2 STUDY OBJECTIVE

The aim of this study was to validate the CEMAS analytical method CAM 0097/draft 'Analytical Method for the Determination of Residues of Methyl Isothiocyanate in Surface Water'. This method is identical in methodology to CAM 0084/001, which has already been validated for MITC in groundwater. Therefore CAM 0097/draft has been superseded and the MITC in surface water validated method is described in CAM 0084, which has been reissued as CAM 0084/002 'Analytical Method for the Determination of Residues of Methyl Isothiocyanate in Drinking, Surface and Groundwater'. (Appendix 4).

This method has been developed at CEMAS for the determination of residues of methyl isothiocyanate (MITC) in surface water to a limit of quantitation (LOQ) of 0.1 µg/L. This method is deemed to be also appropriate for the determination of methyl isothiocyanate (MITC) in drinking water and groundwater. The validation data in surface water covers also the requirements for the determination of MITC in drinking water for the following reasons, this method is validated at the required level of 0.1 ug/L and surface water could be considered as the most complex water type[1].

This study was also designed to evaluate the stability of MITC residues in surface water specimens stored under freezer storage conditions for up to 33 days.

3 MATERIALS / TEST SYSTEM

3.1 SPECIMENS

Fresh surface water was taken from a river in Belstone (Devon). A unique sample number (CCON/037/005) 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)".

Electrical conductivity was determined using CEMAS Standard Operating Procedure CEM-3454 "Determination of the Electrical Conductivity and TDS of Water, Soil and Sediment".

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[1]SANCO/825/00 rev 8.1, section 6, lines 506 and 507, "provided that the method has been successfully validated for surface water at the LOQ required for the drinking water, no further validation in drinking water is required"

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 0.45 µm filter.

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

Alkalinity was determined using CEMAS Standard Operating Procedure CEM-3384 "Determination of Alkalinity of Water - Carbonate, Bicarbonate and Carbonate Hardness".

Total suspended solids was determined using CEMAS Standard Operating Procedure CEM-3448 "Determination of Total Suspended and Volatile Suspended Solids in Waters".

Results of the GLP characterisation were as follows:

CEMAS Specimen Reference: CCON/037/005	Analysis Results
pH	5.8
Electrical Conductivity	36.7 µS/cm
Silt Content	<1.0 mg/L
Dissolved Organic Carbon	2.27 mg/L
Total Hardness (EDTA Titration) as $CaCO_3$	5.0 mg/L
Total Alkalinity as $CaCO_3$	11.0 mg/L
Total Suspended Solids	0.1 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 5.

4 VALIDATION OF MITC IN SURFACE WATER

4.1 Experimental Procedures

4.1.1 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

4.1.2 Methods of Analysis

Specimens were analysed using CAM 097/draft "Analytical Method for the Determination of Methyl Isothiocyanate in Surface Water". This method is identical in methodology to CAM 0084/001, which has already been validated for MITC in groundwater. Therefore CAM 0097/draft has been superseded and the MITC in surface water validated method is described in CAM 0084, which has been reissued as CAM 0084/002 'Analytical Method for the Determination of Residues of Methyl Isothiocyanate in Drinking, Surface and Groundwater' (Appendix 4).

Residues are extracted from surface 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 ANALYTICAL METHOD CAM 0084/002

CEMAS ANALYTICAL METHOD

METHOD No. CAM-0084/002

TITLE ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF METHYL ISOTHIOCYANATE IN DRINKING, SURFACE AND GROUNDWATER

Maria G Alix — 13 March 2014
Maria Garcia-Alix, Author — Effective Date

S Bernardo — 13 March 2014
Sandra Bernardo, QA Review — Date

Lisa Jutsum — 18 March 2014
Lisa Jutsum, Technical Review — Date

DATA REQUIREMENTS

ENV/JM/MONO(2007)17
SANCO/825/00 rev 8.1(2010)
SANCO/3029/99 rev 4 (2000)
OPPTS 850.6100 (2012)

REVISIONS TO PREVIOUS VERSIONS

Version	Reason for Re-issue
CEM-3564/001	To move the method from the SOP system to the CAM system (Original author: M Garcia-Alix)
CAM-0084/001	To incorporate the surface water validation data

CEMAS
METHOD No. CAM-0084/002

1 INTRODUCTION

This SOP describes the procedure for the determination of methyl isothiocyanate (MITC) in drinking water, surface water and groundwater 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 GLP Compliance

This analytical method is applicable for determination of MITC in different water types (groundwater, surface water and drinking water).
The method validation data for the determination of MITC in groundwater are included in the CEMAS GLP Study CEMS-5667.
The method validation data for the determination of MITC in surface water are included in the CEMAS GLP Study CEMS-6314. The validation data in surface water covers also the requirements for the determination of MITC in drinking water for the following reasons, this method is validated at the required level of 0.1 ug/L and surface water could be considered as the most complex water type[1].

3 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.

4 REAGENTS AND SOLUTIONS

1.1 Reagents

Methanol
Deionised water
Sodium Chloride

1.2 Calibration 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 flask (25 mL). Dissolve in methanol and make 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.

[1] SANCO/825/00 rev 8.1, section 6, lines 506 and 507, "provided that the method has been successfully validated for surface water at the LOQ required for the drinking water, no further validation in drinking water is required".

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.

1.2.2 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

1.3 Procedural Recovery Reference Item Solutions

4.1.1 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.

4.1.2 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

No sample preparation is required for water samples. Samples should be stored frozen until required for analysis. It is recommended to analyse the samples the same day of the extraction.

5.1 Include at least one control sample and two procedural recovery samples with each analytical batch using the relevant type of control water 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.

Note: It is critical that the samples are extracted immediately after thawing, while still chilled.

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.

6 CALIBRATION STANDARDS & 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.

The magnitude of the matrix effects were between 10-20% in the waters tested. Although, the matrix effect is not very significant, matrix-matched standards may be used to compensate for any matrix effects, at the discretion of the Study Director. If matrix-matched standards are used for the calibration, relevant control water samples are fortified with standard prepared in methanol, to give a range from 0.03 ng/mL to 10 ng/mL. If matrix-matched standards are not used, calibration standards are prepared with ultrapure water.

6.1 Allow the fortification and calibration solutions in methanol to warm to room temperature.
6.2 Add 5 g of sodium chloride to a headspace vial.
6.3 Accurately pipette 10 mL of ultrapure water or relevant control water sample that has been cooled to 4°C into the headspace vial for calibration standards.
6.4 Accurately pipette 10 mL of relevant control water sample that has been cooled to 4°C into the headspace vial for the procedural recovery samples.
6.5 Fortify the water with 5 µL of the appropriate calibration solution (4.2.2).
6.6 Fortify the water with 5 µL of the appropriate fortification solution (4.3.2).
6.7 Immediately cap the vial tightly with a septum crimp cap.
6.8 Transfer the vial to the headspace carousel for analysis.

7 INSTRUMENTATION AND OPERATING CONDITIONS

7.7 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 µm 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: Straight-through liner
  - Inlet mode: Split
  - Injection port temperature: 220°C
  - Pressure: 21 psi (approximately)
  - Slit ratio: 2:1

- Slit flow: 5.0 mL/min
- Total flow: 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

7.8 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 determinec 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 validation data for the determination of MITC in groundwater are included in the CEMAS GLP Study CEMS-5667.
The method validation data for the determination of MITC in surface water are included in the CEMAS GLP Study CEMS-6314.
The validation data in surface water covers also the requirements for the determination of MITC in drinking water for the following reasons, this method is validated at the required level of 0.1ug/L and surface water could be considered as the most complex water type[1].

[1]SANCO/825/00 rev 8.1, section 6, lines 506 and 507, "provided that the method has been successfully validated for surface water at the LOQ required for the drinking water, no further validation in drinking water is required".

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APPENDIX 1: APPARATUS

Recommended Suppliers

General glassware	General glassware	www.thermofisher.com/global/en/home.asp
40 ml Glass Vials	General glassware	www.thermofisher.com/global/en/home.asp
Gilson pipettes	Various sizes	www.thermofisher.com/global/en/home.asp
2 mL autosampler vials	General labware	www.waters.com
GC-MS system	Agilent 6890 series gas chromatograph	www.agilent.com
Headspace system	Agilent 7694 series Headspace sample introduction system	www.agilent.com
GC column	DB 624, 30 m x 0.25 mm x 1.40 µm film	www.agilent.com
GC column	RTX-35 Amine 30 m x 0.25 mm x 1.0 µm film	www.restek.com
20 mL headspace vials and caps	General glassware	www.agilent.com

Note: Equivalent equipment may be substituted where appropriate.

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APPENDIX 2: REAGENTS AND SOLUTIONS

Recommended Suppliers

Reagent	Description	Supplier
Methanol	HPLC grade	www.thermofisher.com/global/en/home.asp
Water	HPLC grade	www.thermofisher.com/global/en/home.asp
Sodium Chloride	Technical	www.thermofisher.com/global/en/home.asp

Note: Reagents of equal purity may be substituted where appropriate.