Picogram determination of \(N\)-nitrosodimethylamine in water
Ruikang Hu, Lifeng Zhang and Zhaoguang Yang

ABSTRACT

\(N\)-nitrosodimethylamine (NDMA) persistence within surface waters is a major concern for downstream communities exploiting these waters as drinking water supplies. The objective of this study is to develop a novel and efficient analytical method for NDMA via different technologies: pulsed splitless gas chromatography–nitrogen phosphorus detector (GC–NPD), large volume injection (LVI) gas chromatography–mass spectrometry (GC/MS) via programmable temperature vaporizer (PTV) inlet or PTV-gas chromatography– triple quadruple mass spectrometry (GC-MS/MS) and continuous liquid–liquid extraction. It was found that the sensitivity required for NDMA analysis by GC–NPD can only be achieved when the NPD bead is extremely clean. LVI via PTV can greatly improve GC-MS system sensitivity for analyzing NDMA. With the help of DB-624 (25 m \(\times\) 200 \(\mu\)m \(\times\) 1.12 \(\mu\)m) connected with DB-5MS (30 m \(\times\) 250 \(\mu\)m \(\times\) 0.25 \(\mu\)m) in series, PTV–GC/MS could overcome the matrix interference for the trace analysis of NDMA. Variable instrument conditions were studied in detail, with the optimized process being validated via precision and accuracy studies. PTV- triple quadruple GC-MS/MS system could efficiently remove the interference on a single DB-5MS (30 m \(\times\) 250 \(\mu\)m \(\times\) 0.25 \(\mu\)m) column with good sensitivity and selectivity. The developed methods have been successfully applied to test NDMA in different types of water samples with satisfactory results.

**Key words** | GC-MS, large volume injection (LVI), NDMA, programmable temperature vaporizing (PTV), water

INTRODUCTION

Many \(N\)-nitrosamines, especially NDMA, are potent carcinogens. They have been found in many food products, soils and wastewater, as well as drinking water. Rocket fuel (incomplete oxidation of hydrazines), polymers, plasticisers, batteries and other industrial products can be pointed out as the main anthropogenic sources of \(N\)-nitrosamines (Gunnison et al. 2000; Richardson 2003).

\(N\)-nitrosamines can be released directly into the environment from industrial sources or can be formed from different precursors and under a wide variety of conditions by nitrosation or oxidation reactions (Valentine 2000; Jurado-Sánchez et al. 2007). \(N\)-nitrosamines can be formed through the reaction of chlorination and chloramination in drinking water between chloramines, chlorine and organic nitrogen precursors (Choi & Valentine 2002; Mitch & Sedlak 2002, 2004; Schreiber & Mitch 2005) and, once formed, they are very difficult to destroy (O’Donnell 2003). The organic nitrogen containing organic matter can include poorly characterised substances such as common humic material (one form of natural organic matter), as well as substances produced during biological waste treatment (Chen & Valentine 2006). Other precursors, such as dimethylamine or other organic amines, N-based cationic polyelectrolytes (Wilczak et al. 2003), or ion exchange resins may also be important in certain situations (San Francisco Public Utilities Commission 2007). Research in California and Canada indicate that NDMA can be formed in drinking water as a consequence of treatment (California Department; Graham et al. 1995).
As drinking water utilities are considering switching from chlorine to chloramine disinfection to meet new standards on trihalomethane and haloacetic acids, similar concerns apply to unreacted nitrosamine precursors in these effluents (USEPA 1998). The US Environmental Protection Agency (US EPA) has classified these compounds in group B2, thus indicating their probable carcinogenic effect on humans. The maximum admissible concentrations of NDMA, NMEA and NDEA in water were established at very low concentration levels (Andrzejewski and Nawrocki 2005; US EPA 2005). EPA has set $10^{-6}$ life time risk at 0.7 ng/L for NDMA (Munch 2005). CDHS established the notification level for NDMA of 0.002 mg/L in 1998. Although the CDHS notification level was revised to 0.01 mg/L in 2002, 2 ng/L was considered as the necessary method detection limit (MDL) for NDMA analysis. In December 2006, Office of Environmental Health Hazard Assessment (OEHHA) established the public health goal (PHG) for NDMA at 3 ng/L (California Department 2006).

Various methods have been developed in NDMA analysis based on isotope-dilution, chemical ionisation, large volume injection or GC/CI/MS/MS. Determination of NDMA at part-per-quadrillion levels using positive chemical ionisation and large-volume injection, automated solid-phase extraction and high-performance liquid chromatographic determination of nitrosamines using post-column photolysis and tris(2,2’-bipyridyl) ruthenium(III) chemiluminescence and GC/CI/MS/MS methods have been previously reported (Presta & Hermannb 1999; Mitch et al. 2003; McLean et al. 2005; Perez-Ruiz et al. 2005; Schreiber & Mitch 2005; Munch & Bassett 2005).

Using these approaches can satisfy the 2 ng/L action level for NDMA set by the State of California (1998) and the 700 ppq regulatory standard promulgated by US EPA. However, very special reagent gas, such as ammonia, methanol or acetonitrile, is used in chemical ionisation methods, or expensive instruments (GC-MS/MS) have to be used. In this study, a practical and efficient analysis method for NDMA and other N-nitrosamines have been developed based on traditional GC-MS with a programmable temperature inlet at electron impact (EI) mode through large volume injection, and compared with the methods on traditional GC-NPD and more advanced equipment of triple quadruple PTV-GC/MS/MS.

### EXPERIMENTAL

#### Chemicals and reagents

The NDMA standard (2 mg/mL) and internal standard, N-nitrosod-n-propylamine (1 mg/mL), were purchased from Accustandard (New Haven, CT, USA). Surrogate standard, 1 mg/mL of N-nitrosodimethylamine-d6 (98%) in methylene chloride-d2 (P/N: DLM-2130-S) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Dichloromethane was ABSOLV grade and obtained from Tedia Company Inc. (Fairfield, OH, USA). NDMA and NDMA-d6 standard mixture solutions were prepared in dichloromethane, and subsequently diluted to achieve a calibration standard from 0.5 to 20 ng/mL for LVI-PTV-GC/MS and PTV-GC-MS/MS. Deionised water was obtained by passing tap water through an USF-ELGA option 15 system and an USF Maxima system (Vivendi-Water, UK) with the resistance greater than 18.2 M·cm$^{-1}$ and on-line TOC less than 2 μg/L.

#### Equipment

**Pulsed splitless GC/NPD**

The gas chromatograph used for this study was an Agilent 6890 series GC coupled with NPD. In order to achieve the best sensitivity, the whole NPD system must be maintained in extremely clean condition. The columns used were DB-624 (25 m × 200 μm × 1.12 μm) connected with DB-1701 (30 m × 250 μm × 0.25 μm) in series to completely separate NDMA peak with other interference.

**PTV-GC/MS**

The gas chromatograph used for this study was an Agilent 6890 series GC coupled with 5973 N series mass spectral detector. An Agilent programmable temperature vapouriser inlet (G2619A Septumless head) with PTV liner (HP 5183-2039 PTV liner) was applied as the sample injector. The GC column used was DB-624 (30 m × 1.12 mm × 0.2 μm) and DB-5MS (30 m × 0.25 mm × 0.25 μm). NDMA-d6 was used as a surrogate standard to monitor the recovery of process and NDPA-d14 was used as the internal standard to
adjust the variation of instrument. The instrument conditions are shown in Table 1.

**PTV-triple quadruple MS/MS**

The gas chromatograph used for this study was a Quattro Micro™ GC system consisting of an Agilent 6890 series GC coupled with a Micromass® Quattro Micro mass spectrometer. An Agilent programmable temperature vapouriser inlet (G2619A Septumless head) with PTV liner (HP 5183-2039 PTV liner) was applied as the sample injector. The GC column used was DB-5MS 30 m × 0.25 mm × 0.25 μm. NDMA-d6 was used as the surrogate standard and NDPA-d14 was used as the internal standard.

**Extraction procedure**

**Continuous liquid–liquid extraction**

A 1 L water sample was added into a continuous liquid–liquid extractor, and extracted with 400 mL dichloromethane (to minimise the interference from trace residues of solvent, solvent is re-distilled) for 5 hours. The apparatus used for current continuous liquid–liquid extraction was made by UFO Pte Ltd., Singapore. The temperature of the water bath was controlled by a magnetic digital ceramic hotplate/stirrer SM26 (Stuart Scientific, Staffordshire, UK). The temperature of the water bath was set at 65°C, stirred at 700 rpm. After drying over anhydrous sodium sulphate, the extract was concentrated to 1 mL via nitrogen blowdown.

**RESULTS AND DISCUSSION**

Among the techniques of GC-NPD, PTV-GC/MS and PTV-GC/MS/MS, GC-NPD is the easiest way in terms of operation and maintenance, as well as equipment cost. GC-NPD can be applied in the determination of organo-nitrogen or organo-phosphorus compounds. At the beginning of the current NDMA analysis study, it was tried on the GC-NPD system. Continuous liquid–liquid extraction was applied to extract NDMA from the water sample with a mean recovery of above 60%. Compared with the normal split injection mode, the pulsed-splitless injection method was found to be able to greatly improve the sensitivity and peak shape. In order to achieve the best sensitivity, the whole NPD system must be maintained in an extremely clean condition. In such clean conditions, it is capable of analysing NDMA by GC-NPD with 2 ng/L of detection limit (Figure 1). As NDMA and NDMA-d6 could not be separated by GC-NPD, NDMA-d6 could not be used as a surrogate in the GC-NPD method.

**LVI using PTV-GC/MS technique**

The programmed temperature sample introduction technique was first described by Vogt in 1979. This technique was used to introduce large sample volumes into capillary GC columns at a temperature slightly below the boiling point of solvent. Based on this idea, Poy developed the programmed temperature vapouriser inlet (PTV) (Vogt et al. 1979; Poy et al. 1981). LVI using PTV can improve GC system detection limits by one to two orders of magnitude over conventional split/splitless inlet. The PTV inlet has the same basic functions as the split/splitless inlet except that it is temperature programmable from –60°C (using CO₂ cooling) or –160°C (using liquid N₂ cooling) to 450°C at rates up to 720°C/min. At the “solvent vent” mode with multiple injections, system sensitivity can be greatly improved. LVI with PTV is ideal for trace analysis of later eluting solutes with boiling points approximately 100°C higher than solvent and for samples with a dirty matrix.

Generally, LVI via PTV consists of three steps: sample injection and solvent elimination, splitless sample transfer to the GC column, and chromatographic separation. However, due to the complicated PTV injection process,
many factors can affect the performance and efficiency of this system, such as liner type, injection volume, initial inlet temperature and temperature ramp rate etc. Figure 2 shows ion chromatograms of 10 μL × 5 of 10 μg/L NDMA and NDMA-d6 mixture standard.

PTV-triple quadruple GC-MS/MS

The Triple Quadruple GC-MS/MS is a tandem quadrupole mass spectrometer equipped with dedicated electron impact (EI) and chemical ionisation (CI⁺ / CI⁻) sources. Samples can be introduced via gas chromatography (GC/MS/MS), direct insertion probe (DIP) or desorption chemical ionisation (DCI) probes. It is a powerful mass spectrometry tool for quantitative trace analysis.

At multiple reaction monitoring (MRM) mode with electron impact ionisation source (EI) and the collision energy of 5 eV and injection volume of 25 μL, transitions 73.8 → 44, and 73.8 → 43 were chosen for the quantitative analysis and identification. Figure 3 shows the chromatograms of NDMA on PTV-GC/MS/MS analysis.

The above chromatograms clearly indicate that all the above techniques can be used for NDMA analysis in terms of sensitivity. However, compared to the GC-NPD method, it is very troublesome to keep the NPD bead in an extremely...
clean condition. At normal conditions, the method detection limit of NDMA only can be 0.015 μg/L by GC-NPD (US-EPA method 8070A). To maintain the high sensitivity, the NPD parts, such as the base weldment, lid stop, block insulation, metal C-ring, alumina insulator and NPD bead, need to be cleaned or changed frequently. As NDMA and NDMA-d6 could not be well separated, NDMA-d6 could not be used as the surrogate standard. The PTV-GC/MS/MS is an excellent tool for NDMA analysis with good sensitivity and selectivity, the instrument detection limit (IDL) can be as low as 0.1 μg/L (IDL, based on signal/noise = 3). However, it is significantly more expensive than a normal PTV-GC/MS in terms of equipment cost and maintenance. During the method development, more parameters of PTV and MS/MS must be optimised to achieve the high sensitivities and, under MRM mode, the sample matrix has been shown to have a more effective response than single quadruple GC-MS. Therefore, in the current study, we will focus on the PTV-GC/MS technique for the NDMA analysis.

**Effect of different type of liners**

There are four PTV liners provided by Agilent Technologies Pte Ltd. (Table 2). The volume of PTV liner is less than 200 μL, while the volume of traditional split/splitless line could be up to 1,000 μL. The PTV liner has a smaller internal diameter and volume than a normal liner, which allows sharp peaks to be generated after column separation to improve separation and enhance sensitivity. The proper liner to be used is very dependent on the application. Volume, activity and the packing material are also important considerations for choosing a suitable liner. In general, the most appropriate liner must be determined through experimentation.

<table>
<thead>
<tr>
<th>PTV Liner</th>
<th>Part no.</th>
<th>Description</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5183-2037</td>
<td>Multi baffle, deactivated 1.5 mm id, 150 μL</td>
<td>Active compounds, drugs, pesticides</td>
</tr>
<tr>
<td>B</td>
<td>5183-2041</td>
<td>Fritted glass, deactivated, 1.5 mm id, 150 μL</td>
<td>Large volume injections</td>
</tr>
<tr>
<td>C</td>
<td>5183-2038</td>
<td>Single baffle, glass wool, deactivated, 2 mm id, 180 μL</td>
<td>Large volume injection</td>
</tr>
<tr>
<td>D</td>
<td>5183-2036</td>
<td>Single baffle, deactivated, 2 mm id, 200 μL</td>
<td>General purpose</td>
</tr>
</tbody>
</table>

The design of many liners consists of deactivated glass wool packing. The glass wool provides additional surface area for complete volatilisation of the sample to minimise sample discrimination, trap non-volatile components and septum particles before they reach the column and wipes any sample from the syringe needle, thereby increasing reproducibility and preventing sample residue build-up at the Merlin Microseal. Glass wool is generally not recommended for the following compounds: phenols, organic acids, pesticides, slightly acidic, drugs of abuse, reactive polar compounds, thermally labile. The disadvantages of glass wool packed liners are that the wool could adsorb analytes or contribute to analytes decomposition. The second type of liner is baffle liner. It is used for labile samples, analytes with narrow range of boiling points. Turbulent flow is created by internal baffles, to aid in mixing the vaporised sample with carrier gas before it reaches the column inlet. Proper mixing ensures that a representative
part of the sample enters the column. The third type of liner is fritted liner, which is used for minimising sample discrimination and effectively traps particles and non-volatiles. In this liner a high surface area and complex flow path is created through a porous ceramic frit. The turbulent flow is needed for sample volatilisation. The disadvantages are expensive and ceramic frit can adsorb analytes or contribute to their decomposition. In this study, 1 μg/mL × 5 μL NDMA and NDMA-d6 mixtures standard were introduced into different liners at splitless mode, only the glass wool packed liner showed the best sensitivity and was selected in the following experiments.

Effect of injection volume

Large volume injection can be performed via the PTV inlet at solvent vent mode by multiple injections. Large volume injection could greatly increase the response intensities of analytes. When the sample is introduced into the PTV liner, the solvent is vented via a split line while the analytes are trapped and pre-concentrated. As the solvent has been swept out of the liner, there is enough space that could be used for the next injection. At the end of the injection process the split vent is closed and the liner of the PTV is heated rapidly to introduce the adsorbed analytes into the GC column. It was found that with the increase of injection volume, the response intensities increased stably (as shown in Figure 4). When the injection volume of standard mixture was increased from 10 to 100 μL, their response intensities increased as well. However, when the injection volume was over 50 μL, the rate of increase of response intensities dropped. That means that when the injection volume is over 50 μL, the effect of injection volume is less than that at a small injection volume. Therefore, in the current study, the optimal injection volume was set at 50 μL.

Effect of initial inlet temperature

One of the advantages of the PTV inlet is that the sample can be introduced in an initial cool inlet. As the sample is introduced into the cool liner, the evaporation, decomposition and the thermal degradation during the injection process will be minimised. This will help to achieve a high response intensity of analytes. In our study, experiments were conducted at the same running program with different initial inlet temperature at splitless mode. It was found (see Figure 5) that the initial cool inlet could result in a 3–4-fold increase of peak area over that of a traditional hot inlet. If
the sample was injected into a hot inlet, some analytes will be vaporised and swept out of the inlet immediately, resulting in a loss of sensitivity.

**Effect of inlet temperature program and inlet vent flow**

For most of the PTV application, a slow ramp rate can be applied to minimise the thermal decomposition of the labile analytes. However, in practice, too low a ramp rate will result in a long instrumental analysis time. If sample analytes are thermal stable, the inlet may be heated as fast as 720°C/min.

In this study, ramp rates of 100, 200, 300, 400 and 500°C/min were studied to evaluate the effect of the ramp rate to system sensitivity. Figure 6 indicates that the inlet ramp rate set at 400°C/min shows the best sensitivity for NDMA. Four hundred °C/min is the optimum inlet temperature program for NDMA analysis. If the inlet ramp rate is above 400°C/min, some of the analytes will be decomposed, this is proven by the decreased response intensity.

The inlet vent flow is another factor which will affect the system sensitivity. If the vent flow is too low, some solvent will remain in the liner. If the vent flow is too high, some analytes will be swept out of the liner. Our study showed that the vent flow of 70 mL/min generated the best sensitivity for low boiling point N-nitrosamine compounds, NDMA.

**Optimised method**

Based on the above investigations, the optimised PTV condition in our subsequent study is as follows: at solvent vent mode, the PTV inlet temperature ramp rate was set at 400°C/min. The total injection volume was 50 μL. Sample extraction was conducted using continuous liquid–liquid extraction by dichloromethane for 5 h at pH = 7.

**Application to real water sample analysis**

Four different types of water samples, waste water (sample A), raw water (sample B), treated water (sample C & D) and a matrix spiked sample (sample D) were analysed by this validated method.

After a 1 L water sample were extracted by continuous liquid–liquid extraction and concentrated to 1 mL, the samples were analysed by PTV-GC/MS with an optimised
method. The results obtained are listed in Table 3. The overlaid chromatograms of sample D and its matrix spike sample (sample D-MS) are indicated in Figure 7. Target compounds were identified based on both retention time and relative intensity. The major fragments (relative intensities > 10% of the most abundant ion) in the mass spectrum should be present in the sample spectrum, with the relative intensities agreeing within ±20%. For sample D, the relative intensity of fragments of m/e 42 was 70% of m/e 74 for NDMA.

### CONCLUSIONS

LVI-GC/MS via PTV has been proven to be an efficient technology in the analysis of NDMA in water at a sub parts-per-trillion level. Various factors affecting this technique have been studied in detail. It was found that the PTV ramp rate at 400°C/min and a total injection volume of 50 μl with constant column pressure are the most suitable conditions to achieve good results. The method detection limit of NDMA is 0.52 ng/L. The relative standard deviation at the quantification limit level is 7.2% and the recovery is 67.2%. This method has been successfully applied to test NDMA in different types of water samples with satisfactory results.

### REFERENCES


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Munch J. W. 2005 Method 521 Determination of nitrosamines in drinking water by solid phase extraction and capillary column Gas chromatography with Large volume injection and Chemical Ionization tandem Mass Spectrometry (MS/MS), http://oaspub.epa.gov/eims/xmlreport.display?deid = 103912 &z_chk = 30513


### Table 3 | NDMA analysis results of real water samples (ng/L)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample A</th>
<th>Sample B</th>
<th>Sample C</th>
<th>Sample D</th>
<th>Sample D-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMA</td>
<td>1.4</td>
<td>0.8</td>
<td>0.7</td>
<td>3.0</td>
<td>12.6</td>
</tr>
</tbody>
</table>

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