LC-MS/MS Quantitative Analysis of NDMA in Ranitidine Active Pharmaceutical Ingredient (API) and Drug Product using the SCIEX® 4500 QTRAP™

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Introduction

The U.S. Food and Drug Administration has learned that some ranitidine medicines, including some products commonly known as the brand-name drug Zantac®, contain low levels of the genotoxic nitrosamine impurity N-nitrosodimethylamine (NDMA).

Nitrosamines such as NDMA are classified as probable human carcinogens based on results from laboratory tests. Nitrosamines have been found to be present in water and foods, including cured meats, dairy products, and vegetables. Nitrosamines have the potential to be intermediates in organic synthesis and due to their potent genotoxicity, it has become a requirement to accurately quantitate nitrosamines in pharmaceuticals during drug development and manufacturing.

NDMA is a hydrophilic impurity and such compounds present retention challenges for the C18 columns typically used as they offer little interaction and retention for polar molecules. The accurate quantitative analysis of a polar compound like NDMA requires good retention and separation from potential matrix interferences, in order to minimize potential matrix generated signal suppression. In order to achieve enhanced retention and separation, a high efficiency Kinetex® core-shell column containing a Biphenyl stationary phase was used for the determination of NDMA in ranitidine samples. This column is different from traditional C18 columns as it offers enhanced polar selectivity, which should be useful for improved retention of the polar NDMA compound.

The LC-MS/MS method described here is similar to the LC-MS/MS based analytical method recommended by USFDA[1] as other methods such as GC-MS, which use elevated temperatures have been reported to generate NDMA via reaction of ranitidine with itself, and this will result in an overestimation of NDMA present in ranitidine API and drug products[2].

Materials

NDMA standard was obtained as a kind gift from a customer. LC-MS grade Methanol was obtained from Biosolve, and LC-MS grade Water was obtained from Merck. Sample diluent was 10% Methanol in Water, by volume.

Standards Preparation

A 1 mg/mL standard stock solution of NDMA was prepared in diluent and stored at -20 °C.

The working standards were prepared from the stock solution by serial dilution in diluent to obtain the concentrations of 500, 100, 50, 25, 12.5, 6.25, 4.5, 1.5, 0.75, and 0.5 ng/mL.

Sample Preparation for API

The API sample was accurately weighed (100 mg) and the volume was made up to 2 mL with diluent and vortexed until completely dissolved. This solution was used for analysis.

Sample Preparation for Drug Product

The tablets equivalent to 600 mg ranitidine were accurately weighed and crushed. The powdered tablets were taken, weighed and dissolved in appropriate volume of diluent to achieve a target concentration of 50 mg/mL of ranitidine. The suspension was then vortexed for 15 minutes followed by sonication for another 10 minutes. Finally, this solution was centrifuged at 4000 rpm for 5 minutes and the resulting supernatant filtered through a PVDF syringe filter. The first 1 mL of filtrate was discarded, and the remainder of the filtered solution was used for analysis.

The HPLC and MS/MS conditions are noted below. Data acquisition and processing was performed using Analyst® 1.6.3 and MultiQuant™ 3.0.3 was used for data analysis. A 1/x2 weighted linear regression was used to generate the calibration curve.

LC-MS/MS Conditions

Analytical Column:	Kinetex 2.6 µm Biphenyl		
Dimensions:	100 x 4.6 mm		
Part No.:	00D-4622-E0		
Mobile Phase:	A: 0.05% Formic acid in Water B: 0.05% Formic acid in Methanol		
Gradient:	**Time (min)**	**% B**	**Flow Rate (mL/min)**
	0	5	0.4
	6	5	0.4
	6.1	80	0.4
	6.5	80	0.4
	6.6	80	1.0
	7.6	80	1.2
	13.5	80	1.0
	13.6	5	0.4
	17	5	0.4
Valco Valve:	**Time (min)**	**Position**	
	6.9	B (out)	
	13.5	A (in)	
Temperature:	35 °C		
Injection Volume:	20 µL		
HPLC System:	Shimadzu® NEXERA® (Shimadzu Corporation)		
Mass Spectrometer:	SCIEX® 4500 QTRAP™ (AB SCIEX Pte. Ltd.)		
MS Mode:	APCI Positive		
Scan Type:	MRM		
Nebulizer Current:	3 µA		
Curtain Gas:	30 psi		
GS1:	40 psi		
CAD:	40 °C		
Source Temperature:	350 °C		
Dwell Time:	100 msec		
UV Detection:	254 nm		

Compound Retention Times and Dependent Mass Spectrometer Values

Name	Q1	Q3	DP (V)	EP (V)	CE (V)	CXP (V)	RT (min)
NDMA-1	75.05	58.1	45	10	25	10	5.6
NDMA-2	75.05	43	45	10	15	10	5.6
Ranitidine	315	229.1	45	10	25	10	7.3

Results and Discussion

Figure 1 shows the MRM chromatogram of the impurity NMDA (75.05/58.1 & 75.05/43) and ranitidine (315.1/229.9). The corresponding UV chromatogram is shown at the bottom of **Figure 1**. The separation between the NDMA and ranitidine peaks was optimized using the gradient mobile phase conditions detailed in the LC-MS/MS conditions. The MS/MS parameters and analyte retention times are also noted above.

Linearity Range, LOD & LOQ

The calibration curve (**Figure 2**) consisted of 10 calibration points covering the concentration range from 0.5 ng/mL to 500 ng/mL. The calibration curves were constructed by fitting the analyte concentrations versus the peak area of the analyte with a regression line with $1/x^2$ weighting. The regression coefficient, r^2, determined from the calibration curves was 0.99. The linearity was obtained over the concentration range of 0.5 ng/mL to 500 ng/mL, corresponding to 0.01 – 10 ppm in 50 mg API. The LOQ and LOD for NDMA were 1.5 ng/mL and 0.5 ng/mL, corresponding to 0.03 and 0.01 ppm, respectively, with respect to 50 mg/mL API (**Figure 3**).

Accuracy, precision and recovery

The accuracy and precision were demonstrated by spiking a known concentration of NDMA at three different concentrations (LOD, LOQ and specification limit). The accuracy and precision of the method was evaluated based on recovery studies using standard addition to API. Recovery of NDMA was determined in fortified samples. The overall recoveries observed at all three levels were between 80 to 120% as summarized in **Table 1**. The results demonstrate that this method is suitable for the accurate analysis of NDMA in API and drug product.

Figure 1.
Chromatogram of Ranitidine sample containing NDMA contamination, upper chromatogram showing 3 transition of MS/MS for NDMA (75/43 - red, 75/58.1- blue) and Ranitidine (315.1/229.9 - green), and the lower chromatogram showing UV detection at 254 nm with ranitidine eluting at 7.01 – 8.7 in Valco waste line.

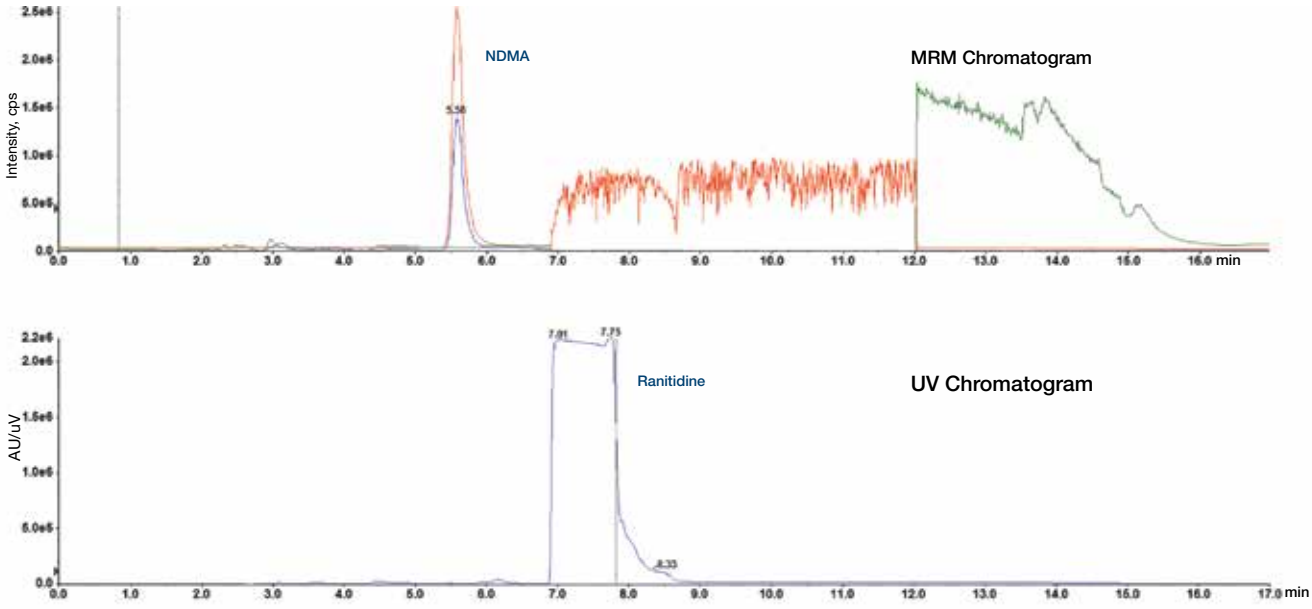

Figure 2.
Calibration Curve for NDMA over the calibration range 0.5 to 500 ng/mL for m/z 75.0 ▸ 58.1 (top) and 75.0 ▸ 43.0 (bottom).

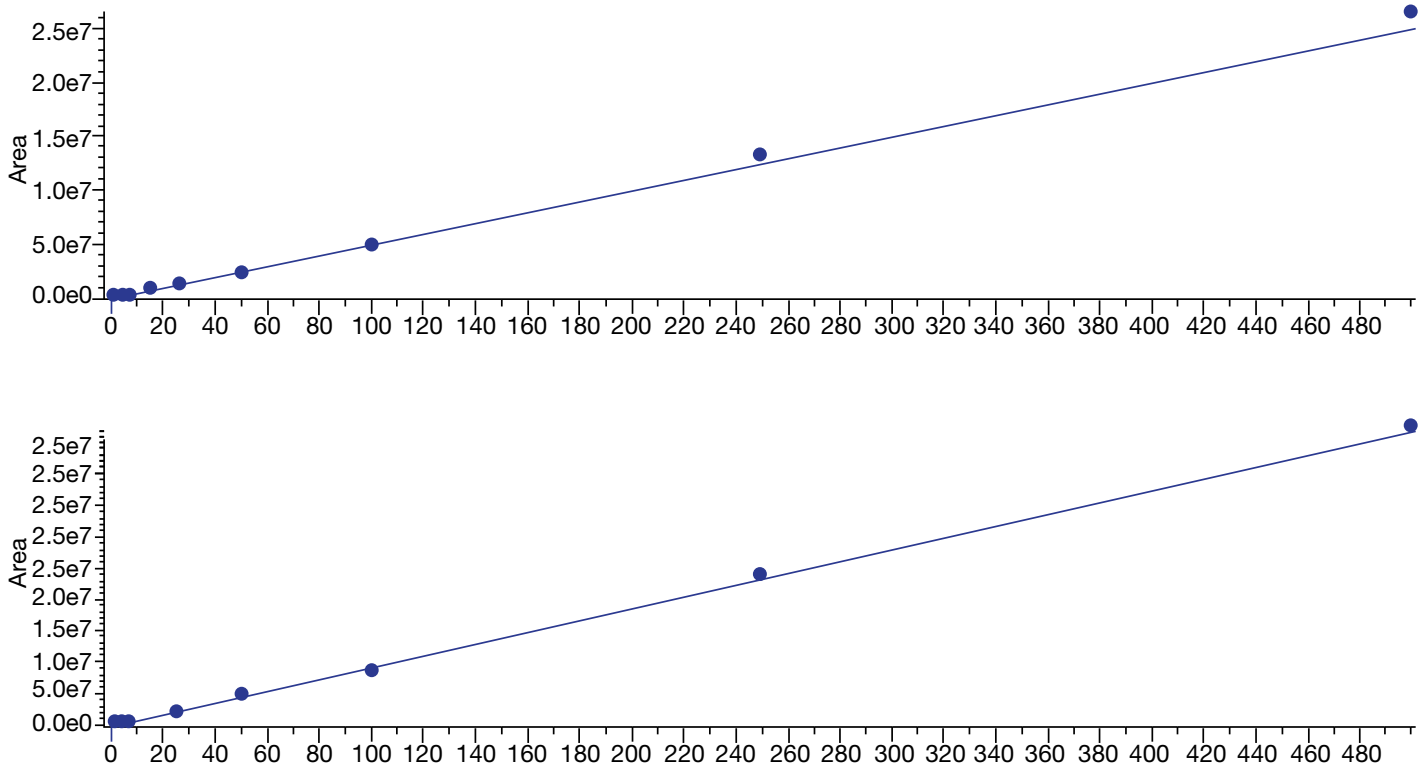

Figure 3.
Extracted Ion Chromatograms of NDMA for diluent, specification, LOQ and LOD level.

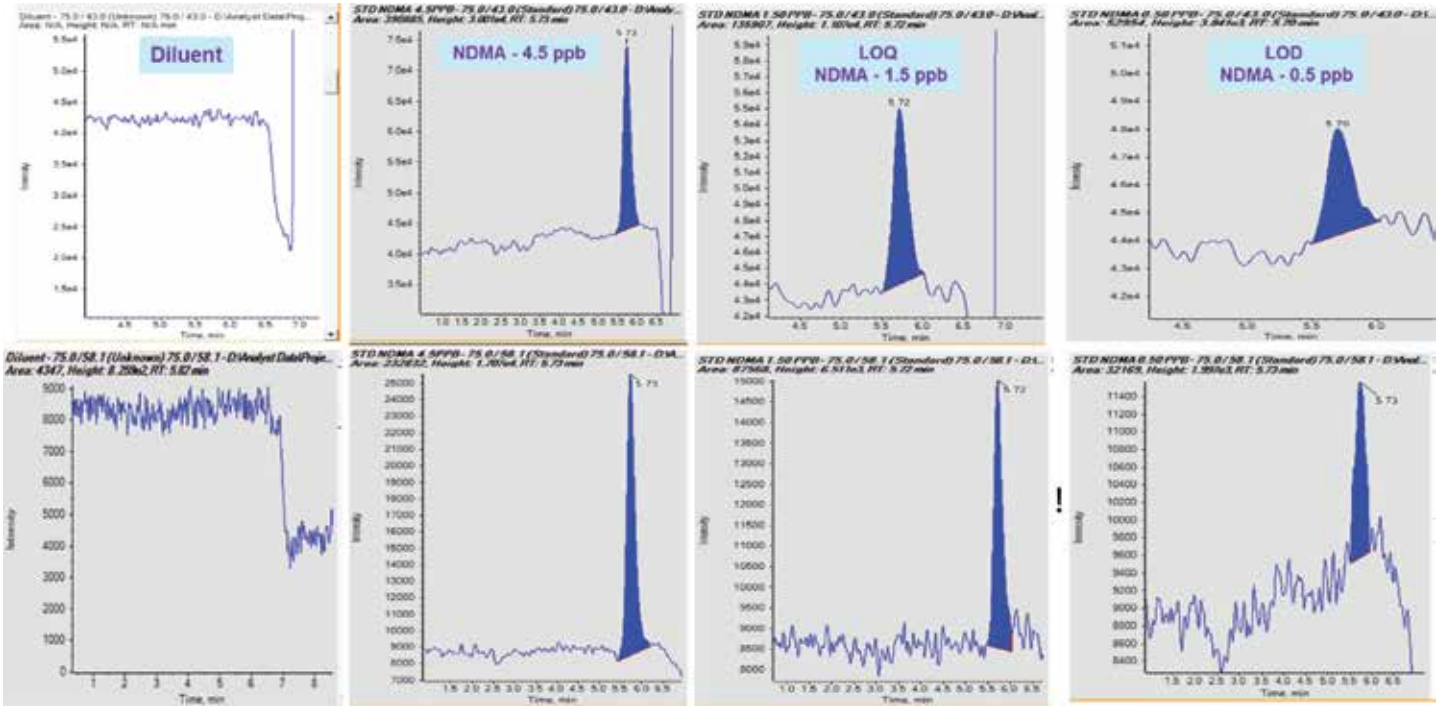

Table 1.
Accuracy, precision and recovery in spiked sample

Concentration Sample (ppm)	NDMA (in 50 mg/mL API)			
	Concentration relative to 50 mg/mL ranitidine	Accuracy (n=6)	Precision (% RSD, n=6) (Spiked Standard)	% Recovery (n=6)
Control sample (50 mg/mL ranitidine)	5 ng/mL	101.18%	3.19	(80-120)
LOD (0.01 ppm)	0.5 ng/mL	101.04%	1.49	(80-120)
LLOQ (0.03 ppm)	1.5 ng/mL	102.77%	2.5	(80-120)
Spec Level (0.09 ppm)	4.5 ng/mL	104.07%	1.43	(80-120)

Conclusion

The LC-MS/MS method for analysis for NDMA in ranitidine was successfully developed and applied to the analysis of NDMA impurities in pharmaceutical API and drug product. The method accurately quantifies the levels of NDMA below the levels required by USFDA and EMA, ensuring the safety of the API and pharmaceutical drug product, while avoiding the use of high temperatures (as in the GC method) which can result in overestimation of NDMA in ranitidine samples. The choice of the Kinetex Biphenyl column was demonstrated to give retention for the polar NDMA impurity, while providing sufficient resolution between NDMA and Ranitidine to minimize any potential for matrix interference.

References

1. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Method for the Determination of NDMA in Ranitidine Drug Substance and Solid Dosage Drug Product; https://www.fda.gov/media/131868/download.
2. https://www.valisure.com/wp-content/uploads/Valisure-Ranitidine-FDA-Citizen-Petition-v4.12.pdf

Kinetex® LC Column Ordering Information

2.6 µm Analytical Columns (mm)			SecurityGuard ULTRA Cartridges‡
Phases	100 x 4.6	150 x 4.6	3/pk
Biphenyl	00D-4622-E0	00F-4622-E0	AJ0-9207 for 4.6 mm ID

‡SecurityGuard ULTRA Cartridges require Holder, Part No.: AJ0-9000

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