Application Note

Pharma & Biopharma

Agilent

Trusted Answers

Determination of a Genotoxic NDMA Impurity Using the High-Resolution Agilent 6546 LC/Q-TOF in Ranitidine Drug Substance and Drug Products

Authors

Chander Mani and
Saikat Banerjee
Agilent Technologies, Inc.

Abstract

Impurities in medicines are of great concern to patients and consumers who rely on safe and effective medicines approved by the FDA. Ranitidine and nizatidine are H2 receptor blockers that decrease the amount of acid in the stomach, and are used to treat gastritis, inflamed stomach, and peptic ulcers. It was found that ranitidine drug substance and drug products contained a carcinogenic nitrosamine impurity; as a result, many such products were recalled. There is a requirement for analytical methods capable of detecting problematic nitrosamine impurities. The most recent among the recalled drugs include ranitidine for the suspected presence of N-nitrosodimethylamine (NDMA). This application note describes a sensitive, high-resolution LC/MS/MS method using the Agilent 6546 LC/Q-TOF for the detection and quantification of NDMA in ranitidine drug substance and products.

Introduction

An NDMA impurity (Figure 1) has become a focus for regulatory agencies such as the FDA and EMA, when the FDA announced a recall of ranitidine drug products due to the potential for these products to contain nitrosamine impurities. These nitroso compounds are classified as probable human carcinogens, and are believed to have been introduced into finished medicines as trace-level byproducts of the manufacturing process. USFDA recently found NDMA in certain batches of ranitidine products.

Liquid chromatography mass spectrometry-based methods are generally very specific and sensitive, and have served as the basis for development of methods to detect and quantify NDMA in ranitidine substance and drug products. This application note describes a method that was performed on the 6546 LC/Q-TOF, providing a comprehensive analysis of NDMA impurity at very low detection limits. This application note also demonstrates the sensitivity of the high-resolution 6546 LC/Q-TOF.

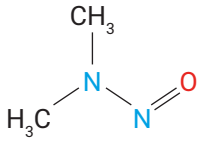

Figure 1. Chemical structure of NDMA.

Experimental

Chemicals and reagents

An NDMA standard used in this study was locally sourced from PS3 Labs LLP (Hyderabad, TS, India). Other LC/MS-grade solvents (for example, methanol, water) were purchased from Honeywell (Charlotte, NC, USA). Formic acid was purchased from Fluka (now of Honeywell).

LC configuration and parameters

Table 1. UHPLC configuration and settings.

Parameter	Value
Instruments	Agilent 1290 Infinity II high-speed pump (G7120A)
	Agilent 1290 Infinity II multisampler (G7167B)
	Agilent 1290 Infinity II multicolumn thermostat (G7116B)
	Agilent 1290 Infinity II variable wavelength detector (G7114B)
Needle Wash	Methanol:water (80:20 v/v)
Sample Diluent	Water:methanol (95:5 v/v)
Multisampler Temperature	6 °C
Injection Volume	20 µL
Analytical Column	Agilent InfinityLab Poroshell HPH-C18, 4.6 × 150 mm, 2.7 µm (p/n 693975-702)
Column Temperature	40 °C
Mobile Phase A	0.2% formic acid in water
Mobile Phase B	Methanol
Flow Rate	0.3 mL/min
Gradient	Time (min) % A % B Flow (mL/min) 0 95 5 0.3 6 92 8 0.3 6.1 92 8 0.5 11 5 95 0.5 11.1 5 95 0.3 11.2 95 5 0.3 14 95 5 0.3
Stop Time	14 minutes
Wavelengths	300 nm, 320 nm

Q-TOF mass spectrometer configuration and parameters

Table 2. Mass spectrometer configuration and source settings.

Instrument	Agilent 6546 LC/Q-TOF
Ion Source	Atmospheric pressure chemical ionization (APCI)
MS Mode	MS
Ionization Mode	Positive
Drying Gas Temperature	300 °C
Drying Gas Flow	6 L/min
Nebulizer Pressure	45 psi
APCI Heater	350 °C
APCI Needle Positive	4 µA
Capillary Voltage, Positive	3,000 V
Mass Range	*m/z* 70 to 170

MS compound information for analytes

Table 3. Detailed MS settings for the Agilent 6546 LC/Q-TOF.

Time Segment	Start Time (min)	Mass Range (*m/z*)	Fragmentor Voltage (V)	Diverter Valve Position
1	0	70 to 170	120	Waste
2	6.5	70 to 170	120	MS
3	8.2	70 to 170	120	Waste

Data analysis

Data were acquired and analyzed using Agilent MassHunter LC/MS Data Acquisition Software version 10 for data collection from the 6546 LC/Q-TOF. The quantification of NDMA was performed using Agilent Masshunter Quantitative Analysis for TOF, version 10.

Results and discussion

The calibration concentrations ranged from 0.25 to 100 ng/mL (Table 4). R^2 values were greater than 0.9997 for NDMA (*m/z* 75.0553) and displayed linear responses throughout the concentration range. Figure 2 shows the representative NDMA extracted ion chromatogram at 5 ng/mL for NDMA along with the ranitidine UV chromatogram showing the elution pattern. The diverter valve program, as detailed in Table 3, was used to divert high concentrations of ranitidine to waste.

Table 4. Results summary for the Agilent 6546 LC/Q-TOF. Data include signal-to-noise (S/N), calculated limit of quantitation (LOQ), coefficient of regression, calibration curve fit, and linearity range. NDMA used a linear function and 1/x weighted calibration curve.

Compound	Detection Limit (ng/mL)	Detection Limit (S/N)	LOQ (ng/mL)	LOQ (S/N)	R^2	Cal. Curve	Linearity Range (ng/mL)
NDMA	0.15	18.8	0.25	47.48	0.9997	Linear	0.25 to 100

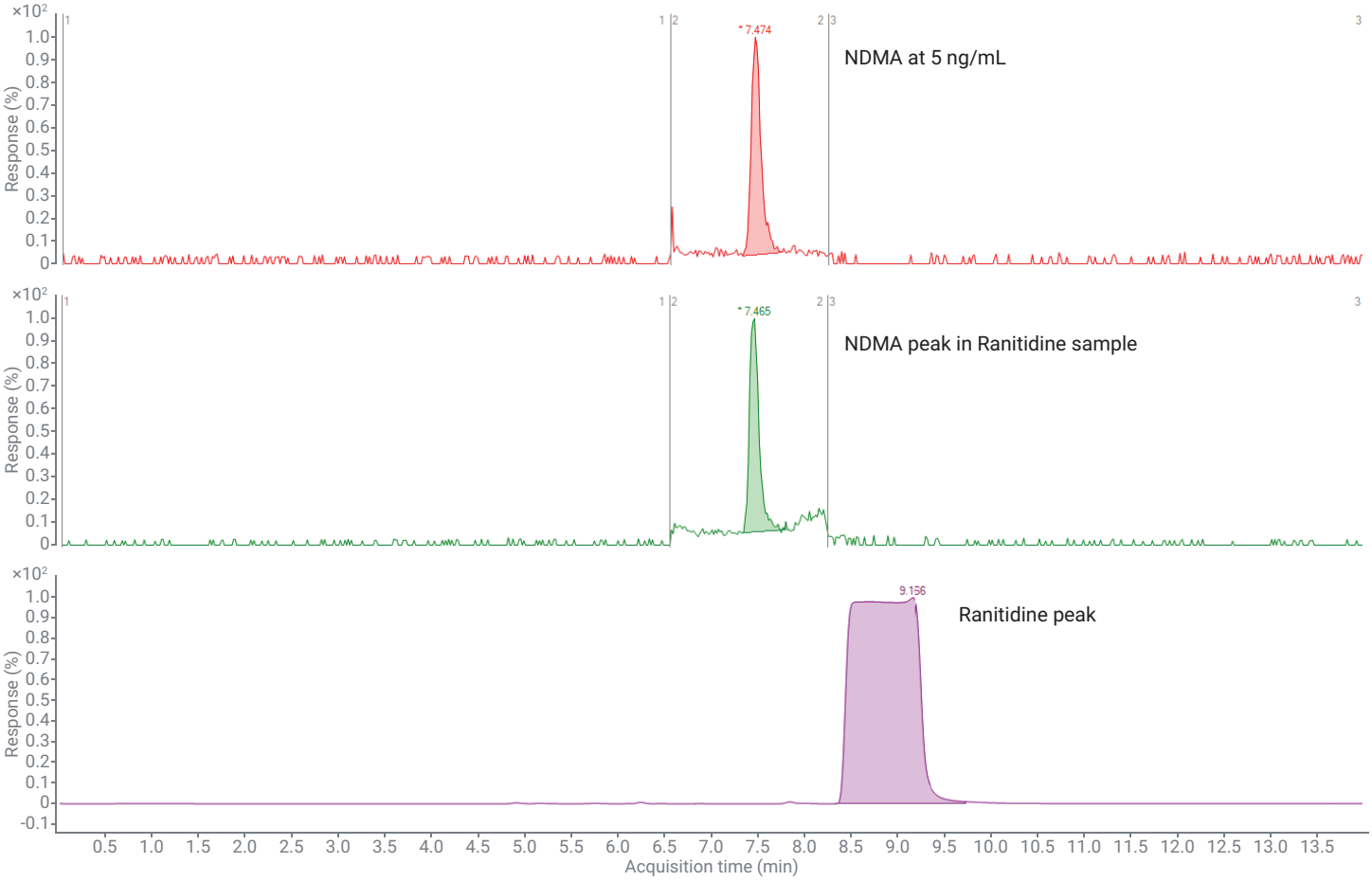

Figure 2. Representative NDMA (5 ng/mL) MRM chromatogram along with the ranitidine UV chromatogram showing elution pattern.

Accuracy and reproducibility

The calibration curve for NDMA demonstrated accuracies of within 20% of the expected concentrations at each calibration level, and reproducibility across all levels exhibited Coefficient Variances (CVs) less than 15% (Figure 3). Figures 4A, 4B, and Table 5 show a detailed comparison of accuracy and reproducibility at different concentration levels.

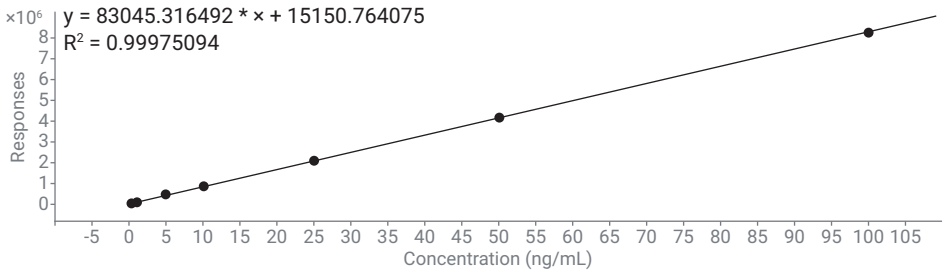

Figure 3. Representative calibration curve for NDMA dispersed throughout the chromatograms. The NDMA calibration curve used a linear function and 1/x weighting factor.

Sample					NDMA M...	NDMA Results							
Name	Data File	Type	Level	Dil.	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Mass Accuracy	S/N
Blank	Blank-r002.d	Blank		1.0000				☐					
0.25 ppb	0.25 ppb-r001.d	Cal	1	1.0000	0.2500	7.496	33707	☐	0.2061	0.2061	82.4	-1.3770	47.46
0.5 ppb	0.5 ppb-r001.d	Cal	2	1.0000	0.5000	7.497	53629	☐	0.4461	0.4461	89.2	-0.2417	92.22
0.5 ppb	0.5 ppb-r002.d	Cal	2	1.0000	0.5000	7.465	53993	☐	0.4505	0.4505	90.1	-2.0896	140.60
1 ppb	1 ppb-r001.d	Cal	3	1.0000	1.0000	7.476	104775	☐	1.0625	1.0625	106.2	-2.0565	184.65
1 ppb	1 ppb-r002.d	Cal	3	1.0000	1.0000	7.480	104836	☐	1.0632	1.0632	106.3	-2.0449	230.68
1 ppb	1 ppb-r003.d	Cal	3	1.0000	1.0000	7.481	104316	☐	1.0569	1.0569	105.7	-1.7189	368.90
1 ppb	1 ppb-r004.d	Cal	3	1.0000	1.0000	7.482	100169	☐	1.0070	1.0070	100.7	-2.2917	99.90
1 ppb	1 ppb-r005.d	Cal	3	1.0000	1.0000	7.483	100928	☐	1.0161	1.0161	101.6	-1.2478	357.81
1 ppb	1 ppb-r006.d	Cal	3	1.0000	1.0000	7.480	105196	☐	1.0675	1.0675	106.8	-1.3829	160.20
5 ppb	5 ppb-r001.d	Cal	4	1.0000	5.0000	7.474	452997	☐	5.2586	5.2586	105.2	-0.8311	1209.04
5 ppb	5 ppb-r002.d	Cal	4	1.0000	5.0000	7.475	445129	☐	5.1637	5.1637	103.3	-1.3531	560.45
10 ppb	10 ppb-r001.d	Cal	5	1.0000	10.0000	7.474	856882	☐	10.1254	10.1254	101.3	-0.9374	1438.92
10 ppb	10 ppb-r002.d	Cal	5	1.0000	10.0000	7.489	864754	☐	10.2203	10.2203	102.2	-1.7301	1477.89
25 ppb	25 ppb-r001.d	Cal	6	1.0000	25.0000	7.471	2063008	☐	24.6593	24.6593	98.6	-1.2186	2690.34
25 ppb	25 ppb-r002.d	Cal	6	1.0000	25.0000	7.469	2114326	☐	25.2777	25.2777	101.1	-0.8898	2937.14
50 ppb	50 ppb-r001.d	Cal	7	1.0000	50.0000	7.470	4190567	☐	50.2965	50.2965	100.6	-1.0925	4673.75
50 ppb	50 ppb-r002.d	Cal	7	1.0000	50.0000	7.470	4149707	☐	49.8042	49.8042	99.6	-1.9279	6299.63
100 ppb	100 ppb-r001.d	Cal	8	1.0000	100.0000	7.468	8266473	☐	99.4115	99.4115	99.4	-1.4094	12159.20
100 ppb	100 ppb-r002.d	Cal	8	1.0000	100.0000	7.468	8286854	☐	99.6571	99.6571	99.7	-1.5509	11701.05

Figure 4A. Representative accuracy and reproducibility for different concentration levels as determined using the Agilent 6546 LC/Q-TOF.

Sample					NDMA M...	NDMA Results							
Name	Data File	Type	Level	Dil.	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Mass Accuracy	S/N
Ranitidine Tablet	Ranitidine Tablet-r001.d	Sample		0.0333		7.468	664819	☐	7.8110	0.2601		-2.3708	839.15
Ranitidine Tablet	Ranitidine Tablet-r002.d	Sample		0.0333		7.452	668811	☐	7.8591	0.2617		-2.6682	568.31
Ranitidine Tablet	Ranitidine Tablet-r003.d	Sample		0.0333		7.466	663197	☐	7.7915	0.2595		-2.5419	989.69
Ranitidine Tablet	Ranitidine Tablet-r0001.d	Sample		0.0333		7.465	692599	☐	8.1458	0.2713		-2.7911	825.24
Ranitidine Tablet	Ranitidine Tablet-r0002.d	Sample		0.0333		7.449	685728	☐	8.0630	0.2685		-0.2582	893.10
Ranitidine Tablet	Ranitidine Tablet-r0003.d	Sample		0.0333		7.450	698539	☐	8.2173	0.2736		-2.6450	1686.68
1 ppb	1 ppb-r008.d	Sample		1.0000		7.463	102906	☐	1.0399	1.0399		-1.9600	133.72
1 ppb	1 ppb-r009.d	Sample		1.0000		7.464	100057	☐	1.0056	1.0056		-1.0172	232.91
1 ppb	1 ppb-r010.d	Sample		1.0000		7.481	101921	☐	1.0281	1.0281		-1.7069	157.19
API 1	API 1-r001.d	Sample		0.0333		7.461	174701	☐	1.9051	0.0634		-1.4023	339.36
API 1	API 1-r002.d	Sample		0.0333		7.452	175971	☐	1.9204	0.0639		-1.6121	296.57
API 2	API 2-r001.d	Sample		0.0333		7.451	330214	☐	3.7790	0.1258		-2.0371	603.20
API 2	API 2-r002.d	Sample		0.0333		7.452	331673	☐	3.7966	0.1264		-2.2190	813.07
API 3	API 3-r001.d	Sample		0.0333		7.449	272822	☐	3.0874	0.1028		-1.4437	500.36
API 3	API 3-r002.d	Sample		0.0333		7.450	271769	☐	3.0748	0.1024		-1.5514	482.95
API 4	API 4-r001.d	Sample		0.0333		7.451	203712	☐	2.2547	0.0751		-0.9700	351.73
API 4	API 4-r002.d	Sample		0.0333		7.449	206482	☐	2.2880	0.0762		-1.4015	360.87
Tablet 1	Tablet 1-r001.d	Sample		0.0333		7.448	1781536	☐	21.2675	0.7082		-0.6552	3700.62
Tablet 1	Tablet 1-r002.d	Sample		0.0333		7.447	1790194	☐	21.3719	0.7117		-0.7234	2645.69
Tablet 2	Tablet 2-r001.d	Sample		0.0333		7.448	4235224	☐	50.8346	1.6928		-0.8068	9141.63
Tablet 2	Tablet 2-r002.d	Sample		0.0333		7.449	4232781	☐	50.8052	1.6918		-0.1469	7276.58

Figure 4B. Representative reproducibility for different sample concentration levels as determined using the Agilent 6546 LC/Q-TOF. **Note:** The API 1, API 2, API 3, and API 4 are different lots of drug substance, where API 1 was used for spiking for the recovery study. Similarly, Ranitidine tablet, Tablet 1, and Tablet 2 are different lots of ranitidine tablets, and Tablet 1 was used for spiking for the recovery study.

Table 5. Representative reproducibility for 1 ng/mL level as determined using the Agilent 6546 LC/Q-TOF.

Number	Response for 1 ng/mL
1	104775
2	104836
3	104316
4	100169
5	100928
6	105196
7 (Bracketing)	102906
8 (Bracketing)	100057
9 (Bracketing)	101921
Average	102789
SD	2086.22
RSD (%)	2.03

Sample					NDMA M...	NDMA Results							
Name	Data File	Type	Level	Dil.	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Mass Accuracy	S/N
API 1	API 1-r001.d	Sample		0.0333		7.461	174701	☐	1.9051	0.0634		-1.4023	339.36
API 1	API 1-r002.d	Sample		0.0333		7.452	175971	☐	1.9204	0.0639		-1.6121	296.57
Tablet 1	Tablet 1-r001.d	Sample		0.0333		7.448	1781536	☐	21.2675	0.7082		-0.6552	3700.62
Tablet 1	Tablet 1-r002.d	Sample		0.0333		7.447	1790194	☐	21.3719	0.7117		-0.7234	2645.69
Spike Standard 6 ppb	Spike Standard 6 ppb-r001.d	Sample		1.0000		7.466	457476	☐	5.3125	5.3125		-0.3589	882.27
Spike Standard 6 ppb	Spike Standard 6 ppb-r002.d	Sample		1.0000		7.464	485174	☐	5.6463	5.6463		-1.0769	1221.93
Spike Standard 48 ppb	Spike Standard 48 ppb-r001.d	Sample		1.0000		7.462	3653515	☐	43.8250	43.8250		0.5924	6178.42
Spike Standard 48 ppb	Spike Standard 48 ppb-r002.d	Sample		1.0000		7.461	3625056	☐	43.4821	43.4821		0.6249	6459.37
Blank	Blank-r00001.d	Sample		1.0000				☐					
API 1_Spike 6 ppb	API 1_Spike 6 ppb-r001.d	Sample		0.0333		7.434	615840	☐	7.2208	0.2405		1.6787	934.18
API 1_Spike 6 ppb	API 1_Spike 6 ppb-r002.d	Sample		0.0333		7.434	620887	☐	7.2816	0.2425		0.9507	1130.88
API 1_Spike 6 ppb	API 1_Spike 6 ppb-r003.d	Sample		0.0333		7.432	616494	☐	7.2287	0.2407		1.5689	1995.76
Tablet1_Spike 48 ppb	Tablet1_Spike 48 ppb-r001.d	Sample		0.0333		7.428	5197274	☐	62.4274	2.0788		1.6808	10813.26
Tablet1_Spike 48 ppb	Tablet1_Spike 48 ppb-r002.d	Sample		0.0333		7.429	5216955	☐	62.6646	2.0867		1.7166	10814.71
Tablet1_Spike 48 ppb	Tablet1_Spike 48 ppb-r003.d	Sample		0.0333		7.435	5224481	☐	62.7553	2.0898		1.4057	6946.74

Figure 5. Representative recovery data for different concentration levels as determined using the Agilent 6546 LC/Q-TOF.

Note: Recovery experiments were performed at higher concentrations, as both the drug substance and drug product already contained NDMA in reasonable amounts.

Table 6A. Summary of recovery experiment at 6 ng/mL concentration in ranitidine drug substance.

Nitrosamine Impurity	Spiked Concentration (ng/mL) Ranitidine API (30 mg/mL)	Recovery %
NDMA	6	93.86

Table 6B. Summary of recovery experiment at 48 ng/mL concentration in ranitidine drug product.

Nitrosamine Impurity	Spiked Concentration (ng/mL) Ranitidine API (30 mg/mL)	Recovery %
NDMA	48	94.16

Conclusion

The Agilent 6546 LC/Q-TOF high-resolution LC/MS/MS can analyze an NDMA nitrosamine impurity at low concentration levels. High resolution mass spectrometry reliably detects the presence of the nitrosamine compound in the ranitidine drug substance and drug products. This application note demonstrates the sensitivity of the 6546 LC/Q-TOF instrument for detecting NDMA at low concentration levels. The excellent mass accuracy values in this method make it extremely reliable in quantifying NDMA in ranitidine. The presence of high concentrations of NDMA found during the analysis of the ranitidine tablets makes this method relevant to addressing the USFDA requirement for the detection of NDMA at low levels in ranitidine drug substance and drug products.

References

1. USFDA guidance document: Development and Validation of a RapidFire-MS/MS Method for Screening of Nitrosamine Impurities.
2. USFDA guidance document: Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs.
3. Determination of Nitrosamine Impurities Using the High-Resolution Agilent 6546 LC/Q-TOF. Agilent Technologies Application Note, publication number 5994-1372EN, **2019**.
4. FDA guidance document: Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of NDMA in Ranitidine Drug Substance and Drug Product.
5. https://www.fda.gov/news-events/press-announcements/statement-alerting-patients-and-health-care-professionals-ndma-found-samples-ranitidine

Acknowledgments

We sincerely acknowledge and thank PS3 Labs LLP, Hyderabad, TS, India for providing the NDMA standard.

www.agilent.com/chem

Printed in the USA, December 19, 2019
5994-1626EN
DE.4242476852

Agilent
Trusted Answers