Application Note 10315

High Sensitivity Analysis of Nitrosamines Using GC-MS/MS

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Introduction

Nitrosamines is the common term used for compounds of the class of N-nitrosodialkylamines. A large variety of compounds are known and described with different alkyl moieties [1]. The simplest N-nitrosodialkylamine with two methyl groups is the N-nitrosodimethylamine (NDMA). Nitrosamines are in common highly toxic compounds with high cancerogenity for humans and animals, in higher doses leading to severe liver damage with internal bleeding [2,3].

Nitrosamines in food are mainly produced from nitrites. Nitrites are added to food as preservatives in meat and meat products preventing the Botulinus poisoning. Antioxidant food additives like vitamin C can prevent the formation of nitrosamines from nitrites [4]. Another source of nitrosamines is described by the reaction of nitrogen oxides with alkaloids as it is reported from the drying process of the germinated malt in beer production [5]. As nitrosamine levels in malt and beer have been significantly reduced in the brewing process, high analytical performance is required. In addition to the regular control of other food products for daily consumption, malt in beer is also monitored for low levels of nitrosamines.

The “classical” nitrosamine analysis was performed for many years by gas chromatography using a thermal energy analyzer (TEA) as detector. This special TEA detector was used due to its selectivity for nitrosamines with to the specific chemilumniscent reaction of ozone with the detector generated NO from nitrosamines. Today, with increased sensitivity requirements, the detection limits of the TEA, and also its complex operation, no longer comply with the required needs for low detection limits and sample throughput. Mass spectrometric methods have increasingly replaced the TEA.

The EPA method 521 by Munch and Bassett from 2004 provided at that time a suitable GC-MS method based on chemical ionization (CI) using an ion trap mass spectrometer with internal ionization [6, 7], in contrast

to standard quadrupole or ion trap mass spectrometers using a dedicated (external) ion source design. Current developments in GC-MS triple quadrupole technology deliver today very high sensitivity and selectivity also in the small molecule mass range and allow the detection of nitrosamines at very low concentration levels even in complex matrix samples. This is made possible by using a much simpler and standard approach with the regular electron impact ionization (EI) for a very straightforward method for low level nitrosamine analysis.

This application note describes a turn-key GC-MS/MS method for routine detection and quantitation of food borne nitrosamine compounds. The food matrix in this work has been different malt beer products and as a final food product the commercial beer. Special focus in the method development has been made to provide the required high sensitivity for the detection of the nitrosamine compounds for a fast, easy to implement routine method.

The sample preparation is adapted and slightly modified from AOAC Official Method (2000), 982.11 [8]. An SPE column extraction method using a celite column and elution with DCM to isolate the nitrosamines from the beer samples was developed.

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Experimental Conditions
GC-MS/MS Instrument

TRACE 1310 GC

iC Injector Module	Split/Splitless Injector
Injector Temperature	250 °C
Injection mode	splitless
Surge mode	300 KPa
Splitless Time	1.0 min
Analytical Column	TG-WAX MS, 30m×0.25mm×0.5µm
Carrier gas	He （99.999% purity）
Flow rate	1.0 mL/min, constant flow
Oven Program	45 °C for 3 min,
	25 °C/min to 130 °C,
	12 °C/min to 230°C, 1min hold
Transfer line Temperature	250°C
Total analysis time	14.7 min
Total cycle time	18.4 min

TriPlus RSH Autosampler

Injection Volume	1 µL
Solvent	dichloromethane
Standard runs	3 replicate of injections each
Dilution of standard mix	1ppb, 5ppb, 10ppb, 25ppb, 100ppb, 250ppb, 500ppb
Internal standard	NDPA added to each calibration level at 50ppb

TSQ 8000 Triple Quadrupole GC-MS/MS system

Ionization mode	EI
Mass resolution setting	normal
Source temperature	220 °C
Scan mode	MRM, retention time-based SRM mode

MRM Method Setup

The triple quadrupole MS method setup was performed by using the AutoSRM software which is part of the Thermo Scientific TSQ™ 8000 GC-MS/MS software suite. The method generated by AutoSRM was used without any additional manually modification. One autosampler vial containing a standard solution of the nitrosamine compounds to be analyzed has been used only for the AutoSRM process.

The AutoSRM procedure automatically runs the following three steps:

1. First a full scan analysis of the standard solution (Figure 1). Get the most intense ions of the full scan spectra to be used as the precursor ions.
2. Run a next analysis acquiring the product ion spectra from the selected precursor ions (the number of precursor ions to be used can be configured to the analytical needs). Get the most intense product ions from each precursor ion (optionally the desired precursor ions can be selected manually for further optimization).

Table 1. MRM method setup using AutoSRM

	Precursor	Product	Collision Energy (eV)	Retention Time (min)	Time Window (min)
NDMA	74	42.1	15	7.89	1
	74	43.8	5	7.89	1
NDEA	102	44.1	10	8.56	1
	102	85.1	5	8.56	1
NDPA (ISTD)	130	42.9	10	9.76	1
	130	113.1	5	9.76	1
NDBA	158	99.1	5	11.35	1
	158	141.1	5	11.35	1
NPIP	114	41.5	15	11.80	1
	114	83.9	5	11.80	1
NPYR	100	43	10	12.06	1
	100	55.1	5	12.06	1
NMOR	116	56.1	10	12.47	1
	116	86.1	5	12.47	1

** The transitions marked as grey color are quantitation ions*

3. Optimize for all compounds the collision energy of the selected precursor/product ion transitions for maximized compound response and best method sensitivity (Figure 2).

Initiated by the AutoSRM procedure as many as necessary autosampler injections from the one standard vial are scheduled.

As a result of the AutoSRM program, the generated SRM transition table shown in Table 1 has been automatically built. The table represents at the same time the TSQ 8000 GC-MS/MS system MRM acquisition method using the timed-SRM mode with a short acquisition window of 60 s around the compound retention time. No other setting of scan segments is necessary, or will be necessary in case additional compounds need to be added to the acquisition, other than the compound retention time.

Sample Measurements

From the large variety of potential nitrosamines the compounds that had been included in this method are those that are reported to be of relevance in the germinated malt drying process. Samples analyzed included malt beer as unspiked samples and 4% ethanol as sample blanks. In case of the analysis of other food matrices, additional compounds can be added to this method easily at any time as described in the method setup by AutoSRM [9, 10].

Results

The chromatograms of the nitrosamines included in this method show a quick elution of the compounds from 7.87 min NDMA to 12:47 min allowing a short cycle time for increased sample throughput. The peak intensities are retained in Figure 3 at the lowest calibration level of 1 ppb. NDMA can be detected with good S/N values.

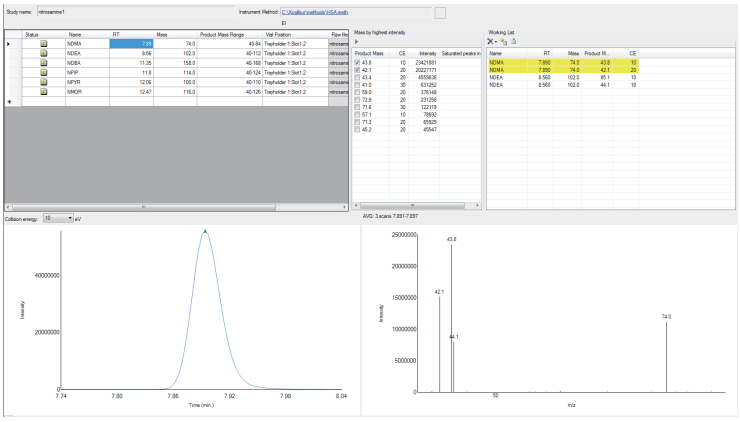

Figure 1. AutoSRM Precursor Ion Selection for NDMA from EI full scan spectra

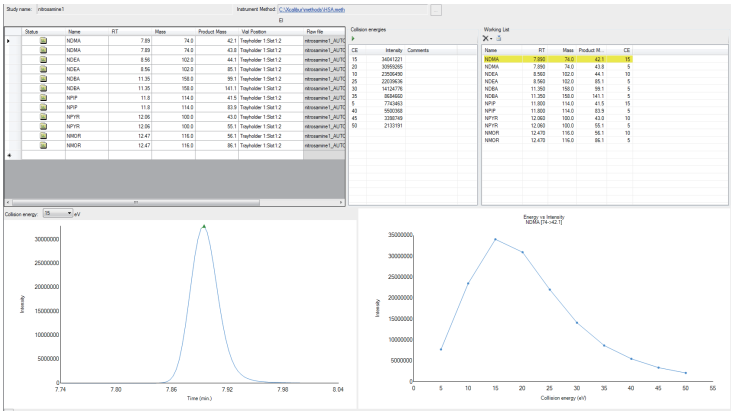

Figure 2. AutoSRM collision energy optimization for all nitrosmaine precursor ions

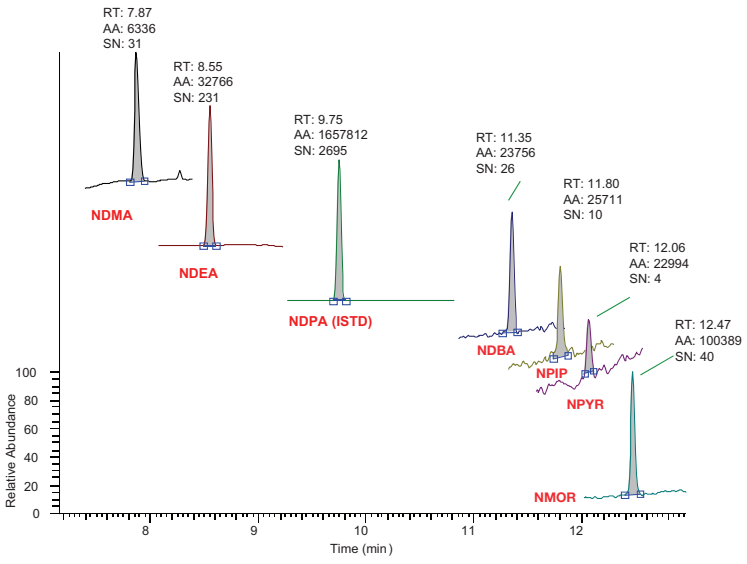

Figure 3. Chromatogram of the standard mix at 1 ppb

The quantitative calibration has been performed in a wide concentration range from 1 ppb to 500 ppb. Figure 4 shows the chromatogram peaks of NDMA from all the calibration runs. In all cases the NDMA peak shape is perfectly symmetrical, no tailing occurs and the peak area integration provides very reliable values without the need for any further manual corrections. The linear calibration of NDMA used to quantify the samples is shown in Figure 5 with very good correlation of R^2 better than 0.99. The same good calibration precision is achieved for all nitrosamines in this TSQ 8000 GC-MS/MS method.

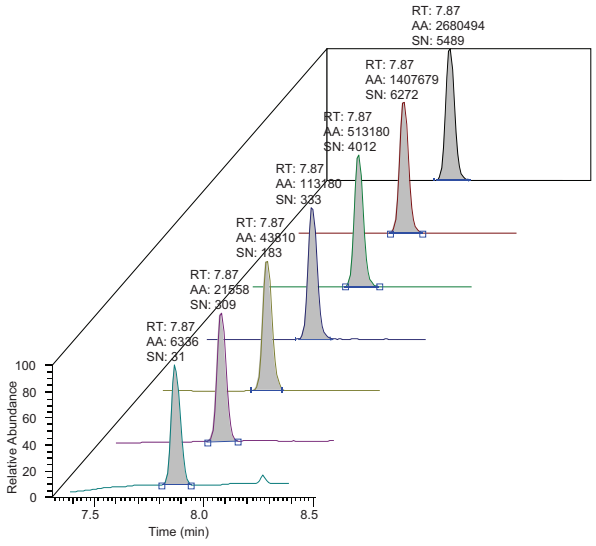

Figure 4. NDMA calibration runs from 1 ppb (bottom) to 500 ppb (top)

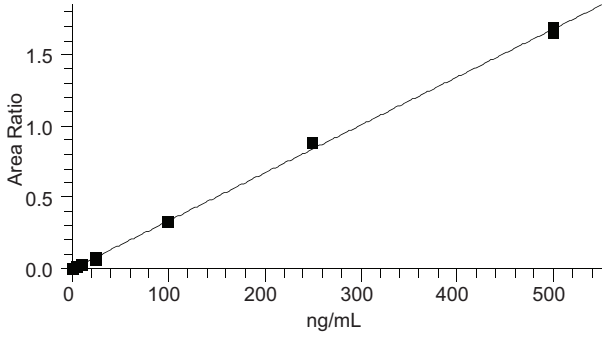

Figure 5. Linear calibration function for NMDA from 1 ppb to 500 ppb

LOQ Determination

The calculation of the LOQ and LOD was based on the S/N achieved for a chromatographic peak.The LOQ calculation is based on the level of S/N 10, and LOD values are calculated based on a S/N of 3.

Table 2. Calculation of the method LOQ and LOD

Compound	S/N @ 1ppb	Calculated LOQ (ppb)	Calculated LOD (ppb)
NDMA	13	1.0	0.25
NDEA	231	0.05	0.02
NDBA	23	0.5	0.20
NPIP	10	1.0	0.50
NMOR	40	0.3	0.10
NPYR	24	3	1.0

Confirmation

For compound confirmation the ion ratio check provided by the Thermo Scientific TraceFinder™ quantitation software was used by comparing the ion intensity of the second acquired SRM transition with the first SRM used for quantitation. The precision for the ion ratio was calculated using the three replicate standard runs over the complete concentration range from 1 ppb to 500 ppb and is shown in Table 3. Although the detected ions all are in the low mass range and potentially subject to many interferences the precision of the product ion ratio is very good in the range of 1-4%.

For quality control purposes in sample analyses the confirmation of a positive result is done by the ion ratio check during the quantitation data processing in TraceFinder software. The ion ratio of the two acquired product ions is required to stay within +/- 5% (10%) for all compounds, compared to the calibrated value from the standard runs. This provides a solid safety margin for routine sample measurements. Table 3 indicates the used average value (AVG) of the ion ratio for all nitrosamines investigated.

Sample Measurements

A number of samples have been measured, including blanks and spiked beer samples. The results of a blank sample are shown in Table 4. The found low NDMA concentration in this sample has been calculated below the calibration, and also below LOQ. The blank sample could be confirmed to be free from nitrosamine compounds at the given LOQ.

Another sample was prepared from beer that has been spiked with different amounts of nitrosamines. All nitrosamine compounds have been detected and quantified in a low concentration range of 9 – 13 ppb, see Table 5. Each quantified peak passed the ion ratio quality control and could be positively confirmed at this low level by calculating the product ion ratios for each of the compounds.

Conclusions

With the described GC-MS/MS method on the TSQ 8000 system all nitrosamine compounds under investigation could be safely detected and precisely quantified at the required low levels for a safe food control.

The LODs of all compounds have been determined to be below 1 ppb, using 1 ppb as the lowest concentration for the quantitative calibration.

The TSQ 8000 GC-MS/MS shows a wide linearity in the range of 1-500 ppb with very good precision. All calibration curves have been shown to be strictly linear with R^2 better than 0.99.

The TSQ 8000 GC-MS/MS shows great ion ratio stability for the confirmation of positive samples. The RSD% of the ion ratio of all compounds is lower than 4% even at LOQ level.

The use, setup and maintenance of a GC-MS/MS method for nitrosamines is easy. The unique AutoSRM software finds and optimizes the SRM transitions and collision energy automatically, even facing new and yet unknown components.

Based on the demonstrated GC-MS/MS method, the TSQ 8000 GC-MS/MS can successfully quantify the concentration of nitrosamine components in real samples without any uncertainty.

The described GC-MS/MS method for food nitrosamines on the TSQ 8000 GC-MS/MS can serve as a turnkey method for routine use in food safety control. It is using standard GC-MS/MS triple quadrupole instrumentation which is also common for many other areas of regular food safety control, e.g. pesticides, POPs or polyaromatic hydrocarbons. The presented method is fast, allows high sample throughput, and provides results with very high sensitivity and precision. With this standard EI ionization method setup this presented method for low level nitrosamine quantitation is recommended to be employed as a productive alternative to the earlier described chemical ionization ion trap procedure using liquid CI reagents.

Table 3. Precision of the confirming ion ratios from 1 ppb – 500 ppb

Concentration (ppb)	1	5	10	25	100	250	500	AVG	RSD (%)
NDMA	70.7	67.9	68.0	69.8	69.1	71.9	69.6	69.6	2.01
NDEA	20.8	22.1	22.5	22.4	22.5	22.5	22.5	22.2	2.84
NDBA	102.4	102.4	98.2	98.6	96.1	93.4	99.2	98.6	3.28
NPIP	6.1	5.5	6.2	5.9	6.0	6.1	6.2	6.0	3.88
NPYR	-	64.6	62.4	66.2	66.9	68.1	66.7	65.8	3.06

Table 4. Results of a blank sample

Compound	Area	ISTD Area	Area Ratio	Ion Ratio Confirmation	Calculated Amount (ppb)
NDMA	2591.368	2028129.842	0.001	Pass (65.1%)	0.74*
NDEA	1875.386	2028129.842	0.001	Fail (0%)	N/A
NDBA	6806.996	2028129.842	0.003	Fail (81.1%)	N/A
NPIP	N/A	2028129.842	N/A	N/A	N/A
NPYR	N/A	2028129.842	N/A	N/A	N/A
NMOR	4415.782	2028129.842	0.002	Fail (0%)	N/A

**Below LOQ*

Table 5. Results from a spiked beer sample

Compound	Area	ISTD Area	Area Ratio	Ion Rato	Calculated Amount (ppb)
NDMA	91318.135	2282168.009	0.040	Pass (68.3%)	12.0
NDEA	480955.478	2282168.009	0.211	Pass (22.0%	9.4
NDBA	402754.561	2282168.009	0.176	Pass (96.8%)	13.2
NPIP	280162.125	2282168.009	0.123	Pass (5.9%)	10.1
NPYR	318081.273	2282168.009	0.139	Pass (68.9%)	13.3
NMOR	1145719.054	2282168.009	0.502	Pass (67.9%)	10.1

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