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FULLY AUTOMATED SYSTEM FOR PREPARING AND ANALYSING SAMPLES FOR NDMA IN POTABLE WATER USING GC-QQQ

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INTRODUCTION

The formation of N-Nitrosodimethylamine (NDMA), in treated sewage and environmental waters has been known for around 40 years [1]. NDMA is formed as a disinfection byproduct during chloramination of wastewaters and drinking waters [2-4].

It has not been recognized as an important drinking water contaminant until quite recently but regulation of N-nitrosamines in drinking waters is rapidly increasing in many parts of the world as a consequence.

N-Nitrosamines, which include NDMA, were among the highest ranked emerging disinfection by products in a recent prioritization process for future public health regulation [5].

NDMA has been classified as "probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC) and similarly by the US Environmental Protection Agency (US EPA). A guideline value for NDMA in drinking water of 100 ng/l has been set by the World Health Organization (WHO) [6].

Five N-nitrosamines including NDMA have now been included in the final US EPA Drinking Water Contaminants List 3. The California Department of Public Health (CDPH) has established a notification level for NDMA of 10 ng/l [7]. The Ontario Ministry of the Environment (MOE) has set a Maximum Acceptable Concentration (MAC) for NDMA at 9 ng/l [8].

NDMA has been measured in the UK, during a Department for Environment Food & Rural Affairs (DEFRA) funded Drinking Water Inspectorate (DWI) study [9]. Forty-one treatment works in England and Wales were selected for sampling because of the presence of key factors known to be associated with the formation of NDMA. NDMA was detected in final waters at three of these works in four quarterly surveys at concentrations up to 5.8 ng/l. Final water NDMA concentrations were within the current concentrations of concern in North America (9-10 ng/l) and substantially lower than the future WHO guideline value. NDMA was also detected in the supernatant recycled to water treatment at 31.5 and 39.1 ng/l in one treatment works.

DWI guidelines indicated that suitable, fit for purpose methods for determining NDMA in water should be capable of detecting and quantifying 1 ng/l of NDMA in water [10].

One of the major objectives of this work was to develop a method based on electron ionisation (EI) rather than most published methods which use chemical ionisation (CI) and are variations on the US EPA Method 521 Trace analysis of N-nitrosamines in drinking water [11].

Presented in this application note is a fully automated solution incorporating the solid phase extraction, to enrich NDMA from water samples. Large volume injection (LVI) is also automated to a GC-QqQ.

Initial work was completed at Anatune in 2012 and this is a continuation of this early work [12].

INSTRUMENTATION

- Agilent 7000 GC-Triple Quadrupole with extractor lens and EI source (Agilent GC 7890A).
    - GERSTEL MPS 2 XL-xt Dual Head HS-enabled.
    - 2.5 ml headspace syringe fitted with 57 mm SPE needle
- 10 µl GC Syringe ITSP Hardware kit.
- CIS 4 PTV fitted with septum less head. Maestro software (1.4.20.6/3.5) – Integrated. MassHunter Data Acquisition (B.06.00.1116).

Figure 1: Analytical solution for automated NDMA analysis.

METHODOLOGY

Commercially available solutions of NDMA (5000 µg/ml) and NDMA-d6 (100 ng/µl) were purchased from Sigma-Aldrich (Dorset, UK) and QMX Laboratories Ltd (Thaxted, UK).

Dichloromethane (DCM) (Pesticide Residue Grade), Methanol (LC-MS Chromasolv Grade) and Water (LC-MS Chromasolv Grade) were purchased from Sigma-Aldrich (Dorset, UK).

Coconut Charcoal Instrument Top Sample Preparation (ITSP) cartridges (25 mg, 80 to 120 mesh size.) were purchased from ITSP Solutions (Hartwell, USA).

Primary stock standards were prepared for NDMA and NDMA-d6 in methanol (50 µg/ml and 2 µg/ml respectively) in amber volumetrics and then diluted with methanol to obtain working standard solutions of lower concentrations. All standard solutions were stored at – 18 °C. Working solutions of NDMA and NDMA-d6 at lower concentrations were stored a 4 °C. A seven-point calibration in DCM was prepared encompassing the typical range of NDMA determined in water samples; NDMA-d6 was kept constant throughout the series. (See Table 1). A second seven-point calibration series was also prepared in methanol encompassing the same range; this was used for spiking water samples (See Table 2). The structure of NDMA is presented in Figure 2.

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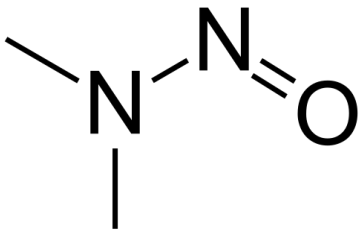

Figure 2: Structure of NDMA

Table 1: DCM calibration standards.

Std	NDMA ng/ml	NDMA-d6 ng/ml	NDMA-d6 ng/l equiv. in Water	NDMA ng/l equiv. in Water
1	0	1	0	40
2	0.00625	1	0.25	40
3	0.125	1	0.5	40
4	0.025	1	1	40
5	0.0625	1	2.5	40
6	0.125	1	5	40
7	0.25	1	10	40
8	0.375	1	15	40

Table 2: Methanol calibration standards

Std	NDMA ng/ml	NDMA-d6 ng/ml	NDMA-d6 ng/l equiv. in Water	NDMA ng/l equiv. in Water
1	0	200	0	40
2	1.25	200	0.25	40
3	2.5	200	0.5	40
4	5	200	1	40
5	12.5	200	2.5	40
6	25	200	5	40
7	50	200	10	40
8	75	200	15	40

40 ml of water sample is pipetted into a 40 ml EPA vial with polytetrafluoroethylene (PTFE) lined screw caps QMX Laboratories Ltd (Thaxted, UK). Drinking water was collected from a regular domestic supply tap at Anatune Ltd. River Water was collected from the River Cam in central Cambridge, UK and stored in the fridge at 4 °C before analysis.

Samples were spiked with 8 µl of the appropriate methanol calibration standards for linearity, method recovery and detection level determination. Spiked ultrapure water, tap and river water were extracted without any further treatment or processing. All samples were extracted within 24 hours of collection and spiking.

All other aspects of sample preparation are handled automatically using the MPS.

Using the 2.5 ml headspace syringe fitted with SPE needle, the coconut charcoal ITSP cartridges are conditioned and solvated with 1000 µl of DCM followed by 500 µl of methanol, the syringe is then rinsed with 250 µl of LC-MS water. 500 µl of water is then applied to the cartridge to equilibrate in preparation for sample loading. A sample volume of 10 ml is then loaded onto the cartridge at a flow rate of 25 µl/sec. After loading, the cartridge is dried for 15 minutes using a flow of nitrogen gas delivered by the headspace syringe.

The syringe is then rinsed with 500 µl of DCM, before elution with 400 µl of DCM into a 2 ml GC vial (x 25 concentration factor). Elution is performed at 10 µl/sec to ensure reproducible recoveries. No further sample preparation is performed.

Samples were analyzed on an Agilent 7890A gas chromatograph (GC) coupled to an Agilent 7000B triple quadrupole mass spectrometer (MS/MS).

A CIS 4 programmed temperature vaporization (PTV) inlet, with Universal Peltier Cooling (UPC) GERSTEL (Mulheim, Germany) was used as the injection port and speed programmed injections were performed using the MPS.

The inlet was operated in solvent vent mode and lined with a deactivated glass beads inlet liner GERSTEL (Mulheim, Germany). The inlet was operated in traditional solvent vent mode with an initial temperature of 10 °C. These conditions were maintained during sample injection and for 0.5 minutes after start, at which point the temperature was ramped at 12 °C/sec to 250 °C and held for 9 minutes.

During solvent vent conditions the vent pressure was 12.2 psi with a vent flow of 43 ml/min a vent time of 0.2 minutes was applied to the method.

An injection volume of 10 µl was used. Analytes were separated on an J&W DB-WAX (30 m x 0.25 mm x 0.5 µm film thickness) column using 1.5 ml/min ultra-high purity helium under constant flow conditions. The oven temperature program was as follows; 35 °C held for 0.5 min then raised at 20 °C/min to 165 °C the secondary ramp was raised at 50 °C/min to 240 °C and held for 2 min (total run time 10.5 min). The transfer line temperature was maintained at 240 °C.

Mass spectrometric ionization was accomplished in electron ionization (EI) mode with an EI voltage of 70 eV and source temperature of 300 °C. The triple quadrupole was operated in multiple reaction monitoring (MRM) mode with a gain setting of 100.

In order to identify the most suitable transitions for MRM, NDMA and NDMA-d6 were analyzed in scan mode to generate EI spectra to identify suitable precursor ions in MS1.

Fragmentation of the precursor ions in the collision cell was assessed by performing product ion scan experiments. Product ion intensity was optimized for each transition by repeated injections at different collision energies (CE). All samples were run with a solvent delay of 2.0 minutes. Dwell times were 25 ms for all analytes to achieve sufficient point across each peak for good quantification. All ions were monitored at wide resolution (1.2m amu at half height). Developed MRM transitions with collision energies are presented in table

Table 3: MRM method parameters

Compound	Precursor Ion (m/z)	MS1 Res	Product Ion (m/z)	MS2 Res	Dwell (ms)	Dwell (ms)
NDMA-d6	80.1	Wide	50.1	Wide	25	5
NDMA-d6	80.1	Wide	46.1	Wide	25	15
NDMA	74.1	Wide	44.1	Wide	25	5
NDMA	74.1	Wide	42.1	Wide	25	20

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Two MRM transitions of a single precursor ion were monitored for each compound. Analysis of the acquired data was undertaken using Agilent Masshunter software.

RESULTS

Calibration

Quantitative determination of the analytes in DCM (non-extracted standards) was achieved using internal standard calibration. Calibration curves were comprised of seven points (0.006 to 0.375 ng/ml in DCM), equivalent to the on-column concentrations derived from extracting 10 ml of water samples spiked with 0.25 to 15 ng/l prepared as above. These standards were prepared in 2 ml GC auto sampler vials. Each calibration standard included 1 ng/ml of NDMA-d6 equivalent to the on-column concentrations derived from extracting 10 ml of water samples spiked with 40 ng/l. A calibration curve of the relative response versus relative concentration ratio of the NDMA to NDMA-d6 was generated from these standards.

The calculated concentration of each of the calibration standards was required to be within 80 to 120 % of its true value for the batch to be considered to have passed quality control criteria.

For the DCM calibration a correlation coefficient of 0.999 for NDMA was achieved. The calculated accuracy of all calibration points ranged from 94.6 to 104.0 %.

For the extracted LC-MS water calibration, sample preparation was performed as described above. Water samples were spiked between 0 to 15 ng/l with NDMA and NDMA-d6 at 40 ng/l, to generate a seven-point calibration curve.

A correlation coefficient of 0.999 for NDMA was achieved. The calculated accuracy of all calibration points ranged from 95.5 to 103.3 %.

Recovery

Background contamination of NDMA can present problems for method validation at low ng/l concentrations. Sources of contamination can include rubber consumable products such as latex gloves and pipette bulbs. Blank (un-spiked) water samples were extracted and analysed to assess background concentrations of NDMA in LC-MS, tap and river water. A small background concentration of NDMA was found in LC-MS water. This was small background was overcome by exposing LC-MS water samples to sunlight for at least 30 h after which the background level of NDMA was below to bottom calibration point and not statistically significant.

The calculated method recoveries both absolute and relative in all water types are shown in table 4.

It was observed that the use of the NDMA-d6 internal standard successfully compensated for any losses during sample preparation, matrix effects and instrument variation leading to accurate quantification in all tested matrices.

For LC-MS, tap and river water samples spiked at 1 ng/l (n=7), (tap and river water background subtracted) absolute method recoveries ranged from 63 to 84 %.

Relative recoveries for LC-MS, tap and river water samples spiked at 1 ng/l (n=7), (tap and river water background subtracted) corrected with the use of NDMA-d6 for losses during extraction ranged from 98 to 115 %. Overall absolute recovery was higher in tap water than in LC-MS water suggesting that dissolved inorganics and/organic carbon in the matrix may enhance the SPE recovery.

Table 4: Absolute & Relative recoveries for all water matrices.

Recovery %	LC-MS	Tap Water*	River Water*
Absolute Recovery	77	84	63
Relative Recovery	98	111	115

* Background levels subtracted.

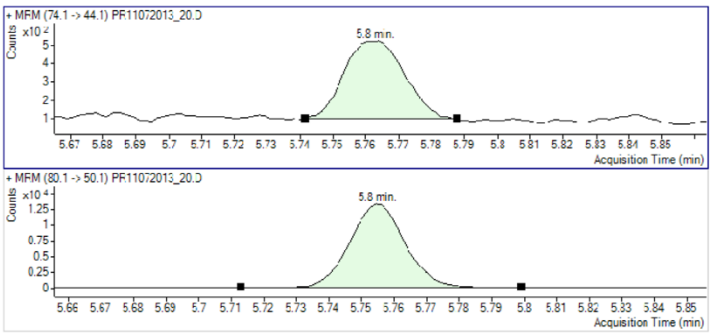

Figure 3: MRM chromatogram for a 1 ng/l spike of river water.

Precision

Replicate analysis (n=7) of 1 and 10 ng/l DCM standards yielded calculated average values of 1.04 ng/l and 10.01 respectively. % RSD's for analysis of these standards were 2.7 and 0.4 respectively. Average calculated accuracy was 103.8 % for the 1 ng/l standard and 100.4 % for the 10 ng/l.

Replicate analysis (n=7) of 1 ng/l spiked LC-MS water samples yielded calculated average values of 0.98 ng/l. % RSD's for analysis of these standards were 4.3. Tap and river water samples (n=7) were also spiked with 1 ng/l of NDMA, calculated values were 10.7 and 7.2 respectively. See table 5 below.

Table 5: Precision data from all water matrices.

	LC-MS	Tap Water	River Water
Spike (ng/l)	1.00	1.00	1.00
Average (ng/l)	0.98	1.16	1.30
SD	0.042	0.124	0.094
% RSD	4.3	10.7	7.2

EI Optimisation

Experiments were conducted to assess the impact of source temperature on the detected abundance of NDMA. Conversely to recently published data [13] this change in temperature between 150 and 350 °C afforded no significant change in abundance of NDMA. See Figure 4.

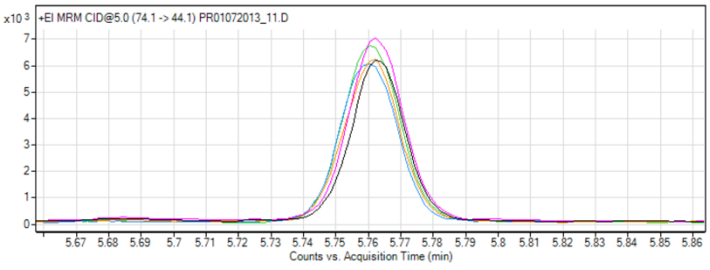

Figure 4: NDMA abundance upon varying source temperature between 150 and 350 °C.

Additionally, the effect of the election ionisation energy was evaluated. Normally 70 eV is default and all EI spectra are generated using this energy. EI is regarded as being a 'hard' ionisation technique, compared to CI. This means that EI tends to result in greater fragmentation of the molecular ion compared to CI. The thinking behind this process was that decreasing this energy may reduce the degree of fragmentation of the molecular ion during ionisation especially for a small molecule such as NDMA. It was observed that changing this voltage did not cause decreased fragmentation of the molecular ion, but just a decrease in overall ionisation, eV experiments were conducted between 10 and 80 eV (Data not shown).

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Detection Limits

When determining detection limits one of the most common procedures is to identify the analyte concentration which gives a signal to noise ratio of > 3. This approach yields the limit of detection (LOD), for quantification a signal to noise > 10 must be achieved which is the limit of quantification (LOQ).

For this application we have chosen to use method detection level (MDL). MDL is used to describe the concentration of an analyte, that when processed through the entire method, produces a signal with a 99 % probability that it is different from the blank. MDL's were determined for NDMA in all of the matrices according to method 1030C from standard methods for the analysis of water and wastewater [14].

For all matrices seven samples were spiked with NDMA at 1 ng/l and NDMA-d6 at 40 ng/l. The samples were then extracted and analysed as above. MDLs were calculated by multiplying the standard deviation of seven replicates by Student's T value of 3.14 (one-side T distribution for six degrees of freedom at the 99% level of confidence).

MDLs for LC-MS, tap and river water were calculated using this method as 0.13, 0.39 and 0.30 ng/l respectively. See below for extracted MRM chromatogram of a 0.5 ng/l spike, see Figure 5.

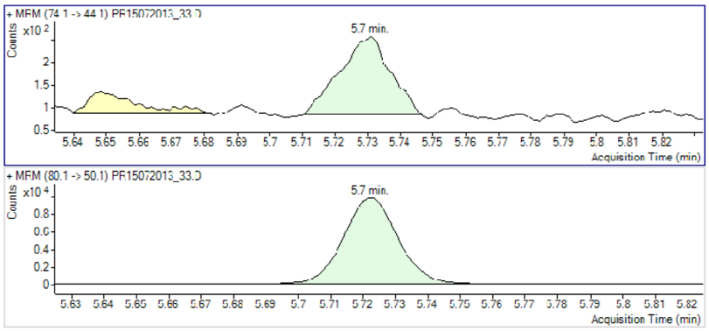

Figure 5: NDMA spike at 0.5 ng/l in LC-MS water ~ derived MDL value.

Overall instrument sensitivity was assessed as instrument detection level (IDL). Instrument detection level (IDL) is the mass on analyte that produces SN >3 [14]. For NDMA IDL was assessed to be 0.25 pg on column, which is in line with recently published data [13].

Notes

Significant amounts of work were completed during this study to remove sources of NDMA interference. Without removing these interferences, the determined MDLs would not have been possible to achieve, and as a consequence the developed method would not be suitable for the automated determination of NDMA in real water samples. Presented below is a chromatogram for an extracted un-spiked LC-MS water sample, to illustrate the absence of NDMA background, see Figure 6.

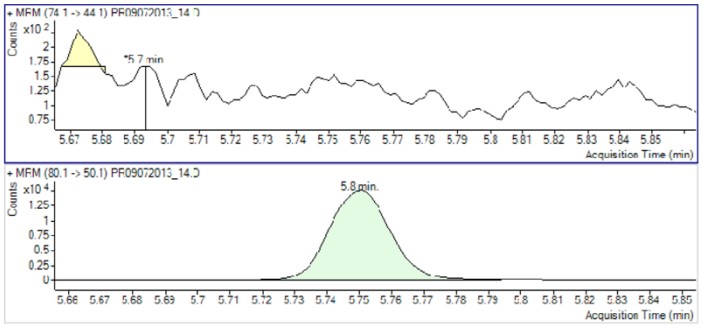

Figure 6: Extracted un-spiked LC-MS water sample.

In the next few months an inter-comparison exercise in collaboration with Anglian Water Services Ltd. will be completed, whereby a selected number of samples will be extracted and analysed blind and compared to the results obtained with their current manual procedure.

CONCLUSIONS

This application note shows how the extraction and analysis of NDMA from water samples can be fully automated using the MPS in combination with ITSP and an Agilent 7000B triple quadrupole mass spectrometer using electron impact ionisation.

The high specificity and sensitivity gained by using tandem MS has allowed unambiguous identification and quantification of NDMA in real water samples.

The use of NDMA-d6 allows accurate quantification and corrects for extraction variability, chromatography, ionisation or detection.

The established MDL for NDMA in tap and river water (0.39 and 0.30 ng/l respectively) means that the method is fit for purpose to fulfil the DWI requirements for the detection of NDMA in water.

Further work will be completed shortly to incorporate the full suite of additional nitrosamines into this automated procedure.

If you are interested in the automated extraction of NDMA or other analytes from water, please contact Anatune.

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