Journal of Chromatography A, 1217 (2010) 2171–2178

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Headspace-trap gas chromatography–mass spectrometry for determination of sulphur mustard and related compounds in soil

Bent T. Røen[a,b,*], Erik Unneberg[a], John Aa. Tørnes[a], Elsa Lundanes[b]

[a] Norwegian Defence Research Establishment (FFI), P.O. Box 25, NO-2027 Kjeller, Norway
[b] Department of Chemistry, University of Oslo, P.O. Box 1033 Blindern, NO-0315 Oslo, Norway

ARTICLE INFO

Article history:
Received 6 December 2009
Received in revised form 25 January 2010
Accepted 29 January 2010
Available online 6 February 2010

Keywords:
Headspace-trap
Mass spectrometry
Soil
Sulphur mustard
Cyclic sulphur compounds

ABSTRACT

Methods for trace determination of sulphur mustard (HD) and some related cyclic sulphur compounds in soil samples have been developed using headspace-trap in combination with gas chromatography–mass spectrometry (GC–MS). Two quite different types of soil were employed in the method optimisation (sandy loam and silty clay loam). Prior to analysis, water saturated with sodium chloride was added to the samples, at a water to soil ratio of 1:1. A detection limit of 3 ng/g was achieved for HD, while the cyclic sulphur compounds 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane could be detected at 0.2–0.7 ng/g. The methods were validated in the concentration range from the limit of quantification (LOQ) to hundred times LOQ. The within assay precision at fifty times LOQ was 6.9–7.3% relative standard deviation (RSD) for determination of the cyclic sulphur compounds, and 15% RSD for determination of HD. Recoveries were in the range of 43–60% from the two soil types. As the technique requires very little sample preparation, the total time for sample handling and analysis was less than 1 h. The technique was successfully employed for the determination of cyclic sulphur compounds in a sediment sample from an old dumping site for chemical munitions, known to contain HD degradation products.

1. Introduction

The use of chemical warfare agents (CWA) in armed conflicts has been banned since the Geneva Convention entered into force in 1928, and more recently through the Chemical Weapons Convention (CWC) from 1997 [1]. Still, the use of CWA poses a possible threat from non-state parties or terrorist attacks, as in 1994 (Matsumoto City) and in 1995 (Tokyo) [2]. Another concern is the large amounts of chemical weapons that were disposed of in specific ocean areas or abandoned after World War II [3]. Hence, reliable and sensitive methods for determination of CWA and related chemicals in environmental samples are of great importance, both for verification purposes and for environmental concerns.

The skin damaging agent bis(2-chloroethyl) sulphide (sulphur mustard, military designated HD) is probably the most employed CWA in history. HD was frequently used in World War I, and more recently in the Iran–Iraq war and during the campaign against the Iraqi Kurdish population in 1987–1988 [4]. HD hydrolyses in the environment to a set of sulphides, disulphides, sulphoxides, sulphones, and thiols [5]. In addition, munition grade HD often contains impurities that can survive in the environment longer than the agent itself. Thus, determination of several common degradation products and impurities may act as a reliable proof of the original presence of HD. Besides determination of HD, this study includes two of the most common degradation products, 1,4-thioxane and 1,4-dithiane [5], and in addition 1,3-dithiolane (structures shown in Table 1). The latter has been found in water and soil samples near an old destruction site for HD [6,7].

Amongst various environmental matrices, soil is probably the most employed sample specimen for the identification of HD. Soil has high adsorption capacity, making it able to retain organic compounds for a long time [8]. In addition, HD is rather persistent in soil and can remain intact for several years [5]. One example of this was soil samples taken from a village in the northern Iraq four years after a reported CWA attack. Several samples still contained traces of HD, in addition to common degradation products such as 1,4-thioxane and 1,4-dithiane [9]. Traces of HD have also been found in sediment samples taken from the seabed near wrecks loaded with chemical munitions in Skagerrak [10]. In several other cases, HD and related compounds have been found in soil samples, giving evidence to the use of the agent [11–13].

The combination of gas chromatography (GC) and mass spectrometry (MS) has been extensively used for the identification of HD and related compounds in environmental samples [9–15]. For determination of CWA in soil, a recommended protocol from sample treatment to final instrumental analysis is available [16]. This includes extractions both with an organic solvent and with

* Corresponding author at: Norwegian Defence Research Establishment (FFI), P.O. Box 25, NO-2027 Kjeller, Norway. Tel.: +47 6380 7881; fax: +47 6380 7509.
E-mail address: Bent-Tore.Roen@ffi.no (B.T. Røen).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.01.088

water, followed by filtration and concentration steps, and analysis by GC–MS or liquid chromatography (LC)–MS. Hancock et al. have reported an HD limit of detection (LOD) of 0.2 μg/g with dichloromethane extraction of the soil, followed by GC–MS in full scan mode [6]. For the determination of 1,4-thioxane and 1,4-dithiane in soil, Tomkins et al. have used pressurised liquid extraction at elevated temperature followed by GC with flame photometric detection (FPD) [17]. The LODs of this technique were quite high, however (1.5–1.6 μg/g). For the polar and longer chain degradation products of HD, water extraction followed by LC–MS is more suited [18,19]. Alternatively, on-matrix derivatisation–extraction has been performed for the polar degradation product bis(2-hydroxyethyl) sulphide (TDG), followed by GC–MS determination [20].

Several headspace (HS) extraction and sample introduction techniques have been applied for determination of HD and related compounds in soil samples. Stach et al. used both a modified dynamic HS system coupled to ion mobility spectrometry (IMS)–MS, and static HS–GC–MS for analyses of soil from an old German production site [21]. Kimm et al. have developed a method using headspace-solid phase microextraction (HS-SPME) in combination with GC–MS for determination of HD in soil [22]. An LOD of 0.2 μg/g was achieved with the MS in full scan mode.

In the present study, the headspace-trap (HS-trap) technique in combination with GC–MS has been applied for the first time for determination of CWA in soil samples. This work is a continuation of a former investigation for determination of HD and related compounds in water by HS-trap GC–MS [23]. The HS-trap technique patented by Tipler and Mazza [24], is an enhanced static HS system which was commercialised in 2004. This system allows focusing and concentration of the analytes prior to chromatographic analysis, thus enhancing the sensitivity compared to conventional static HS. The technique has shown a great potential for trace determination of various volatile organic compounds in water [25–27]. To our knowledge, trace determination of organic compounds in soil samples by this technique has not been published.

2. Experimental

2.1. Chemicals

HD (98.5%) was purchased from Netherlands Organisation for Applied Scientific Research (TNO, Delft, The Netherlands). 1,4-Thioxane (98%) and 1,3-dithiolane (97%) were obtained from Sigma–Aldrich Inc., MO, USA, while 1,4-dithiane was obtained from Sigma–Aldrich, U.K. 1,2,4-Trimethylbenzene (1,2,4-TMB) (98%) was purchased from Acros Organics, NJ, USA. Ultra resi-analysed acetone (≥99.4%) was obtained from J.T. Baker, Deventer, The Netherlands. Analytical grade sodium chloride (≥99.5%) was purchased from Merck, Darmstadt, Germany. Laboratory type II water (classified according to the American Society of Testing and Materials, D1193-91) was delivered in-house by RIOS 30 Laboratory-Grade Water Systems from Millipore, France.

Table 2
Selected properties of the soil types employed in the method development.

	Soil A	Soil B
Particle size distribution (%)		
<0.002 mm	9.3	33.4
0.002–0.05 mm	29.1	55.0
0.05–2.0 mm	61.6	11.6
Soil type (USDA classification)	Sandy loam	Silty clay loam
pH-value	6.2	5.6
TOC (%)	1.0	0.4

Structural formulas and some physical properties of HD and the cyclic sulphur compounds are shown in Table 1. The vapour pressure of the compounds is of high relevance for the sensitivity in HS analysis, as well as the water solubility when water is present in the sample matrix.

2.2. Soil samples for method development

Two types of characterised soil were applied in the method development. Soil A was purchased from LUFA Speyer in Germany, sieved to a grain size of 2 mm and characterised by the supplier. Soil B was collected at Kjeller, Norway, and homogenised and sieved with a 2 mm screen. Measurements for characterisation of soil B were performed by the Norwegian Center for Soil and Environmental Research (Ås, Norway). Both soils were dried in nitrogen at 50 °C for 24 h prior to use. The particle size distribution, pH values and total organic carbon (TOC) content of the soils are listed in Table 2. Classification of the soil types is given according to the United States Department of Agriculture (USDA) [28].

2.3. Preparation of solutions and samples

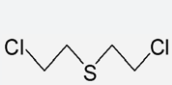
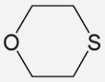
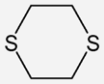
2.3.1. Spiking solutions

Stock solutions of HD were prepared by diluting (1.00 ± 0.02) μl of the neat agent in acetone to concentrations of 0.1–0.3 mg/ml, using a calibrated plunger-in-needle syringe from Hamilton (Bonaduz, Switzerland). Spiking solutions were made in acetone at concentrations from 0.03 to 90 μg/ml depending upon the experiment, by appropriate dilution of the stock solutions. Joint stock solutions of 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane were prepared by diluting 50–100 mg of the neat agents in acetone to concentrations of 0.2–1 mg/ml. Spiking solutions with concentrations from 0.006 to 1 μg/ml were made by appropriate dilutions of the stock solutions in acetone. The internal standard (IS) stock solution was prepared by diluting 200 mg 1,2,4-TMB in 100 ml acetone. This solution was further diluted in type II water to a concentration of 0.4 μg/ml. All solutions were stored at 4 °C.

2.3.2. Soil spiking and sample handling

Each soil sample was weighed directly into the HS-vial. The spiking solution was added to the soil at an amount of 40 μl per g soil. The spiking levels for method development ranged from 21

Table 1
Structural formula, vapour pressure and water solubility of HD, 1,4-thioxane, 1,4-dithiane (data from Munro et al. [5]) and 1,3-dithiolane (data is calculated using Advanced Chemistry Development (ACD/Labs) Software V9.04 for Solaris (©1994–2008 ACD/Labs).

	HD	1,4-Thioxane	1,4-Dithiane	1,3-Dithiolane
Structural formula				
Vapour pressure (mmHg)	0.1	3.9	0.8	1.6
Water solubility (g/l)	1.0	167	3.0	9.1

to 230 ng/g for HD, and from 25 to 43 ng/g for the cyclic sulphur compounds.

Both the soil and the added spiking solution were weighed with an accuracy of 0.1 mg. The vial was immediately capped and homogenised on a whirlmixer for 1 min, and stored at 4 °C for 1 h. Then, the vial was decapped and vented for 3 min at room temperature (22–24 °C). Slurry samples were prepared by adding NaCl saturated type II water. The vial was capped, and sample thermostatting was initialised within 1 min after water addition.

2.3.3. Samples for method validation

For method validation, aliquots of 2.0 g soil were weighed into the vials and 80 μl of the spiking solution was added. The samples were treated in the same way as described in Section 2.3.2. After addition of salt saturated water, the samples containing cyclic sulphur compounds were added to 50 μl of the IS spiking solution, at an aqueous concentration of 10 ng/ml.

Samples for linearity tests were prepared at six concentration levels in the range of 8.8–860 ng/g for HD and 0.70–69, 2.0–196 and 1.0–97 ng/g for 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane, respectively. Two replicates were prepared at each level in the linearity test of HD, while one replicate was used for the cyclic sulphur compounds. Samples for investigation of the repeatability were prepared at two concentration levels: 9 and 450 ng/g (HD), 0.7 and 35 ng/g (1,4-thioxane), 2 and 100 ng/g (1,3-dithiolane), 1 and 50 ng/g (1,4-dithiane). Precision was investigated with preparation and analysis of six replicates within one day (within assay), and one replicate for six consecutive days (between assay). Samples for investigation of robustness and recovery were prepared at the same concentrations as described for the repeatability tests (high levels). The recovery was calculated as the amount of soil-spiked analytes that was extracted into the water phase of the slurry:

$$\text{Recovery } (\%) = \frac{\text{Peak area of analyte spiked into the soil}}{\text{Peak area of analyte spiked into the slurry}} \times 100.$$

2.3.4. Sediment sample

The sediment sample was collected during an environmental investigation of an old dumping area of ships loaded with chemical munition in Skagerrak, performed by FFI in 2002. Since then, the sample has been stored in a closed glass container at −20 °C.

After allowing the sample to reach room temperature, it was filtrated through an S&S 597 filter (Schleicher & Schuell GmbH, Dessel, Germany) to remove excess of water. The water content after filtration was approximately 40% (w/w). Sample aliquots of 2.0 g each were weighed into HS-vials and added 2.00 ml of NaCl saturated water. The samples were then analysed according to the developed methods for determination of HD in soil, and for cyclic sulphur compounds in soil. Identification of the compounds was made by matching of mass spectra with those of authentic standards, or NIST library mass spectra.

2.4. Instrumentation

A TurboMatrix HS 110 Trap (PerkinElmer instruments, CT, USA) connected to a Clarus 500 GC–MS with quadropole analyser (also PerkinElmer) was used. The HS-trap system was controlled by an internal graphical user interface, while the GC and MS were controlled by the Turbomass software, version 5.1.0. The adsorbent tube was a Tenax trap with a bed size of 2.7 mm × 25 mm, delivered by PerkinElmer. A DB-5MS column from J&W Scientific (Folson, CA, USA), 30 m × 0.25 mm ID and 0.25 μm film thickness, was coupled directly to the HS through a heated transfer line. Helium was used as carrier gas with a constant inlet pressure of 15 psi, giving a flow rate of 1 ml/min at 100 °C. The transfer line temperature was set to 150 °C, in order to avoid sample condensation on possible cold spots at the connection between the transfer line and the HS sampler (recommended by the manufacturer). The GC temperature program was: 40 °C (1 min), then 10 °C/min to 140 °C (0 min) and 20 °C/min to 300 °C (1 min). The MS was operated in electron ionisation (EI) mode with ionisation energy of 70 eV. Mass spectra were collected over the m/z range 35–300 with a scan time of 0.2 s, and an inter-scan delay of 0.05 s. Peak areas were measured as the sum of three characteristic ions of each compound (Table 4), extracted from the total ion current (TIC) chromatograms.

Headspace-vials (22 ml), together with septa of polytetrafluoroethylene (PTFE)/silicone were delivered by PerkinElmer. Preliminary experiments had shown poor repeatability for water analyses when the HS-vials were used several times. All method development and validation analyses were therefore performed with new HS-vials.

2.4.1. Headspace-trap working principle

The HS-trap system works as a conventional static HS analyser in the first step, by heating the vial until the analytes approach equilibrium between the sample matrix and the vapour phase (thermostatting). Thereafter, the vial is pressurised, and the pressure is released by leading the vapour phase through an adsorbent tube where the analytes are focused (trap load). The adsorbed water is then removed by purging helium through the trap (dry purge). Finally, the trap is rapidly heated and backflushed (trap desorption), and the analytes are desorbed and led into the chromatographic system in a narrow band. In this way, a much larger amount of the analytes is introduced onto the GC, compared to conventional static HS. Furthermore, the pressurising and trap load steps can be repeated to utilise an even larger fraction of the vapour phase.

2.5. Experimental design

Factorial design experiments were employed in the robustness tests of the developed methods. Statistical data from the factorial design experiments were treated in Minitab®, version 15.1.1.0.

3. Results and discussion

3.1. Drying of soils

Optimisation of analytical conditions was performed for two quite different types of well characterised soils to ensure that the method was suited for a wide range of soil types. To avoid any influence of water content on the results during method development, the soils were dried prior to use. Drying with nitrogen gave lower chromatographic background signal compared to drying in air atmosphere. Fig. 1 shows chromatograms of soil A dried in both ways for 24 h at 50 °C. Drying at 110 °C in air atmosphere increased the background signal compared to drying at 50 °C. However, after charring of the soil by heating it to 550 °C in ambient air, the background signal was at the level of a non-dried soil. Investigations on the consequences of soil drying on the chromatographic background signal have to our knowledge not been reported. Based on our observations, the soil samples that were used for method development were dried in nitrogen for 24 h at 50 °C.

3.2. Trap parameters

The trap parameters should be set to give optimum sample transfer into the GC column, and to ensure an efficient removal of water from the trap without loss of analytes. These conditions were thoroughly investigated in a previous study for determination of the analytes in water [23]. The trap drying conditions are dependent on the amount of water adsorbed on the trap, which for

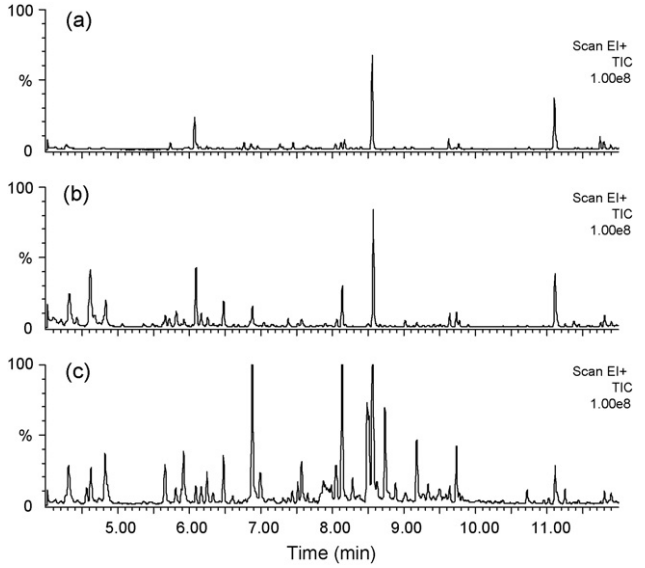


Fig. 1. TIC chromatograms of soil A: (a) soil not dried; (b) dried in nitrogen at 50 °C; (c) air dried at 50 °C.

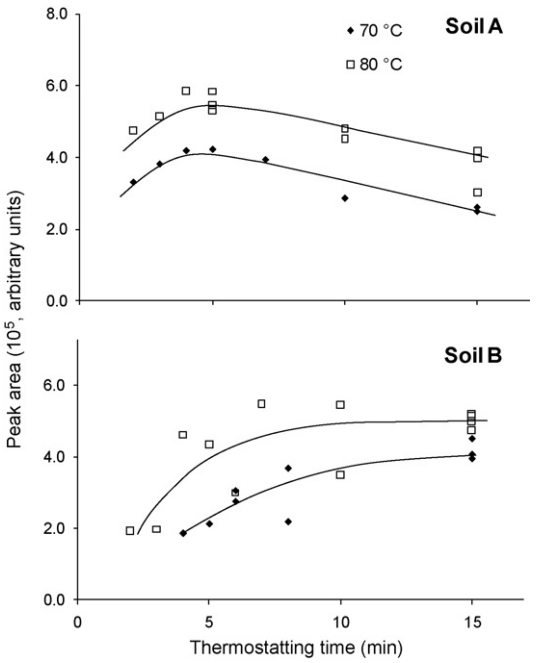


Fig. 2. Extraction yields of 1,3-dithiolane measured as peak areas as a function of thermostatting time, thermostatted at 70 °C and 80 °C. Each point represents one replicate, and trend lines are manually inserted. Sample amount was 1.0 g and concentration was 25 ng/g. The samples were added to 1.0 ml salt saturated solution prior to analyses.

water and slurry samples is determined by the thermostatting temperature and the number of vial extractions. As the HS conditions turned out to be identical for determination of the cyclic sulphur compounds in both water and soil, the trap parameters optimised in the former study were applied in this work as well.

3.3. Sample agitation

Shaking of the sample helps to shorten the time needed to establish equilibrium between the sample matrix and the vapour phase. It has been shown that sample agitation also improves precision when analysing soil/water slurry samples [29]. In the present study, shaking was activated in all experiments.

3.4. Thermostatting temperature and thermostatting time

The thermostatting temperature and thermostatting time are amongst the conditions of most importance to the sensitivity in HS determination. Extraction yields of the compounds were investigated at thermostatting temperatures of 70 and 80 °C, with increasing thermostatting time from 2 to 15 min. The extraction profiles of 1,3-dithiolane from both soil types are shown in Fig. 2. Equal profiles were found for 1,4-thioxane and 1,4-dithiane. From soil A, the highest extraction yields were achieved after 4–5 min. A decrease in peak areas was found when increasing from 5 to 15 min thermostatting time. The decreasing tendency could be due to oxidation of the analytes at elevated temperatures. Opstad and Tørnes showed that 1,4-dithiane was oxidised to the respective sulphoxide and sulphone after long time storage in seawater at 25 °C [30]. For soil B, a longer thermostatting time (10–15 min) was required to reach equilibrium between the soil/water and vapour phase. Furthermore, large variations in peak areas were found at thermostatting times shorter than 15 min. It has been shown that the clay content of the soil plays an important role for the extraction recovery of organic compounds, both by use of HS [31], and with solvent extraction [32]. Thus, the high clay content of soil B may be the reason for making the extraction more challenging.

The extraction profiles of HD (Fig. 3) differed from those of the cyclic sulphur compounds, probably due to the low stability of HD in aqueous environment and at elevated temperatures. Highest extraction yields were achieved at thermostatting times from 2 to 6 min, and a rapid decrease in peak area was observed at extended thermostatting time, especially at 80 °C. Similar to that for the cyclic sulphur compounds, large variations in the extraction yields from soil B were observed.

The differences in extraction profiles for the two soil types demonstrate the importance of including soils of various charac-

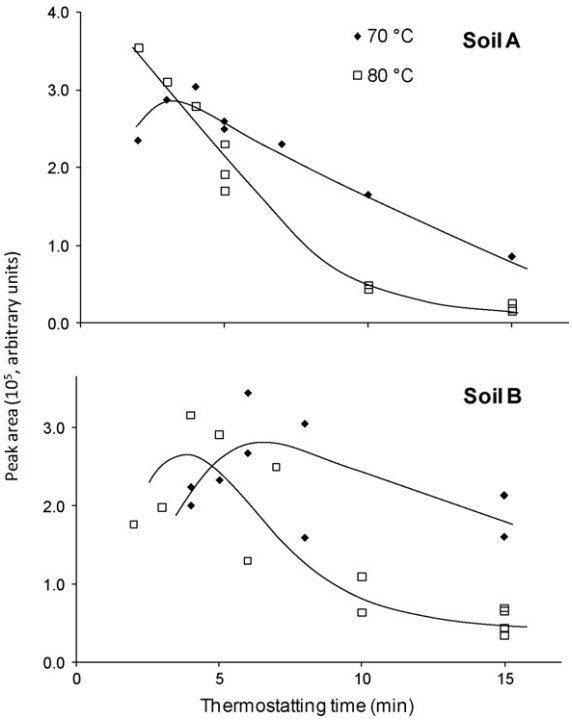


Fig. 3. Extraction yields of HD measured as peak areas as a function of thermostatting time, thermostatted at 70 °C and 80 °C. Each point represents one replicate, and trend lines are manually inserted. Sample amount was 1.0 g and concentration was 230 ng/g. The samples were added to 1.0 ml salt saturated solution prior to analyses.

teristics when performing method optimisation. Since the optimal analysis conditions differed between the two soil types, a compromise had to be chosen. For determination of the cyclic sulphur compounds, a thermostatting time of 15 min at 80 °C was chosen, to ensure equilibrium between the soil/water sample and the vapour phase. Due to the differences in optimal analysis conditions for HD and the cyclic sulphur compounds, further method development on HD was carried out separately. A thermostatting time of 5 min at 70 °C was preferred, due to the rapid degradation of HD at longer thermostatting time.

3.5. Determination of the cyclic sulphur compounds

3.5.1. Addition of salt saturated water

Addition of water to solid adsorbent samples is a widely employed matrix modification technique to increase sensitivity in static HS sampling. The technique has proven to be effective for determination of HD in soil as well [22]. Moreover, salt saturation (NaCl) of the water may be used to increase partitioning of the analytes into the headspace [23], and was hence used in the present study.

The effect of water addition on extraction efficiency of the cyclic sulphur compounds was investigated at various water to soil ratios, as shown in Fig. 4. Highest extraction yields were achieved with a water to soil ratio of 1:1 for both soil types. The effect was most evident for 1,4-dithiane, with a two- to four-fold increase compared to that of dry soil. The lowest effect was seen for 1,4-thioxane, probably because of the higher water solubility of this compound (Table 1). The partitioning between the vapour and the water phase will be favoured towards the vapour phase for the less water soluble compounds. Furthermore, the water addition had somewhat larger effect on extraction from the silty clay loam (soil B) compared to the sandy loam.

3.5.2. Sample amount

In conventional static HS, the sensitivity is proportional to the concentration of analytes in the vapour phase. It has been shown that this concentration may be affected by the ratio between the sample matrix volume and HS volume [33]. In HS-trap, the sensitivity is proportional to the total amount of analytes present in the vapour phase, which in turn is dependent upon both the concentration of analytes in the vapour phase and the available HS volume. Thus, the total amount of soil and water applied in the sample vial could affect the extraction recovery, as it influences the HS volume and may influence the analyte concentration.

The effect of sample amount was investigated for both soil types by comparing the extraction yields from aliquots of 1.0, 2.0 and 3.0 g soil weighed into the HS-vials. All samples were added to the salt saturated solution at a water to soil ratio of 1:1. Hence, the added water represented the largest part of the sample matrix volume. No significant differences in extraction yields of the analytes were seen, however, in both soil types (t-test at $\alpha = 0.05$, $n = 3$). Thus, a sample amount variation in the range 2.0 ± 1.0 g was not critical for the extraction recovery. For the further studies, a sample amount of 2.0 g was chosen.

3.5.3. Repeated vial extractions

The pressurisation and trap load steps can be repeated up to four times, in order to achieve a more complete vapour extraction from the vial. This option could be valuable for enhancing the sensitivity in trace analyses. However, as more water will be adsorbed on the trap for each repeated vial extraction, it also requires more extensive drying prior to trap desorption. The effect of repeated vial extractions on recovery was investigated with soil A as the sample matrix. Peak areas and relative standard deviations (RSDs) were compared for analyses with use of one, two and three successive vial extractions. An increase in peak areas of 35–40% was seen by use of a second vial extraction, and the RSD values were overall low (<5%, $n = 4$). By use of a third extraction, the further increase in peak areas was negligible, and hence a procedure with two vial extractions was chosen.

3.6. Determination of sulphur mustard

The optimal amount of salt saturated water on extraction of HD was investigated for both soil types, with sample amounts of 2.0 g (Fig. 5). Even though HD is highly unstable in water, a distinct positive effect was seen when salt water was added to the soil. A five- and nine-fold increase was seen in the extraction yields from soil A and soil B, respectively (water to soil ratio 1:1), compared

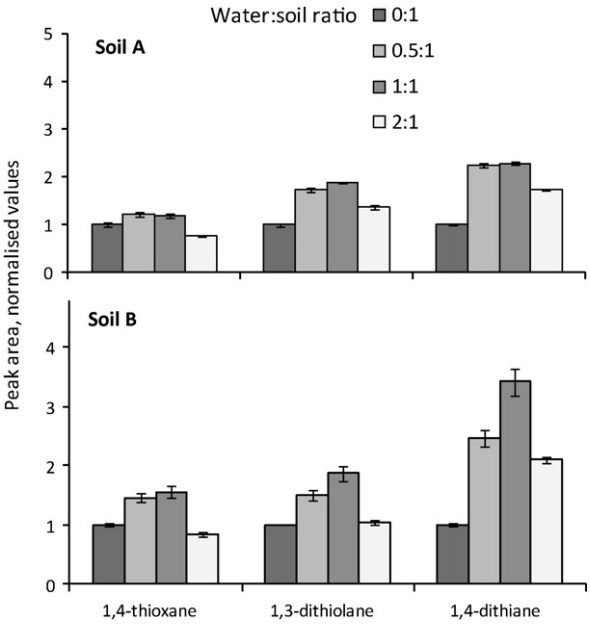


Fig. 4. Effect of water to soil ratio on extraction efficiency of the cyclic sulphur compounds. Peak areas are presented as mean values ± one standard deviation ($n = 3$), normalised to those of the samples with no water added. Various amounts of NaCl saturated water (0.5, 1 and 2 ml) were added to 1.0 g dried soil, as well as soil with no water addition. Analyte concentrations were in the range of 25–43 ng/g. The samples were thermostatted for 15 min at 80 °C, and one vial extraction was performed.

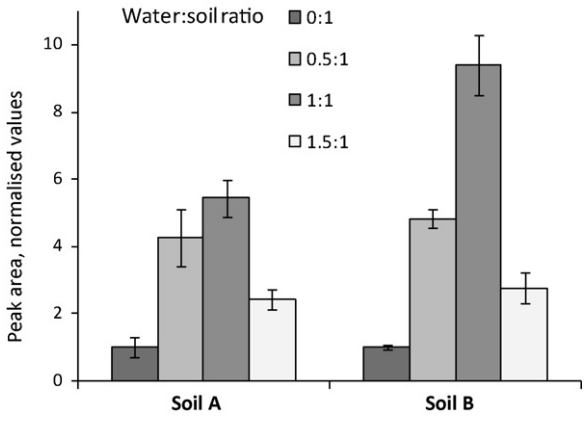


Fig. 5. Effect of water to soil ratio on extraction efficiency of HD. Peak areas are presented as mean values ± one SD ($n = 4$), normalised to those of the samples with no water added. Various amounts of NaCl saturated water (1, 2 and 3 ml) were added to 2.0 g dried soil, as well as soil with no water addition. HD concentration was 21 ng/g. The samples were thermostatted for 5 min at 70 °C, and one vial extraction was performed.

Table 3
Instrument parameter values for determination of the analytes in soil. The values for determination of HD are given in parenthesis where the methods diverge.

	Parameter values
Trap parameters	
Trap low temperature	50 °C
Trap high temperature	280 °C
Dry purge time	7 min
Desorption time	0.5 min
Trap hold time	3 min
Desorption pressure	30 psi
Needle purge split flow	13 ml/min
HS parameters	
Thermostatting temperature	80 °C (HD: 70 °C)
Needle temperature	90 °C
Transfer line temperaure	150 °C
Thermostatting time	15 min (HD: 5 min)
Pressurisation time	1.0 min
Decay (trap load) time	2.0 min
Number of cycles	2
Vial pressure	40 psi
Column pressure	15 psi
Shaker (on/off)	On

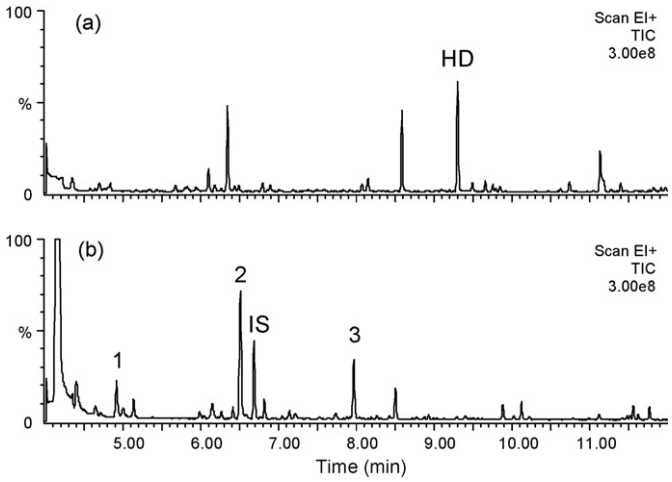


Fig. 6. TIC chromatograms of soil A, spiked with HD (a) and the cyclic sulphur compounds (b). Analysis conditions were as described in Table 3. Concentration of HD was 268 ng/g. The cyclic sulphur compounds eluted in the order: (1) 1,4-thioxane, (2) 1,3-dithiolane, (3) 1,4-dithiane, and their concentrations were 34, 96 and 48 ng/g, respectively. The IS was added into the aqueous slurry to a concentration of 10 ng/ml.

to the analyses of dry soil. The main reason of the large effect is probably the low water solubility of HD (Table 1). Furthermore, Røen et al. showed that salt saturation of the water was essential for a high extraction yield of HD [23]. The salt content not only increases the partitioning of the analyte into the vapour phase, but also decreases the degradation rate of HD in water [34]. However, an abrupt decrease in recovery was seen for both soil types when the water to soil ratio was further increased. Hence, the added water volume should be reduced when analysing soil samples with a high water content.

The effect of a second vial extraction on HD recovery was investigated with soil A as the sample matrix. The average peak area increased by a factor of 1.9, while the relative SD increased from 11 to 13% ($n = 4$). Hence, a procedure with two vial extractions was chosen.

3.7. Method validation

The optimised parameter values for determination of the analytes in soil are listed in Table 3. Examples of chromatograms from analyses of the cyclic sulphur compounds and of HD in soil are shown in Fig. 6. The developed methods were validated with soil A as the sample matrix; however, the recovery was investigated for both soil types. The LOD of HD was also investigated for both soil types. The IS (1,2,4-TMB) was applied for determination of the cyclic sulphur compounds. The method for determination of HD gave poor precision of 1,2,4-TMB, probably due to the short thermostatting time of 5 min. Hence, the external standard procedure was preferred for this compound. Data from the method validation are given in Table 4.

3.7.1. Detection limit, quantification limit, linearity and repeatability

The LODs were established from reconstructed ion chromatograms (RICs), extracted from the TIC chromatograms. The RICs were plotted as the sum of signals of three characteristic ions (listed in Table 4) for each compound. The LODs were chosen as the concentrations giving a signal to noise (S/N) ratio of ten. In addition, a requirement was that all three ions should be visible in the mass spectrum at these concentrations. Thus, the selectivity of the method was ensured by performing full scan GC–MS and by the requirement of molecular mass presence. The quantification limits (LOQs) were calculated as three times the detection limits.

An LOD of 3 ng/g was found for HD, which is two orders of magnitude lower than what has been reported by HS-SPME GC–MS [22], and solvent extraction GC–MS [6] (both 2×10^2 ng/g). This proves the superior sensitivity of the technique for determination of HD in soil samples. The obtained LODs of the more stable cyclic sulphur compounds were at the sub ppb levels (0.2–0.7 ng/g). The linearity and precision of the methods were investigated within the concentration range from LOQ to 100 times LOQ. Good linearity and within assay repeatability were found for the cyclic sulphur compounds within the investigated range, while the between assay repeatabil-

Table 4
Method validation.

		1,4-Thioxane	1,3-Dithiolane	1,4-Dithiane	HD
m/*z*-ratios for quantification and determination of LOD		46, 61, 104	60, 78, 106	46, 61, 120	109, 111, 158
LOD (ng/g)		0.2	0.7	0.3	3
LOQ (ng/g)		0.7	2	1	9
Linearity (R^2) LOQ—100·LOQ		0.995	0.996	0.991	0.95
Repeatability (RSD) ($n = 6$)					
LOQ	Within assay	9.9	8.0	5.6	18
	Between assay	5.8	17	7.8	25
50·LOQ	Within assay	6.9	7.2	7.3	15
	Between assay	9.5	7.8	13	19
% Recovery ± SD ($n = 4$)					
50·LOQ	Soil A	53 ± 4	51 ± 4	46 ± 3	58 ± 9
	Soil B	43 ± 4	48 ± 4	51 ± 4	60 ± 3

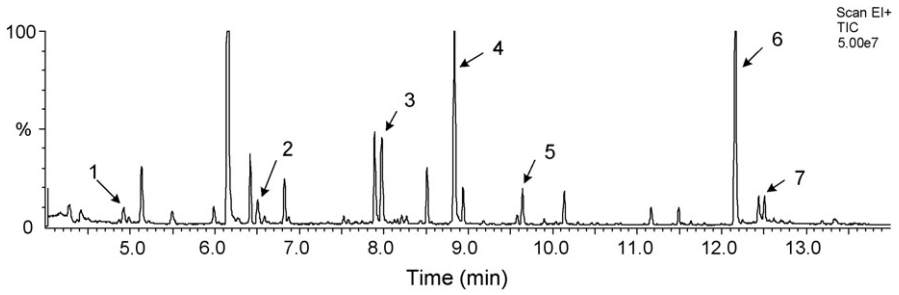


Fig. 7. TIC chromatogram of a sediment sample collected from an old dumping site for chemical munitions in Skagerrak 2002. The sample was analysed according to the method for determination cyclic sulphur compounds (Table 3). Seven compounds related to HD were identified: (1) 1,4-thioxane, (2) 1,3-dithiolane, (3) 1,4-dithiane, (4) 1,2,3-trithiolane, (5) 1,4,5-oxadithiephane, (6) 1,2,3,4-tetrathiane, and (7) 1,2,5-trithiephane.

ity was above 10% RSD in two of six cases. However, taking into consideration the complexity of the sample matrix, the precision must be regarded as acceptable. Somewhat higher variations had to be accepted for HD, due to the instability in aqueous environment and at elevated temperatures. Moreover, the IS peak area correction could not be applied for determination of HD.

3.7.2. Recovery

The recovery study of the cyclic sulphur compounds shows that approximately one half of the analytes were retained in the soil during thermostatting. Some of the loss of analytes may also be attributed to evaporation during sample preparation. Somewhat higher recoveries were achieved for HD. This may be due to less volatilisation loss during sample preparation, as HD has lower vapour pressure than the other compounds.

3.7.3. Robustness

Robustness of both methods was investigated for three of the most important parameters, at analyte concentrations of 50 times LOQ. A two-level factorial design experiment was set up with high and low values for the thermostatting temperature (±2 °C), water to soil ratio (0.90 and 1.2) and percentage salt saturation (90 and 100%). The reduced salt content was included to simulate a dilution due to the possible natural water content in a soil sample. The samples that were prepared with a water to soil ratio of 1.2 and 90% salt saturation represent a soil sample of 2.0 g originally containing approximately 10% water (to which 2.00 ml of NaCl saturated water has been added).

The method for determination of the cyclic sulphur compounds was not vulnerable to the variations in thermostatting temperature or salt content. On the other hand, when the water to soil ratio increased similar to a 10% water content of the soil, the recoveries decreased in the range of 13–19% (significant at $\alpha = 0.05$). The recovery of HD was negatively influenced both by the higher water to soil ratio and the lower salt concentration (significant at $\alpha = 0.05$). Variations in the thermostatting temperature of ±2 °C did not influence the HD recovery. To secure a high recovery of the analytes with both methods, the amount of salt solution may be reduced if the soil sample has a high water concentration. Furthermore, additional salt may be added to the slurry to ensure complete saturation of the sample.

3.8. Analysis of a sediment sample

The developed methods were employed for analysis of a sediment sample, collected from an old dumping site for chemical munitions in Skagerrak in 2002 [10]. When examined in 2002, sample aliquots were extracted with dichloromethane and analysed by GC–MS, according to the recommended procedure for determination of CWA in soil [16]. Six cyclic sulphur compounds related to HD, and three arsenic compounds related to what is known as vomiting agents were identified in the sample by the solvent extraction GC–MS method in 2002.

Fig. 7 shows the TIC chromatograms of the sediment sample, analysed by the present method for determination of the cyclic compounds. All the former identified HD related compounds were seen in the TIC chromatogram. In addition, a seventh HD related compound (1,3-dithiolane) was identified. The other significant compounds were mostly aldehydes and ketones, presumably from decomposition of organic material, in addition to some system contaminants. The S/N levels of the identified compounds were significantly higher than those in the corresponding chromatogram by the liquid extraction GC–MS method. Hence, the applicability of the HS-trap technique for volatile and semi-volatile compounds was also proven for determination of analytes after many years of retention in the sample. The three arsenic compounds (diphenylchloroarsine, triphenylarsine and bis(diphenylarsine) oxide) were not detected with the HS-trap technique. This is most probably due to the low vapour pressures of the arsines ($<1 \times 10^{-3}$ mmHg), making them unsuitable for HS extraction. Analysis of the sample with the method for determination of HD showed no detectable signal of this compound, in agreement with the results from the solvent extraction GC–MS technique.

Concentrations of the identified compounds were semi quantitatively determined to be in the range of 1–10 ng/g. No quantitative comparison of the two methods was performed, since minor changes of the sample composition during storage could not be excluded. An extended investigation including HS-trap analyses of several sediment samples from the chemical munitions dumping area will be published.

4. Conclusion

Methods for trace determination of HD and related compounds in soil by HS-trap GC–MS have been developed. Two soil types were employed in the method development; sandy loam and silty clay loam. Due to the low stability of HD in aqueous environment and at elevated temperatures, the optimal analysis conditions for determination of this compound differed from those of the cyclic sulphur compounds. Therefore, separate methods were developed for determination of HD and for the cyclic compounds. Optimisation of the thermostatting time and temperature showed that it was important to include soils of different characteristics, as the optimal analysis conditions differed between the soil types. Addition of NaCl saturated water to the soil increased the recovery of all analytes considerably, with the greatest improvement achieved for HD.

The LOD of HD was determined to 3 ng/g. This is two orders of magnitude lower than what has been obtained by the HS-SPME technique, or by the recommended solvent extraction procedure followed by GC–MS. The present analysis technique showed to be

even more sensitive for the more stable cyclic sulphur compounds 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane, with LODs of 0.2, 0.7 and 0.3 ng/g, respectively. The method for determination of the cyclic sulphur compounds showed good linearity ($R^2 > 0.990$), and within assay precision (10% RSD or lower) within the investigated range from LOQ to 100 times LOQ. For determination of HD in soil, a somewhat poorer linearity ($R^2 > 0.95$) and within assay repeatability (15–18% RSD) had to be accepted, due to the low stability of HD in aqueous environment. The technique proved to be very simple in use, as the only sample preparation needed was the addition of salt saturated water to the samples. Thus, the total time of sample handling and analysis was less than 1 h. In contrast, the recommended solvent extraction procedure prior to GC–MS analysis requires sample handling times of typically 4-5 h.

The developed method for determination of cyclic sulphur compounds was used for analysis of a sediment sample collected from an old dumping site for chemical munition in Skagerrak in 2002. All cyclic sulphur compounds that were detected in the sample in 2002, were also found by the developed HS-trap GC–MS method. In addition, 1,3-dithiolane was unambiguously identified. The result of this analysis demonstrated that the extraction technique worked successfully for determination of the analytes after many years of retention in the sediment.

References

[1] Convention on the Prohibition of the Development, Production, Stockpiling, use of Chemical Weapons and their Destruction, Technical Secretariat of the Organization of Prohibition of Chemical Weapons, The Hague, 1997. http://www.opcw.org/.
[2] A.T. Tu, Toxin Rev. 26 (2007) 231.
[3] MEDEA, Ocean Dumping of Chemical Munitions: Environmental Effects in Arctic Seas, MEDEA, McLean, VA, USA, 1997.
[4] L. Szinicz, Toxicology 214 (2005) 167.
[5] N.B. Munro, S.S. Talmage, G.D. Griffin, L.C. Waters, A.P. Watson, J.F. King, V. Hauschild, Environ. Health Perspect. 107 (1999) 933.
[6] J.R. Hancock, P.A. D'Agostino, C.L. Jackson Lepage, C.L. Chenier, H.D.M. Wyatt, Eight International Symposium on Protection against Chemical and Biological Warfare Agents, Gothenburg, 2004.
[7] P.A. D'Agostino, L.R. Provost, A.S. Hansen, G.A. Luoma, Biomed. Environ. Mass Spectrom. 18 (1989) 484.
[8] M.B. McBride, Environmental Chemistry of Soils, Oxford University Press, Inc., New York, NY, 1994, p. 372.
[9] R.M. Black, R.J. Clarke, R.W. Read, M.T.J. Reid, J. Chromatogr. A 662 (1994) 301.
[10] J.Aa. Tørnes, Ø.A. Voie, M. Ljønes, Aa.M. Opstad, L.H. Bjerkeseth, F. Hussain, Investigation and risk assessment of ships loaded with chemical ammunition scuttled in Skagerrak, FFI/RAPPORT-2002/04951, Norwegian Defence Research Establishment, 2002.
[11] R.M. Black, R.J. Clarke, D.B. Cooper, R.W. Read, D. Utley, J. Chromatogr. 637 (1993) 71.
[12] P.A. D'Agostino, L.R. Provost, Biomed. Environ. Mass Spectrom. 15 (1988) 553.
[13] J. Lippert, I. Swahn, Proceedings of the ERDEC Scientific Conference on Chemical and Biological Defense Research, Aberdeen Proving Ground, MD, 1994, p. 705.
[14] P.A. D'Agostino, J.R. Hancock, C.L. Chenier, Eur. J. Mass Spectrom. 9 (2003) 609.
[15] P.A. D'Agostino, L.R. Provost, J. Chromatogr. 436 (1988) 399.
[16] M.L. Kuitunen, in: M. Mesilaakso (Ed.), Chemical Weapons Convention Chemical Analysis, John Wiley & Sons, Ltd., Chichester, 2005, p. 163.
[17] B.A. Tomkins, G.A. Sega, S.J. Macnaughton, Anal. Lett. 31 (1998) 1603.
[18] P.A. D'Agostino, J.R. Hancock, C.L. Chenier, J. Chromatogr. A 1058 (2004) 97.
[19] E.W.J. Hooijschuur, C.E. Kientz, A.G. Hulst, U.A.Th. Brinkman, Anal. Chem. 72 (2000) 1199.
[20] D.K. Dubey, D. Pardasani, M. Palit, A.K. Gupta, R. Jain, J. Chromatogr. A 1076 (2005) 27.
[21] J. Stach, J. Flachowsky, J. Adler, M. Brodacki, A. Loudon, H.R. Doring, Field Screening Methods for Hazardous Wastes and Toxic Chemicals Proceedings of an International Symposium, Las Vegas, NV, 1995, p. 1053.
[22] G.L. Kimm, G.L. Hook, P.A. Smith, J. Chromatogr. A 971 (2002) 185.
[23] B.T. Røen, E. Unneberg, J.Aa. Tørnes, E. Lundanes, J. Chromatogr. A 1217 (2010) 761.
[24] A. Tipler, C. Mazza (PERKIN ELMER LAS, INC.), System and Method for Extracting Headspace Vapor. International Application Number PCT/US2004/011487 (2004).
[25] F. Barani, N. DellïAmico, L. Griffone, M. Santoro, C. Tarabella, J. Chromatogr. Sci. 44 (2006) 625.
[26] H. Griffith, Measuring environmental volatile organic compounds by U.S. EPA Method 8260B with headspace trap GC/MS, PerkinElmer Life and Analytical Sciences, Shelton, CT, 2004.
[27] H. Grecsek, LC GC N Am. 23 (2005) 47.
[28] R.B. Brown, Soil Texture; Fact Sheet SL-29, Soil and Water Science Department, Institute of Food and Agricultural Sciences, University of Florida, FL, 2003.
[29] T.C. Voice, B. Kolb, Environ. Sci. Technol. 27 (1993) 709.
[30] Aa.M. Opstad, J.Aa. Tørnes, Identification and quantification by GC–MS of sulphur mustard and related compounds after long time storage in sea water, FFI-RAPPORT-2002/03237, Norwegian Defence Research Establishment, 2004.
[31] A. Serrano, A. Gallego, J. Chromatogr. A 1118 (2006) 261.
[32] F. Hussain, J.Aa. Tørnes, A. Strømseng, Seventh International Symposium on Protection Against Chemical and Biological Warfare Agents, Stockholm, 2001.
[33] L.S. Ettre, B. Kolb, Chromatographia 32 (1991) 5.
[34] S.L. Hoenig, Compendium of chemical warfare agents, Springer Science + Business Media, LLC, New York, NY, 2007, p. 1.