SHIMADZU
Excellence in Science

Application News

LCMS™-8060NX Liquid Chromatograph Mass Spectrometer

Simultaneous Analysis of 10 Nitrosamines in an Active Pharmaceutical Ingredient Using a Triple Quadrupole Mass Spectrometer

Miho Kawashima and Ryo Kubota

User Benefits

- Meet Japanese, U.S., and European risk assessment standards for contamination with nitrosamines.
- Reliable quantitative analysis of nitrosamines in an active pharmaceutical ingredient.

■ Introduction

Nitrosamines can be highly genotoxic, and the nitrosamines NDMA and NDEA are classified as group 2A carcinogens (probably carcinogenic to humans) by the International Agency for Research on Cancer (IARC). ICH M7 provides guidance for the assessment and control of mutagenic impurities in drug products and describes N-nitroso compounds as high-potency carcinogens (cohort of concern) that must be controlled at or below compound-specific acceptable limits. Acceptable intake limits are also being established by regulatory agencies in various countries for an increasing number of nitrosamines, so there is a demand for analysis methods that can quantitate a larger number of nitrosamine compounds.

This article presents a case example in which the LCMS-8060NX triple quadrupole mass spectrometer was used to perform a simultaneous analysis of ten components including nine nitrosamines regulated by the U.S. Food and Drug Association (FDA) and European Medicines Agency (EMA), and N-Nitrosodi-n-propylamine (NDPA) regulated by the European Pharmacopoeia (Ph. Eur.) Commission.

■ Samples

Nitrosamine Standard

A mixed standard stock solution was prepared by dissolving and combining commercially available nitrosamine standards in methanol. The mixed standard stock solution was diluted stepwise with ultrapure water/methanol (9:1) to prepare calibration curve samples in the range of 0.1 to 25 ng/mL.

Active Pharmaceutical Ingredient

An active pharmaceutical ingredient sample ("API i") was prepared for analysis based on the method described by the Taiwan Food and Drug Administration (TFDA).[2] Extraction times and other details of the TFDA method were altered based on the use of an external standard for evaluation instead of an internal standard. The preparation workflow for the API sample is shown in Table 1. (Concentration of extracted API: 50 mg/mL.)

Weigh 250 mg API in a 25 mL centrifuge tube
↓
[Non-spiked sample] Add 0.25 mL of methanol
[Spiked sample] Add 0.25 mL of standard solution in methanol
↓
Add 0.25 mL of methanol
↓
Vortex for 10 sec.
↓
Sonicate for 15 min.
↓
Add 4.5 mL of water
↓
Sonicate for 15 min.
↓
Centrifuge (at 10,000 g for 5 min., in 4 °C)
↓
Filter the supernatant using 0.22 µm PTFE syringe filter

Fig. 1 API Sample Preparation Workflow

■ Analytical Conditions

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

Table 1 LCMS Analytical Conditions

HPLC conditions (Nexera™ X3)	
Column:	Shim-pack Velox™ Bipheny*1 (100 mmL. × 2.1 mm I.D., 2.7 µm)
Mobile Phases:	A) H_2O/Formic acid = 1000 : 0.5 B) Methanol/Formic acid = 1000 : 0.5
Gradient Program:	B conc. 1.0 % (0.00 - 1.20 min.) – 35.0 % (2.00 min.) – 60.0 % (5.30 - 7.00 min.) – 98.0 % (7.01 - 10.00 min.) – 1.0 % (10.01 - 14.00 min.) Valve position : loading (0 min.) - analysis (0.25 min.) - loading (4.65 min.) - analysis (4.9 min.) - loading (7.0 min.)
Flowrate:	0.60 mL/min.
Column Temp.:	45 °C
Injection Volume:	20 µL
Detection:	254 nm (SPD-M40, Semi micro cell)
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: 227-32015-03
*2 Hydrocarbon filter used on nitrogen gas line (P/N: 225-42793-01)

Table 2 MS/MS Parameters

Compound	Polarity	Precursor ion *m/z*	Product ion *m/z*	Collision Energy (V)
MeNP	+	130.1	58.0	-21
		130.1	100.1	-11
NDMA	+	75.1	42.9	-16
		75.1	57.6	-15
NMOR	+	117.1	87.2	-12
		117.1	73.1	-13
NMBA	+	147.1	43.6	-16
		147.1	116.4	-10
NDEA	+	103.1	29.1	-14
		103.1	74.8	-12
NEIPA	+	117.1	75.2	-10
		117.1	43.0	-16
NDIPA	+	131.1	43.1	-13
		131.1	89.2	-11
NDPA	+	131.1	89.2	-10
		131.1	43.1	-15
NMPA	+	137.1	66.1	-21
		137.1	107.1	-16
NDBA	+	159.1	57.2	-14
		159.1	41.2	-22

■ Analysis of Mixed Nitrosamine Standard

The calibration curve samples were analyzed six times and the linearity and repeatability of the results were investigated.

Fig. 2 shows a typical chromatogram obtained from the 10 ng/mL mixed nitrosamine standard. Calibration curves prepared using an external standard method are also shown in Fig. 3 and calibration curve ranges and correlation coefficients (R) are shown in Table 3. This analysis method provided good linearity as shown by a correlation coefficient (R) > 0.99 for all nitrosamines.

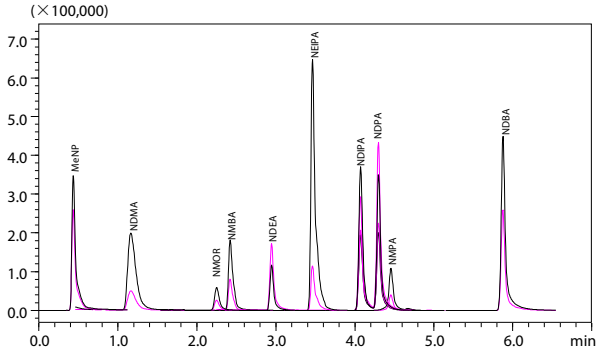


Fig. 2 Chromatogram of 10 ng/mL Mixed Nitrosamine Standard

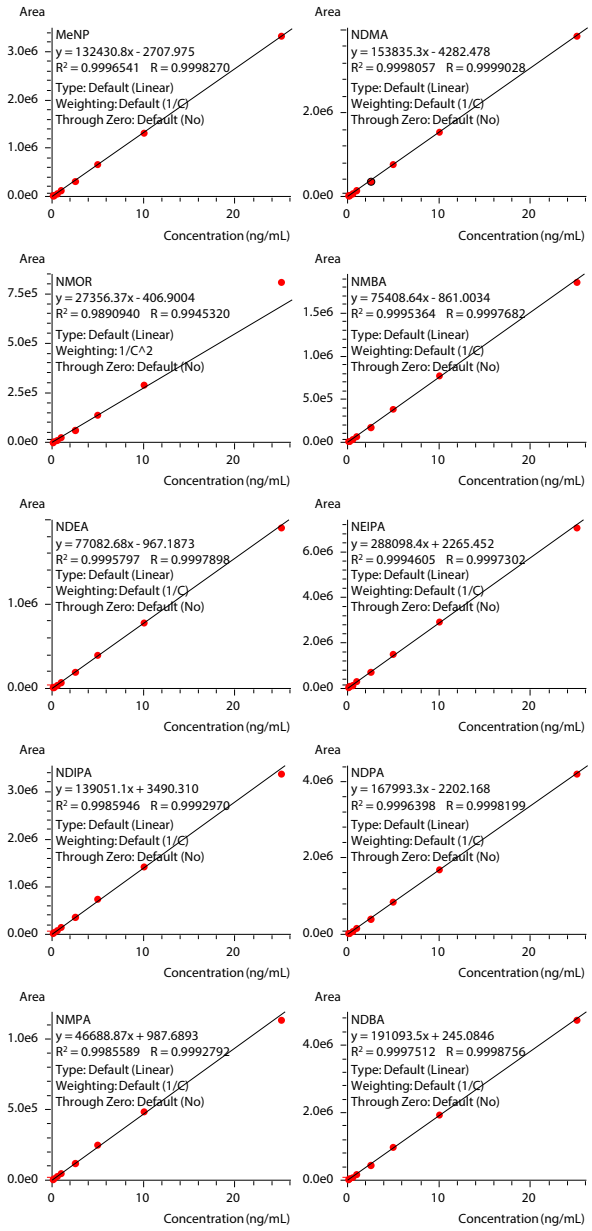


Fig. 3 Calibration Curves

Table 3 Calibration Curve Range and Correlation Coefficient (R)

Compound	Calibration curve (ng/mL)			Correlation coefficient (R)
MeNP	0.1	-	25	0.999
NDMA	0.1	-	25	0.999
NMOR	0.1	-	25	0.994
NMBA	0.1	-	25	0.999
NDEA	0.1	-	25	0.999
NEIPA	0.1	-	25	0.999
NDIPA	0.1	-	25	0.999
NDPA	0.1	-	25	0.999
NMPA	0.1	-	25	0.999
NDBA	0.1	-	25	0.999

* Weighting factor of $1/C^2$ on NMOR calibration curve and 1/C on all other calibration curves.

Table 4 shows repeatability (conc. %RSD) and accuracy after analyzing the 0.1 ng/mL mixed nitrosamine standard six times. Accuracy was between 80 and 120 % and repeatability was within 10 % for all nitrosamines.

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

Compound	Concentration (ng/mL)	Repeatability (Conc. %RSD, n = 6)	Accuracy (Average, n = 6)
MeNP	0.1	7.7	110.5
NDMA	0.1	6.9	109.3
NMOR	0.1	9.6	104.3
NMBA	0.1	6.4	105.2
NDEA	0.1	8.3	104.7
NEIPA	0.1	4.0	97.0
NDIPA	0.1	7.1	85.8
NDPA	0.1	3.3	109.4
NMPA	0.1	7.8	83.7
NDBA	0.1	7.2	101.5

■ Analysis of Nitrosamines in an Active Pharmaceutical Ingredient

Nitrosamines were added to an API sample (“API i”) at concentrations of 0.01 ppm, 0.02 ppm, and 0.1 ppm and a spike recovery test was performed. The PDA and MS chromatograms of the 0.1 ppm spiked sample are shown in Fig. 4. The chromatograms show complete separation between API i and nitrosamines under these analytical conditions.

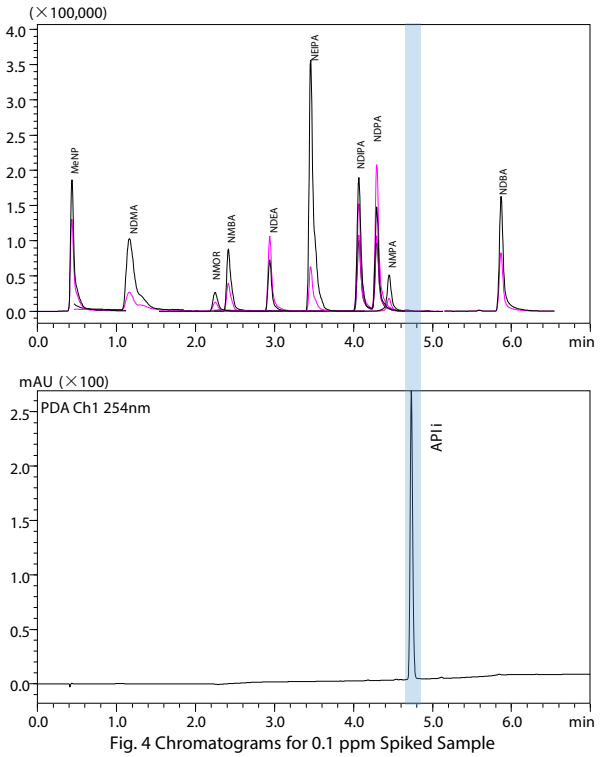
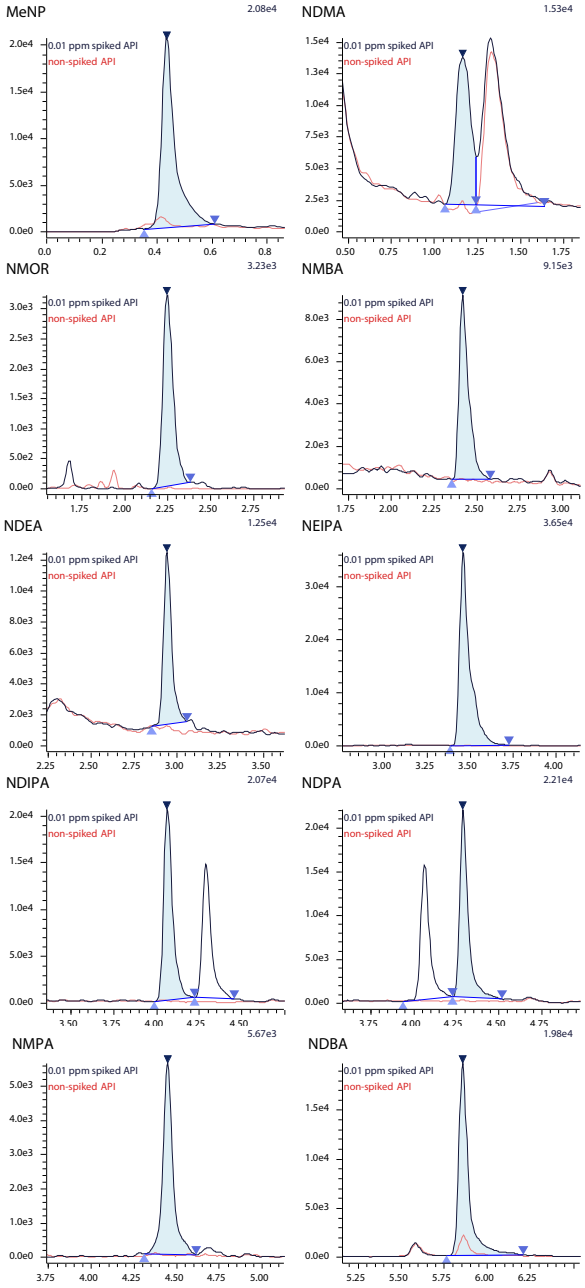


Fig. 4 Chromatograms for 0.1 ppm Spiked Sample

Fig. 5 shows the chromatogram for the extracted API sample superimposed with the chromatogram for the extracted API sample spiked with nitrosamines at 0.01 ppm. In the MRM transition for NDMA a contaminant peak attributed to the API was detected, but separation was achieved using a Shim-pack Velox Biphenyl column. NDMA was identified and quantitated by dividing the NDMA peak and the contaminant peak vertically.



Black: Extracted API spiked with 0.01 ppm of nitrosamine
Red: Unspiked extracted API

Fig. 5 Chromatograms (MRM) of Extracted API and Extracted API Spiked with 0.01 ppm of Nitrosamine

The results of the spike recovery test at each spiked concentration are shown together in Tables 5 and 6. Good spike recoveries within ±15 % were obtained for all nitrosamines other than NDBA. Spike recovery was in the 70 % range for NDBA and further investigation into sample preparation is required to improve this result.

Repeatability was also excellent with an RSD below 10 % for all nitrosamines at all concentrations in the API.

Table 5 Spike Recovery Rates

Compound	0.5 ng/mL (in vial) 0.01 ppm (in API)	1 ng/mL (in vial) 0.02 ppm (in API)	5 ng/mL (in vial) 0.1 ppm (in API)
MeNP	105.0	104.2	104.4
NDMA	96.0	95.3	92.0
NMOR	93.6	94.5	98.7
NMBA	92.0	95.6	98.6
NDEA	102.5	101.3	100.6
NEIPA	98.4	101.3	100.1
NDIPA	96.8	98.9	99.2
NDPA	90.9	90.0	91.3
NMPA	91.6	92.3	89.0
NDBA	77.0	73.4	70.9

Table 6 Repeatability of Spike Recovery Test (n = 6, Conc. %RSD)

Compound	0.5 ng/mL (in vial) 0.01 ppm (in API)	1 ng/mL (in vial) 0.02 ppm (in API)	5 ng/mL (in vial) 0.1 ppm (in API)
MeNP	3.0	2.1	3.0
NDMA	1.8	2.2	2.1
NMOR	6.2	5.6	4.0
NMBA	4.5	3.7	1.6
NDEA	7.2	8.7	6.7
NEIPA	6.6	4.6	6.0
NDIPA	7.6	4.4	6.6
NDPA	6.6	5.4	5.8
NMPA	3.8	6.5	4.9
NDBA	5.6	2.8	2.7

■ Conclusion

- The LCMS-8060NX triple quadrupole mass spectrometer was used to develop a method for the quantitative analysis of 10 nitrosamines in an API.
- The analysis method provided good linearity for all nitrosamines in the range of 0.1 to 25 ng/L (equivalent to 0.0002 to 0.5 ppm in API), as shown by correlation coefficients of R > 0.99.
- Accuracy was between 80 and 120% and repeatability was within 10 % at a nitrosamine concentration of 0.1 ng/mL, showing this method can be used to analyze very low nitrosamine levels.
- The results show that this analysis method enabled the quantitative analysis of all 10 targeted nitrosamines at levels of 0.03 ppm and below in the API sample (“API i”).

■ References

1) International Council for Harmonisation M7 (R1), Addendum: Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk
2) Method of Test for Nitrosamines in Medicines - Multiple Analysis (LC-MS/MS Method) (RA01I004.002) https://www.fda.gov.tw/tc/includes/GetFile.ashx?id=f637417502730979011 (Accessed on May 15, 2022)

* Results will vary depending on the analytical setup, etc.
The data presented herein is a case example and not a guarantee of results.

01-00400-EN First Edition: Sep. 2022

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/