



ENVIRONMENT AGENCY

The determination of N-nitrosodimethylamine in drinking waters (2014)

24 November 2014

Methods for the Examination of Waters and Associated Materials

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This booklet contains 3 methods for the determination of N-nitrosodimethylamine in drinking waters.

Limited performance data are available for the methods described in this booklet. Each method has been validated or tested in only one laboratory and consequently details are included for information purposes only, as examples of the type of procedures that are available to analysts. Information on routine multi-laboratory use of these methods would be welcomed to assess their full capabilities.

Whilst specific commercial products may be referred to in this document, this does not constitute an endorsement of these products but serves only as illustrative examples of the types of products available. Equivalent products may be available and it should be understood that the performance of the method might differ when other materials are used and all should be confirmed by validation of the method.

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About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, waste water and effluents as well as sewage sludges, sediments, soils (including contaminated land) and biota. In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets within the series "Methods for the Examination of Waters and Associated Materials" and their continuing

revision is the responsibility of the Standing Committee of Analysts (established 1972 by the Department of the Environment). At present, there are seven working groups, each responsible for one section or aspect of water quality analysis. They are

- 1 General principles of sampling and accuracy of results
- 2 Microbiological methods
- 3 Empirical, Inorganic and physical methods, Metals and metalloids
- 4 Solid substances
- 5 Organic impurities
- 6 Biological, biodegradability and inhibition methods
- 7 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with these methods are listed at the back of this booklet.

Publication of new or revised methods will be notified to the technical press. If users wish to receive copies or advanced notice of forthcoming publications or obtain details of the index of methods then contact the Secretary on the SCA's web-page:-
<http://www.standingcommitteeofanalysts.co.uk/Contact.html>

Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary. Users should ensure they are aware of the most recent version they seek.

Rob Carter
Secretary
June 2017

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 2002 (SI 2002/2677). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety. These should be consulted and be readily accessible to all analysts. Amongst such resources are; HSE website [HSE: Information about health and safety at work](http://www.hse.gov.uk) ; RSC website <http://www.rsc.org/learn-chemistry/collections/health-and-safety> "Safe Practices in Chemical Laboratories" and "Hazards in the Chemical Laboratory", 1992, produced by the Royal Society of Chemistry; "Guidelines for Microbiological Safety", 1986, Portland Press, Colchester, produced by Member Societies of the Microbiological Consultative Committee; and "Safety Precautions, Notes for Guidance" produced by the Public Health Laboratory Service. Another useful publication is "Good Laboratory Practice" produced by the Department of Health.

The determination of N-nitrosodimethylamine in drinking waters by GC-MS

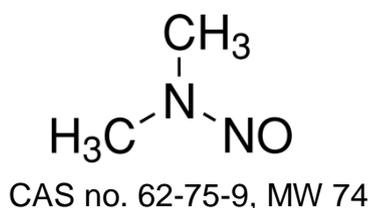
1 Introduction

The presence of N-nitrosodimethylamine (NDMA) is frequently associated with the practice of chloramination and in this respect NDMA is considered to be a disinfection by-product. Factors implicated in the formation of NDMA include raw water source, influence of sewage effluent, agricultural and industrial discharges (i.e. as sources of precursors) and chemicals used in water treatment processes, particularly ion exchange and chloramination. Also, NDMA may be formed as a result of coagulation. A common factor where NDMA is detected is the use of particular ferric coagulants. Analyses on samples of coagulants have indicated that NDMA is a possible contaminant of particular coagulants. NDMA concentrations in post-granular activated carbon (GAC) samples are often lower than in pre-GAC samples, suggesting possible removal as a result of biological activity and/or biodegradation in the carbon bed. Concentrations of NDMA in samples from distribution are generally comparable to values measured in final waters. There is no clear evidence to indicate continued formation of NDMA in distribution.

NDMA may be detected⁽¹⁾ in final waters at concentrations of up to about 10 ng/l. Should future UK drinking water regulations restrict NDMA to similar levels, this would have only a minor impact on the UK water industry. A guideline value⁽²⁾ for NDMA in drinking water is under consideration by the World Health Organization and is likely to be in the region of 100 ng/l.

The chemical structure of N-nitrosodimethylamine (NDMA, C₂H₆N₂O) is shown in Figure 1.

Figure 1 Chemical structure of N-nitrosodimethylamine



Formation of NDMA can result from pre-formed chloramine or from the separate addition of ammonia and chlorine. This is significant in relation to both treatment and distribution where chloramination is used. It may also be significant where ammonia occurs naturally in the raw water and is removed by chlorination.

Some poly-electrolytes used in water treatment contain the precursor dimethylamine. However, this and similar chemicals are not believed to be widely used for drinking water treatment in England and Wales. Strong base anion exchange resins used for nitrate removal may provide a source of precursors for NDMA formation, particularly when new. NDMA is a very hydrophilic compound, very soluble in water, hence is poorly adsorbed onto activated carbon. NDMA removal observed in some GAC systems may be due to biological activity and biodegradation.

Methods are based^(3, 4) on spiked samples passed through coconut charcoal solid extraction cartridges, eluted with dichloromethane and determined using GC-MS detection. The methods described in this booklet are very similar but subtle differences are reported in the procedures.

2 References

- 1 NDMA - Levels in drinking water and factors affecting its formation (CSA7240 / WT02049) Defra 7348 - March, 2008.
- 2 N-nitrosodimethylamine in drinking water. World Health Organization Guidelines for Drinking-Water Quality, 3rd edition including 1st and 2nd addenda, 2008. (http://www.who.int/water_sanitation_health/dwq/chemicals/ndmasummary_2ndadd.pdf)
- 3 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). J W Munch and M V Bassett, 2004. Version 1.0. EPA Document No. EPA/600/R-05/054. National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268 (www.epa.gov/nerlcwww/m_521.pdf).
- 4 Comparison of alternative nitrosamine analyses for water reuse samples. Water Reuse Association, R C Cheng, C Andrews-Tate, C J Hwang, Y Guo, J E Grebel and I H Suffet, 2005. California Section, 2005 Annual Conference, San Diego, CA. (www.watereuse.org/ca/2005conf/papers/A1_rcheng.pdf).

A The determination of N-nitrosodimethylamine in drinking waters by GC-MS

A1 Performance characteristics of the method

A1.1	Substance determined	N-nitrosodimethylamine (NDMA).
A1.2	Type of sample	Drinking waters.
A1.3	Basis of method	A deuterated internal standard is added to the sample which is then passed through a conditioned solid phase extraction cartridge. The dried cartridge is eluted with dichloromethane and the volume reduced. The extract is then ready for gas chromatography (GC) with mass spectrometric (MS) detection.
A1.4	Range of application	Typically, up to 0.04 µg/l, but can be extended, see section A7.18.
A1.5	Calibration curve	Linear over the range of application.
A1.6	Interferences	Any substance which is co-extracted under the conditions used and is not removed by the clean-up procedure and which exhibits similar chromatographic behaviour to NDMA, by producing the same mass ion fragments and has the same retention time as NDMA, will interfere.
A1.7	Total standard deviation	See Table A1.
A1.8	Limit of detection	Typically, 0.0008 µg/l of NDMA can be detected.
A1.9	Bias	See Table A1.

A2 Principle

Deuterated-NDMA (as an internal standard) is added to the sample. The spiked sample is then passed through a conditioned charcoal solid phase extraction cartridge. The cartridge is dried under vacuum and the NDMA eluted from the cartridge with dichloromethane and the volume of solvent reduced. The extract is then ready for gas chromatography (GC) with mass spectrometric (MS) detection using electron impact (EI) ionisation mode. Quantification is achieved by internal standardisation. Calibration, blank and AQC samples are extracted as with the samples.

A3 Hazards

N-nitrosodimethylamine is poisonous if swallowed, inhaled or absorbed through the skin, and is probably carcinogenic. Methanol is toxic and flammable. Dichloromethane is a suspect carcinogen. Skin contact, ingestion and inhalation of these compounds should be avoided by

using appropriate protective equipment and working within a fume cupboard, when appropriate.

A4 Reagents

All reagents should be of analytical grade quality and distilled or deionised water should be used throughout.

A4.1 Dichloromethane – HPLC grade.

A4.2 Methanol – HPLC grade.

A4.3 Anhydrous sodium sulphate – Heat granular anhydrous sodium sulphate at 500 °C for approximately 4 hours. Allow the sodium sulphate to cool and transfer to a suitable container. Seal the container. Dried sodium sulphate may be stored at 25°C in the sealed container for up to 4 weeks.

A4.4 Ascorbic acid – Analytical reagent grade.

A4.5 Ascorbic acid solution (80 g/l) – Dissolve 4 g of ascorbic acid in approximately 30 ml of water. Mix well and make to 50 ml with water. Mix well. This solution may be stored at room temperature for up to 1 month.

A4.6 Stock internal standard solution (1000 mg/l) – For example, dissolve 10 mg of deuterated-NDMA (D₆-NDMA) in approximately 9 ml of methanol and mix well. Make to 10 ml with methanol. Mix well. This solution may be stored at -15 °C for up to 1 year. The solution may be available commercially and should be stored according to manufacturer's instructions.

A4.7 Intermediate internal standard solution (50 mg/l) – Add 500 µl of stock internal standard solution (A4.6) to approximately 5 ml of methanol contained in a 10 ml volumetric flask and mix well. Make to 10 ml with methanol. Mix well. This solution may be stored at -15 °C for up to 6 months.

A4.8 Working internal standard solution (0.4 mg/l) – Add 200 µl of intermediate internal standard solution (A4.7) to approximately 15 ml of methanol contained in a 25 ml volumetric flask and mix well. Make to 25 ml with methanol. Mix well. This solution may be stored at -15 °C for up to 6 months.

A4.9 N-nitrosodimethylamine stock standard solution (1000 mg/l) – Dissolve 10 mg of N-nitrosodimethylamine (NDMA) in approximately 9 ml of methanol and mix well. Make to 10 ml with methanol. Mix well. This solution may be stored at -15°C for up to 1 year. The solution may be available commercially and should be stored according to manufacturer's instructions.

A similar AQC solution should also be prepared, preferably by different staff, from a separate lot number and ideally using reagents from different manufacturers.

A4.10 N-nitrosodimethylamine intermediate standard solution (10 mg/l) – Add 100 µl of NDMA stock standard solution (A4.9) to a 10 ml volumetric flask and make to 10 ml with methanol. This solution may be stored at -15 °C for up to 1 year.

A similar AQC solution should also be prepared, preferably by different staff, from a separate lot number and ideally using reagents from different manufacturers.

A4.11 N-nitrosodimethylamine spiking standard solution (0.2 mg/l) – Add 200 µl of NDMA intermediate standard solution (A4.10) to a 10 ml volumetric flask and make to 10 ml with methanol. This solution may be stored at -15 °C for up to 6 months.

A similar AQC solution should also be prepared, preferably by different staff, from a separate lot number and ideally using reagents from different manufacturers.

A4.12 N-nitrosodimethylamine calibration standard solutions – For example, for a five point calibration, prepare a series of five 1000 ml volumetric flasks containing 1000 ml of water and 0.5 ml of ascorbic acid solution. Mix well.

To each flask add 100 µl of working internal standard solution (A4.8). This is equivalent to 0.04 µg of D₆-NDMA.

To these flasks, add 0.0, 20.0, 50.0, 100.0 and 200.0 µl of NDMA spiking standard solution (A4.11). The flasks contain 0.0, 0.004, 0.01, 0.02 and 0.04 µg of NDMA, respectively. Mix well. These solutions should be prepared on the day of use.

An AQC solution should be prepared by adding 50.0 µl of the spiking AQC standard (A4.11) to 1000ml of water containing 0.5ml of ascorbic acid solution.

A5 Apparatus

In addition to normal laboratory glassware the following will be required.

A5.1 SPE cartridges – For example, 6 ml, 2g activated coconut charcoal cartridges, or equivalent.

A5.2 Evaporating system – For example, Zymark Turbovap operated at 25 °C, or equivalent.

A5.3 Amber glass vials

A5.4 GC-MS equipment – The suitability of the equipment will need to be evaluated.

The following conditions have been used in generating performance data.

Column: RTx5-Amine, 30 m x 0.25 mm diameter, 1 µm film thickness, with 1 m 0.53 mm diameter deactivated pre-column, or equivalent.

Carrier gas: Helium, constant flow at 0.8 ml per minute.

Injection volume: 3µl (PTV split-less injection mode)

Temperature programmes:

Oven: Initial temperature at 35 °C for 5 minutes, then 120°C per minute to 320 °C, hold time for 8 minutes.

Injector: Initial temperature at 20 °C for 0.1 minute, then 720 °C per minute to 300 °C.

Using these conditions, the following apply

Compound	Approximate retention time (minutes)	Ions monitored	
		Target	Qualifier
D ₆ -NDMA	7.1	80	-
NDMA	7.1	74	42

Equivalent equipment and conditions may be used. See Figure A1 for typical chromatograms.

A6 Sample collection and preservation

Samples should be collected in 1-litre plastic polyethyleneterephthalate (PET) bottles containing 40 mg of ascorbic acid or 0.5 ml of ascorbic acid solution. In stability trials, spiked NDMA samples were maintained at room temperature for approximately 17 hours prior to refrigeration. On receipt into the laboratory, samples were stored in the dark at 5 ± 3 °C prior to extraction. Samples containing ascorbic acid have been shown to be stable for up to 3 weeks when stored in the dark at 5 ± 3 °C. When extracted, sample extracts have been shown to be stable for up to 3 weeks when stored in the dark at -15 °C.

A7 Analytical procedure

A7.1 Allow the sample to reach room temperature before proceeding. To 1000 ml of sample, add 100 µl of working internal standard solution (A4.8). Mix well. This is equivalent to 0.04 µg of D₆-NDMA.

A7.2 Using vacuum, wash an SPE cartridge by passing 3 ml of dichloromethane through the cartridge. Discard the washings. Repeat this process with another 3 ml of dichloromethane.

A7.3 Repeat step A7.2 using 3 ml quantities of methanol. Turn off the vacuum.

A7.4 Condition the cartridge by allowing 3 ml of methanol to drain through the cartridge under gravity. Discard the solvent. Repeat this process with another 3 ml of methanol.

Note:- Do not let the meniscus of the solvent to go below the level of the cartridge packing material.

A7.5 Further condition the cartridge by allowing 3 ml of water to drain through the cartridge under gravity (see note A7.4). Discard the water.

A7.6 Repeat step A7.5 a further four times using 3 ml quantities of water (see note A7.4).

A7.7 Using vacuum, pass the spiked sample (A7.1) through the cartridge. Rinse the cartridge with 3 ml of deionised water. Turn off the vacuum. The flow rate should be approximately 20 ml per minute (also see note A7.4).

A7.8 When all of the sample has passed through the cartridge, dry the cartridge. This process should take about 30 minutes and may be assisted with vacuum or by passing air through the cartridge.

A7.9 Elute the cartridge with 3 ml of dichloromethane and apply vacuum to draw out all liquid, collecting the eluate in a suitable container. Turn off the vacuum.

A7.10 Add another 3 ml of dichloromethane to the cartridge. Under gravity, allow the solvent to soak into the cartridge packing material and leave for approximately 2 minutes.

A7.11 Continue the elution by adding a further 2 x 3 ml quantity of dichloromethane collecting the eluate in the same container.

A7.12 Remove the container and add sufficient sodium sulphate to dry the dichloromethane such that a clear solution remains. Any traces of moisture will be noticeable as turbidity within the container.

A7.13 Transfer the dichloromethane to a second container taking care not to transfer any sodium sulphate. Rinse the sodium sulphate with 1-2 ml of dichloromethane and transfer the washings to the second container.

A7.14 Reduce the dichloromethane to a low volume (but do not evaporate to dryness) and transfer the dichloromethane to a vial rinsing as appropriate, so that the final volume is about 200 µl. The extract is now ready for GC determination.

A7.15 Extract the N-nitrosodimethylamine AQC and calibration standard solutions (A4.12), in place of the volume of sample, as per the procedures described in steps A7.2 - A7.14.

A7.16 Set up the GC-MS system according to manufacturer's instructions and construct a calibration graph of response versus amount of NDMA in the vial, monitoring for the ion fragments under the instrument conditions given in section A5.4.

This equates to 0.0, 0.004, 0.01, 0.02 and 0.04 µg of NDMA, respectively and 0.04 µg of internal standard. Since standards are passed through the cartridge an automatic recovery correction is obtained. If recovery estimates were required, spiked and un-spiked aqueous solutions would need to be analysed.

A7.17 Analyse blank, sample and AQC extract solutions using the instrument conditions given in section A5.4 and the calibration curve generated in step A7.16.

A7.18 From the calibration graph, obtain the amount, A_v , of NDMA in the vial and then calculate the concentration, C_s , of NDMA in the sample.

If the response or calculated concentration exceeds the calibration range, the analysis may be repeated using a smaller amount of sample and making the volume to 1000 ml with water.

A8 Calculations

Determine the concentration, C_s , in the sample using the equation:

$$C_s = (A_v \times 1000) / V_s \text{ } \mu\text{g/l}$$

where

C_s is the concentration (µg/l) of NDMA in the sample;

A_v is the amount (µg) of NDMA obtained from the graph;

V_s is the volume (ml) of sample analysed (A7.1);

and taking into account any dilution of the sample (see A7.18)

Table A1 NDMA Performance data

Solution	Precision (%)	Trueness (%)	LOD (ng/l)	Recovery (%)	Degrees of freedom
Blank			0.8		20
Low standard	8.4	96.5			21
High standard	6.1	99.9			20
Final water	7.4	97.5		97.5	21

Low standard was water spiked with 0.01 µg/l NDMA

High standard was water spiked with 0.03 µg/l NDMA.

Final water was a typical drinking water spiked at 0.01 µg/l.

Limit of detection (LOD) = 3 x within batch standard deviation of blank spiked at 0.004 µg/l.

Precision = 2 x Relative Total standard Deviation

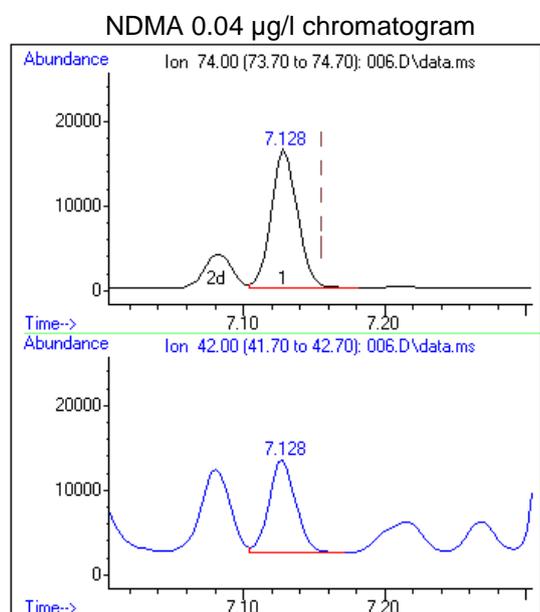
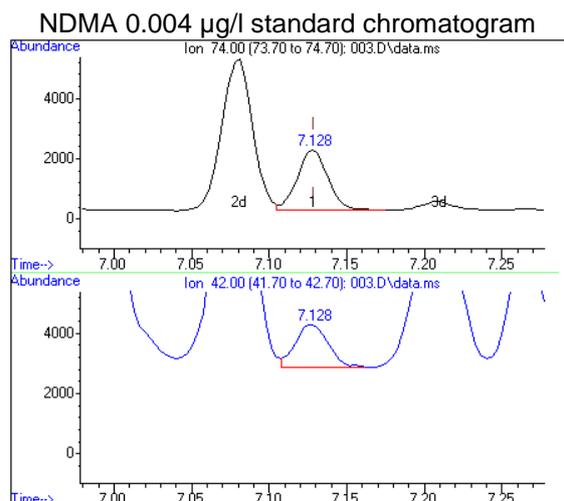
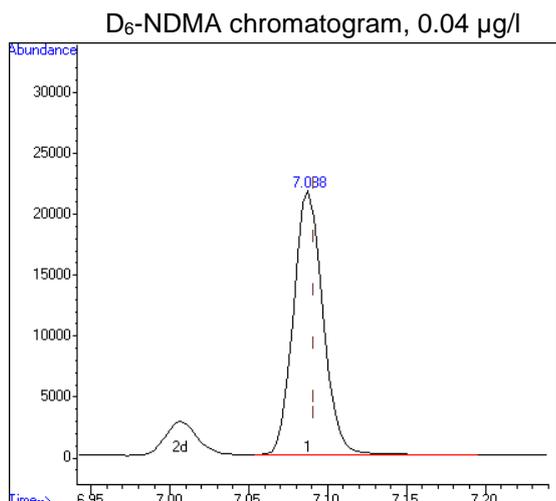
Trueness = ((True conc – derived conc)/True conc) x 100

These terms are based on the DWI prescribed characteristics as detailed in the regulations.

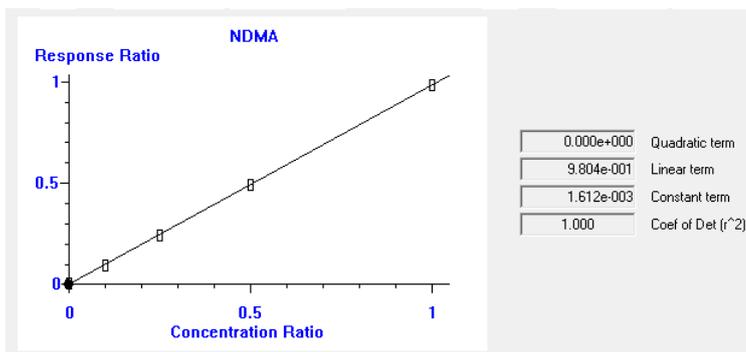
Precision is a measure of the variation in results (random error) whilst trueness is how far the mean of results is from the target value (systematic error).

Data provided by **Thames Water**.

Figure A1 Typical chromatograms



NDMA calibration curve



B The determination of N-nitrosodimethylamine in drinking waters by GC-MS

B1 Performance characteristics of the method

B1.1	Substance determined	N-nitrosodimethylamine (NDMA).
B1.2	Type of sample	Drinking waters. The method may (with suitable adaptation) be used for untreated source waters and other types of water.
B1.3	Basis of method	A deuterated internal standard is added to the sample which is then passed through a conditioned solid phase extraction cartridge. The dried cartridge is eluted with dichloromethane and the volume reduced. The extract is then ready for gas chromatography (GC) with mass spectrometric (MS) detection.
B1.4	Range of application	Typically, up to 0.04 µg/l, but can be extended, see section B7.14
B1.5	Interferences	Any substance which is co-extracted under the conditions used and is not removed by the clean-up procedure and which exhibits similar chromatographic behaviour to NDMA, by producing the same mass ion fragments and has the same retention time as NDMA, will interfere.
B1.6	Calibration curve	Linear over the range of application.
B1.7	Total standard deviation	See Tables B1 and B2.
B1.8	Limit of detection	Typically, 0.0005 µg/l of NDMA can be detected.
B1.9	Bias	See Tables B1 and B2.

B2 Principle

Deuterated-NDMA (as an internal standard) is added to the sample. The spiked sample is then passed through a conditioned charcoal solid phase extraction cartridge. The cartridge is dried under vacuum and the NDMA eluted from the cartridge with dichloromethane and the volume of solvent reduced. The extract is then ready for gas chromatography (GC) with mass spectrometric (MS) detection using electron impact (EI) ionisation mode. Quantification is achieved by internal standardisation.

Calibration standards are unextracted.

B3 Hazards

N-nitrosodimethylamine is poisonous if swallowed, inhaled or absorbed through the skin, and is probably carcinogenic. Methanol is toxic and flammable. Dichloromethane is a suspect carcinogen. Skin contact, ingestion and inhalation of these compounds should be avoided by using appropriate protective equipment and working within a fume cupboard, when appropriate.

B4 Reagents

All reagents should be of analytical grade quality and distilled or deionised water should be used throughout.

B4.1 Dichloromethane – HPLC Grade.

B4.2 Methanol – HPLC Grade.

B4.3 Anhydrous sodium sulphate – Analytical reagent grade.

B4.4 Ascorbic acid – Analytical reagent grade.

B4.5 Stock internal standard solution (1000 mg/l) – For example, dissolve 20 mg of deuterated-NDMA (D₆-NDMA) in 15 ml of methanol and mix well. Make to 20 ml with methanol. Mix well. This solution may be stored at -18 °C for up to 12 months. The solution may be available commercially and should be stored according to manufacturer's instructions.

B4.6 Intermediate internal standard solution (100 mg/l) – Add 1000 µl of stock internal standard solution (B4.5) i.e. 1 mg of D₆-NDMA, to 5 ml of methanol contained in a 10-ml volumetric flask and mix well. Make to 10 ml with methanol. Mix well. This solution may be stored at -18 °C for up to 12 months.

B4.7 Spiking internal standard solution (0.4 mg/l) – Add 40 µl of intermediate internal standard solution (B4.6) i.e. 4 µg of D₆-NDMA, to approximately 8 ml of methanol contained in a 10-ml volumetric flask and mix well. Make to 10 ml with methanol. Mix well. This solution may be stored at -18 °C for up to 12 months.

B4.8 N-nitrosodimethylamine stock standard solution (2000 mg/l) – Dissolve 20 mg of NDMA in approximately 9 ml of methanol and mix well. Make to 10 ml with methanol. Mix well. This solution may be stored at -18 °C for up to 12 months. The solution may be available commercially and should be stored according to manufacturer's instructions.

A similar AQC solution should also be prepared, preferably by different staff, from a separate lot number and ideally using reagents from different manufacturers.

B4.9 N-nitrosodimethylamine intermediate standard solution (10 mg/l) – Add 50 µl of NDMA stock standard solution (B4.8) i.e. 100 µg of NDMA, to a 10 ml volumetric flask and make to 10 ml with methanol. This solution may be stored at -18 °C for up to 12 months.

A similar AQC solution should also be prepared, preferably by different staff, from a separate lot number and ideally using reagents from different manufacturers.

B4.10 N-nitrosodimethylamine spiking AQC standard solution (0.1 mg/l) – Add 100 µl of intermediate standard solution (B4.9) i.e. 1 µg of NDMA, to a 10 ml volumetric flask and

make to 10 ml with methanol. This solution may be stored at -18 °C for up to 12 months.

B4.11 N-nitrosodimethylamine calibration standard solutions – For example, for a seven point calibration, prepare a series of seven 10 ml volumetric flasks, and add approximately 5ml of dichloromethane to each flask. Add to each flask, 40 µl of intermediate internal standard solution (B4.6). This is equivalent to 4 µg of D₆-NDMA. The concentration of D₆-NDMA is 0.4 mg/l.

To these flasks, add 0.0, 10.0, 20.0, 50.0, 100.0, 200.0 and 400.0 µl of NDMA intermediate standard solution (B4.9). The flasks contain 0.0, 0.1, 0.2, 0.5, 1.0, 2.0 and 4.0 µg of NDMA, respectively. Mix well. Make to 10 ml with dichloromethane. Mix well. The concentration of NDMA in these flasks is 0, 10, 20, 100, 200 and 400 µg/l, respectively. These solutions should be prepared on the day of use.

B4.12 Working AQC standard (0.01µg/l) – Add 100 µl of spiking AQC solution (B4.10) to 1000 ml water that has been pre-treated with 40 mg Ascorbic acid. Then add 100 µl of spiking internal standard solution (B4.7). Mix well. This solution should be prepared on the day and extracted as per a sample in B7.1. This solution contains 0.01µg of NDMA and 0.04µg D₆-NDMA.

B5 Apparatus

In addition to normal laboratory glassware the following will be required.

B5.1 SPE cartridges – For example, 6 ml, 2 g (80 - 120) mesh activated coconut charcoal cartridges, or equivalent.

B5.2 Evaporating system – For example, Zymark Turbovap, or equivalent.

B5.3 Glass vials.

B5.4 Nitrogen blow-down apparatus.

B5.5 GC-MS equipment – The suitability of the equipment will need to be evaluated.

The following conditions have been used in generating performance data.

Columns: Rtx5, 30 m x 0.25 mm diameter, 1 µm bonded film of diphenyldimethylsilicone deactivated pre-column, or equivalent. DB-624, 30 m x 0.25 mm diameter, 1.4 µm bonded film of polyphenylmethylsilicone deactivated column, or equivalent, connected in series.

Carrier gas: Helium, constant flow at 1.5 ml per minute.

Injection volume: 5 µl (pulsed split-less injection mode)

Temperature programmes:

Oven Initial temperature at 45 °C for 1 minute, then 10 °C per minute to 90 °C, hold for 5 minutes then 20 °C per minute to 150 °C, hold for 2 minutes, minutes then 40 °C per minute to 250 °C and hold for 10 minutes.

Injector 250 °C.

Using these conditions, the following apply

Compound	Approximate retention time (minutes)	Ions monitored	
		Target	Qualifier
D ₆ -NDMA	11.3	80	
NDMA	11.3	74	42, 43

Equivalent equipment and conditions may be used. See Figure B1 for typical chromatograms.

B6 Sample collection and preservation

Samples should be collected in 1-litre plastic polyethyleneterephthalate (PET) bottles containing 40 mg of ascorbic acid. In stability trials, samples containing ascorbic acid have been shown to be stable for up to 3 weeks when stored in the dark at 5 ± 3 °C. When extracted, sample extracts have been shown to be stable for up to 3 weeks when stored in the dark at -15 °C.

B7 Analytical procedure

B7.1 Allow the sample to reach room temperature before proceeding. To 1000 ml of sample, add 100 µl of spiking internal standard solution (B4.7). Mix well. This is equivalent to 0.04 µg of D₆-NDMA in 1000 ml of sample.

B7.2 Using vacuum, wash an SPE cartridge by passing 15 ml of methanol through the cartridge. Discard the washings. Repeat this process with 15 ml of dichloromethane.

Note:- Do not let the meniscus of the solvent to go below the level of the cartridge packing material in either stage.

B7.3 Repeat step B7.2.

B7.4 Repeat step B7.2 using 6 ml quantities of methanol followed by 6 ml of water. Turn off the vacuum (see note B7.2).

B7.5 Using vacuum, pass the spiked sample (B7.1) through the cartridge (see note B7.2). The flow rate should be approximately 10 ml per minute.

B7.6 When all of the sample has passed through the cartridge, turn off the vacuum, add 5 ml of water to the reservoir, and pass through the cartridge. Discard the eluate.

B7.7 Dry the cartridge which should take about 30 minutes and may be assisted with vacuum or by passing nitrogen through the cartridge. Ensure the cartridge is thoroughly dry before continuing

B7.8 Add 8 ml of dichloromethane to the cartridge. Stop the elution once the solvent reaches the bottom of the cartridge packing material. Allow the dichloromethane to soak into the cartridge packing material for about 1 minute. Continue the elution under gravity, collecting the eluate in a suitable container (see note A7.2).

B7.9 Add a further 3 ml of dichloromethane collecting the eluate in the same container (see note A7.2).

B7.10 Repeat step B7.9 three more times.

B7.11 Prepare a glass column packed with 5 g of anhydrous sodium sulphate. Pre-wet the sodium sulphate with a small volume of dichloromethane prior to passing the extract (B7.10) through it. Collect the dried extract in a clean centrifuge tube.

Note:- Improvements in true recovery have been noted when reducing the drying time and using dry disk cartridges to remove excess water from the eluate. This would need to be tested and validated prior to changing existing methodologies.

After passing the extract through the drying column, wash the sodium sulphate with at least 3 ml of dichloromethane and collect the washings in the same collection tube.

Concentrate the extract to approximately 1 ml using a turbovap or suitable equivalent at 40°C and transfer to a vial.

Concentrate the extract to a final volume of 100 µl using a nitrogen blow down apparatus. The extract is now ready for GC determination and contains 0.04 µg of D₆-NDMA.

B7.12 Set up the GC-MS system according to manufacturer's instructions. Using the N-nitrosodimethylamine calibration standard solutions (B4.11) and the conditions described in section B5.5, construct a calibration graph of response versus amount of NDMA.

Note:- This equates to 0.0, 0.001, 0.002, 0.005, 0.01, 0.02 and 0.04 µg of NDMA respectively and 0.04 µg of internal standard, D₆-NDMA, in 100 µl. Since these standards are not passed through the cartridge a recovery correction may need to be considered. If recovery estimates were required, spiked and un-spiked aqueous solutions would need to be analysed.

B7.13 Analyse blank, sample and AQC extract solutions using the procedures described in step B7.12.

B7.14 From the calibration graph, obtain the amount, Av, of NDMA in the vial and then calculate the concentration, Cs, of NDMA in the sample. If the response exceeds the calibration range, the analysis should be repeated using a smaller amount of sample (B7.1) and making the volume to 1000 µl with water.

B8 Calculations

Determine the concentration, Cs, in the sample using the equation:

$$Cs = (Av \times 1000) / Vs \text{ } \mu\text{g/l}$$

where

Cs is the concentration (µg/l) of NDMA in the sample;

Av is the amount (µg) of NDMA obtained from the graph;

Vs is the volume (ml) of sample analysed (B7.1);

and taking into account any dilution of the sample (note g)

Table B1 NDMA Performance data - Standards

Solution	Precision (%)	LOD (µg/l)	Recovery (%)	Degrees of freedom
Blank		0.00045		16
Low standard	4.9		99.0	14
High standard	3.9		107.5	14

Low standard was water spiked with 0.008 µg/l NDMA

High standard was water spiked with 0.032 µg/l NDMA.

Limit of detection (LOD) = 3 x within batch standard deviation of blank water samples.

Data provided by Severn Trent Services - Analytical Services.

The development and validation of this method was carried out by Severn Trent Services and was funded by the Department for Environment, Food and Rural Affairs, under a contract (WT1219/DWI 70/2/239) managed by WRc plc.

Table B2 NDMA Performance data - Samples

Sample	Precision (%)	LOD (µg/l)	Recovery (%)	Degrees of freedom
Blank		0.00077		12
Low soft water spike	9.7		93.6	13
High soft water spike	5.5		94.8	13
Low medium hardness water	8.8		91.9	19
High medium hardness water	6.5		95.9	11
Low hard water spike	9.3		93.5	21
High hard water spike	6.0		96.9	13

Low sample was water spiked with 0.01 µg/l NDMA

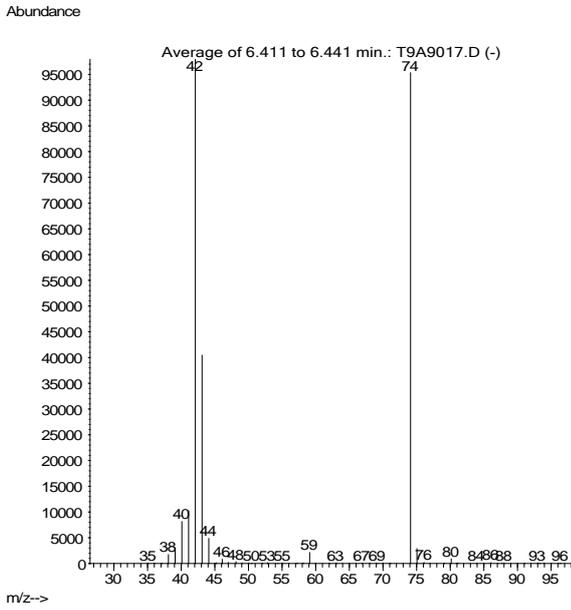
High sample was water spiked with 0.1 µg/l NDMA.

Limit of detection (LOD) = 3 x within batch standard deviation of "blank" water samples (spiked at 0.001 µg/l).

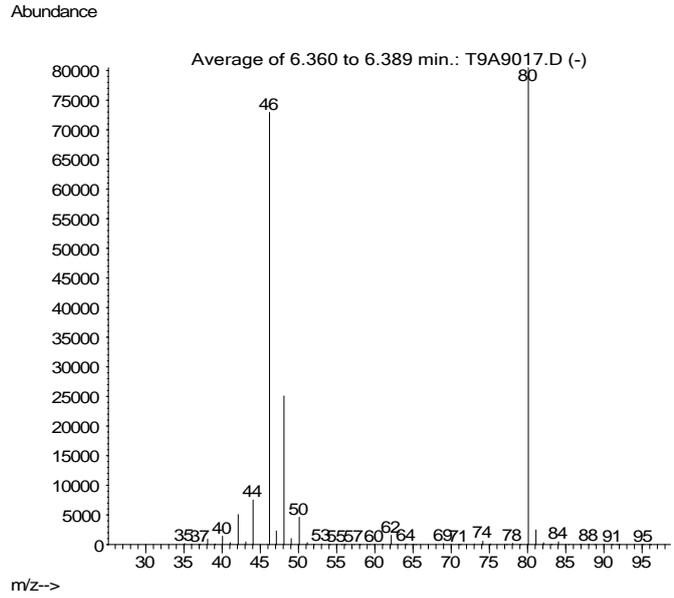
Data provided by Severn Trent Water.

Figure B1 Typical spectra/chromatograms

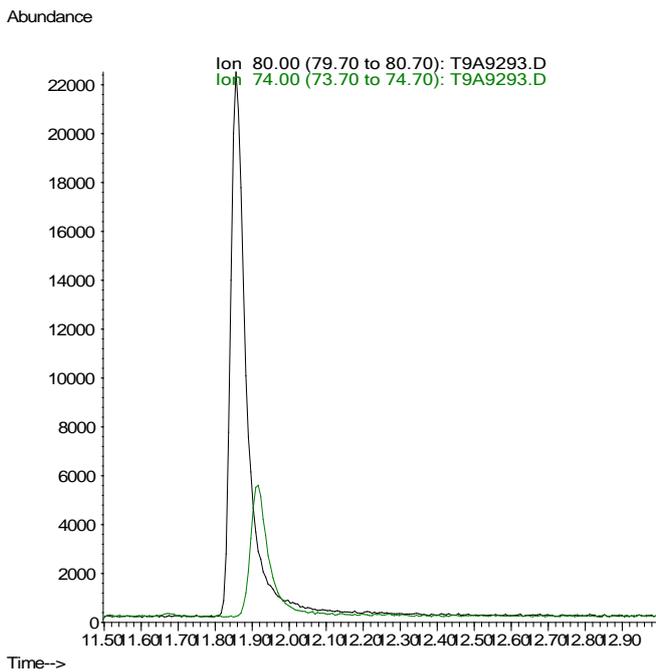
NDMA mass spectrum



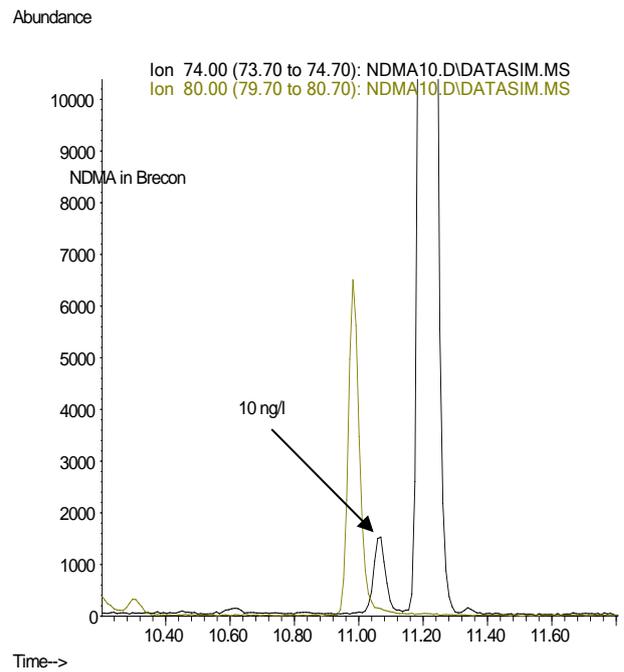
D₆-NDMA mass spectrum



Calibration standard 0.01 µg/l NDMA (0.04 µg/l D₆-NDMA)



Spiked Sample (0.01 µg/l in Medium Hardness Water)



C The determination of N-nitrosodimethylamine in drinking waters by GC-MSMS

C1 Performance characteristics of the method

C1.1	Substance determined	N-nitrosodimethylamine (NDMA).
C1.2	Type of sample	Drinking waters. The method may (with suitable adaptation) be used for raw and river waters.
C1.3	Basis of method	A deuterated internal standard is added to an acidified sample which is then passed through a conditioned solid phase extraction cartridge. The dried cartridge is eluted with dichloromethane and the volume reduced. The extract is then ready for gas chromatography (GC) with MS-MS detection in multiple reaction monitoring mode.
C1.4	Range of application	Typically, up to 0.04 µg/l, but can be extended, see section C7.10.
C1.5	Interferences	Any substance which is co-extracted under the conditions used and is not removed by the clean-up procedure and which exhibits similar chromatographic behaviour to NDMA, by producing the same mass ion fragments and has the same retention time as NDMA, will interfere.
C1.5	Calibration curve	Linear over the range of application.
C1.6	Total standard deviation	See Table C1.
C1.7	Limit of detection	Typically, 0.0005 µg/l of NDMA can be detected.
C1.8	Bias	See Table C1.

C2 Principle

Deuterated-NDMA (as an internal standard) is added to an acidified sample. The spiked sample is then passed through a conditioned solid phase extraction cartridge. The cartridge is dried under vacuum and the NDMA eluted from the cartridge with dichloromethane and the volume of solvent reduced. The extract is then ready for gas chromatography (GC) with MS-MS detection using multiple reaction monitoring mode. Quantification is achieved by internal standardisation. Calibration standards are un-extracted.

C3 Hazards

N-nitrosodimethylamine is poisonous if swallowed, inhaled or absorbed through the skin, and is probably carcinogenic. Hydrochloric acid is corrosive and may cause burns. Methanol is

toxic and flammable. Acetone is flammable. Dichloromethane is a suspect carcinogen. Skin contact, ingestion and inhalation of these compounds should be avoided by using appropriate protective equipment and working within a fume cupboard, when appropriate.

C4 Reagents

All reagents should be of analytical grade quality and distilled or deionised water should be used throughout.

C4.1 Dichloromethane – HPLC Grade.

C4.2 Methanol – HPLC Grade.

C4.3 Concentrated hydrochloric acid (SG 1.18).

C4.4 Ascorbic acid – Reagent Grade.

C4.5 Acetone – HPLC Grade.

C4.6 Stock internal standard solution (100 mg/l) – For example, dissolve 20 mg of deuterated-NDMA (D₆-NDMA) in approximately 50 ml of acetone and mix well. Make to 200 ml with acetone. Mix well. This solution may be stored in the dark at room temperature for up to 12 months. The solution may be available commercially and should be stored according to manufacturer's instructions.

C4.7 Working internal standard solution (2.5 mg/l) – Add 500 µl of intermediate internal standard solution (C4.6) i.e. 50 µg of D₆-NDMA, to approximately 10 ml of methanol contained in a 20-ml volumetric flask and mix well. Make to 20 ml with methanol. Mix well. This solution may be stored in the dark at room temperature for up to 12 months.

C4.8 N-nitrosodimethylamine stock standard solution (2000 mg/l) – Dissolve 20 mg of NDMA in approximately 9 ml of methanol and mix well. Make to 10 ml with methanol. Mix well. This solution may be stored at -18 °C for up to 12 months. The solution may be available commercially and should be stored according to manufacturer's instructions.

A similar AQC solution should also be prepared, preferably by different staff, from a separate lot number and ideally using reagents from different manufacturers.

C4.9 N-nitrosodimethylamine intermediate standard solution (2 mg/l) – Add 40 µl of NDMA stock standard solution (C4.8) i.e. 80 µg of NDMA, to a 40 ml volumetric flask and make to 40 ml with dichloromethane. This solution may be stored in the dark at room temperature for up to 12 months.

A similar AQC solution should also be prepared, preferably by different staff, from a separate lot number and ideally using reagents from different manufacturers.

C4.10 N-nitrosodimethylamine calibration standard solutions – For example, for a four point calibration, prepare four 40 ml volumetric flasks, adding approximately 40ml of Dichloromethane (DCM) to each flask. Add 0.0, 40.0, 400.0 and 800.0 µl of NDMA intermediate standard solution (C4.9) into separate flasks. The flasks contain 0.0, 0.08, 0.8 and 1.6 µg of NDMA, respectively. Make to 40 ml with dichloromethane. Mix well. The concentration of NDMA in these flasks is 0, 2.0, 20.0 and 40.0 µg/l, respectively. These solutions may be stored in the dark at room temperature for up to 12 months.

Transfer 1.0 ml of each solution to separate vials and, to each, add 10 µl of working internal standard solution (C4.7). These solutions contain 0.025 µg of D₆-NDMA, and 0.0, 0.002, 0.02 and 0.04 µg of NDMA. These solutions should be prepared on the day of use.

An AQC spiked standard should be prepared by spiking 5 µl of the AQC intermediate standard solution (C4.9) into 1000 ml water treated with 50mg Ascorbic acid. This contains 0.01 µg of NDMA.

C5 Apparatus

In addition to normal laboratory glassware the following will be required.

C5.1 SPE cartridges – For example, 6 ml, 1 g Biotage Isolute ENV cartridges, or equivalent.

C5.2 Evaporating system – For example, Zymark Turbovap, or equivalent.

C5.3 Glass vials.

C5.4 Nitrogen blow-down apparatus.

C5.5 GC-MSMS equipment – The suitability of the equipment will need to be evaluated.

The following conditions have been used in generating performance data.

Columns: Rtx5, 30 m x 0.25 mm diameter, 1 µm bonded film of polyphenylmethylsilicone, or equivalent.

Carrier gas: Helium, constant flow at 1 ml per minute.

Injection volume: 1 µl

Temperature programmes:

Oven Initial temperature at 32 °C for 12 minutes, then 20 °C per minute to 260 °C, and hold for 28 minutes.

Injector 300 °C.

Using these conditions, the following apply

Compound	Transitions monitored		
D ₆ -NDMA	80	→	50
	46	→	42
NDMA	74	→	44
	42	→	41

Equivalent equipment and conditions may be used. See Figure C1 for typical chromatograms.

C6 Sample collection and preservation

Samples should be collected in 1-litre plastic polyethyleneterephthalate (PET) bottles containing 50 mg of ascorbic acid. Samples may be stored at room temperature but should be protected from direct sunlight. Extracts should be stored in a refrigerator.

C7 Analytical procedure

C7.1 To 1000 ml of sample, add 1 ml of hydrochloric acid. Mix well. To the acidified sample, add 10 µl of working internal standard solution (C4.7). Mix well. This is equivalent to 0.025 µg of D₆-NDMA in 1000 ml of sample.

C7.2 Using vacuum, wash an SPE cartridge by passing 10 ml of methanol through the cartridge. Discard the washings. Repeat this process with another 10 ml of methanol. Discard the washings. Wash the cartridge with 5 ml of water. Discard the washings. Turn off the vacuum.

Note:- Do not let the meniscus of the solvent to go below the level of the cartridge packing material at any stage.

C7.3 Using vacuum, pass 500 ml of the spiked sample (C7.1) through the cartridge (see note C7.2). The flow rate should be approximately 10 ml per minute. Turn off the vacuum.

C7.4 When the sample has passed through the cartridge, add 5 ml of water to the reservoir, and pass through the cartridge. Discard the eluate.

C7.5 Dry the cartridge, ensuring that the cartridge is thoroughly dry before continuing. This process should take about 30 minutes and may be assisted with vacuum or by passing nitrogen through the cartridge. Failure to dry the cartridge properly may result in the cartridge appearing darker in appearance than normally expected.

C7.6 Under gravity, elute the dried cartridge with 4 ml of dichloromethane until the flow of solvent stops. Repeat this process with two further 4 ml quantities of dichloromethane, collecting the eluate in a suitable container.

C7.7 Concentrate the extract to approximately 2 ml (see C5.2) and transfer to a vial. Concentrate the extract to a final volume of about 250 µl. This extract contains 0.025 µg of D₆-NDMA and is now ready for GC determination.

C7.8 Set up the GC-MS system according to manufacturer's instructions. Using the N-nitrosodimethylamine calibration standard solutions (C4.10) and the conditions described in section C5.5, construct a calibration graph of response versus amount of NDMA.

This equates to 0.0, 0.002, 0.02 and 0.04 µg of NDMA respectively and 0.025 µg of internal standard, D₆-NDMA. Since these standards are not passed through the cartridge a recovery correction may need to be considered. If recovery estimates were required, spiked and un-spiked aqueous solutions would need to be analysed.

C7.9 Analyse blank, samples and spiked AQC standards using the procedures described in steps C7.1 - C7.8.

C7.10 From the calibration graph, obtain the amount, A_v , of NDMA in the vial and then calculate the concentration, C_s , of NDMA in the sample.

If the response exceeds the calibration range, the analysis should be repeated using a smaller amount of sample (C7.1) and making the volume to 1000 ml with water.

C8 Calculations

Determine the concentration, C_s , in the sample using the equation:

$$C_s = 2x (A_v \times 1000) / V_s \mu\text{g/l}$$

where

C_s is the concentration ($\mu\text{g/l}$) of NDMA in the sample;
 A_v is the amount (μg) of NDMA obtained from the graph;
 V_s is the volume (ml) of sample analysed (C7.1/C7.3);
and taking into account any dilution of the sample (see A7.10)

Table C1 NDMA Performance data

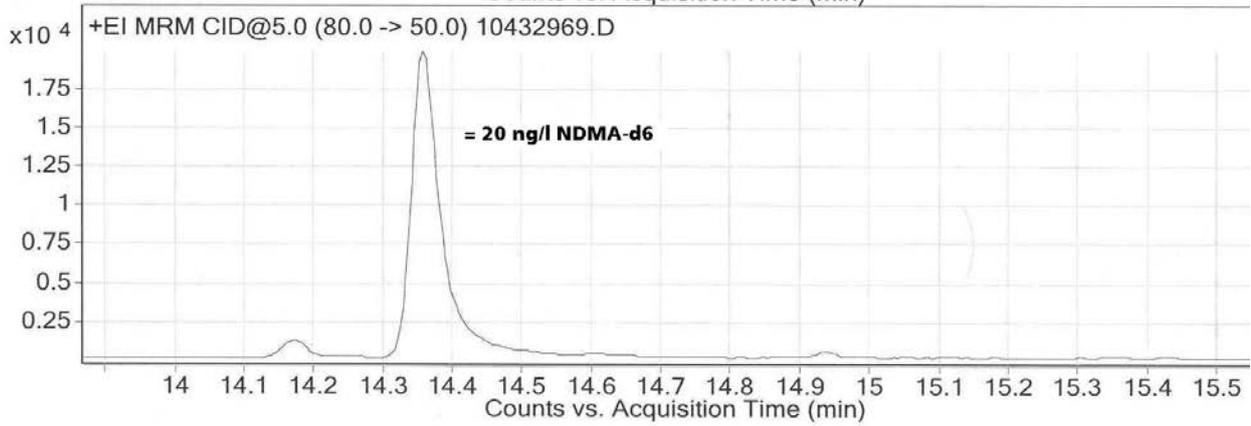
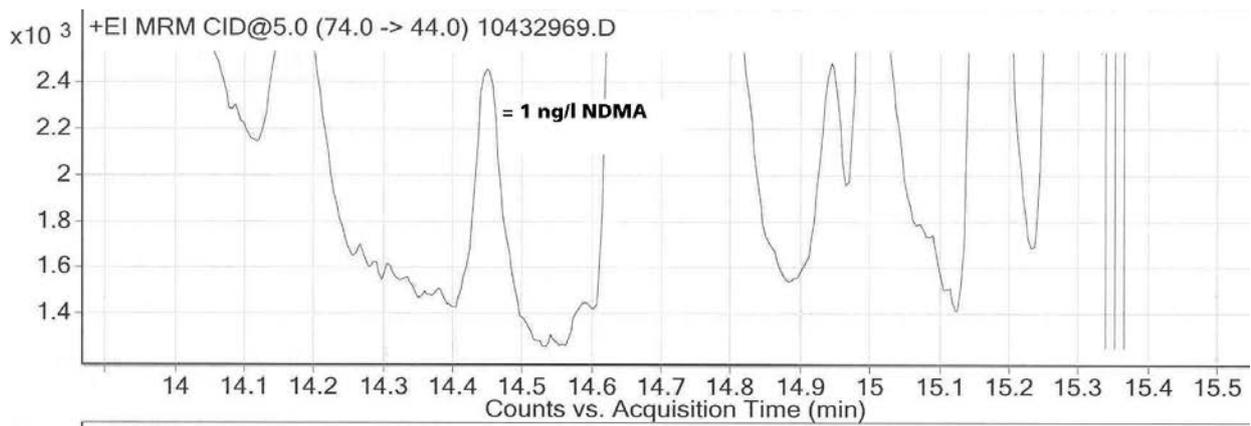
Number of determinations	51
Mean value ($\mu\text{g/l}$)	0.0106
Standard deviation ($\mu\text{g/l}$)	0.0011
Precision %	20.8%
Trueness	6.0%
Limit of detection ($\mu\text{g/l}$)	0.0001

Water spiked with 0.01 $\mu\text{g/l}$ NDMA

Limit of detection (LOD) from within batch standard deviation of 11 blank water samples spiked at 0.0004 $\mu\text{g/l}$.

Data provided by Anglian Water.

Figure C1 Typical chromatograms



Address for correspondence

However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts at the address given below. In addition, if users wish to receive advance notice of forthcoming publications, please contact the Secretary.

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