Application Note

Pharma/Biopharma

Determination of Nitrosamine Impurities Using the Agilent 6475 Triple Quadrupole LC/MS System

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Abstract

Nitrosamine impurities are by-products that are produced in trace amounts during the manufacturing process of pharmaceutical drugs. These impurities are classified as potentially genotoxic impurities and 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 evaluates the quantification performance of eight nitrosamine impurities using the Agilent 6475 triple quadrupole LC/MS (LC/TQ) system coupled with the Agilent 1290 Infinity II Bio LC system and atmospheric pressure chemical ionization (APCI) source.

Introduction

A recent announcement for the recall of some angiotensin II receptor blocker (ARB) drug products made nitrosamine impurities a focus for regulatory agencies.[1] Nitrosamine impurities are by-products in the manufacturing process of pharmaceutical drugs. They are a member of the "cohort of concern," which classifies 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 6475 LC/TQ coupled with the 1290 Infinity II Bio LC system and APCI source. It is demonstrated that the following compounds can be determined at 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

Chemicals 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 (MilliporeSigma, Billerica, MA, U.S.). Standards containing eight nitrosamines NDMA, NDEA, NMOR, NMEA, NPYR, NPIP, NDPA, and NDBA were from Agilent (part number US-113N-1).

Sample preparation

Nitrosamine standards were spiked into solvent blank (10:90 methanol:water) at nine different concentration levels ranging from 0.0125 to 10 ng/mL. The preparation of active pharmaceutical ingredient (API) matrix was carried out following these steps: 100 mg losartan potassium drug product was dissolved in 2 mL of solvent (50:50 methanol:water), followed by sonication for 30 minutes. Then, samples were centrifuged at 12,000 rpm for 10 minutes. Supernatants were collected and then diluted with water at a ratio of 1:5. Nitrosamine standards were spiked into the prepared API matrix at concentrations ranging from 0.05 to 1 ng/mL.

Equipment

Sample separation was performed using the Agilent 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 LC system was coupled to the Agilent 6475 triple quadrupole LC/MS (G6475AA) equipped with the Agilent APCI source (G1947B). Agilent MassHunter Workstation software 12.0 was used for data acquisition.

Methods

Liquid chromatography/mass spectrometry (LC/MS) conditions and parameters are provided in Tables 1 and 2. The multiple reaction monitoring (MRM) settings for the compounds are listed in Table 3. For calibration curve analysis, linear fitting with origin ignored and 1/x weighting was used.

Table 1. Agilent 1290 Infinity II Bio LC method.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 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

Table 2. Agilent 6475 LC/TQ parameters.

Parameter	Value
Ion Source	Agilent APCI source
Polarity	Positive
Gas Temperature	300 °C
Drying Gas Flow	7 L/min
Nebulizer	25 psi
APCI Vaporizer Temperature	350 °C
Capillary Voltage	4,000 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 diverter to MS)

Table 3. Detailed multiple reaction monitoring (MRM) settings and compound information on the Agilent 6475 LC/TQ.

Compound Name	Precursor *m/z*	Product *m/z*	Dwell (ms)	Fragmentor (V)	Cell Accelerator Voltage (V)	Collision Energy (V)	Polarity	Measured Retention Time (min)
NDMA	75	43	50	95	3	16	+	2.51
	75	58	50	95	3	10	+	2.51
NMOR	117	45	50	80	4	21	+	3.99
	117	87	50	80	4	11	+	3.99
NMEA	89	43	50	75	3	12	+	5.35
	89	61	50	75	3	10	+	5.35
NPYR	101	41	50	90	3	24	+	5.70
	101	55	50	90	3	19	+	5.70
NDEA	103	47	50	80	4	20	+	7.48
	103	75	50	80	4	12	+	7.48
NPIP	115	41	50	90	3	24	+	7.90
	115	69	50	90	3	12	+	7.90
NDPA	131	43	50	80	4	10	+	10.10
	131	89	50	80	4	16	+	10.10
NDBA	159	41	50	80	3	20	+	13.50
	159	57	50	80	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 0.0125 to 10 ng/mL in solvent.

The analysis results are summarized here:

- Good chromatographic separation and peak shapes for all the analytes (Figure 1)
- Outstanding precision and accuracy observed at all tested levels including the lower limit of quantitation (LLOQ) levels (Table 4)
- High analytical sensitivity with an LLOQ ≤0.025 ng/mL for all targeted analytes (Table 4)
- Excellent quantitation linearity for the tested levels, with R^2 >0.99 for all eight analytes (Figure 2)

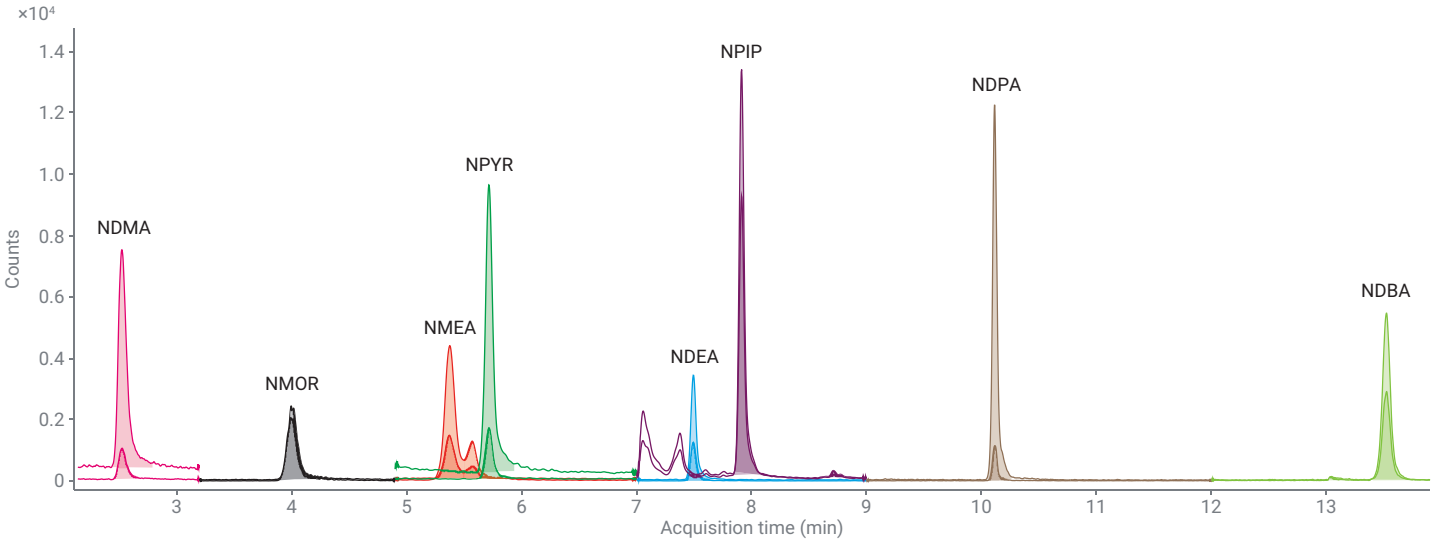

Figure 1. Total MRM chromatograms of the eight nitrosamine compounds at 1 ng/mL.

Table 4. Average response, signal-to-noise ratio (S/N) of quantifier ion, %RSD, and accuracy (%) at 0.025 and 0.05 ng/mL using the Agilent 6475 LC/TQ system (n = 3).

Concentration	0.025 ng/mL (n = 3)				0.05 ng/mL (n = 3)				LLOQ* (ng/mL)
Name	Response	S/N	RSD (%)	Accuracy (%)	Response	S/N	RSD (%)	Accuracy (%)	
NDMA	1,145.08	14.63	1.28	113.86	2,154.26	23.08	1.07	105.41	0.025
NMOR	328.76	52.40	15.94	104.90	705.55	105.41	14.94	103.87	0.025
NMEA	1,057.38	40.79	3.86	116.86	1,909.29	91.84	4.81	101.00	0.0125
NPYR	918.54	24.31	2.65	115.47	1,697.56	41.59	2.45	89.61	0.025
NDEA	382.15	31.11	8.14	107.67	606.87	52.10	8.58	87.91	0.025
NPIP	890.01	57.47	6.46	97.45	2,106.81	111.72	5.30	98.29	0.025
NDPA	795.93	95.62	12.01	106.13	1,647.60	177.47	10.77	95.97	0.025
NDBA	892.36	67.66	7.58	112.05	1,581.52	123.78	7.83	101.33	0.025

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

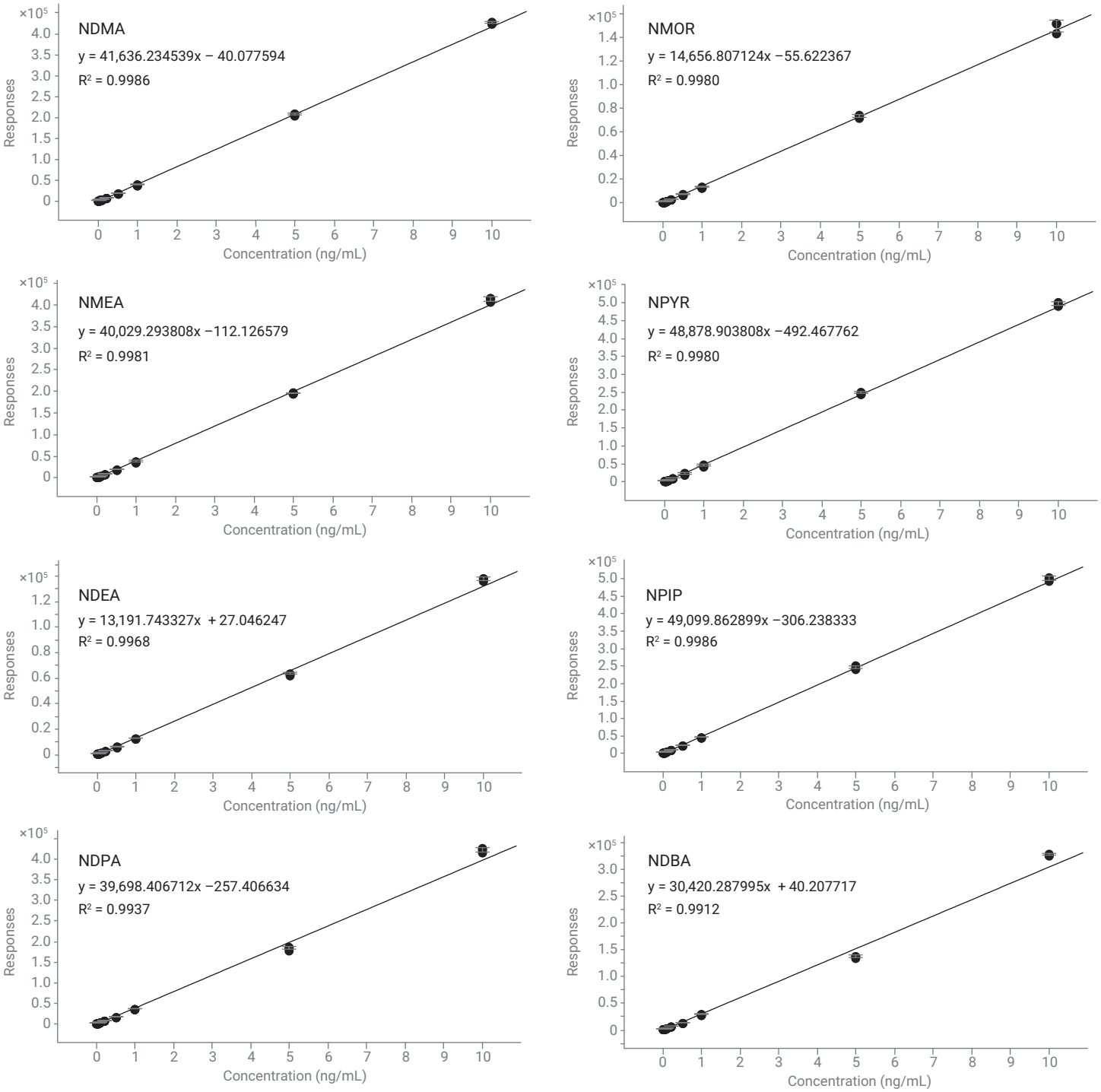

Figure 2. Calibration curves on the Agilent 6475 LC/TQ for the eight nitrosamine compounds in the concentration range of 0.0125 to 10 ng/mL.

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 of 0.05 to 1 ng/mL. The results show that all eight nitrosamine compounds could be quantified with high confidence at 0.05 ng/mL in drug matrix. The analytical sensitivities for all eight analytes were well below the acceptable nitrosamine content that is stated by regulatory agencies.[2, 3]

NDMA is commonly a challenging compound for nitrosamine impurity analysis. Figure 3 shows the representative chromatograms of NDMA at matrix blank, and at 0.05 and 0.1 ng/mL in 10 mg/mL drug extract with replicates. Excellent response, S/N, and reproducibility of NDMA at the low concentrations in drug matrix were demonstrated on the 6475 LC/TQ.

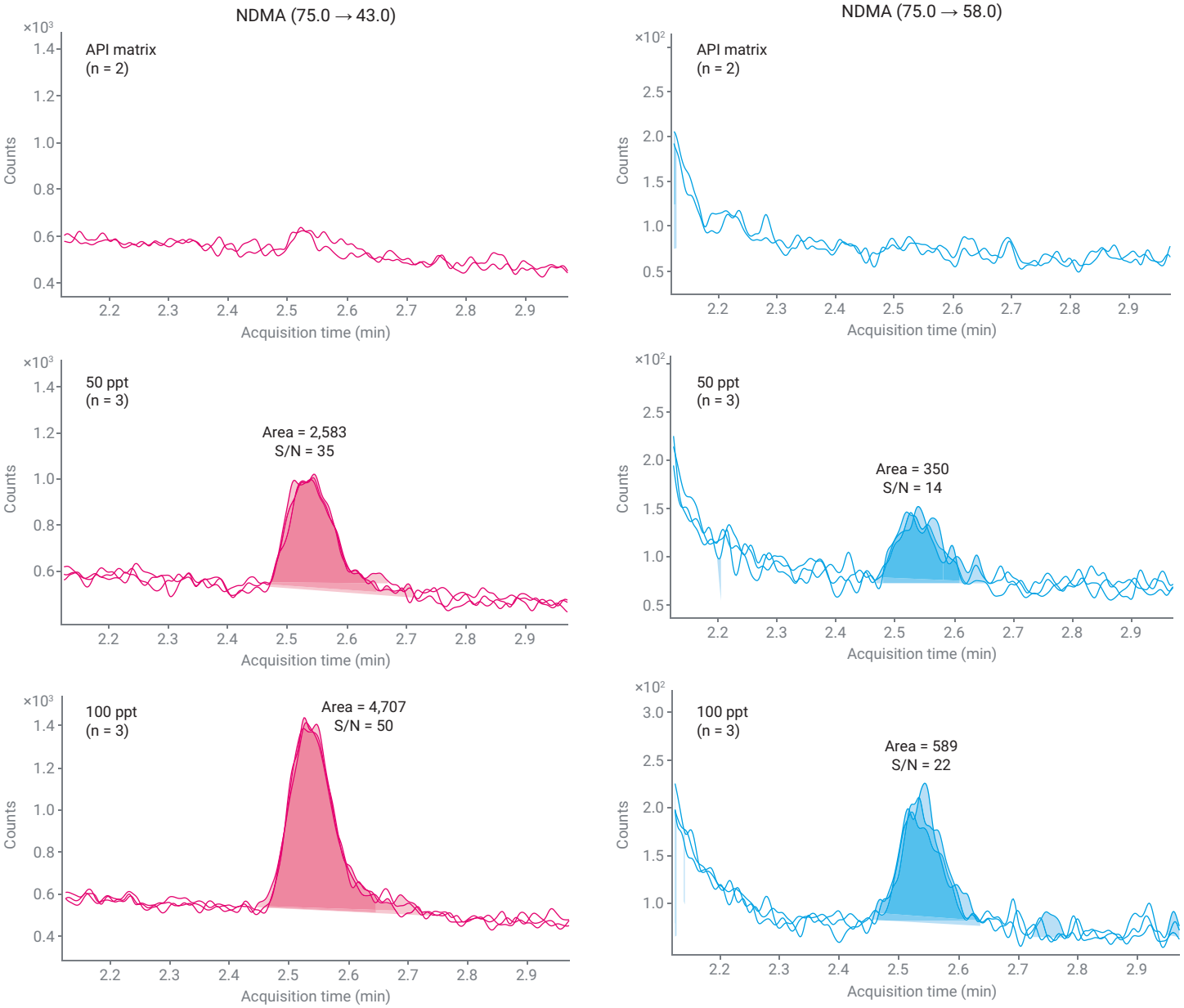

Figure 3. Replicate chromatograms of NDMA product ions in losartan potassium drug matrix at low concentration levels.

Conclusion

This application note demonstrates that the Agilent 6475 triple quadrupole LC/MS system can confidently quantify nitrosamine impurities at the low concentration levels specified by regulatory requirements. This method can be used to quantify these impurities in different ARB drug products, with some changes in chromatographic conditions based on the elution pattern of the drug product.

References

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