

# Determination of Nitrosamine Impurities Using the High-Resolution Agilent 6546 LC/Q-TOF



## Authors

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## Abstract

Angiotensin II receptor blocker (ARB) drug products are commonly used to treat high blood pressure and heart failure. Recently, it was found that some ARB drug products contained carcinogenic nitrosamine impurities; as a result, many such products were recalled. Therefore, there is a clear requirement for analytical methods capable of detecting problematic nitrosamine impurities. This Application Note describes a sensitive, high-resolution LC/MS/MS method using the Agilent 6546 LC/Q-TOF for the detection and quantification of the six nitrosamines listed by the USFDA, and also demonstrates the simultaneous detection of 11 nitrosamine impurities.

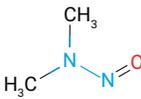
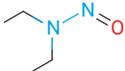
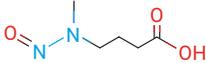
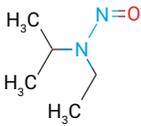
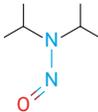
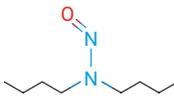
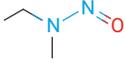
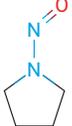
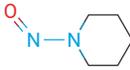
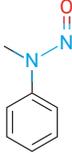
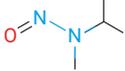
## Introduction

N-nitroso impurities recently became a focus for regulatory agencies such as the FDA and EMA when the FDA announced a recall of some ARB medicines such as valsartan, Losartan, Irbesartan, and so forth, due to the potential for these products to contain nitrosamine impurities. These nitroso compounds are classified as probable human carcinogens, and are believed to have been introduced into the finished pharmaceutical products as trace-level by-products of the manufacturing process.

This Application Note presents a comprehensive report of the analysis of 11 nitrosamine impurities at low detection limits using the 6546 LC/Q-TOF. These impurities have low molecular weight ranging from 74 to 158. These include:

- 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)

Liquid chromatography mass spectrometry (LC/MS)-based methods are generally very specific and sensitive, and served as the basis for the development of a single method that can detect and quantify all of these impurities. This Application Note demonstrates the sensitivity of the high-resolution 6546 LC/Q-TOF.

Nitrosamine Compound	Chemical Structure
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)	

## Experimental

### Chemicals and reagents

All 11 nitrosamine standards used for the study were locally sourced from PS3 Labs LLP, Hyderabad, TS, India. Other LC/MS-grade solvents (for example, methanol and water) were purchased from Honeywell. Formic acid was purchased from Fluka.

### Data analysis

Data were acquired and analyzed using Agilent MassHunter software suite 10 for data collection from the 6546 LC/Q-TOF. The personal compound database and library (PCDL) was customized and used for the identification using the Find by Formula algorithm, followed by quantitation.

### 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 v/v)												
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	<table border="1"><thead><tr><th>Time (min)</th><th>%B</th></tr></thead><tbody><tr><td>0</td><td>1</td></tr><tr><td>2</td><td>13</td></tr><tr><td>5.5</td><td>80</td></tr><tr><td>8</td><td>95</td></tr><tr><td>10</td><td>95</td></tr></tbody></table>	Time (min)	%B	0	1	2	13	5.5	80	8	95	10	95
Time (min)	%B												
0	1												
2	13												
5.5	80												
8	95												
10	95												
Stop Time	10 minutes												
Post Time	3 minutes												

### Q-TOF mass spectrometer configuration and parameters

Table 2. MS configuration and source settings.

Instrument	Agilent 6546 LC/Q-TOF
Ion Source	Atmospheric pressure chemical ionization (APCI)
MS Mode	MS
Ionization Mode	Positive
Drying Gas Temperature	300 °C
Drying Gas Flow	6 L/min
Nebulizer Pressure	45 psi
APCI Heater	350 °C
APCI Needle Positive	4 µA
Capillary Voltage, Positive	3,000 V
Mass Range	70 to 170 <i>m/z</i>

### MS compound information for analytes

Table 3. Detailed MS settings for the Agilent 6546 LC/ Q-TOF.

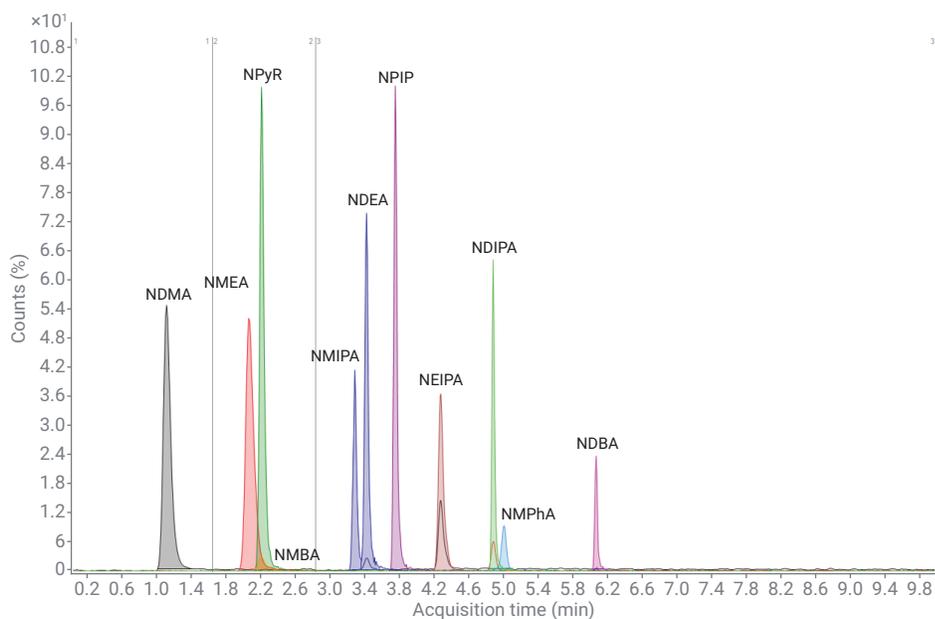
Time Segment	Start Time (min)	Mass Range (m/z)	Acquisition Rate (spectra/sec)	Fragmentor Voltage (V)
1	0	70-170	1.5	120
2	1.6	70-170	1.5	90
3	2.8	70-170	2	120

## Results and discussion

The calibration concentrations ranged from 0.05 to 100 ng/mL (Table 4).  $R^2$  values were greater than 0.996 for all the analytes, and displayed linear responses throughout the concentration range. Figure 1 shows a representative overlaid extracted ion chromatogram at 10 ng/mL for all nitrosamine compounds tested.

**Table 4.** Results summary for the Agilent 6546 LC/Q-TOF. Data include signal-to-noise (S/N), calculated LOQ, coefficient of regression, calibration curve fit, and linearity range. All standards used a linear function and 1/x weighted calibration curve.

Compound	Detection Limit (ng/mL)	Detection Limit (S/N)	LOQ (ng/mL)	LOQ (S/N)	$R^2$	Cal. Curve	Linearity Range (ng/mL)
NDMA	0.1	16.54	0.25	35.72	0.999	Linear	0.1 to 100
NDEA	0.05	29.56	0.1	79.2	0.999	Linear	0.1 to 100
NMBA	0.25	12.16	0.5	27.88	0.996	Linear	0.5 to 100
NEIPA	0.1	11.22	0.25	80.6	0.998	Linear	0.1 to 100
NDIPA	0.075	16.65	0.15	21.99	0.999	Linear	0.075 to 100
NDBA	0.1	44.60	0.25	61.56	0.997	Linear	0.1 to 100
NMEA	0.05	18.42	0.1	23	0.998	Linear	0.05 to 100
NPYR	0.1	29.73	0.15	50.39	0.999	Linear	0.1 to 100
NPIP	0.075	12.18	0.1	25.30	0.998	Linear	0.075 to 100
NMPhA	0.25	24.22	0.5	32.30	0.997	Linear	0.5 to 100
NMIPA	0.075	29.79	0.1	47.46	0.997	Linear	0.075 to 100



**Figure 1.** Representative overlaid extracted ion chromatogram, showing elution of 11 nitrosamine compounds.

### Accuracy and reproducibility

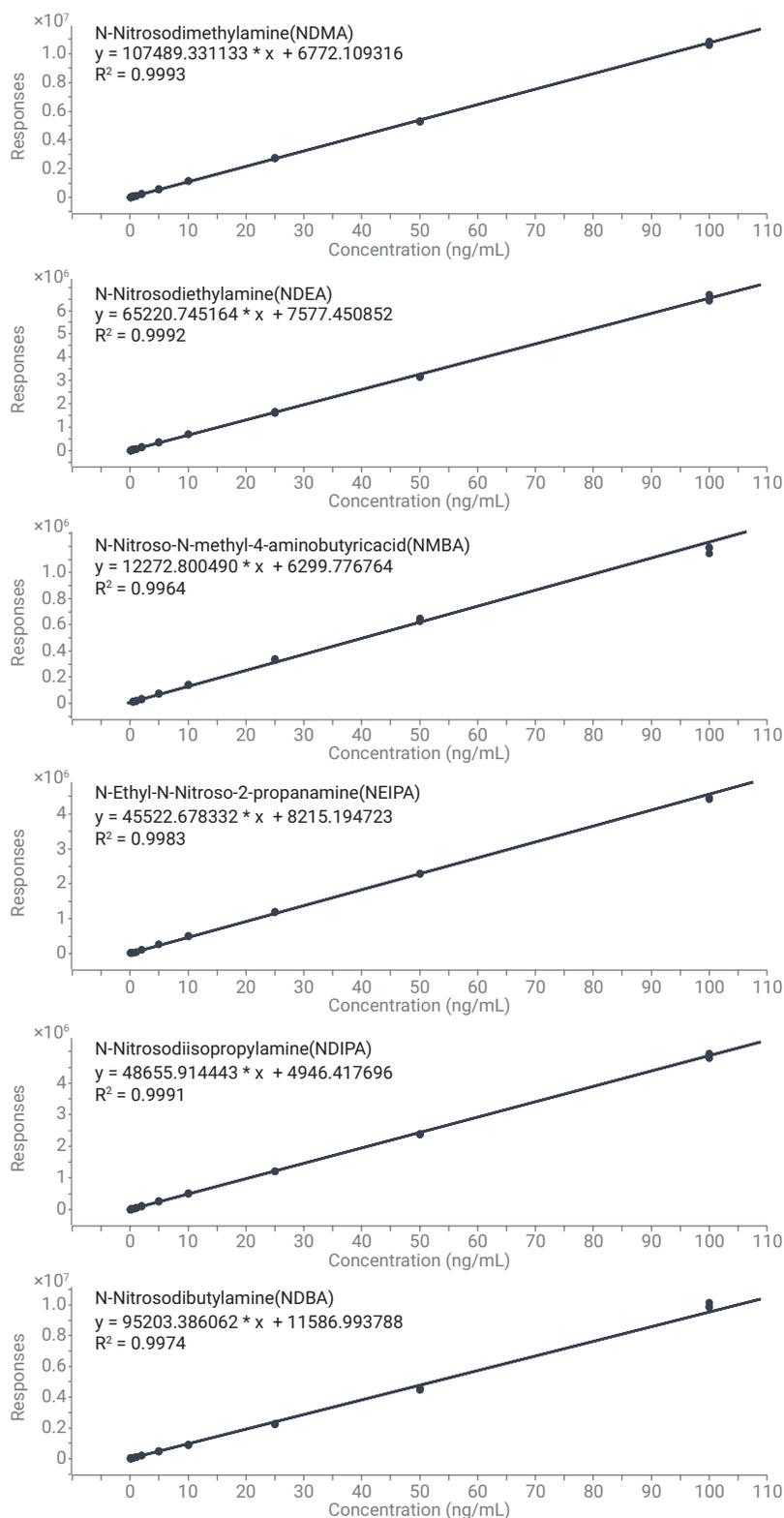
Calibration curves for each of the 11 compounds demonstrated accuracies within 20% of the expected concentrations at each calibration level, and reproducibility across all levels exhibited CVs <15% (Figure 4). Table 5 shows a detailed comparison of accuracy and reproducibility at different concentration levels.

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS
N-Nitrosodimethylamine(NDMA)	C2H6N2O	74.04801	<input type="checkbox"/>	<input checked="" type="checkbox"/>	1.131	<a href="#">62-72-9</a>
N-Nitrosoethylmethylamine(NMEA)	C3H8N2O	88.06366	<input type="checkbox"/>	<input checked="" type="checkbox"/>	2.075	<a href="#">10595-95-6</a>
N-Nitrosopyrrolidine(NPyR)	C4H8N2O	100.06366	<input type="checkbox"/>	<input checked="" type="checkbox"/>	2.222	<a href="#">930-55-2</a>
N-Nitrosodiethylamine(NDEA)	C4H10N2O	102.07931	<input type="checkbox"/>	<input checked="" type="checkbox"/>	3.426	<a href="#">55-18-5</a>
N-Isopropylmethyl nitrosamine(NMIPA)	C4H10N2O	102.07931	<input type="checkbox"/>	<input checked="" type="checkbox"/>	3.297	<a href="#">30553-08-5</a>
N-Nitrosopiperidine(NPIP)	C5H10N2O	114.07931	<input type="checkbox"/>	<input checked="" type="checkbox"/>	3.766	<a href="#">100-75-4</a>
N-Ethyl-N-Nitroso-2-propanamine(NEIPA)	C5H12N2O	116.09496	<input type="checkbox"/>	<input checked="" type="checkbox"/>	4.289	<a href="#">16339-04-1</a>
N-Nitrosodiisopropylamine(NDIPA)	C6H14N2O	130.11061	<input type="checkbox"/>	<input checked="" type="checkbox"/>	4.886	<a href="#">601-77-4</a>
N-methyl-n-nitrosoaniline(NMPhA)	C7H8N2O	136.06366	<input type="checkbox"/>	<input checked="" type="checkbox"/>	5.009	<a href="#">614-00-6</a>
N-Nitroso-N-methyl-4-aminobutyricacid(NMBA)	C5H10N2O3	146.06914	<input type="checkbox"/>	<input checked="" type="checkbox"/>	2.219	<a href="#">61445-55-4</a>
N-Nitrosodibutylamine(NDBA)	C8H18N2O	158.14191	<input type="checkbox"/>	<input checked="" type="checkbox"/>	6.059	<a href="#">924-16-3</a>

Figure 2. The PCDL used for Find by Formula for identification and quantification.

Name	Transition
N-Nitrosodimethylamine(NDMA)	75.0552
N-Nitrosoethylmethylamine(NMEA)	89.0709
N-Nitrosopyrrolidine(NPyR)	101.0710
N-Nitrosodiethylamine(NDEA)	103.0866
N-Isopropylmethyl nitrosamine(NMIPA)	103.0866
N-Nitrosopiperidine(NPIP)	115.0866
N-Ethyl-N-Nitroso-2-propanamine(NEIPA)	117.1022
N-Nitrosodiisopropylamine(NDIPA)	131.1179
N-methyl-n-nitrosoaniline(NMPhA)	137.0709
N-Nitroso-N-methyl-4-aminobutyricacid(NMBA)	147.0766
N-Nitrosodibutylamine(NDBA)	159.1492

Figure 3. *m/z* Extracted for the quantification of different nitrosamine impurities.



**Figure 4.** Representative calibration curves for compounds dispersed throughout the chromatograms. All calibration curves used a linear function and 1/x weighting factor.

**Table 5.** Representative accuracy and reproducibility for different concentration levels determined using the Agilent 6546 LC/Q-TOF (n = 3).

Concentration (ng/mL)	NDMA		NDEA		NMBA		NDIPA		NDBA		NMEA	
	Average	CV										
0.1	86.67	5.57	83.53	1.59	NA	NA	91.30	0.55	101.03	6.19	93.93	1.67
0.25	87.00	3.96	88.33	2.58	NA	NA	91.33	4.67	95.13	5.69	89.40	4.55
0.5	97.23	4.51	101.17	3.62	92.40	1.96	94.6	2.27	102.30	3.38	102.53	3.28
1.0	104.93	2.44	110.80	2.47	98.37	5.80	109.83	2.13	101.80	1.15	103.23	0.78

## Conclusion

The Agilent 6546 LC/Q-TOF high-resolution LC/MS/MS can analyze nitrosamine impurities at low concentration levels; high-resolution mass spectrometry reliably detects the presence of nitrosamine compounds in the drug products. This Application Note demonstrates the sensitivity of the 6546 LC/ Q-TOF instrument for detecting these nitrosamine impurities at low concentration levels. The 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 drug product to ensure that the drug product peak may be diverted to waste to avoid mass spectrometer contamination.

## References

1. USFDA guidance document: Development and validation of a RapidFire-MS/MS method for screening of nitrosamine impurities.
2. USFDA guidance document: Liquid chromatography-high resolution mass spectrometry (LC/HRMS) method for the determination of Six nitrosamine impurities in ARB drugs.

## Acknowledgments

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