1. INTRODUCTION

Background and Objective:

The objective of the present study was to independently validate an analytical method developed and validated by CEMAS[3,4] for the determination of Methyl Isothiocyanate (MITC) in water by headspace HS-GC/MS. The method was reported to have a limit of quantitation (LOQ) of 0.10 µg/L.

MITC (Methyl Isothiocyanate)

H_3C-N=C=S

Analytical Method Principles:

An aliquot of the water samples is dosed into a headspace vial, the water is saturated with NaCl, the vial sealed and then the vial is agitated and heated in the headspace oven. Then a given aliquot of the headspace is injected and MITC analysed by thick-film capillary GC/MS monitoring the 73 m/z molecular ion for quantitation and the 72 m/z $[M-H]^+$ ion for confirmation.

The calibration range covered by the CEMAS study was given with 0.03 µg/L to 10 µg/L and was demonstrated to be linear. For evaluation and calculation, the original method used calibration line/functions.

Confirmation in the original method was performed with a different stationary GC phase.

The present ILV study only used one column, but for confirmation additionally the MS/MS mode to additionally monitor two mass transitions was used, to provide full confirmatory results. One mass transition was the characteristic 72 m/z $[M-H]^+$ ion forming the 45 m/z daughter and the molecular ion 73 m/z forming 72 m/z. Both parents were used in the original method in the selected ion monitoring.

Independent Method Validation (ILV):

In the present study the ILV for drinking water was performed with fortifications to obtain fortification levels at the 0.10 µg/L and at 1.0 µg/L.

2. EXPERIMENTAL

2.1 Test Systems

The analytical method was validated with drinking water.

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[3] Maria Garcia Alix. 02-Apr-13. Validation of an Analytical Method for the Determination of MITC in Ground Water. CEMAS Report No. CEMR-5667.

[4] Maria Garcia-Alix. 13-Mar-14. Analytical Method for the Determination of Residues of Methyl Isothiocyanate in Drinking, Surface and Groundwater. CEMAS Analytical Method No. CAM-0084/002.

Tap water from a local source in Ulm was used. The tap water was characterized by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods), resulting in the following (non-GLP, see Appendix 2):

pH (DIN 38 404-C5)	7.23
DOC (diluted organic carbon, EN 1484: 1997):	0.75 mg/L
Silt content (EN 872 Whatman GF 6):	<0.1 mg/L
Electric conductivity (at 25 °C):	776 µS/cm
Total water hardness (calculated)	3.10 mmol/L

2.2 Test and Reference Item

MITC (Methyl Isothiocyanate) **H_3C-N=C=S**

Chemical formula: C_2H_3NS Molar mass: 73.12 g/mol

See Appendix 1 for information provided on the analytical reference items used.

2.3 Equipment and Instrumentation

2.3.1 Equipment

Balances: Sartorius ED 2202S-CW (used for specimens) and Mettler-Toledo XP205DR (used for preparation of stock solutions).
Ultrasonic bath: USC 600 T, VWR international.
20 mL headspace vials with Sil/PTFE (1.3 mm) septa equipped screw caps, Chromtech.
Typical glassware and laboratory equipment. All the glassware was cleaned in a laboratory dishwasher and air-dried.

2.3.2 HS-GC/MS Instrumentation

Thermo Trace 1310 gas chromatograph equipped with a TriPlus RSH Base liquid + HS Autosampler, split/splitless injector and TSQ 8000 GC/MS/MS triple quadrupole mass spectrometric detection, helium as carrier gas, and Xcalibur Software.

GC column: Agilent DB-624 fused silica capillary column (30 m length, 0.25 mm inner diameter, 1.4 µm film thickness).

2.4 Reagents, Chemicals and Miscellaneous

Methanol, Promochem, HPLC grade.
Millipore water, PTRL Europe.
Sodium chloride, >99%, Th Geyer.

2.5 Standard Solutions

2.5.1 MITC Solutions

MITC stock solutions in methanol:

A standard stock solution of MITC (2.0 mg/mL) was prepared by accurately weighing approximately 50 mg of reference item (taking into account its purity) into a 25 mL volumetric flask and bringing to volume with methanol (storage: frozen).

MITC fortification solutions in methanol:

The MITC stock solution was diluted with methanol to an intermediate solution of (20 µg/mL). Spiking solutions were prepared by accurate dilution of the intermediate solution with methanol resulting in 2.0 µg/mL and 0.20 µg/mL (storage: frozen).

Fortifications were performed by adding 5 µL of MITC fortification solutions to control specimens just prior to analysis. These fortified specimens were analyzed along with control specimens.

2.5.2 MITC Calibration Solutions

MITC standard solutions in methanol:

MITC standard solutions were prepared by accurate dilution of the MITC stock solution (2.0 mg/mL) and further dilution steps in methanol, resulting in 20000, 10000, 4000, 2000, 1000, 400, 200, 100 and 60 ng/mL.

MITC calibration solutions in ultrapure water:

For the calibration solutions, 5 µL of each MITC standard solution (20000 to 60 ng/mL) were dosed into a headspace vial containing 10 mL Millipore water and 5 g NaCl. This resulted in concentrations of 10.0, 5.0, 2.0, 1.0, 0.50, 0.20, 0.10, 0.050 and 0.030 µg/L.

MITC matrix matched calibration solutions in tap water:

For the matrix matched calibration solutions, 5 µL of MITC standard solutions (2000 and 200 ng/mL) were dosed into a headspace vial containing 10 mL tap water and 5 g NaCl. This resulted in concentrations of 1.0 and 0.10 µg/L.

3. Analytical Procedure

3.1 Sample preparation

5 g of NaCl were added to a headspace vial, then the (refrigerated) tap water sample was added (10 mL) and the headspace vial was immediately capped. Then the vial was directly placed in the autosampler rack for analysis.

3.2 HS-GC/MS Determination

GC-MS analysis parameters were taken from the CEMAS Report No. CEMR-5667, with adaptations made due to different HS-GC/MS instrumentation.
The adaptations were necessary as the headspace autosampler used in this study uses a heated syringe in contrast to the sample loop injection system used in the original method. Hence, the injection volume was reduced to 2.5 mL (original method 3.0 mL) corresponding to the maximum sample capacity of the heated syringe. Additionally two MS/MS mass transitions were monitored to obtain full confirmatory results.

3.2.1 HS-GC/MS Method

The following HS-GC/MS method was used:

GC/MS System	Thermo Trace 1310 gas chromatograph equipped with a TriPlus RSH Base liquid + HS Autosampler, split/splitless injector and TSQ 8000 GC/MS/MS triple quadrupole mass spectrometric detection, helium as carrier gas, and Xcalibur Software.
Carrier gas	Helium at 2.5 mL/min constant flow.
Injection technique	Headspace split injection (split: 1/2) at 220 °C, injection volume: 2500 µL. Syringe temperature: 105 °C.
Incubation parameters	Incubation time: 10 min at 80 °C.
GC capillary column	Agilent DB-624 fused silica capillary column. (30 m length, 0.25 mm inner diameter, 1.4 µm film thickness)
Oven temperature program	50 °C, 5 min hold, then with 15 °C/min to 100 °C, then with 35 °C/min to 200 °C, 1 min hold.
MS detection	6.0 min filament delay. Emission current: 50 µA. Electron energy: -70 eV. Mass spectrometric detection, using selected ion monitoring at 73 m/z and 72 m/z and MS/MS transitions 73 m/z -> 72 m/z and 72 m/z -> 45 m/z at 230 °C ion source temperature. EI ion volume installed. Positive ions detected.
Retention time	MITC ≈ 7.9 min

3.2.2 Calibration and Evaluation Procedures

Calculation of results is based on peak areas and external calibration. Calibration functions were obtained by injections of standards from 0.03 to 10 µg/L MITC; (≥ 7 levels) using linear regression calculation by Xcalibur. Typical calibration diagrams with functions and coefficients of determination R^2 as well as response factors versus concentration diagrams are shown in Figure 2 and Figure 3. Typical calibration, sample and blank chromatograms with retention times and peak areas are given in Figure 4 to Figure 10.

3.3 Calculation of Results

3.3.1 Calculation

Calculation of results is based on peak area measurements and external linear calibration functions using calibrations in ultrapure water. The residue water concentrations C in the specimens [in µg/L] are obtained directly as a result of the GC/MS measurements.

Recoveries (in %) are calculated as follows:

Rec. = $(R / R_{fortified}) \times 100\ \%$.

Calculations were performed by Excel with full precision, but displayed rounded, thus re-calculation with hand-held calculator may result in slightly different results.