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Application News

GC-MS GCMS-TQ™8050 NX

High Sensitive and Accurate Analysis of Nitrosamines Using GC/MS/MS

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User Benefits

- Analyze trace quantities of nitrosamines with state-of-the-art detector.
- MRM measurements eliminate matrix interferences to ensure reliable target compound detection.
- Superior reproducibility helps to ensure reliable laboratory operations.

■ Introduction

In 2018, more than the permitted quantities of carcinogenic components, N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), were detected in a valsartan drug substance. Since then, a demand for analyzing nitrosamines, not only in drug ingredients but also in pharmaceuticals, has been increasing.

According to the International Agency for Research on Cancer (IARC), NDMA and NDEA belong in Group 2A substances that are probably carcinogenic to humans. In the case of the ICH M7 guideline[1)], NDMA and NDEA are equivalent to a class 1 as carcinogenic controlled impurities that must be controlled within allowable limit values specified specifically for the respective compounds.

In the case of GC/MS for analysis, direct injection is well-suited for simultaneous analysis of NDMA, NDEA, and other nitrosamines. This Application News describes simultaneous analytical method for five kinds of nitrosamines using a triple quadrupole gas chromatograph mass spectrometer system and indicates the corresponding results.

■ Samples

• Standards

10 points calibration standards (0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, and 200 ng/mL concentrations of the following nitrosamines) were prepared with dichloromethane.

NDMA: N-nitrosodimethylamine
NDEA: N-nitrosodiethylamine
NEIPA: N-nitrosoethylisopropylamine
NDIPA: N-nitrosodiisopropylamine
NDBA: N-nitrosodibutylamine

• Drug Substance Samples

A total of five kinds of drug substance were prepared (API i to iii and insoluble substances iv and v). 5 mL of dichloromethane was added to 0.1 g of the drug substance which was weighed into a glass centrifuge tube. After that, the samples were mixed in a vortex mixer for one minute, followed by centrifugal separation (2.5 minutes at 4000 rpm). Then a glass syringe was used to filter 1 mL of the dichloromethane layer through a PTFE filter (0.45 µm).

To compare the intensity to the standards, the extract solution was spiked with standard solution to prepare solutions with 0.5, 5, and 50 ng/mL (concentration in the solution) nitrosamine concentrations. That resulted in 0.025, 0.25, and 2.5 ppm concentrations in the drug substance.

■ Analytical Conditions

Table 1 Instruments Used and Analytical Conditions

Instruments Used	
GCMS:	GCMS-TQ8050 NX
Autosampler:	AOC-20i+s Plus
Column:	SH-I-624Sil MS (30 m × 0.25 mmI.D., df = 1.4 µm)
Insert Liner:	Split-less Deactivated Liner w/ Low Wool
GC Conditions	
Injection Unit Temp.:	250 °C
Injection Volume:	2 µL
Injection Mode:	Splitless (high pressure injection for 1.5 min at 250 kPa)
Carrier Gas Control:	Linear speed (39.7 cm/sec)
Column Oven Temp.:	50 °C (1 min) → (20 °C/min) → 250 °C (3 min)
MS Conditions	
Interface Temp.:	250 °C
Ion Source Temp.:	230 °C
Ionization Method:	EI
Measurement Mode:	MRM
Measurement *m/z*:	NDMA 74>44 CE = 6, 74>42 CE = 18 NDEA 102>85 CE = 6, 102>56 CE = 16 NEIPA 116>99 CE = 6, 116>70 CE = 14 NDIPA 130>88 CE = 6, 130>42 CE = 12 NDBA 158>99 CE = 10, 158>141 CE = 4
Event Time:	0.3 sec
Resolution:	Unit – Unit (Q1 – Q3)

Fig. 1 GCMS-TQ™8050 NX

■ Analysis Results

• Standards (Calibration Curves and Reproducibility)

For calibration curves, excellent results were obtained with calibration curve correlation coefficients, R, of 0.999 or higher for all five types of nitrosamines (Fig. 2). Reproducibility (n = 3) was also checked for each calibration point, which resulted in an area %RSD value of 8 % or less for all calibration points (Table 2). These results showed that nitrosamines can be analyzed with high sensitivity and high accuracy despite the wide dynamic range from 0.2 to 200 ng/mL.

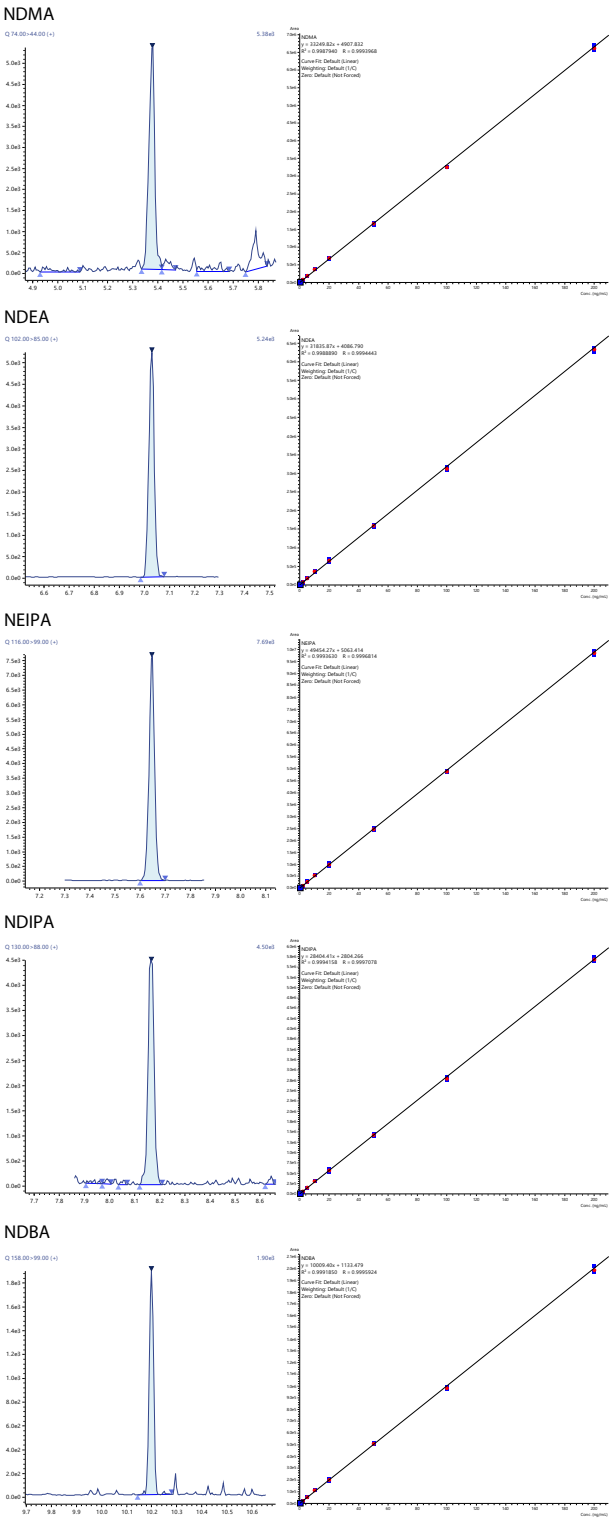

Fig. 2 Chromatograms of 0.2 ng/mL Standard and Calibration Curves

Table 2 Area Repeatability for Standards (%RSD)

Standard Conc.	NDMA	NDEA	NEIPA	NDIPA	NDBA
0.2 ng/mL	0.4	6.3	3.4	6.7	7.9
0.5 ng/mL	2.4	0.8	2.1	1.6	4.6
1 ng/mL	1.1	2.3	2.3	4.2	1.7
2 ng/mL	1.3	4.5	0.8	1.7	2.9
5 ng/mL	1.6	0.8	1.2	1.5	2.2
10 ng/mL	1.2	0.9	1.2	1.6	1.2
20 ng/mL	2.4	5.8	5.4	4.7	3.9
50 ng/mL	1.7	1.5	1.6	1.9	1.1
100 ng/mL	0.3	1.2	0.7	1.0	0.7
200 ng/mL	1.0	0.8	0.7	0.9	1.3

- Drug Substance Extract Solution
  (Separation and Reproducibility)

To check the ion suppression and the impact on the baseline caused by matrices originating from the drug substance, the signal intensities from samples prepared by adding nitrosamines to each drug substance extract solution (0.5, 5, and 50 ng/mL) were compared with those of the standards. Fig. 3 shows the chromatogram comparison for the 0.5 ng/mL spiked samples and standard.

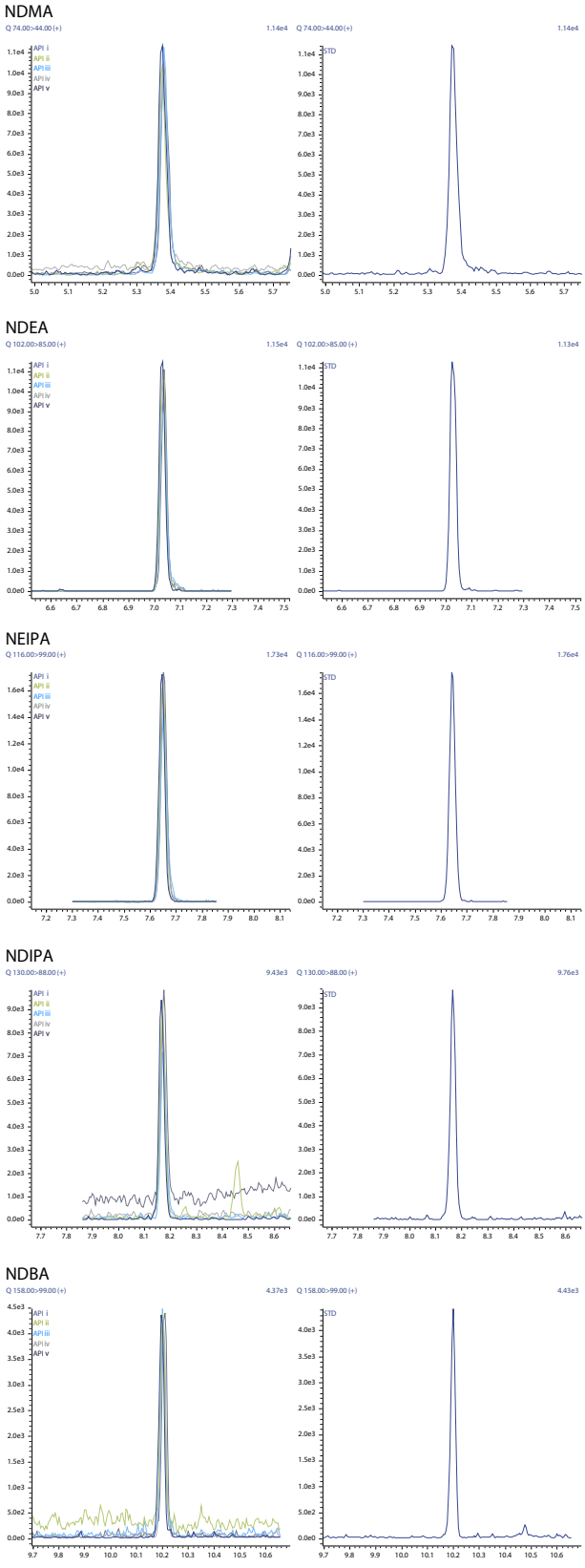

Fig. 3 Chromatograms from 0.5 ng/mL Spiked Samples (Left) and Standard (Right)

The results indicated excellent area ratio values (spiked sample/standard) between 75 and 106 %, which confirmed that matrix interferences were minimal (Table 3). The area reproducibility (%RSD, n = 3) was also checked.

For all compounds in all solutions, accurate results were shown with %RSD of 10 % or less. This method enabled reliable trace amount analysis (Table 4).

Table 3 Area Ratio (%) for Spiked Samples and Standards

	Conc. in Solution	Conc. in Drug Substance	NDMA	NDEA	NEIPA	NDIPA	NDBA
API i	0.5 ng/mL	0.025 ppm	94	94	94	96	102
	5 ng/mL	0.25 ppm	85	88	90	85	97
	50 ng/mL	2.5 ppm	91	94	95	86	97
API ii	0.5 ng/mL	0.025 ppm	102	94	97	95	101
	5 ng/mL	0.25 ppm	90	90	96	87	101
	50 ng/mL	2.5 ppm	94	95	98	88	105
API iii	0.5 ng/mL	0.025 ppm	91	91	90	84	98
	5 ng/mL	0.25 ppm	88	89	89	77	95
	50 ng/mL	2.5 ppm	94	91	89	75	93
API iv	0.5 ng/mL	0.025 ppm	96	93	100	95	105
	5 ng/mL	0.25 ppm	90	87	93	84	101
	50 ng/mL	2.5 ppm	94	90	94	81	100
API v	0.5 ng/mL	0.025 ppm	87	91	100	104	106
	5 ng/mL	0.25 ppm	79	82	90	84	95
	50 ng/mL	2.5 ppm	85	83	90	80	98

Table 4 Area Repeatability for Spiked Samples (%RSD)

	Conc. in Solution	Conc. in Drug Substance	NDMA	NDEA	NEIPA	NDIPA	NDBA
API i	0.5 ng/mL	0.025 ppm	4.8	5.7	3.4	3.9	5.8
	5 ng/mL	0.25 ppm	4.2	0.6	2.0	0.6	4.8
	50 ng/mL	2.5 ppm	0.8	0.6	0.2	0.4	0.6
API ii	0.5 ng/mL	0.025 ppm	4.2	3.1	9.4	4.5	4.1
	5 ng/mL	0.25 ppm	0.5	1.0	0.7	2.2	1.9
	50 ng/mL	2.5 ppm	1.5	2.1	2.4	2.7	1.2
API iii	0.5 ng/mL	0.025 ppm	7.6	3.0	3.9	3.3	3.3
	5 ng/mL	0.25 ppm	1.4	0.2	0.7	2.1	3.8
	50 ng/mL	2.5 ppm	1.1	0.5	1.3	2.1	1.7
API iv	0.5 ng/mL	0.025 ppm	1.7	3.5	2.3	7.3	2.1
	5 ng/mL	0.25 ppm	1.4	2.0	1.1	2.5	2.4
	50 ng/mL	2.5 ppm	7.0	7.9	8.6	9.5	7.9
API v	0.5 ng/mL	0.025 ppm	6.8	2.8	1.3	1.2	2.1
	5 ng/mL	0.25 ppm	2.1	1.5	0.8	0.8	0.6
	50 ng/mL	2.5 ppm	0.7	0.8	0.7	0.6	0.3

■ Conclusion

Results from using the GCMS-TQ8050 NX system showed excellent linearity and reproducibility even over a wide dynamic range. The results also showed that the system can be used for accurate quantitative analysis without matrix interferences.

Nitrosamines include some highly polar components with low volatility. High performance liquid chromatograph mass spectrometer (LC/MS/MS) systems are well-suited to measuring such components.

Also refer to the Application News entitled High-Sensitivity Quantitative Analysis of Nitrosamines Using Triple Quadrupole LC/MS/MS (01-00188-EN), which describes an example of analyzing nitrosamines using an LC/MS/MS system.

References
1) International Council for Harmonisation M7 (R1), Addendum: Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk

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