Application Note

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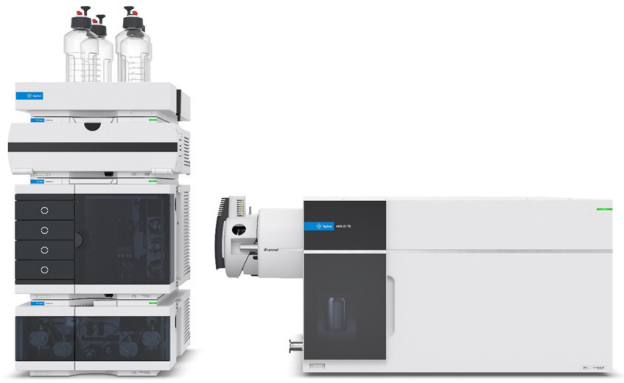
Determination of Nitrosamine Impurities Using the Agilent 6495D Triple Quadrupole LC/MS System



Authors

Lee Bertram and Linfeng Wu
Agilent Technologies, Inc.

Abstract

Nitrosamine impurities are byproducts produced in trace amounts during the manufacture of pharmaceutical drugs. These impurities are classified as potentially genotoxic, and as probable carcinogens with long-term intake. Therefore, it is important to determine their levels in final drug products with a high level of sensitivity and confidence.

This application note describes the evaluated quantification performance of eight nitrosamine impurities using an Agilent 6495D triple quadrupole LC/MS (6495D LC/TQ) system coupled with an Agilent 1290 Infinity II bio LC system and Agilent APCI source.

Introduction

The recall of several pharmaceutical drugs, including Angiotensin II receptor blocker (ARB) drug products, made nitrosamine impurities a focus for regulatory agencies.[1] Nitrosamine impurities are byproducts that arise during the manufacturing of pharmaceutical drugs. They are a member of the "cohort of concern," classifying them as potentially genotoxic impurities and probable carcinogens with long-term intake.

In this application note, a comprehensive analysis of eight nitrosamine compounds was carried out on the Agilent 6495D triple quadrupole LC/MS system coupled with the Agilent 1290 Infinity II LC system and Agilent APCI source. It demonstrated that the following compounds can be determined at very low levels:

- N-Nitrosodimethylamine (NDMA)
- N-Nitrosomorpholine (NMOR)
- N-Nitrosomethylethylamine (NMEA)
- N-Nitrosopyrrolidine (NPYR)
- N-Nitrosodiethylamine (NDEA)
- N-Nitrosopiperidine (NPIP)
- N-Nitrosodi-n-propylamine (NDPA)
- N-Nitrosodi-n-butylamine (NDBA)

Experimental

Chemical and standards

All reagents and solvents used for the analysis were LC/MS grade. Ultrapure water was produced with a Milli-Q Integral system equipped with a LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Standards containing eight nitrosamines NDMA, NDEA, NMOR, NMEA, NPYR, NPIP, NDPA, and NDBA were purchased from Agilent (part number US-113N-1).

Sample preparation

Nitrosamine standards were spiked into solvent blank (methanol:water 10:90) at nine different concentration levels ranging from 0.0125 to 10 ng/mL. Preparation of the active pharmaceutical ingredient (API) matrix was carried out following these steps: 100 mg losartan potassium drug product was dissolved in 2 mL of solvent (methanol: water 50:50), followed by sonication for 30 minutes. The samples were then centrifuged at 12,000 rpm for 10 minutes. Supernatants were collected and then 1:5 diluted with water. Nitrosamine standards were spiked into the prepared API matrix at concentrations ranging from 0.005 to 20 ng/mL.

Equipment

Sample separation was performed using the 1290 Infinity II bio LC system, consisting of the following modules:

- Agilent 1290 Infinity II bio high-speed pump (G7132A)
- Agilent 1290 Infinity II bio multisampler with thermostat (G7137A)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)

The liquid chromatography system was coupled to the Agilent 6495D triple quadrupole LC/MS (G6495D) mass spectrometer equipped with the APCI source (G1947B). Agilent MassHunter Workstation software version 12.1 was used for data acquisition.

The testing in this application note can be carried out using either a 1290 Infinity II bio LC system or a 1290 Infinity II LC system.

Methods

The LC/MS conditions and parameters are provided in Tables 1 and 2. The MRM settings for the compounds are listed in Table 3. Using the MassHunter 12.1 MRM-specific iFunnel mode feature, it was determined that the Fragile mode setting allowed for the highest abundance for all compounds in this study. For calibration curve analysis, linear fitting with origin ignored and 1/x weighting was used.

Liquid chromatography

Table 1. Agilent 1290 Infinity II bio LC method.

Agilent 1290 Infinity II Bio LC System	
Column	Agilent InfinityLab Poroshell 120 column EC-C18, 3.0 × 150 mm, 2.7 µm (p/n 693975-302)
Sampler Temperature	4 °C
Mobile Phase A	ddH_2O + 0.1% formic acid
Mobile Phase B	MeOH + 0.1% formic acid
Flow Rate	0.5 mL/min
Injection Volume	20 µL
Column Temperature	40 °C
Gradient Program	Time (min) %B 0.0 5 3.5 5 7.0 45 9.0 60 11.0 60 15.0 65 16.0 90 16.1 5 20.0 5

Mass spectrometry

Table 2. LC/TQ parameters.

Agilent 6495D Triple Quadrupole LC/MS System	
Ion Source	Agilent APCI source
Polarity	Positive
Gas Temperature	290 °C
Drying Gas Flow	11 L/min
Nebulizer	25 psi
APCI Vaporizer Temperature	350 °C
Capillary Voltage	1,000 V
Corona Current	4.0 µA
Scan Type	MRM with time segments
Detector Gain Factor (+)	10
LC Diverter to Waste	0 to 2 min; 12 to 13 min; 14 to 18 min (the remaining time diverted to MS)

Compound information and MRM settings

Table 3. Detailed MRM settings and compound information for the Agilent 6495D triple quadrupole LC/MS.

Compound Name	Precursor *m/z*	Product *m/z*	Dwell (ms)	iFunnel Mode	CAV (V)	CE (V)	Polarity	Measured Retention Time (min)
NDMA	75	43	50	Fragile	3	16	+	2.51
NDMA	75	58	50	Fragile	3	10	+	2.51
NMOR	117	45	50	Fragile	4	21	+	3.99
NMOR	117	87	50	Fragile	4	11	+	3.99
NMEA	89	43	50	Fragile	3	12	+	5.35
NMEA	89	61	50	Fragile	3	10	+	5.35
NPYR	101	41	50	Fragile	3	24	+	5.70
NPYR	101	55	50	Fragile	3	19	+	5.70
NDEA	103	47	50	Fragile	4	20	+	7.48
NDEA	103	75	50	Fragile	4	12	+	7.48
NPIP	115	41	50	Fragile	3	24	+	7.90
NPIP	115	69	50	Fragile	3	12	+	7.90
NDPA	131	43	50	Fragile	4	10	+	10.10
NDPA	131	89	50	Fragile	4	16	+	10.10
NDBA	159	41	50	Fragile	3	20	+	13.50
NDBA	159	57	50	Fragile	3	12	+	13.50

Results and discussion

Calibration curve analysis

To evaluate the quantification performance of nitrosamines, the calibration curves of the eight nitrosamine compounds were analyzed with concentrations ranging from 0.005 to 20 ng/mL in solvent. The analysis results were as follows:

Excellent chromatographic separation and peak shapes for all analytes were achieved, as shown in Figure 1. Excellent quantitation linearity for the tested levels with R^2 >0.99 for all eight analytes (Figure 2) was achieved. Outstanding precision and accuracy were observed at all tested levels, including the lower limit of quantitation (LLOQ) levels (Table 4). High analytical sensitivity, with an LLOQ ≤0.01 ng/mL (10 ppt) for all the targeted analytes (Table 4) was observed.

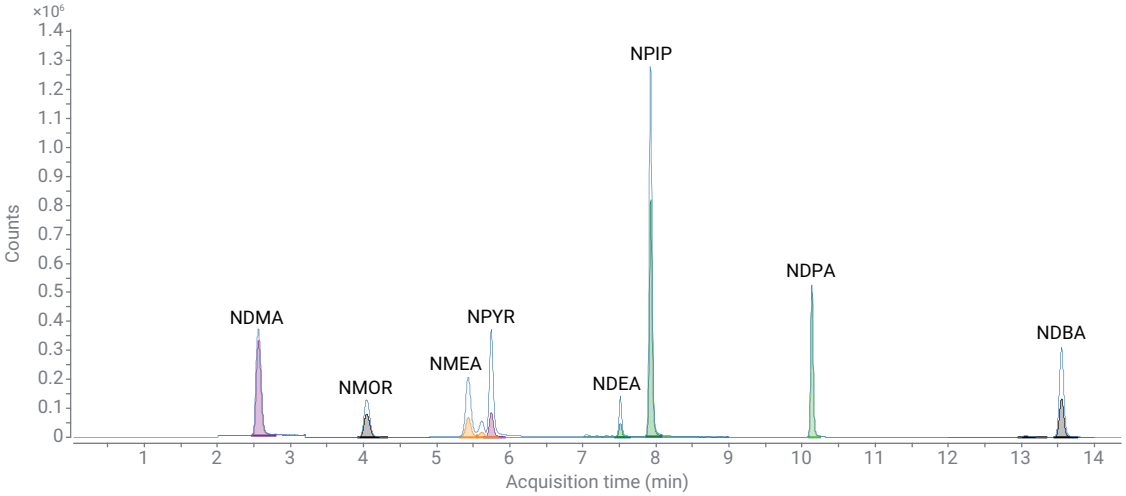


Figure 1. Total MRM chromatograms of the eight nitrosamine compounds at 0.6 ng/mL.

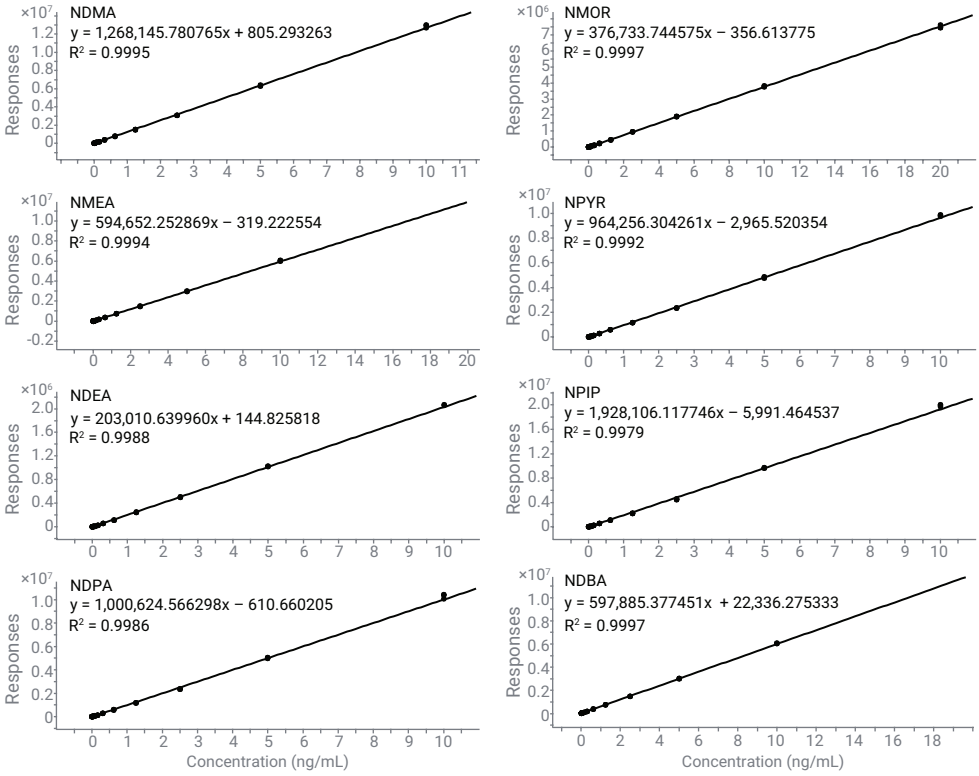


Figure 2. Calibration curves on the Agilent 6495D triple quadrupole LC/MS for the eight nitrosamine compounds.

Table 4. Average response, signal-to-noise ratio (S/N) of the quantifier ion, %RSD, and percent accuracy at 0.01 and 0.04 ng/mL using the Agilent 6495D triple quadrupole LC/MS (n = 3).

	Concentration								
	0.01 ng/mL (n = 3)				0.04 ng/mL (n = 3)				
Name	Response	S/N	RSD (%)	Accuracy (%)	Response	S/N	RSD (%)	Accuracy (%)	LLOQ (ng/mL)
NDMA	13,035	48.8	1.30	103.0	46,851	130.7	0.001	92.6	0.01
NMOR	3,286	102.1	0.01	89.4	12,779	357.7	0.009	86.9	0.01
NMEA	5,121	136.7	0.02	93.8	20,411	436.3	0.019	89.2	0.01
NPYR	6,792	66.9	0.01	103.6	31,451	317.3	0.030	91.4	0.01
NDEA	1,752	149.51	0.05	81.3	6,921	374.2	0.012	85.5	0.01
NPIP	14,630	162.5	0.02	109.5	64,325	858.2	0.008	93.4	0.01
NDPA	8,745	305.6	0.02	95.8	34,844	1,316.8	0.002	90.7	0.01
NDBA	28,666	586.8	0.02	108.4	46,273	769.8	0.005	102.5	0.01

LLOQ was determined as S/N >10 for quantifier, S/N >3 for qualifier, RSD <20%, and accuracy within 80 to 120%.

Quantification in losartan potassium drug matrix

To examine the analytical performance of nitrosamine impurities in pharmaceutical drug products, the nitrosamine standards were spiked into the losartan potassium drug extract at concentrations ranging from 0.005 to 20 ng/mL. The results showed that all eight nitrosamine compounds could be quantified with high confidence at 0.03 ng/mL in drug matrix. The analytical sensitivities for all eight analytes were well below the accepted nitrosamine content required by regulatory agencies.[2,3]

NDMA is often a challenging compound for nitrosamine impurity analysis. Figure 3 shows the representative chromatograms of NDMA at matrix blank, 0.03 ng/mL, and 0.12 ng/mL in 10 mg/mL drug extract with replicates. The system demonstrated excellent response, signal-to-noise ratio (S/N), and reproducibility of NDMA at low concentrations in drug matrix on the 6495D LC/TQ.

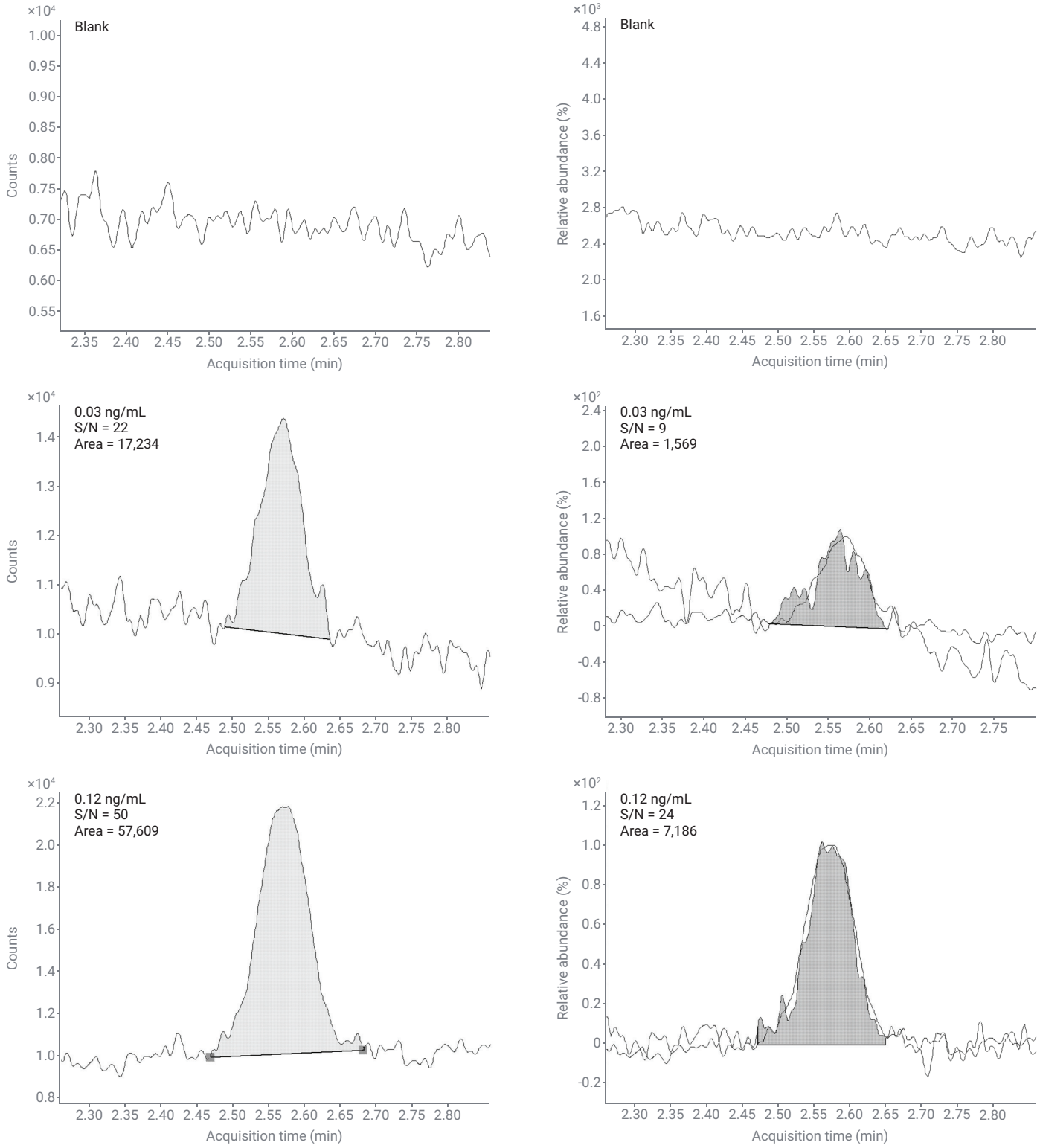


Figure 3. Representative chromatograms of NDMA product ions in losartan potassium drug matrix at low concentration levels.

Conclusion

This application note demonstrates that the Agilent 6495D triple quadrupole LC/MS system can quantify nitrosamine impurities at levels as low as 0.01 ng/mL or 10 ppt, which is below the concentration levels required by regulatory requirements, with high confidence. This method can be used to quantify these impurities in different ARB drug products with some alterations in chromatographic conditions, based on the elution pattern of the intended drug substance or drug product of interest.

References

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2. Control of Nitrosamine Impurities in Human Drugs Guidance for Industry. U.S. Food & Drug Administration (updated 24 February **2021**).
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