Unparalleled Performance of Advanced Electron Ionization GC-MS/MS Technology for the Determination of Nitrosamines in Drinking Water

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GOAL

The aim of the study was to assess the quantitative performance of the new Thermo Scientific™ TSQ™ 9000 triple quadrupole system with the Thermo Scientific™ Advanced Electron Ionization (AEI) source for the analysis of nitrosamines in drinking water at low concentrations.

INTRODUCTION

Nitrosamines are semi-volatile compounds that are an emerging class of drinking water contaminants. N-nitrosodimethylamine (NDMA) is the main nitrosamine of concern and is classified as a potent carcinogen by the U.S. Environmental Protection Agency (EPA) due to its tumor inducing properties through ingestion or inhalation.[1] Nitrosamines are used in various industries to manufacture cosmetics, pesticides, or rubber products. In water, nitrosamines are formed as by-products during industrial processes such as chloramination of wastewater and drinking water.[2] Due to their potency as carcinogens, nitrosamines are considered as priority pollutants and various countries around the world have already introduced maximum acceptable concentrations of 9 ng/L and notification levels at 10 ng/L.[3, 4]

GC-MS is the analytical technique of choice for nitrosamine determination and, in particular the use of triple quadrupole GC-MS/MS instrumentation has recently become popular for this application due to its high selectivity and sensitivity provided through selective reaction monitoring (SRM). High selectivity and sensitivity are required to (i) reduce interferences from matrix and background chemical ions that can result in false positive detection and erroneous quantification of nitrosamines (ii) detect ultra-trace levels of these toxic compounds.

In this work, the analytical performance of the new TSQ 9000 triple quadrupole GC-MS/MS system using the advanced electron ionization (AEI) source was tested for the ultra-trace analysis of nitrosamines in drinking water from 17 drinking water testing facilities across Europe.

MATERIALS AND METHODS

Calibration Standard and Sample Preparation

17 drinking water samples were obtained from water treatment stations across Europe. To test the limit of detection (LOD) and to assess the linearity of the method individual nitrosamine standards including NDMA d-6 surrogate (LGC Ltd, UK) were used to prepare nine calibration levels: 0.0, 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10, 20, 50, 100 pg/µL in DCM* (corresponding to 0.05–100 ng/L in drinking water after concentrating x1000 with SPE). NDPA-d14 was also spiked in as an internal standard at 25 pg/µL (corresponding to 25 ng/L in drinking water).

Solid phase extraction (SPE) was performed using activated charcoal SPE based on modified EPA 521 methodology. The summary of the SPE method can be seen in Figure 1. In addition to this the limit of quantitation (LOQ) was assessed by fortifying ultra-pure water with nitrosamines at 0.1 and 0.5 ng/L (step 2). Similarly, recovery was assessed by fortifying water at 50 ng/L (step 2).

Figure 1. SPE method used for drinking water analysis. *DCM = dichloromethane

Data Analysis

Data were acquired using timed-SIM, processed and reported using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software, which allows instrument control, method development, quantitative/qualitative analysis and customizable reporting all within one platform. Data review is highly customizable, allowing the user to display the information required on screen in real time and the software is 21 CFR part 11 compliant. All instrument conditions can be seen in Table 1.

Table 1. Instrument conditions for Trace 1310 GC system and TSQ 9000 mass spectrometer.

TRACE 1310 GC System Parameters

Injection Volume:	2.0 µL
Liner:	Restek® CarboFrit® liner (P/N 20294)
Inlet:	240 °C
Carrier Gas:	He, 1.3 mL/min.
Injector Injection Mode:	Splitless with surge (surge pressure 25 psi for 1.01 min., split flow 80 mL/min. after 1 min.)
Column:	TraceGOLD TG-1701MS (30 m × 0.25 mm, 0.5 µm P/N 26090-2230)

Oven Temperature Program:

	RT (min)	Rate (°C/min)	Target Temperature (°C)	Hold Time (min)
Ramp			35	
Initial	0.0	—	35	1.0
1	4.8	25.0	130	0.0
Final	12.8	20.0	250	2.0
Run Time	12.8	—	—	—

TSQ 9000 Mass Spectrometer Parameters

Transfer Line:	250 °C
Source Used:	Thermo Scientific™ Advanced Electron Ionization (AEI)
Ionization Type, eV, Emission Current:	Electron Ionization (EI), 50, 100 µA
Ion Source:	300 °C
Acquisition Mode:	Timed SRM
Tune Type:	AEI SmartTune
Collision Gas and Pressure:	Argon at 70 psi
Peak Width:	0.7 Da at FWHM (both Q1 and Q3)

RESULTS

Carryover Assessment

Carryover can be a problem for this application, in order to assess the performance of this effect a dichloromethane (DCM) blank was injected immediately after the highest concentration standard. In Figure 2 (below) an example extracted ion chromatogram of the highest concentration injected standard for NDMA 200 pg on column (oc) (left chromatogram) and the consecutive DCM blank (right chromatogram) demonstrates that there is no carryover.
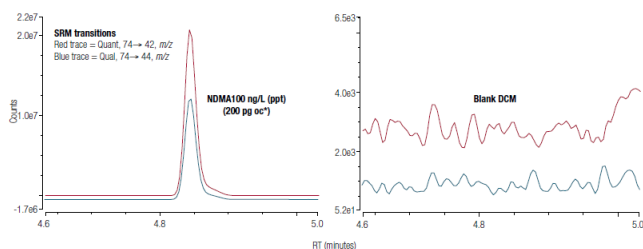

Figure 2. NDMA overlaid quantification ion and qualification ions for the highest standard in DCM 100 pg/µL corresponding to 200 pg OC* (left chromatogram) and a consecutive DCM blank (right chromatogram). No data smoothing was used and data was acquired in SRM mode. *OC = On column amount pg.

Chromatography

All compounds were separated in <9 minutes which is 3x faster than what is suggested in certain methodology such as EPA 521. This will allow for high sample throughput and reduced cost per analysis. Using the TG-1701 MS column good chromatographic peak shape was obtained for all compounds, even for NDMA which is particularly challenging for this analysis due to its polarity (Figure 3).
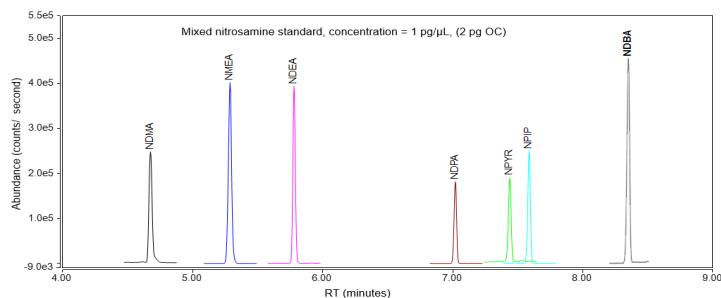

Figure 3. Chromatogram showing the quantitation SRM transition ions for nitrosamines in a 1 pg/µL solvent standard (equivalent to 1 ng/L in sample) with excellent chromatographic peak shapes for all compounds. (NDMA-d6 was not displayed to show peak shape for NDMA).

Determination of IDL

The enhanced sensitivity of the new AEI source is demonstrated for NDMA in Figure 4. Here a 0.01 pg/µL (0.02 pg oc) solvent standard shows excellent signal precision with peak area repeatability <10 % RSD at low ppt levels (equivalent to low ppq [0.01 ng/L] in sample extracts). To practically assess the instrument detection limit (IDL), 15 consecutive injections were performed using a 0.01 and 0.1 pg/µL calibration standard. The instrumental detection limit for each individual compound was then calculated by taking into account the injected amount, % RSD, and t-score of 2.624, corresponding to 14 degrees of freedom at 99% confidence (Table 2).
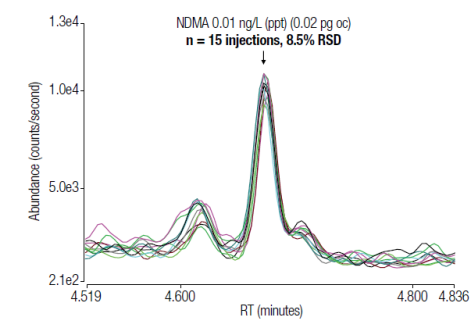

Figure 4. Overlaid quantification SRM transitions (74 → 44 m/z) from 15 consecutive injections of a 0.01 pg/µL NDMA solvent standard corresponding to 0.01 ng/L in sample. This shows excellent instrument precision and sensitivity at 0.02 pg on column. No data smoothing was used and data was acquired in timed-SRM mode.

Table 2. Calculated instrument detection limit (IDLs) and absolute peak area repeatability (as % RSD) for nitrosamines determined from n=15 injections of either a 0.01 pg/µL or 0.1 pg/µL solvent standards where the peak area % RSD was lower than 15%.

Component	Concentration Injected (pg OC*)	Peak Area % RSD	IDL (pg OC*) Equivalent to ng/L in Sample
NDMA	0.02	8.5	0.005
NMEA	0.02	5.2	0.003
NDEA	0.02	7.9	0.004
NDPA	0.20	7.7	0.040
NPYR	0.20	10.9	0.060
NPIP	0.02	12.0	0.006
NDBA	0.02	9.9	0.005

*oc = on column, t-score = 3.787 and n=14 degrees of freedom

Linearity

Linearity was determined using dichloromethane solvent standards at concentrations of 0.05–20 pg/µL (corresponding to 0.05–20 ng/L in water extracts). The calibration of each nitrosamine was performed calibrated using average calibration factor (AvCF) function in Chromeleon CDS and three injections were made at each concentration. All compounds showed excellent linear response with coefficient of determination R2 > 0.999, and average response factor values (RF, % RSD) across this calibration range were all below 5% (Figure 5).
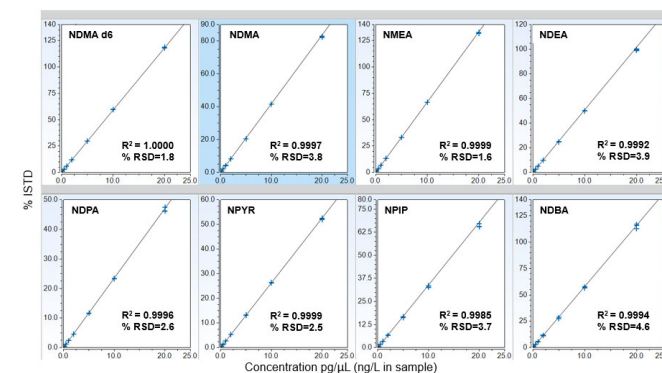

Figure 5. Linearity of targeted compounds demonstrated using a solvent-based calibration curve ranging from 0.05 to 20 pg/µL(corresponding to 5–20 ng/L in drinking water). NDPA d-14 ISTD, AvCF calibration, triplicate injections per level.

Calculated LOQ in Sample

The LOQ was determined as the lowest concentration of nitrosamines with the following:

- Ion ratios within ± 30% of the expected values calculated as an average across a calibration curve ranging from 0.05 to 100 pg/µL (corresponding to 0.05–100 ng/L in drinking water)
- Measured ion ratio % RSD < 15%
- Ion co-elution within ± 0.01 minutes
- Peak area repeatability of < 15% RSD

To demonstrate the method LOQs, water was fortified with nitrosamines prior to extraction at 0.1 and 0.5 pg/µL. These were injected 10 times, and based on satisfaction of criteria above, the LOQs for compounds ranged from 0.1 to 0.5 ng/L (Table 3).

Table 3. Method LOQ values derived for nitrosamines in drinking water from injecting n=10 times 0.1 ng/L and 0.5 ng/L fortified water extracts. The criteria used to assess individual nitrosamine LOQ values were ion ratio % deviation from theoretical, measured ion ratio % RSD, peak area % RSD and ion co-elution.

Component	Concentration Injected (pg OC*)	Measured Ion Ratio % RSD	Ion Ratio Abundance % Deviation	Pass Criteria	Peak Area % RSD	Pass Criteria	LOQ (ng/L)
NDMA	0.2	6.6	6.9	± 30%	1.5	<15%	0.1
NMEA	0.2	9.5	8.1	± 30%	3.1	<15%	0.1
NDEA	0.2	6.2	5.0	± 30%	3.4	<15%	0.1
NDPA	1.0	4.8	5.5	± 30%	4.0	<15%	0.5
NPYR	1.0	9.4	13.3	± 30%	3.8	<15%	0.5
NPIP	0.2	10.6	9.7	± 30%	4.9	<15%	0.1
NDBA	0.2	1.7	1.5	± 30%	1.6	<15%	0.1

Method Accuracy

The method performance was assessed by evaluating the compound recoveries determined from three separate extractions of a 50 ng/L nitrosamine fortified water sample. The results show that the average recovery values ranged between 80.7% and 111.1% (Table 4). This was well within the 70–130% criteria set for this method, demonstrating that the extraction procedure had outstanding recovery for nitrosamines in drinking water.

Table 4. % Recovery determined from three separate nitrosamine fortified water extractions at 50 ng/L. NDMA d-6 and NDEA d-10 surrogate standards were spiked into 1 L of water at 25 ng/L to correct recoveries for NDMA and NDEA.

Compound	RT (min)	Concentration (ng/L)	Calculated (ng/L)	% Recovery	Pass/ Fail	Limits Recovery %
NDMA	4.7		54.2	108.4	PASS	
NMEA	5.3		41.5	83.0	PASS	
NDEA	5.8		55.5	111.1	PASS	
NDPA	7.0	50.0	40.4	80.7	PASS	70–130
NPYR	7.4		48.3	96.5	PASS	
NPIP	7.6		45.0	90.0	PASS	
NDBA	8.4		42.2	84.3	PASS	

Quantification of Nitrosamines in Drinking Water

Seventeen drinking water samples were obtained from water testing facilities across Europe and the total nitrosamine content was quantified as total nitrosamines in ng/L, taking into account any nitrosamine present above the LOQ (as defined in Table 3). All drinking water samples contained nitrosamines with values ranging between 0.9 and 4.5 ng/L (Figure 6). Out of the nitrosamines present in drinking water, NDMA, NDBA and NDEA were the most prevalent with calculated NDMA amounts ranging from 0.2 to 3.5 ng/L. For all of the samples, the amount of nitrosamines was below the threshold value of 10 ng/L.[3,4] This demonstrates that the TSQ 9000 GC-MS/MS system with the AEI source is capable of detecting and quantifying nitrosamines in drinking water easily down to sub ppt levels, and if regulation arises, is ideally positioned for this type of analysis.
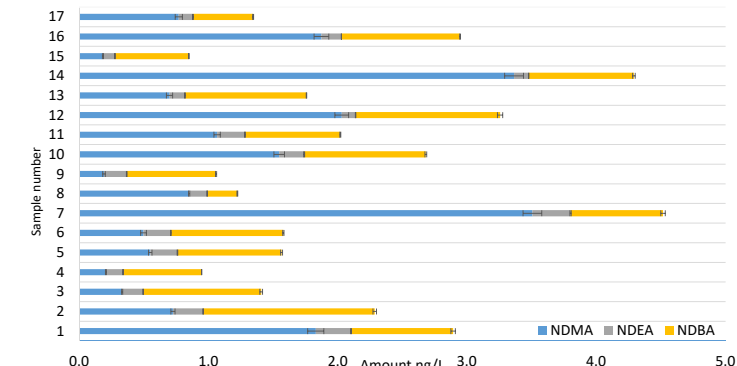

Figure 6. Total quantified nitrosamine content (ng/L) from 17 drinking water samples sourced from separate water testing facilities across Europe. Triplicate injections per sample and standard deviation was added to the stacked bar chart. NDMA d-6 and NDEA d-10 surrogate standards were spiked to 1 L of water pre-extraction at 25 ng/L to correct recoveries for NDMA and NDEA. Deuterated NDBA was not available for the analysis so the values are not corrected.

CONCLUSIONS

The results of the experiments described here demonstrate:

- Excellent sensitivity with unrivaled instrument detection limits for nitrosamines in solvent standards down to low ppt levels 0.003 pg oc translating to 0.003 ng/L (low ppq w/v) in sample.
- Outstanding linearity used for the quantification of nitrosamines in 17 drinking water samples analyzed was demonstrated over a range of 0.05 to 20 pg/µL (corresponding to 0.05–20 ng/L (ppt w/v) in drinking water). All compounds showed excellent linear responses with coefficient of determination R2 > 0.999 and average response factor % RSDs < 5%.
- The method detection limit for nitrosamines was calculated to be between 0.008 and 0.045 ng/L (ppt w/v).
- The LOQ for the method was set at between 0.1 and 0.5 ng/L (ppt w/v) for nitrosamines in drinking water with data from n=10 injections of LOQ standard, having ion ratio % deviation from the average of the calibration standards within ± 15%, peak area % RSD < 15%, and ion co-elution within 0.01 minutes.
- Compound recoveries were found to be between 80.7% and 111.1%, well within the set method performance limits of 70–130%.
- Seventeen drinking water samples from separate water testing facilities across Europe were quantified and total nitrosamine content ranged between 0.9 and 4.5 ng/L.

Taken together these results demonstrate that the TSQ 9000 GC-MS/MS system configured with the AEI source provides unparalleled levels of quantitative performance making it an ideal analytical tool for routine laboratories.

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