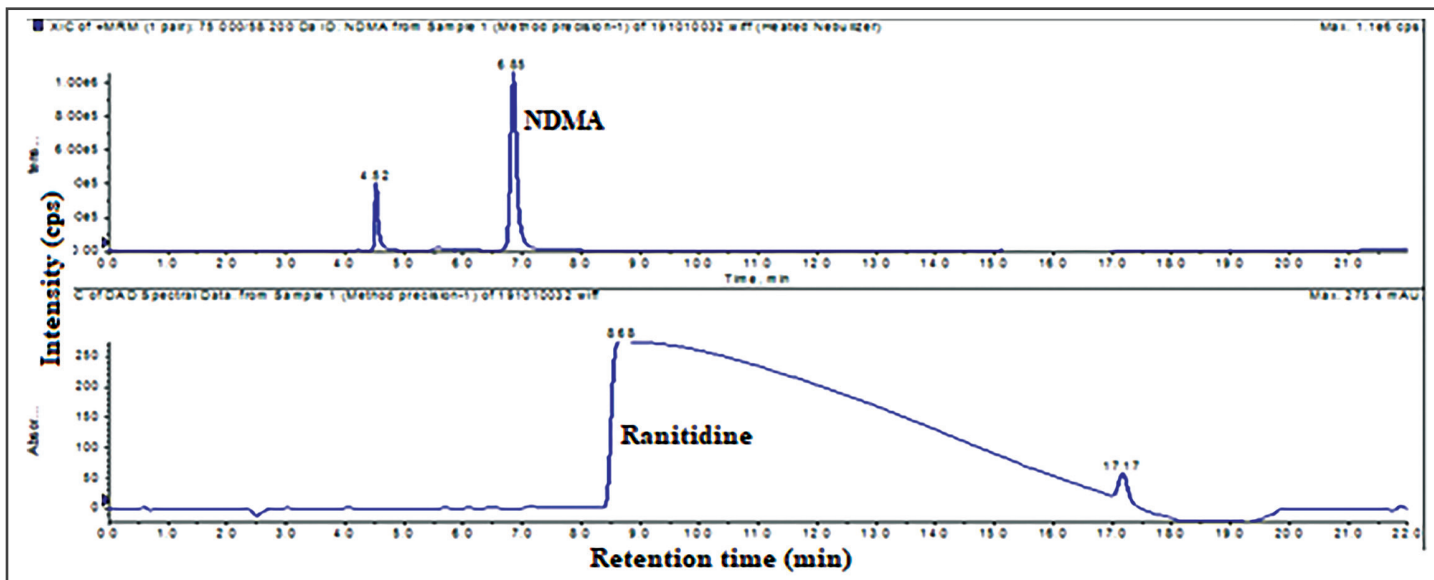


Figure 3. Typical method precision total ion chromatogram and UV chromatogram of NDMA and ranitidine HCl drug substance.

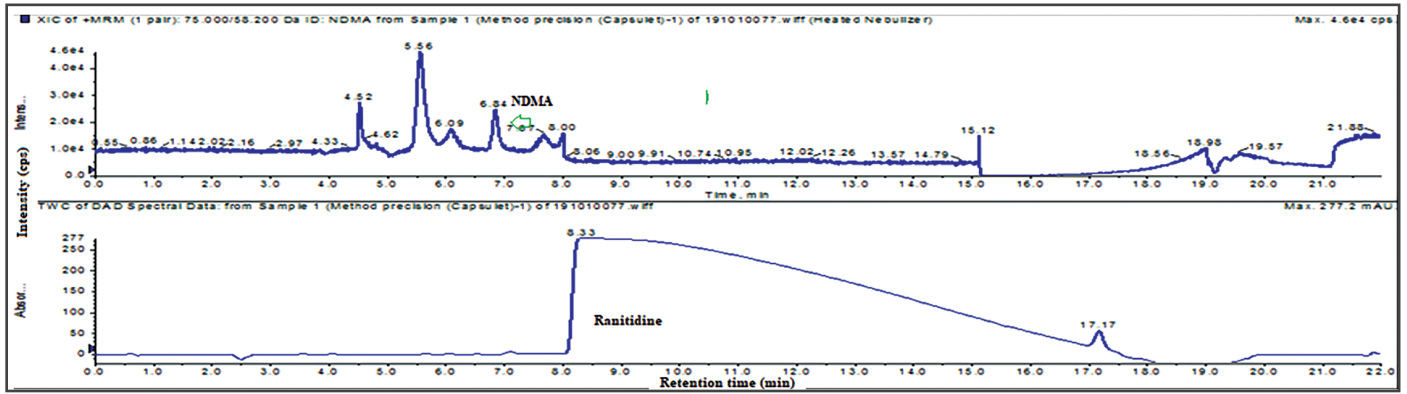


Figure 4. Typical method precision total ion chromatogram and UV chromatogram of NDMA and ranitidine HCl drug product (tablets).

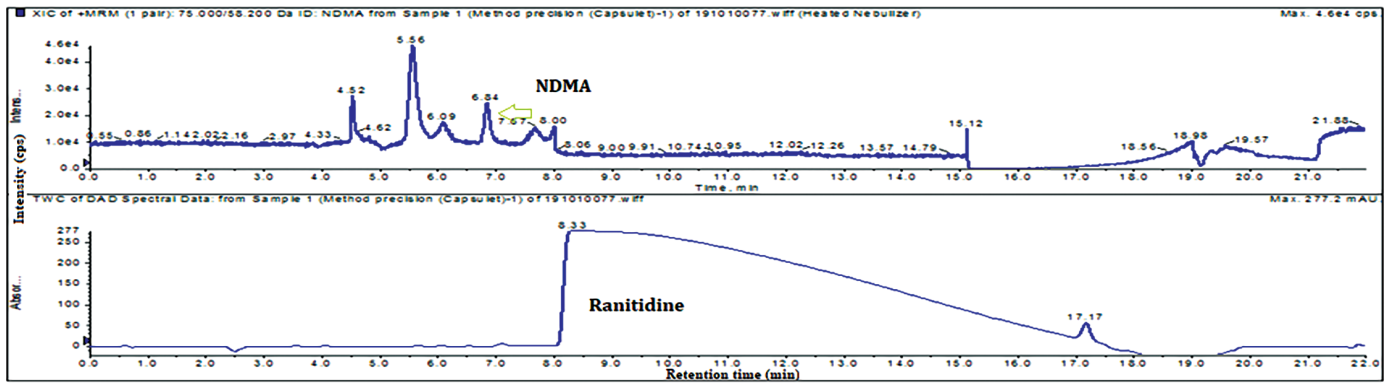


Figure 5. Typical method precision total ion chromatogram and UV chromatogram of NDMA and ranitidine HCl drug product (capsules).

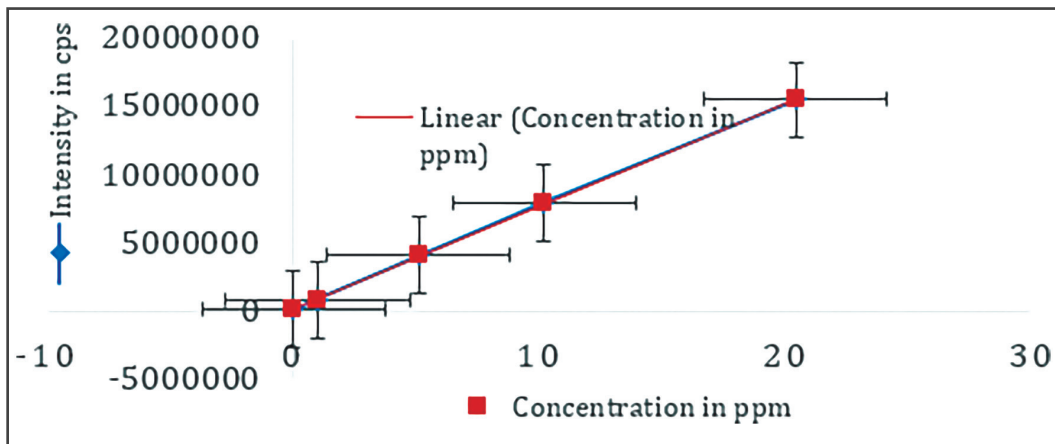


Figure 6. Linearity plot of NDMA.

Table 3. Results of LOD and LOQ.

Parameter	Concentration (ppm)	NDMA area	S/N
LOD	0.01	11,500	5.2
LOQ	0.03	21,800	10.8

**Table 4.** Summary of validation.

Parameter	Results
LOD and LOQ establishment	
LOQ in ppm (10.8: S/N)	0.03 (Area: 21,800)
LOD in ppm (5.2: S/N)	0.01 (Area: 11,500)
LOQ precision (% RSD)	6.9
Linearity	
Correlation coefficient ( <i>r</i> )	0.9999
Intercept ( <i>c</i> )	109,127.015
Slope ( <i>m</i> )	753,761.6369
Method precision	
Method precision (% RSD): drug substance	2.07
Method precision (% RSD): drug product (tablets)	2.22
Method precision (% RSD): drug product (capsules)	2.61
LOQ precision (% RSD)	6.9
Accuracy/recovery	
Accuracy at 10 ppm: drug substance	86.6
Accuracy at 0.2 ppm: drug product (tablets)	72.3
Accuracy at 0.2 ppm: drug product (capsules)	99.6
Accuracy at LOQ: drug substance	97.4
Accuracy at LOQ: drug product (tablets)	82.4
Accuracy at LOQ: drug product (capsules)	78.4
Solution stability	
Solution stability: % variation after 12 hours: DS	-7.1
Solution stability: % variation after 12 hours: DP (tablets)	5.8
Solution stability: % variation after 12 hours: DP (capsules)	-9.4
Specificity (drug substance-form I)	
Acid stress (1 N HCl)	No degradation
Base stress (1 N NaOH)	No degradation
Water stress	No degradation
Peroxide stress (3% H <sub>2</sub> O <sub>2</sub> )	No degradation
Thermal stress (105°C)	10.7 ppm
Specificity (drug substance-form II)	
Acid stress (1 N HCl)	No degradation
Base stress (1 N NaOH)	No degradation
Water stress: NDMA content in ppm	No degradation
Peroxide stress (3% H <sub>2</sub> O <sub>2</sub> )	No degradation
Thermal stress (105°C)	111.0
Specificity (drug product-tablets)	
Acid stress (1 N HCl)	No degradation
Base stress (1 N NaOH)	No degradation
Water stress: NDMA content in ppm	No degradation
Peroxide stress (3% H <sub>2</sub> O <sub>2</sub> )	No degradation
Thermal stress (105°C)	27.4 ppm

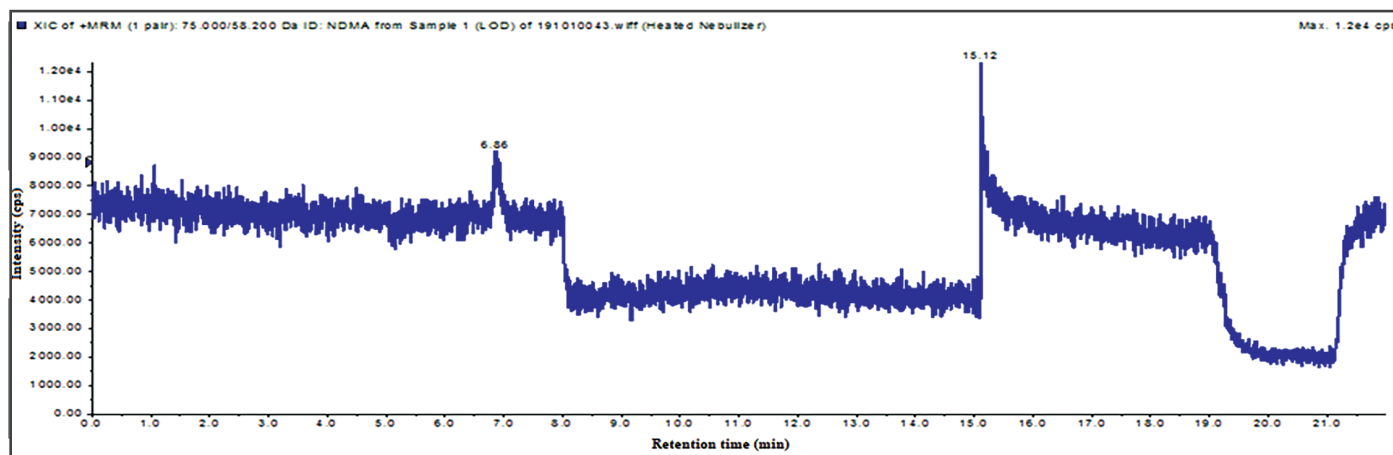


Figure 7. The total ion chromatogram of LOD solution.

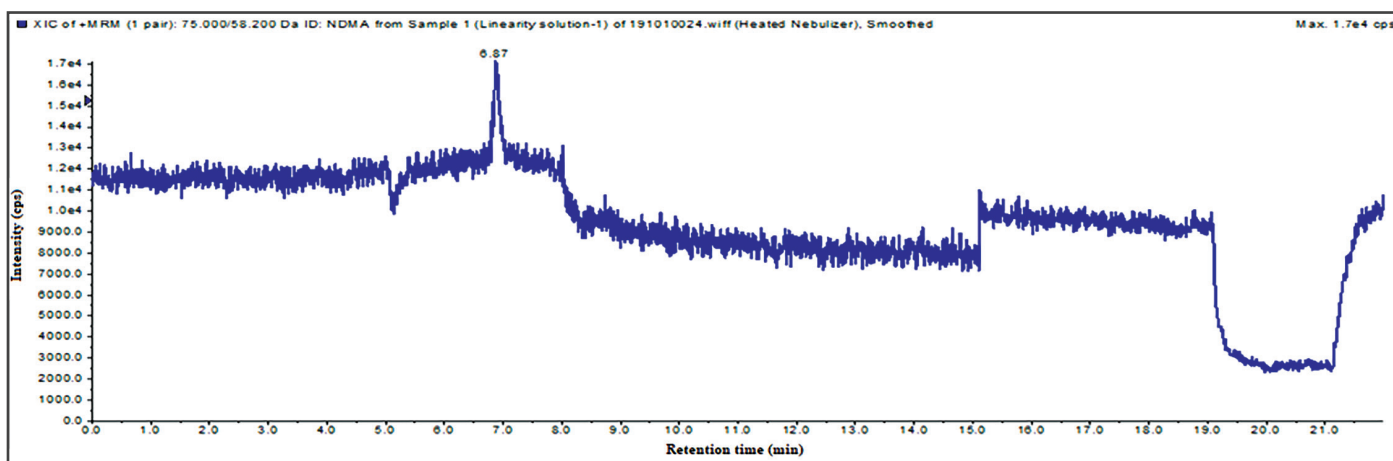


Figure 8. The total ion chromatogram of LOQ solution.

was captured in Figures 7 and 8, and results were captured in Table 3.

#### Precision and accuracy at LOQ

Injected the blank, precision at six LOQ solutions single time into LCMS. Recorded the peak area of NDMA and calculated the % RSD for the area from precision at LOQ solutions. The LOQ recovery was performed by injecting the three spiked solutions against the blank solution.

#### Solution stability

The solution stability for NDMA standard and spiked sample was established up to the 12 hours. The solutions were injected into LC-MS at zero hour (initial) and after 12 hours. At each interval the NDMA content was recorded and the difference with respect to NDMA obtained at initial interval was calculated. The obtained results were within 10% variation.

#### Selectivity

Injected actual quantity of placebo present in capsules and tablets in to the LC-MS/MS instrument as per method of analysis and also injected diluent. The validation summary was captured in Table 4.

## CONCLUSION

From the above data of method development and validation, it is concluded that the method which is designed to determine NDMA content in Ranitidine drug substance and drug product found to be precise, accurate, and linear. LOQ of the NDMA impurity was found to be 0.03 ppm. The method found to be precise and accurate at LOQ level. LOD of the NDMA impurity found to be 0.01 ppm. Solution stability of test sample solution was established up to 12 hours in room temperature, and it was found stable and specific with placebo. The degradation study was performed to understand the NDMA formation from ranitidine drug substance and drug product. The NDMA formation from ranitidine drug substance and drug product was found under thermal degradation. This LC-MS/MS method can be employed for regular analysis, based on the development and validation of NDMA in drug substance and drug product as well as stress study of ranitidine drug substance and drug product by LC-MS/MS.

## AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising



it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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#### CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

#### ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

#### DATA AVAILABILITY

All data generated and analyzed are included within this research article.

#### PUBLISHER'S NOTE

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