

Nitrosamine analysis in a variety of food matrices

Lakshmanan Deenadayalan¹, Sashank Pillai¹ and Craig M. Butt²
¹SCIEX, India; ²SCIEX, USA

The technical note describes the analysis of 11 nitrosamine compounds in 6 food matrices (milk, oats, chicken, fried chicken, fish, and prawn) achieving low ng/g detection limits. Using the SCIEX 6500+ QTRAP system, matrix spikes at 10 and 20 ng/g levels showed good accuracy (recovery $\pm 30\%$) and precision (%CV <20%), see Figure 1. Comparison of pre- and post-extraction spikes showed minimal matrix effects demonstrating good data quality when quantifying against the solvent-based calibration curve.

Nitrosamines are formed during food processing through the reaction of organic amines and nitrosating agents¹. Nitrosamines have been detected in a variety of foods, most notably cured meats and fish, beer and cheese². Some nitrosamines have been shown to damage DNA and cause cancer, such as N-nitrosodimethylamine (NDMA)³. A recent risk assessment from the European Food Safety Authority (EFSA) for 10 nitrosamines identified a slight cancer risk from dietary exposure but currently there are no regulatory limits in the European Union for food. However, sensitive, accurate and robust analytical methods for nitrosamines in food are necessary to ensure food safety and protection of human health.

Key benefits of nitrosamine analysis using the QTRAP 6500+ system

- **Low ng/g sensitivity in food matrices.** Good sensitivity observed for 10 and 20 ng/g spikes into 6 diverse food matrices (milk, oats, fried chicken, chicken, fish, and prawn)
- **Broad analyte coverage.** 11 nitrosamine compounds included in the analysis method
- **Method recovery across a diverse range of food matrices.** 10 ng/g matrix spike recoveries ranged from 69% to 123%, indicating good method performance
- **Simple sample preparation results in negligible matrix effects.** Post-spike analyte accuracies responses ranged near 100% when quantified against the solvent-calibration standards, indicating minimal matrix effects

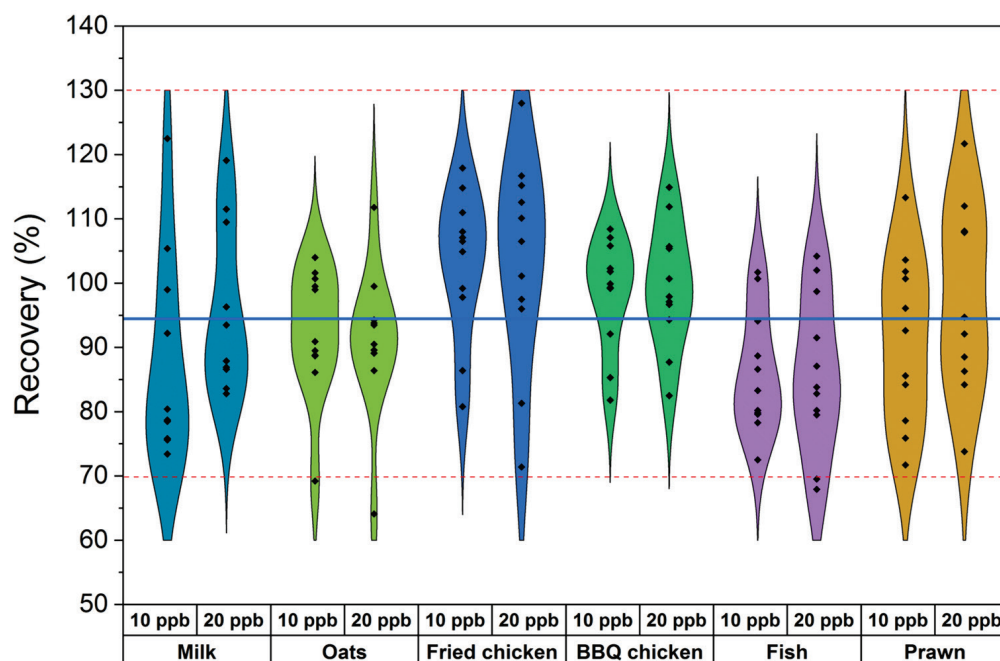


Figure 1. Violin plots showing nitrosamine recovery (%) in 6 food matrices spiked at 10 and 20 ng/g (ppb). Data points represent recoveries for individual nitrosamine compounds. Dashed red line indicate 70% and 130%, solid blue line indicates overall mean recovery of 94%. p 1

Methods

Standard preparation: Neat standards were purchased from VIVAN Life Sciences. Individual stock solutions were prepared in methanol and the final mixed stock solution (1000 ng/mL) was prepared in water.

Sample preparation: Various food items were purchased from a local market (fried chicken, BBQ chicken, fish, prawn, milk, oats). For the solid matrices, 1.0 g was weighed, and 1 mL of acetonitrile (containing 50 ng/mL of internal standard) was added. For the oats, 2 ml of acetonitrile was added. The vials were shaken, vortexed for 15 minutes, and centrifuged at 4500 rpm for 5 minutes. The supernatant was removed, filtered through a 0.2 µm PTFE filter, and then diluted in a 1:9 ratio using water before transferring to an autosampler vial for analysis.

Matrix spiking experiments: Method recovery and matrix effects were evaluated by performing pre- and post-extraction spikes at 10 ng/g and 20 ng/g levels. Samples were prepared in triplicate as described above.

Chromatography: An ExionLC system was used with a [Phenyl Hexyl column \(3.0 x 100 mm, 3.0 µm, 100 Å\)](#) for chromatographic separation. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid methanol. Table 1 summarizes the gradient conditions. The mobile phase flow rate was 0.500 mL/min, the column oven temperature was 40°C and the injection volume was 10 µL.

Table 1. LC gradient conditions for the analysis of 11 nitrosamines in food.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	98	2
1.5	98	2
10	2	98
12	2	98
12.1	98	2
14	98	2

Table 2. Optimized source and gas parameters for analysis of 11 nitrosamines in food.

Parameter	Value
Curtain gas	30 psi
CAD gas	High
Nebulizing current	1 µA
Source temperature	375°C
Ion source gas 1	35 psi

Table 3. MRM conditions and compound-specific parameters for the analysis of nitrosamines in food using the QTRAP 6500+ system. For compounds with two stable transitions the first Q3 and CE values represent the quantifier ion, the second values represent the qualifier ion. Compounds with one Q3 and CE value have one stable transition only.

Compound	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (V)
<i>N</i> -Nitrosodimethylamine (NDMA)	75.0	43.1	40	21
<i>N</i> -Nitrosodibutylamine (NDBA)	159.0	103.0/57	40	15/19
<i>N</i> -Nitrosodi- <i>n</i> -propylamine (NDIPA)	131.1	89.0/43.0	45	12/20
<i>N</i> -Nitrosomethylethylamine (NMEA)	89.0	61.0	20	16
<i>N</i> -Nitrosodiethylamine (NDEA)	103.0	75.0/47.0	70	14/21
4-Nitrosomorpholine (NMO)	117.1	87.1/86	40	23/25
1-Nitrosopiperidine (NPIP)	115.1	69.0/41.0	40	28/33
<i>N</i> -Nitrosomethylphenylamine (NMPA)	137.1	107.2	45	16
Ethyl(nitroso)(propan-2-yl)amine (NEIPA)	117.0	75.0	60	14
4-[methyl(nitroso)amino]butanoic acid (NMBA)	147.0	117.0	60	9
<i>N</i> -Nitrosodiisopropylamine (NDPA)	131.1	89.0/43.0	65	12/20
<i>N</i> -Nitrosodimethylamine D6 (NDMA D6)	81.0	46.0	22	60
4-[methyl(nitroso)amino]butanoic acid D5 (NMBA D5)	150.0	120.0	9	17

Mass spectrometry: The QTRAP 6500+ system was operated in positive ion mode using APCI ionization. The data was collected using MRM acquisition. Table 2 shows the source and gas parameters, and the compound-specific MRM parameters are displayed in Table 3.

Data processing: All data was processed using SCIEX OS software v 2.2. The MQ4 algorithm was used for the quantitation.

Quantitative performance of nitrosamine solvent standards

Calibration standards were injected in triplicate to evaluate the sensitivity, accuracy, precision and linear dynamic range. Considering all calibration levels among the 11 nitrosamine compounds, the mean accuracy was between 89% and 112%, and the %CV was between 3.6% and 17% (Table 5). The solvent standard LOQ concentrations ranged from 0.10 to 0.25 ng/mL demonstrating sub-ng/mL sensitivity for nitrosamines on the QTRAP 6500+ system. Calibration curves showed correlation coefficient values (r^2) >0.998, demonstrating good linearity across the calibration range (Table 5).

Accurate and precise quantitation of nitrosamines in food at 10 and 20 ng/g

Method recovery was evaluated by performing matrix spike experiments before (“pre-spike”, n=3) and after sample preparation (“post-spike”, n=3) at 10 and 20 ng/g. The recovery was calculated as the ratio of the pre- to post-spike area counts. Considering the 10 ng/g matrix spikes the recoveries ranged from 69% to 123%, indicating good method performance across the diverse range of food matrices (milk, oats, fried and bbq

chicken, fish, and prawns). Full recovery data is presented in Table 6 for the 10 ng/g spikes, and XICs for the 10 ng/g spikes in oats are shown in Figure 2. Violins plots summarizing the 10 and 20 ng/g spike recoveries per matrix are shown in Figure 1. Considering the 11 nitrosamine analytes in 6 food matrixes, at two spiking levels (n=132), only 4 of the mean recovery values were outside of the 70-130% criteria. These results highlight the broad applicability of the sample preparation and instrumental analysis methods. Further, the %CV was generally less than 10% for the triplicate samples in both the pre- and post-spikes, indicating good method precision (Table 6).

The method used solvent-based external calibration standards to quantify the matrix spikes. That is, internal standards were not used to correct for matrix effects or recovery losses. Post-spike accuracies were ~100%, demonstrating negligible matrix effects (Table 6). The matrix effects were reduced through modifications to the simple sample preparation procedure including the 10-fold dilution step.

Developing the extraction protocol for the diversity of food matrices in this technical note was challenging, specifically the milk and fish matrices. Regarding the milk samples, the matrix volume was reduced and the extraction time was increased. However, despite this optimization an unknown NMPA interference in milk was observed. In fish samples, interferences were observed when homogenizing the whole fish which we suspected was due to the high calcium concentration from the bones. These interferences were not observed when analyzing the fillet only.

Table 5. Linearity, LOQ, %CV and average accuracy for the analysis of 11 nitrosamines in the solvent standards (n=3).

Compound name	LOQ (ng/mL)	CV (%)	Average accuracy (%)	Linearity range (ng/mL)	Correlation coefficient (r^2)
NDMA	0.25	3.6	97	0.25-50	0.999
NDBA	0.25	4.6	112	0.25-50	0.999
NDIPA	0.25	15	97	0.25-50	0.999
NMEA	0.25	9.2	89	0.25-50	0.999
NDEA	0.10	5.9	96	0.10-50	0.999
NMO	0.25	9.7	104	0.25-50	0.999
NPIP	0.25	17	99	0.25-50	0.998
NMPA	0.10	13	99	0.10-50	0.998
NEIPA	0.25	3.7	105	0.25-50	0.999
NMBA	0.25	15	100	0.25-50	0.999
NDPA	0.10	17	103	0.10-50	0.999

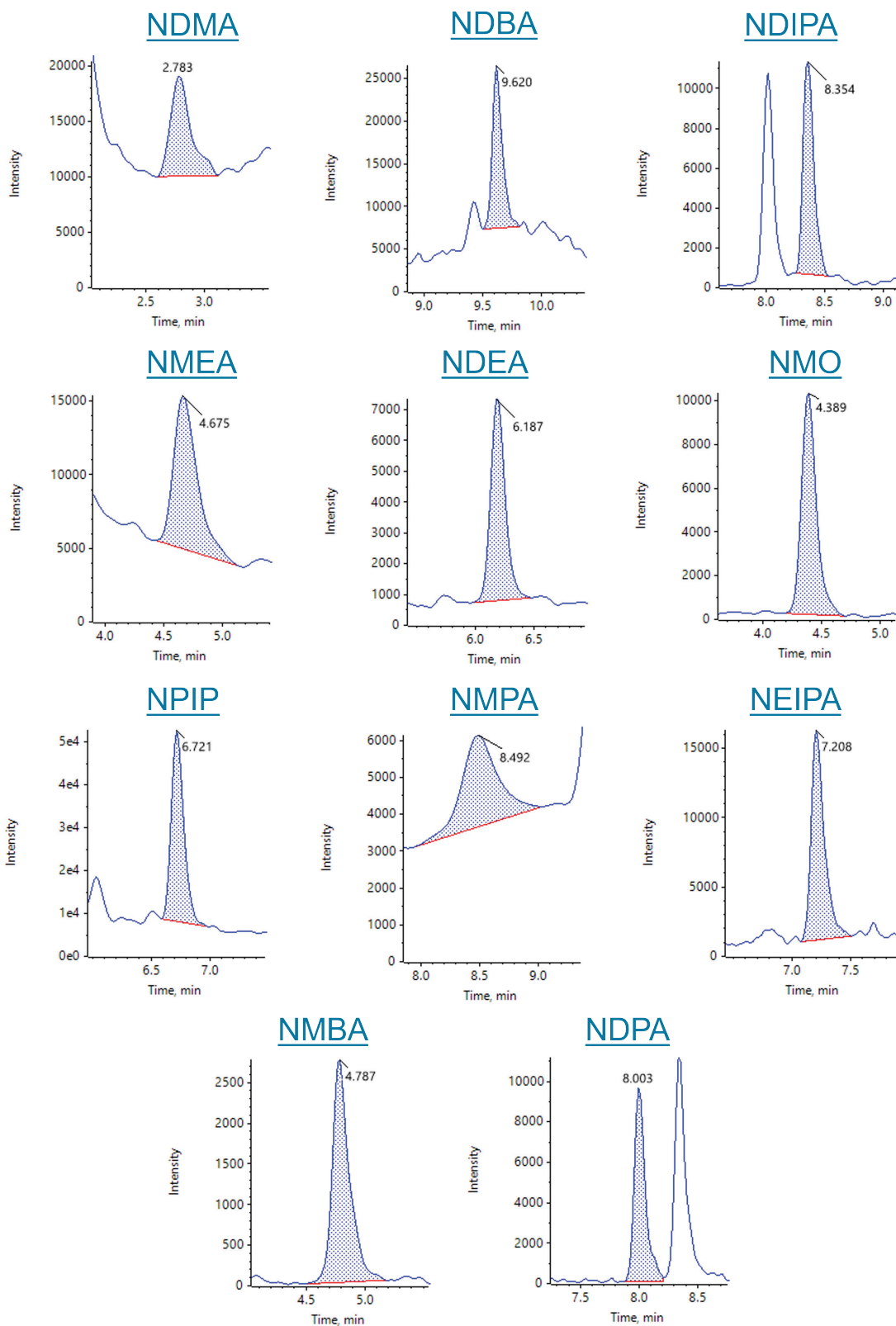


Figure 2. Extracted ion chromatograms (XICs) of 10 ng/g spike for 11 nitrosamine compounds into oats.

Table 6. Average accuracy (%CV) and recovery for the analysis of 11 nitrosamines in various food matrices spiked at 10 ng/g.

Food matrix		Nitrosamine compound										
		NDMA	NDEA	NMBA	NDPA	NDIPA	NDBA	NEIPA	NMPA	NMO	NPP	NMEA
Oats	Pre-spike	96 (1.8)	102 (5.9)	78 (7.9)	100 (4.8)	105 (7.9)	109 (4.5)	103 (5.0)	105 (11)	98 (3.2)	100 (2.8)	102 (3.5)
	Post-spike	95 (1.7)	115 (2.6)	113 (2.6)	101 (2.5)	101 (2.6)	110 (1.7)	114 (1.1)	105 (8.9)	111 (0.8)	117 (3.8)	114 (2.2)
	Recovery	101	88.7	69.0	99.0	104	99.1	90.4	100	88.3	85.5	89.5
BBQ Chicken	Pre-spike	90 (1.6)	106 (8.6)	87 (9.3)	120 (0.4)	120 (2.8)	117 (2.4)	125 (3.2)	111 (4.5)	101 (1.7)	100 (6.2)	110 (4.8)
	Post-spike	88 (4.9)	107 (3.3)	107 (0.5)	111 (0.2)	118 (2.5)	111 (2.7)	116 (2.4)	111 (11)	110 (1.7)	118 (2.3)	111 (1.8)
	Recovery	102	99.1	81.3	108	102	105	108	100	91.8	84.7	99.1
Fried chicken	Pre-spike	93 (11.4)	122 (2.9)	88 (10)	120 (1.1)	118 (3.0)	122 (2.2)	115 (18)	86 (17)	120 (5.8)	121 (2.2)	123 (1.6)
	Post-spike	89 (1.6)	114 (3.1)	109 (1.5)	105 (4.8)	106 (6.0)	104 (2.5)	118 (4.9)	100 (5.5)	112 (4.4)	122 (3.5)	114 (3.4)
	Recovery	105	107	80.7	114	111	117	97.5	86.0	107	99.2	108
Milk	Pre-spike	93 (6.4)	89 (8.4)	86 (12)	105 (7.0)	114 (15)	93 (6.3)	88 (5.6)	n/a	86 (6.1)	91 (10)	85 (2.1)
	Post-spike	93 (3.1)	121 (2.8)	109 (2.8)	100 (3.7)	93 (2.4)	101 (2.6)	116 (2.2)	n/a	109 (2.3)	114 (1.7)	113 (2.2)
	Recovery	100	73.6	78.9	105	123	92.1	75.9	n/a	78.9	79.8	75.2
Prawn	Pre-spike	94 (4.8)	114 (12)	90 (8.1)	117 (3.2)	115 (8.0)	115 (5.7)	103 (6.6)	84 (15)	88 (8.0)	83 (3.9)	93 (2.7)
	Post-spike	90 (0.15)	101 (1.5)	105 (1.2)	115 (1.9)	119 (0.4)	114 (1.9)	112 (1.6)	110 (4.3)	112 (0.2)	116 (1.7)	110 (1.7)
	Recovery	104	113	85.7	102	96.6	101	92.0	76.4	78.6	71.6	84.5
Fish	Pre-spike	93 (6.2)	84 (2.9)	78 (0.8)	109 (1.7)	114 (12)	116 (4.8)	102 (2.5)	88 (14)	88 (1.8)	92 (11)	93 (3.4)
	Post-spike	92 (3.0)	104 (2.3)	107 (3.2)	116 (4.5)	120 (5.0)	114 (4.6)	115 (1.6)	112 (8.7)	111 (2.7)	116 (2.0)	112 (1.2)
	Recovery	101	80.8	72.9	94.0	95.0	102	88.7	78.6	79.3	79.3	83.0

Conclusions

- Broad analyte coverage – 11 different nitrosamine compounds – applied to 6 unique food matrices (milk, oats, fried chicken, chicken, fish, and prawn)
- Good sensitivity observed in matrix spikes at 10 and 20 ng/g across a diverse range of food items
- 10 ng/g matrix spike recoveries ranged from 69% to 123%, indicating good method performance
- Simple sample preparation procedure yielded negligible matrix effects with post-spike analyte accuracies near 100% when quantified against the solvent-calibration standards
- Sub-ng/mL sensitivity in solvent standards with LOQs ranging from 0.10 to 0.25 ng/mL on the QTRAP 6500+ system

The SCIEX clinical diagnostic portfolio is For In Vitro Diagnostic Use. Rx Only. Product(s) not available in all countries. For information on availability, please contact your local sales representative or refer to <https://sciex.com/diagnostics>. All other products are For Research Use Only. Not for use in Diagnostic Procedures.

Trademarks and/or registered trademarks mentioned herein, including associated logos, are the property of AB Sciex Pte. Ltd. or their respective owners in the United States and/or certain other countries (see www.sciex.com/trademarks).

© 2023 DH Tech. Dev. Pte. Ltd. MKT-29889-A



Headquarters
 500 Old Connecticut Path | Framingham, MA 01701 USA
 Phone 508-383-7700
sciex.com

International Sales
 For our office locations please call the division headquarters or refer to our website at sciex.com/offices