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Application News

LCMS™-8060NX Liquid Chromatograph Mass Spectrometer

High-Sensitivity Quantitative Analysis of Nitrosamines Using Triple Quadrupole LC/MS/MS

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User Benefits

- High-sensitivity analysis supports the strict risk assessment required for nitrosamine impurities.
- LC/MS/MS enables accurate quantitative analysis by providing a wide range of calibration curve concentrations and excellent linearity.

■ Introduction

The nitrosamine compounds NDMA and NDEA are classified as probably carcinogenic to humans (Group 2A) by the International Agency for Research on Cancer (IARC). This corresponds to a Class 1 impurities classification by the ICH M7 guideline on controls for impurities[1)] and requires control at or below a compound-specific acceptable limit.

LC/MS/MS methods are included among analysis methods used for detecting nitrosamines published by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA). This article describes an example high-sensitivity analysis of six nitrosamines, including NDMA and NDEA, performed by LC/MS/MS.

■ Analysis Conditions

The analysis conditions used are shown in Table 1 and Table 2. The mass spectrometer used for analysis was the LCMS-8060NX (Fig. 1), and the ionization unit used was the APCI probe.

Table 1 LCMS Analysis Conditions

[HPLC conditions] (Nexera™ X3)	
Column	: Shim-pack Scepter™ C18-120*1 (100 mm × 2.1 mm I.D., 1.9 µm)
Mobile Phases	: A) 0.05 % formic acid in H_2O B) 0.05 % formic acid in Methanol
Gradient Program	: B conc. 1.0 % (0.00-1.50 min) – 40.0 % (2.50 min) – 80.0 % (7.00-8.50 min) – 1.0 % (8.51-12.50 min)
Flowrate	: 0.40 mL/min
Column Temp.	: 45 °C
Injection Volume	: 10 µL
[MS conditions] (LCMS -8060NX*2)	
Ionization	: APCI (Positive mode)
Probe Voltage	: 4.0 kV
Mode	: MRM
Nebulizing Gas Flow	: 4.0 L/min
Drying Gas Flow	: 3 L/min
DL Temp.	: 150 °C
Heat Block Temp.	: 200 °C
Interface Temp.	: 300 °C

*1 P/N: 227-31011-05
*2 Hydrocarbon filter (P/N: 225-42793-01) used on nitrogen gas line

Fig. 1 External View of LCMS™-8060NX

Table 2 MS/MS Parameters

Compound	Ret. Time (min)	Precursor ion *m/z*	Product ion *m/z*	Collision Energy (V)
NDMA	1.968	75.05	43.0	-17.0
		75.05	58.1	-12.0
NMBA	3.361	147.05	43.4	-16.0
		147.05	116.9	-11.0
NDEA	4.053	103.10	28.8	-16.0
		103.10	74.7	-13.0
NEIPA	4.637	117.10	75.0	-12.0
		117.10	43.1	-21.0
NDIPA	5.269	131.10	43.0	-15.0
		131.10	88.9	-9.0
NDBA	7.121	159.10	57.0	-13.0
		159.10	41.2	-23.0

■ Results from Analysis of Standard Sample

Calibration standards were prepared and analyzed six times to confirm the linearity of the calibration curve and the repeatability.

A typical chromatogram obtained from analyzing a 10 ng/mL standard sample is shown in Fig. 2. Chromatograms close to the lower limit of quantification (LLOQ) and calibration curves prepared using an external standard method are also shown in Fig. 3. Calibration curve ranges and coefficients of determination (R^2) are shown in Table 3.

Good linearity was obtained as shown by a coefficient of determination (R^2) of ≥ 0.998 for all compounds.

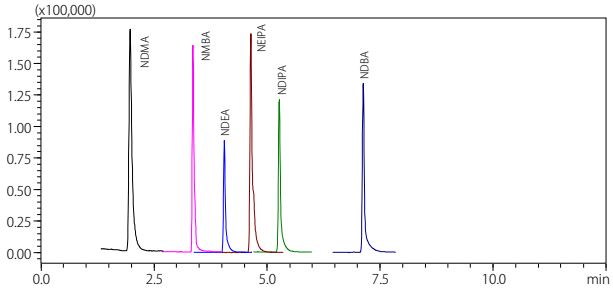

Fig. 2 Chromatogram of 10 ng/mL Standard Sample

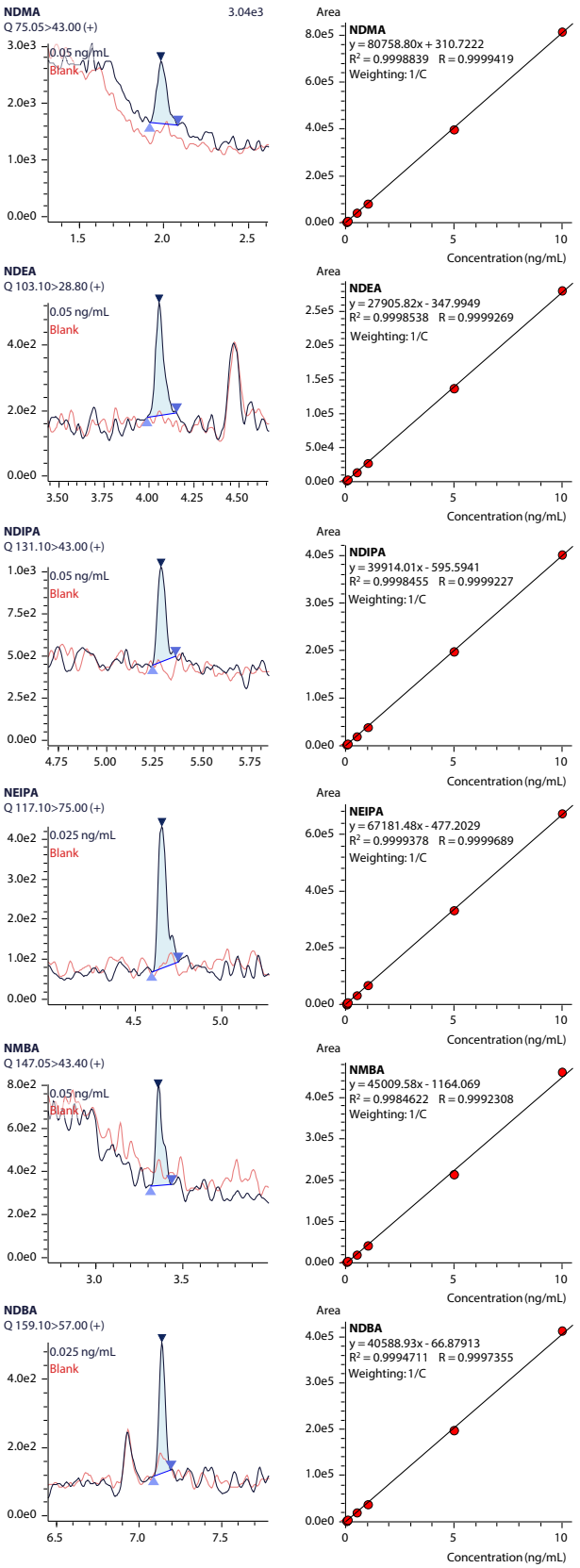

Fig. 3 Chromatograms Close to LLOQ and Calibration Curves

Table 3 Calibration Curve Range and Coefficient of Determination (R^2)

Compound	Calibration curve (ng/mL)	Contribution ratio (R^2)
NDMA	0.05 - 10	0.999
NDEA	0.05 - 10	0.999
NDIPA	0.05 - 10	0.999
NEIPA	0.025 - 10	0.999
NMBA	0.05 - 10	0.998
NDBA	0.025 - 10	0.999

■ Repeatability and Accuracy

The repeatability (conc.%RSD) and accuracy of analyzing the lowest concentration on the calibration curve six times are shown in Table 4.

A repeatability of ≤ 10 % and accuracy of 102.0–114.5 % for all compounds show these concentrations are reliable enough to be used as lower limits of quantification.

Table 4 Repeatability (Conc.%RSD) and Average Accuracy

Compound	Concentration (ng/mL)	Repeatability (Conc.%RSD, n=6)	Accuracy (Average, n=6)
NDMA	0.05	4.99	106.1
NDEA	0.05	2.17	102.0
NDIPA	0.05	5.30	108.5
NEIPA	0.025	3.48	104.7
NMBA	0.05	3.70	114.5
NDBA	0.025	6.49	107.4

■ Conclusion

- Results confirm that all six nitrosamines can be measured at a limit of quantification of ≤ 0.05 ng/mL from an injection volume of 10 µL.
- Excellent linearity was obtained as shown by a calibration curve coefficient of determination (R^2) of ≥ 0.998 for all nitrosamines.

<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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