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A New ASTM Method for the Determination of Nitrosamines in Water by Direct Injection LC-MS/MS

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Why would we need a LC-MS/MS nitrosamines method?

OSHA Hazard Information Bulletins
N-Nitrosamine in the Rubber Industry

Discovery of nitrosamine impurities in Active Pharmaceutical Ingredients

Distribution of Seven N-Nitrosamines in Food

Potential for the Formation of N-Nitrosamines During the Manufacture of Active Pharmaceutical Ingredients: An Assessment of the Risk Posed by Trace Nitrite in Water

Current Water Methods for Nitrosamines – Method 521

250 mL sample

Ion Trap GCMS

Table 1. Detection Limits and Lowest Concentration Minimum Reporting Levels [a, b, c]

Analyte	DL (ng/L)	LCMRL (ng/L)
NDMA	0.28	1.6
NMEA	0.28	1.5
NDEA	0.26	2.1
NPYR	0.35	1.4
NDPA	0.32	1.2
NPIP	0.66	1.4
NDBA	0.36	1.4

a. DLs determination from LFBs fortified at 1.0 ng/L (N = 8).
b. LCMRLs determined from LFBs fortified at 1.0, 2.0, 3.0, and 4.0 ng/L (N = 5 or 6 at each concentration).
c. DL and LCMRL data were obtained on a Varian Saturn 4 GC/MS/MS.

Current Water Methods for Nitrosamines – Method 625.1

1000 mL sample

Single Quadrupole GCMS

Current Water Methods for Nitrosamines – Method 607

1000 mL sample

GC with Nitrogen Detector

USP 1469 – Extraction then Direct Injection LC-MS/MS

1 gram + 5 ml Extraction solution

Cleanup if necessary

Extracted sample

Triple Quadrupole LCMS

ASTM D8456 – Direct Injection LC-MS/MS (also meets USP 1649)

~ 3 mL sample

Triple Quadrupole LCMS

ASTM D8456 Determination of Nitrosamines in Water by Liquid Chromatography Tandem Mass Spectrometry

(LC-MS/MS) detection and quantitation of N-nitrosamines after direct injection.

groundwater, surface water, wastewater influents, and wastewater effluents.

Not limited to these aqueous matrices; however, the applicability of this test method to other aqueous matrices must be demonstrated

Table 1 lists the compounds that have been validated

method is not limited to the compounds listed in Table 1

Method LOQ dependent on instrument and dilution

0.05 µg/L up to 5 µg/L

Lower concentrations may be possible with a more sensitive LC-MS/MS

Shimadzu’s least sensitive LC-MS/MS used for this validation

Comparison of the nitrosamine method LOQs

	Method 521	625.1	607	USP 1469	D8456
	LCMRL (µg/L)	MDL (µg/L)	MDL (µg/L)	LOQ (µg/L)	LOQ (µg/L)
NDMA	0.0016	No data	0.15	1.3	0.05
NMEA	0.0015	No data			0.05
NDEA	0.0021	No data		0.66	0.05
NDPA	0.0014	No data	0.46		0.05
NDBA	0.0012	No data	0.81	1.3	0.05
NPYR	0.0014	No data			0.05
NPIP	0.0014	No data			0.05
NMOR		No data			0.05
NEIPA		No data		1.3	0.05
NDIPA		No data		1.3	0.05
NMBA		No data		1.3	0.05
NMPA		No data			0.05
NDPhA		No data			0.05

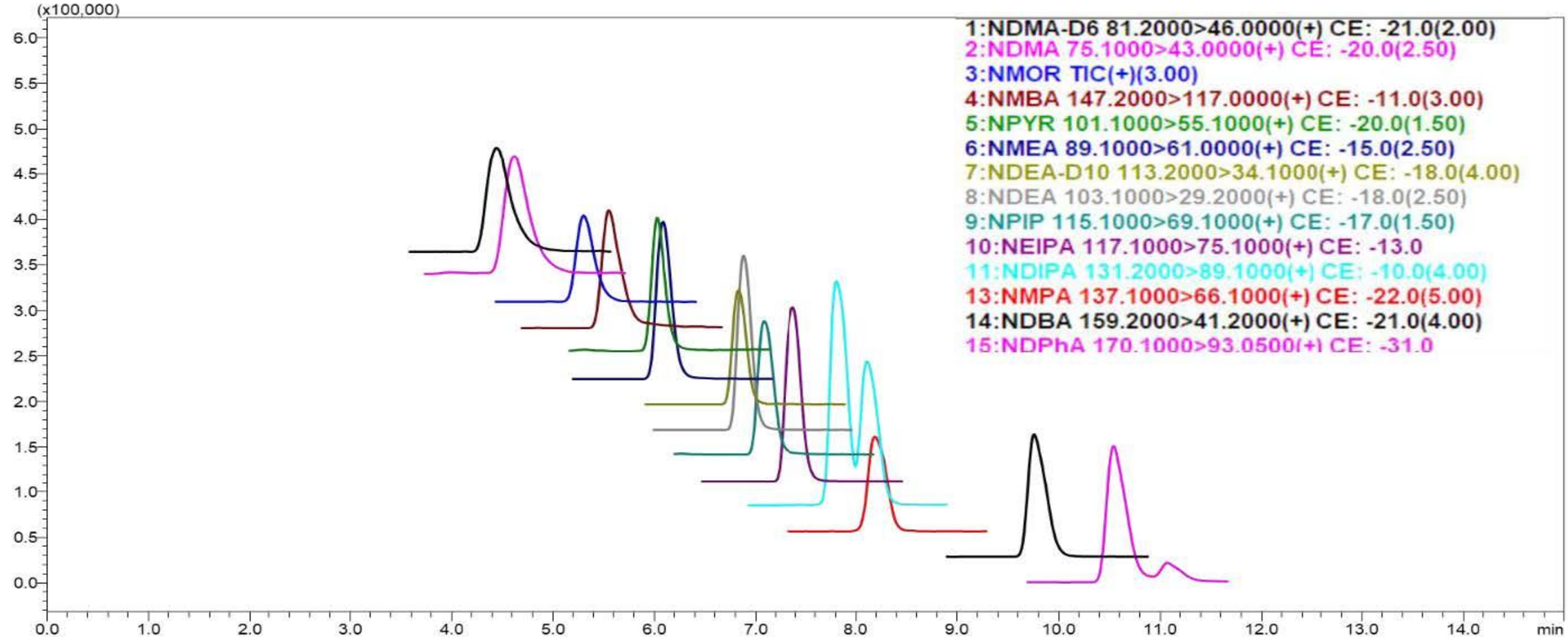
ASTM D8456 Chromatographic conditions

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
	: 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

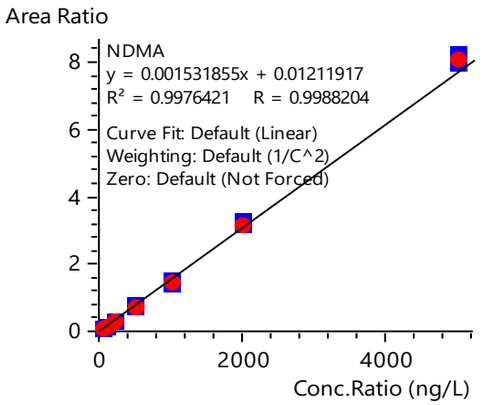
MS/MS conditions for ASTM D8456

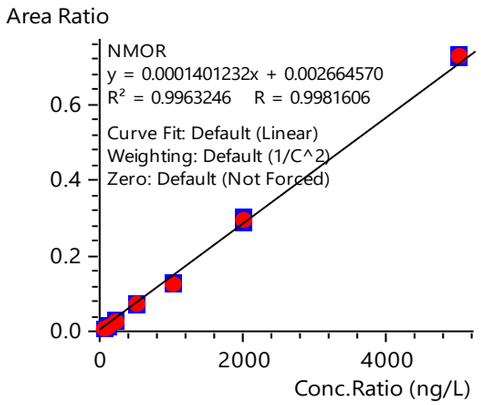
MRM Transitions				
Nitrosamine Impurity	Type	ISTD Group	MRM (Quantifier)	MRM (Qualifier)
NDMA	Target	1	75>43	75>58
NMOR	Target	2	TIC (117>87, 117>45,117>28)	-
NMBA	Target	1	147>117	147>44
NPYR	Target	2	101>55	101>41
NMEA	Target	2	89>61	89>43
NDEA	Target	2	103>29	103>45
NPIP	Target	2	115>69	115>41
NEIPA	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	-

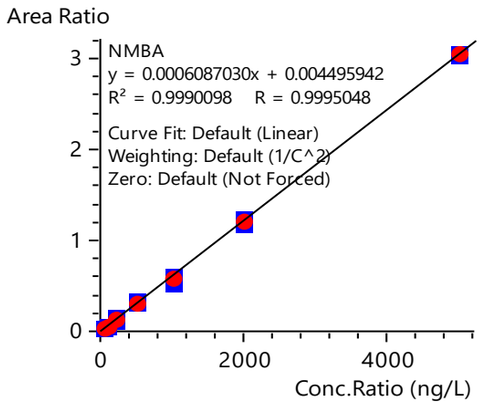
MRM Chromatograms. MRMs, and CE for D8456

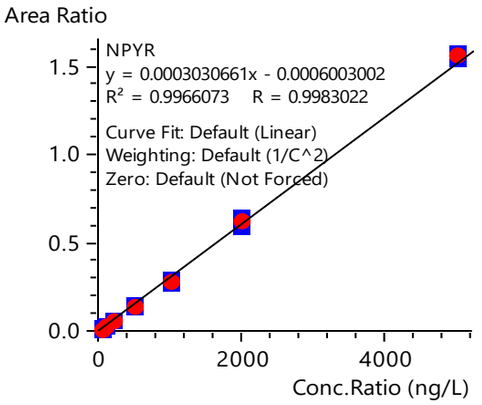


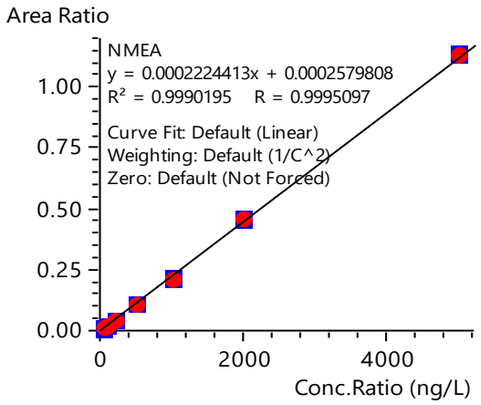
Calibration curves showing linearity in the range of the method

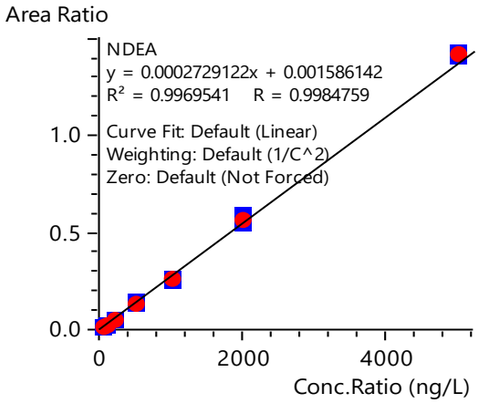


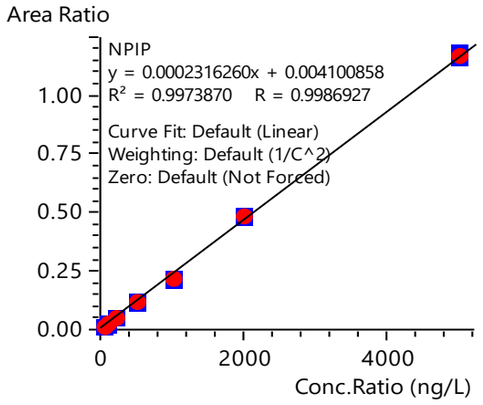


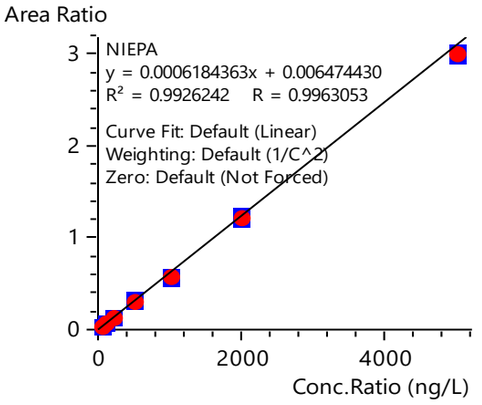




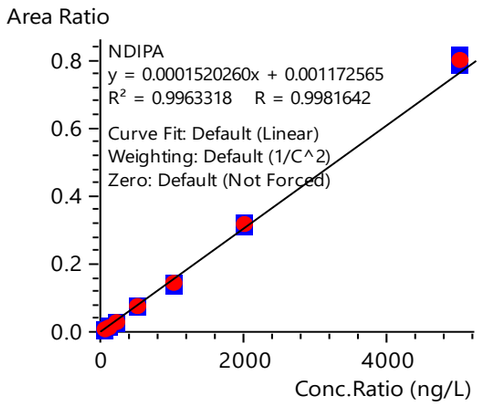


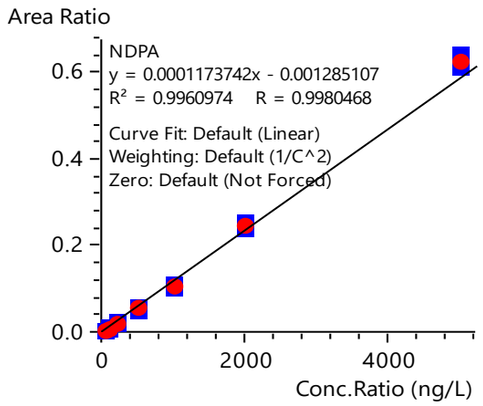


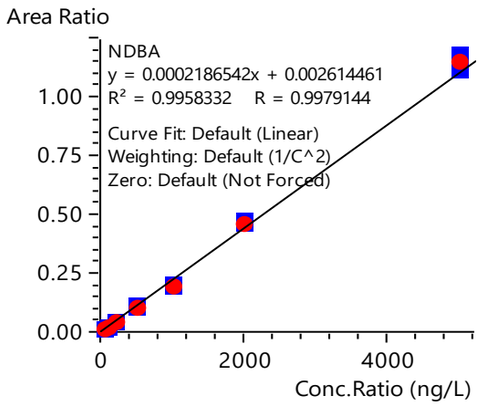


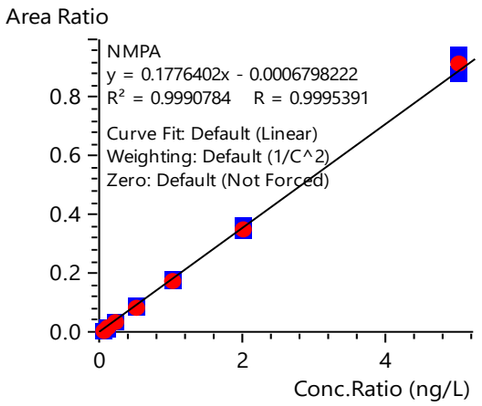


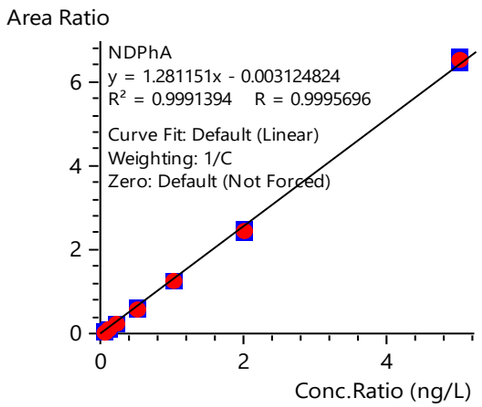
Calibration curves showing linearity in the range of the method - continued











Isotope dilution allowed if there are standards

Internal standard is added to a known volume of sample.

The sample is injected into LC-MS/MS system operated with an APCI source.

Alternatively, extract an isotopically labelled analog of each analyte (isotope dilution), if available, and correct for recovery.

Sample Collection and Preservation

Collect all chlorinated samples in 40 mL amber glass bottles containing ~3 mg of sodium thiosulfate crystals.

Unchlorinated samples do not require sodium thiosulfate.

The samples must be chilled to above freezing but not to exceed 6 °C

Store samples at above freezing to 6 °C until analysis.

Analyze all samples within 14 days of collection.

Sample preparation for D8456

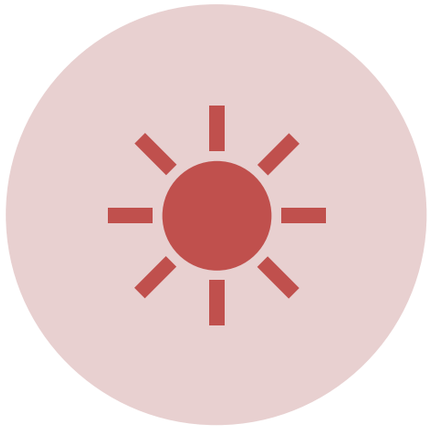
60 µL ISTD

2940 µL sample

Initial Demonstration of Capability

5 – 7 REPLICATES AT 2 UG/L

70 – 130% RECOVERY



≤ 20% RSD

Required Batch QC samples

Calibration Verification	Method Blank	RL Check	LCS (potentially LCS Duplicate)	Sample 1	Sample 1 spike	Sample 1 duplicate spike (optional)	Sample 2 – 20	Calibration Verification

Precision at the 0.05 µg/L LOQ

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

Single laboratory repeatability in 3000 mg/L TDS (from seawater salts)

Nitrosamines	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

Comparison of QC requirements of Methods

	Method 521	625.1	607	USP 1469	D8456
Accuracy (%) Mid range	70-130	60 – 140?	13 – 109 D - 139 45 - 146	70 - 130	70 - 130
Accuracy (%) LOQ	50 - 150				50 - 150
RSD (%)	30	No data	17 30.5 28.5	25	20
Surrogates Recovery (%)	70 - 130	No data	NA	NA	70 - 130
Internal Standard Area (%)	50 - 150	No data	NA	50 - 150	50 - 150

Conclusions and next steps

- A LC-MS/MS method has been developed for the direct determination of nitrosamines in water.
- Recovery and repeatability well within the acceptable criteria at each level.
- Recovery study was performed by spiking the four different levels of the nitrosamine in synthetic wastewater.
- The recoveries of all nitrosamines were found to be well within 70 – 130%.

- Next Step is an Interlaboratory Study

Questions?

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