Application Note

Pharmaceuticals

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Determination of Nitrosamine Impurities Using the Ultivo Triple Quadrupole LC/MS

Detection of regulated genotoxic impurities from the drug manufacturing process

Authors

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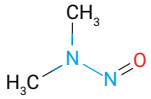
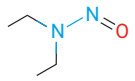
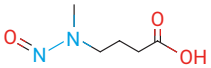
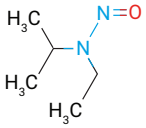
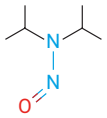
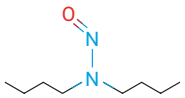
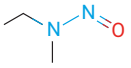
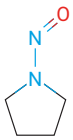
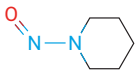
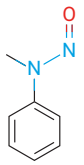
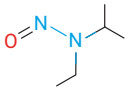
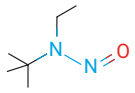
Abstract

Recent findings show that some Angiotensin II receptor blocker (ARB) drug products contain carcinogenic nitrosamine impurities.[1] As a result, many of these products were recalled, demonstrating a need for analytical methods capable of detecting problematic nitrosamine impurities above target concentration levels. This Application Note describes how the sensitive Agilent Ultivo LC/TQ based method works, not only for the six nitrosamines listed by the U.S. Food and Drug Administration (FDA), but also for the simultaneous detection of six other potentially mutagenic nitrosamine impurities.

Introduction

The announcement for the recall of ARB medicines Valsartan, Losartan, and Irbesartan made N-Nitroso impurities a focus for regulatory agencies including the FDA and the European Medicines Agency (EMA). Nitrosamine impurities are byproducts produced in trace amounts during the manufacturing processes of these medicines. These impurities are classified as probable carcinogens (that is, potentially genotoxic impurities). The LC/MS-based method described in this Application Note was performed on an Ultivo triple quadrupole LC/MS. It presents a comprehensive analysis of 12 nitrosamine compounds at very low detection limits. All nitrosamine impurities are of small molecular weight, ranging from 74 to 158. The compounds included:

- N-nitrosodimethylamine (NDMA)
- N-nitrosodiethylamine (NDEA)
- N-nitroso-4-methyl-4-aminobutyric acid (NMBA)
- N-nitrosoethylisopropylamine (NEIPA)
- N-nitrosodiisopropylamine (NDIPA)
- N-nitrosodibutylamine (NDBA)
- N-nitrosoethylmethylamine (NMEA)
- N-nitrosopyrrolidine (NPyR)
- N-nitrosopiperidine (NPIP)
- N-methyl-N-nitrosoaniline (NMPhA)
- N-isopropylmethyl nitrosamine (NMIPA)
- N-*tert*-butyl-N-ethylnitrosamine

Nitrosamine Compound	Chemical Structure
NDMA	
NDEA	
NMBA	
NEIPA	
NDIPA	
NDBA	
NMEA	
NPyR	
NPIP	
NMPhA	
NMIPA	
N-*tert*-butyl-N-ethylnitrosamine	

Experimental

Chemicals and reagents

All 12 nitrosamine standards used for the study were arranged from PS3 Labs LLP, Hyderabad, TS, India. LC/MS grade solvents such as methanol and water were purchased from Honeywell. The formic acid used for the analysis was purchased from Fluka.

Data analysis

Data were acquired and analyzed using Agilent MassHunter software version 1.1 for Ultivo. MS/MS transitions were obtained and optimized using the Agilent MassHunter Acquisition Optimizer software to determine optimal precursor and product ions, fragmentor voltages, and collision energies upon injection of a neat solution (of each individual compound at a concentration level of 1,000 ng/mL, 1 µL injection volume in flow injection mode).

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)
Needle Wash	Methanol: Water/ 80:20
Sample Diluent	Water
Multisampler Temperature	6 °C
Injection Volume	40 µL
Analytical Column	Agilent InfinityLab Poroshell HPH C18, 2.1 × 100 mm 1.9 µm (p/n 695675-702)
Column Temperature	40 °C
Mobile Phase A	0.2 % formic acid in water
Mobile Phase B	Methanol
Flow Rate	0.4 mL/min
Gradient	Time (min) %B 0 1 2 13 5.5 80 8 95 10 95
Stop Time	10 minutes
Post Time	3 minutes

Triple quadrupole mass spectrometer configuration and parameters

Table 2. MS configuration and source settings.

Parameter	Value
Instrument	Agilent Ultivo triple quadrupole mass spectrometer
Ion Source	Atmospheric pressure chemical ionization (APCI)
MS/MS Mode	Dynamic MRM (dMRM)
Ion Mode	Positive
Drying Gas Temperature	300 °C
Drying Gas Flow	6 L/min
Nebulizer Pressure	55 psi
APCI Heater	350 °C
APCI Needle Positive	4 µA
Capillary Voltage, Positive	3,000 V
MS1/MS2 Resolution	0.7/0.7 (unit/unit)
Dwell Time	Variable

MS/MS compound information for analytes

Table 3. Detailed MRM settings in dynamic MRM mode in Ultivo.

Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Retention Time (min)	Retention Time Window (Min)	Fragmentor (V)	Collision Energy (V)	Polarity
NDEA	103.1	75.1	3.484	1.5	85	8	+
NDEA	103.1	47.1	3.484	1.5	85	16	+
NDMA	75.1	58	1.143	1.24	65	10	+
NDMA	75.1	43.1	1.143	1.24	65	17	+
NMBA	147.1	44.2	2.247	1.2	50	7	+
NMBA	147.1	87.2	2.247	1.2	50	7	+
NEIPA	117.1	75.1	4.325	1.0	70	7	+
NEIPA	117.1	47.1	4.325	1.0	70	15	+
NDIPA	131.1	89.1	4.916	1.0	50	5	+
NDIPA	131.1	43.1	4.916	1.0	50	7	+
NDBA	159.1	57.2	6.096	1.0	70	7	+
NDBA	159.1	41.1	6.096	1.0	70	24	+
NMEA	89.1	61.1	2.109	1.37	75	10	+
NMEA	89.1	43.1	2.109	1.37	75	10	+
NPyR	101.1	55.1	2.248	1.43	55	18	+
NPyR	101.1	41	2.248	1.43	55	26	+
NPIP	115.1	69.1	3.809	1.0	85	9	+
NPIP	115.1	41.2	3.809	1.0	85	26	+
NMPhA	137	66.1	5.029	1.32	45	18	+
NMPhA	137	107	5.029	1.32	45	10	+
NMIPA	103.1	61	3.358	1.0	60	8	+
NMIPA	103.1	43	3.358	1.0	60	8	+
N-*tert*-butyl-N-ethylnitrosamine	131	75.1	4.897	1.0	40	4	+
N-*tert*-butyl-N-ethylnitrosamine	131	57.1	4.897	1.0	40	6	+

Results and discussion

The calibration concentrations ranged from 0.05 to 100 ng/mL with specific details mentioned in Table 4. R^2 values were greater than 0.996 for all the analytes, displaying linear responses throughout the concentration range. Figure 1 shows the representative overlaid dMRM chromatogram from Ultivo TQ showing elution of all 12 nitrosamine compounds at 10 ng/mL.

Table 4. Result summary of the Agilent Ultivo TQ. Data include signal-to-noise (S/N), calculated LOQ, coefficient of regression, and calibration curve fit. All standards used 1/x weighted calibration curve.

Compound	Agilent Ultivo TQ					
	LOD (ng/mL)	LOD (S/N)	LOQ (ng/mL)	LOQ (S/N)	R^2	Linearity Range (ng/mL)
NDMA	0.05	9.74	0.1	13.49	0.997	0.05 to 100
NDEA	0.025	22.68	0.05	53.82	0.998	0.025 to 100
NMBA	0.05	23.88	0.1	31.18	0.998	0.05 to 100
NEIPA	0.025	57.37	0.05	69.67	0.999	0.025 to 100
NDIPA	0.025	15.72	0.05	45.18	0.998	0.025 to 100
NDBA	0.05	225.18	0.1	323.11	0.999	0.025 to 100
NMEA	0.075	7.84	0.1	12.10	0.999	0.1 to 100
NPyR	0.075	30.02	0.1	41.72	0.999	0.1 to 100
NPIP	0.1	11.60	0.15	14.11	0.998	0.15 to 100
NMPhA	0.075	29.58	0.1	45.34	0.994	0.075 to 100
NMIPA	0.025	42.17	0.05	208.86	0.996	0.025 to 100
N-*tert*-butyl-N-ethylnitrosamine	0.075	54.92	0.1	81.62	0.999	0.075 to 100

* S/N was calculated using the Auto-RMS algorithm, noise reference selected as sample using Agilent MassHunter Quantitaive 10 software.

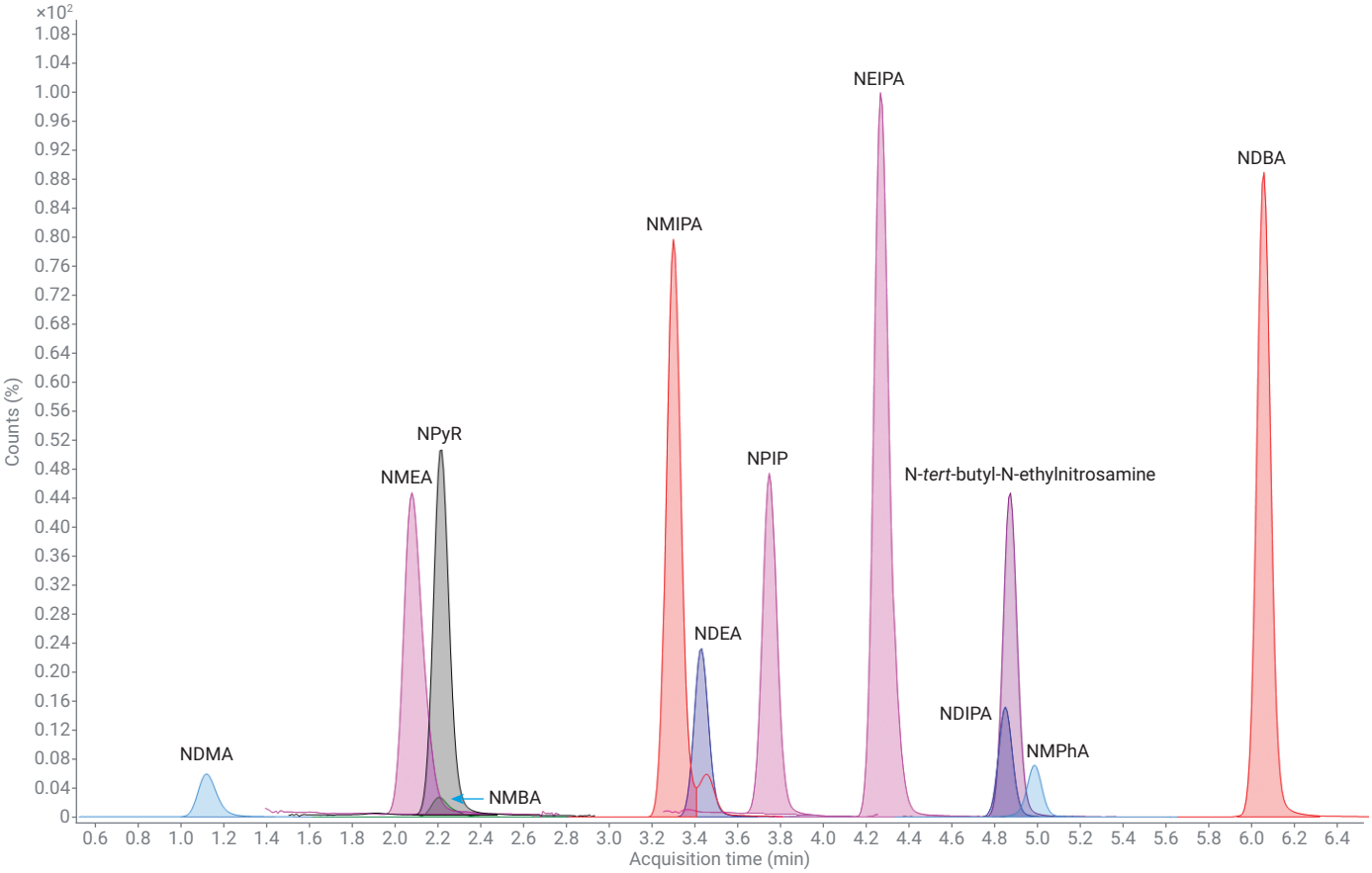


Figure 1. Overlaid dMRM chromatogram, showing elution of 12 nitrosamine compounds.

Accuracy and reproducibility

Calibration curves for each of the 12 compounds demonstrated an accuracy rate within 20% of the expected concentration limit at the limit of quantitation (LOQ). Detection limits, quantitation limits, and calculated statistics are shown for the measured linear calibration range provided in Table 4, demonstrating reproducibility across all calibration points with CV <15%. Table 5 shows a detailed summary of accuracies and reproducibility of results at different concentration levels. Figure 2 shows the representative calibration curves generated from the Ultivo LC/TQ system.

Table 5. Accuracy and reproducibility for different concentration levels in Agilent Ultivo LC/TQ (n = 3).

Concentration (ng/mL)	NDMA		NDEA		NMBA		NEIPA		NDIPA		NDBA	
	Average	CV	Average	CV	Average	CV	Average	CV	Average	CV	Average	CV
0.1	95.53	5.63	96.23	5.78	84.73	0.00	96.37	5.82	107.9	9.01	99.63	6.47
0.25	102.47	2.79	98.53	4.77	95.53	4.31	101.43	5.16	101.6	3.59	105.53	3.60
0.5	100.00	2.68	98.53	2.15	105.67	1.15	95.30	3.15	95.30	1.06	95.77	2.07
1.0	104.27	4.70	99.47	1.22	105.67	1.80	98.47	4.59	97.63	3.88	95.77	2.39

Concentration (ng/mL)	NMEA		NPyR		NPIP*		NMPhA		NMIPA		N-*tert*-butyl-N-ethyl nitrosamine	
	Average	CV	Average	CV	Average	CV	Average	CV	Average	CV	Average	CV
0.1	110.97	7.27	97.7	6.56	112.27	4.52	100.6	8.81	105.23	2.58	105.27	4.56
0.25	105.1	5.64	103.63	6.66	101.43	4.79	106.43	6.59	107.5	7.97	106.47	5.27
0.5	91.87	6.20	96.20	3.19	93.07	5.83	84.40	3.35	90.50	5.61	96.77	0.26
1.0	93.80	5.39	99.97	2.36	102.67	0.79	108.03	2.25	100.23	2.94	94.83	1.10

* For NPIP, the first concentration is 0.15 ng/mL, not 0.1 ng/mL.

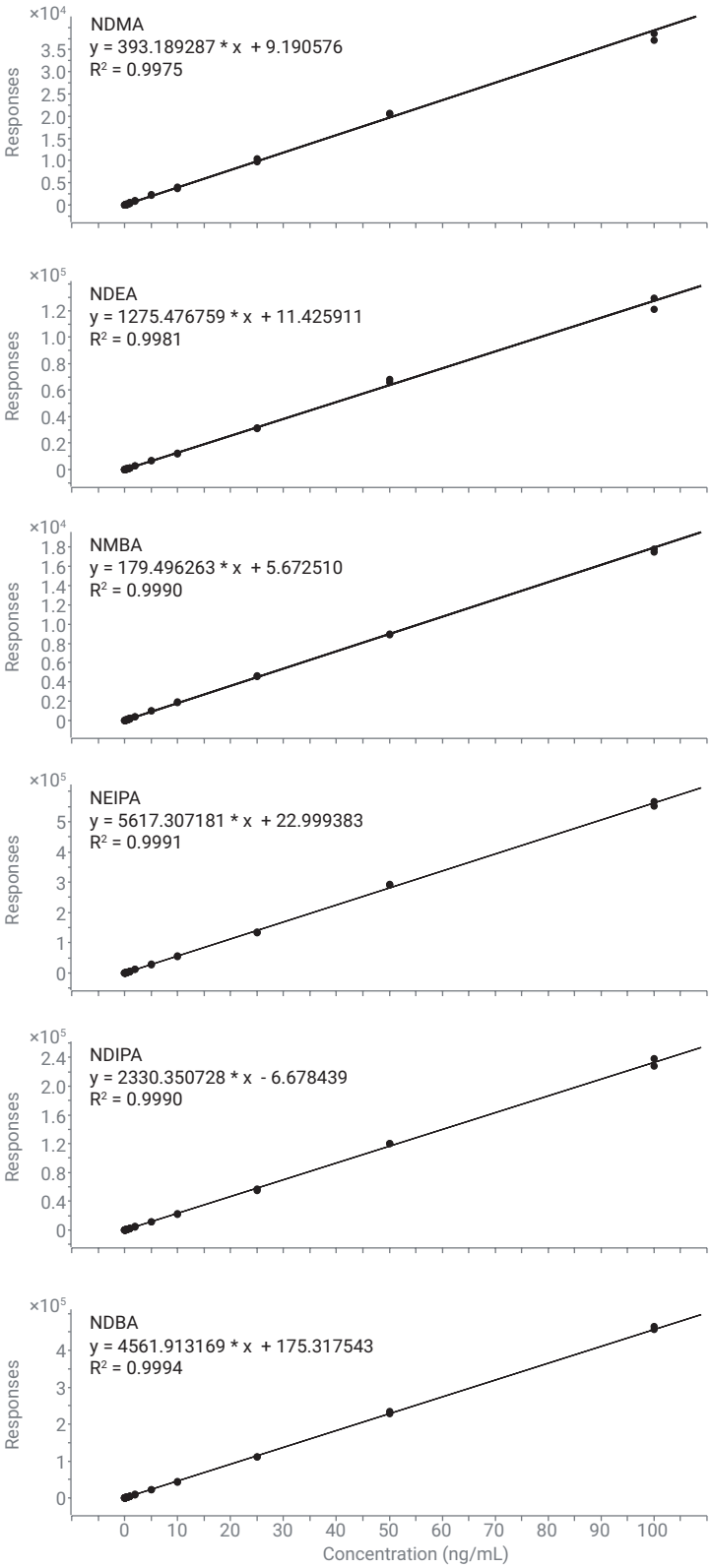


Figure 2. Representative calibration curves from the Agilent Ultivo LC/TQ for compounds observed throughout the chromatogram. All calibration curves used a 1/x weighting factor.

Conclusion

The Agilent Ultivo triple quadrupole LC/MS can analyze nitrosamine impurities at the low concentration levels demanded by regulatory requirements. This Application Note demonstrates the sensitivity of the Ultivo LC/TQ in the detection of certain nitrosamine impurities at low concentration levels. This method can be used in the quantitation of these impurities in different ARB drug products with some alterations in chromatographic conditions, according to the elution pattern of the drug product.

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.

Acknowledgement

We sincerely acknowledge and thank PS3 Labs LLP, Hyderabad, TS, India for providing us nitrosamine standards.

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Printed in the USA, November 8, 2019
5994-1383EN

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