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Improvement Upon a Multi-Residue Method for Nitrosamine Analysis in Losartan Drug Product Using an Enhanced LC/MS/MS System

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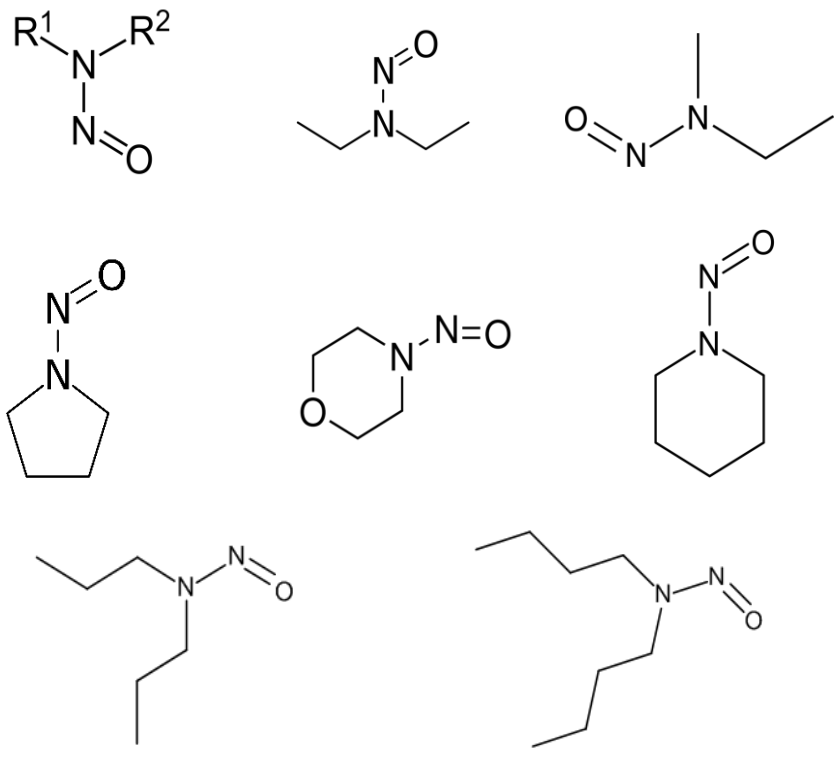
Introduction

Since 2018, nitrosamine impurities have been recognized as byproducts produced in trace amounts during manufacture of pharmaceutical drugs. They are members of the “cohort of concern” classifying them as potentially genotoxic impurities and probable carcinogens if they exceed the maximum allowable intake of 96 ng/kg for a treatment with long-term intake. A recent announcement for the recall of some angiotensin II receptor blocker (ARB) drug products made nitrosamine impurities a focus for regulatory agencies.[1] Therefore, it is important to determine their levels in final drug products with a high level of sensitivity and confidence.

In this study, a comprehensive analysis of eight nitrosamine compounds was carried out on the new 6495 LC/TQ (G6495D) 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

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:

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

The LC system was coupled to the Agilent 6495 triple quadrupole LC/MS (G6495D) equipped with the Agilent APCI source (G1947B). Agilent MassHunter Workstation software 12.0 was used for data acquisition.

The Agilent 6495 triple quadrupole LC/MS system with 1290 Infinity II Bio LC

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Experimental

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.

Parameter	Value	
Column	Agilent Poroshell 120 EC-C18, 3.0 X 150mm, 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 1. 1290 Infinity II Bio LC Method.

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	1000 V
Corona Current	4.0 µA
Scan Type	MRM with time segments
Detector gain factor (+)	10
Ion Funnel Mode	Fragile
LC diverter to waste	0 – 2 min; 12 – 13 min; 14 – 18 min (the remaining time diverter to MS)

Table 2. 6495 LC/TQ Parameters.

Compound name	Precursor *m/z*	Product *m/z*	Dwell (ms)	CAV (V)	CE (V)	Polarity
NDMA	75	43	50	3	16	+
NDMA	75	58	50	3	10	+
NMOR	117	45	50	4	21	+
NMOR	117	87	50	4	11	+
NMEA	89	43	50	3	12	+
NMEA	89	61	50	3	10	+
NPYR	101	41	50	3	24	+
NPYR	101	55	50	3	19	+
NDEA	103	47	50	4	20	+
NDEA	103	75	50	4	12	+
NPIP	115	41	50	3	24	+
NPIP	115	69	50	3	12	+
NDPA	131	43	50	4	10	+
NDPA	131	89	50	4	16	+
NDBA	159	41	50	3	20	+
NDBA	159	57	50	3	12	+

Table 3. Detailed Multiple Reaction Monitoring Settings.

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 0.0012 to 10 ng/mL in solvent.

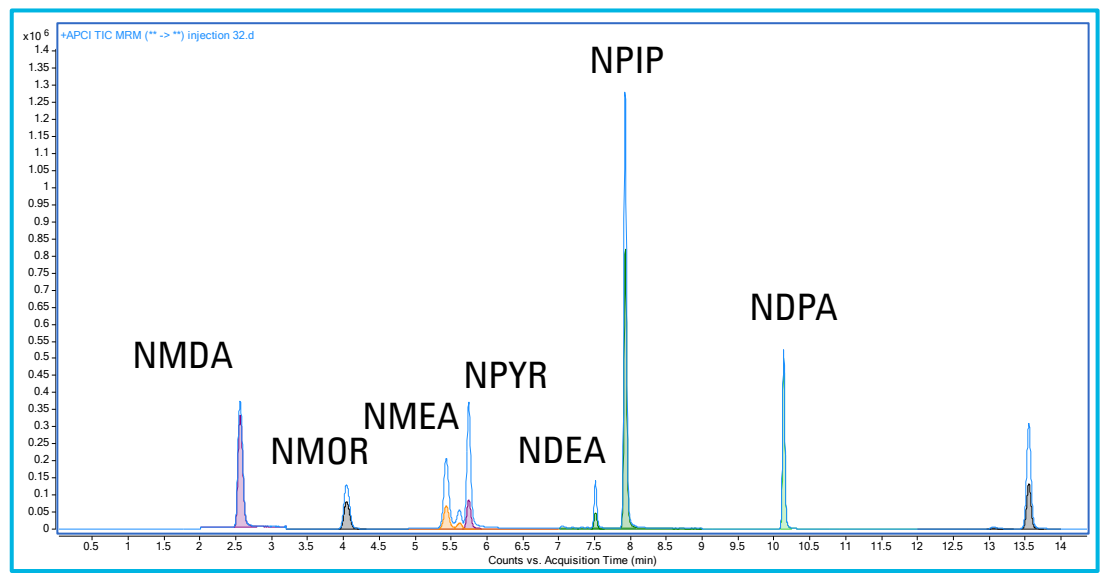

Figure 1. Total MRM Chromatogram of all Compounds at 0.6 ppb.

Results and Discussion

Name	Concentration								LLOQ (ng/mL)
	0.01 ng/mL (n=3)				0.04 ng/mL (n=3)				
	Response	S/N	RSD (%)	accuracy (%)	Response	S/N	RSD (%)	accuracy (%)	
NDMA	13035	48.8	1.30	103.0	46851	130.7	0.001	92.6	0.01
NMOR	3286	102.1	0.01	89.4	12779	357.7	0.009	86.9	0.01
NMEA	5121	136.7	0.02	93.8	20411	436.3	0.019	89.2	0.01
NPYR	6792	66.9	0.01	103.6	31451	317.3	0.030	91.4	0.01
NDEA	1752	149.51	0.05	81.3	6921	374.2	0.012	85.5	0.02
NPIP	14630	162.5	0.02	109.5	64325	858.2	0.008	93.4	0.01
NDPA	8745	305.6	0.02	95.8	34844	1316.8	0.002	90.7	0.01
NDBA	28666	586.8	0.02	108.4	46273	769.8	0.005	102.5	0.04

Table 4. Average Response, Signal to Noise (S/N), %RSD and Accuracy at 0.01 and 0.04 ng/mL using the Agilent 6495 LC/TQ.

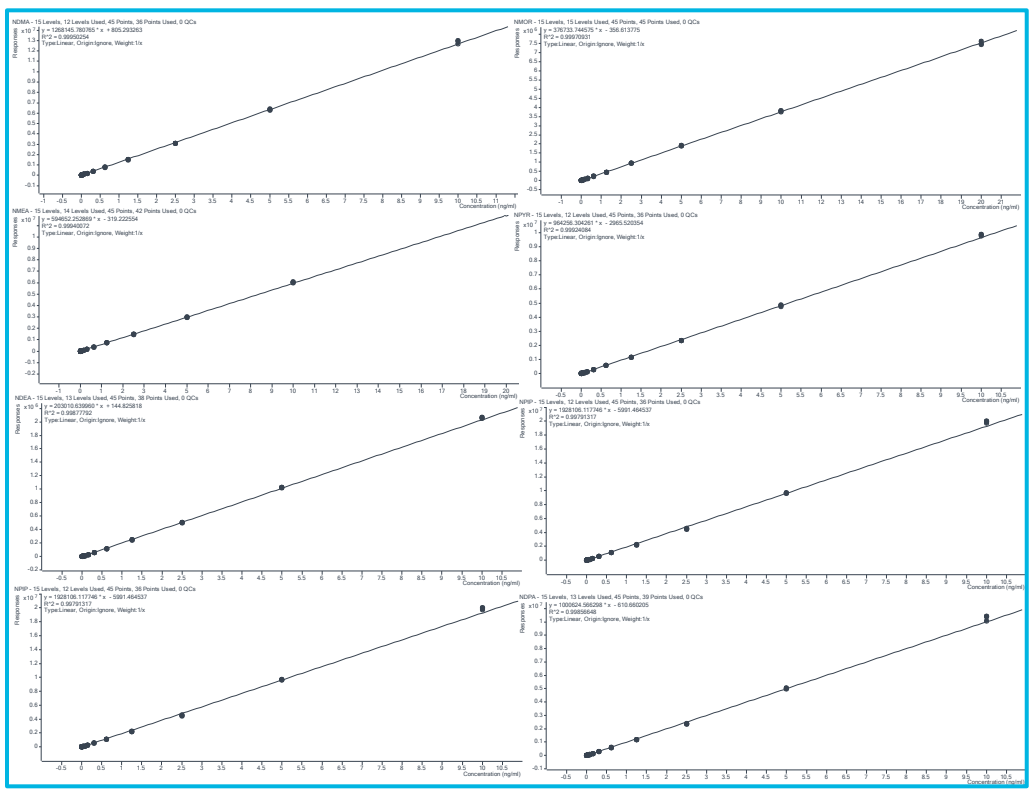

Figure 2. Calibration Curves for the Eight Nitrosamine Compounds on the Agilent 6495 LC/TQ.

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 0.0015 to 1 ng/mL. The results show 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 acceptable nitrosamine content that is stated by regulator agencies.[2, 3]

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NDMA is commonly a challenging compound for nitrosamine impurity analysis. Figure 3 shows the representative chromatograms of NDMA at matrix blank, and at 0.03 and 0.12 ng/mL in 10 mg/mL drug with the 6495 LC/TQ.

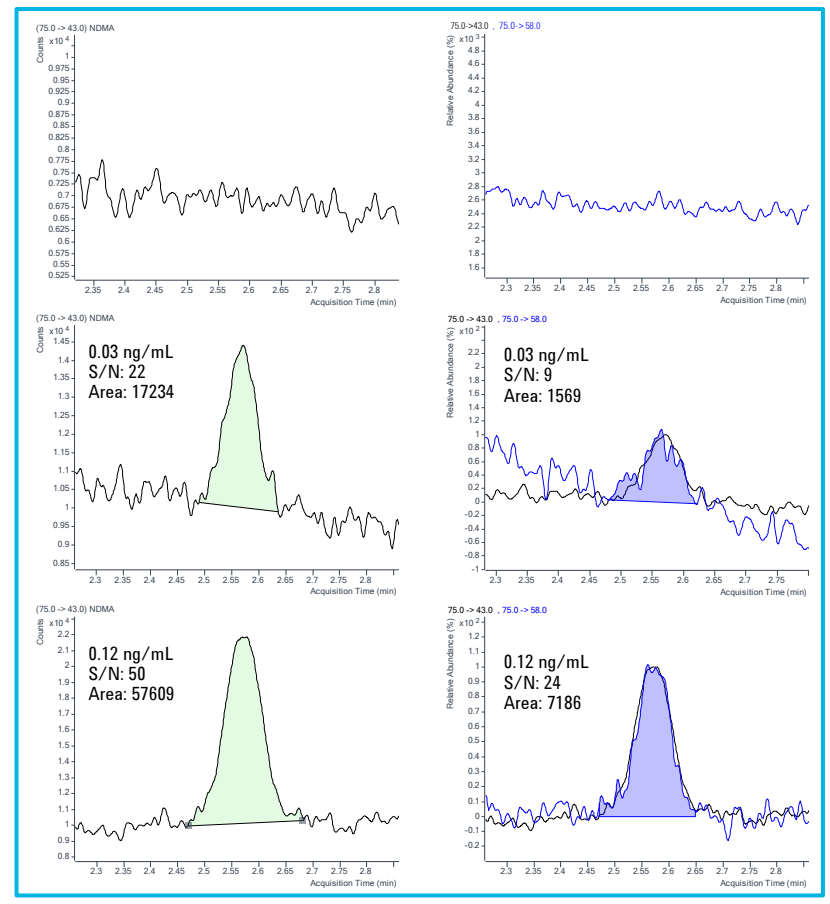

Figure 3. Representation Chromatograms of NMDA in Losartan Potassium Drug Matrix at Low Concentrations

Conclusions

This poster demonstrates that the Agilent 6495 triple quadrupole LC/MS system shows increased analytical sensitivity of nitrosamine impurities at the low concentration levels specified by regulatory requirements. Ability to detect lower than 0.01 ng/mL for most nitrosamine impurities. The 6495 LC/TQ system along with 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

[1]U.S. Food and Drug Administration. FDA Updates and Press Announcements on Angiotensin II Receptor Blocker (ARB) Recalls (Valsartan, Losartan, and Irbesartan). Last revised March 2023.

2 Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Control of Nitrosamine Impurities in Human Drugs: Guidance for Industry. Last revised February 2021. Control of Nitrosamine Impurities in Human Drugs | FDA.

3 Committee for Medicinal Products for Human Use, European Medicines Agency. Procedure under Article 5(3) of Regulation EC (No) 726/2004. Procedure number: EMEA/H/A-5(3)/1490.

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