Application Note

Pharma & Biopharma

Determination of NDMA Impurity in Ranitidine Using the Agilent 6470 Triple Quadrupole LC/MS

Detection of regulated genotoxic impurity from the drug manufacturing process

Authors

Chander Mani and
Saikat Banerjee
Agilent Technologies, Inc.

Abstract

Impurities in prescription medicines are of great concern to patients and consumers who rely on safe and effective pharmaceutical compounds approved by the FDA. Ranitidine and nizatidine are both histamine-2 (H2) receptor blockers, that decrease the amount of acid in the stomach, and are used to treat gastritis (inflammation of the stomach lining) and peptic ulcers. For ranitidine, it was found that its drug substance and drug products contained a carcinogenic nitrosamine impurity called N-nitrosodimethylamine (NDMA). As a result, many such drug products were recalled. In addition, nizatidine, being structurally similar to ranitidine, is also prone to the presence of NDMA. Therefore, there is a requirement for analytical methods capable of detecting problematic nitrosamine impurities in such drugs. This application note describes a sensitive, LC/MS/MS method using the Agilent 6470 triple quadrupole LC/MS for the detection and quantification of NDMA in ranitidine drug substance and drug products.

Introduction

The drug manufacturing impurity NDMA (Figure 1) falls under the class of nitrosamine compounds, and can be introduced into finished medicines as trace-level manufacturing by-products. Nitrosamine compounds are classified as probable human carcinogens, and have become a focus for regulatory agencies due to their potential danger. The US FDA recently found NDMA in certain batches of ranitidine products, resulting in widespread recall of these drugs. It was concluded that there was a high potential for ranitidine and its derivatives to contain NDMA. Therefore, there has been a focus on monitoring by regulatory agencies worldwide such as the US FDA, China FDA, and EMA.

Triple quadrupole LC/MS-based methods are very specific and highly sensitive, serving as the base technique for methods developed to detect and quantify NDMA in ranitidine drug substances and drug products. The method described in this application note was carried out on the 6470 triple quadrupole LC/MS, providing a comprehensive analysis of NDMA at very low detection limits.

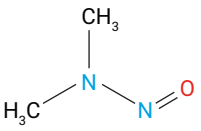

Figure 1. Chemical structure of N-nitrosodimethylamine (NDMA).

Experimental

Chemicals and reagents

The NDMA standard used in this study was locally sourced from PS3 Labs LLP (Hyderabad, TS, India). LC/MS-grade solvents (e.g., methanol, water) were purchased from Honeywell (Charlotte, NC, USA). Analytical-grade formic acid was purchased from Fluka (now of Honeywell).

Sample preparation

Drug substance: A 120 mg amount of drug substance was accurately weighed into a 15 mL centrifuge tube and diluted into 4 mL of water. The solution was mixed using a vortex mixer until completely dissolved.

Drug product: An appropriate number of tablet(s) was crushed to obtain a target concentration of 30 mg/mL of API in water. The powdered drug product was transferred to a 15 mL centrifuge tube, and an appropriate volume of water was added to meet the target concentration. The solution was mixed for approximately one minute using a vortex mixer, then placed on a mechanical shaker for 40 minutes. After extraction, the sample was centrifuged for 15 minutes at 4,500 rpm. The supernatant was collected after filtration using a 0.2 µm nylon syringe filter.

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	80:20, methanol:water
Sample Diluent	Water
Multisampler Temperature	6 ±2 °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	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in 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
Post Time	1 minute
UV Wavelengths	230 nm, 300 nm

Triple quadrupole mass spectrometer configuration and parameters

Table 2. Mass spectrometer configuration and source settings.

Parameter	Value
Instrument	Agilent 6470A triple quadrupole LC/MS
Ion Source	Atmospheric pressure chemical ionization (APCI)
MS/MS Mode	MRM
Ion Mode	Positive
Drying Gas Temperature	300 °C
Drying Gas Flow	5 L/min
Nebulizer Pressure	35 psi
APCI Heater	350 °C
APCI Needle Positive	4 µA
Capillary Voltage, Positive	4,000 V
MS1/MS2 Resolution	0.7/0.7 (unit/unit)
Dwell Time	200 ms

MS/MS compound information for analytes

Table 3. Detailed MRM settings in MRM mode in the Agilent 6470 triple quadrupole LC/MS.

Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Fragmentor (V)	Collision Energy (V)	CAV (V)	Polarity
NDMA (Quantifier)	75.1	43.1	75	18	1	+
NDMA (Qualifier)	75.1	58.1	75	10	1	+

Method development and data analysis

Method development was done by injecting 1 µL of neat solution at a concentration level of 1,000 ng/mL in flow injection mode. MRM transitions were obtained and optimized using the Agilent MassHunter Acquisition Optimizer software to determine optimal precursor and product ions, fragmentor voltages, and collision energies. Data were acquired and analyzed using Agilent MassHunter software version 10.

Results and discussion

The calibration concentrations ranged from 0.1 to 100 ng/mL, with results mentioned in Table 5. R^2 values were greater than 0.9997 for NDMA, displaying good linear response throughout the concentration range. Figure 2 is the representative extracted ion MRM chromatogram from 6470 triple quadrupole LC/MS acquisition, showing elution of NDMA in a 1 ng/mL calibration standard and ranitidine (30 mg/mL). A diverter valve program (Table 4) was used to divert the high concentration of ranitidine to waste.

Table 4. Diverter valve program used to divert ranitidine peak to waste.

#	Start Time (minutes)	Scan Type	Diverter Valve
1	0	MRM	Waste
2	6	MRM	MS
3	8.2	MRM	Waste

Table 5. Result summary of the Agilent 6470 triple quadrupole LC/MS. Data include signal-to-noise ratio (S/N), calculated limit of quantitation (LOQ), limit of detection (LOD), coefficient of regression, and calibration curve fit. NDMA used a 1/x weighted calibration curve.

Agilent 6470 Triple Quadrupole LC/MS						
Compound	LOD (ng/mL)	LOD (S/N)	LOQ (ng/mL)	LOQ (S/N)	R^2	Linearity Range (ng/mL)
NDMA	0.1	20.85	0.25	45.11	0.9997	0.1 to 100

*S/N was calculated using the Auto-RMS algorithm, a noise reference selected as sample, and a noise width of 0.2 minute using MassHunter Quantitative 10 software.

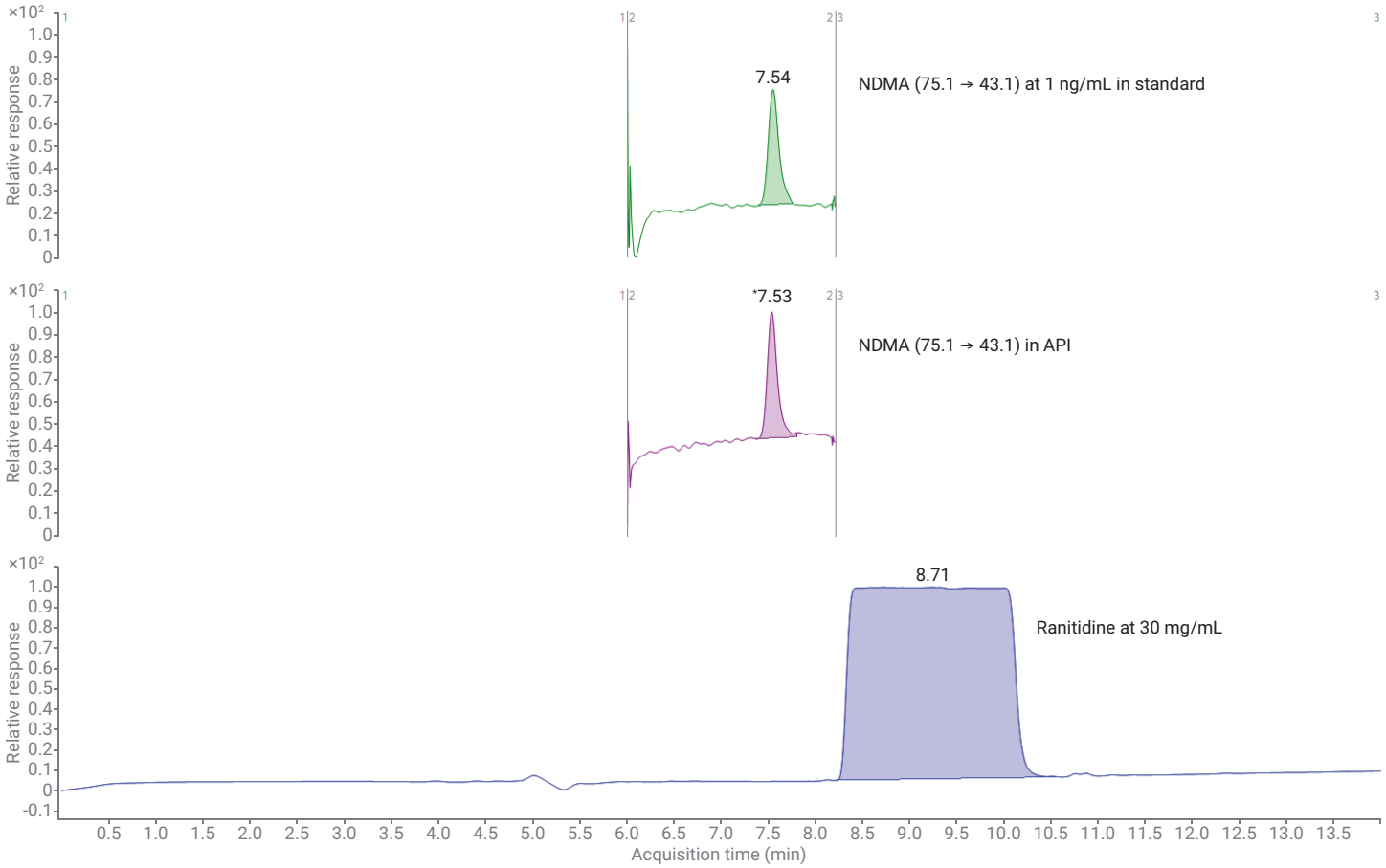

Figure 2. Representative NDMA (1 ng/mL) MRM chromatogram along with ranitidine UV chromatogram showing elution pattern.

Accuracy and reproducibility

The calibration curve for NDMA (Figure 4) demonstrated an accuracy rate within 20% of the expected concentration level at the LOQ. Calibration levels are shown in Table 6B, and reproducibility across all levels exhibited CVs less than 15%.

Table 6A. Representative replicate reproducibility for 1 ng/mL NDMA for ranitidine.

Number	Response for 1 ng/mL
1	17,404
2	17,560
3	17,656
4	17,337
5	17,412
6	17,497
7 (Bracketing)	17,643
8 (Bracketing)	17,488
9 (Bracketing)	17,464
Average	17,495.67
SD	107.93
RSD (%)	**0.62**

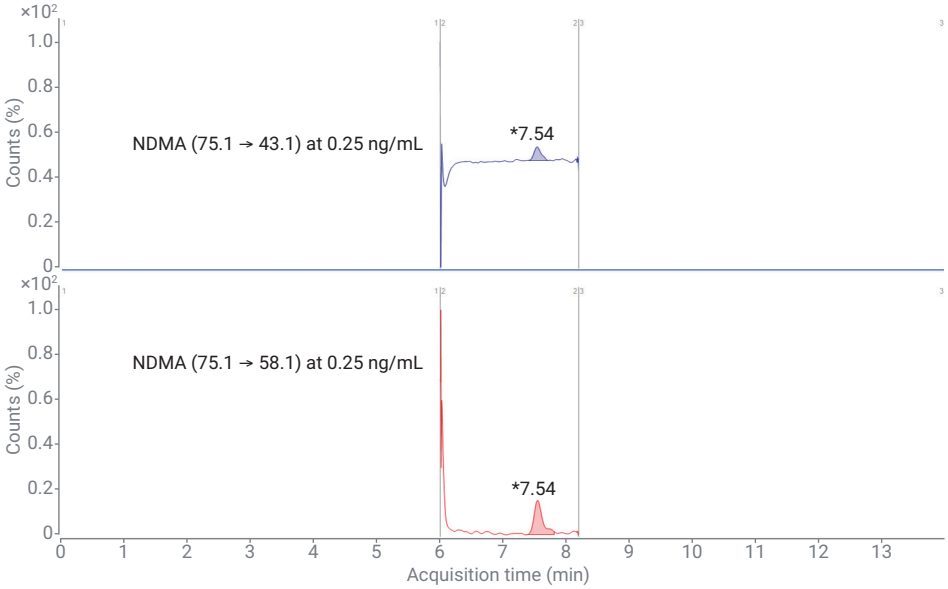

Figure 3. Representative extracted ion MRM chromatogram of NDMA (0.25 ng/mL).

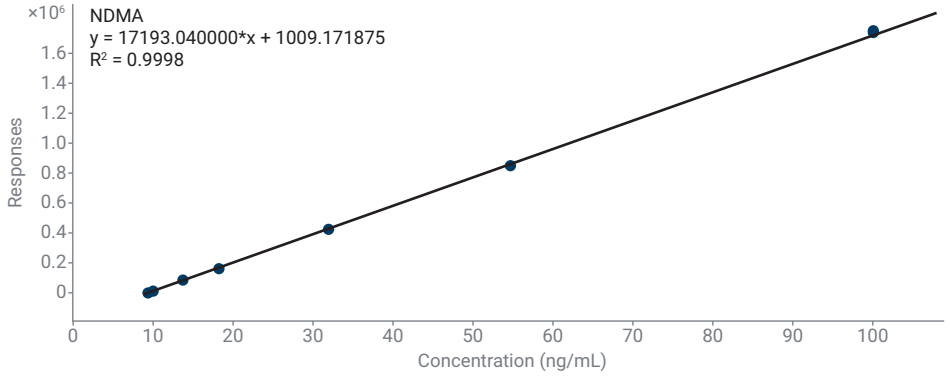

Figure 4. Representative calibration curve for NDMA dispersed throughout the chromatogram. The calibration curve fit used a 1/x weighting factor.

Table 6B. Representative accuracy and reproducibility for different concentration levels of NDMA.

Sample					NDMA M...	NDMA Results							Qualifier (75.1 -> 58.1) Resul...	
Name	Data File	Type	Level	Dil.	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	S/N	Accuracy	Ratio	MI
Blank	Blank-r001.d	Blank		1.0000		7.46	83	☐	0.00	0.00	2.66		0.2	☐
0.1 ppb	0.1 ppb-r01.d	Cal	1	1.0000	0.100	7.55	2840	☐	0.11	0.11	25.58	108.7	12.1	☐
0.1 ppb	0.1 ppb-r02.d	Cal	1	1.0000	0.100	7.54	2850	☐	0.11	0.11	51.45	109.3	12.5	☐
0.1 ppb	0.1 ppb-r03.d	Cal	1	1.0000	0.100	7.54	2762	☐	0.10	0.10	20.85	104.1	12.7	☐
0.15 ppb	0.15 ppb-r01.d	Cal	2	1.0000	0.150	7.55	3873	☐	0.17	0.17	36.42	112.5	12.0	☐
0.15 ppb	0.15 ppb-r02.d	Cal	2	1.0000	0.150	7.56	3885	☐	0.17	0.17	30.03	113.0	12.8	☐
0.15 ppb	0.15 ppb-r03.d	Cal	2	1.0000	0.150	7.54	3807	☐	0.16	0.16	51.22	110.0	11.9	☐
0.25 ppb	0.25 ppb-r001.d	Cal	3	1.0000	0.250	7.54	4913	☐	0.23	0.23	45.11	91.7	15.2	☐
0.25 ppb	0.25 ppb-r002.d	Cal	3	1.0000	0.250	7.54	4967	☐	0.23	0.23	77.74	93.0	14.5	☐
0.5 ppb	0.5 ppb-r001.d	Cal	4	1.0000	0.500	7.54	9215	☐	0.48	0.48	105.09	95.9	15.3	☐
0.5 ppb	0.5 ppb-r002.d	Cal	4	1.0000	0.500	7.54	9175	☐	0.48	0.48	70.79	95.4	15.5	☐
1 ppb	1 ppb-r001.d	Cal	5	1.0000	1.000	7.54	17404	☐	0.96	0.96	247.40	95.6	15.7	☐
1 ppb	1 ppb-r002.d	Cal	5	1.0000	1.000	7.54	17560	☐	0.96	0.96	151.33	96.5	16.4	☐
1 ppb	1 ppb-r003.d	Cal	5	1.0000	1.000	7.54	17656	☐	0.97	0.97	183.09	97.0	15.2	☐
1 ppb	1 ppb-r004.d	Cal	5	1.0000	1.000	7.54	17337	☐	0.95	0.95	157.95	95.2	15.3	☐
1 ppb	1 ppb-r005.d	Cal	5	1.0000	1.000	7.54	17412	☐	0.96	0.96	195.77	95.6	15.2	☐
1 ppb	1 ppb-r006.d	Cal	5	1.0000	1.000	7.54	17497	☐	0.96	0.96	156.49	96.1	15.0	☐
5 ppb	5 ppb-r001.d	Cal	6	1.0000	5.000	7.54	84350	☐	4.85	4.85	916.65	97.0	15.7	☐
5 ppb	5 ppb-r002.d	Cal	6	1.0000	5.000	7.54	86047	☐	4.95	4.95	767.70	99.0	15.2	☐
10 ppb	10 ppb-r001.d	Cal	7	1.0000	10.000	7.54	170350	☐	9.85	9.85	1559.19	98.5	15.7	☐
10 ppb	10 ppb-r002.d	Cal	7	1.0000	10.000	7.54	169259	☐	9.79	9.79	1959.80	97.9	15.7	☐
25 ppb	25 ppb-r001.d	Cal	8	1.0000	25.000	7.54	426448	☐	24.75	24.75	3071.92	99.0	16.0	☐
25 ppb	25 ppb-r002.d	Cal	8	1.0000	25.000	7.54	425667	☐	24.70	24.70	4586.77	98.8	15.9	☐
50 ppb	50 ppb-r001.d	Cal	9	1.0000	50.000	7.54	849615	☐	49.36	49.36	11449.61	98.7	16.3	☐
50 ppb	50 ppb-r002.d	Cal	9	1.0000	50.000	7.54	852273	☐	49.52	49.52	9389.86	99.0	16.3	☐
100 ppb	100 ppb-r001.d	Cal	10	1.0000	100.000	7.54	1732267	☐	100.70	100.70	17270.41	100.7	16.4	☐
100 ppb	100 ppb-r002.d	Cal	10	1.0000	100.000	7.54	1750803	☐	101.78	101.78	26786.79	101.8	16.5	☐
Blank	Blank0001.d	Blank		1.0000		7.38	58	☐	0.00	0.00	1.77		3.3	☐
API 1	API 1-r001.d	Sample		0.0333		7.53	42993	☐	2.44	0.08	271.83		14.6	☐
API 1	API 1-r002.d	Sample		0.0333		7.52	43152	☐	2.45	0.08	242.10		13.7	☐
API 2	API 2-r001.d	Sample		0.0333		7.52	77665	☐	4.46	0.15	573.65		14.5	☐
API 2	API 2-r002.d	Sample		0.0333		7.52	77600	☐	4.46	0.15	562.52		14.8	☐
API 3	API 3-r001.d	Sample		0.0333		7.53	78169	☐	4.49	0.15	480.63		14.9	☐
API 3	API 3-r002.d	Sample		0.0333		7.52	78036	☐	4.48	0.15	602.97		14.6	☐
API 4	API 4-r001.d	Sample		0.0333		7.53	45485	☐	2.59	0.09	305.13		14.6	☐
API 4	API 4-r002.d	Sample		0.0333		7.52	45776	☐	2.61	0.09	298.66		15.1	☐
Tablet 1	Tablet 1-r001.d	Sample		0.0333		7.53	418616	☐	24.29	0.81	2490.74		16.0	☐
Tablet 1	Tablet 1-r002.d	Sample		0.0333		7.52	412148	☐	23.92	0.80	1636.55		16.0	☐
Tablet 2	Tablet 2-r001.d	Sample		0.0333		7.52	935299	☐	54.35	1.81	2625.31		16.2	☐
Tablet 2	Tablet 2-r002.d	Sample		0.0333		7.52	968082	☐	56.25	1.87	3133.06		16.2	☐
1 ppb	1 ppb-r008.d	QC	5	1.0000	1.000	7.54	17643	☐	0.97	0.97	155.79	97.0	14.4	☐
1 ppb	1 ppb-r009.d	QC	5	1.0000	1.000	7.54	17488	☐	0.96	0.96	215.70	96.1	17.2	☐
1 ppb	1 ppb-r010.d	QC	5	1.0000	1.000	7.54	17464	☐	0.96	0.96	228.05	95.9	15.1	☐

Table 7A. Representative recovery data for different concentration levels.

Sample					NDMA M...	NDMA Results							Qualifier (75.1 -> 58.1) Resul...	
Name	Data File	Type	Level	Dil.	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	S/N	Accuracy	Ratio	MI
Blank	Blank0001.d	Blank		1.0000		7.38	58	☐	0.00	0.00	1.77		3.3	☐
API 1	API 1-r001.d	Sample		0.0333		7.53	42993	☐	2.44	0.08	271.83		14.6	☐
API 1	API 1-r002.d	Sample		0.0333		7.52	43152	☐	2.45	0.08	242.10		13.7	☐
Tablet 1	Tablet 1-r001.d	Sample		0.0333		7.53	418616	☐	24.29	0.81	2490.74		16.0	☐
Tablet 1	Tablet 1-r002.d	Sample		0.0333		7.52	412148	☐	23.92	0.80	1636.55		16.0	☐
1 ppb	1 ppb-r008.d	QC	5	1.0000	1.000	7.54	17643	☐	0.97	0.97	155.79	97.0	14.4	☐
1 ppb	1 ppb-r009.d	QC	5	1.0000	1.000	7.54	17488	☐	0.96	0.96	215.70	96.1	17.2	☐
1 ppb	1 ppb-r010.d	QC	5	1.0000	1.000	7.54	17464	☐	0.96	0.96	228.05	95.9	15.1	☐
Spike Standard 1.2 ppb	Spike Standard 1.2 ppb-r001.d	Sample		1.0000		7.54	19878	☐	1.10	1.10	172.32		15.8	☐
Spike Standard 1.2 ppb	Spike Standard 1.2 ppb-r002.d	Sample		1.0000		7.54	20055	☐	1.11	1.11	153.59		14.6	☐
Spike Standard 3 ppb	Spike Standard 3 ppb-r001.d	Sample		1.0000		7.54	47768	☐	2.72	2.72	375.00		15.6	☐
Spike Standard 3 ppb	Spike Standard 3 ppb-r002.d	Sample		1.0000		7.54	48768	☐	2.78	2.78	694.55		15.5	☐
Spike Standard 6 ppb	Spike Standard 6 ppb-r001.d	Sample		1.0000		7.54	91959	☐	5.29	5.29	734.28		15.8	☐
Spike Standard 6 ppb	Spike Standard 6 ppb-r002.d	Sample		1.0000		7.54	91015	☐	5.24	5.24	572.61		15.8	☐
Spike Standard 24 ppb	Spike Standard 24 ppb-r001.d	Sample		1.0000		7.54	389055	☐	22.57	22.57	2923.45		15.9	☐
Spike Standard 24 ppb	Spike Standard 24 ppb-r002.d	Sample		1.0000		7.54	389143	☐	22.58	22.58	5110.55		16.1	☐
Spike Standard 48 ppb	Spike Standard 48 ppb-r001.d	Sample		1.0000		7.54	776451	☐	45.11	45.11	6201.45		16.1	☐
Spike Standard 48 ppb	Spike Standard 48 ppb-r002.d	Sample		1.0000		7.53	773383	☐	44.93	44.93	4509.49		16.2	☐
Blank	Blank-r00001.d	Blank		1.0000		7.30	41	☐	0.00	0.00	1.76		2.7	☐
API 1_Spike 1.2 ppb	API 1_Spike 1.2 ppb-r001.d	Sample		0.0333		7.53	59441	☐	3.40	0.11	293.24		14.8	☐
API 1_Spike 1.2 ppb	API 1_Spike 1.2 ppb-r002.d	Sample		0.0333		7.52	58789	☐	3.36	0.11	487.66		15.4	☐
API 1_Spike 1.2 ppb	API 1_Spike 1.2 ppb-r003.d	Sample		0.0333		7.52	60290	☐	3.45	0.11	278.26		14.7	☐
API 1_Spike 3 ppb	API 1_Spike 3 ppb-r001.d	Sample		0.0333		7.53	89357	☐	5.14	0.17	586.84		14.4	☐
API 1_Spike 3 ppb	API 1_Spike 3 ppb-r002.d	Sample		0.0333		7.53	84080	☐	4.83	0.16	595.11		14.6	☐
API 1_Spike 3 ppb	API 1_Spike 3 ppb-r003.d	Sample		0.0333		7.52	87804	☐	5.05	0.17	445.13		13.8	☐
API 1_Spike 6 ppb	API 1_Spike 6 ppb-r001.d	Sample		0.0333		7.53	122493	☐	7.07	0.24	800.46		14.7	☐
API 1_Spike 6 ppb	API 1_Spike 6 ppb-r002.d	Sample		0.0333		7.52	123129	☐	7.11	0.24	721.82		14.6	☐
API 1_Spike 6 ppb	API 1_Spike 6 ppb-r003.d	Sample		0.0333		7.52	118258	☐	6.82	0.23	827.85		15.3	☐
Tablet1_Spike 24 ppb	Tablet1_Spike 24 ppb-r001.d	Sample		0.0333		7.52	785466	☐	45.63	1.52	3496.02		15.0	☐
Tablet1_Spike 24 ppb	Tablet1_Spike 24 ppb-r002.d	Sample		0.0333		7.52	785694	☐	45.64	1.52	4132.82		15.3	☐
Tablet1_Spike 24 ppb	Tablet1_Spike 24 ppb-r003.d	Sample		0.0333		7.52	779892	☐	45.31	1.51	4947.35		14.9	☐
Tablet1_Spike 48 ppb	Tablet1_Spike 48 ppb-r001.d	Sample		0.0333		7.52	1093565	☐	63.55	2.12	4758.37		15.8	☐
Tablet1_Spike 48 ppb	Tablet1_Spike 48 ppb-r002.d	Sample		0.0333		7.52	1102529	☐	64.07	2.13	4654.77		15.7	☐
Tablet1_Spike 48 ppb	Tablet1_Spike 48 ppb-r003.d	Sample		0.0333		7.52	1102972	☐	64.10	2.13	8666.73		15.8	☐

Table 7B. Summary of experiment recovery in ranitidine drug substance.

Nitrosamine Impurity	Spiked Concentration (ng/mL) Mixed with Ranitidine API (30 mg/mL)	Recovery %
NDMA	1.2	86.4
	3	93.3
	6	86.5

Table 7C. Summary of recovery experiment in ranitidine drug product in tablet form.

Nitrosamine Impurity	Spiked Concentration (ng/mL) in 30 mg/mL of Ranitidine Tablet	Recovery %
NDMA	24	94.9
	48	87.4

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

Conclusion

The Agilent 6470 triple quadrupole LC/MS can analyze nitrosamine impurities at the low concentration levels demanded of regulatory requirements. This application note demonstrates the sensitivity of the 6470 triple quadrupole LC/MS for the detection of the NDMA nitrosamine impurity in ranitidine drug substance and drug products. As ranitidine is chromatographically well separated (even at a very high concentration level of 30 mg/mL), a diverter valve program can be used to exclude the API. As a result, this LC/MS method is highly sensitive and very reproducible in nature, because ranitidine does not enter the mass spectrometer.

References

1. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-angiotensin-ii-receptor-blocker-arb-recalls-valsartan-losartan
2. FDA guidance document: Development and Validation of a RapidFire-MS/MS Method for Screening of Nitrosamine Impurities.
3. FDA guidance document: Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs.
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
6. Determination of Nitrosamine Impurities Using the Ultivo Triple Quadrupole LC/MS. *Agilent Technologies application note*, publication number 5994-1383EN, **2019**.
7. https://www.registrarcorp.com/fda-drugs/definitions/

Definitions

Drug Substance: "Active Pharmaceutical Ingredient." Any component of a drug product intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of humans or other animals. Active ingredients include those components of the product that may undergo chemical change during the manufacture of the drug product and be present in the drug product in a modified form intended to furnish the specified activity or effect.

Drug Product: A finished dosage form, for example, a tablet, capsule or solution that contains an active pharmaceutical ingredient, generally, but not necessarily, in association with inactive ingredients. Reference: Manufacturing, Processing, or Holding Active Pharmaceutical Ingredients FDA Guidance

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