## 2 STUDY OBJECTIVE

The aim of this study was to validate the analytical method described in the Standard Operating Procedure CEM-3564/draft. This method has been developed at CEMAS for the determination of residues of methyl isothiocyanate (MITC) in ground water to a limit of quantitation (LOQ) of 0.1 µg/L.

## 3 MATERIALS / TEST SYSTEM

### 3.1 SPECIMENS

Fresh ground water was taken from a well in Fawley Lodge, Henley-on-thames. A unique sample number (CCON/038/002) was assigned to the water specimen to track it during receipt, storage and analysis. On receipt the specimen was stored at approximately 4°C.

The water specimen was GLP characterised at CEMAS.

The pH was determined using CEMAS Standard Operating Procedure CEM-3373 "Determination of the pH of Water, Soil and Sediment Samples in Water and/or Salt Solutions (0.01M Calcium Chloride, 0.1M Potassium Chloride, 1.0M Potassium Chloride)".

Silt content was determined using CEMAS Standard Operating Procedure CEM-3385 "Determination of Particle Size Distribution in Water – Fractionation/Sedimentation Method".

Total Organic Carbon and Dissolved Organic Carbon was determined using CEMAS Standard Operating Procedure CEM-3396 "Determination of the Total and Dissolved Organic Carbon, Inorganic Carbon and Carbon in Water". The dissolved organic carbon was determined as the sample was filtered through a 0.45µm filter.

Total hardness was determined using CEMAS Standard Operating Procedure CEM-3060 "Determination of Total Hardness by EDTA Titration in Water".

Results of the GLP characterisation were as follows:

| CEMAS Specimen Reference: CCON/038/002 (Well at Fawley Lodge, Henley-on-thames) | Analysis Results |
|---|---|
| pH | 7.4 |
| Silt Content | <1 mg/L |
| Dissolved Organic Carbon | 0.41 mg/L |
| Total Hardness (EDTA Titration) as $CaCO_3$ | 291 mg/L |

### 3.2 REFERENCE ITEM

**Table 4: Reference Item**

| | |
|---|---|
| Analyte | Methyl isothiocyanate (MITC) |
| Batch Number | SZB9154XV |
| Purity | 98.5% |
| Expiry Date | 03 Jun 2015 |
| Storage: | Coldroom |

The reference item will be retained until expiry and then disposed of. A copy of the Certificate of Analysis is given in Appendix 3.

## 4 EXPERIMENTAL PROCEDURES

### 4.1 FORTIFICATION OF SPECIMENS

Control specimens were fortified with MITC as detailed below:

| Untreated Replicates | Replicates at Fortification Level (mg/kg) | |
|---|---|---|
| | LOQ | Higher Level |
| 2 | 5 at 0.1 µg/L | 5 at 1.0 µg/L |

### 4.2 METHODS OF ANALYSIS

Specimens were analysed using CEMAS SOP CEM-3564/draft "Determination of Methyl Isothiocyanate in Ground Water by Headspace Introduced Gas Chromatography with Mass Selective Detection".

Residues are extracted from ground water samples by sweeping the headspace from a septum capped vial into a gas chromatograph-mass spectrometer (GC-MS)

During method validation, acceptable recoveries were generated for samples fortified at LOQ (0.1 µg/L) and at a higher level (1.0 µg/L). Results from the GC-MS method validation are summarised in Tables 1 and 2.
For a detailed description of the method see Appendix 4. Quantitation was performed by external standardisation with linearity.

The limit of quantitation (LOQ) for this method is 0.1 µg/L.

## APPENDIX 4: CEMAS SOP CEM 3564/001

**CEMAS**

CEM Analytical Services Ltd, Glendale Park, Fernbank Road, North Ascot, Berkshire SL5 8JB

### STANDARD OPERATING PROCEDURE

**SOP No** CEM-3564/001

**TITLE** DETERMINATION OF METHYL ISOTHIOCYANATE IN GROUND WATER BY HEADSPACE INTRODUCED GAS CHROMATOGRAPHY WITH MASS-SELECTIVE DETECTION

**COPY No** 2 **ISSUE DATE** 28 MAR 2013

Maria G. Alla — 28 March 2013.
M Garcia Allx — Date
Author/Reviewer

S Campbell — 28 March 2013
S Campbell — Date
QA Review

L Jutsum — 28 March 2013
L Jutsum — Date
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**CEMAS**

SOP No. CEM-3564/001

## 1 INTRODUCTION

This SOP describes the procedure for the determination of methyl isothiocyanate (MITC) in ground water samples by sweeping the headspace from a septum capped vial into a gas chromatograph-mass spectrometer (GC-MS). During validation, this method gave typical linearity correlation coefficients of >0.995 and mean recoveries of between 70 and 120%. The limit of quantification (LOQ) has been established as 0.1 µg/L.

## 2 PRINCIPLE

Water samples (10 mL) are pipetted into a 20-mL headspace vial. Sufficient sodium chloride is added to saturate the solution. The vial is immediately septum capped. The target analyte is encouraged into the headspace (vapour phase) by warming and agitating the vial in a headspace oven. The headspace in the vial is then swept into a sample loop connected to the GC inlet. The analyte is detected using capillary gas chromatography with mass-selective detection. Quantitation is performed by the external standard method using calibration solutions prepared concurrently with the samples.

## 3 MATERIALS AND EQUIPMENT

The following list details the materials and equipment that were used for the validation of this method. Common laboratory supplies are assumed to be readily available and are, therefore, not listed.

| | |
|---|---|
| Headspace vials (20 mL) Agilent | Cat. No. 5182-0837 |
| Headspace vial septa Agilent | Cat. No. 9301-0719 |
| Headspace crimp caps Agilent | Cat. No. 9301-0721 |
| Injection Port liner | Cat. No. 5181-8818 |

Headspace vial crimper Agilent
Eppendorf Pipetter, air displacement, single channel with variable volume disposable plastic tips
Gilson Microman pipettes and tips, available from SLS
GC-MSD system (see Section 7)
Headspace system (see Section 7)

## 4 REAGENTS AND SOLUTIONS

### 4.1 REAGENTS

Methanol
Deionised water
Sodium Chloride

4.2 REFERENCE ITEM SOLUTIONS

4.2.1 Weigh accurately, using an analytical balance, 0.050 g (weight adjusted for purity) of reference material into a glass volumetric flasks (25 mL). Dissolve in methanol up to the mark. The stock solution contains 2000 µg/mL of analyte. Transfer the solutions into amber 40 mL vials with PTFE caps. The reference item solutions should always be stored in a refrigerator to prevent decomposition and/or concentration of the analyte. The preparation of these standard solutions may be achieved by the use of alternative dilutions if necessary and alternative concentrations may be used as appropriate to the analysis.

4.2.2 Prepare the following standards in methanol in volumetric flasks:

| Parent conc. µg/mL | Volume taken mL | Final Volume mL | Standard conc. µg/mL |
|---|---|---|---|
| 2000 | 0.1 | 10 | 20 |
| 2000 | 0.05 | 10 | 10 |
| 20 | 2 | 10 | 4 |
| 20 | 1 | 10 | 2 |
| 20 | 0.5 | 10 | 1 |
| 20 | 0.2 | 10 | 0.4 |
| 20 | 0.1 | 10 | 0.2 |
| 2 | 0.5 | 10 | 0.1 |
| 2 | 0.3 | 10 | 0.06 |

4.3 PROCEDURAL RECOVERY REFERENCE ITEM SOLUTIONS

4.3.1 Weigh accurately, using an analytical balance, 0.050 g (weight adjusted for purity) of the reference material into a glass volumetric flasks (25 mL). Dissolve in methanol up to the mark. The stock solution contains 2000 µg/mL of analyte. Transfer the solutions into amber 40 mL vials with PTFE caps. The reference item solution should always be stored in a refrigerator to prevent decomposition and/or concentration of the analyte.

4.3.2 Prepare the following fortification standards in methanol:

| Parent conc. µg/mL | Volume taken mL | Final Volume mL | Standard conc. µg/mL |
|---|---|---|---|
| 2000 | 0.1 | 10 | 20 |
| 20 | 1.0 | 10 | 2 |
| 20 | 0.1 | 10 | 0.2 |

## 5 SAMPLE PREPARATION

5.1 Include at least one control and two procedural recovery samples with each analytical batch using groundwater known not to contain methyl isothiocyanate. The procedural recoveries should be prepared AFTER the samples (see Section 6).
5.2 Add 5 g of sodium chloride to a headspace vial.
5.3 Remove samples from the refrigerated storage immediately before analysis. Do not allow them to warm up.
5.4 Accurately transfer 10 mL of sample into the headspace vial. Procedural recoveries should be fortified at this stage (see Section 6).
5.5 Immediately cap the vial tightly with a septum crimp cap.
5.6 Transfer the vial to the headspace carousel for analysis.

It is recommended to analyse the samples the same day of the extraction.

## 6 CALIBRATION STANDARD & PROCEDURAL RECOVERY PREPARATION

Calibration standards should be prepared after the samples have been transferred to headspace vials and capped to avoid any possibility of cross-contamination.
6.1 Allow the fortification and calibration solutions to warm to room temperature.
6.2 Add 5 g of sodium chloride to a headspace vial.
6.3 Accurately pipette 10 mL of deionised water that has been cooled to 4°C into the headspace vial for calibration standards.
6.4 Accurately pipette 10 mL of groundwater that has been cooled to 4°C into the headspace vial for the procedural recovery samples.
6.5 Accurately pipette 10 mL of ultrapure water that has been cooled to 4°C into the headspace vial for the calibration standards.
6.6 Fortify the water with 5 µL of the appropriate fortification solution (4.3.2).
6.7 Fortify the water with 5 µL of the appropriate calibration solution (4.2.2).
6.8 Immediately cap the vial tightly with a septum crimp cap.
6.9 Transfer the vial to the headspace carousel for analysis.

## 7 INSTRUMENTATION AND OPERATING CONDITIONS

### 7.1 GC Instrument: Agilent 6890 series gas chromatograph

- GC Column:
  DB 624 30 m x 0.25 mm x 1.40 µm film. (Quantitation)
  RTX-35 Amine 30 m x 0.25 mm x 1.0 um film (Confirmation)
- Oven
  - Initial temperature: 50°C
  - Initial time: 5.00 min
  - Ramp: 15°C/min to 100°C then 35°C/min to 200°C and hold for 1 min

- Injection system and pneumatics
  - Carrier gas: Helium
  - Liner type: Splitless double taper
  - Inlet mode: Split
  - Injection port temperature: 220°C
  - Pressure: 21 psi (approximately)
  - Slit ratio: 2:1
  - Slit flow: 5.0 mL/min
  - Total #slit: 9.7 mL/minute (approximately)
  - Column mode: Constant flow.
  - Column flow: 2.5 mL/min
  - Average velocity: 58cm/sec (approximately)
  -
- MSD interface: 260°C
- MS Quad temperature: 150°C
- MS Source: 230°C
- EM Volts: Rel +400
- SIM windows and ion assignment as shown in the tables below;

| Analyte | Target ion | Confirmatory ion |
|---|---|---|
| methyl isothiocyanate | 73 | 72 |

**7.2 Instrument: Agilent 7694 series Headspace sample introduction system**

- Oven temperature: 80°C
- Sample loop temperature: 105°C
- Transfer line temperature: 120°C
- GC Cycle time: 20 minutes (approximately)
- Vial equilibrium time: 10 minutes
- Pressurisation time: 0.2 minutes
- Loop fill time: 0.05 minutes
- Loop equilibrium time: 0.2 minutes
- Inject time (3 mL loop): 3 minutes
- Carrier gas transfer line flow: 20 mL/min* see procedure below
- Vial pressure: 13.5 psi (approximately)

*The carrier gas transfer line flow should be set after all other headspace and GC-MS conditions as follows;
Connect a flow meter to the back injector split flow vent
Shut off the carrier gas flow on the transfer line using the knob on the top of the headspace instrument
Change the split ratio on the inlet to 50:1 using Chemstation and note the flow rate on the flow meter
Turn on the flow from the transfer line.
Continue increasing the transfer line flow and measuring the flow rate from the split vent until the flow has increased by 20 mL/min.
Return the split ratio to the original setting.

## 8 ANALYSIS AND CALCULATIONS

- Using a calibration standard inject aliquots of an appropriate concentration to obtain a reproducible response before proceeding.
- Bracket samples with calibration standards (0.03 ng/mL to 10 ng/mL). The calibration should have a minimum of 5 points and all samples should be within the calibration range.

Prepare an appropriate calibration curve by plotting peak area versus concentration expressed in ng/mL. Using appropriate regression analysis, determine the equation of the line and the coefficient of determination ($r^2$).

For example;

If using linear regression, generate the following equation:

y = mx
y = peak area
x = concentration in ng/mL

Calculate the residue in the extract:

$$\text{Residue (ng/mL)} = \frac{\text{peak area}}{m}$$

Calculate the residue in the sample:

Residue (µg/L) = Residue in extract (ng/L) ÷ Sample conc. (mL/mL)

The extraction efficiency of procedural recovery specimens should be determined as follows:

$$\% \text{ Recovery} = \frac{\text{Residue in sample (µg/L) - Residue in control (µg/L)}}{\text{Fortification level (µg/L)}} \times 100$$

## 9 VALIDATION

The method was validated in the CEMAS Study CEMS-5667

### 9.1 Linearity

At least eight batch standard solutions were prepared over a range of concentrations. The detector response for GC-MS was plotted against standard concentration. The lowest concentration injected was at 30 % of the LOQ of the method (0.03 ng/mL based on a 1.0 mL/mL final sample concentration). The highest concentration level injected (10 ng/mL) was at least 20% higher than the 10 x LOQ fortifications.

### 9.2 Specificity

The analytical method developed for the determination of the methyl isothiocyanate in ground water has been shown to be highly specific due to the instrumentation used (GC-MS). A confirmation to the primary detection (*m/z* 73) was used by monitoring an additional fragment ion (*m/z* 72) using DB-624 GC column. Another column with different stationary phase (RTX-35 Amine) was also used for confirmation of the compound identity and it was quantitatively monitored (*m/z* 73).