



LCMS[™]-8060NX Liquid Chromatograph Mass Spectrometer

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

Compound

NDMA

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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 impurities¹⁾ 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 $	imes$ 2.1 mm l.D., 1.9 μ m) | | | |
| Mobile Phases | : A) 0.05 % formic acid in H ₂ O | | | |
| | 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}) | | | | |
| lonization | : 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



| NMBA | 3.361 | 147.05 | 43.4 | -16.0 |
|-------|-------|--------|-------|-------|
| | | 147.05 | 116.9 | -11.0 |
| NDEA | 4.053 | 103.10 | 28.8 | -16.0 |
| NDEA | 4.055 | 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 |
| NDIFA | | 131.10 | 88.9 | -9.0 |
| NDBA | 7.121 | 159.10 | 57.0 | -13.0 |
| | | 159.10 | 41.2 | -23.0 |

Table 2 MS/MS Parameters

Precursor ion

m/z

75.05

75.05

Product ion

m/z

43.0

58.1

Collision

Energy (V)

-17.0

-12.0

Ret. Time

(min)

1.968

es 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 (R²) are shown in Table 3.

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

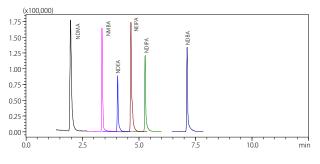


Fig. 2 Chromatogram of 10 ng/mL Standard Sample

Fig. 1 External View of LCMS[™]-8060NX

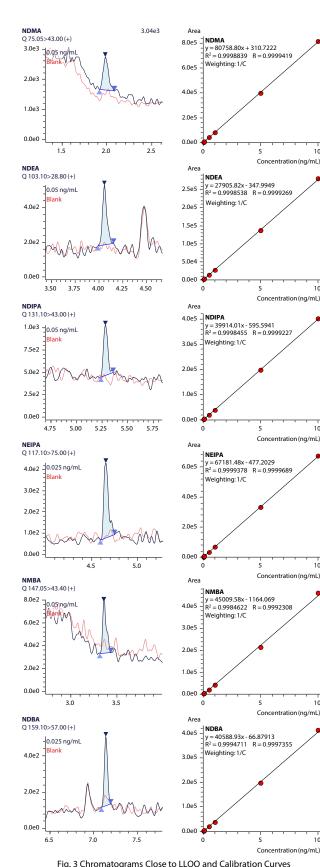


Table 3 Calibration Curve Range and Coefficient of Determination (R²)

| Compound | Calibration curve (ng/mL) | e 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

10

10

10

10

10

nL)

10

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 \leq 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 | | | | | |
|--|---|---|--|--|--|
| Concentration (ng/mL) | Repeatability (Conc.%RSD, n=6) | Accuracy (Average, n=6) | | | |
| 0.05 | 4.99 | 106.1 | | | |
| 0.05 | 2.17 | 102.0 | | | |
| 0.05 | 5.30 | 108.5 | | | |
| 0.025 | 3.48 | 104.7 | | | |
| 0.05 | 3.70 | 114.5 | | | |
| 0.025 | 6.49 | 107.4 | | | |
| | Concentration (ng/mL) 0.05 0.05 0.05 0.025 0.05 | Concentration (ng/mL) Repeatability (Conc.%RSD, n=6) 0.05 4.99 0.05 2.17 0.05 5.30 0.025 3.48 0.05 3.70 | | | |

Conclusion

- Results confirm that all six nitrosamines can be measured at a limit of quantification of \leq 0.05 ng/mL from an injection volume of 10 µL.
- Excellent linearity was obtained as shown by a calibration curve coefficient of determination (R²) of \geq 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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