Unparalleled performance of Advanced Electron Ionization GC-MS/MS technology for the determination of nitrosamines in drinking water

Authors

Aaron Lamb,[1] Cristian Cojocariu,[1] Sara Insa,[2] and Maria José Farré[2]

[1]Thermo Fisher Scientific, Runcorn, UK

[2]Catalan Institute for Water Research (ICRA), Parc Científic i Tecnològic de la Universitat de Girona, 17003 Girona, Spain

Keywords

Environmental analysis, nitrosamines, NDMA, trace analysis, gas chromatography, TSQ 9000, triple quadrupole mass spectrometry, selected reaction monitoring, drinking water, advanced electron ionization

Goal

The aim of the study was to assess the quantitative performance of the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system with advanced electron ionization 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]

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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 and (ii) detect ultra-trace levels of these toxic compounds.

In this work, the analytical performance of the TSQ 9000 triple quadrupole GC-MS/MS system using the Thermo Scientific™ 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.

Experimental

Preparation of solvent calibration curve

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: 0.05, 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10, 20, 50, and 100 pg/µL (corresponding to 0.05–100 ng/L in drinking water after concentrating ×1000 with SPE). NDPA-d14 was also spiked in as an internal standard at 25 pg/µL (corresponding to 25 ng/L in drinking water).

Preparation of samples

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, 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).

GC-MS/MS analysis

A TSQ 9000 triple quadrupole GC-MS/MS instrument equipped with an AEI source and coupled with a Thermo Scientific™ TRACE™ 1310 GC system was used. The AEI source provides a highly efficient electron ionization of analytes and a more tightly focused ion beam that leads to an unparalleled level of sensitivity.

Liquid injections of the sample extracts were performed using a Thermo Scientific™ TriPlus™ RSH autosampler and chromatographic separation was achieved by a Thermo Scientific™ TraceGOLD™ TG-1701 MS 30 m × 0.25 mm I.D. × 0.50 µm film capillary column. Additional details of instrument parameters are displayed in Table 1.

Data processing

Data were acquired using timed-SRM, 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.[5] Data review is highly customizable, allowing the user to display the information required on screen in real time and the software is FDA 21 CFR part 11 compliant.
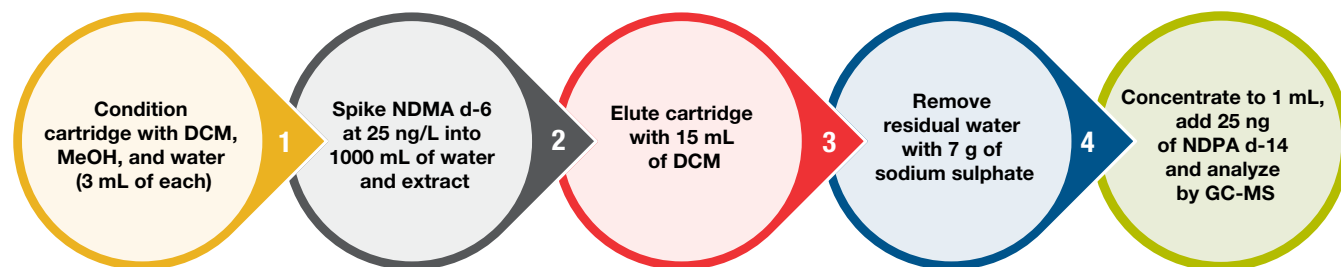

Figure 1. SPE steps used for drinking water samples

Table 1. Instrument parameters used in the drinking water analysis. A full list of consumables and instrument conditions including SRM transitions can be found in the AppsLab library.

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:

Ramp	RT (min)	Rate (°C/min)	Target Temperature (°C)	Hold Time (min)
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)

See SRM transitions in Appendix.

Results and discussion

The objective of the analysis was to test the TSQ 9000 triple quadrupole GC-MS/MS system performance for the targeted analysis of nitrosamines in drinking water samples. To accomplish this, solvent standards and real drinking water samples were analyzed.

Nitrosamines chromatography, selectivity, sensitivity, linearity, and peak area repeatability were evaluated using solvent-based standards. This was followed by validation of the method using fortification of water samples prior to SPE and concentration to assess compound LOQs and recoveries. The method was then applied to quantify nitrosamines in several drinking water samples obtained from water treatment stations across Europe.

Carryover can be a problem for this application, to assess the performance of this effect a dichloromethane (DCM) blank was injected immediately after the highest concentration standard. In Figure 2 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.

Chromatography

All compounds were separated in less than 9 minutes, which is 3× faster than what is suggested in certain methodology such as EPA Method 521. This will allow for high sample throughput and reduced cost per analysis. Using the TraceGOLD 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).
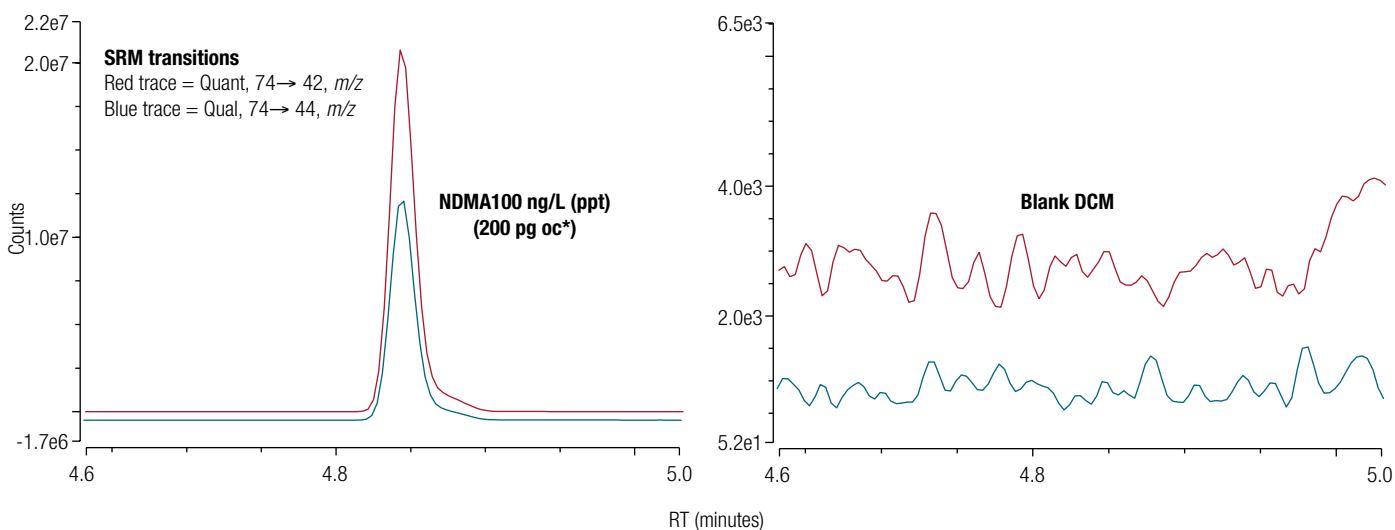

Figure 2. NDMA overlaid quantification ion and qualification ions for the highest standard in dichloromethane 100 pg/µL corresponding to 200 pg on-column (oc*) (left chromatogram) and a consecutive DCM blank (right chromatogram). Data is unsmoothed and was acquired in timed-SRM mode.
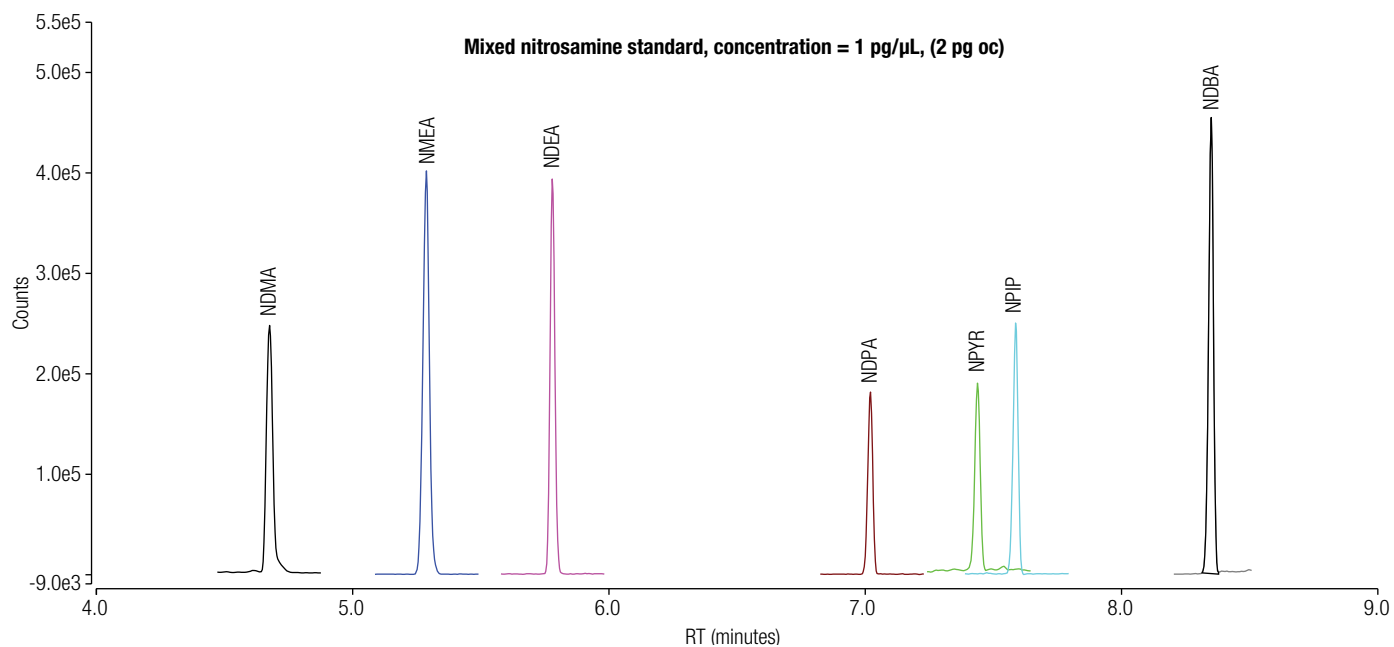

Figure 3. Chromatogram showing the quantitation SRM transition ions for nitrosamines in a 1 pg/µL solvent standard (equivalent to 1 ng/L in sample) with excellent chromatographic peak shapes for all compounds. (NDMA-d6 was not displayed to show peak shape for NDMA).

Sensitivity

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).
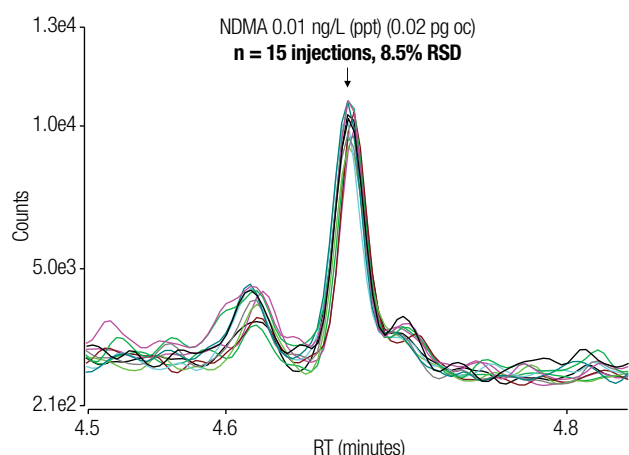
Figure 4. Overlaid quantification SRM transitions (74 → 44 *m/z*) from n=15 consecutive injections of a 0.01 pg/µL NDMA solvent standard corresponding to 0.01 ng/L in sample. No data smoothing was used and data was acquired in timed-SRM mode.

To assess the instrument detection limit (IDL), 15 consecutive injections were performed using the 0.01 and 0.1 pg/µL solvent standards. The IDL for each individual compound was then calculated by taking into account the on-column amount, % RSD of peak area repeatability from n=15 injections, and *t*-score of 2.624, corresponding to 14 degrees of freedom at 99% confidence (Table 2).

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	Calculated IDL values		
	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 = 2.624, n=14 degrees of freedom

Linearity

Nitrosamines linearity was determined using dichloromethane solvent standards at concentrations ranging from 0.05 to 20 pg/µL (corresponding to 0.05–20 ng/L in water extracts). Linear regression curves were plotted as average values of n=3 injections per calibration level. All compounds showed excellent linear response with coefficient of determination $R^2 > 0.999$, and average response factor values (RF % RSD) across this concentration range < 5% (Figure 5).

Method Detection Limit (MDL) determination

The method detection limit was derived in the same way as for the solvent standard derived IDL except that 1 L ultra-pure water was fortified with nitrosamines prior to extraction at 0.1 and 0.5 ng/L. Excellent limits of detection were demonstrated down to low ppq levels in sample. The results for the method detection limit are outlined below with values ranging from 0.008 to 0.045 ng/L (Table 3).

Calculated LOQ in sample

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

- 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 4).

A

B
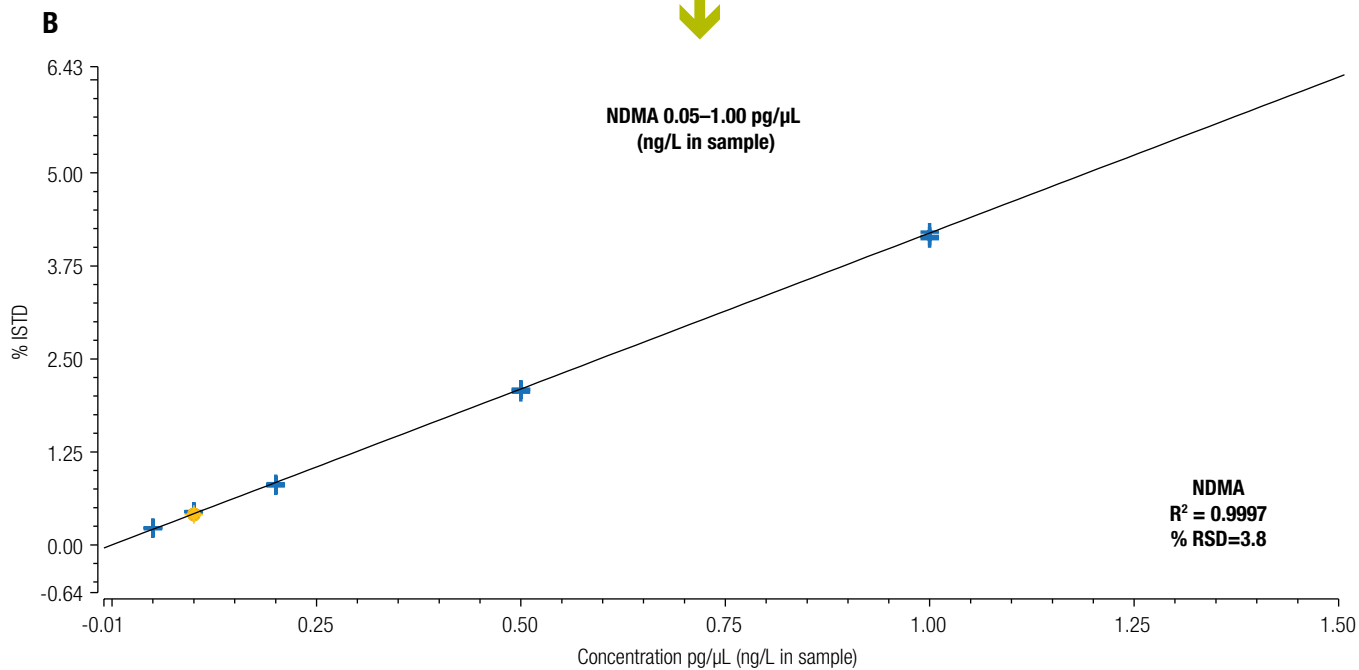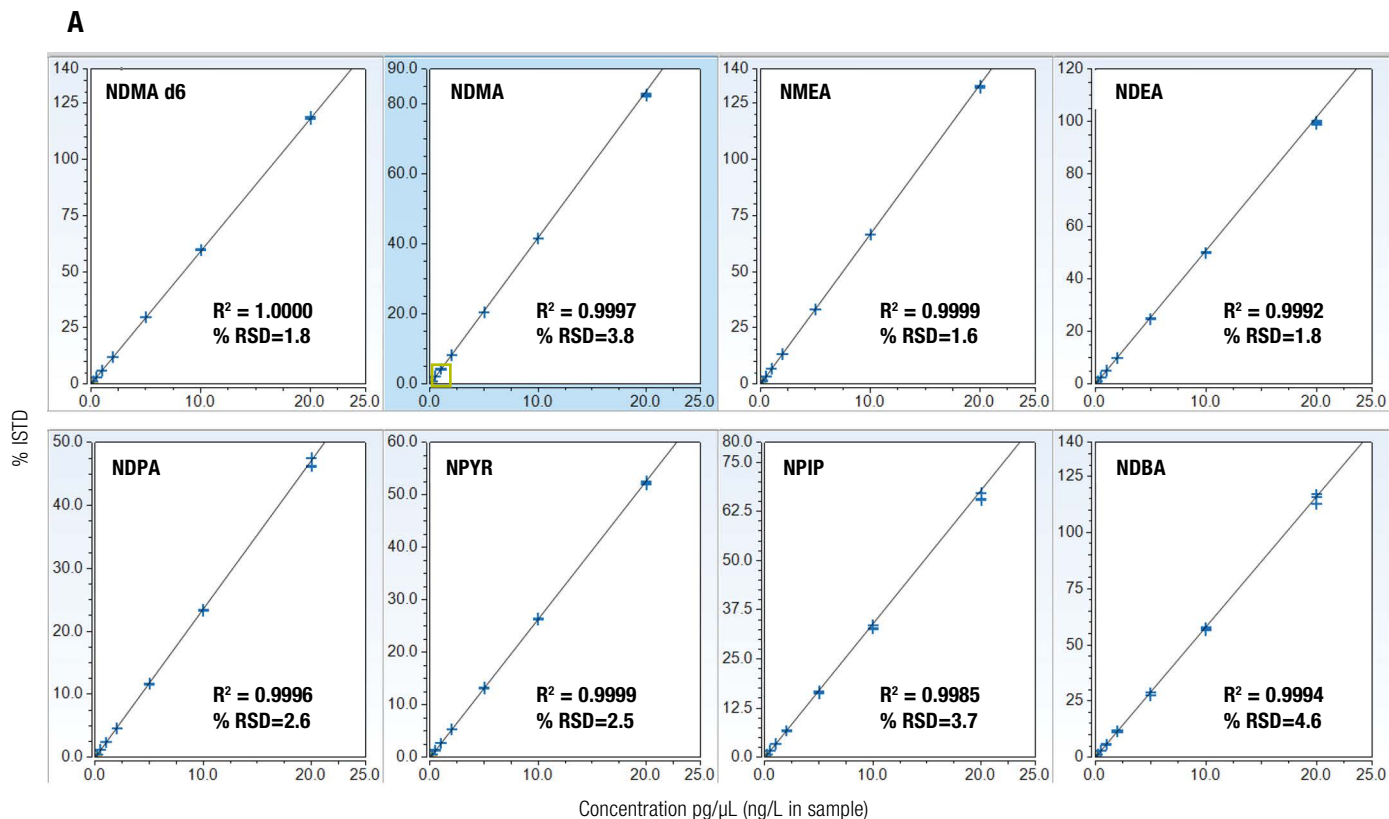
Figure 5. (A) Linearity of targeted compounds demonstrated using a solvent-based calibration curve ranging from 0.05 to 20 pg/µL (corresponding to 5–20 ng/L in drinking water). Average calibration factor (AvCF) function was used in Chromeleon CDS software with three replicate injections at each concentration and internal standard adjustment was conducted using NDPA d-14. Coefficient of determination (R^2) and average response factor values (RF % RSD) are displayed. (B) Expanded region of calibration for NDMA from 0.05–1.00 pg/µL (corresponding to 0.05–1.00 ng/L in drinking water) showing excellent precision for triplicate injections per point.

Table 3. Calculated method detection limit (MDLs) and absolute peak area repeatability (as % RSD) for nitrosamines determined from n=10 injections of water fortified with nitrosamines prior to extraction at 0.1 and 0.5 ng/L.

Component	Calculated MDL values		
	Concentration injected (pg oc*)	Peak area % RSD	MDL (pg oc*) equivalent to ng/L in sample
NDMA	0.2	1.5	0.03
NMEA	0.2	3.1	0.01
NDEA	0.2	3.4	0.01
NDPA	1.0	4.0	0.02
NPYR	1.0	3.8	0.02
NPIP	0.2	4.9	0.05
NDBA	0.2	1.6	0.01

*oc = on column, *t*-score = 2.821, n=9 degrees of freedom, 99% confidence level, peak area % RSD < 15%

Table 4. 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 coelution.

Component	RT	Conc. injected (pg oc*)	Target ion ratio** %	Mean measured % ion ratio	Measured ion ratio % RSD	Mean ion ratio abundance % deviation	Pass criteria	Peak area % RSD	Pass criteria	LOQ (ng/L)
NDMA	4.8	0.2	164	154	6.6	6.9	±30%	1.5	<15%	0.1
NMEA	5.5	0.2	50	50	9.5	8.1	±30%	3.1	<15%	0.1
NDEA	6.0	0.2	33	34	6.2	5.0	±30%	3.4	<15%	0.1
NDPA	7.2	1.0	35	33	4.8	5.5	±30%	4.0	<15%	0.5
NPYR	7.6	1.0	37	41	9.4	13.3	±30%	3.8	<15%	0.5
NPIP	7.8	0.2	91	94	10.6	9.7	±30%	4.9	<15%	0.1
NDBA	8.5	0.2	21	21	1.7	1.5	±30%	1.6	<15%	0.1

*oc = on column, **derived from average ion ratio across calibration range 0.05-20 ng/L, n=10 injections of tap water spiked at 0.1 ng/L pre-extraction, *t*-score= 2.821, n=9 degrees of freedom. peak area % RSD <15%, criteria for ion coelution ±0.01 min deviation

Due to the unrivaled sensitivity and selectivity of the new TSQ 9000 AEI GC-MS/MS system, accurate quantitation of nitrosamines down to low ppq (ng/L) levels in sample is now achievable. The chromatograms for individual nitrosamines at the relevant LOQ in extracted water are shown with confirmed qualifier within ±15% (Figure 6).

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 5). This was comfortably within the 70–130% criteria set for this method, showing that the extraction procedure had excellent recovery for nitrosamines in drinking water.
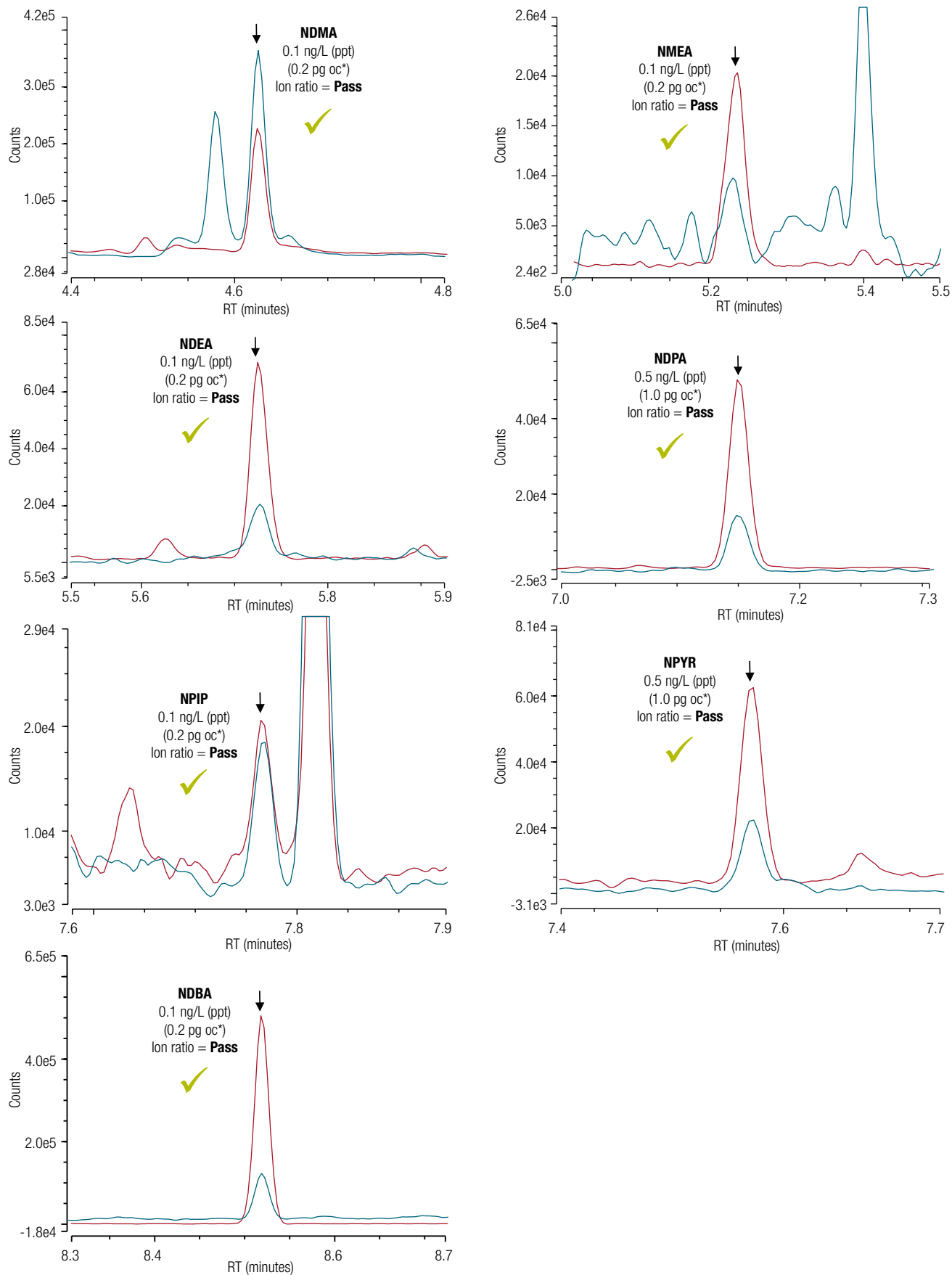
Figure 6. Individual chromatograms of nitrosamines with overlaid quantitation and qualifier ions at the LOQ in sample at between 0.1 ng/L and 0.5 ng/L in ultra-pure water. All the qualification ion ratios were found to be within ±15% of the average value calculated across the range of calibration standards 0.05 to 100 pg/µL (corresponding to 0.05–100 ng/L in drinking water).

Table 5. % 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 samples

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 4). All drinking water samples contained nitrosamines with values ranging between 0.9 and 4.5 ng/L (Figure 7). 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 AEI GC-MS/MS system 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.
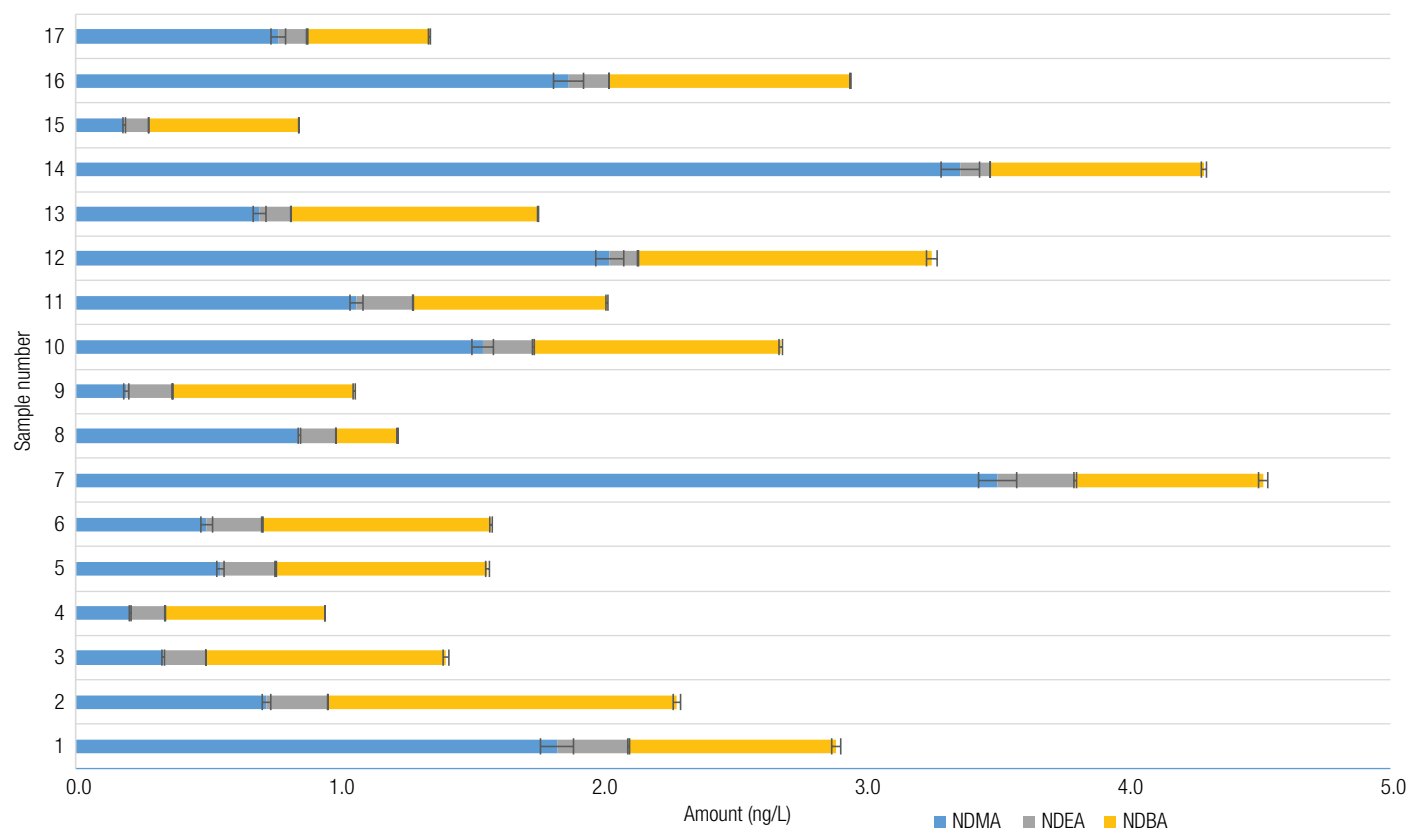
Figure 7. Total quantified nitrosamine content (ng/L) from 17 drinking water samples sourced from separate water testing facilities across Europe. NDMA d-6 and NDEA d-10 surrogate standards were spiked to 1 L of water pre-extraction at 25 ng/L to correct recoveries for NDMA and NDEA. Deuterated NDBA was not available for the analysis so the values are not corrected. The mean and standard deviation for triplicate injections per sample are presented in the chart.

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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 determinations $R^2 > 0.999$ and average response factor % RSDs < 5%.

- The MDL 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.

Appendix. SRM transitions

Name	RT (min)	(SRM) m/z		
		Mass (*m/z*)	Product Mass (*m/z*)	Collision energy V
NDMA-d6	4.7	80	50	5
		80	46	15
NDMA	4.8	74	42	15
		74	44	5
NMEA	5.5	88	71	5
		88	42	15
NDEA-d10	5.9	112	34	5
		112	50	10
NDEA	6.0	102	85	5
		102	44	10
NDPA-d14	7.1	78	46	10
		110	78	5
NDPA	7.2	130	113	5
		130	43	10
NPYR	7.6	100	55	5
		100	70	5
NPIP	7.8	114	84	5
		114	97	5
NDBA	8.5	116	99	5
		158	99	5

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