Liquid Chromatograph Mass Spectrometer LCMS™-8045

Application News

A Direct Method for the Determination of 13 Nitrosamines in Water by LCMS-8045 System

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User Benefits

◆ Enables estimation of nitrosamines in water without any sample pretreatment
◆ This method can also be employed for the waste-water containing high organic loads

■ Introduction

Overview : Nitrosamines (especially NDMA) have been monitored by FDA since 2018, because Nitrosamines may increase the risk of cancer. There are several recalls regarding NDMA detection in pharmaceutical products. Also, the presence of nitrosamines, primarily NDMA, is common in water and foods, including cured and grilled meats, dairy products, and vegetables.

Occurrence of NDMA in water (including drinking, recycled and non-potable waters) is primarily driven by chloramination and ozonation of water that contains NDMA precursors. Industrial wastewater effluents from the manufacturing of cosmetics, pharmaceuticals, personal care products, rubber and tires, food and beverages, among others, contribute to the presence of NDMA precursors in municipal wastewater and other non-potable waters. Reported NDMA concentrations in these samples range from 5 – 20 ng/L all the way up to 1000 ng/L[1] .The World Health Organization (WHO) has set a world guideline for NDMA at 100 ng/L in drinking water and similar guidelines have been established in different countries. However, industrial or municipal effluent guidelines for nitrosamines have not been established yet.

US EPA Method 521 uses SPE followed by GC-MS/MS and is used for low level nitrosamine analysis in drinking water.

However, SPE methods present several drawbacks for the extraction of nitrosamines from water samples containing suspended solids as particulates may clog the cartridge and prevent elution of the sample.

In addition, high organic loads consume active sites of the SPE cartridge lowering the adsorption and recovery of analytes by SPE. Also, the concentration of the SPE eluent may cause the loss of NDMA due to its high volatility. A method is therefore needed for water samples with high TOC (Total Organic Carbon) and TSS (Total Suspended Solids) content.

This application note describes ASTM D8456 a method for the analyses of thirteen nitrosamines using LC-MS/MS directly from water without any SPE pretreatment.

■ Experimental

Thirteen nitrosamines (Table 1) were analyzed using an Ultra High Performance Liquid Chromatograph (UHPLC) Nexera™ XS coupled with an LCMS-8045, a Triple Quadrupole Mass Spectrometer from Shimadzu Corporation, Japan (Fig. 1).
LCMS-8045, sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity™), ultra fast scanning speed of 30,000 u/sec (UFscanning™) and polarity switching speed of 5 msec (UFswitching™). This system ensures highest quality of data, with very high degree of reliability.

All nitrosamines were easily ionized by Atmospheric Pressure Chemical Ionization (APCI) interface.

Fig. 1 Nexera™ XS with LCMS™-8045 system

Table 1 List of Nitrosamines

Sr.No.	Name	Abbreviations	CAS NO
1	N-Nitroso dimethylamine	NDMA	62-75-9
2	N-Nitroso morpholine	NMOR	59-89-2
3	N-Nitroso-N-methyl-4-aminobutyric acid	NMBA	61445-55-4
4	N-Nitroso pyrrolidine	NPYR	930-55-2
5	N-Nitroso ethylmethylamine	NMEA	10595-95-6
6	N-Nitroso diethylamine	NDEA	55-18-5
7	N-Nitroso piperidine	NPIP	100-75-4
8	N-Nitroso isopropylethylamine	NEIPA	16339-04-1
9	N-Nitroso diisopropylamine	NDIPA	601-77-4
10	N-Nitroso di n-propyl amine	NDPA	621-64-7
11	N-Nitroso dibutylamine	NDBA	924-16-3
12	N-Nitrosomethylphenylamine	NMPA	614-00-6
13	N-Nitrosodiphenylamine	NDPhA	86-30-6
14	N-Nitroso dimethylamine-d6	NDMA-d6	17829-05-9
15	N-Nitroso diethylamine-d10	NDEA-d10	1219794-54-3

■ Method

The MRM transitions of 13 nitrosamines and 2 internal standards are shown in Table 2 and analytical conditions in Table 3. The diverter valve setting is shown in Table 4.

Table 2 MRM transitions of nitrosamines

Nitrosamine Impurity	Type	ISTD Group	MRM (Quantifier)	MRM (Qualifier)
		MRM Transitions		
NDMA	Target	1	75>43	75>58
NMOR	Target	2	TIC (117>87, 117>45,117>28)	-
NMBA	Target	1	147>117	147>44
NPYR	Target	2	101>55	101>41
NMEA	Target	2	89>61	89>43
NDEA	Target	2	103>29	103>45
NPIP	Target	2	115>69	115>41
NEIPA	Target	2	117>75	117>27
NDIPA	Target	2	131>89	131>43
NDPA	Target	2	131>89	131>43
NDBA	Target	2	159>41	159>29
NMPA	Target	2	137>66	137>107
NDPhA	Target	2	170>93	170>65
NDMA-d6	ISTD	1	81>46	-
NDEA-d10	ISTD	2	113>34	-

Table 3 Analytical conditions

HPLC System	: Nexera XS
Column	: Shim-pack™ GIST C18 (150 mm x 4.6 mm, 5µ) (P/N :227-30017-07)
Column Oven	: 40 °C
Mobile Phases	: A-0.1% Formic acid in Water;
Flow Rate	B-0.1% Formic acid in Methanol
Gradient program	: 0.7 mL/min
(B%)	: 0.01-1.5 min →15(%); 1-3.5 min → 15-70(%); 3.5-11 min →70-90 (%);11-11.1 min→90-15 15 min→STOP
Injection Volume	: 300 µL
Autosampler Temperature	: 15 °C
LCMS System	: LCMS-8045
Interface	: APCI
Temperature	: Interface: 300 °C Desolvation Line: 180 °C Heater Block: 200 °C
Gas Flow	: Nebulizing Gas: 4 L/min Drying Gas: 5 L/min

Table 4 Diverter Valve Settings

Time (Min)	Divert Valve Position
0.0	To waste
3.4	To Mass Spectrometer

■ Linearity of the nitrosamines

A representative MRM chromatogram for 13 nitrosamines with 2 ISTDs (Internal standards) is shown in Fig. 2.

1:NDMA-D6 81.2000>46.0000(+) CE: -21.0(2.00)
2:NDMA 75.1000>43.0000(+) CE: -20.0(2.50)
3:NMOR TIC(+)(3.00)
4:NMBA 147.2000>117.0000(+) CE: -11.0(3.00)
5:NPYR 101.1000>55.1000(+) CE: -20.0(1.50)
6:NMEA 89.1000>61.0000(+) CE: -15.0(2.50)
7:NDEA-D10 113.2000>34.1000(+) CE: -18.0(4.00)
8:NDEA 103.1000>29.2000(+) CE: -17.0(1.50)
9:NPIP 115.1000>69.1000(+) CE: -13.0
10:NEIPA 117.1000>75.1000(+) CE: -13.0
11:NDIPA 131.2000>89.1000(+) CE: -10.0(4.00)
12:NMPA 137.1000>66.1000(+) CE: -22.0(5.00)
14:NDBA 159.2000>41.2000(+) CE: -21.0(4.00)
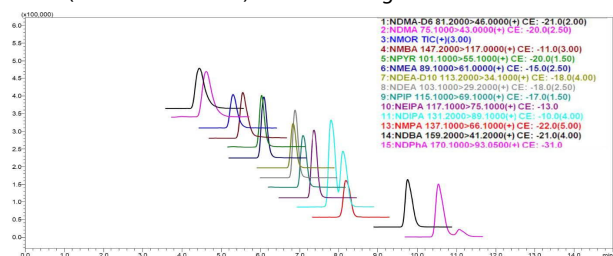15:NDPhA 170.1000>93.0500(+) CE: -31.0

Fig. 2 Representative MRM chromatogram of 13 nitrosamines and 2 ISTDs

The calibration curves for 13 nitrosamines were prepared from 0.05 µg/L to 5.00 µg/L. The calibration curves of the nitrosamines are shown in the Fig. 3 to Fig. 7. Results of nitrosamines linearity and repeatability at 0.05 µg/L is shown in Table 5.
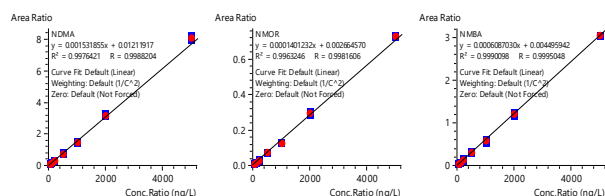

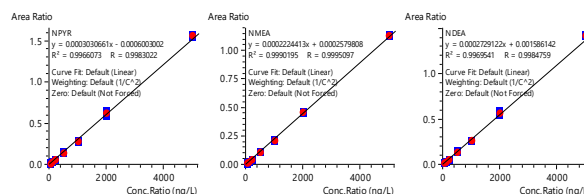
Fig.3 Calibration Curves of NDMA, NMOR and NMBA

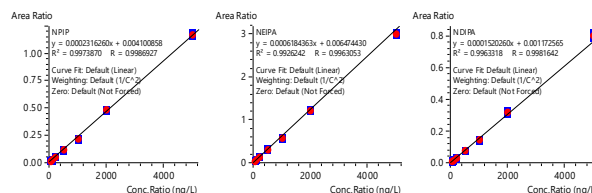Fig.4 Calibration Curves of NPYR, NMEA and NDEA

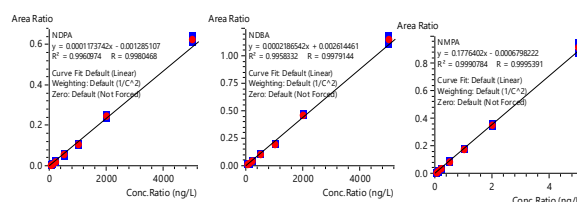Fig.5 Calibration Curves of NPIP, NEIPA and NDIPA
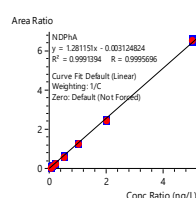

Fig.6 Calibration Curves of NDPA, NDBA and NMPA

Fig.7 Calibration Curve of NDPhA

Out of all these nitrosamines, NDPhA is unique molecule due to its fragile nature. The bond energy between the two nitrogen atoms is only 11 kcal/mole. This value is quite small when compared to the N-NO bond strength of dimethylnitrosamine which is 52 kcal/mole and the 70 to 90 kcal/mole bond dissociation energies of the C-N, C-C and C-H bonds in most organic molecules[2].

The comparative instability of NDPhA is demonstrated by the relative ease with which the agent can transfer its nitroso (NO) moiety to other amines. This process is known as transnitrosation and it can occur in dilute acid or organic solvents upon mild heating as mentioned in Fig. 8 [3].
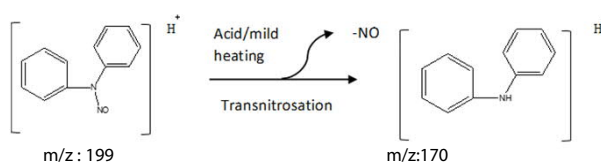

m/z : 199   m/z:170

Fig.8 Transnitrosation of NDPhA

Hence, due to the instability of m/z : 199 under LCMS conditions; for the MRM optimization m/z : 170 was taken as a precursor for NDPhA.

Table 5 Nitrosamines linearity and repeatability at 0.05 µg/L

Nitrosamines	Linearity	%RSD of area at 0.05 µg/L (n=6)
NDMA	0.997	12.92
NMOR	0.996	9.46
NMBA	0.999	8.40
NPYR	0.996	6.72
NMEA	0.999	5.82
NDEA	0.996	7.43
NPIP	0.997	5.05
NEIPA	0.992	6.85
NDIPA	0.996	7.13
NDPA	0.996	6.47
NDBA	0.996	4.14
NMPA	0.999	9.97
NDPhA	0.999	5.22

■ Sample Analysis

Synthetic effluent samples prepared in accordance to ASTM were used to demonstrate the method performance in samples. The effluent sample was spiked with four levels of nitrosamines: 0.05 µg/L, 0.10 µg/L, 0.50 µg/L and 1.70 µg/L, and then recovery was calculated. All samples were spiked with two ISTDs, NDMA-d6 and NDEA-d10, at concentration of 2 µg/L. Samples were filtered through 0.2 µm syringe filter into HPLC vials. Recovery results are shown in Table 6.

Table 6 The sample spiked study at four levels

Nitrosamines	% Recovery			
	0.05 µg/L	0.1 µg/L	0.5 µg/L	1.7 µg/L
NDMA	96.1	96.1	101.0	96.2
NMOR	59.1	90.2	96.2	91.2
NMBA	76.1	95.9	102.0	92.6
NPYR	109.0	93.6	96.9	91.2
NMEA	93.8	104.0	96.9	93.5
NDEA	113.0	103.0	106.0	98.9
NPIP	67.4	85.0	105.6	91.4
NEIPA	70.9	91.8	93.0	88.1
NDIPA	100.0	101.0	102.8	99.1
NDPA	135.0	96.5	119.4	96.5
NDBA	85.5	90.3	146.2	93.2
NMPA	122.0	108.0	113.0	102.0
NDPhA	86.5	81.7	85.8	83.5

■ Conclusions

▪ A direct LC-MS/MS quantification method for 13 nitrosamines in non-potable water has been successfully developed. The performance of Shimadzu LCMS-8045 system in demonstrated.

▪ A seven levels linearity study was performed for all 13 nitrosamines by an internal standard method.

▪ Correlation coefficient was greater than 0.99 for all the nitrosamines.

▪ The repeatability (n=6) at 0.05 µg/L level was found to be less than 25 %RSD.

■ References

1. Stuart W. Krasner and Ruth Marfil-Vega, Occurrence of nitrosamines and their precursors in NorthAmerican drinking waters, AWWA Water Science, (2020).
2. Waltham, Massachusetts, Instruction Manual - Thermal Energy Analyzer, Model TEA 502/LC, Thermal Electron Corporation,12-77 (1985).
3. Buglass, A.J., Challis, B.C. and Osborne, M.R. "IARC Scientific Publication No. 9" Bogovski, P., and Walker, E. A. Eds., 94-100 (1974).

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