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Trace determination of sulphur mustard and related compounds in environmental samples by headspace-trap GC-MS

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English summary

New methods for trace determination of sulphur mustard (HD) by headspace-trap GC-MS have been developed for water and soil samples. As HD is unstable, especially in water, methods for determination of some of the cyclic decomposition compounds have also been investigated. Several parameters showed to influence the detection of the compounds, and statistical experimental design was applied to optimise instrumental parameters of the methods. Furthermore, it was found that salt saturation of the water samples and addition of salt saturated water to the soil samples improved the recovery of all analytes considerably, and in particular of HD.

The developed methods made it possible to determine HD in water and soil at the ppb level. For soil samples, this was an improvement in sensitivity by two orders of magnitude compared to literature values. The present technique showed to be even more sensitive for the cyclic decomposition compounds, with detection limits at sub-ppb level.

The headspace-trap extraction technique requires almost no sample preparation, and the total sample handling time was less than one hour for determination of the analytes in water as well as in soil samples. This is a great improvement compared to the prevailing procedures using solvent extraction, which requires several hours sample handling time.

The application of the method was demonstrated by analysing an environmental sample known to contain several HD related compounds. All compounds were found at a signal to noise level higher than what was obtained with solvent extraction. In addition, one HD related sulphur compound that had not been detected previously was found.

In the present work, it is concluded that the headspace-trap GC-MS technique has a great potential for determination of HD and HD related compounds in environmental samples.

Sammendrag

Det er utviklet metoder for bestemmelse av sennepsgass (HD) i vann- og jordprøver ved hjelp av headspace-trap GC-MS. Siden HD er ustabil i vandige prøver, er det også utviklet metode for bestemmelse av nedbrytningsprodukter fra HD. Statistisk forsøksplanlegging ble brukt i metodeutviklingen. Dette viste seg å være et effektivt hjelpemiddel, siden flere av analyseparameterne påvirket hverandre. Det ble funnet at tilsetting av salt til vannprøvene, og mettet saltløsning til jordprøvene, økte gjenfinningen av alle analyttene. Dette var spesielt effektivt for gjenfinningen av HD.

Analyseteknikken gjorde det mulig å detektere HD på ppb-nivå i både vann og jordprøver. For jordprøver er dette en forbedring i følsomheten med to størrelsesordener, sammenlignet med hva som er oppgitt i litteraturen. Metoden viste seg å være enda mer følsom for de sykliske nedbrytningsproduktene, med deteksjonsgrenser under en ppb.

Headspace-trap analyseteknikken krever nesten ingen prøveopparbeidelse, og den totale analysetiden med prøvehåndtering var mindre enn en time. Dette er betydelig kortere enn analysetiden for de metodene som brukes i dag, hvor selve prøveopparbeidelsen tar flere timer.

Anvendelsen av metoden ble demonstrert ved å analysere en sedimentprøve som var kjent å inneholde flere forbindelser relatert til HD. Alle forbindelsene ble påvist, og med et bedre signal-støy forhold enn det som tidligere var oppnådd med løsemiddelekstraksjon og GC-MS analyse. Det ble også identifisert ytterligere en forbindelse relatert til HD, som tidligere ikke var funnet i prøven.

Denne studien konkluderer med at headspace-trap GC-MS teknikken har et stort potensial for bestemmelse av HD og relaterte forbindelser i miljøprøver.

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Preface

This report presents the work of my MSc thesis performed at the Norwegian Defence Research Establishment (FFI), Protection Division, in the period of September 2007 to September 2008.

I would like to thank the Department Director Dr. Jan Ivar Botnan and Research Director Dr. Bjørn Arne Johnsen at FFI for giving me the opportunity to define and accomplish the present study at the institute. The work with the thesis has given me valuable practical and theoretical experience within my scientific field. Thanks also to my colleagues at FFI, for their support and encouragement, especially during the writing of the thesis.

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Finally, special thanks to MSc Fatima Hussain for convincing me to start this study, and guiding me through the scary corridors of the Department of Chemistry at the University of Oslo.

Kjeller, December 2008

Bent Tore Røen

Abbreviations

CWA	chemical warfare agents
CWC	Chemical Weapons Convention
ESI	electrospray ionisation
FID	flame ionisation detection
FPD	flame photometric detection
GC	gas chromatography
HD	bis(2-chloroethyl) sulphide (sulphur mustard)
HF-LPME	Hollow fibre-mediated liquid-phase microextraction
HS	headspace
IMS	ion mobility spectrometry
IS	internal standard
LC	liquid chromatography
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantification
MECK	micellar electrokinetic chromatography
MS	mass spectrometry
NIST	National Institute of Standards and Technology
OPCW	Organisation for the Prohibition of Chemical Weapons
PTFE	polytetrafluoroethylene
RI	retention indicies
ROP	recommended operating procedure
SD	standard deviation
SDME	single drop microextraction
SPE	solid phase extraction
SPME	solid phase microextraction
1,2,4-TMB	1,2,4-Trimethylbenzene
TDG	thiodiglycol
TIC	total ion current

TOC	total organic carbon
TOF	time-of-flight
USDA	United States Department of Agriculture
VOC	volatile organic carbon
WWI	World War I
WWII	World War II

1 Introduction

The use of chemical warfare agents (CWA) in armed conflicts has been banned since the Geneva Convention entered into force in 1928 [1]. The treaty does not, however, ban the production and stockpiling of chemical munitions. Hence, there has been an extensive research and development of CWA. In some cases these agents have also been used, against soldiers in armed conflicts as well as against civilians. The more comprehensive Chemical Weapons Convention (CWC) entered into force on April 29th 1997 [2], and was at June 2008 signed by 188 countries. This treaty prohibits the development, production, stockpiling and use of CWA. Even though most countries have signed the CWC, use of CWA is still concerned as a possible threat. This threat could be from non-state parties or connected to terrorist attacks, as occurred in Japan in 1994 (Matsumoto City) and in 1995 (Tokyo) [3].

If the use of CWA is suspected in a conflict or by terrorists, it is important to be able to give unambiguous verification of possible use. This can be done by taking samples from the site, like soil, water or vegetation, and perform trace determination of the CWA or their typical degradation products. Within an ongoing project at Norwegian Defence Research Establishment (FFI), sampling and trace determination of CWA are main issues. One of the areas of interest is to develop less labour demanding and more sensitive analysis techniques for CWA determination in environmental samples. The headspace-trap sample introduction system combined with gas chromatography and mass spectrometric detection (HS-trap-GC-MS) is a new and sensitive analysis technique, which requires little or no sample preparation. The technique was introduced on a commercial available instrument in 2003, and has a great potential for trace determination of volatile and semi-volatile components in environmental samples [4,5].

In the present work, the HS-trap-GC-MS technique has been investigated for trace determination of bis(2-chloroethyl) sulphide (commonly known as sulphur mustard or mustard gas) and some related compounds in water and soil. The related compounds are 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane, which can be present as impurities, or be formed from degradation of sulphur mustard [6,7].

1.1 Use of sulphur mustard as a chemical warfare agent

The vesicant sulphur mustard is one of the CWA of most historical and current interest (Figure 1.1). The compound is also called *yperite* from the name of the city Ypres in Belgium, close to where it was used for the first time by Germans in World War I (WWI), in 1917 [8]. A common designation is agent H, which originates from the first letter of the English slang word “Huns” used for Germans during WWI [9]. Distilled and purified agent H is called HD. Distilled sulphur mustard is an oily liquid, colourless and odourless in its pure form. If it contains small quantities of impurities, it is yellowish and with a characteristic odour resembling oil of mustard, hence the name mustard gas. During WWI and World War II (WWII), other types of mustard agents were also used in munitions, like agent Q and agent T shown in Figure 1.1.

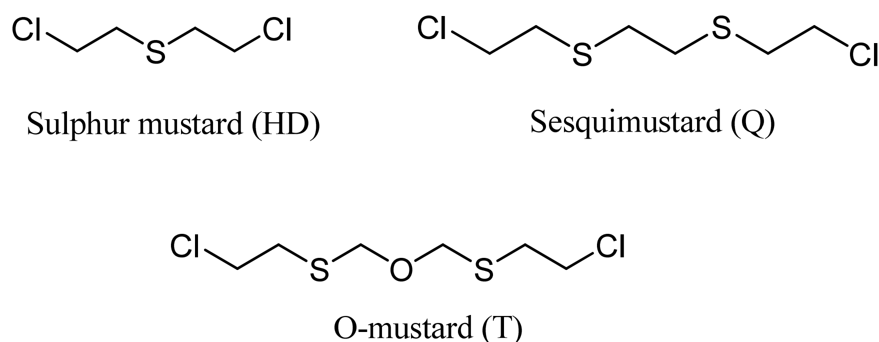


Figure 1.1 Chemical structures of three mustard agents used in munition grade mustard.

Munition grade HQ is a mixture of 75% HD and 25% agent Q, whereas HT has typically 60% HD and 40% agent T [7,10]. Later on, mainly the distilled preparation of agent H has been used.

In the last period of WWI, mustard agents were also used both by British and French forces [9]. Subsequent documented use of mustard agents includes use in Ethiopia in 1935, in China between 1937 and 1945 and in the Iran-Iraq war 1983-1988 [9]. Iraqi forces also used HD against their own Kurdish population in 1988 [11]. Mustard agents were not reported used during WWII, except from in China. However, large quantities were produced and stockpiled both by Germany and many countries of the Allied forces [9].

Today, one of the concerns with mustard agents is related to the large amount of sea dumped or abandoned weapons from WWII. During the following years, allied forces disposed captured German and Japanese CWA stockpiles in which HD was one of the main components, by dumping them into dedicated sea areas [12]. Sulphur mustard has low aquatic solubility, and blocks of HD can stay intact at the sea bead for several decades after the artillery shell is corroded [13]. Accidents have been reported both in the Baltic Sea and along the coast of Japan, involving fishermen who inadvertibly have snared mustard agent with their net [12]. In China, another concern has been from large amounts of abandoned chemical weapons left behind during Japanese retreat in the closing stages of WWII. It has been estimated that abandoned CWA in China have caused 2000 casualties or fatalities since the end of the war [14]. Examples of such incidents are construction workers digging beneath city streets or riverboat workers who brought CWA up from the water during dredging operations. Many of the casualties are associated with mustard agents or a mixture of mustard agents and another type of blistering agents, known as lewisites.

1.2 Toxicity of sulphur mustard

As HD appears as an oily liquid at room temperature, the name mustard *gas* is somewhat misleading. However, the vapour pressure and toxicity values are sufficient to reach dangerous dosages from vaporisation of even small liquid amounts at ambient temperature. Skin, eyes and the respiratory system are the principal target organs of HD [8]. Skin effects caused by HD

vapour are dependent upon ambient temperature [15], and wide concentration ranges are quoted for specified effects. Toxicity values within these typical ranges are shown in Table 1.1. Blisters generally appear 18-24 hours after exposure, and they often contain large volumes of fluid. Erythema appears within 2-4 hours and causes extreme itching, which diminishes as the blisters appear [15]. Eye exposure causes intense irritation with watering, conjunctival swelling and erythema [15]. Inhalation of HD causes damages to the respiratory system, and vomiting and diarrhea when absorbed [16]. Also, HD is classified as a human carcinogen by the International Agency for Research on Cancer [16].

Table 1.1 Vapour exposure toxicity values for sulphur mustard, given as cumulative exposure in mg·min/m³. References are given in parenthesis.

ECt_{50} level [1)]	Eye [15]	Skin [15]	Inhalation
Threshold [2)]	50	100 - 400	50 [8]
Incapacitation	200	200 - 1000	300 [8]
Lethal	-	750 – 10 000	1500 [17]
1)	The ECt_{50} concept means the cumulative exposure (concentration multiplied by time, C·t), causing a specific defined effect (E) in 50% of the exposed population.		
2)	The threshold level is the lowest cumulative exposure where minor irritation and/or erythema may occur.		

Two examples of injuries caused by HD exposure are shown in Figure 1.2. The left picture shows an Iranian soldier exposed to HD in the Iran-Iraq war. He was treated for mustard agent burns in a Swedish hospital [18]. The other picture shows a Baltic fisherman, exposed to HD from old ammunition brought up from the water by a fishing net [18].

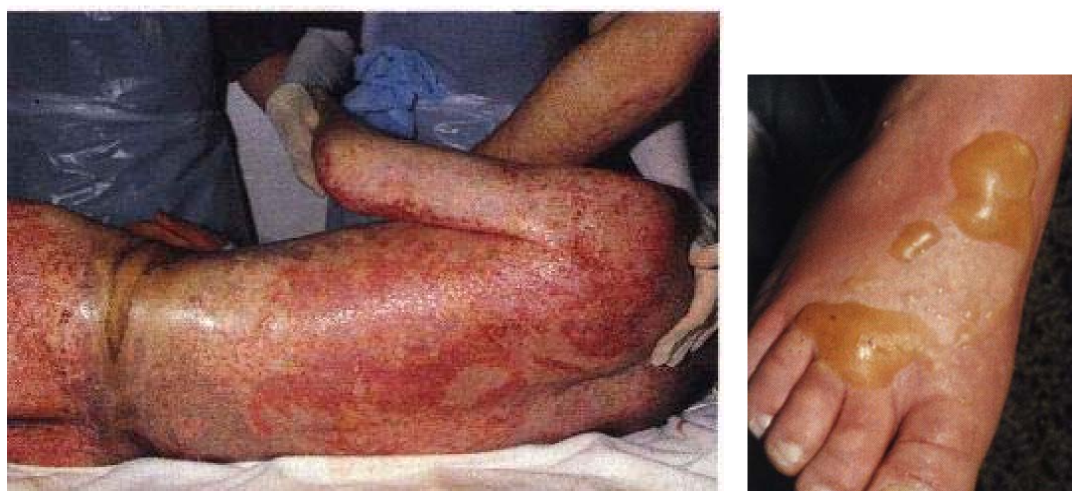
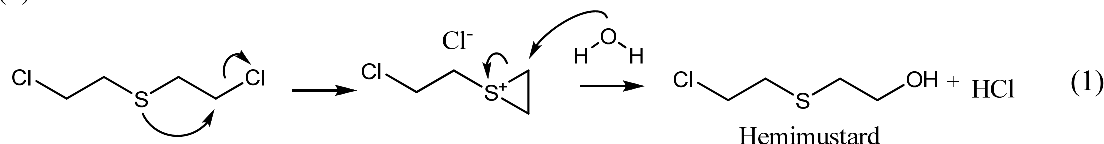


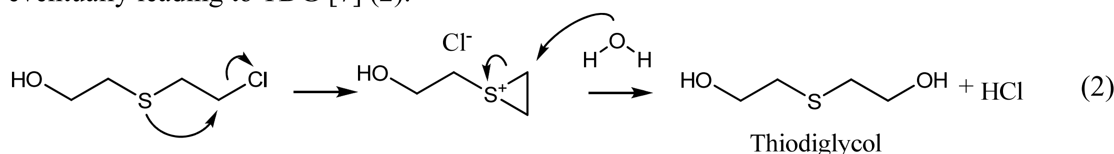
Figure 1.2 Examples of injuries caused by HD exposure. Left picture: Iranian soldier, several weeks after exposure. Photo taken at a Swedish hospital [18]. Right picture: Baltic fisherman with a relatively fresh injury. Photo taken at Bornholm hospital, Denmark [18].

1.3 Hydrolysis of sulphur mustard

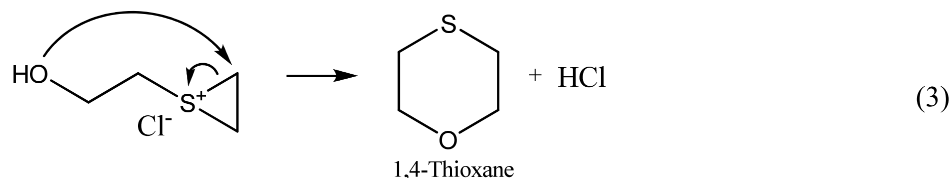
When solved in freshwater, HD easily hydrolyses with a half-life of 4-8 min at 25 °C [7], giving thiodiglycol (TDG) as the main product. The first step in the hydrolysis process is a neighbouring group nucleophilic attack of the sulphide to form a sulphonium ion intermediate. The sulphonium ion then reacts quickly with water to form 2-chloroethyl 2-hydroxyethyl sulfide (hemimustard) (1).



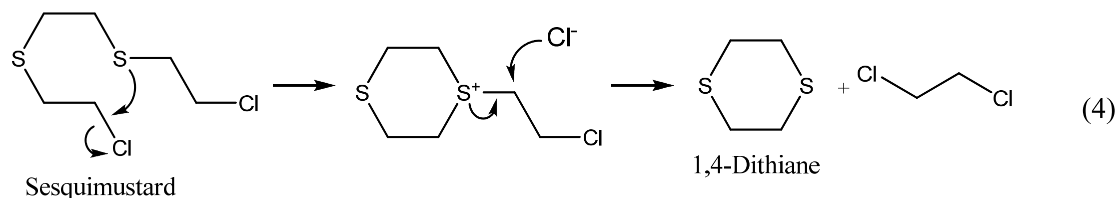
As the HD molecule contains two chlorine atoms, the hemimustard can react in the same way, eventually leading to TDG [7] (2).



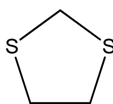
Two other common degradation products of HD are the cyclic sulphur compounds 1,4-thioxane and 1,4-dithiane. 1,4-Thioxane is formed from an internal displacement of the hemimustard sulphonium ion (3) [19].



The formation of 1,4-dithiane is proposed to occur from degradation of sesquimustard (Q). Q can form a 6-ring sulphonium chloride through an internal reaction, which forms 1,4-dithiane upon attack by the chloride ion (4) [20].



Q can be formed from degradation of HD [20], or be present as an impurity. In addition, Q can be present as an additive in what is known as munition grade HQ [10].

The mechanism of formation of 1,3-dithiolane 

from HD is not described. This compound occurs less frequently in the literature than 1,4-thioxane and 1,4-dithiane, but it has been detected several times in trace determination of sulphur mustard [6,21,22].

In addition to the compounds discussed above, HD can form a variety of degradation products, both cyclic and open longer chain compounds [7]. A range of intermediate sulphonium ions can react with water, with other HD molecules, or through internal reactions. Furthermore, both HD and many of the hydrolysis products can be oxidized to sulphoxides or sulphones [23]. One example is soil samples taken from a Kurdish village in Iraq in 1988. HD and 22 different degradation products were found in the soil [24]. In a block of old munition grade mustard from a dumping site in the Baltic Sea, 16 different degradation products or contaminants from production were found [25]. Likewise, analyses of abandoned munition grade mustards in China showed traces of HD and 27 related compounds [26].

1.4 Physical properties of sulphur mustard and related compounds

The vapour pressure and water solubility are the two most important physical properties when determining compounds in water or soil by the headspace sample introduction technique. The values for HD and the compounds described in the previous chapter are listed in Table 1.2.

Table 1.2 Vapour pressure and water solubility of HD, TDG and three of the cyclic degradation products. Data is collected from Munro et al. [7], except from the data for 1,3-dithiolane [1].

Compound	**Vapour pressure mmHg**	**Water solubility g/l**
HD	0.1	1.0
TDG	$0.2 \cdot 10^{-4}$	miscible
1,4-thioxane	3.9	167
1,3-dithiolane	1.6	9.1
1,4-dithiane	0.8	3.0

1.5 Sampling and Identification

Obviously, the most reliable way to confirm the existence of HD in a sample is to identify the intact compound. If HD is not found, the next step will be to search for the most common degradation products, like TDG. However, this compound is used in the manufacture of several commercial products including pulp, paper products, paints and coatings as well as in the manufacture of furniture [27]. Thus, traces of TDG in the environment could originate from production and processing, or from one of the manufactured products. Therefore, several of the common degradation products should be identified to give a reliable and trustworthy verification for the original existence of HD.

The high water solubility and the low vapour pressure of TDG make the HS analysis technique quite unsuitable, as will be discussed below. On the other hand, the technique should be well

[1] Calculated using Advanced Chemistry Development (ACD/Labs) Software V9.04 for Solaris (©1994-2008 ACD/Labs).

suited for determination of HD and many of the volatile and semi-volatile degradation products, like the cyclic sulphur compounds. One example is analyses of soil samples taken from an HD contaminated area [24]. Before further treatment of the samples, the headspace inside the storing containers was drawn through an adsorbent tube and analysed by thermal desorption combined with GC-MS. The headspace samples were found to contain HD, 1,4-thioxane, 1,4-dithiane and nine other volatile and semi-volatile degradation products.

1.6 Water samples

HD has low water solubility (Table 1.2), and hydrolyses rapidly when dissolved. Therefore, it is not very likely to identify the compound in water. However, some of the cyclic degradation products are more water soluble, and will stay intact in the water for a long time. For example, 1,4-dithiane has been found in the ground water near an old storage site for mustard agents in the Rocky Mountains, USA [7]. Likewise, ground water samples obtained from locations near an old HD destruction site in Canada revealed the presence of both 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane, amongst several other degradation products [21]. Furthermore, trace determination of HD and related compounds in seawater was part of an investigation at an old dumping site for chemical munition in Skagerrak [13]. In addition, aqueous hydrolysates have been subjected to analyses after HD destruction, for determination of remaining HD and identification of degradation products [6,28].

1.6.1 Analysis techniques

The recommended operating procedure (ROP) for determination of CWA in water samples refers to several techniques to cover the range of CWA and their degradation products [29]. This includes liquid-liquid extraction (LLE) with an organic solvent or solid phase extraction (SPE). Both extraction techniques are followed by filtration and concentration steps, and analysis by GC-MS or liquid chromatography-MS (LC-MS). For determination of HD and the cyclic degradation products, the LLE or SPE procedure followed by GC-MS is most suitable. D'Agostino *et al.* have used LLE with hexane, followed by GC analysis with flame ionisation detection (FID) for the determination of HD, with a limit of detection (LOD) of 50 μg/l [6]. Kanaujia *et al*. have employed SPE followed by GC-MS for HD determination, giving LOD of 50 μg/l with the MS in full scan mode [30]. No work has been reported for trace determination of the cyclic sulphur compounds using SPE or LLE procedures, but the LODs would presumably be in the same range as for HD. For the determination of non-volatile degradation products from HD in the extracts, a derivatisation step must be included prior to the GC-MS analysis [31-33]. For the water-soluble non-volatile degradation products from HD, LC-MS is a good alternative [34-36]. Microcolumn LC with MS or flame photometric detection (FPD), with large volume injection and peak compression has also been applied for the longer chain compounds [37,38].

The sensitivity of the analysis techniques is of great importance when performing trace determinations of CWA. In the later years, several micro extraction techniques have been developed to improve the sensivity. Palit *et al.* have used single drop microextraction (SDME) followed by GC-MS analysis in full scan mode, with a reported LOD for HD of 30 μg/l [39].

Hollow fibre-mediated liquid-phase microextraction (HF-LPME) has also been applied, followed by GC-MS analysis. The reported LOD for HD with single ion monitoring was 1.0 µg/l [40]. An even more sensitive technique is the hollow fibre-*protected* LPME combined with GC-MS, giving an LOD for HD of 0.1 µg/l in full scan mode [41]. Another sensitive technique is the solid phase microextraction (SPME). Hussain has obtained an LOD for HD in water of 1.7 µg/l, using SPME combined with GC-FID determination [42].

The HS technique has also been applied for the determination of HD in water. Johnsen *et al.* have used static HS coupled to a GC-FID to determine HD in several sample matrices, including water [43]. The detection limit in water was estimated to be in the range of 50-500 µg/l. Wils *et al.* employed dynamic headspace followed by GC-MS to determine TDG in water and urine, where TDG was converted to HD prior to the analysis by addition of HCl. The reported LODs for TDG were 0.1 µg/l in water and 1.0 µg/l in urine [44,45].

Less work has been reported on the determination of the cyclic sulphur compounds in water. Cheicante *et al.* have used micellar electrokinetic chromatography (MECK) with UV detection for determination of 1,4-thioxane and 1,4-dithiane in water [46,47]. The reported LODs were quite high however (2-8 mg/l). Another method using ion mobility spectrometry (IMS) with mass selected detection has been employed for determination of 1,4-thioxane, with a reported LOD of 1.3 mg/l [48]. A more sensitive technique is the electrospray ionisation (ESI) IMS with a time-of-flight (TOF) MS. Steiner *et al.* have used this technique for determination of several HD degradation products in water, with a reported LOD for 1,4-dithiane of 51 µg/l [49].

1.7 Soil samples

Soil is an omnipresent material, and is probably the most employed sample matrix for the identification of HD. An important property of soil is the high adsorbing capacity, making it able to retain organic compounds for a long time [50]. Also, HD is rather persistent in soil and can remain undecomposed for years [7]. 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 as 1,4-thioxane and 1,4-dithiane [51]. Traces of HD have also been found in sediment samples taken from the seabed near wrecks in Skagerrak, loaded with chemical munition [13]. In several other cases, HD and related compounds have been found in soil samples, giving evidence to the use of the agent [10,24,52,53]. Soil samples have also been applied in the environmental examination of an old destruction site for HD [22,54].

Soil can be classified according to the distribution of mineral particle sizes, where the amount of sand (50-2000 µm), silt (2-50 µm) and clay (< 2 µm) are determined [55]. Other important parameters are the total organic carbon (TOC) amount, ion exchange capacity and pH. Adsorption and desorption of organic molecules in soil are controlled by the chemical properties of the molecules and the surface properties of the particular soil. For the adsorption of nonpolar or weakly polar organic compounds in soil, the octanol/water partition coefficient (K_{ow}), and the fraction of organic carbon in soil are important factors [50]. Also, Hussain *et al.* have shown that

the extraction recovery of HD correlates negatively to the quantity of clay and silt, when extracting with dichloromethane [56].

1.7.1 Analysis techniques

Similar to determination of CWA in water, the ROP for soil samples include several techniques to cover the range of CWA and degradation products [29]. This includes extractions both with an organic solvent and with water, followed by filtration and concentration steps, and analysis by GC-MS or LC-MS. The analytes in the water fraction may also be derivatised prior to determination. HD and the cyclic degradation products will be extracted to the organic fraction and should be determined by GC-MS. As discussed, the extraction recovery of organic compounds is dependent on the surface properties and the amount of organic fraction of the particular soil. However, Hancock *et al.* have reported an LOD for HD of 0.2 µg/g with dichloromethane extraction of the soil, followed by GC-MS in full scan mode [22]. No values are reported for the cyclic degradation products with this method. Tomkins *et al.* have used pressurised liquid extraction at elevated temperature followed by GC-FPD for determination of 1,4-thioxane and 1,4-dithiane in soil, with LODs of 1.5 and 1.6 µg/g, respectively [57]. For TDG and the open longer chain degradation products, water extraction followed by LC-MS is more suited [22,58]. On-matrix derivatisation of the more polar degradation products combined with extraction, followed by GC-MS analysis, has also been reported [59].

Johnsen *et al.* have employed static HS coupled to a GC-FID to determine HD in soil. The detection limit was estimated to be in the range of 0.05-0.5 µg/g [43]. Stach *et al.* used both a modified dynamic HS system coupled to IMS-MS, and static HS-GC-MS for detection of HD and related compounds in soil samples from an old German production site [60]. No LOD was reported for the methods, but 1,4-thioxane and 1,4-dithiane were detected in several soil samples, with both techniques.

A technique that has obtained increasing attention the later years is the headspace solid phase microextraction (HS-SPME). Kimm *et al.* have developed a method using HS-SPME combined with GC-MS for determination of HD in soil [61]. A detection limit of 0.2 µg/g was achieved with the MS in full scan mode. Hancock *et al.* have employed HS-SPME-GC-MS for analysis of soil samples from an old HD storage site. With this technique, five cyclic degradation products were detected in the soil, including 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane [22]. It is interesting to notice that two of the cyclic compounds (1,3-dithiolane and 1,2,5-trithiapane) were not detected in the soil when using solvent extraction followed by GC-MS analysis.

1.8 Headspace - gas chromatography

Headspace – gas chromatography (HS-GC) is an analysis technique for determination of volatile and semi volatile compounds in solid or liquid samples. The HS-GC technique has two distinct benefits compared to most other techniques: Little or no sample preparation is required, and only the vapour phase above the sample matrix is introduced into the chromatographic system. Thus,

the technique requires little labour, both regarding the sample handling and the instrument maintenance.

1.8.1 Static and dynamic headspace

There are two types of HS techniques commercially available: static and dynamic HS. In both techniques, the sample is placed in a closed vial having a gas volume above it and thermostatted at a constant temperature. In static HS, the vial is usually thermostatted until equilibrium is reached between the two phases for the analyte of interest. Thereafter an aliquot of the gas phase (the *headspace*) is transferred into a GC column for analysis. In dynamic HS, the gas phase is removed continuously, not allowing equilibrium to establish. At the end, the total amount of the volatile compounds could thus be removed from the sample. The gas effluent is guided through an adsorbent where the volatile compounds are trapped. When extraction is completed, the analytes are transferred into the GC column by rapidly releasing them from the adsorbent, usually by heating and backflushing. The static HS technique usually offers good repeatability. However, the sensitivity is limited by the equilibrium of analytes established between the two phases, and the fact that only a fraction of the gas phase is analysed. In general, the dynamic HS offers better sensitivity, but has usually not so good repeatability. The dynamic HS also requires more maintenance, and is subject to problems such as carryover effects and foaming of the sample [62].

To enhance sensitivity of static HS, a method for transferring a larger aliquot of the gas phase has been applied. With this method, a cryogenic sample-focusing unit traps the analytes before they are introduced into the GC column. However, the technique has shown problems when water is present in the sample matrix, by icing and clogging of the system, and peak distortion [63][2]. The introduction of water into the chromatographic system is especially undesired when a mass spectrometric detector is used. Another version of static HS was introduced in the 1990s: HS-solid phase microextraction (HS-SPME) [64]. Here, a fiber coated with a stationary phase like polyacrylate, is kept in the headspace above the sample during thermostatting. The analytes are extracted from the vapour phase by adsorption on the fiber. After extraction, the fiber is transferred to a GC injection port where the analytes are thermally desorbed and transferred into the column. This technique has shown better sensitivity than ordinary static HS for volatile compounds in e.g. soil [65]. However the extraction of analytes is limited by the fact that both the partitioning between the sample and headspace, and between the headspace and stationary phase are temperature dependent, and will counteract. This effect has been avoided by internally cooling the fiber [66], but such a system is not commercially available.

1.8.2 Headspace-trap technique

The new HS-trap sample introduction technique was introduced on a commercial available instrument by Perkin Elmer in 2003. The technique combines some of the benefits from both static and dynamic HS. The HS-trap system acts similar to a conventional static headspace analyser until sample transfer from the vial. In the sample transfer step, the gas phase is guided through an adsorbing tube where the analytes are trapped. This is similar to the cryofocusing

[2] Chapter 3, Page 92

technique, but while the cryofocusing adsorbing system is connected in series with the GC column, the outlet of the HS-trap is coupled to atmosphere. In this way a drying step is allowed, avoiding the introduction of large amounts of water into the chromatographic system. After the drying step, the adsorbing tube is rapidly heated, and the trapped analytes are thermally desorbed into the GC column.

The sample introduction by thermal desorption leads to a quick release of the adsorbed components. This gives a narrow “sample plug” into the transfer line, which helps focusing the analytes at the beginning of the column. Thus, more narrow chromatographic peaks are obtained with enhanced signal height compared to conventional HS. The technique is presented in detail in Section 2.2.1.

1.8.3 Static headspace theory

The static HS theory described in this chapter is collected from Kolb and Ettre [63], Chapter 2. In static HS, the intention is to force as much as possible of the analytes into the vapour phase.The concentration of an analyte in the vapour phase at equilibrium can be expressed as follows:

$$C_v = \frac{C_0}{\beta + K} \qquad (5)$$

where C_v is the analyte concentration in the vapour phase

C_0 is the original analyte concentration in the sample

β is the phase ratio

K is the distribution coefficient of the analyte between sample and vapour phase

The phase ratio represents the relative amount of the sample matrix in the vial:

$$\beta = \frac{V_v}{V_s} \qquad (6)$$

where V_v is the vapour volume

V_s is the sample matrix volume

The distribution coefficient (K) describes the concentration ratio of analyte between the sample and the vapour phase (C_s/C_v). There are two factors influencing K: the vapour pressure (p^0) and the activity coefficient (γ). K can be expressed as:

$$K = \frac{1}{p^0 \cdot \gamma} \qquad (7)$$

The activity coefficient (γ) is dependent on the nature of the analyte and reflects the intermolecular interaction between the analyte and other sample components, particularly the matrix.

In ordinary static headspace, where a fixed volume is utilised, the extraction yield correlates to the analyte concentration. In headspace-trap analysis, however, a certain fraction of the vapour phase is utilised. Thus, the extraction yield correlates to the amount of analyte in the vapour phase (N_v), which can be expressed:

$$N_v = V_v \cdot C_v = V_v \cdot \frac{C_0}{V_v/V_s + K} \tag{8}$$

From equation (8), it is clear that a small value of K is advantageous for the analyte amount in the vapour phase. The easiest way to influence K is to alter the temperature, on which p^0 is highly dependent. Another common procedure is to change the activity coefficient by adding a matrix modifier. For example, addition of salt in water matrices is frequently used to lower the solubility of polar analytes.

The optimal sample matrix volume, which also decides the vapour volume, is dependent on the value of K. This is shown in Figure 1.3, where the amount of analyte in the vapour phase is given as a function of the sample volume for three compounds with various K-values. The calculations are based on a standard HS-vial volume of 22 ml, and K-values are given for the compounds in water at 60 °C. Note that the y-axis is logarithmic.

The curves clearly show that the distribution coefficient (K) has the largest influence on the analyte amount in vapour phase. It is however, also seen that the optimal sample volume decreases considerably with increasing K-value. The optimal sample volume for toluene is 10 ml, and not more than 1.5 ml for isopropanol. However, it is important to be aware of some disadvantages with a large sample matrix volume. Longer time will be needed to establish equilibrium between the sample matrix and the vapour phase, and a larger sample amount must be available. In general, the optimal conditions should always be found experimentally, but it is important to be aware of the influence of both the distribution coefficient and the sample matrix volume.

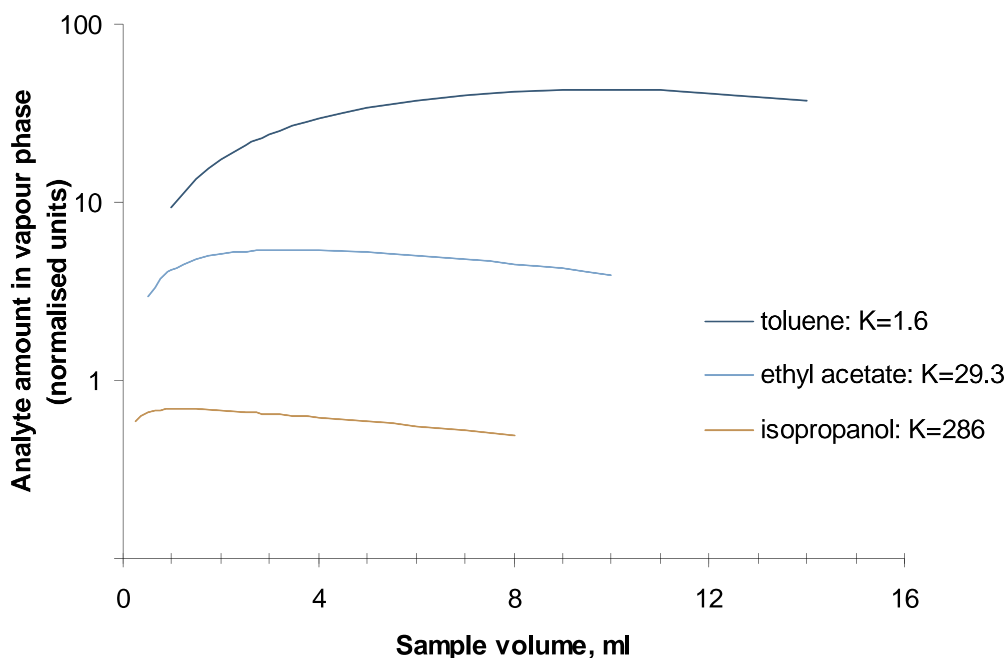


Figure 1.3 Influence of sample volume on the analyte amount in vapour phase for compounds with various distribution coefficients in water at 60 °C. The calculations are based on a vial volume of 22 ml.

1.9 Experimental design

There are often many variables to consider when performing method development in analytical chemistry. Several variables may influence each other, and should therefore not be considered independently. In such cases, experimental design is a systematic and labour saving tool to be used in the optimisation process. In the present study, two experimental design tools have been used: *factorial design*, and *simplex optimisation*.

Factorial design can be employed to check the influence of several variables, and possible interactions between them, with use of few experiments. The most common way to set up a factorial design is to choose two levels for each variable (high and low), and perform experiments with all possible combinations of the variables. For a factorial design with 3 variables, the number of experiments will be 2^3. By treating the results in a Yate's algorithm [67], it is possible to identify the effect of each variable, and if there are interactions between any of them. An example of setup and interpretation of the responses from a 2^3 factorial design is given in Appendix A.1.

If several variables influence each other, the simplex optimisation procedure can be used to find the optimal value for each of the variables [68]. The principle is to find the optimal region on a response surface by performing few experiments. An example of a basic simplex procedure for a set of two variables is given in Appendix A.2.

1.10 Aim of the study

When performing trace determination of HD and degradation products in environmental samples, large variations in analyte concentration and sample composition must be expected. The aim of this study was to develop a robust and sensitive method for trace determination of HD and some of its cyclic degradation products in water and soil, by headspace-trap GC-MS. An important issue was to develop a method with minimal need for sample cleanup or other time-consuming sample preparation procedures.

If the developed method for trace determination of HD and degradation products tourned out to be applicable for soil samples, it would also be interesting to demonstrate the method by analysing a sediment sample, collected from an old dumping site for chemical munition in Skagerrak in 2002 [13].

2 Experimental

2.1 Chemicals and equipment

Chemicals
Bis(2-chloroethyl)sulphide (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 (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 III water was delivered in-house by RIOS 30 Laboratory-Grade Water Systems from Millipore, France.

Safety regulations
All handling with neat and diluted HD was subjected to internal safety regulations for working with CWA. Storage and handling of neat HD was restricted to declared areas with limited access, according to regulations set by the Organisation for the Prohibition of Chemical Weapons (OPCW). All consumption of HD was logged for annual declarations to the OPCW.

Equipment
For transfer of neat HD, a 1 µl plunger-in-needle syringe from Hamilton (Bonaduz, Switzerland) was used. Headspace vials (22 ml), together with septa of polytetrafluoroethylene (PTFE)/silicone were delivered by Perkin Elmer instruments, CT, USA. Previous works 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. The adsorbent tube was a Tenax trap with a bed size of 2.7 x 25 mm, delivered by Perkin Elmer instruments, CT, USA.

Statistical data from factorial design experiments were treated in Minitab®, version 15.1.1.0. Presentations of the data in geometric figures were drawn in Microsoft® office Visio® Professional 2003.

2.2 Instrumentation

2.2.1 Headspace-trap system

The HS system was a TurboMatrix HS 110 Trap from Perkin Elmer. The system was controlled by an internal graphical user interface. A schematic presentation of the HS-trap technique is shown in Figure 2.1.

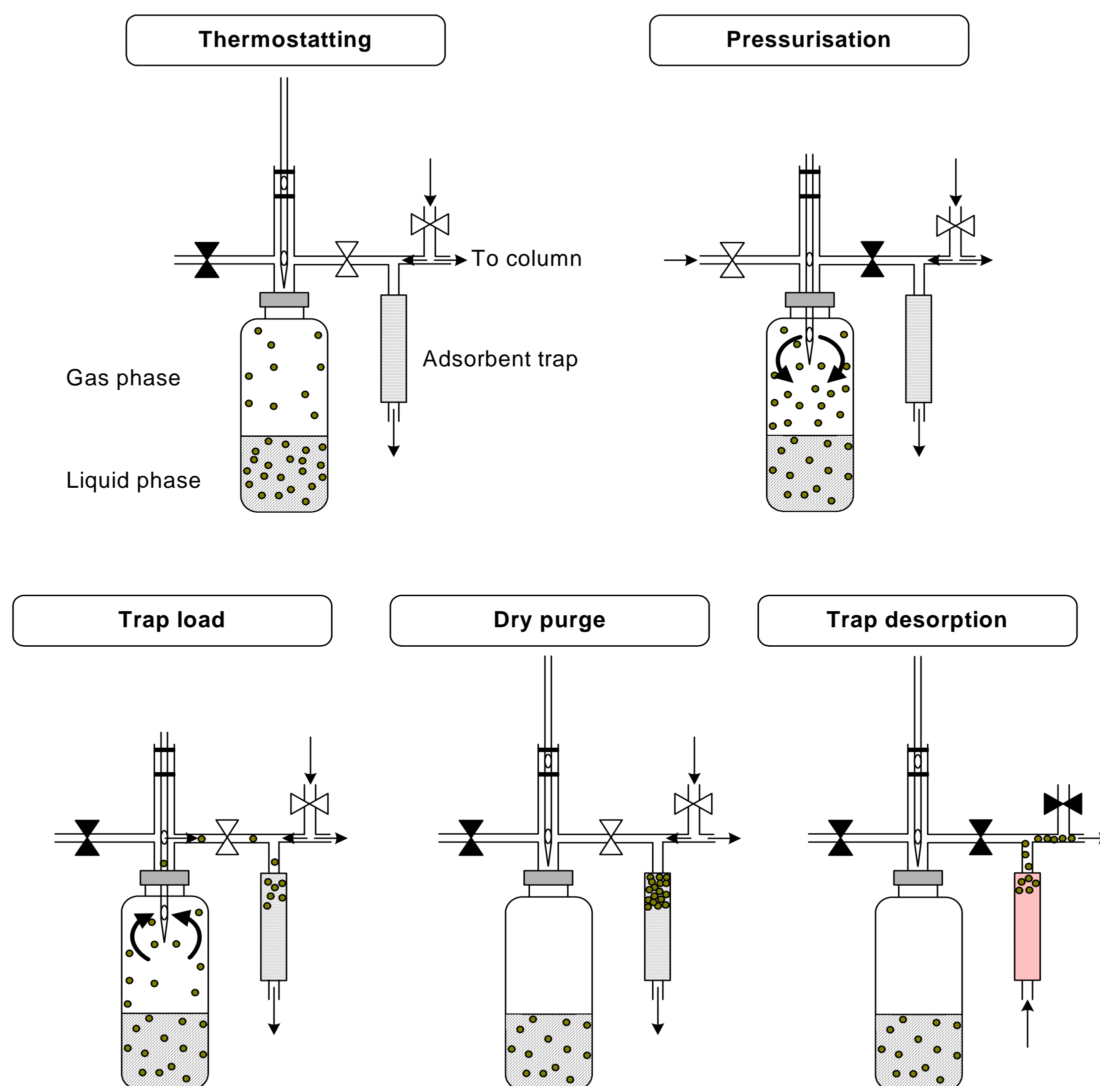


Figure 2.1 Schematic presentation of the HS-trap technique.

First, the sample was thermostatted until the analytes approached equilibrium between the sample matrix and the vapour phase (thermostatting). Thereafter, the vial was pressurised for a defined time (pressurisation). This pressure was released by leading the vapour through an adsorbent tube

where the analytes were trapped (trap load). Then, helium was purged through the adsorbent for removal of water vapour (dry purge). Finally, the trap was rapidly heated and backflushed (trap desorption). In that way the analytes were desorbed and led into the chromatographic system.

After thermostatting, the pressurisation and trap load steps can be repeated up to four times before dry purge and trap desorption are activated. This way, the sensitivity can be increased by utilising a larger amount of the vapour phase. The vapour residue after *n* cycles of pressurisation and trap load can be expressed as:

$$\text{Vapour residue}\,(\%) = 100 \cdot \left(\frac{\text{Vial final pressure}}{\text{Vial initial pressure}} \right)^{n} \tag{9}$$

The vial final pressure is equal to atmospheric pressure of 14.7 psi. With a vial initial pressure of 40 psi, the theoretical vapour residue will be 37% after one pressurisation and trap load cycle. This means that 63% of the vapour phase is utilised. After four cycles, as much as 98% of the vapour phase is utilised.

The HS-trap instrumental parameters are listed in Table 2.1. Parameter values were set at a default level, as a starting point for the method development.

Table 2.1 Instrumental parameters with values used as a starting point for the HS-trap method development

	Starting values
HS parameters	
Thermostatting temperature	80 °C
Needle temperature	90 °C
Transfer line temperature	150 °C
Thermostatting time	20 min
Pressurisation time	1.0 min
Decay (trap load) time	1.6 min
Number of cycles	1
Vial pressure	25 psi
Column pressure	15 psi
Shaker (on/off)	on
Trap parameters	
Trap low temperature	40 °C
Trap high temperature	280 °C
Dry purge time	4 min
Desorption time	0.3 min
Trap hold time	3 min
Desorption pressure	25 psi
Needle purge split flow	13 ml/min

2.2.2 Gas chromatograph – mass spectrometer

The HS-trap system was coupled to a Clarus 500 GC and a quadropole Clarus 500 MS from Perkin Elmer. Both the GC and MS were controlled by the Turbomass software, version 5.1.0. The software contains an MS library from the National Institute of Standards and Technology (NIST). The GC column was a DB-5MS from J&W Scientific, with 30 m length, 0.25 mm inner diameter and 0.25 μm film thickness. The column was coupled directly to the HS through a heated transfer line, with a constant helium inlet pressure of 15 psi. This gave a flow rate of 1 ml/min at 100 °C. 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 ionization energy was set to 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.

2.3 Preparation of solutions for water analyses

All stock solutions were prepared with acetone as solvent. Neat agents were weighed into the stock solutions with an accuracy of 0.1 mg. Due to the low viscosity of acetone, it was difficult to perform volumetric transfers with high accuracy. Hence, the transferred amounts were weighed, with an accuracy of 0.1 mg, and converted to ml using the specific density. When the transferred amounts were diluted in less than 20 ml acetone, also the diluted amounts were weighed. All solutions were stored in refrigerator (4-6 °C) when not used.

2.3.1 Stability test solutions

The three cyclic sulphur compounds were prepared in one stock solution, where 50-100 mg of each of the neat agents were diluted in 50 ml acetone. HD was handled separately as it may decompose in water into the cyclic sulphur compounds. A stock solution was made by pipetting 1.0 μl of HD into 5 ml of acetone. 1,2,4-Trimethylbenzene (1,2,4-TMB) was used as internal standard (IS) for the quantifications. A separate stock solution was made for 1,2,4-TMB, where 30 mg was diluted in 25 ml acetone. The working solutions were prepared by appropriate dilutions of the stock solutions in acetone. Concentrations of stock and working solutions are shown in Table 2.2.

Table 2.2 Solutions for stability tests of the analytes in water. Concentrations are given in μg/ml.

		1,4-thioxane	**1,3-dithiolane**	**1,4-dithiane**	**1,2,4-TMB**	**HD**
Stock solution	1	$2.08 \cdot 10^3$	$1.02 \cdot 10^3$	$1.02 \cdot 10^3$		
	2				$1.19 \cdot 10^3$	
	3					$2.55 \cdot 10^2$
Working solution	1	15.9	7.79	7.82	1.07	
	2				0.720	86.8

2.3.2 Method development solutions

A stock solution containing the three cyclic sulphur compounds was made by diluting 50-100 mg of the neat agents in 50 ml acetone. A separate stock solution of HD was prepared by pipetting 1.0 µl into approximately 4 ml acetone. The working solutions were made by further dilution of one or both of the stock solutions in acetone. Concentrations in stock and working solutions are shown in Table 2.3.

Table 2.3 Solutions used in the method development for trace determination of the analytes in water. Concentrations are given in µg/ml.

		1,4-thioxane	**1,3-dithiolane**	**1,4-dithiane**	**HD**
Stock solution	1	$2.04 \cdot 10^3$	$9.72 \cdot 10^2$	$9.88 \cdot 10^2$	
	2				$3.04 \cdot 10^2$
Working solution	3	3.50	1.67	1.69	32.1
	4	0.677	0.322	0.327	
	5	3.60	1.72	1.74	18.7
	6	18.9	8.98	9.13	90.7
	7	3.84	1.83	1.86	
	8				0.761
	9				0.647

2.3.3 Validation solutions

The solutions prepared for determination of the detection limits are given in Table 2.4. A stock solution containing the three cyclic sulphur compounds was made by diluting 30-36 mg of the neat agents in 100 ml acetone. The working solution was prepared by diluting 50 µl of the stock solution in 250 ml water. The validation solutions were prepared by further dilutions of the working solution in water. The HD stock solution was prepared by pipetting 1.0 µl of the neat agent into 8 ml acetone, followed by a 100:1 dilution in acetone. The two working solutions were prepared by further dilutions in acetone. The validation solutions were prepared by addition of 25 µl of the respective working solutions in 2.0 ml water, directly into the HS-vials.

Table 2.4 Solutions for determination of the detection limits in water. All concentrations are given in ng/ml.

		1,4-thioxane	1,3-dithiolane	1,4-dithiane	HD
Stock solution	1	$3.20 \cdot 10^5$	$3.58 \cdot 10^5$	$3.03 \cdot 10^5$	
	2				$1.59 \cdot 10^3$
Working solution		61.1	68.3	57.8	
Validation solution	1	0.0517	0.0578	0.0489	
	2	0.103	0.116	0.978	
	3	0.154	0.172	0.146	
Working solution					41.1
Validation solution	4				0.531
Working solution					79.9
Validation solution	5				0.996
	6				0.960
	7				0.930

The solutions prepared for determination of linearity, within assay repeatability and recovery from natural water samples are given in Table 2.5. A stock solution with the three cyclic sulphur compounds was made by diluting 24-42 mg of the neat agents in 100 ml acetone. A separate stock solution was made for the internal standard by diluting 200 mg 1,2,4-TMB in 100 ml acetone. Working solution 1 and 2 were prepared by diluting 100 µl of stock solution 1 in 50 ml water, and 50 µl of stock solution 2 in 250 ml water, respectively. The validation solutions were prepared by diluting various amounts of working solution 1 in 100 ml water, where each solution was added 1 ml of IS solution (working solution 2). The transferred amounts of working solutions were also weighed, with accuracy 0.1 mg. The spiking solution was used for adding the cyclic sulphur compounds into the natural water samples, and was prepared by diluting 100 µl of stock solution 1 in 50 ml water. The IS solution was prepared by diluting 50 µl of stock solution 2 in 250 ml water.

Table 2.5 Solutions for determination of linearity and repeatability in water. The spiking solution and IS solution were used in the the recovery test of natural water samples. All concentrations are given in ng/ml.

		1,4-thioxane	**1,3-dithiolane**	**1,4-dithiane**	**1,2,4-TMB**
Stock solution	1	$4.19 \cdot 10^5$	$2.41 \cdot 10^5$	$3.60 \cdot 10^5$	
	2				$2.00 \cdot 10^6$
Working solution	1	819	471	704	
	2				398
Validation solution	8	0.402	0.231	0.345	3.98
	9	4.08	2.34	3.50	3.97
	10	12.2	7.04	10.5	3.98
	11	20.5	11.8	17.6	3.99
	12	30.9	17.8	26.6	3.85
	13	40.9	23.5	35.2	3.97
Spiking solution		833	479	715	
IS solution					369

For determination of the between assay repeatability, new working solutions were made from stock solutions 1 and 2 each day. From the working solutions, new validation solutions of no 8, 11 and 13 were made.

2.4 Natural water samples

The rainwater sample was collected from a rain pool inside the area of FFI on 7th April 2008, approximately one hour after raining had stopped. The river water sample was taken from Leira on 11th April 2008, close to the bridge where the road Fetveien crosses the river. At this time of year, the river has a high content of mud, and the total residue on evaporation was determined to $(2.7 \pm 0.3) \cdot 10^2$ mg/l (n=3). The seawater sample was collected at "Aker brygge" situated in Oslo harbour, on 8th April 2008. The sample was collected some centimeters below the surface, to avoid contamination from the water surface. All samples were kept in borosilicate glass 3.3 (Duran) bottles with screw caps having Teflon gaskets (Schott, Mainz, Germany), and stored in a refrigerator at 2-6 °C.

2.5 Soil samples

Two soil types were used in the method development for trace determination of the analytes. Soil A was a standard soil purchased from LUFA Speyer in Germany, characterised and sieved with a 2 mm screen. Soil B was collected inside the area of FFI, pulverised and sieved to a grain size of 2 mm. Measurements for characterisation of soil B were performed by the Norwegian Center for Soil and Environmental Research (Ås, Norway). Both soil samples were dried in nitrogen atmosphere at 50 °C for 24 hours prior to use. The particle size distribution, pH values and TOC of the soils are listed in Table 2.6. Classification of the soil types is given according to the United States Department of Agriculture (USDA) [55].

Table 2.6 Parameters for the soil types used in the method development for trace determination of analytes in soil.

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

2.5.1 Sample preparation

Each soil sample was weighed directly into the HS-vial. The analytes were added to the soil solved in acetone, at an amount of 40 µl per g soil. Both the soil and the amount of spiking solution were weighed with an accuracy of 0.1 mg. The vial was immediately capped and homogenised on a whirlmixer for one minute, after which it was stored in a refrigerator at 4-6 °C for one hour. Then, the vial was decapped and vented for three min at room temperature (22-25 °C). Slurry samples were prepared by adding NaCl saturated type III water. The vial was capped, and sample thermostatting was initialised within one minute after water addition.

2.5.2 Spiking solutions

The stock, working and spiking solutions were prepared with acetone as solvent. All volumetric transferred fractions were weighed (accuracy of 0.1 mg), due to the difficulty of pipetting acetone with high accuracy.

Concentrations of the stock, working and spiking solutions for method development are shown in Table 2.7. Stock solution 1 was identical to the stock solution for preparation of validation solutions in Table 2.5. The HD stock solution was prepared by pipetting 1.0 µl of the neat agent into 8 ml acetone. Spiking solution 1 was prepared by appropriate dilutions of the stock solutions, via a working solution. Spiking solution 2 was prepared by further dilution of spiking solution 1.

Table 2.7 Solutions prepared for method development for determination of the analytes in soil samples. All concentrations are given in µg/ml.

	1,4-thioxane	**1,3-dithiolane**	**1,4-dithiane**	**HD**
Stock solution 1	419	241	360	
Stock solution 2				157
Working solution 1	9.70	5.58	8.33	51.3
Spiking solution 1	1.07	0.616	0.921	5.68
Spiking solution 2				0.522

Concentrations of the validation solutions for determination of the detection limits in soil are shown in Table 2.8. The validation solutions for determination of linearity, repeatability, recovery and robustness are shown in Table 2.9. Validation solution 14 and the IS working solution were prepared with water as solvent. The other validation solutions were prepared with acetone as solvent.

Table 2.8 Solutions for determination of the detection limits in soil. All concentrations are given in ng/ml.

		1,4-thioxane	**1,3-dithiolane**	**1,4-dithiane**	**HD**
Stock solution 1		$4.81 \cdot 10^5$	$5.20 \cdot 10^5$	$4.97 \cdot 10^5$	
Stock solution 2					$1.28 \cdot 10^5$
Working solution		$1.95 \cdot 10^3$	$2.11 \cdot 10^3$	$2.02 \cdot 10^3$	
Validation solution	1	5.77	6.24	5.96	
	2	9.20	9.95	9.51	
	3	17.6	19.0	18.2	
	4	27.8	30.0	28.7	
Working solution					$2.83 \cdot 10^3$
Validation solution	5				2.52
	6				5.11
	7				7.56

Table 2.9 Solutions for determination of linearity, repeatability, recovery and robustness in soil. All concentrations are given in µg/ml.

		1,4-thioxane	**1,3-dithiolane**	**1,4-dithiane**	**1,2,4-TMB**	**HD**
Stock solution 1		$3.55 \cdot 10^2$	$1.00 \cdot 10^3$	$4.97 \cdot 10^2$		
Stock solution 2					$2.01 \cdot 10^3$	
Stock solution 3						$3.18 \cdot 10^2$
Working solution					0.4[3]	
Validation solution	8	0.0178	0.0501	0.0249		
	9	0.184	0.520	0.258		
	10	0.535	1.51	0.749		
	11	0.870	2.45	1.22		
	12	1.33	3.74	1.86		
	13	1.78	5.03	2.50		
	14	0.916	2.58	1.28		
Validation solution	15					0.225
	16					2.30
	17					6.77
	18					11.2
	19					17.1
	20					22.3
	21					90.5

3 Results and discussion

3.1 Trace determination of CWA in water

In this section, the method development for trace determination of HD and the cyclic degradation products in water are reported. A complete method validation has been performed for trace determination of the degradation products in water. In addition, recovery tests from three types of natural water samples are reported.

3.1.1 Stability of the compounds in water

Before starting the method development, the stability of the compounds in water was investigated within the expected time range from sample preparation to analysis. Some preliminary analyses showed that the addition of salt was favourable for the extraction yield. Therefore, the water samples were saturated with NaCl before the compounds were added. The stability of the three cyclic sulphur compounds was investigated by analyses performed from 0 to 40 hours after preparation in water. Samples analysed within 6 hours after preparation were stored in the HS autosampler at room temperature. Samples stored for more than 6 hours were kept in an incubator

[3] New working solutions were made each day for 1,2,4-TMB in water at approximately 0.4 µg/ml

at 30 °C. Figure 3.1 shows the peak areas relative to the peak areas of the IS, plotted as a function of time after preparation in water. Raw data are given in Table B.1 in Appendix.

Stability of HD in salt water was investigated by analysis immediately after preparation, and with successive analyses for approximately 4 hours. Peak areas relative to IS are presented in Figure 3.2, as a function of time after preparation. All samples were stored in the HS autosampler at room temperature until the start of analysis. Raw data are given in Table B.2 in Appendix.

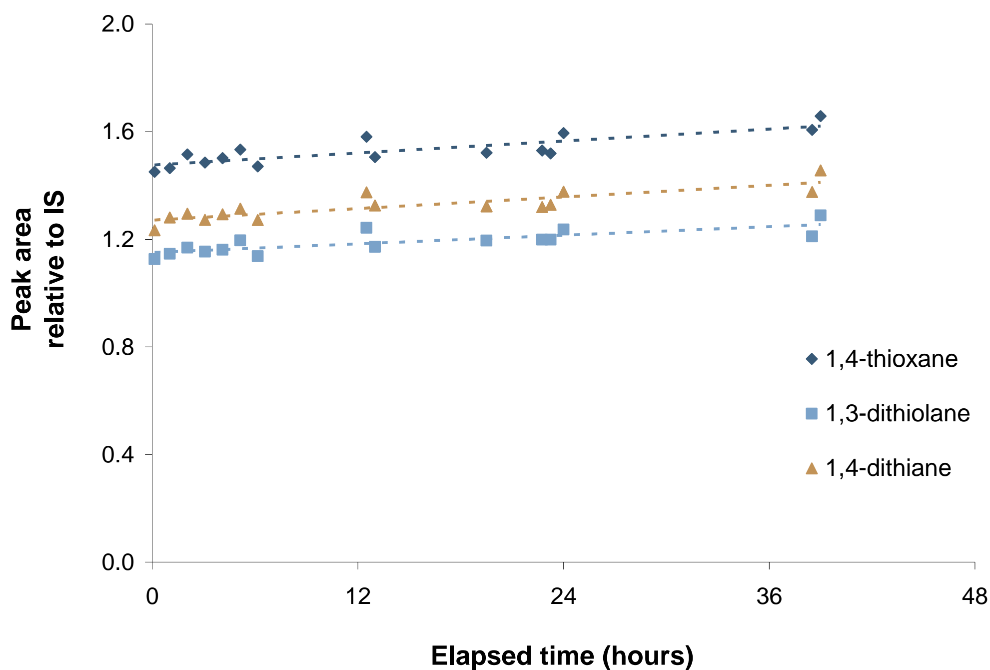


Figure 3.1 Relative peak areas of the cyclic sulphur compounds in salt saturated water, presented as a function of time after preparation.

Each sample was prepared directly in HS vials by diluting 25 µl of working solution 1 (Table 2.2) in 2.00 ml water, saturated with 0.80 g NaCl. The resulting concentrations were 0.20 µg/ml for 1,4-thioxane, and 0.10 µg/ml for both 1,3 dithiolane and 1,4 dithiane.

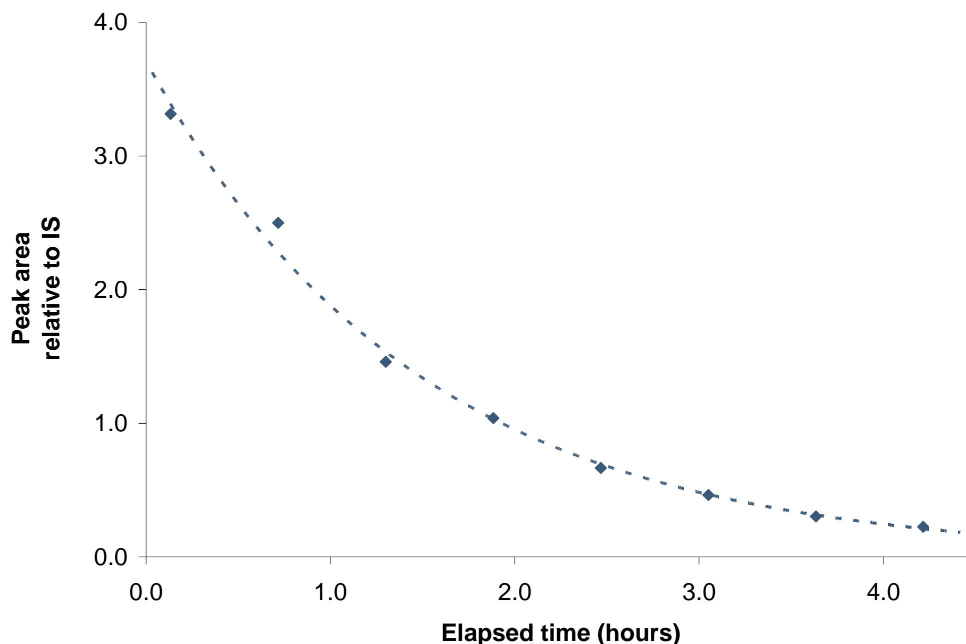


Figure 3.2 Relative peak areas of HD in salt saturated water, presented as a function of time after preparation.

The start concentration was 1.1 µg/ml. Samples were prepared by diluting 25 µl of working solution 2 (Table 2.2) in 2.00 ml of water, saturated with 0.80 g NaCl. The instrumental conditions were as listed in Table 2.1, except from the thermostatting temperature and needle temperature, which were set to 50 °C and 60 °C, respectively.

All three cyclic sulphur compounds showed a slight increase in peak area relative to IS, indicating that 1,2,4-TMB was less stable in water. Since 1,2,4-TMB does not react with water, this could be due to adsorption on the vial surface, or to septa. However, the peak areas of the cyclic sulphur compounds relative to each other were constant. This shows that the cyclic sulphur compounds were stable in water at temperatures below 30 °C, within the time range of interest. As expected, HD decomposed rapidly in water, with a half-life of approximately 60 min. This is in agreement with Hoenig, who reported a half-life of 60 min of HD in salt water at 25 °C [16]. Because of the rapid degradation, all samples containing HD were prepared immediately prior to analysis.

3.1.2 Effect of salt addition

As discussed in Section 1.8.3, the activity coefficient of the analytes can be altered by adding salt to aqueous sample matrices. The technique (commonly called ”salting out”) has shown to be especially effective for determination of polar compounds in water [63][4]. It is important to saturate the sample to maximise the effect of the salt and also to avoid variations in salt concentrations from sample to sample, which may affect the repeatability of the analyses [62]. The salting out effect was investigated for HD and the cyclic sulphur compounds by comparing the extraction yields between samples with no salt added, and samples saturated with NaCl. Figure 3.3 shows the extraction yields for each compound with and without salt added, as the

[4] Chapter 2, page 30

average of three replicates ± one standard deviation (SD). Peak areas of all replicates are given in Table B.3 in Appendix.

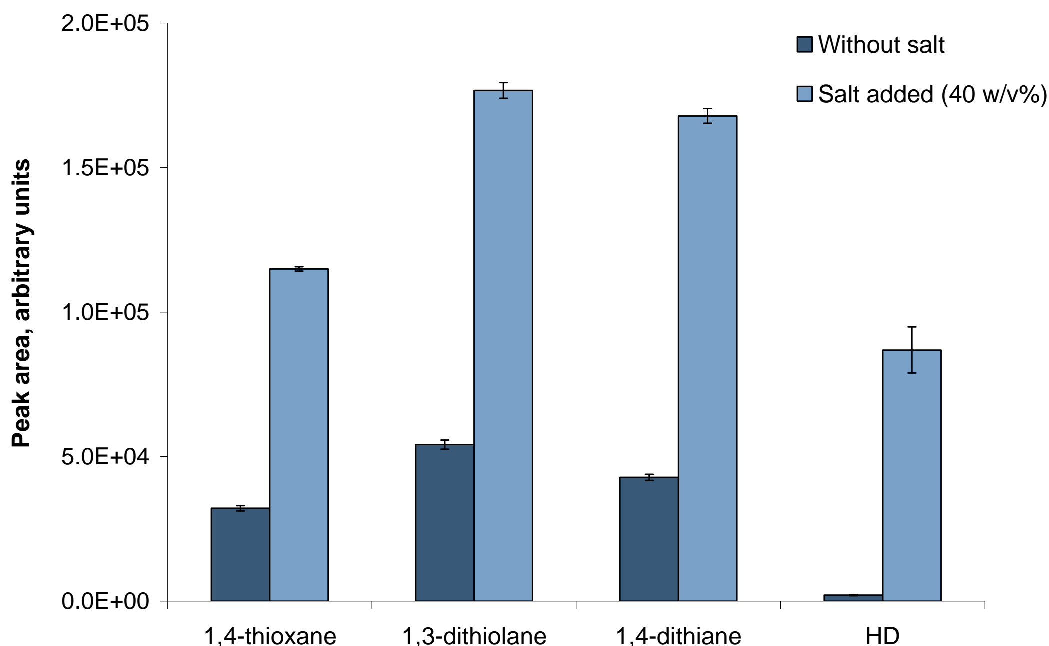


Figure 3.3 Comparison of extraction yields between water samples with no salt added, and samples saturated with NaCl. The columns represent the average peak area of three replicates, and the error bars indicate ± one SD.

Each sample was prepared by adding 25 µl of working solution 3 (Table 2.3) into 2.00 ml of water, obtaining concentrations of 0.02-0.04 µg/ml for the cyclic sulphur compounds and 0.4 µg/ml for HD. The salt saturated samples were obtained by adding 0.80 g of NaCl into the vials. Each sample was prepared 2-3 min prior to analysis. The thermostatting temperature and needle temperature were 60 °C and 70 °C, respectively. The other instrumental conditions were as listed in Table 2.1.

The salt saturation showed to give a great improvement in recovery of all analytes, and in particular of HD. The recoveries were three to four times higher for the cyclic sulphur compounds, and approximately forty times higher for HD. According to the theory presented in Section 1.8.3, HD should actually be less affected by salt addition, due to its low water solubility. Thus, the main reason for the large effect was probably a considerable decrease in degradation of HD during thermostatting, as a result of the high salt content [69]. Due to the significantly positive effect on all the analytes, all further determinations of the compounds in water were performed with salt saturated solutions.

3.1.3 Trap settings

The adsorbing material in the trap was Tenax, which is a porous polymer resin based on 2,6-diphenyl oxide. Tenax is widely used for trapping volatiles and semi-volatiles from air, and in purge and trap devices. It has low water affinity, which makes it especially useful for purging and trapping of organic volatiles from water. The highest recommendable temperature for the Tenax

material during desorption is 280 °C. To avoid carry-over between samples, the analytes must be completely desorbed during trap desorption. Possible carry-over was checked with analyses performed at a trap high temperature of 280 °C, and desorption time of 3 min. No carry-over of analytes was observed when blank samples (salt saturated solutions) were analysed subsequent to water samples with the analytes (data not shown).

The trap parameters should be set to give optimum transfer of the analytes into the GC column, and to ensure an efficient removal of water from the trap. The sample transfer efficiency from the trap into the GC column is dependent on the desorption pressure and the desorption time. The water removal efficiency is influenced by the dry purge time, trap low temperature and desorption pressure. It is possible to maximise the sample transfer efficiency by closing the needle purge split flow during trap desorption. However, this gives larger background signal and broader chromatographic peaks, especially for the early eluting compounds. Therefore, it is not recommended by the manufacturer. Instead, the introduced sample amount is optimised by adjusting the desorption pressure and desorption time.

The peak areas of the three cyclic sulphur compounds were measured at various desorption pressure and desorption time. HD was not included in these experiments, as the effect of various settings should be independent of the analytes. The needle purge split flow was set to 13 ml/min (recommended 10-15 ml/min). Figure 3.4 shows the peak area of 1,3-dithiolane as a function of desorption time, at a desorption pressure of 25 psi. Only slight changes in the response were observed at desorption times from 0.3 to 0.7 min. However, an abrupt decrease in peak area was observed when the desorption time was decreased from 0.3 to 0.2 min.

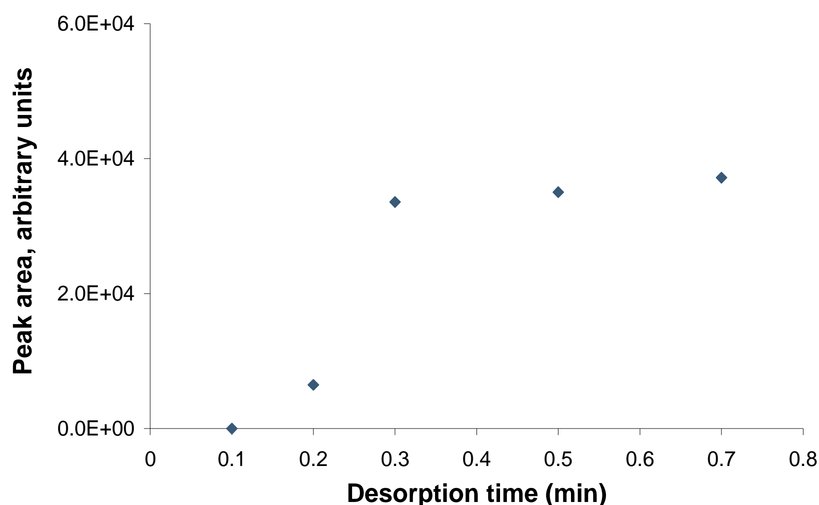


Figure 3.4 Peak area of the m/z 106 ion (molecular ion) of 1,3-dithiolane (4 ng/ml), as a function of desorption time. Desorption pressure was 25 psi.

Sample solutions were obtained by adding 25 µl of working solution 4 (Table 2.3) into 2.00 ml NaCl saturated water solutions.

Figure 3.5 shows a surface diagram for the peak area of 1,3-dithiolane where both the desorption pressure and desorption time were altered. Raw data are given in Table B.4 in Appendix.

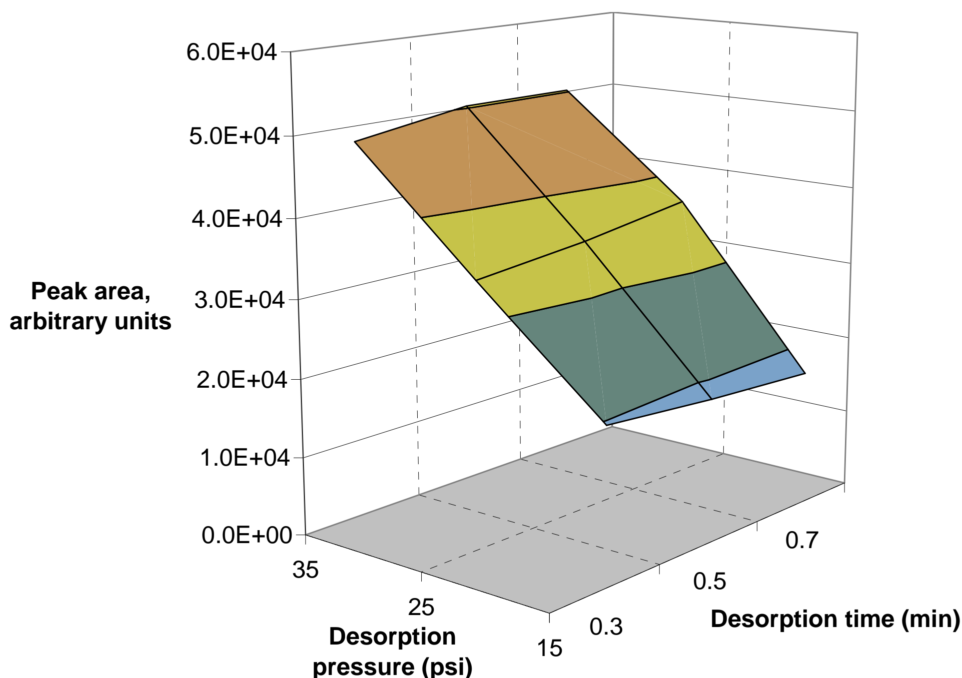


Figure 3.5 Surface diagram of the peak area of the m/z 106 ion of 1,3-dithiolane (4 ng/ml). Desorption pressure was set to 15, 25 and 35 psi, and desorption time was set to 0.3, 0.5 and 0.7 min. One analysis was performed at each setting.

Sample solutions were obtained by adding 25 µl of working solution 4 (Table 2.3) into 2.00 ml NaCl saturated water solutions.

The diagram shows a relatively plane surface, where the peak area increased linearly with increasing desorption pressure from 15 to 35 psi. The desorption time (0.3 to 0.7 min) did not influence the response significantly.

Figure 3.5 clearly shows that a high desorption pressure is desirable to obtain a high recovery. However, the desorption pressure is also activated during trap dry purge, and a high pressure gives a less effective water removal. This is illustrated in Figure 3.6, where the *m/z* 18 ion of water is extracted from the chromatogram of three analyses performed at variable desorption pressure. A considerable improvement in the water removal was seen when the desorption pressure was decreased from 35 to 25 psi. Less difference was observed when the pressure was further decreased.

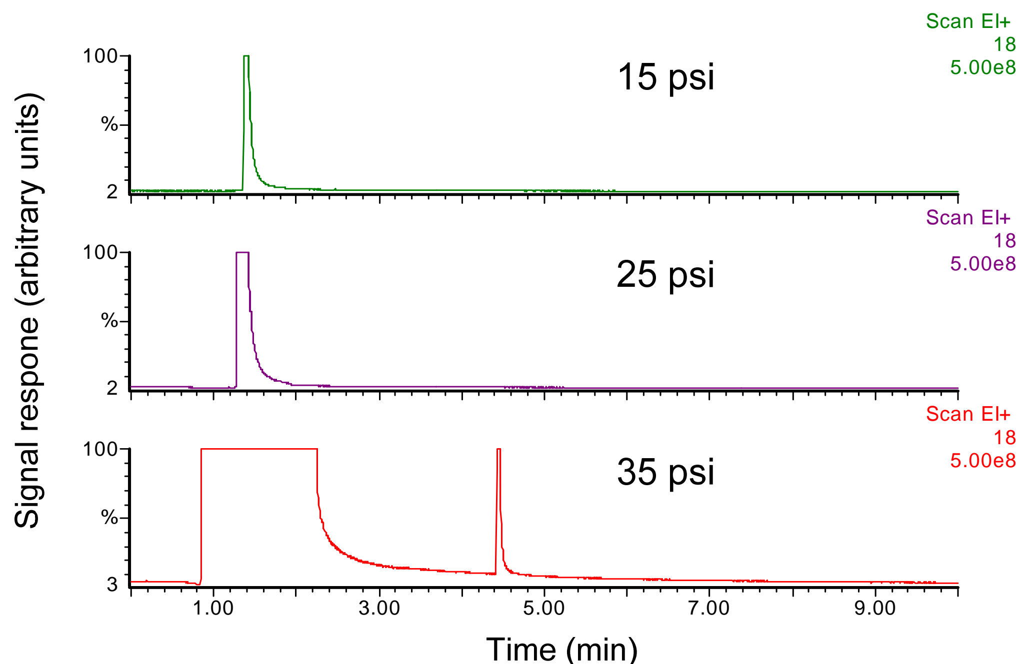


Figure 3.6 Signal intensity of the m/z 18 ion of water, at desorption pressures of 15, 25 and 35 psi. Desorption time was 0.5 min, trap low temperature was 40 °C and dry purge time was 4 min. The MS scan range was set to m/z 15-300.

Introduction of large amounts of water into the chromatographic system will result in peak broadening and can be harmful to the column at elevated temperatures. It will also affect the signal intensity, due to collisions between electrons and water molecules. Thus, an effective drying step is important to achieve high accuracy and repeatability. However, minor water amounts are not harmful to the system, as seen in the upper chromatogram (15 psi desorption pressure) in Figure 3.6.

A factorial design at two levels was set up to examine the efficiency of the water removal with various settings of the dry purge time, trap low temperature and desorption pressure. The HS vials contained 2.00 ml salt saturated solutions. The amount of water introduced on the column was measured as the peak area of the extracted *m/z* 18 ion. The high and low values for each parameter, and the peak areas from each analysis are shown in Figure 3.7. Three replicate analyses were also performed at the intermediate values of the parameters. The average peak area ± one SD is given in the middle of the geometric figure.

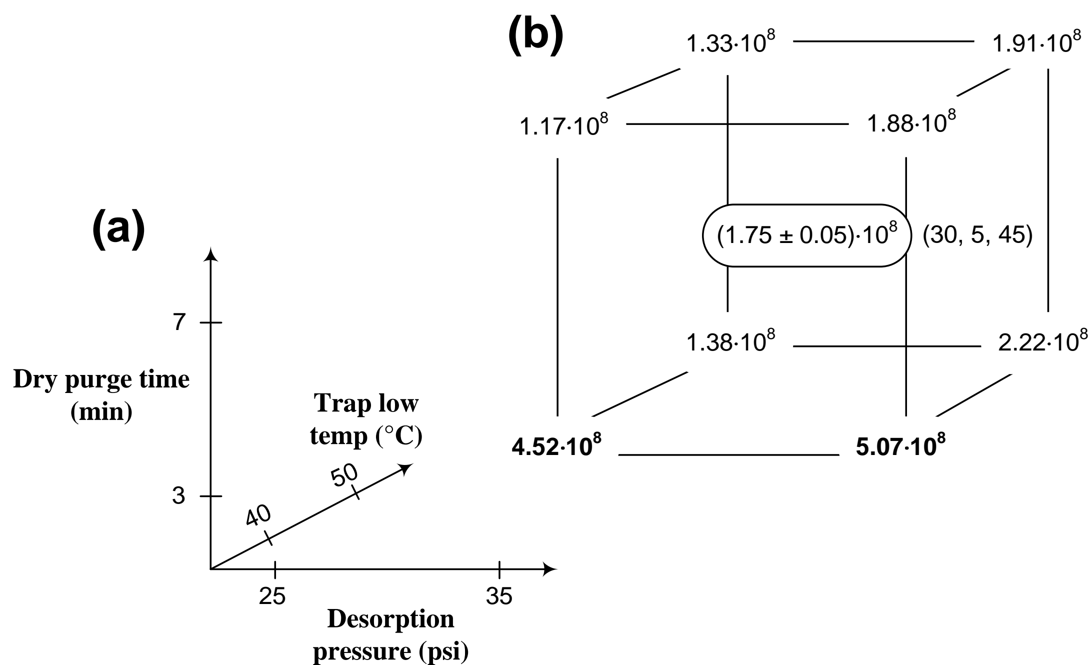


Figure 3.7 Two-level factorial design experiment for the desorption pressure, dry purge time and trap low temperature. The high and low values are given on the axes in the figure (a). The numbers in the geometric figure (b) represent the peak areas of the extracted m/z 18 ion of water.

A lower desorption pressure was favourable for the water removal step, independent of the other parameters. A higher trap low temperature had a positive effect on the water removal at 3 min dry purge time, but had no significant effect at 7 min dry purge time. Likewise, a longer dry purge time had a positive effect at 40 °C trap low temperature, but no significant effect at 50 °C. Complete water removal was not achieved for any of the analyses. However, all values are acceptable, except for those achieved with trap low temperature of 40 °C at 3 min dry purge time (in bold).

Hence, a compromise was made for the trap conditions, where both an effective sample transfer and a reasonable effective water removal from the trap were ensured. For the following analyses, the desorption pressure was set to 30 psi, desorption time to 0.5 min, trap low temperature to 40 °C, and dry purge time to 5 min. However, the conditions needed for an acceptable water removal is highly dependent on the water amount introduced on the adsorbent. For that reason, the dry purge parameters must be reconsidered if the HS parameters are altered considerably from those given in Table 2.1.

3.1.4 Headspace analysis conditions

In this section, the investigations of sample volume, thermostatting temperature, thermostatting time, vial pressure and sequential extractions are presented. As a starting point, the HS parameters were set at the default level given in Table 2.1. During the optimisation prosess, the tested parameters were continuously changed to the new preferred values before continuing the work.

The HS parameters with less influence on the sensitivity were set as recommended from the manufacturer, or adjusted according to the optimised parameters. The needle temperature is usually set 10-20 °C higher than the thermostatting temperature, in order to avoid condensation on the needle surfaces during trap load. The temperature difference should not be too high, as this could disturb temperature equilibrium when the needle is introduced in the headspace during pressurisation [63][5]. 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 pressurisation time should be set long enough to assure homogenisation between the incoming gas and the air in the headspace, before trap load is activated [63][e]. In the present study, the pressurisation time was set to 1 min. The decay time (trap load) must be long enough to just allow the vial pressure to be decreased to atmospheric pressure. The decay time is dependent on vial pressure setting and sample matrix volume, and is calibrated with a blank sample, where the vial pressure is monitored during trap load.

Shaking of the sample helps to shorten the time needed to establish equilibrium between the sample and vapour phase. It has been shown that sample agitation also improves precision when analysing soil/water slurry samples [70]. In the present study, shaking was activated in all experiments.

Sample volume, thermostatting temperature and thermostatting time
Among the conditions of most importance to the sensitivity in HS analysis are the sample volume, thermostatting temperature and thermostatting time. A two-level factorial design experiment was performed for the three parameters, as shown in Figure 3.8. Because of the low stability of HD in water, each sample was prepared immediately prior to analysis. Figure 3.8 shows the peak areas of the molecular ions of 1,3-dithiolane and HD, presented in geometric figures. Three replicate analyses were also performed with intermediate values for the parameters, and the average peak area ± one SD is given in the middle of the geometric figures. A complete table with peak areas of all compounds is given in Table B.5 in Appendix.

The cyclic sulphur compounds showed very similar behavior in response, hence the figures of 1,3-dithiolane represents the general trends for all of them. A thermostatting temperature of 80 °C gave considerably higher recoveries for the cyclic sulphur compounds, while an increase of the sample volume affected the peak areas in a negative way. An increase in the thermostatting time from 15 to 30 min did not affect the peak areas, which indicated that equilibrium was achieved already after 15 min.

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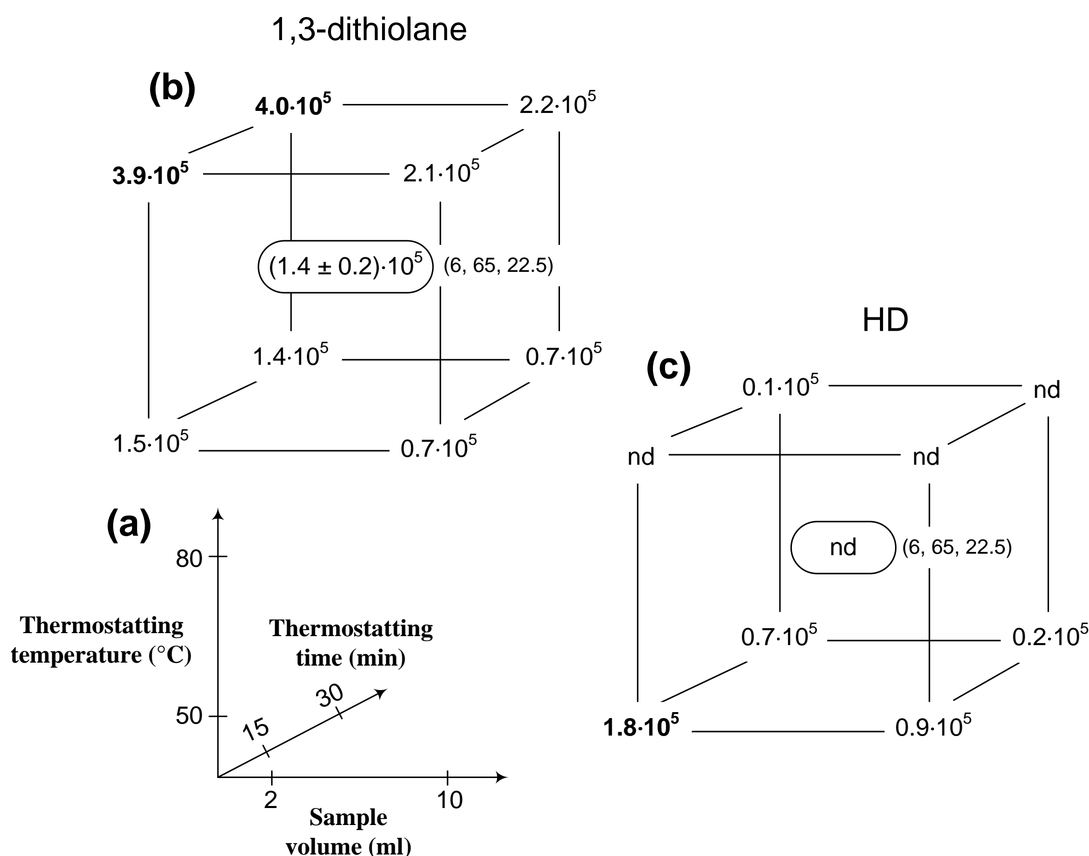

Figure 3.8 Two-level factorial design experiment for the sample volume, thermostatting temperature and thermostatting time. The high and low values are given on the axes (a). Peak areas from each experiment are presented in geometric figures for the m/z 106 ion of 1,3-dithiolane (b) and the m/z 158 ion of HD (c).

The sample solutions were prepared by adding 25 µl of working solution 5 (Table 2.3) into each of the 2.00 ml samples, and 25 µl of working solution 6 into each of the 10.0 ml samples. This gave concentrations of 22-47 ng/ml for the cyclic sulphur compounds, and approximately 230 ng/ml for HD.

For HD, the recovery varied in a quite different way. Only one of the analyses performed at 80 °C showed detectable peak area of HD. Considerably higher peak areas were achieved at 50 °C, but the recoveries decreased with increased thermostatting time. This was probably due to degradation of HD in the vapour phase during thermostatting. Because of the different behaviour of HD relative to the cyclic sulphur compounds, the approach of developing one method for determination of all analytes was abandoned. Thus, further optimisation of the conditions for HD determination was performed separately.

The sample volume, thermostatting temperature and thermostatting time were further investigated for the cyclic sulphur compounds by performing three analysis series. Even though the 2 ml samples gave higher recoveries than the 10 ml samples, the optimal sample volume could be between these values (see Section 1.8.3). Two sample series were therefore analysed with sample volumes of 2 ml and 4 ml water, respectively, and with a thermostatting temperature of 80 °C. The samples were analysed with increasing thermostatting time, from 2.5 to 20 min. A third

sample series was performed with a thermostatting temperature of 90 °C, and with a sample volume of 2 ml. Four samples were analysed at thermostatting times from 5 to 20 min. Since the cyclic sulphur compounds showed to be stable in water for at least 40 hours, a single solution could be made for preparation of all samples. The peak areas of 1,4-thioxane from the three analysis series are presented in Figure 3.9, as a function of the thermostatting time. Complete raw data for all compounds are given in Table B.6 in Appendix.

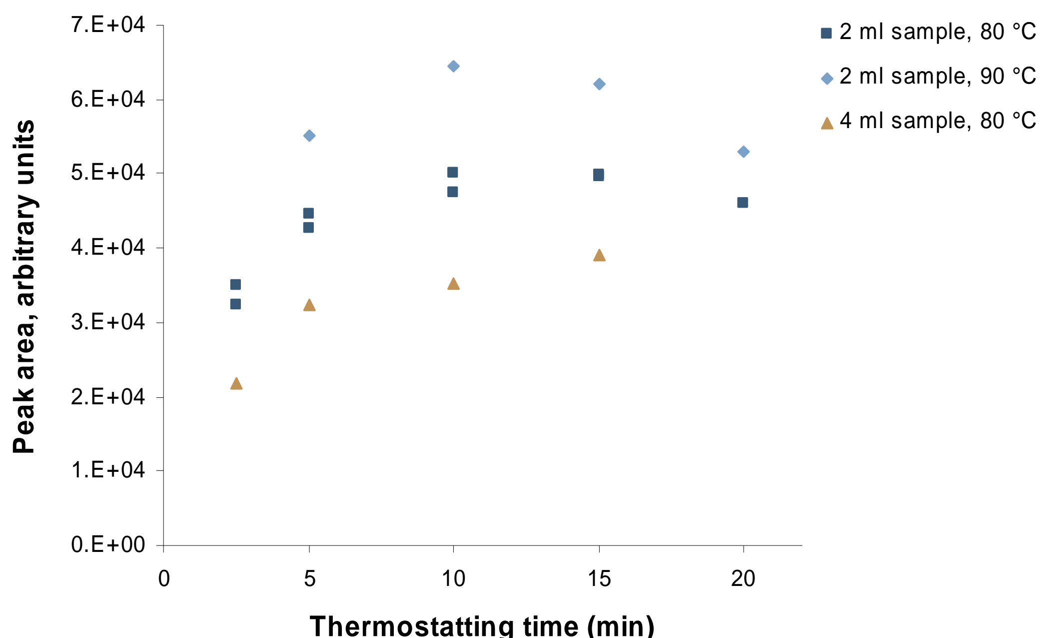

Figure 3.9 Peak areas of the m/z 104 molecular ion of 1,4-thioxane from three analysis series, as a function of thermostatting time, analysed with various sample volume and thermostatting temperature.

The sample solution was prepared by diluting 100 µl of working solution 7 (Table 2.3) in 50 ml water, obtaining concentrations of 8 ng/ml for 1,4-thioxane, and 4 ng/ml for 1,3-dithiolane and 1,4-dithiane. Samples of 2.00 and 4.00 ml were transferred to the HS vials and saturated with NaCl.

For the 2 ml samples analysed at 80 °C, equilibrium between the water phase and headspace was reached after 10-15 min. Recoveries from the 4 ml samples were lower compared to the 2 ml samples at the investigated thermostatting times. Thus, no further analyses were performed with 4 ml samples. The peak areas of 1,4-thioxane were highest at a thermostatting temperature of 90 °C, but a decreasing trend were observed when thermostatting for more than 10 min. For 1,3-dithiolane and 1,4-dithiane, little or no increase in peak areas was observed at a thermostatting temperature of 90 °C compared to 80 °C. In addition, higher variations in peak area were observed compared to the analyses at 80 °C (Table B.6).

Based on these experiments, the analysis conditions for the cyclic sulphur compounds were set to a sample volume of 2 ml, thermostatting temperature of 80 °C and thermostatting time of 15 min.

Effect of vial pressure

The vial pressure affects how efficiently the vapour phase is transferred from the vial headspace to the trap. A higher vial pressure improves the sample transfer, but it also increases the risk of leakage between the septum and the vial, or in the septum puncture at the needle. The effect of vial pressure was investigated at 30 psi and 45 psi, where the measured peak areas and standard deviations were compared. Table 3.1 shows the peak areas from three replicate analyses at each vial pressure setting.

Table 3.1 Peak areas of the molecular ions, m/z 104, 106 and 120 of 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane, respectively, at two different vial pressures.

Vial pressure	**Replicates**	**Peak area (10^4)**		
psi		**1,4-thioxane**	**1,3-dithiolane**	**1,4-dithiane**
30	1	4.57	5.32	5.56
	2	4.65	5.64	5.72
	3	4.79	5.64	5.92
	Mean	4.67	5.53	5.73
	SD	0.11	0.18	0.18
45	1	5.47	6.45	6.81
	2	5.59	6.70	6.81
	3	5.73	6.70	7.04
	Mean	5.60	6.62	6.89
	SD	0.13	0.14	0.14

The sample solution was prepared by diluting 100 µl of working solution 7 (Table 2.3) in 50 ml water, obtaining concentrations of 8 ng/ml for 1,4-thioxane, and 4 ng/ml for 1,3-dithiolane and 1,4-dithiane. Samples of 2.00 ml were transferred to the HS vials and saturated with NaCl.

The peak areas were approximately 20% higher for the analyses performed with a vial pressure of 45 psi. No increase in standard deviations was observed with the higher pressure, which indicates that no vial leakage occurred. Since the effect of a high vial pressure is limited and it is important to ensure that no vial leakage occurs, a vial pressure of 40 psi was chosen for the method.

Sequential extractions

As discussed in Section 2.2.1, it is possible to repeat the pressurisation and trap load steps up to four times, in order to achieve a more complete vapour extraction from the vial. Since the use of several extractions also introduces more water vapour onto the trap, the drying step had to be reconsidered. The desorption pressure was kept at 30 psi, while the trap low temperature and dry purge time were investigated. Figure B.1 in Appendix shows that a dry purge time of 7 min and a trap low temperature of 50 °C was necessary to achieve an acceptable water removal. Generally, the trap low temperature should be as low as possible, to prevent loss of analyte during the trap drying step. To ensure that no loss of analytes occurred using the new trap settings, extraction yields were compared with those at the former settings. Four replicate analyses were performed at each setting. The peak areas of the molecular ions of the three cyclic sulphur compounds are given in Table B.7 in Appendix. No significant decrease in peak areas was observed when the trap trap drying conditions was changed (t-test at $\alpha=0.05$), and the standard deviations were overall low at both settings. Hence, no loss of analytes occurred at a trap low temperature of 50 °C and a dry purge time of 7 min.

The effect of sequential extractions was investigated by comparing the peak areas from analyses with one, two and three successive vial extractions. Figure 3.10 presents the average peak areas from each experiment. Raw data are given in Table B.8 in Appendix.

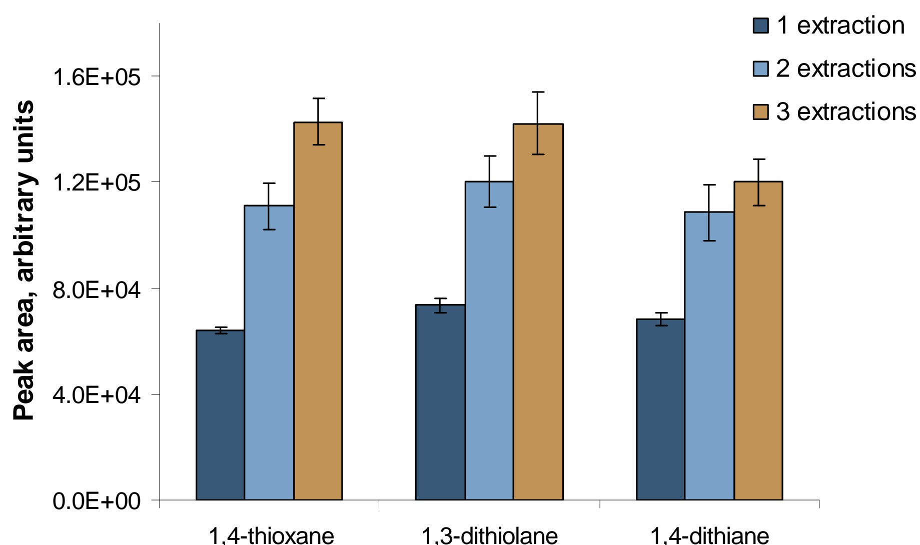

Figure 3.10 Peak areas of the molecular ions, m/z 104, 106 and 120 of 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane respectively, with one, two and three successive extractions from the HS vial. The error bars represent ± one SD of four replicates.

The sample solution was prepared by diluting 100 µl of working solution 7 (Table 2.3) in 50 ml water, resulting in concentrations of 8 ng/ml for 1,4-thioxane, and 4 ng/ml for 1,3-dithiolane and 1,4-dithiane. Aliquots of 2.00 ml were transferred to the HS vials and saturated with NaCl.

The average peak areas increased whith 59-74% when two vial extractions were applied. With a third extraction, the additional increase were 11% for 1,4-dithiane, 18% for 1,3-dithiolane and 29% for 1,4-thioxane. According to equation (9) in Section 2.2.1, the recovery should not increase by more than 37% with a second extraction, and additional 10% with a third one. The reason for the discrepancy is probably because the theoretical model assumes no analyte transfers between the sample and vapour phase during the pressurisation and trap load steps. However, further transfer of the analytes into the headspace should be expected, as the system was no longer in equilibrium after the first vial extraction. The standard deviations were also higher with use of two and three extractions, compared to one. This was probably because the second and third trap load steps were activated before new equilibriums were established in the HS-vial.

Since the aim of the investigation was to develop a method for trace determination of the cyclic sulphur compounds, the sensitivity was of higher interest than the accuracy and precision. Thus, the higher variatons in peak area with sequential extractions were not considered to be critical. However, each successive extraction led to a prolonged analysis time, and also introduced larger amount of water vapour on the trap. All factors considered, a procedure with two vial extractions was preferred.

Repeated vial thermostattings

The original purpose of sequential extractions is to utilise a larger fraction of the vapour phase with one vial thermostatting. However, if the pressurisation time was set long enough, the vial should be thermostatted a second time until equilibrium was re-established. Figure 3.9 shows that more than 80% of the extracted amount at equilibrium was in the vapour phase after 5 min thermostatting. If this relationship recurs in subsequent extractions, the overall extracted amount should be higher with repeated vial thermostattings at 5-10 min compared to one thermostatting until equilibrium. This possibility was investigated by setting a thermostatting time of 2 min and a pressurisation time of 8 min, when two vial extractions was applied. The time of the first thermostatting was then the sum of the thermostatting and pressurisation time, of 10 min. The time for a second thermostatting was equal to the pressurisation time of 8 min.

However, during the analyses an unexpected consequence from the use of longer pressurisation time was observed. The intensity of the chromatographic background signal increased by a factor varying from one to two orders of magnitude, and large fluctuations in the signal were observed, including sudden falls and rises. The source of the background signal showed to be the *m/z* ratios typical for acetone, in which the analytes were dissolved before they were diluted in the water samples. The instrument manufacturer had no experience from the use of this long pressurisation time. A proposed explantion was that somehow, the vapour phase in the sample vial had diffused into the chromatographic system during pressurisation. No further investigations were made on the appearance of the background. Regardless of the reason, the consequence was that the system could not be operated with pressurisation times long enough to re-establish equilibrium in the vial. Thus, the HS was operated with one thermostatting until equilibrium, followed by pressurisation for 1 min, and with two vial extractions.

Optimisation of analytical condititons for sulphur mustard

From the experimental design presented in Figure 3.8, the highest extraction yield of HD was achieved with a thermostatting temperature of 50 °C at 15 min thermostatting time, and with a sample volume of 2 ml. A simplex optimisation procedure (see Section 1.9) was performed for the further investigation of the thermostatting temperature and time, whith a sample volume of 2 ml. The results are presented in Figure 3.11, where the values noted at each point show the peak area of the *m/z* 158 ion.

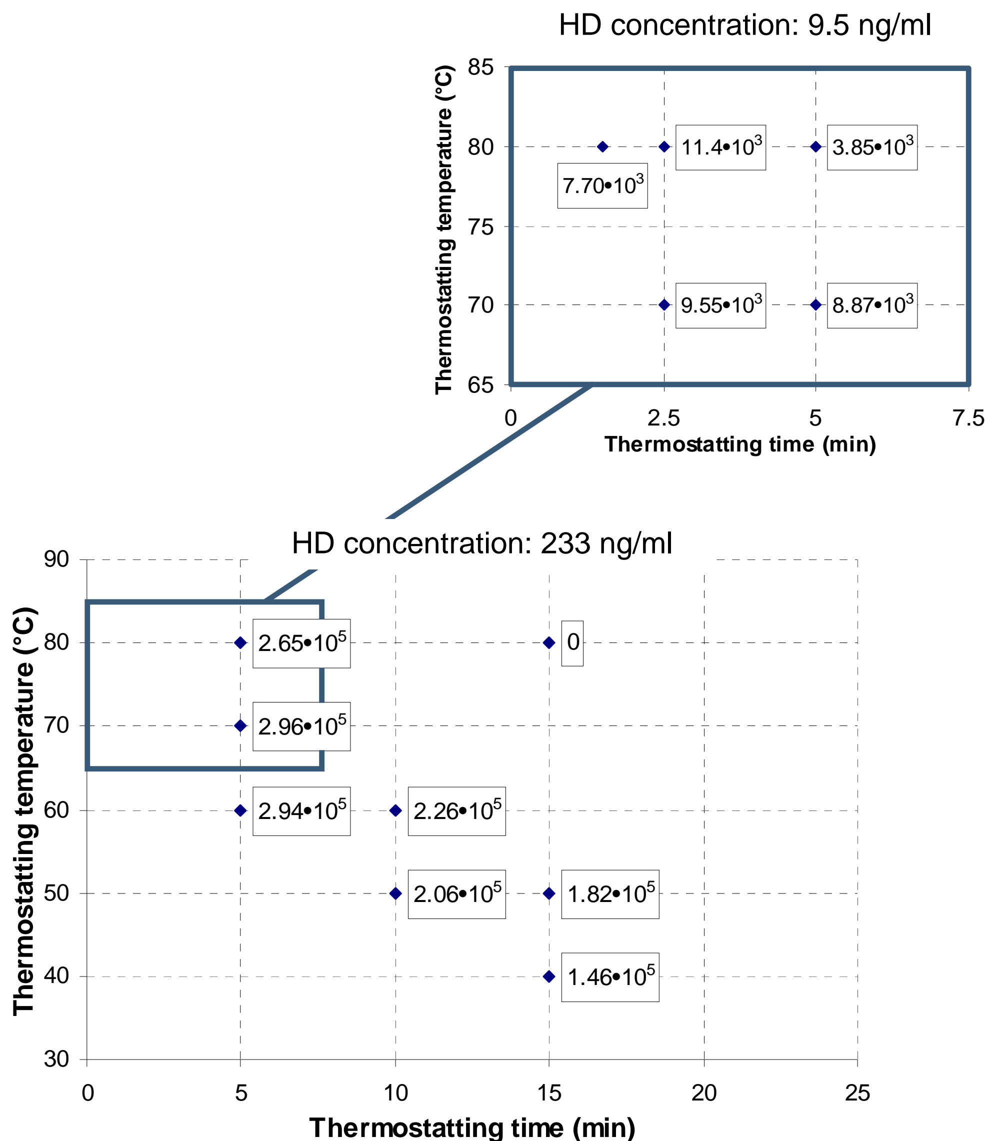

Figure 3.11 Simplex optimisation of thermostatting time and thermostatting temperature for the determination of HD in water (lower diagram). The numbers at each point represent the peak areas of the molecular m/z 158 ion. The upper diagram shows peak areas from a fine-tuning of the parameters.

The samples referred to in the lower (233 ng/ml) and upper diagram (9.5 ng/ml) were prepared by transferring 25 µl of working solution 5 and 8 (Table 2.3), respectively, into 2.00 ml salt saturated solutions. Each sample was prepared immediately prior to analysis.

The starting simplex consists of the experiments at 40 °C/15 min, 50 °C/15 min and 50 °C/10 min. From this, the response increased continuously towards decreasing thermostatting time and increasing temperature. New samples with lower concentrations were prepared, and further analyses were performed within the region marked with a frame. The maximum extraction yield was achieved with a thermostatting time of 2.5 min and a thermostatting temperature of 80 °C. This very short thermostatting time is presumably a compromise between the transfer rate into the vapour phase, and the rapid degradation of HD at 80 °C. Thus, the analyte is most probably not in equilibrium between the water and the vapour phase, as is the intention with the HS extraction technique. However, with the low stability of HD in water, it is not possible to achieve stable conditions in the vial in any case.

The effect of sequential extractions was investigated for HD by comparing the extraction yields from analyses with one and two vial extractions. Peak areas from three replicate analyses at each setting are shown in Table 3.2.

Table 3.2 Peak areas of the extracted m/z *158 molecular ion of HD, determined with one and two vial extractions.*

	Peak area (10^3)	
Replicate nr.	**One extraction**	**Two extractions**
1	2.6	2.8
2	2.9	5.1
3	2.0	1.8
Mean	2.5	3.2
SD	0.4	1.7

The samples were prepared by adding 25 µl of working solution 9 (Table 2.3) into 2.00 ml of salt saturated solutions, giving an HD concentration of 8.1 ng/ml. The thermostatting time was 80 °C, and the thermostatting time was 2.5 min.

The analyses with two vial extractions gave 30% higher peak areas in average, but large variations in peak area appeared. The large variations could be due to further degradation of HD on the adsorbent during the second trap load step. Because of the large variations in peak area after two extractions, a procedure with one sample extraction was preferred.

3.1.5 Summary, method development

Due to the very short thermostatting time optimal for determination of HD in water, separate methods are recommended for trace determination of HD and for its degradation products. The HS-trap parameters for the two developed methods are summarised in Table 3.3.

Table 3.3 Analysis parameters for trace determination of cyclic sulphur compounds in water, and for HD in water. Differences between the two methods are indicated in bold.

	Determination of cyclic sulphur compounds	**Determination of HD**
HS parameters		
Thermostatting temperature	80 °C	80 °C
Needle temperature	90 °C	90 °C
Transfer line temperaure	150 °C	150 °C
Thermostatting time	**15 min**	**2.5 min**
Pressurisation time	1.0 min	1.0 min
Decay (trap load) time	2.0 min	2.0 min
Number of cycles	**2**	**1**
Vial pressure	40 psi	40 psi
Column pressure	15 psi	15 psi
Shaker (on/off)	on	on
Trap parameters		
Trap low temperature	50 °C	50 °C
Trap high temperature	280 °C	280 °C
Dry purge time	**7 min**	**5 min**
Desorption time	0.5 min	0.5 min
Trap hold time	3 min	3 min
Desorption pressure	30 psi	30 psi
Needle purge split flow	13 ml/min	13 ml/min

The total analysis time was 30.5 min for HD determination, and 48 min for determination of the cyclic sulphur compounds. However, the only sample preparation needed was addition of salt to the HS-vials, giving a total sample handling time of less than one hour. The minimal need for sample preparation is an important improvement compared to the recommended LLE or SPE procedures [29], which are both time consuming (typically 4-5 hours) and labour demanding.

An example of a total ion current (TIC) chromatogram of the cyclic sulphur compounds analysed with the developed method is given in Figure 3.12 (lower chromatogram). A blank sample (2 ml salt saturated water) is shown in the upper chromatogram. Some system contaminants were seen in the blank sample, and one of these was coeluting with 1,3-dithiolane. However, this was not problematic for the method validation, since the MS could be used to extract some characteristic *m/z* ratios of 1,3-dithiolane from the chromatogram.

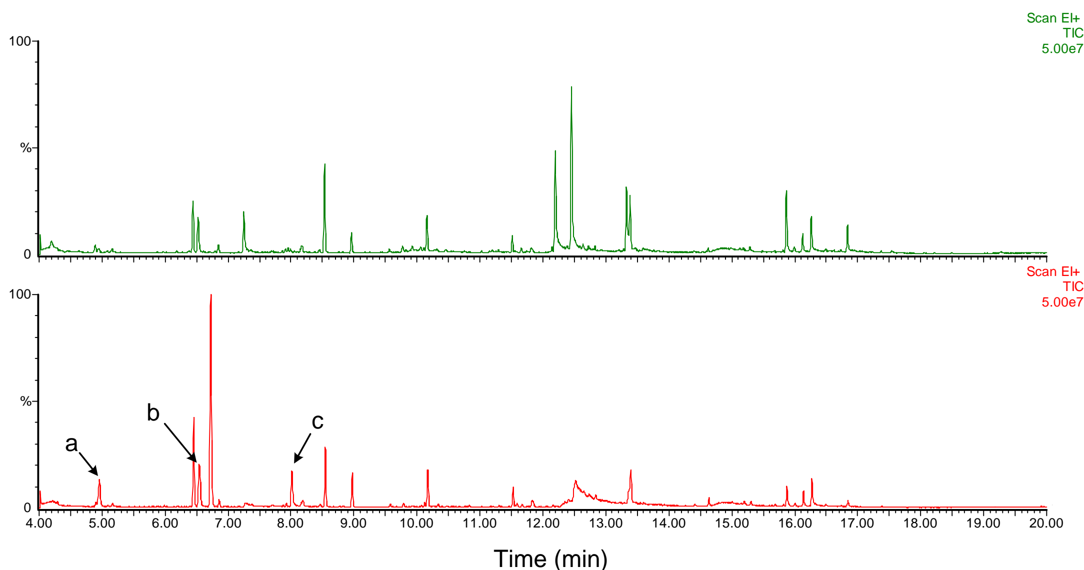

Figure 3.12 Example of a TIC chromatogram of a water sample, spiked with the cyclic sulphur compounds (lower chromatogram). The compounds eluted in the order: a) 1,4 thioxane, b) 1,3-dithiolane, c) 1,4-dithiane, and the concentrations were 4.1, 2.3 and 3.5 ng/ml, respectively. Analysis of a blank sample is shown in the upper chromatogram.

3.1.6 Method validation

A full method validation was performed only for trace determination of the cyclic sulphur compounds. Because of the low stability of HD in water, quantitative measurements of high precision were of limited value. Therefore, only the detection limit was investigated for this compound.

Detection limits and quantification limits

From the TIC chromatogram, three characteristic *m/z* ratios were chosen for each compound. The LODs were calculated as the concentrations giving a signal to noise (S/N) ratio of 10 for the sum of the three extracted ions. In addition, all three ions should be visible in the mass spectrum at these concentrations. The quantification limits (LOQs) were calculated as three times the detection limits. Figure 3.13 shows the mass spectrum of each compound, where the selected *m/z* ratios are indicated.

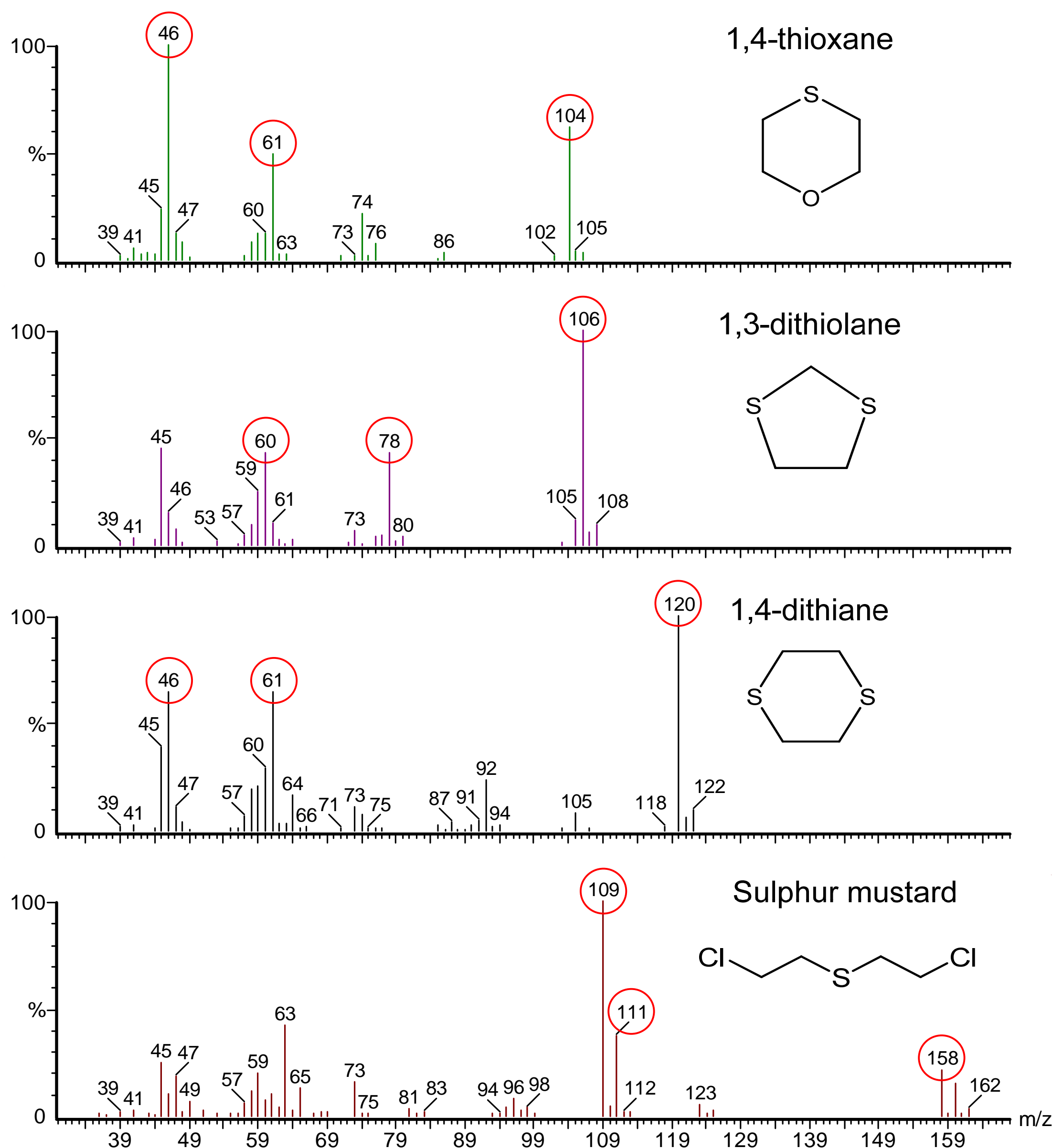

Figure 3.13 Mass spectra of the analytes. The m/z *ratios used for determination of detection limits are marked with circles.*

Validation solutions with the three cyclic sulphur compounds were prepared at concentrations from 0.05 ng/ml to 0.17 ng/ml (validation solutions 1-3, Table 2.4). Two or three replicates were

analysed at each concentration level. The selected *m/z* ratios were extracted from the TIC chromatograms, and S/N values were calculated. Figure B.2 in Appendix shows the S/N values plotted against concentration. The LODs were estimated from linear regressions of the plots. Table B.9 shows the linear regression equations with standard error of the plots, and also the calculated LODs for an S/N ratio of 10. A chromatogram of 1,4-dithiane, giving an S/N ratio of 10 for the extracted *m/z* ratios is shown in Figure 3.14 (lower chromatogram). Upper chromatogram is from a blank sample.

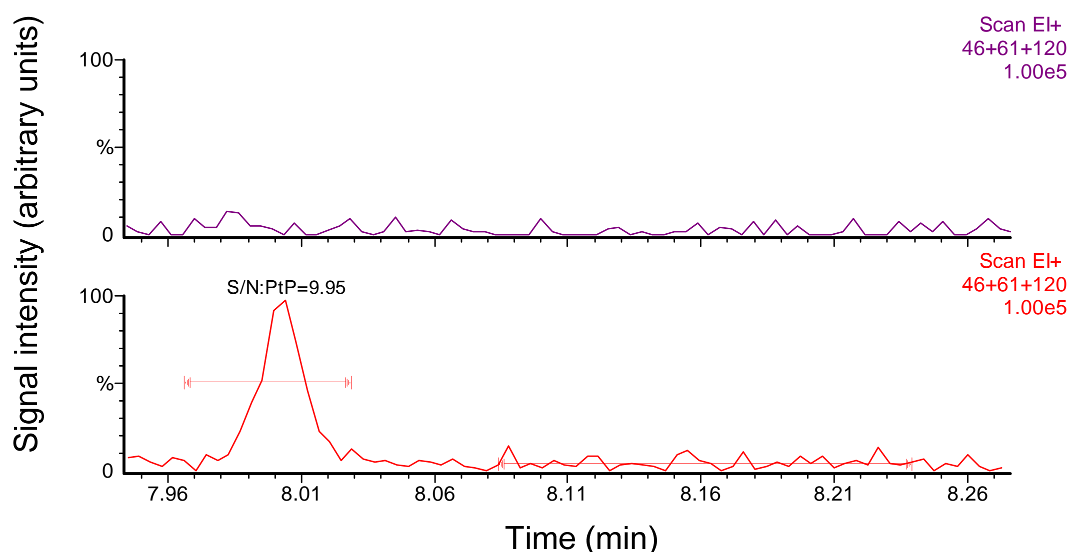

Figure 3.14 Chromatograms of the extracted m/z 46+61+120 of 1,4-dithiane from TIC chromatograms. Upper chromatogram is from a sample of water. Lower chromatogram is from a sample spiked with 1,4-dithiane (0.10 ng/ml), giving an S/N ratio of 10.

For HD, the LOD was limited by the signal of the molecular *m/z* 158 ion, which disappeared in the spectrum at concentrations below 1.0 ng/ml. The S/N ratio for the summarised extracted ions was still above 10, even at a concentration of 0.5 ng/ml. Figure B.3 in Appendix shows the mass spectra of HD at concentrations of 0.5 ng/ml and 1.0 ng/ml. The LOD requirements were met in three consecutive analyses with HD concentrations of 0.9-1.0 ng/ml (validation solutions 5-7). Table 3.4 gives the obtained LODs and LOQs of the compounds.

Table 3.4 Detection limits and quantification limits of HD and the three cyclic degradation products in water.

Compound	**1,4-thioxane**	**1,3-dithiolane**	**1,4-dithiane**	**HD** [1)]
LOD (ng/ml)	0.14	0.08	0.12	1.0
LOQ (ng/ml)	0.42	0.24	0.36	-

[1)] LOQ of HD is not included, as no thorough validation was performed for determination of the compound in water

The obtained LODs of the cyclic sulphur compounds were all determined to approximately 0.1 ng/ml. Little work has been reported on determination of the compounds in water, and the lowest LOD found the litterature is 51 ng/ml for 1,4-dithiane [49]. The obtained LOD of HD was one order of magnitude higher than the cyclic compounds (1.0 ng/g). Nevertheless, this is substantially better than what is reported for the recommended LLE procedure followed by GC-FID [6], or SPE followed by GC-MS [30], both with obtained LODs of 50 ng/ml. The sensitivity of the present method is comparable to what is achieved for determination of HD by the HF-LPME technique [40,41] (0.1-1 ng/ml) and SPME technique [42] (1.7 ng/ml). However, the HF-LPME technique is not commercially available.

Identification of the compounds could be carried out either by matching of the mass spectra with those obtained for an authentic reference standard, or with a respective spectrum from a software library. A common requirement for certified match with a library entry is that the reverse fit factor[6] exceeds a value of 800. When the mass spectra from the samples were compared with the corresponding mass spectra in the NIST software library, this requirement was met at the LOD concentration level for HD, and the LOQ levels for the cyclic sulphur compounds.

Linearity

The linearity of the method was investigated from LOQ to 100 times LOQ for each of the cyclic sulphur compounds. Samples were prepared at six concentration levels, given in Table 2.5. Peak areas of the three selected *m/z* ratios used to establish detection limits, were calculated relative to peak areas of the extracted *m/z* 105+120 of 1,2,4-TMB. The relative peak areas are plotted as a function of concentration in Figure 3.15 to Figure 3.17. Linear regression of the plots gave R^2 values of 0.997 - 0.998. This proves very good linearity of the method within the tested range. However, at concentrations below ten times the LOQs, the polynomial regression was even more suited. The smaller plots in each figure show polynomial regressions from LOQ and even up to 50 times LOQ, which all gave $R^2 > 0.9990$. Raw data are given in Table B.10 in Appendix.

[6] The reverse fit factor indicates how likely it is that the obtained spectrum contains the library entry. Any peaks present in the library spectrum, but not present in the search spectrum, decrease the fit value.

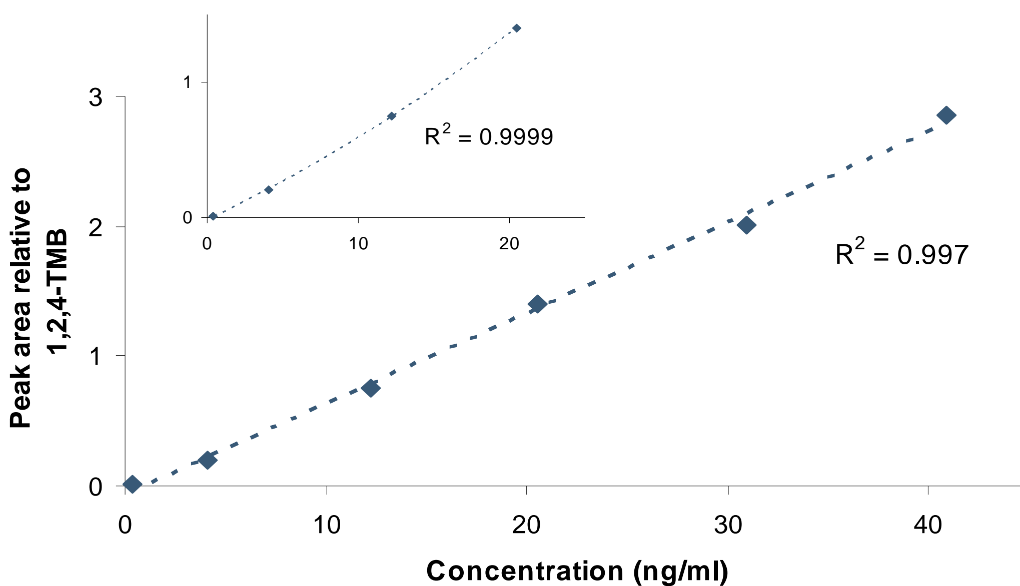

Figure 3.15 Relative peak area as a function of concentration from LOQ to 100 times LOQ for 1,4-thioxane, with linear regression. In the smaller diagram, polynomial regression is used from LOQ to 50 times LOQ ($y = 0.00084 \cdot x^2 + 0.052 \cdot x – 0.013$).

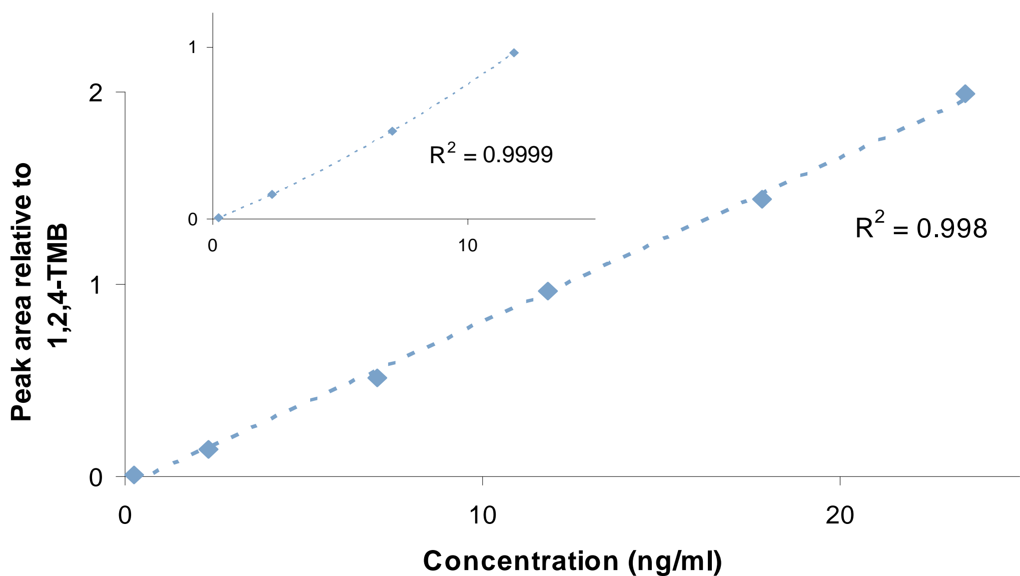

Figure 3.16 Relative peak area as a function of concentration from LOQ to 100 times LOQ for 1,3-dithiolane, with linear regression. In the smaller diagram, polynomial regression is used from LOQ to 50 times LOQ ($y = 0.0019 \cdot x^2 + 0.060 \cdot x – 0.0073$).

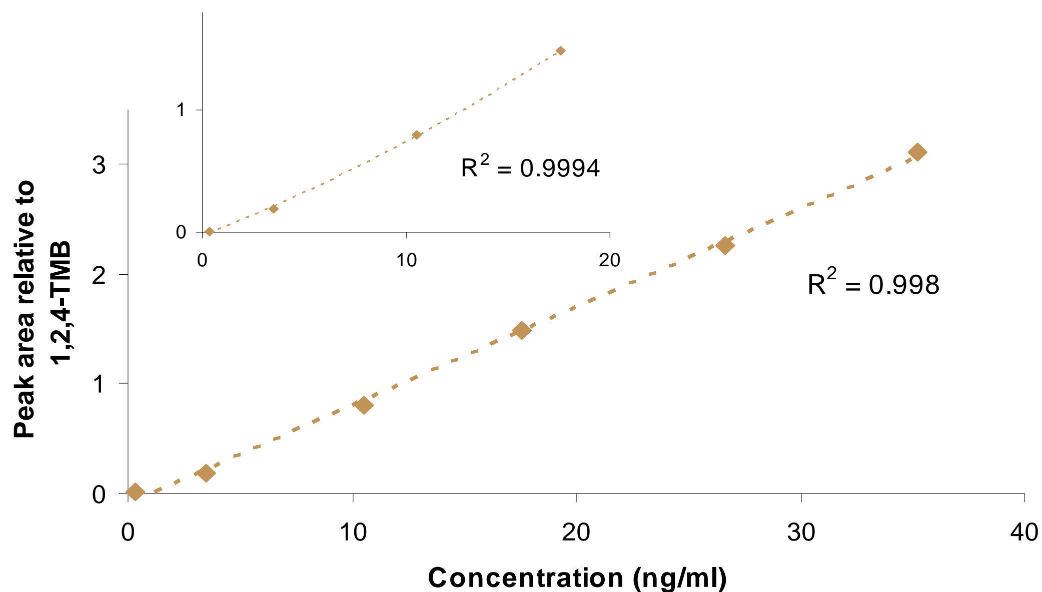

Figure 3.17 Relative peak area as a function of concentration from LOQ to 100 times LOQ for 1,4-dithiane, with linear regression. In the smaller diagram, polynomial regression is used from LOQ to 50 times LOQ ($y = 0.0012 \cdot x^2 + 0.065 \cdot x - 0.027$).

Repeatability

Repeatability was investigated both within assay and between assay, with six replicates at concentration levels of LOQ, 50 times LOQ and 100 times LOQ. Validation solutions 8, 11 and 13 (Table 2.5) were used in the within assay repeatability test. In the between assay repeatability test, new working and validation solutions were made each day from the stock solutions in Table 2.5. The variations in prepared concentrations were corrected for when calculating the peak areas relative to the IS. Every second day, new tuning and calibration of the MS detector were performed. In Table 3.5, the peak areas of the three selected ions used to establish detection limits are given for each compound. The values are relative to the peak areas of *m/z* 105+120 of 1,2,4-TMB.

Table 3.5 Within assay and between assay repeatability tests of the cyclic sulphur compounds in water, at three concentration levels. Peak areas of three selected m/z ratios of each of the cyclic sulphur compounds relative to peak areas of m/z 105+120 of 1,2,4-TMB are given.

		1,4-thioxane		**1,3-dithiolane**		**1,4-dithiane**	
		Within assay	**Between assay**	**Within assay**	**Between assay**	**Within assay**	**Between assay**
LOQ	1	0.0141	0.0140	0.0093	0.0082	0.0133	0.0112
	2	0.0145	0.0142	0.0096	0.0101	0.0126	0.0100
	3	0.0140	0.0145	0.0082	0.0086	0.0112	0.0104
	4	0.0133	0.0156	0.0085	0.0088	0.0102	0.0116
	5	0.0129	0.0131	0.0078	0.0087	0.0088	0.0083
	6	0.0138	0.0129	0.0080	0.0088	0.0100	0.0079
	Mean	0.0138	0.0141	0.0086	0.0089	0.0110	0.0099
	SD	0.0006	0.0010	0.0007	0.0007	0.0017	0.0015
	RSD (%)	4.1	7.0	8.2	7.4	15	15
50 x LOQ	1	1.39	1.37	0.95	0.94	1.55	1.48
	2	1.38	1.26	0.96	0.86	1.56	1.46
	3	1.37	1.16	0.94	0.79	1.48	1.41
	4	1.44	1.32	0.98	0.91	1.53	1.55
	5	1.36	1.40	0.95	0.96	1.48	1.62
	6	1.40	1.23	0.95	0.84	1.55	1.48
	Mean	1.39	1.29	0.96	0.88	1.52	1.49
	SD	0.03	0.09	0.02	0.07	0.04	0.08
	RSD (%)	2.0	6.9	3.5	7.6	2.5	5.6
100 x LOQ	1	2.92	2.84	2.08	2.04	3.24	3.31
	2	2.91	2.49	2.08	1.79	3.30	2.83
	3	2.84	2.41	2.04	1.68	3.31	2.88
	4	2.93	2.72	2.03	1.90	3.18	3.16
	5	2.98	2.56	2.11	1.84	3.42	3.09
	6	2.95	2.34	2.09	1.71	3.30	2.68
	Mean	2.92	2.56	2.07	1.83	3.29	3.0
	SD	0.05	0.19	0.03	0.13	0.08	0.2
	RSD (%)	1.6	7.4	1.5	7.1	2.4	7.9

The within assay repeatability test gave RSD values of 1-4% at concentrations of 50 and 100 times LOQ. At the LOQ levels, the RSD values were higher for 1,3-dithiolane and 1,4-dithiane, with a decreasing trend in the peak areas from replicate one to six. The decreasing trend was not seen at higher concentration levels. Since it was most evident for the least water-soluble compound (1,4-dithiane), this was most likely caused by surface adsorption in the vials during storage. All samples were prepared at the same time, and the last replicate was analysed approximately three hours after preparation. Because of this decreasing tendency, the time between sample preparation and analysis was kept constant when performing day to day repeatability for the sample at LOQ levels. The between assay repeatability test showed higher variations in general. However, all RSD values were less than 10%, except for 1,4-dithiane at the LOQ level, with 15% RSD.

Robustness
A robustness test was performed on the method for determination of the cyclic sulphur compounds in water. The effect of alterations in sample volume, thermostatting temperature and vial pressure were investigated. A two-level full factorial design experiment was performed with high and low values as showed in Table 3.6. Standard deviations were established from three replicates analysed at the method level conditions. A validation solution was prepared from the stock solutions in Table 2.5, at concentrations similar to validation solution 11 (10-20 ng/ml). All samples were added 0.80 g NaCl, regardless of the sample volume used.

Table 3.6 Analysis conditions for robustness test of the method for determination of cyclic sulphur compounds in water.

	Low level	Method	High level
Sample volume (ml)	1.95	2.00	2.05
Thermostatting temperature (°C)	78.0	80.0	82.0
Vial pressure (psi)	39.0	40.0	41.0

The experiment set-up and results are shown in Table B.11 and Figure B.4 in Appendix with peak areas for each compound relative to the peak areas of 1,2,4-TMB. The effect of each parameter was analysed in Minitab. Figure B.4 shows the effects presented in Pareto charts, with a confidence limit of 95%. A change in thermostatting temperature of 2 °C gave a significant effect on the peak areas relative to 1,2,4-TMB for 1,4-thioxane and 1,3-dithiolane, while the effect on 1,4-dithiane was close to the significance level. This indicates that a change in temperature has more effect on the cyclic sulphur compounds than on the internal standard. A change in vial pressure showed a barely significant effect on 1,4-thioxane, while alterations in the sample volume did not affect the peak areas for any of the compounds.

The robustness test showed that variations in thermostatting temperature should be kept well within ± 2 °C. It showed that the sample volume, which is dependent on the operator skill, is not vulnerable to variations of ± 2.5%.

3.1.7 Recovery test of natural water samples

The developed method for trace determination of the cyclic sulphur compounds was tested for analysis of three different types of natural water samples. Water from a rain pool, as well as river water and sea water were investigated. The samples were spiked with the three cyclic sulphur compounds at concentrations of both 3 and 30 times the LOQs. New calibration curves were established for quantification analyses. Preparation of the calibration solutions is described in Appendix B.3, together with the calibration plots.

Preparation of the spiking solution and IS solution for the recovery test is described in Section 2.3.3 (Table 2.5). Two 100 ml aliquots of the water samples were added 0.150 and 1.50 ml of the spiking solution, giving concentrations of approximately 3 and 30 times the LOQ levels. No sample cleanup or other sample preparation was performed. Three replicates of 2.00 ml were

transferred from each solution to HS-vials, and added 0.80 g NaCl. Finally, 20 µl of the IS solution was added. The samples were analysed according to the method described in Table 3.3. The average recovery ± one SD from each sample solution is shown in Table 3.7. Figures of all replicates are given in Table B.13 to B.15 in Appendix.

Table 3.7 Recovery test of natural water samples. Each water sample was spiked at concentrations of approximately 3 and 30 times the LOQ levels.

		% Recovery ± SD (n=3)		
Compound	**Spiking level ng/ml**	**Rain water**	**River water**	**Sea water**
1,4-thioxane	1.2	100 ± 2	90 ± 4	98 ± 2
	12	91 ± 3	89 ± 2	95 ± 2
1,3-dithiolane	0.72	102 ± 5	93 ± 3	98 ± 5
	7.2	92 ± 2	89 ± 2	95 ± 1
1,4-dithiane	1.1	104 ± 1	97 ± 4	101 ± 4
	11	90 ± 2	89 ± 3	96 ± 3

The overall recoveries were high from all samples, varying from 89 to 104%, with standard deviations varying between 1 and 5 %. Actually, the recoveries were higher from the samples spiked at LOQ concentrations. This could be due to the use of two calibration plots. At the lowest concentration levels, recoveries from the river water were sligthly lower than from rain water and sea water. The lower recoveries could be explained by the high clay content of the river water (Section 2.4). However, the high clay content did not affect the precision of the determination.

In conclusion, both the recovery and the precision were high, even at concentrations at the ppb level. This proved that the method was well suited for different types of water samples, and not especially vulnerable to matrix effects like a high content of mud.

3.2 Trace determination of CWA in soil

This section describes the method development for trace determination of the analytes in soil samples. Optimisation of analytical conditions was performed for two types of well characterised soils to ensure that the method was suited for soils of various characteristics. The soil types are classified as sandy loam and silty clay loam (Table 2.6), and are designated soil A and soil B, respectively.

Due to the low stability of HD in aqueous environment and at elevated temperatures, the optimum conditions for some important analysis parameters for HD differed from those of the cyclic sulphur compounds. A separate method was therefore developed for HD determination. Complete validations were performed on both methods, including recovery tests of both soils.

Addition of water may be used to enhance the extraction recovery from solid adsorbents in headspace sampling. This matrix modification technique has also proved to be effective for determination of HD and a simulant in soil [61,71]. Liu *et al.* showed that also salt saturation of the added water was very effective for determination of an HD simulant in soil [71]. If the amount of water is sufficient to form a separate liquid layer, the displaced analytes elute into the liquid phase. Hence, the analytes will be partitioned between the vial headspace and the water phase, and the added water becomes the matrix [63][7]. Some of the analysis conditions for water samples should therefore be applicable for soil samples as well, like the trap parameter values. Another advantage is that the added water solution could be used to prepare internal or external standards.

The parameters optimised especially for soil samples were the thermostatting temperature, thermostatting time, sample amount and volume of added water. In addition, the effect of sequential extractions was investigated. The other parameters were set as listed in Table 3.3.

3.2.1 Soil preparation

The soils were dried at 50 °C under nitrogen atmosphere for 24 hours prior to use. Drying with nitrogen led to lower chromatographic background signal, compared to drying in air atmosphere. Figure C.1 in Appendix shows chromatograms of soil A dried both ways at 50 °C (chromatogram 2 and 3). Chromatogram 4 shows a sample dried at 105 °C under normal atmosphere, where an even higher background signal is seen. Changes in the organic fraction of the soil seem to be the reason for the high chromatographic background signal from samples dried in normal atmosphere. This hypothesis is supported by analyses of the soil after the organic fraction was charred, by heating it to 550 °C under normal atmosphere (chromatogram 5). The background signal of this chromatogram is at the level of a non-treated soil, shown in chromatogram 1.

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It should be noted that no articles have been found on the issue of soil drying effects on chromatographic analyses. Hence, further investigation on the consequences of soil drying may be performed.

Soil B showed to be considerably contaminated with organic compounds. Thus, no significant difference was observed for the background signal of this soil, whether it was dried under nitrogen or at normal atmosphere. Chromatograms of soil B are shown in Figure C.2 in Appendix. The relatively high contaminations made this soil unsuitable for establishing detection limits, due to a high chromatographic background signal. However, soil B was suitable for method optimisation at higher concentrations.

3.2.2 Thermostatting temperature and thermostatting time

The thermostatting temperature and thermostatting time were optimised for determination of the analytes in both soil types. The samples were prepared as described in Section 2.5.1, and salt saturated solutions were added into the vials prior to analyses.

Two analysis series were performed with thermostatting temperatures of 70 °C and 80 °C, respectively, and with thermostatting times from 2 to 15 min. As for the determination in water, the trends in extraction recoveries of HD differed from those of the cyclic sulphur compounds. Thus, the peak areas of HD are presented separately. Figure 3.18 and Figure 3.19 show the extraction recoveries of the cyclic sulphur compounds from soil A and soil B, respectively, at 80 °C thermostatting temperature. The peak areas of the molecular ions are plotted as a function of thermostatting time.

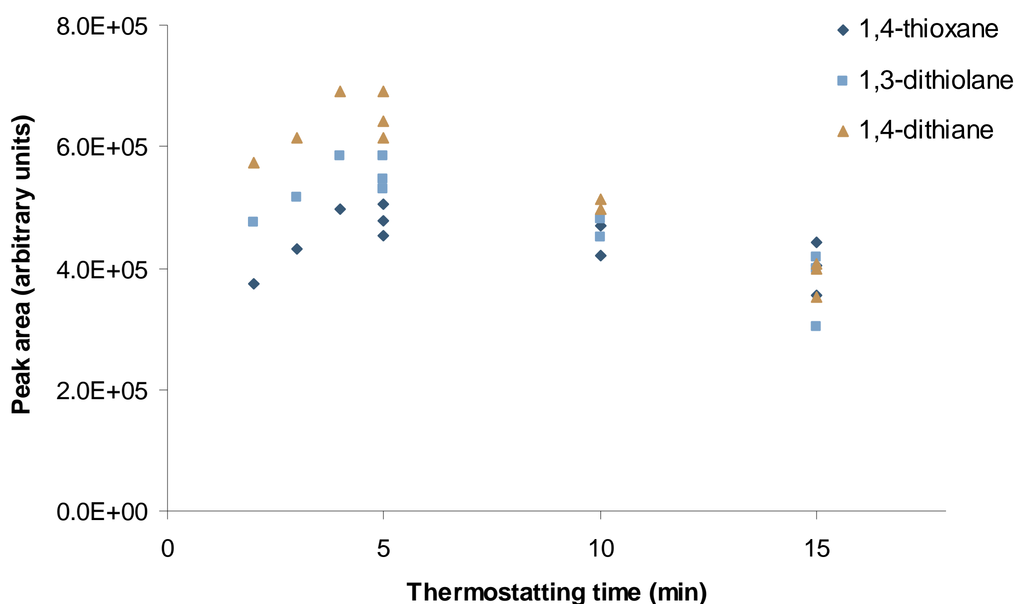

Figure 3.18 Determination of the cyclic sulphur compounds in soil A, at a thermostatting temperature of 80 °C. Peak areas of the molecular ions are given as a function of thermostatting time.

Aliquots of 40 µl of spiking solution 1 (Table 2.7) were added to 1.0 g dried soil, obtaining concentrations between 25 and 43 ng/g. The samples were added 1.00 ml of saturated NaCl solution immediately prior to analysis.

The peak areas from the analyses of soil A indicate that equilibrium was established already after 4-5 min thermostatting. A decrease in peak areas was found when going from 5 to 15 min thermostatting. This effect was most evident for 1,4-dithiane. The decrease could be due to oxidation of the analytes at elevated temperatures. Opstad and Tørnes showed that 1,4-dithiane was oxidised to the respective sulfoxide and sulfone after long time storage in seawater at 25 °C [23].

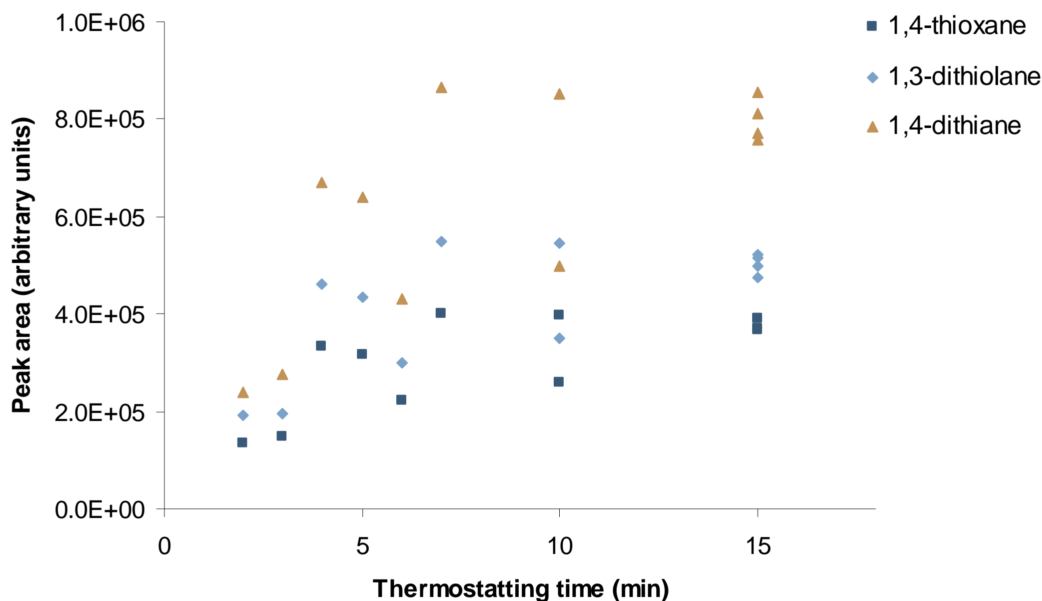

Figure 3.19 Determination of the cyclic sulphur compounds in soil B, at a thermostatting temperature of 80 °C. Peak areas of the molecular ions are given as a function of thermostatting time.

Aliquots of 40 µl of spiking solution 1 (Table 2.7) were added to 1.0 g dried soil, obtaining concentrations between 25 and 43 ng/g. The samples were added 1.00 ml of saturated NaCl solution immediately prior to analysis.

The peak areas from analyses of soil B showed large variations at a thermostatting time shorter than 15 min. Furthermore, a longer thermostatting time (10-15) min was required to reach equilibrium between the soil/water and vapour phase. 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-GC-MS [72], and with solvent extraction [56]. Thus, the high clay content of soil B may be responsible for making the extraction more challenging. The reason for the large variations in peak areas at thermostatting times shorter than 15 min is more uncertain. A possible explanation could be variations in the exposed soil surface when adding the spiking solution. Logically, this should be less pronounced at stronger extraction conditions, like a longer thermostatting time.

The extraction recoveries at thermostatting temperatures of 70 °C and 80 °C were also compared. Figure 3.20 shows the extraction recovery of 1,3-dithiolane from soil A and soil B at both temperatures. The peak areas of the molecular ion are plotted as a function of thermostatting time from 2 to 15 min.

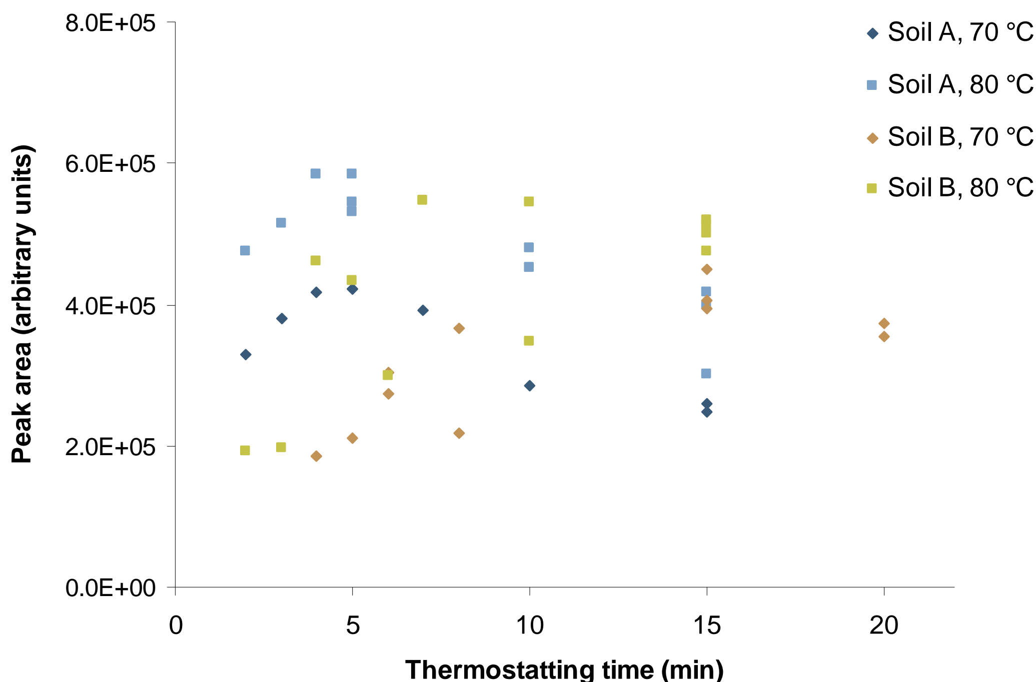

Figure 3.20 Determination of 1,3-dithiolane in soil A and soil B, at thermostatting temperatures of 70 °C and 80 °C. Peak areas of the molecular ions are given as a function of thermostatting time.

Aliquots of 40 µl of spiking solution 1 (Table 2.7) were added to 1.0 g dried soil, obtaining concentrations between 25 and 43 ng/g. The samples were added 1.00 ml of saturated NaCl solution immediately prior to analysis.

The analyses of soil A showed the same trends for the plotted peak areas at both thermostatting temperatures. The recoveries were highest at thermostatting times of 4-5 min, and decreased at longer thermostatting. However, the analyses at 70 °C gave significantly lower recoveries than at 80 °C. The peak areas from the analyses of soil B also showed similar trends at both temperatures, with lower recoveries at 70 °C compared to 80 °C. In order to examine if the recovery from soil B was still increasing at 70 °C with extended thermostatting, additional analyses were performed. However, the recovery decreased when the thermostatting time was prolonged from 15 to 20 min.

The extraction recoveries of HD are presented in Figure 3.21 (soil A) and Figure 3.22 (soil B). Peak areas of the molecular ion at both thermostatting temperatures are plotted as a function of thermostatting time.

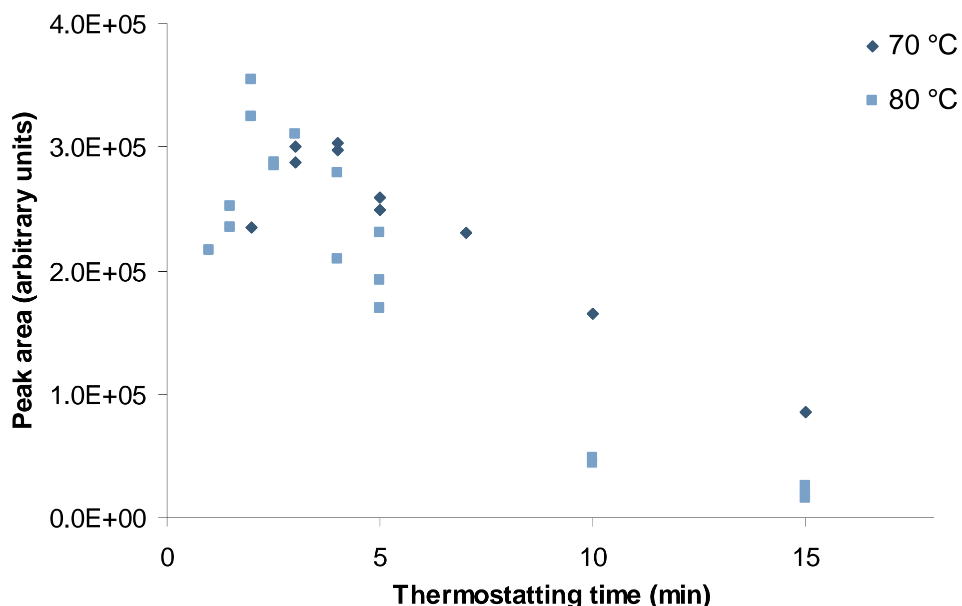

Figure 3.21 Determination of HD in soil A, at thermostatting temperatures of 70 °C and 80 °C. Peak areas of the molecular ion are given as a function of thermostatting time.

Aliquots of 40 µl of spiking solution 1 (Table 2.7) were added to 1.0 g dried soil, obtaining HD concentrations of approximately 230 ng/g. The samples were added 1.00 ml of saturated NaCl solution immediately prior to analysis.

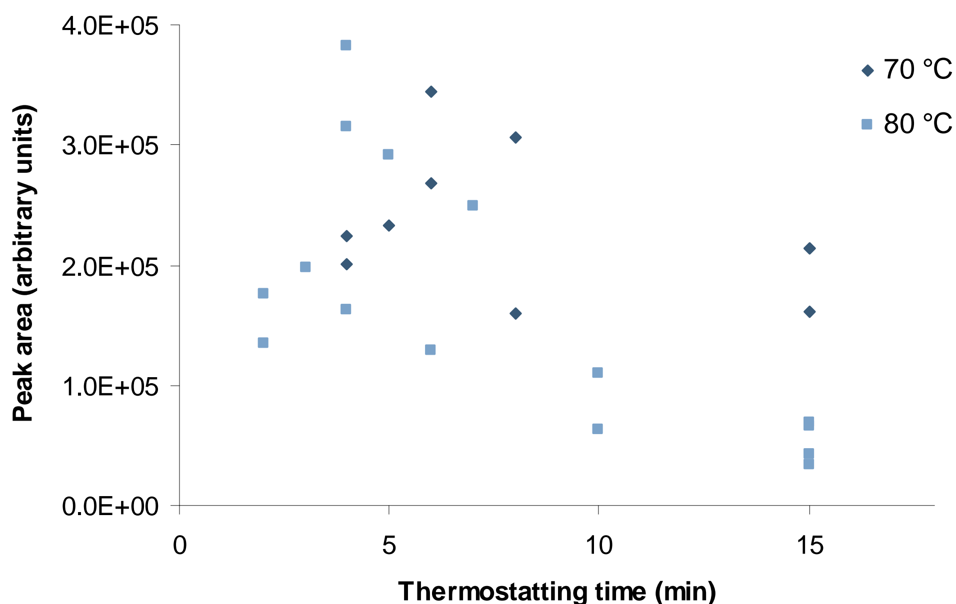

Figure 3.21 Determination of HD in soil B, at thermostatting temperatures of 70 °C and 80 °C. Peak areas of the molecular ion are given as a function of thermostatting time.

Aliquots of 40 µl of spiking solution 1 (Table 2.7) were added to 1.0 g dried soil, obtaining HD concentrations of approximately 230 ng/g. The samples were added 1.00 ml of saturated NaCl solution immediately prior to analysis.

From soil A, the highest recoveries at 80 °C were achieved at a thermostatting time of only 2 min. At 70 °C, a sligthly lower maximum in peak area appeared at approximately 4 min thermostatting time. Due to the degradation of HD, a rapid decrease in peak area was observed at extended thermostatting. In the regions where the highest recoveries were achieved, the replicates at 70 °C had less variation in peak area compared to the replicates at 80 °C. Figure 3.21 shows similar trends in the recoveries from soil B, but with a shift in maxima towards longer thermostatting times. The highest recoveries appeared at thermostatting times of approximately 4 min and 6 min at thermostatting temperatures of 80 °C and 70 °C, respectively. Similar to the cyclic sulphur compounds, large variations in the recoveries were observed from soil B at both temperatures.

Due to the relatively large differences in optimal analysis conditions between HD and the cyclic sulphur compounds, further method development for HD determination was handled separately. However, since the probability of finding the intact compound in soil samples is much higher than in water samples, more attention was paid to the method development and validation for HD determination in soil.

The peak areas presented in Figure 3.18 to Figure 3.20 show that even the cyclic sulphur compounds are somewhat unstable in the soil/water matrix at elevated temperatures. Thus, the optimal thermostatting time was dependent on the soil type. This shows that it is important to include soil types of different characteristics when performing method optimisation on such sample matrices. To ensure equilibrium between the soil/water sample and the vapour phase for most soil types, a thermostatting time of 15 min was chosen for the cyclic sulphur compounds. The thermostatting temperature of 80 °C was preferred, since this gave the highest recoveries from both soils.

For HD, there were no significant differences in the highest extraction recovery between the analyses at 70 °C and 80 °C thermostatting temperature. Since the analyses at 70 °C showed less variations in the replicates, this temperature was preferred. A thermostatting time of 5 min was chosen, which was between the optimum times for soil A and soil B.

3.2.3 Sample amount and volume of added water

Cyclic sulphur compounds

The effect of water addition on extraction recovery was investigated for both soils by adding various amounts of saturated salt solution to the soil samples. Figure 3.22 and Figure 3.23 show the average peak areas of the extracted molecular ions of each analyte from soil A and soil B, respectively. Peak areas for all replicates are given in Table C.1 and C.2 in Appendix.

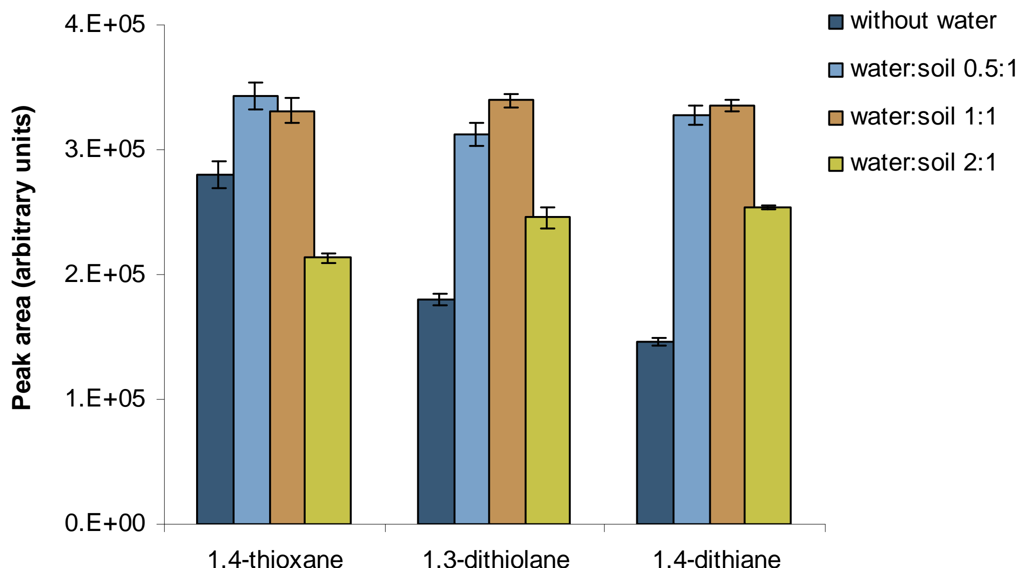

Figure 3.22 Effect of water addition on the extraction recovery of the cyclic sulphur compounds from 1.0 g of soil A. Peak areas of the molecular ions are presented as mean values of three replicates ± one SD.

Aliquots of 40 µl of spiking solution 1 (Table 2.7) were added to 1.0 g dried soil, obtaining concentrations between 25 and 43 ng/g. The samples were added 0.50, 1.00 and 2.00 ml saturated NaCl solution immediately prior to analysis. Samples with no water added were also analysed.

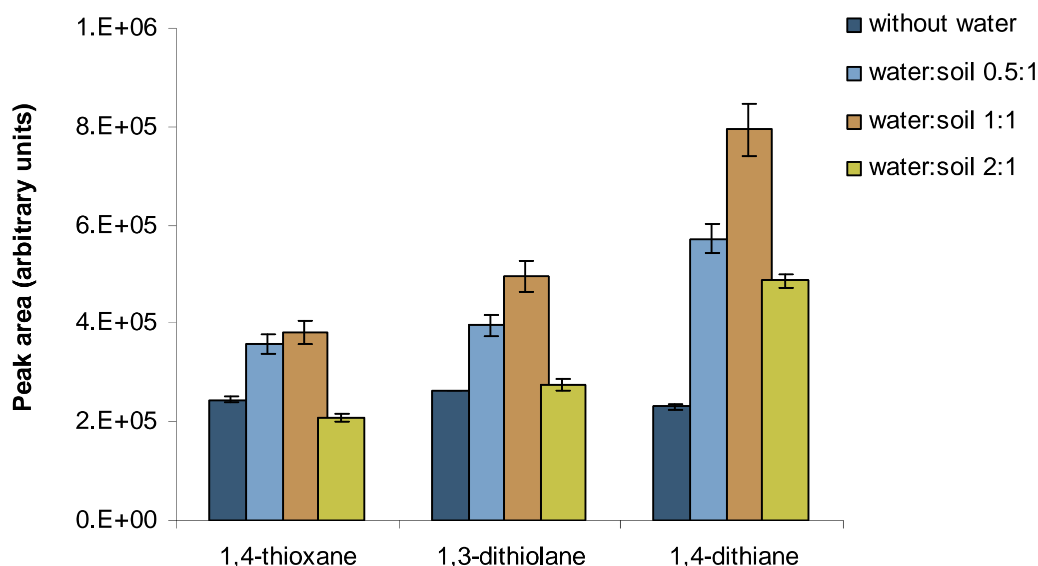

Figure 3.23 Effect of water addition on the extraction recovery of the cyclic sulphur compounds from 1.0 g of soil B. Peak areas of the molecular ions are presented as mean values of three replicates ± one SD.

Aliquots of 40 µl of spiking solution 1 (Table 2.7) were added to 1.0 g dried soil, obtaining concentrations between 25 and 43 ng/g. The samples were added 0.50, 1.00 or 2.00 ml saturated NaCl solution immediately prior to analysis. Samples with no water added were also analysed.

The highest recoveries from soil A were achieved with water to soil ratios of 0.5:1 and 1:1, with small differences between the two settings. Only 1,3-dithiolane showed sligthly higher recoveries in the analyses with 1 ml added water (two tailed t-test, α=0.95). The recoveries of 1,3-dithiolane and 1,4-dithiane from soil B were significantly higher when 1 ml water was added. A water volume of 2 ml gave a significant decrease in recovery for both soil types, probably because of dilution. The smallest effect of salt water addition was observed for 1,4-thioxane, while the largest was observed for 1,4 dithiane. Thus, the effect may be correlated to the water solubility of the compounds (Table 1.2). As 1,4-dithiane has the lowest solubility, this will favour the distribution towards the vapour phase. Due to the higher recoveries of the analytes from soil B at a water to soil ratio of 1:1, this ratio was chosen for the further studies.

The effect of various sample amounts on extraction recovery was investigated with soil samples of 1, 2 and 3 g, and with a water to soil ratio of 1:1. Figure 3.24 shows the normalised peak areas of the molecular ions. Raw data for all replicates are given in Table C.1 and C.2 in Appendix.

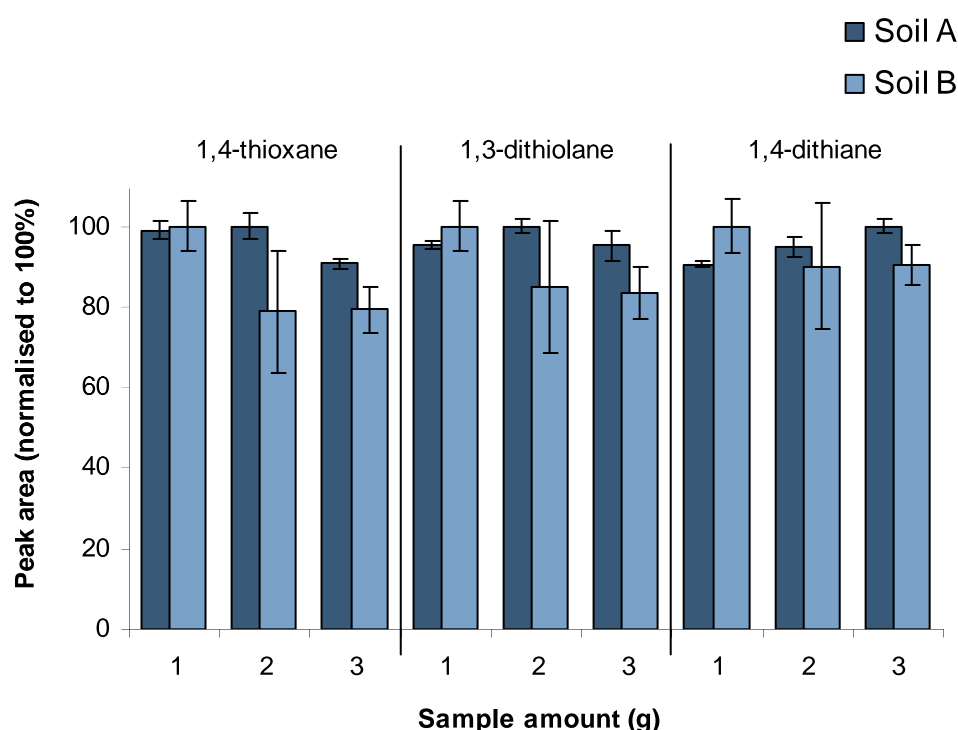

Figure 3.24 Effect of sample amount on extraction recoveries from soil A and soil B, with a water to soil ratio of 1:1. Peak areas are mean values of three replicates with error bars ± one SD (1 and 2 g soil), and of two replicates with error bars indicating high and low value (3 g soil).

Spiking solution 1 (Table 2.7) was added to dried soil samples at an amount of 40 µl per g soil, so that all samples were prepared at the same concentrations, between 25 and 43 ng/g. The samples were added 1.00, 2.00 or 3.00 ml saturated NaCl solution immediately prior to analysis.

No typical trends were observed in the recoveries from soil A, and only small variations between different sample amounts were found. For soil B, slightly higher recoveries were achieved from the samples with 1 g soil, but the differences were not significant at a 95% confidence level. For the further studies, a sample amount of 2 g was chosen. It was then shown that a change in sample amount up to 1 g was not critical for the extraction recovery.

Sulphur mustard

Due to the insignificant effect of sample amount on extraction recovery of the cyclic sulphur compounds, this parameter was not investigated for HD determination. Hence, a sample amount of 2 g was applied also for determination of HD. The effect of water addition on extraction recovery of HD from soil was investigated for both soils. Average peak areas of four replicates from each experiment are presented in Figure 3.25. Peak areas of all replicates are given in Table C.3 in Appendix.

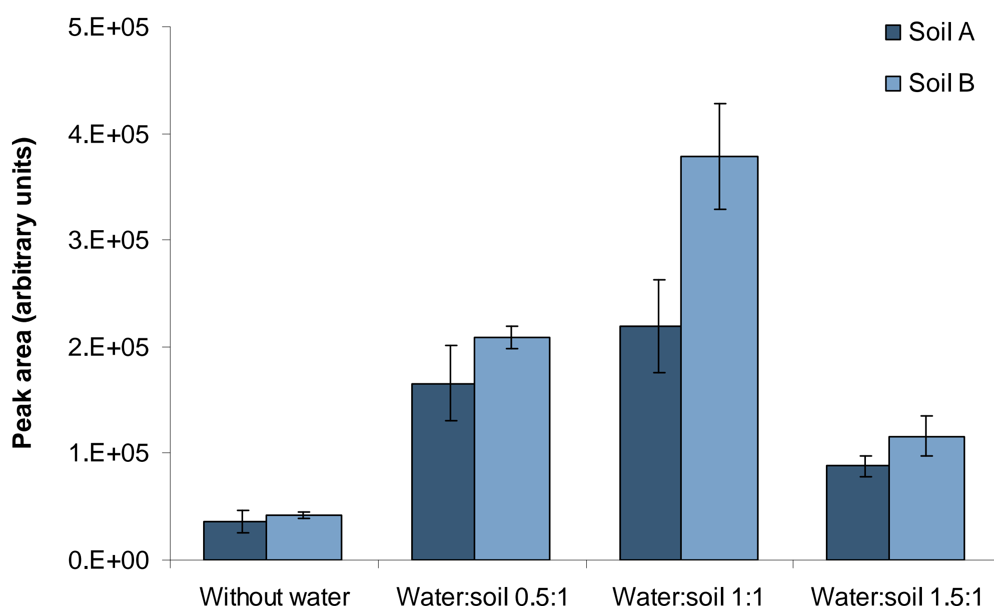

Figure 3.25 Effect of water to soil ratio on the extraction recovery of HD from 2 g soil samples. Peak areas of the molecular ion are presented as mean values ± one SD (n=4).

Aliquots of 80 µl of spiking solution 2 (Table 2.7) were added to 2.00 g dried soil, obtaining HD concentrations of 21 ng/g. The samples were 1.00, 2.00 of 3.00 ml saturated NaCl solution immediately prior to analysis. Samples with no water added were also analysed.

The highest recoveries were achieved with a water to soil ratio of 1:1. This was true for both soils. Compared to the analyses with no water added, a six- and nine-fold increase in peak area was achieved from soil A and soil B, respectively. As for the cyclic sulphur compounds, the difference between water to soil ratios of 0.5:1 and 1:1 was most evident for soil B. When the volume of added water was increased from 2 to 3 ml, an abrupt decrease by 60-70% in recoveries was observed. Based on these experiments, a water to soil ratio of 1:1 was chosen for the method.

However, when analysing soil samples with high water concentrations, the volume of added solution may be reduced.

3.2.4 Effect of sequential extractions

Cyclic sulphur compounds

The effect of sequential extractions on the cyclic sulphur compounds was investigated with soil A as the sample matrix. Peak areas from three replicates of one, two and three successive vial extractions are presented in Figure 3.26. Peak areas from all replicates are given in Table C.4 in Appendix.

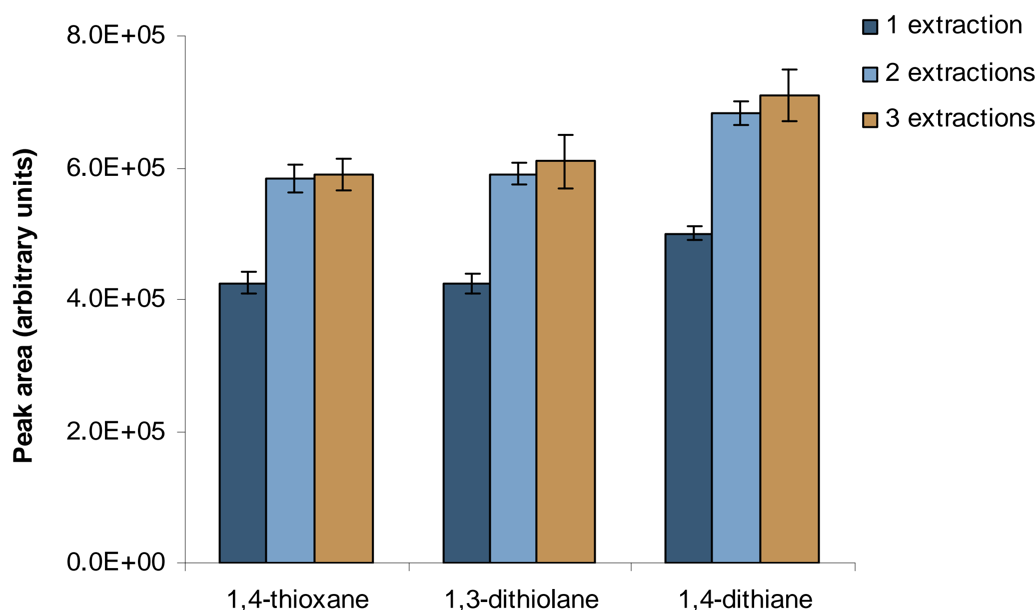

Figure 3.26 Effect of sequential extractions on recovery of the cyclic sulphur compounds from soil. The columns represent the peak areas of the molecular ions, with error bars ± one SD (n=4).

Aliquots of 80 µl of spiking solution 1 (Table 2.7) were added to 2.00 g dried soil, obtaining concentrations between 25 and 43 ng/g. The samples were added 2.00 ml of saturated NaCl solution immediately prior to analysis.

The peak areas increased by 36-39% from one to two vial extractions. The standard deviations were below 5% both with one and two vial extractions. No significant increase in peak area was observed when a third extraction was performed. Hence, a method with two vial extractions was chosen.

Sulphur mustard

Extraction recovery of HD was investigated for one and two vial extractions. Based on the experience from determination of the cyclic sulphur compounds, a third vial extraction was not included. Peak areas from four replicates of each experiment are presented in Table 3.8.

Table 3.8 Comparison of the extraction yield of HD from soil A, with one and two vial extractions. Peak areas are from the extracted m/z *158 molecular ion.*

	Peak area (10^4)	
Replicate	**One extraction**	**Two extractions**
1	1.36	2.08
2	1.37	2.62
3	1.14	2.56
4	1.47	2.04
Mean	1.33	2.32
SD	0.14	0.31
RSD (%)	11	13

Aliquots of 80 µl of spiking solution 2 (Table 2.7) were added to 2.00 g dried soil, obtaining HD concentrations of 21 ng/g. The samples were added 2.00 ml of salt saturated water immediately prior to analyses.

The average peak area increased by 74% from one to two vial extractions, and no significant increase in the relative standard deviation was observed. Thus, the effect of two vial extractions was superior to the corresponding experiment with water samples (Table 3.2). The better outcome for soil samples could be explained by the lower thermostatting temperature, which decreases the degradation rate of HD, both in vapour phase and potentially on the trap between the extractions. Hence, a procedure with two vial extractions was preferred.

3.2.5 Summary, method development

The instrumental parameters obtained from method optmisations are listed in Table 3.9. The parameters obtained for the method for determination of the cyclic sulphur compounds in soil, were identical to those for determination of the analytes in water. Thus, the same method could be applied for both matrices. For HD determination in soil, lower thermostatting temperature and longer thermostatting time were used compared to the method for determination of HD in water. In addition, two vial extractions were applied.

Table 3.9 Analysis parameters for trace determination of cyclic sulphur compounds in soil, and for HD in soil. Differences between the two methods are indicated in bold.

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

The total analysis time was 38 min for HD determination, and 48 min for determination of the cyclic sulphur compounds. However, the only sample preparation required, was to transfer an aliquot of the sample into an HS-vial and adding salt saturated solution. This means that the total sample handling time for one sample was less than one hour. As for the water samples, this is a great improvement compared to the recommended solvent extraction technique [29,29], requiring typically 4-5 hours sample handling.

3.2.6 Method validation, cyclic sulphur compounds

The method for trace determination of the cyclic sulphur compounds in soil was validated with soil A as the sample matrix. Detection limits, linearity, repeatability and robustness of the method were determined. In addition, the recovery from both soil types was investigated. The recoveries were calculated by comparison of peak areas from analyses of spiked samples, and samples where the analytes were added directly into the slurry.

Detection limits and quantification limits

The criteria set for detection limits and quantification limits of the compounds in soil were the same as for the water samples (Section 3.1.6). Soil samples were prepared from validation solutions 1-4 (Table 2.8). Two replicates were prepared from each solution and analysed with the developed method. The three characteristic *m/z* ratios of each compound were summarised, and S/N values were calculated. Figure C.3 in Appendix shows the S/N ratio of each compound, plotted against concentration. Linear regressions of the plots are given in Table C.5, with calculated concentrations at S/N ratios of 10. The calculated LODs and LOQs of the compounds are given in Table 3.10.

Table 3.10 Detection limits and quantification limits for the cyclic sulphur compounds, determined in soil A.

Compound	**1,4-thioxane**	**1,3-dithiolane**	**1,4-dithiane**
LOD (ng/g)	0.2	0.7	0.3
LOQ (ng/g)	0.7	2	1

The obtained LODs were well below 1 ppb for all compounds, and showed that the method is very sensitive for soil samples as well. The peak of 1,3-dithiolane had more tailing than those of 1,4-thioxane and 1,4-dithiane, giving somewhat higher LOD for this compound. This was in contrast to what was obtained when validating the method for water samples, where 1,3-dithiolane had the lowest LOD (Table 3.4). A possible explanation could be deterioration of the stationary phase of the GC column from the time of the water analyses.

The only article found, reporting LODs for any of the cyclic sulphur compounds in soil, describes a method of accelerated solvent extraction followed by GC-FPD [57]. The reported LODs of 1,4-thioxane and 1,4-dithiane were at the ppm level, however. Compared to this, the sensitivity of the HS-trap technique is considerably better.

Linearity

The linearity of the method was investigated from LOQ to 100 times the LOQ for each analyte, with 1,2,4-TMB as internal standard. Spiking solutions were prepared at six concentration levels (validation solutions 8-13, Table 2.9). The soil samples were prepared as described in Section 2.5.1. From the IS working solution, aliquots of 50 µl were added to the slurry, giving a concentration of 10 ng/ml water. Peak areas of the three selected *m/z* ratios applied to establish detection limits, were calculated relative to peak areas of the extracted *m/z* 105+120 of 1,2,4-TMB. Figure 3.27 shows the relative peak areas plotted against concentration, with R^2 values from linear regression. Good correlations were shown, with $R^2 > 0.990$ for all compounds. However, similar to the water analyses, polynomial regression gave the best fit at concentrations below 10 times LOQ. Figure 3.28 shows that polynomial regression was very well suited from LOQ to 50 times LOQ. Raw data are given in Table C.6 in Appendix.

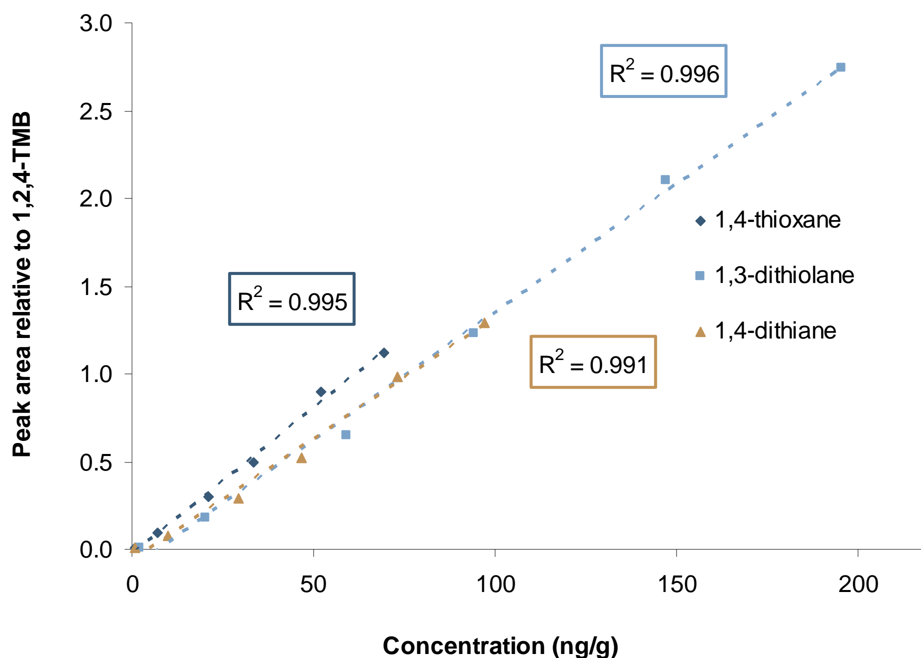

Figure 3.27 Linearity test of 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane in soil. Peak areas relative to IS are given as a function of concentration from LOQ to 100 times LOQ. The R2 values from linear regressions are given in the frames.

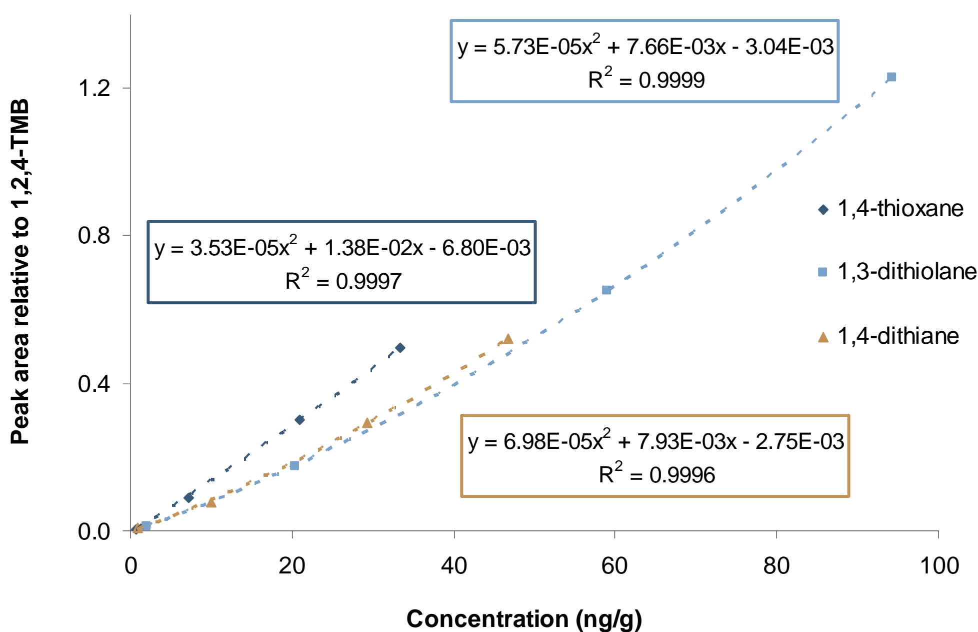

Figure 3.28 Peak areas of the cyclic sulphur compounds relative to IS, plotted as a function of concentration. Polynomial regression is used from LOQ to 50 times LOQ.

Repeatability

Repeatability of the method was investigated with six within assay replicates, and with one replicate for six successive days (between assay), at concentration levels of LOQ and 50 times LOQ. Validation solutions 8 and 11 in Table 2.9 were used for the sample preparations. Aliquots of 50 µl IS working solution were added to the slurry, giving a concentration of 10 ng/g soil. Peak areas of the cyclic sulphur compounds relative to peak areas of IS are given in Table 3.11.

Table 3.11 Within assay and between assay repeatability tests of the cyclic sulphur compounds in soil, at concentration levels of LOQ and 50 times LOQ. The values are given as peak areas of three selected m/z ratios for each of the cyclic sulphur compounds relative to peak areas of m/z 105+120 of 1,2,4-TMB.

		1,4-thioxane		**1,3-dithiolane**		**1,4-dithiane**	
		Within assay	**Between assay**	**Within assay**	**Between assay**	**Within assay**	**Between assay**
LOQ	1	0.0050	0.0052	0.0103	0.015	0.0076	0.0078
	2	0.0050	0.0058	0.0104	0.014	0.0079	0.0078
	3	0.0058	0.0054	0.0097	0.012	0.0083	0.0086
	4	0.0055	0.0050	0.0086	0.010	0.0073	0.0079
	5	0.0044	0.0049	0.0087	0.011	0.0075	0.0081
	6	0.0057	0.0055	0.0098	0.010	0.0083	0.0068
	Mean	0.0052	0.0053	0.0096	0.012	0.0078	0.0078
	SD	0.0005	0.0003	0.0008	0.002	0.0004	0.0006
	RSD (%)	9.9	5.8	8.0	17	5.6	7.8
50 x LOQ	1	0.56	0.55	1.31	1.28	0.68	0.67
	2	0.60	0.64	1.42	1.52	0.72	0.76
	3	0.64	0.52	1.52	1.31	0.76	0.56
	4	0.66	0.52	1.58	1.30	0.81	0.56
	5	0.64	0.63	1.51	1.32	0.75	0.69
	6	0.68	0.56	1.59	1.21	0.83	0.58
	Mean	0.63	0.57	1.49	1.33	0.76	0.64
	SD	0.04	0.05	0.11	0.10	0.06	0.08
	RSD (%)	6.9	9.5	7.2	7.8	7.3	13

The within assay repeatability test gave RSD values below 10% for all compounds at both concentration levels. Except for 1,3-dithiolane at LOQ and 1,4-dithiane at 50 times LOQ, the between assay tests also gave RSD values below 10%. Taken into consideration the complexity of the sample matrix, the precision of the analyses must be regarded as good.

Robustness

Method robustness was investigated with soil A as the sample matrix, for the thermostatting temperature, water to soil ratio and percentage salt saturation. A two-level factorial design experiment was set up with high and low values as listed in Table 3.12. The relatively large difference between high and low value of the water to soil ratio was chosen because these variations must be expected due to different water content of environmental soil samples. A soil sample of 2 g containing 10% water, which is added 2 ml of salt saturated water, will result in an actual water to soil ratio of 1.22. The percentage salt saturation will be 89% when the water from the sample is included. These conditions are simulated by the high level of water to soil ratio, and low level of salt saturation. The low level of percentage salt saturation was prepared by substituting 0.20 ml of the salt saturated water with pure type III water. This resulted in small differences in the percentage salt saturation between the water to soil ratio of 0.9 (89%), and 1.22 (91%).

Table 3.12 Analysis conditions for the robustness test for determination of cyclic sulphur compounds in soil.

	Low level	Method	High level
Thermostatting temperature (°C)	78.0	80.0	82.0
Water to soil ratio (ml:g)	0.900 (1.80:2.00)	1.00 (2.00:2.00)	1.22 (2.20:1.80)
Salt saturation (%)	89/91	100	100

All samples were prepared with an analyte concentration of 50 times LOQ, by adding 72 and 80 µl of validation solution 11 to 1.80 g and 2.00 g soil, respectively. Aliquots of 50 µl of the IS working solution were added to all slurry samples. The experiment set-up is shown in Table C.7 in Appendix, with peak areas relative to IS for each compound. The standard deviation of the analyses was established from four replicate analyses at the method level conditions. The effect of each parameter was calculated by Minitab, and is presented in Pareto charts in Figure C.4. Possible effects of altering analysis conditions on the IS peak area are also presented. It is clear from the figure that the water to soil ratio had a significant effect on the recovery of all analytes. On the other hand, the variations in thermostatting temperature and percentage salt saturation showed negligible effects on recoveries of all analytes. No effect was observed on the peak area of 1,2,4-TMB from any of the parameters. Thus, within the tested range the compound was not influenced by variations in water content of the sample. As the effects of thermostatting temperature and salt content could be neglected, the analyses were divided into three groups by the different water to soil ratios. Statistical t-tests at α=0.05 showed that the recoveries of all analytes were significantly lower at a water to soil ratio of 1.22 compared to the recoveries at water to soil ratios of 0.900 and 1.00. Compared to the method level, the decrease in recoveries varied from (13±6)% for 1,4-dithiane to (19±11)% for 1,4-thioxane.

The robustness test showed that the method was not vulnerable to variations in the thermostatting temperature of ± 2 °C, or in the percentage salt saturation from 89 to 100%. On the other hand, when the water to soil ratio increased to a level similar to 10% water content of the soil, the recoveries decreased by 13-19% (significant at α=0.05). Hence, for quantitative measurements, the added water volume may be reduced if the sample has a high concentration of water.

Recovery

Extraction recoveries were investigated for both soil types at concentrations of 50 times the LOQ levels. Samples simulating 100% recovery were prepared by adding the diluted compounds directly into the slurry. In that way, a complete extraction of the analytes from the soil into the water phase was simulated. For this purpose, 1.90 ml salt solution was added to 2.00 g of soil A, followed by 80 µl of validation solution 14 and 50 µl of the IS working solution (Table 2.9). The samples were analysed immediately after preparation. Validation solution 14 was prepared by dilution of stock solution 1 in water, not to alter the sample matrix conditions by introducing high amounts of acetone into the slurry.

Extraction recovery from soil A was calculated from the first four replicates of the within assay repeatability test. For soil B, four replicates were prepared with 80 µl of validation solution 11

(Table 2.9), and analysed according to the established method. Extraction recoveries of the analytes are given in Table 3.13. Calculations were performed both with and without IS correction of the peak areas. Raw data are given in Table C.8 in Appendix.

Table 3.13 Extraction recoveries of the cyclic sulphur compounds from soil, at concentrations of 50 times LOQ.

	% recovery ± SD (n=4)		
	1,4-thioxane	**1,3-dithiolane**	**1,4-dithiane**
Soil A	53 ± 4	51 ± 4	46 ± 3
Soil A, not corrected for IS	51 ± 1	49 ± 1	44 ± 1
Soil B	43 ± 4	48 ± 4	51 ± 4
Soil B, not corrected for IS	61 ± 5	68 ± 6	72 ± 5

With IS correction, the recoveries were 46-53% from soil A, and 43-51% from soil B. This means that approximately 50% of the added amounts were retained in the soils during thermostatting. Some of the loss of analytes may also be attributed to evaporation during sample preparation. In a comparable study using static headspace for determination of nine volatile organic carbons in various soil types, the average recoveries varied from 68 to 88% [70]. It should though be noted that stronger extraction conditions were applied in the compared investigation (thermostatting at 95 °C for 60 min).

There were good agreements between the calculations with and without IS for soil A. For soil B, however, there were discrepancies in the calculations of approximately 20%. The reason for this deviation was probably that the simulated 100% recovery samples were prepared in slurrys with soil A only. Thus, the calculations do not correct for possible different matrix effects of the two soils. It is seen in Table C.8 that the peak areas of the IS are considerably higher from the analyses of soil B, which gives lower relative peak areas of the compounds. Moreover, the peak areas of the analytes could be infuenced by different matrix effects of the two samples. In order to decide which values that were most correct, experiments with slurrys of soil B with simulated 100% recovery should be performed.

The discrepancy in the calculations with and without use of IS correction shows that care should be taken for quantitative calculations. If possible, calibrations should be performed on blank samples of the identical sample matrix. Alternatively, standard addition may be employed, as this technique is very well suited for correction of possible matrix effects.

3.2.7 Method validation, sulphur mustard

A complete method validation for trace determination of HD in soil was performed. The linearity, repeatability and robustness of the method were determined with soil A as the sample matrix. As the chromatographic background signal of soil B was acceptable at the retention time of HD (see Section 3.2.1), the detection limit was determined for both soils. In addition, recovery from both soils was examined.

Application of IS was problematic for determination of HD in soil, as large variations in the peak area of 1,2,4-TMB were observed. Equilibrium between the sample and vapour phase was probably not reached with the short thermostatting time of 5 min. The IS was included in the linearity and repeatability tests, but insignificant improvements were seen by use of IS correction. Thus, the use of IS was rejected for the present method, and only calculations without IS correction are presented in this work. For quantitative measurements, the use of external standard or standard addititon is a better approach.

Detection limit and quantification limit

The criterion set for detection limit of HD in soil was similar to the criterion described for determination in water (Section 3.1.6). Due to low background contamination in soil B at the retention time of HD, the LOD could be determined for both soils. Validation solutions 5-7 in Table 2.8 were used to establish the detection limits. At concentrations of 1 ng/g, the obtained S/N ratio was higher than ten in analyses of both soils, but the signal of the molecular ion was at the noise level. A sufficient signal intensity of the molecular ion was reached at a concentration of 3 ng/g. The criterion was met in three consecutive analyses of both soils at this concentration. The quantification limit was thus determined to 9 ng/g (three times the LOD).

The obtained LOD by HS-trap is two orders of magnitude better than what has been reported by HS-SPME GC-MS [61] (237 ng/g), and solvent extraction GC-MS [22] (0.2 µg/g). This proves the superior sensitivity of the technique for determination of HD in soil samples, and makes it promising for determination of other CWA as well, like several of the nerve agents.

Linearity

The linearity for determination of HD in soil was investigated within the concentration range of LOQ to 100 times LOQ. Spiking solutions were prepared at six concentration levels (validation solutions 15-20, Table 2.9). The soil samples were prepared as described in Section 2.5.1, with addition of 2.00 ml salt saturated solution immediately prior to analyses. Generally, the variations in peak area were higher for HD determination in soil, compared to determination of the degradation products. Thus, two replicates were analysed at each concentration level. Figure 3.29 shows the peak areas of *m/z* 109+111+158 as a function of concentration. Raw data are given in Table C.9 in Appendix.

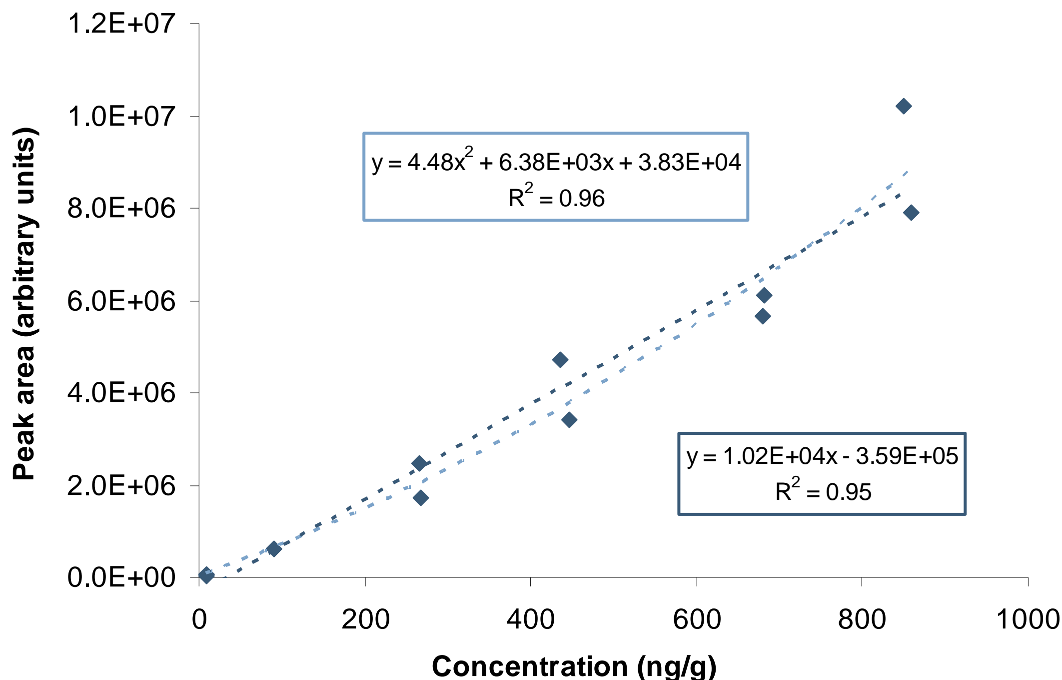

Figure 3.29 Peak area of HD as a function of concentration from LOQ to 100 times LOQ. Linear and polynomial regressions are shown.

In the regression calculations, each replicate is treated as a single point. With linear regression, an R^2 value of 0.95 was obtained. Slightly better correlation was achieved with polynominal regression, with an R^2 value of 0.96. The polynominal regression is more suited, especially at concentrations below ten times the LOQ, as the linear regression gives large errors in this region. As expected, the uncertainty in the plot was significantly higher compared to the linearity tests of the cyclic sulphur compounds.

Repeatability

Repeatability of the method was investigated with six replicates at concentration levels of LOQ and 50 times LOQ, both within assay and between assay. Validation solutions 15 and 18 in Table 2.9 were used for the sample preparations. Peak areas of the *m/z* 109+111+158 of HD are listed in Table 3.14.

Table 3.14 Within assay and between assay repeatability tests for determination of HD in soil, at concentration levels of LOQ and 50 times LOQ.

	Peak area *m/z* 109+111+158 (10^4)			
	LOQ		**50xLOQ**	
	Within assay	**Between assay**	**Within assay**	**Between assay**
1	4.5	4.3	4.0	3.3
2	3.3	3.7	5.2	3.3
3	4.1	3.3	4.7	4.0
4	3.6	5.5	3.4	3.4
5	3.3	4.7	5.1	5.0
6	5.1	2.7	4.4	4.3
Mean	4.0	4.0	4.5	3.8
SD	0.7	1.0	0.7	0.7
RSD (%)	18	25	15	19

The RSD values varied from 15 to 25%, with sligthly better presicion for the within assay repeatability tests. In general, the variatons were higher than what was obtained for the cyclic sulphur compounds. As discussed, HD is unstable in aqueous environment and at elevated temperatures. For this reason, somewhat higher variations have to be accepted for determination of HD with the present technique.

Robustness

The robustness of the method was investigated with a two-level factorial design experiment for the thermostatting temperature, water to soil ratio and percentage salt saturation. Due to the generally high variations in recovery of HD from soil, two replicates were analysed at each level. The replicates were analysed by performing full factorial design experiments on two different days. The overall standard deviation of the analyses was calculated from the variations in the replicates, instead of analysing replicates at the method levels. The high and low values of the thermostatting temperature were set to 72 and 68 °C, respectively. The values for water to soil ratio and percentage salt saturation were set similar to the robustness test for the cyclic sulphur compounds (Table 3.12). The samples were prepared from validation solution 18 (Table 2.9), at concentrations of approximately 450 ng/g. Peak areas from the measurements are shown in Table C.10 in Appendix. The effects of each parameter on the HD recovery are presented in a Pareto chart, shown in Figure 3.30.

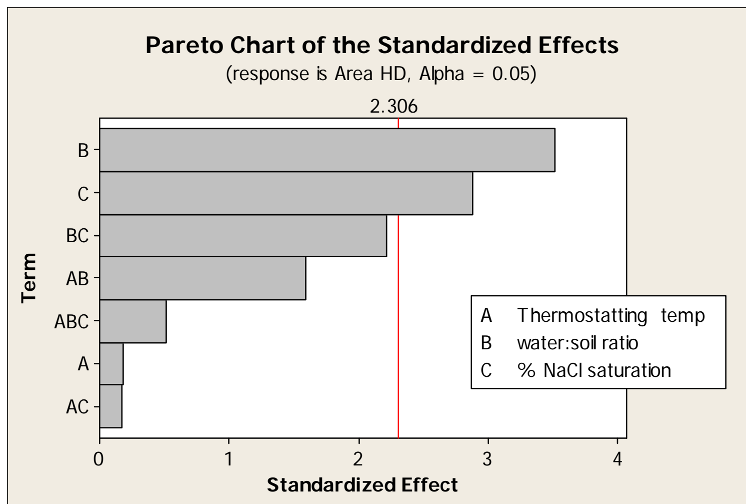

Figure 3.30 Data from the robustness test for HD determination in soil, treated in Minitab. The effects of the parameters on HD recovery are presented in a Pareto chart where the 95% confidencial limit is indicated with a vertical line.

The effects of both the water to soil ratio and the percentage salt saturation exceed the 95% significance level, indcated by the vertical line. No effect was observed from changing the thermostatting temperature. The salt content was more important for the HD recovery than for the recovery of the cyclic sulphur compounds, which is in agreement with the observed effects of salt saturation of the water samples (Figure 3.3).

The robustness test showed that the method precision was not affected by variations in the thermostatting temperature of ± 2 °C. However, the HD recovery was significantly influenced by changes both in the water to soil ratio and in the salt concentration. Hence, to secure a high recovery of HD from soil samples, the added 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.

Recovery
The extraction recovery of HD was investigated for both soils. Samples simulating 100% extraction recovery were prepared by adding an HD solution directly into the slurry of soil A and salt saturated water. Since the HD solution had to be prepared in acetone (validation solution 21, Table 2.9), it was made so that the proper concentration in the slurry was obtaind by addition of only 10 µl. This was done in order to not change the sample matrix conditions too much from the recovery samples. Aliquots of 2.00 g soil were weighed into the HS-vials, followed by 2.00 ml water and 10 µl of the validation solution. The samples were analysed immediately.

The recovery samples were prepared by adding 80 µl of validation solution 18 (Table 2.9) to 2.00 g samples, obtaining concentrations of 450 ng/g. Four replicates were analysed of each soil type. The average recoveries ± SD are presented in Table 3.15. Raw data are given in Table C.11 in Appendix.

Table 3.15 Extraction recovery of HD from soil at concentrations of 450 ng/g.

	% recovery ± SD (n=4)
Soil A	58 ± 9
Soil B	60 ± 3

The recoveries of HD were approximately 60% from both soil types, which was higher than the recoveries of the cyclic sulphur compounds. This shows that the procedure of adding salt saturated solution to the soil is an effective technique for HD determination in soil. The higher recoveries may also be related to less evaporation of HD during sample preparation, due to a lower vapour pressure (Table 1.2).

4 Analysis of a sediment sample from Skagerrak

In 2002, a project was carried out by FFI to investigate some ships loaded with chemical munition, scuttled in Skagerrak in 1945 [13]. A main issue of the investigation was analyses of sediment samples for determination of CWA and related compounds. The samples were collected at various positions around the wrecks. When examined in 2002, the samples were extracted with dichloromethane and analysed on GC-MS, according to the ROP for determination of CWA in soil [29]. Some of the samples were found to contain HD related compounds. After the investigation in 2002, the sediment samples have been stored at FFI in glass containers at –20 °C.

The developed method for trace determination of degradation products in water and soil was tested for analysis of one of the sediment samples. When analysed in 2002, six cyclic sulphur compounds related to HD were identified in this sample. In addition, three arsenic compounds were found, related to what is known as vomiting agents. As the sample contained a large fraction of water, the water phase was filtrated from the sediment and collected. The sediment phase contained approximately 40% water after filtration. An aliquot of 2.0 g of the sediment phase was weighed into an HS vial and added 2.00 ml salt saturated solution. The sample was analysed according to the method for determination of cyclic sulphur compounds in soil. An aliquot of 2.00 ml of the water phase was saturated with 0.80 g NaCl, and analysed by the method for determination of cyclic sulphur compounds in water. Figure 4.1 shows chromatograms from the analyses of both the water and the sediment phase. A chromatogram of a sample containing only salt saturated water is also shown.

An aliquot of the sediment phase was also prepared and analysed with the method for trace determination of HD in soil. The chromatogram showed no detectable amounts of HD in the sediment.

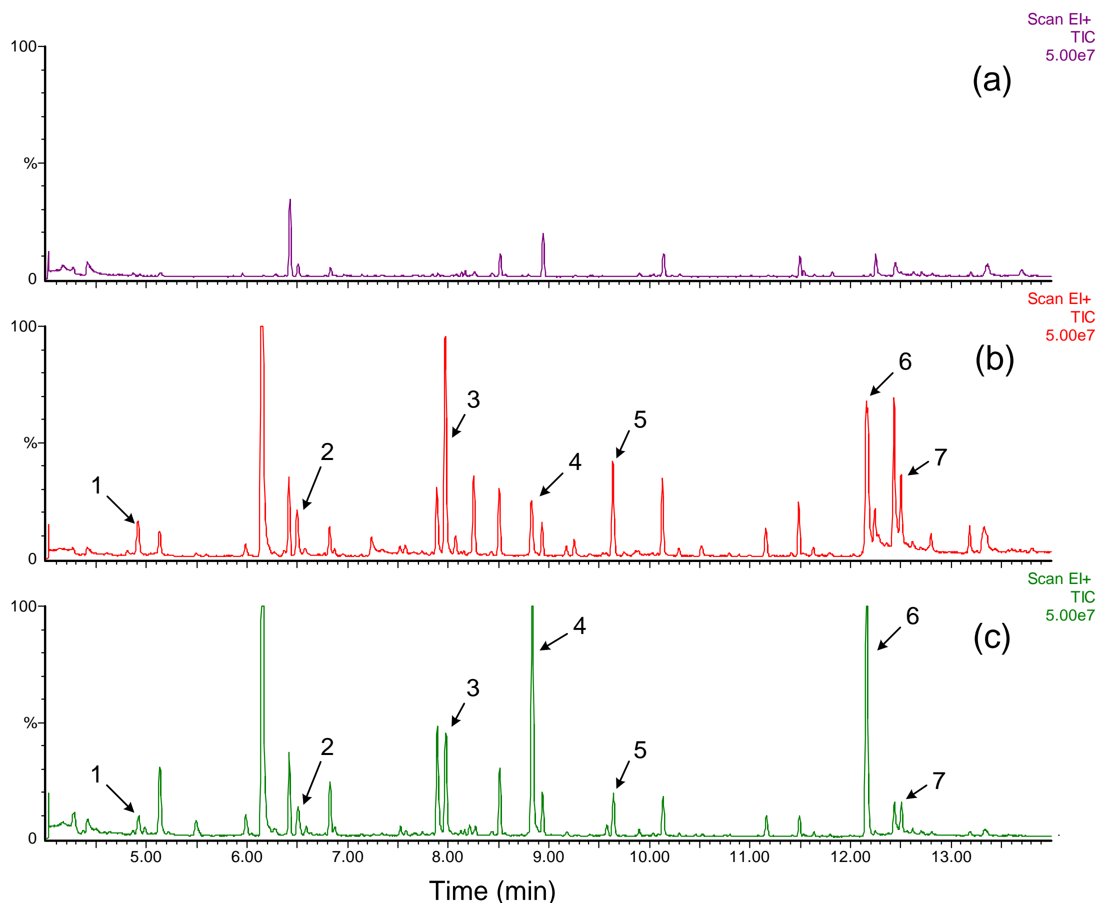

Figure 4.1 TIC chromatograms of a sediment sample collected in Skagerrak in 2002. (a) blank sample (2 ml salt saturated water), (b) water phase of the samlpe, (c) sediment phase. Seven identified cyclic sulphur compounds are marked from 1 to 7 in the chromatograms of both the water and sediment phase.

Seven different sulphur compounds were identified in both the water and the sediment phase, which could be related to sulphur mustard munition. The compounds numbered 1-3 in the chromatograms are identical to the three cyclic sulphur compounds used in the method development in the present study. These were identified by matching of the mass spectra with the compounds in standard solutions, and from retention times. The compounds numbered 4-7 were identified by matching of the mass spectra with respective spectra from the NIST library. All matchings with the library entries gave reverse fit factors above 950 (see Section 3.1.6). In addition, the retention indicies (RI) of the compounds were found in litterature, and the relative retention times were checked according the RIs. Spectra of each of the compounds from the sediment phase, with background subtraction, are shown in Figure 4.2.

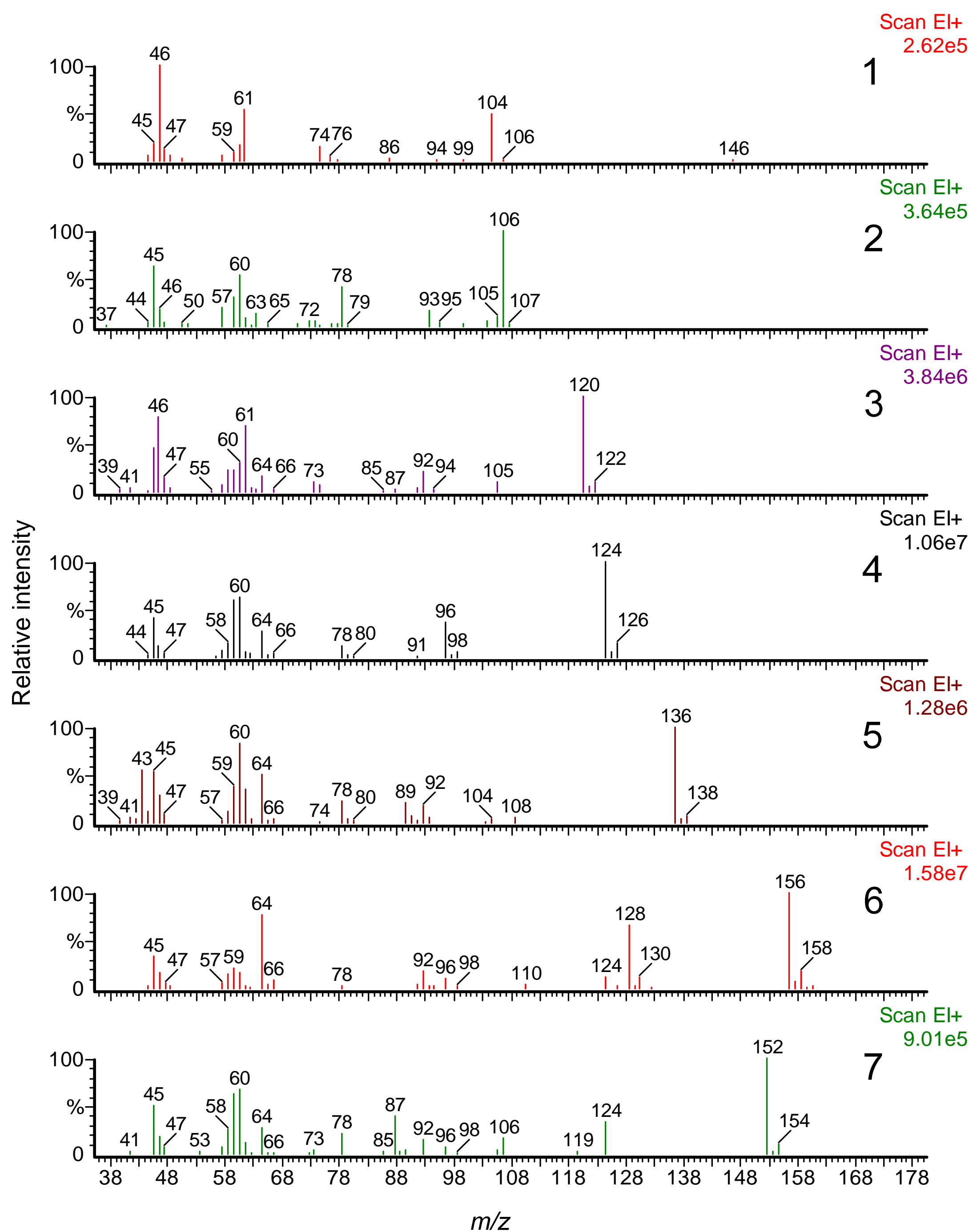

Figure 4.2 Spectra of the compounds numbered from 1 to 7 in the TIC chromatogram of the sediment phase (c) in Figure 4.1. The spectra are obtained with backgrund subtraction. The compounds are identified as 1) 1,4-thioxane, 2) 1,3-dithiolane, 3) 1,4-dithiane, 4) 1,2,3-trithiolane, 5) 1,4,5-oxadithiephane, 6) 1,2,3,4-tetrathiane, 7) 1,2,5-trithiephane.

The relationship to mustard munition for the identified compounds no 1-3 has been thoroughly discussed in Section 1.3-1.7. The two seven-ring compounds 1,4,5-oxadithiephane and 1,2,5-trithiephane, are also frequently reported in trace determination of sulphur mustard

munition [6,21,22,24]. The compounds identified as 1,2,3-trithiolane and 1,2,3,4-tetrathiane are less frequently mentioned. However, they have been reported to be present as impurities in undistilled sulphur mustard munition [7]. Interestingly, the compound 1,3-dithiolane could not be identified from the analyses by solvent extraction and GC-MS [13]. It was also seen that all peaks were higher and sharper in the TIC chromatograms of the present study. This was especially seen for the early eluting peaks, mainly due to a high background from the solvent in the analyses with solvent extraction.

When the sediment sample was analysed by solvent extraction GC-MS in 2002, diphenylchloroarsine, triphenylarsine and bis(diphenylarsine) oxide were found [13]. When analysed with the present developed HS-trap method, none of these compounds could be identified. This is most probably due to the low vapour pressures below $1 \cdot 10^{-3}$ mm Hg of the arsines, making them unsuitable for headspace extraction.

A blank sediment sample, collected some distance from the ships, was also analysed. The main part of the water phase was filtered from the sediment, and the two phases were analysed with the method for determination of cyclic sulphur compounds in water and soil. No detectable amounts of cyclic sulphur compounds were found in this sample.

5 Conclusion

New methods for trace determination of HD and related compounds by HS-trap GC-MS have been developed. Due to the low stability of HD, several possible degradation products were also investigated in this work. The analytes were determined in both water and soil samples.

Statistical design proved to be time saving for method development and validation, as it was possible to draw much information from relatively few experiments. It was especially useful in the robustness tests, to check if the variations in different parameters significantly affected the recovery.

By saturating the water samples with salt, the recovery of all analytes was considerably improved. The greatest improvement was achieved for HD. Likewise, addition of salt saturated solutions to different soil samples increased the recoveries of HD by a factor of six to nine compared to dry samples. The tested Tenax trap worked successfully for concentrating the analytes from the HS vapour phase, and thereafter release the water with no loss of analytes before introducing them into the chromatographic system. With application of the new HS-trap system and utilisation of the matrix modification techniques, HD could be determined even at ppb levels in water as well as in soil.

The obtained LOD for determination of HD in water was found to be 1.0 ng/ml with the MS in full scan mode. This is substantially better than what has been obtained by the recommended SPE or LLE procedures, followed by GC-MS or GC-FID. The LOD in soil was found to be 3 ng/g, which is an improvement in sensitivity by two orders of magnitudes compared to literature. The

present analysis technique showed to be even more sensitive for 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane. The obtained LODs in water and soil were determined to 0.1 ng/ml and 0.2-0.7 ng/g, respectively.

The optimal analysis conditions for HD differed considerably from those of the cyclic sulphur compounds, due to the low stability of HD in aqueous environment and at elevated temperatures. Therefore, separate methods were developed for determination of HD and for the cyclic compounds. For determination of the cyclic sulphur compounds, the same method could be applied for both water and soil, as the optimal instrumental parameters showed to be identical for the two matrices. On the other hand, separate methods were applied for HD determination in water and soil, but it might be possible that a common method could be used for these analyses as well.

Validation of the methods showed very good linearity and repeatability for determination of the cyclic sulphur compounds, within the tested range from LOQ to 100 times LOQ. Linearity for determination of HD in soil proved to be good as well, but due to the low stability in aqueous environment, a within assay precision of 15-20% RSD had to be accepted. The method for determination of HD in water was not validated, as quantitative measurements were of little interest.

The only sample preparation needed for HS-trap, was the addition of salt for saturation of the water samples, and addition of salt saturated solution to the soil samples. The total sample handling time for determination of the analytes, either in water or in soil, was less than one hour. This is a great improvement compared to the recommended SPE or LLE procedures followed by GC-MS analysis, which are both labour demanding and requires sample handling times of typically 4-5 hours.

The developed method for determination of cyclic sulphur compounds was applied on 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 found in this work as well. 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 retention in the soil. Hence, the method could be used for identifying volatile and semi-volatile compounds in similar future investigations.

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Appendix A Experimental design

A.1 Factorial design

This section describes the basic theory of factorial design, found in Box *et al* [71]. An example of a factorial design at two levels and with three variables (2^3 design), is given in Table A.1. The variables A, B and C are set to alter between low (-) and high (+) values, at all possible combinations. The response for each experiment could for instance be the integrated chromatographic peak area of a specific compound.

Table A.1 Example of a full factorial design at two levels and with three variables, where the responses are treated in a Yate's algorithm

	Variables				Yate's algorithm:				
Experiment	**A**	**B**	**C**	**Response**	**(I)**	**(II)**	**(III)**	**Effect values**	**Identi-fication**
1	-	-	-	34	79	152	413	51.6	Average
2	+	-	-	45	73	261	23	5.75	A
3	-	+	-	36	132	12	-9	-2.25	B
4	+	+	-	37	129	11	-23	-5.75	AB
5	-	-	+	60	11	-6	109	27.3	C
6	+	-	+	72	1	-3	-1	-0.25	AC
7	-	+	+	65	12	-10	3	0.75	BC
8	+	+	+	64	-1	-13	-3	-0.75	ABC

The last five columns in the table shows the response treated in a Yate's algorithm. Starting with column (I), the first four entries are obtained by adding pairs from the response column (34 + 45 = 79, 36 + 37 = 73, etc.). The last four entries are obtained by subtracting the bottom value from the top value in each pair (45 - 34 = 11, 37 - 36 = 1, etc.). The values obtained in column (I) are treated the same way in column (II), and this is repeated in column (III) for the values in column (II). Thus, the first entry in column (III) is the total sum of the results, and should be divided by 8 to obtain the average. The other values are divided by 4, to give the effect of a specified variable or the interaction of several variables. The effects are identified in the last column by locating the "+" signs in the design matrix. In the second row, a plus sign occurs only in the A column, so this is the main effect of A. In the fourth row, plus signs occur in both column A and B, so this is the interaction effect of AB.

From the Yate's algorithm, it is seen that alterations of variable C has the largest effect on the response (effect value 27.3), followed by variable A. It is also seen that variable A and B together have a considerable effect (-5.75), which means that they interacts. Further optimisation of variable A and B should therefore be performed by finding the best combination of the

parameters, for example with *simplex optimisation*. The other combinations have negligible effects on the result (effect values < ± 1).

An alternative way to present the responses is in a geometric structure, like the one presented in Figure A.1. This way, it is visualised that a high level of variable C has a positive effect on the results. Furthermore, it can bee seen that the positive effect due to a high level of variable A, is valid only when B is low. Thus, the effect of variable A is dependant on the value of variable B (interaction).

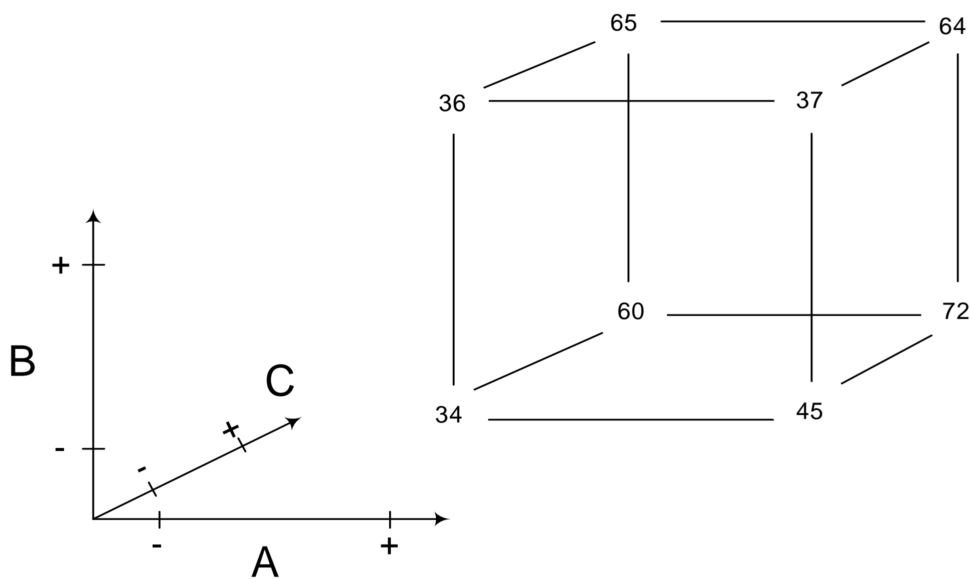

Figure A.1 Responses from a 2^3 factorial design presented in a geometric figure.

A.2 Simplex optimisation

The simplex optimisation procedure can be used in method development to find the values for a set of variables that gives the optimal response. In this section, the very basic simplex method is described, found in Walters *et al.* [72]. A simplex is a geometric figure that has a set of corners equal to one more than the number of dimensions in the factor space. Thus, for a set of two variables, three experiments with different combinations of the variables must be performed to produce a simplex. A two-factor simplex is represented in Figure A.2 with the dark blue lines, where the experiments are noted 1, 2 and 3. The response surface is indicated by the light blue curves. From the obtained response values, a new set of values for variable A and B is determined for the following analysis. This is done by rejecting the set of values that gives the lowest response (experiment 1), and project the point through the average of the two remaining points (2 and 3). Thus, the next simplex is defined by experiment 2, 3 and 4. This procedure is repeated until the response decreases, as is the case for experiment 8.

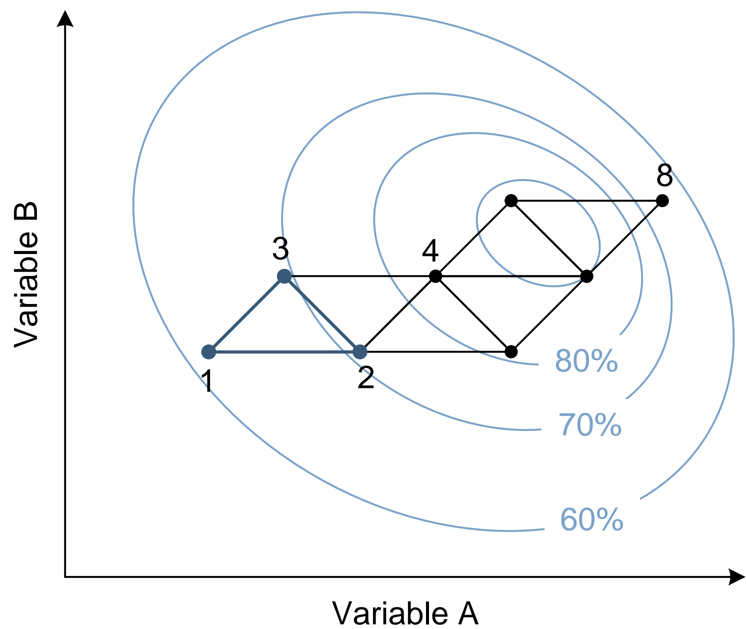

Figure A.2 Example of a two-factor simplex optimisation, starting with experiments number 1, 2 and 3. A total of 8 experiments were performed to find the optimal region in the plane.

Appendix B Trace determination of analytes in water

B.1 Method development

Table B.1 Stability test of the cyclic compounds in water. Peak areas are given relative to IS from the TIC chromatograms.

Elapsed time after preparation	Peak area relative to IS		
hours	1,4-thioxane	1,3-dithiolane	1,4-dithiane
0.133	1.45	1.13	1.23
1.02	1.46	1.15	1.28
2.05	1.52	1.17	1.30
3.07	1.49	1.15	1.27
4.10	1.50	1.16	1.29
5.13	1.53	1.20	1.31
6.15	1.47	1.14	1.27
12.5	1.58	1.24	1.37
13.0	1.51	1.17	1.33
19.5	1.52	1.19	1.32
22.8	1.53	1.20	1.32
23.3	1.52	1.20	1.33
24.0	1.59	1.24	1.38
38.5	1.61	1.21	1.38
39.0	1.66	1.29	1.46

Table B.2 Stability test for HD in water. Peak areas are given relative to IS from the TIC chromatograms.

Elapsed time after preparation hours	Peak area relative to IS
0.133	3.31
0.717	2.50
1.30	1.46
1.88	1.04
2.47	0.665
3.05	0.463
3.63	0.303
4.22	0.225

Table B.3 Peak areas of the molecular ions of the analytes, with and without salt saturation of the water samples.

		Peak area (10^4)			
		1,4-thioxane	**1,3-dithiolane**	**1,4-dithiane**	**HD**
		***m/z* 104**	***m/z* 106**	***m/z* 120**	***m/z* 158**
Without salt		3.16	5.59	4.34	0.20
		3.16	5.28	4.16	0.23
		3.32	5.38	4.35	0.19
	Mean	3.21	5.42	4.28	0.21
	SD	0.09	0.16	0.10	0.02
Salt added (40 w/v%)		11.49	17.5	16.5	9.0
		11.41	17.5	16.9	9.3
		11.57	18.0	16.9	7.8
	Mean	11.49	17.7	16.8	8.7
	SD	0.08	0.3	0.3	0.8

Table B.4 Peak areas of the molecular ions of the cyclic sulphur compounds, determined at various desorption pressure and desorption time.

Desorption pressure	**Desorption time**	**Peak area (10^4)**		
		1,4-thioxane	**1,3-dithiolane**	**1,4-dithiane**
psi	**min**	***m/z* 104**	***m/z* 106**	***m/z* 120**
15	0.3	1.52	1.97	1.58
	0.5	1.39	1.83	1.77
	0.7	1.37	1.73	1.54
25	0.1	nd	nd	nd
	0.2	0.431	0.648	0.449
	0.3	2.69	3.36	2.78
	0.5	2.71	3.50	3.00
	0.7	2.97	3.72	3.43
35	0.3	4.07	4.81	4.23
	0.5	3.97	5.02	4.40
	0.7	4.10	5.02	4.52

Table B.5 Peak areas of the molecular ions of the analytes from a full factorial design experiment for sample volume, thermostatting temperature and thermostatting time. Peak areas from three analyses with intermediate values of the variables are also given.

Sample volume	Therm. temp	Therm. time	Peak area (10^5)			
			1,4-thioxane	1,3-dithiolane	1,4-dithiane	HD
ml	°C	min	*m/z* 104	*m/z* 106	*m/z* 120	*m/z* 158
2	50	15	0.90	1.46	1.34	1.82
10	50	15	0.43	0.71	0.67	0.91
2	80	15	3.12	3.93	3.89	nd
10	80	15	1.49	2.12	2.07	nd
2	50	30	0.87	1.42	1.33	0.70
10	50	30	0.39	0.65	0.59	0.20
2	80	30	3.10	3.99	3.98	0.07
10	80	30	1.52	2.22	2.18	nd
6	65	22.5	0.87	1.43	1.25	nd
6	65	22.5	0.79	1.27	1.10	nd
6	65	22.5	1.01	1.60	1.53	nd
		SD	0.11	0.17	0.2	-

Table B.6 Method optimisation of sample volume, thermostatting temperature and thermostatting time for determination of the cyclic sulphur compounds. Peak areas of the molecular ions are given.

Analysis	Sample volume	Therm. temp	Therm. time	Peak area (10^4)		
				1,4-thioxane	1,3-dithiolane	1,4-dithiane
	ml	°C	min	*m/z* 104	*m/z* 106	*m/z* 120
1	2	80	2.5	3.24	4.32	4.33
2	2	80	2.5	3.50	4.51	4.46
3	2	80	5	4.26	5.29	5.18
4	2	80	5	4.46	5.59	5.49
5	2	80	10	4.74	5.81	5.75
6	2	80	10	5.01	5.86	5.94
7	2	80	15	4.99	5.82	6.04
8	2	80	15	4.96	5.86	6.22
9	2	80	20	4.60	5.51	5.66
10	2	90	5	5.51	6.39	6.61
11	2	90	10	6.45	6.54	6.19
12	2	90	15	6.22	6.23	6.00
13	2	90	20	5.29	6.50	6.39
14	4	80	2.5	2.19	3.07	2.91
15	4	80	5	3.24	4.19	4.19
16	4	80	10	3.53	4.72	4.47
17	4	80	15	4.13	5.36	5.42

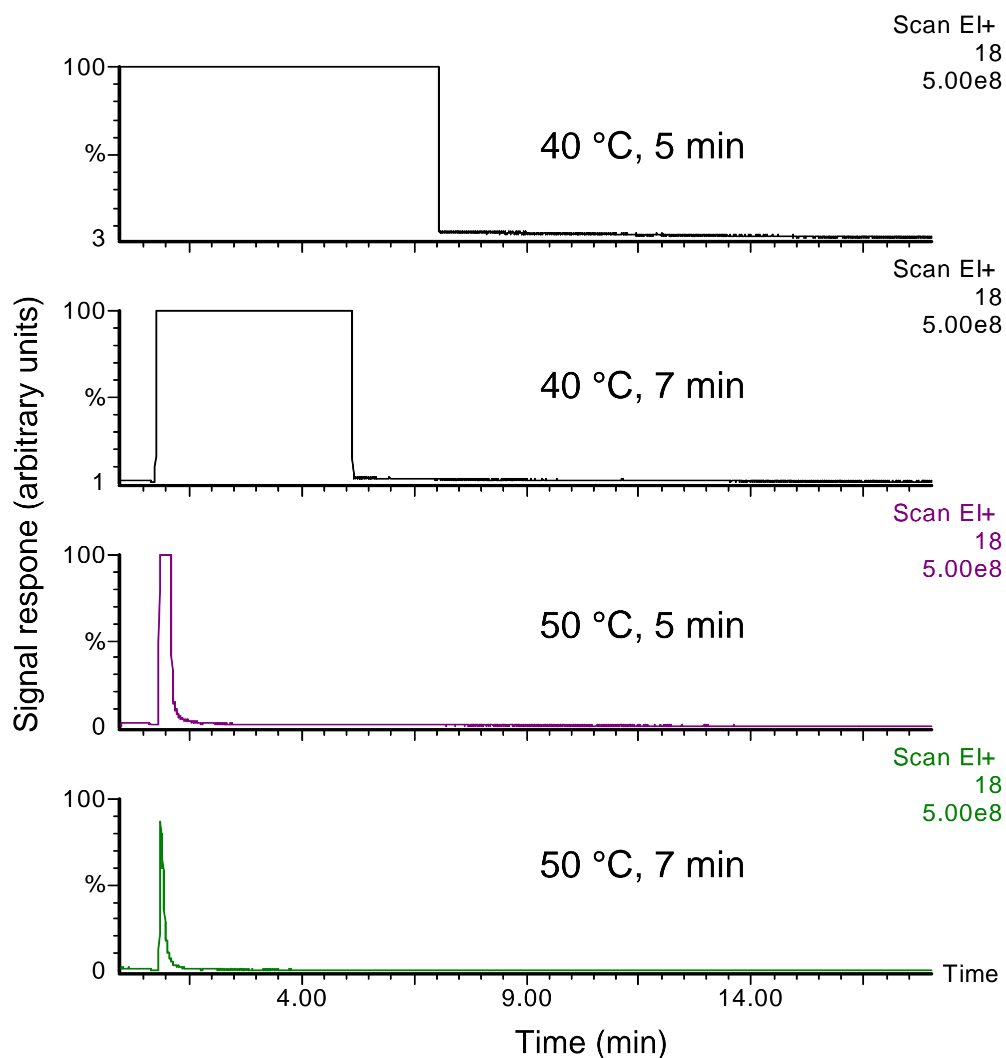

Figure B.1 Responses of the m/z 18 ion of water, at various trap low temperature and dry purge time. The analyses was performed with three successive vial extractions, and a desorption pressure of 30 psi.

Table B.7 Investigation of analyte adsorption on the trap during the dry purge step, at different settings of the dry purge time and trap low temperature.

Dry purge time (min)	Trap low temp (°C)	Replicate	Peak area (10^5) 1,4-thioxane *m/z* 104	1,3-dithiolane *m/z* 106	1,4-dithiane *m/z* 120
5	40	1	3.89	4.75	4.38
		2	3.92	4.76	4.39
		3	3.96	4.81	4.41
		4	4.01	4.89	4.53
		Mean	3.94	4.80	4.43
		SD	0.05	0.06	0.07
7	50	1	3.92	4.84	4.44
		2	4.03	4.86	4.54
		3	4.03	4.86	4.51
		4	3.96	4.89	4.48
		Mean	3.99	4.86	4.49
		SD	0.05	0.02	0.04

The sample solution was prepared by diluting 200 µl of working solution 7 (Table 2.3) in 20 ml water, giving concentrations of 39 ng/ml for 1,4-thioxane, and 19 ng/ml for 1,3-dithiolane and 1,4-dithiane. Sample amounts of 2.00 ml were transferred to the HS vials and saturated with NaCl.

Table B.8 Peak areas from analyses of the cyclic sulphur compounds in water with use of one, two and three successive vial extractions.

Number of extractions	Replicate	Peak area (10^5) 1,4-thioxane *m/z* 104	1,3-dithiolane *m/z* 106	1,4-dithiane *m/z* 120
1	1	0.63	0.70	0.65
	2	0.64	0.75	0.69
	3	0.63	0.75	0.68
	4	0.66	0.74	0.71
	Mean	0.64	0.73	0.68
	SD	0.01	0.03	0.02
2	1	1.00	1.08	0.96
	2	1.11	1.20	1.08
	3	1.11	1.22	1.09
	4	1.22	1.32	1.21
	Mean	1.11	1.20	1.21
	SD	0.09	0.10	0.11
3	1	1.46	1.50	1.22
	2	1.51	1.52	1.29
	3	1.43	1.39	1.21
	4	1.31	1.26	1.08
	Mean	1.43	1.42	1.20
	SD	0.09	0.12	0.09

B.2 Method validation

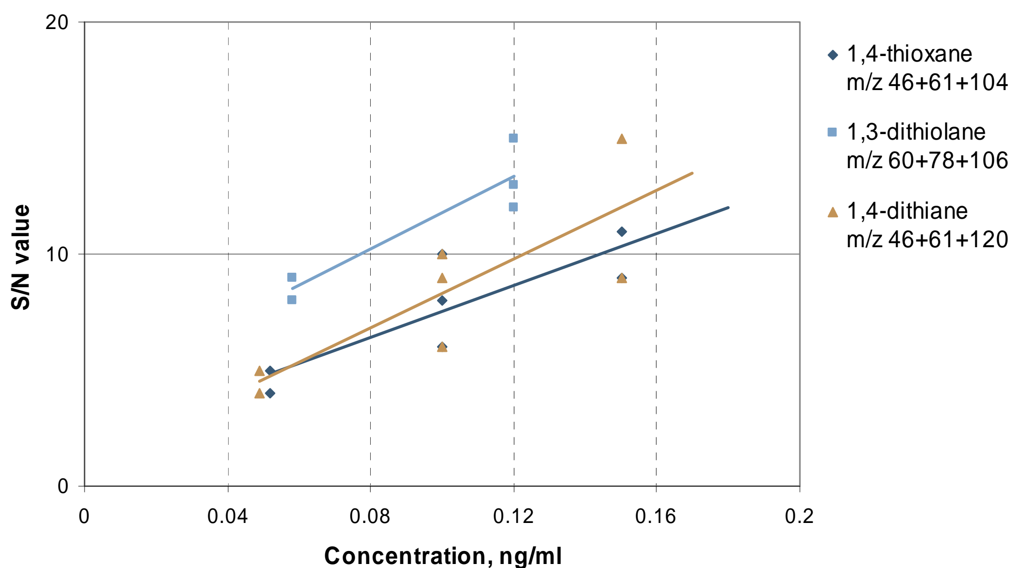

Figure B.2 S/N values of three extracted m/z ratios from analyses of the cyclic sulphur compounds, plotted as a function of concentration. Linear regression curves through the plots are given.

Table B.9 Linear regression of the plots in Figure B.2, with calculated LODs ± one standard error.

Compound	Linear regression	Standard error	Calculated LOD (S/N=10) ± one standard error
1,4-thioxane	$y = 56.0 \cdot x + 1.94$	1.52	0.14 ± 0.03
1,3-dithiolane	$y = 78.0 \cdot x + 3.98$	1.31	0.08 ± 0.02
1,4-dithiane	$y = 74.3 \cdot x + 0.881$	2.33	0.12 ± 0.03

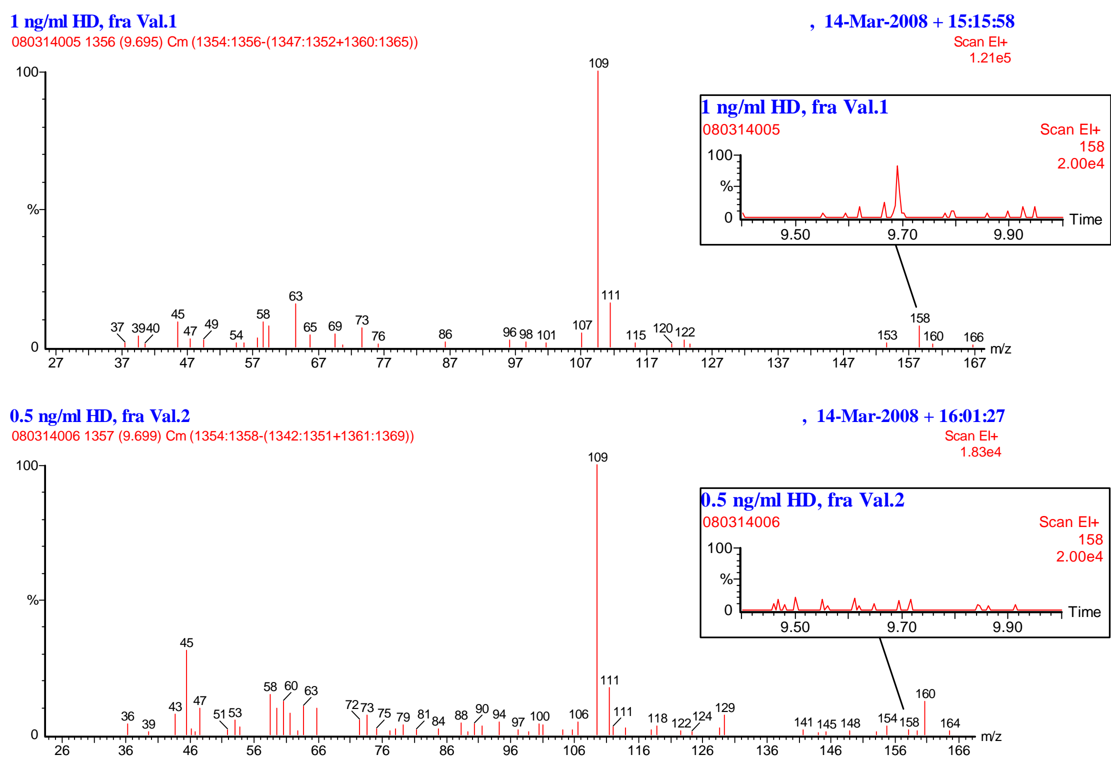

Figure B.3 Spectra of HD with background subtraction, at concentrations of 1.0 ng/ml (upper spectrum) and 0.5 ng/ml (lower spectrum). Chromatograms of the extracted molecular ion (m/z 158) from TIC chromatograms are shown in the frames.

Table B.10 Linearity test of the method for trace determination of cyclic sulphur compounds in water, from LOQs to 100 times LOQs. The peak areas are corrected for the extracted m/z 105+120 of 1,2,4-TMB, at a concentration of 4 ng/ml.

	1,4-thioxane *m/z* 46+61+104		**1,3-dithiolane *m/z* 60+78+106**		**1,4-dithiane *m/z* 46+61+120**	
Concentration level	**ng/ml**	**peak area**	**ng/ml**	**peak area**	**ng/ml**	**peak area**
1	0.402	0.0124	0.231	0.00755	0.345	0.00844
2	4.08	0.204	2.34	0.143	3.50	0.192
3	12.2	0.751	7.04	0.512	10.5	0.801
4	20.5	1.40	11.8	0.967	17.6	1.49
5	30.9	2.00	17.8	1.45	26.6	2.25
6	40.9	2.85	23.5	1.99	35.2	3.11
R^2, linear regression	0.997		0.998		0.998	
R^2, polynomial regression (level 1-4)	0.9999		0.9999		0.9994	

Table B.11 Robustness test of the method for trace determination of cyclic sulphur compounds in water. A 2^3 factorial design experiment was set up for the sample volume, thermostatting temperature and vial pressure. In addition, three replicate analyses were performed with the parameter values established in the method.

Sample volume	**Therm. temp**	**Vial pressure**	**Peak area, relative to 1,2,4-TMB**		
			1,4-thioxane	**1,3-dithiolane**	**1,4-dithiane**
ml	**°C**	**psi**	***m/z* 46+61+104**	***m/z* 60+78+106**	***m/z* 46+61+120**
1.95	78	39	0.908	0.660	1.055
2.05	78	39	0.971	0.708	1.119
1.95	82	39	1.030	0.726	1.145
2.05	82	39	1.015	0.715	1.130
1.95	78	41	0.915	0.660	1.081
2.05	78	41	0.881	0.660	1.071
1.95	82	41	0.989	0.709	1.126
2.05	82	41	0.983	0.706	1.123
2.00	80	40	0.952	0.684	1.077
2.00	80	40	0.931	0.676	1.100
2.00	80	40	0.929	0.670	1.066
		SD (n=3)	0.013	0.007	0.017

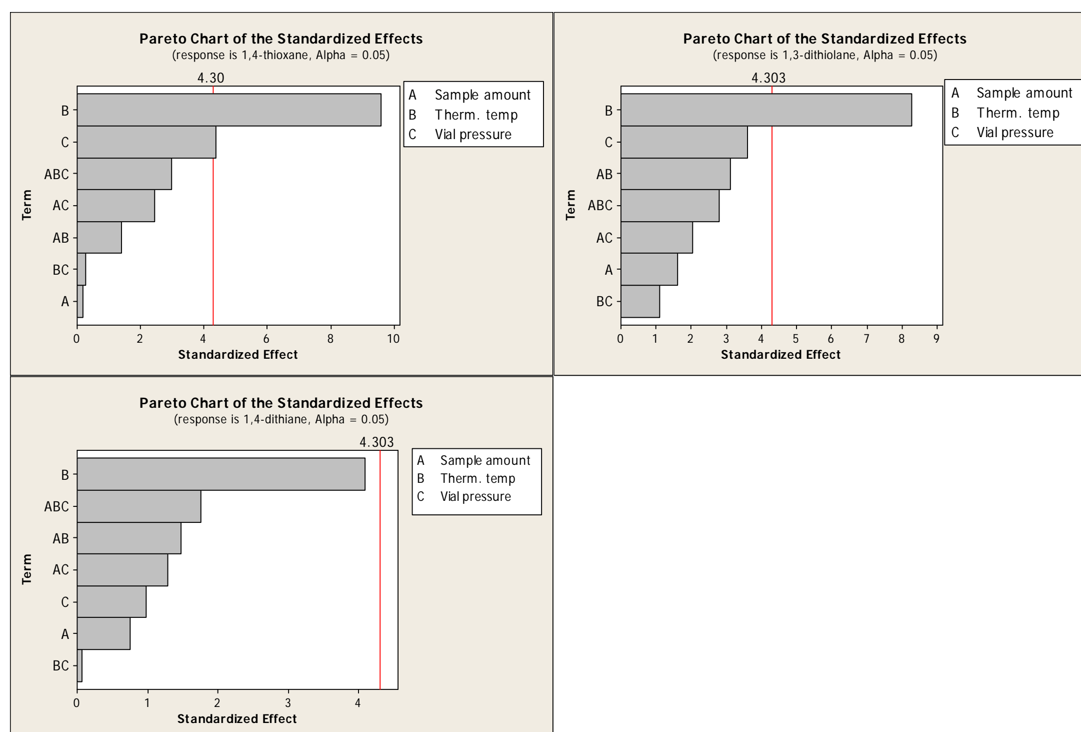

Figure B.4 Data from the robustness test presented in Table B.7, treated in Minitab. The standardised effects on each analyte are presented in Pareto charts, where the 95% confidencial limits are indicated with red lines. SD of the experiments is determined from the analyses at the mehod levels (n=3).

B.3 Natural water samples

The stock solutions used for preparation of calibration solutions are the same as used for the linearity test, described in Section 2.3.3. Working solution 1 and 2 were prepared by diluting 100 µl of stock solution 1 in 50 ml water, and 50 µl of stock solution 2 in 250 ml water, respectively. The transfers of stock solutions were weighed with an accuracy of 0.1 mg. Calibration solutions were prepared at five concentration levels from LOQ to 50 times the LOQ, by diluting various amounts of working solution 1 in 100 ml water. Aliquots of 2.00 ml of the calibration solutions were transferred to HS-vials, and added 20 µl (weighed amount) of the 1,2,4-TMB working solution. The HS vials were added 0.80 g NaCl in advance. Concentrations of the calibration solutions are given in Table B.8, with two replicates at each concentration level.

Table B.12 Preparation of calibration solutions and spiking solutions for recovery tests on natural water samples. All concentrations are in ng/ml.

		1,4-thioxane	**1,3-dithiolane**	**1,4-dithiane**	**1,2,4-TMB**
Stock solution	1	$419 \cdot 10^3$	$241 \cdot 10^3$	$360 \cdot 10^3$	
	2				$1998 \cdot 10^3$
Working solution	1	824	474	708	
	2				355
Calibration solution	1a	0.403	0.232	0.346	3.77
	1b	0.403	0.232	0.346	3.80
	2a	1.252	0.720	1.075	3.88
	2b	1.252	0.720	1.075	3.91
	3a	4.11	2.37	3.53	3.76
	3b	4.11	2.37	3.53	3.80
	4a	12.3	7.09	10.6	3.78
	4b	12.3	7.09	10.6	3.78
	5a	20.6	11.8	17.7	3.80
	5b	20.6	11.8	17.7	3.84

Figure B.5 and B.6 shows the calibration plots with regression curves. Polynomial regression was used from LOQ to 10 times the LOQ, and linear regression was used from 10 to 50 times the LOQ.

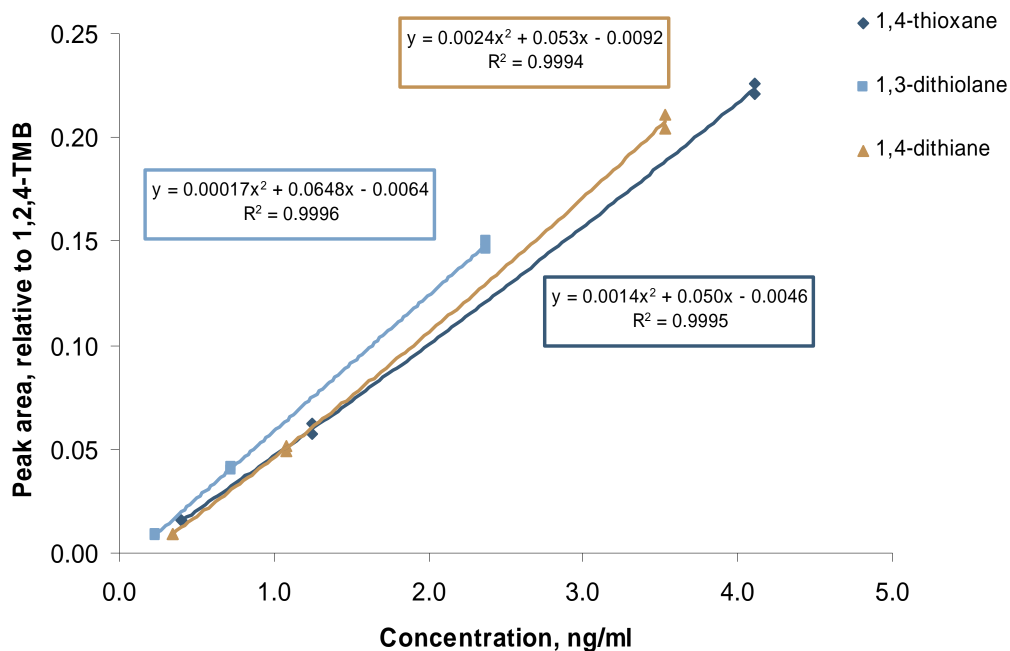

Figure B.5 Calibration plots from LOQ to 10 times LOQ for the cyclic sulphur compounds, with polynomial regressions.

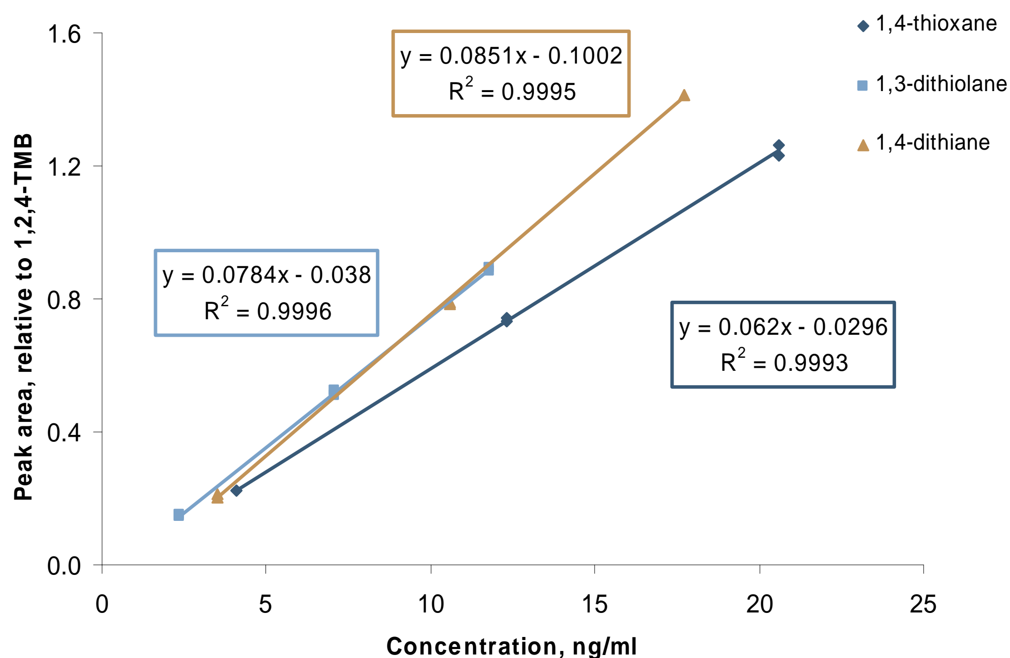

Figure B.6 Calibration plots from 10 to 50 times LOQ for the cyclic sulphur compounds, with linear regressions.

Recoveries of the spiked samples at two concentration levels are given for 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane in Table B.9 to B.11.

Table B.13 Recoveries of 1,4-thioxane from spiked samples at two concentration levels. The spiked and calculated amounts are given in ng/ml.

		Replicate no				
Sample		**1**	**2**	**3**	**Mean**	**SD**
Rain water	Spiked amount	1.236	1.236	1.236		
	Calculated amount	1.203	1.233	1.263		
	% recovery	97.3	99.7	102.2	99.7	2.4
	Spiked amount	12.50	12.50	12.50		
	Calculated amount	10.99	11.57	11.56		
	% recovery	87.9	92.6	92.5	91.0	2.9
River water	Spiked amount	1.256	1.256	1.256		
	Calculated amount	1.130	1.093	1.184		
	% recovery	89.9	87.0	94.3	90.4	3.7
	Spiked amount	12.53	12.53	12.53		
	Calculated amount	10.92	11.34	11.12		
	% recovery	87.1	90.5	88.7	88.8	1.7
Seawater	Spiked amount	1.232	1.232	1.232		
	Calculated amount	1.234	1.185	1.211		
	% recovery	100.2	96.2	98.3	98.2	2.0
	Spiked amount	12.45	12.45	12.45		
	Calculated amount	12.10	11.72	11.74		
	% recovery	97.2	94.1	94.3	95.2	1.7

Table B.14 Recoveries of 1,3-dithiolane from spiked samples at two concentration levels. The spiked and calculated amounts are given in ng/ml.

Sample		Replicate no 1	2	3	Mean	SD
Rain water	Spiked amount	0.7107	0.7107	0.7107		
	Calculated amount	0.6844	0.7368	0.7437		
	% recovery	96.3	103.7	104.6	101.5	4.6
	Spiked amount	7.189	7.189	7.189		
	Calculated amount	6.485	6.667	6.700		
	% recovery	90.2	92.7	93.2	92.0	1.8
River water	Spiked amount	0.7224	0.7224	0.7224		
	Calculated amount	0.6627	0.6559	0.6914		
	% recovery	91.7	90.8	95.7	92.7	2.6
	Spiked amount	7.206	7.206	7.206		
	Calculated amount	6.283	6.497	6.498		
	% recovery	87.2	90.2	90.2	89.2	1.7
Seawater	Spiked amount	0.7083	0.7083	0.7083		
	Calculated amount	0.7060	0.7214	0.6527		
	% recovery	99.7	101.8	92.2	97.9	5.1
	Spiked amount	7.162	7.162	7.162		
	Calculated amount	6.842	6.751	6.914		
	% recovery	95.5	94.3	96.5	95.4	1.1

Table B.15 Recoveries of 1,4-dithiane from spiked samples at two concentration levels. The spiked and calculated amounts are given in ng/ml.

Sample		Replicate no 1	2	3	Mean	SD
Rain water	Spiked amount	1.094	1.096	1.113		
	Calculated amount	1.061	1.061	1.061		
	% recovery	103.1	103.3	104.9	103.8	1.0
	Spiked amount	9.501	9.755	9.881		
	Calculated amount	10.74	10.74	10.74		
	% recovery	88.5	90.8	92.0	90.4	2.0
River water	Spiked amount	1.049	1.003	1.095		
	Calculated amount	1.079	1.079	1.079		
	% recovery	97.2	92.9	101.5	97.2	4.3
	Spiked amount	9.285	9.913	9.578		
	Calculated amount	10.76	10.76	10.76		
	% recovery	86.3	92.1	89.0	89.1	2.9
Seawater	Spiked amount	1.109	1.038	1.050		
	Calculated amount	1.058	1.058	1.058		
	% recovery	104.8	98.1	99.2	100.7	3.6
	Spiked amount	10.57	9.995	10.11		
	Calculated amount	10.70	10.70	10.70		
	% recovery	98.8	93.4	94.4	95.5	2.8

Appendix C Trace determination of analytes in soil

C.1 Chromatographic background of soil A and soil B

The drying conditions had a considerable influence on the chromatographic background signal of soil A. Figure C.1 shows chromatograms of soil A, dried for 24 hours at various conditions.

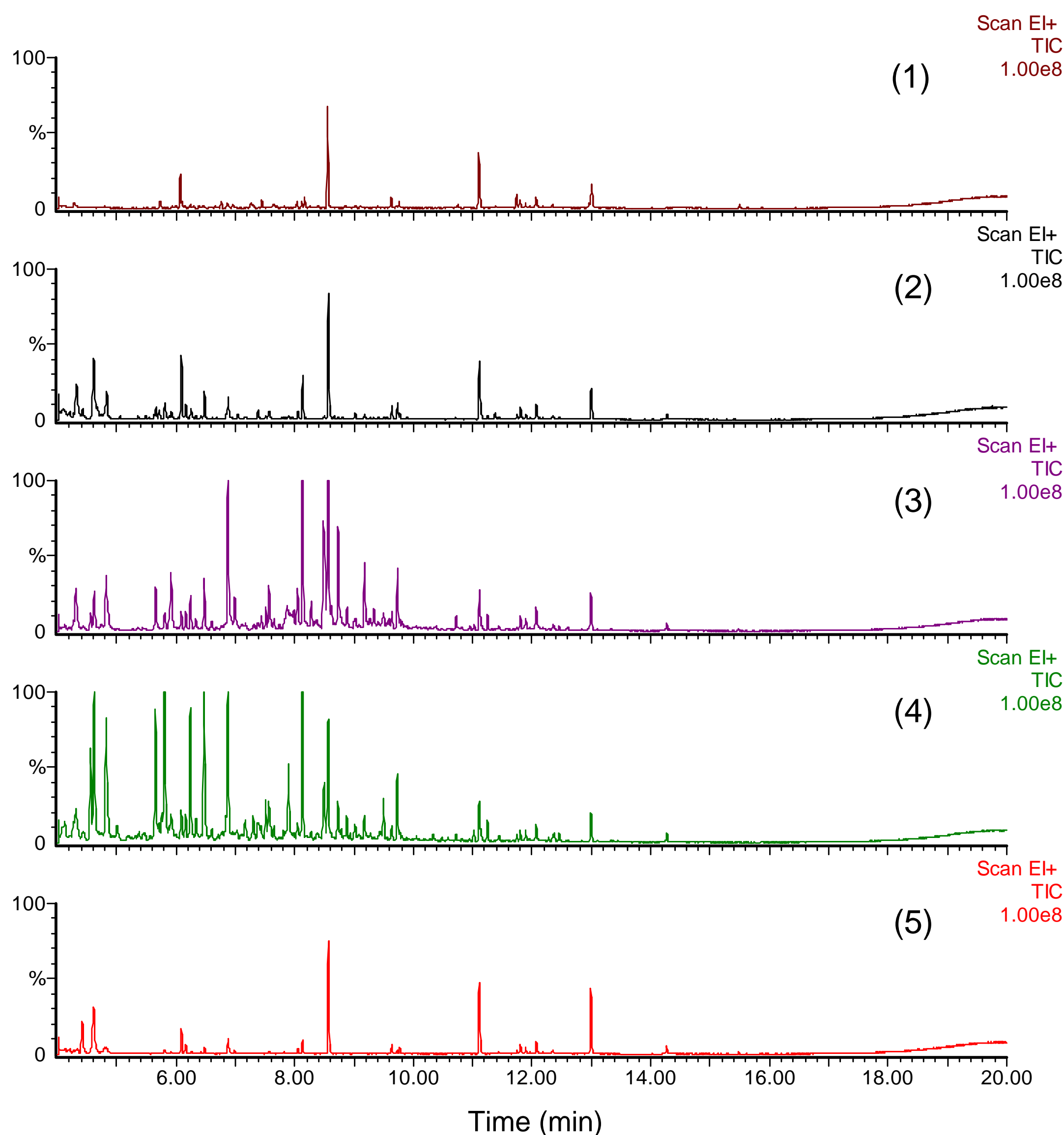

Figure C.1 TIC chromatograms of soil A, dried at various conditions. (1) soil not dried, (2) dried at 50 °C under nitrogen atmosphere, (3, 4, 5) dried in air atmosphere at 50, 105 and 550 °C, respectively.

Aliquots of 1 g soil were weighed into HS vials, and added 1 ml salt saturated water. The samples were analysed at 80 °C thermostatting temperature for 15 min. One vial extraction was performed.

Soil B gave a considerable higher chromatographic background signal, compared to soil A. Figure C.2 shows chromatograms of soil B, not dried (1), and dried at 50 °C under nitrogen atmosphere (2). Note that the intensity scale is increased with a factor of 10, compared to the chromatograms in Figure C.1.

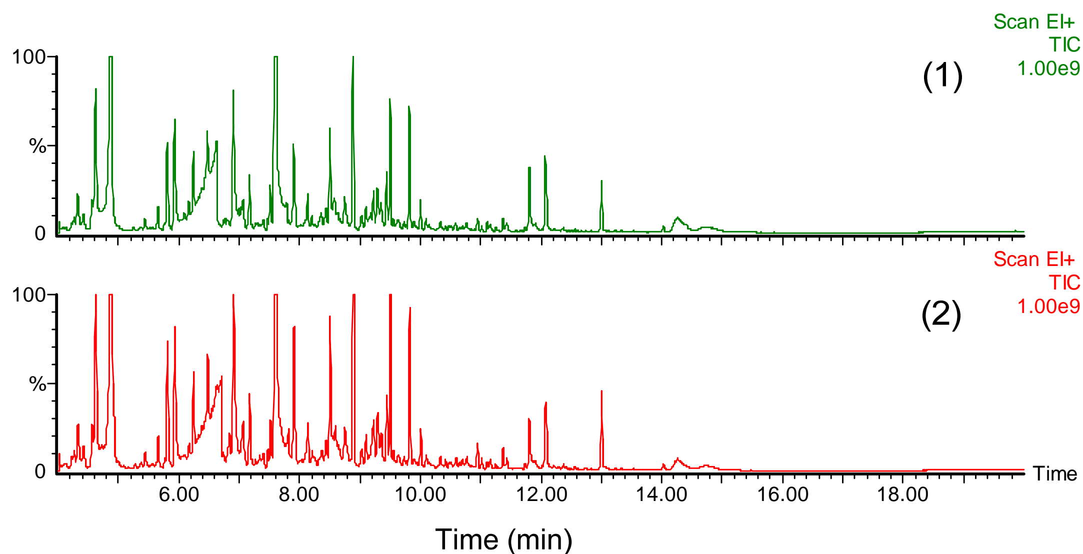

Figure C.2 TIC chromatograms of soil B. (1) soil not dried, (2) dried at 50 °C under nitrogen atmosphere.

Aliquots of 1 g soil were weighed into HS vials, and added 1 ml salt saturated water. The samples were analysed at 80 °C thermostatting temperature for 15 min. One vial extraction was performed.

C.2 Method development

Table C.1 Peak areas from analyses of the cyclic sulphur compounds in soil A, with various amounts of soil and added water (salt-saturated solution).

				Peak area (10^5)	
Amount of soil (g)	**Added water (ml)**	**Replicate**	**1,4-thioxane *m/z* 104**	**1,3-dithiolane *m/z* 106**	**1,4-dithiane *m/z* 120**
1.0	0	1	2.68	1.75	1.43
		2	2.89	1.81	1.49
		3	2.81	1.84	1.47
		Mean	2.79	1.80	1.46
		SD	0.11	0.05	0.03
1.0	0.50	1	3.30	3.02	3.19
		2	3.50	3.14	3.30
		3	3.49	3.21	3.34
		Mean	3.43	3.12	3.28
		SD	0.11	0.09	0.08
1.0	1.0	1	3.25	3.40	3.36
		2	3.26	3.34	3.30
		3	3.43	3.44	3.39
		Mean	3.31	3.39	3.35
		SD	0.10	0.05	0.05
1.0	2.0	1	2.16	2.51	2.52
		2	2.09	2.49	2.56
		3	2.14	2.36	2.54
		Mean	2.13	2.46	2.54
		SD	0.04	0.08	0.02
2.0	2.0	1	3.32	3.53	3.64
		2	3.21	3.48	3.40
		3	3.52	3.65	3.49
		Mean	3.3	3.55	3.51
		SD	0.2	0.09	0.12
3.0	3.0	1	3.08	3.57	3.78
		2	2.98	3.19	3.62
		Mean	3.0	3.4	3.7

Table C.2 Peak areas from analyses of the cyclic sulphur compounds in soil B, with various amounts of soil and added water (salt-saturated solution).

			Peak area (10^5)		
Amount of soil (g)	**Added water (ml)**	**Replicate**	**1,4-thioxane *m/z* 104**	**1,3-dithiolane *m/z* 106**	**1,4-dithiane *m/z* 120**
1.0	0	1	2.38	2.64	2.25
		2	2.49	2.65	2.35
		3	2.50	2.64	2.36
		Mean	2.46	2.65	2.32
		SD	0.07	0.01	0.06
1.0	0.50	1	3.77	4.19	6.06
		2	3.58	3.94	5.62
		3	3.38	3.76	5.49
		Mean	3.6	4.0	5.7
		SD	0.2	0.2	0.3
1.0	1.0	1	4.05	5.31	8.40
		2	3.57	4.69	7.36
		3	3.85	4.87	8.09
		Mean	3.8	5.0	8.0
		SD	0.2	0.3	0.5
1.0	2.0	1	2.05	2.77	4.93
		2	2.02	2.66	4.72
		3	2.17	2.87	4.98
		Mean	2.08	2.76	4.88
		SD	0.08	0.11	0.14
2.0	2.0	1	2.36	3.32	5.72
		2	3.49	4.91	8.08
		3	3.19	4.43	7.66
		Mean	3.0	4.2	7.2
		SD	0.6	0.8	1.3
3.0	3.0	1	3.25	4.45	7.57
		2	2.80	3.82	6.80
		Mean	3.0	4.1	7.2

Table C.3 Peak areas from analyses of HD in 2.00 g soil, with various amounts of salt-saturated water solution added to the soil.

Added water (ml)	**Replicate**	**Peak area *m/z* 158 (10^5)**	
		Soil A	**Soil B**
0	1	0.47	0.42
	2	0.33	0.44
	3	0.28	0.39
	Mean	0.36	0.42
	SD	0.10	0.03
1.0	1	1.97	2.19
	2	1.84	2.07
	3	1.17	2.12
	4	1.64	1.95
	Mean	1.7	2.08
	SD	0.4	0.10
2.0	1	2.81	3.10
	2	2.11	4.30
	3	1.77	3.92
	4	2.09	3.82
	Mean	2.2	3.8
	SD	0.4	0.5
3.0	1	0.87	1.05
	2	0.99	1.06
	3	0.79	1.38
	Mean	0.88	1.2
	SD	0.10	0.2

Table C.4 Peak areas from analyses of the cyclic sulphur compounds in soil A, with use of one, two and three successive vial extractions.

		Peak area (10^5)		
Number of extractions	**Replicate**	**1,4-thioxane *m/z* 104**	**1,3-dithiolane *m/z* 106**	**1,4-dithiane *m/z* 120**
1	1	4.42	4.41	5.07
	2	4.10	4.12	4.90
	3	4.23	4.19	5.04
	Mean	4.2	4.2	5.01
	SD	0.2	0.2	0.09
2	1	6.01	5.99	6.82
	2	5.92	6.01	7.01
	3	5.61	5.71	6.65
	Mean	5.8	5.9	6.8
	SD	0.2	0.2	0.2
3	1	6.18	6.52	7.48
	2	5.72	5.71	6.69
	3	5.80	6.08	7.13
	Mean	5.9	6.1	7.1
	SD	0.2	0.4	0.4

C.3 Method validation, cyclic sulphur compounds

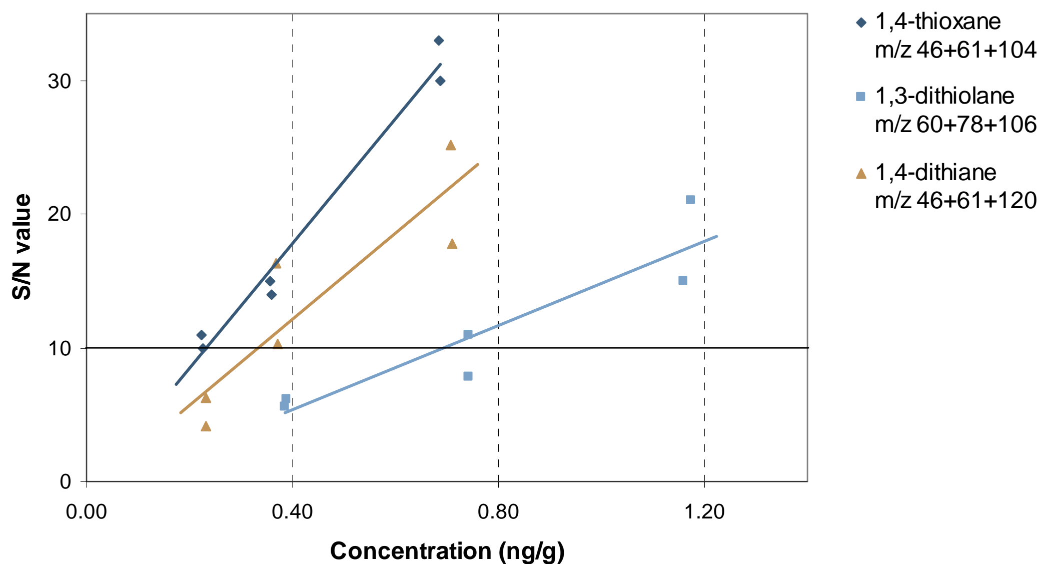

Figure C.3 S/N values of three extracted m/z ratios from analyses of the cyclic sulphur compounds in soil A, plotted as a function of concentration. Linear regression curves through the plots are given.

Table C.5 Linear regression of the plots in Figure C.1 with calculated LODs ± one standard error.

Compound	Linear regression	Standard error	Calculated LOD (S/N=10) ± one standard error
1,4-thioxane	y = 46.7·x - 0.904	1.66	0.23 ± 0.04
1,3-dithiolane	y = 15.8·x - 0.985	2.61	0.70 ± 0.17
1,4-dithiane	y = 32.2·x - 0.721	3.99	0.33 ± 0.12

Table C.6 Linearity test of the method for trace determination of cyclic sulphur compounds in soil A, from LOQs to 100 times LOQs. The peak areas are relative to the extracted m/z 105+120 of 1,2,4-TMB, at a concentration of 10 ng/g.

	1,4-thioxane *m/z* **46+61+104**		**1,3-dithiolane** *m/z* **60+78+106**		**1,4-dithiane** *m/z* **46+61+120**	
Concentration level	**ng/g**	**peak area**	**ng/g**	**peak area**	**ng/g**	**peak area**
1	0.698	0.00534	1.96	0.0121	0.976	0.00819
2	7.19	0.0901	20.3	0.176	10.1	0.0786
3	20.9	0.302	59.1	0.649	29.3	0.294
4	33.5	0.495	94.3	1.23	46.8	0.521
5	52.1	0.893	147	2.11	73.0	0.985
6	69.4	1.12	196	2.74	97.2	1.29
R^2, linear regression	0.995		0.996		0.991	
R^2, polynomial regression (level 1-4)	0.9997		0.9999		0.9996	

Table C.7 Robustness test of the method for trace determination of cyclic sulphur compounds in soil A. Factorial design experiment at two levels was set up for the thermostatting temperature, water to soil ratio and percentage salt saturation. In addition, four analyses were performed at the established method levels.

Therm. temp °C	**Water to soil ratio (ml:g)**	**Salt saturation %**	**Peak area relative to IS** 1,4-thioxane	1,3-dithiolane	1,4-dithiane
78	0.900 (1.80:2.00)	89	0.59	1.36	0.66
82	0.900 (1.80:2.00)	89	0.53	1.40	0.59
78	1.22 (2.20:1.80)	91	0.35	0.99	0.45
82	1.22 (2.20:1.80)	91	0.43	1.11	0.49
78	0.900 (1.80:2.00)	100	0.57	1.39	0.60
82	0.900 (1.80:2.00)	100	0.51	1.34	0.53
78	1.22 (2.20:1.80)	100	0.44	1.14	0.51
82	1.22 (2.20:1.80)	100	0.44	1.15	0.50
80	1.00 (2.00:2.00)	100	0.56	1.31	0.57
80	1.00 (2.00:2.00)	100	0.48	1.32	0.54
80	1.00 (2.00:2.00)	100	0.51	1.39	0.59
80	1.00 (2.00:2.00)	100	0.51	1.25	0.54
		SD. n=4:	0.03	0.06	0.02

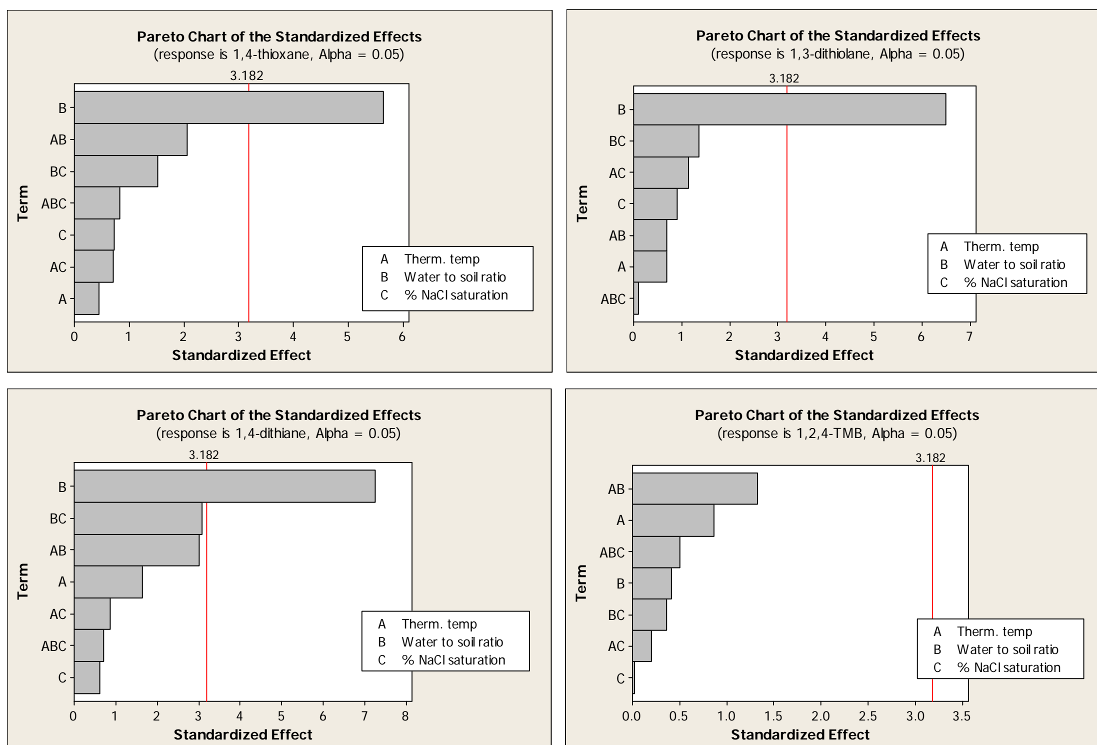

Figure C.4 Data from the robustness test presented in Table C.7, treated in Minitab. The standardised effects on each analyte and on IS are presented in Pareto charts, where the 95% confidencial limits are indicated with red lines. SD of the experiments is determined from the analyses at the mehod levels (n=4).

Table C.8 Recovery test of the cyclic sulphur compounds in soil A and soil B at concentrations of 50 times LOQ. Calculations are based on comparison of the peak areas (a), and peak areas relative to 1,2,4-TMB (b).

		1,2,4-TMB	**1,4-thioxane**		**1,3-dithiolane**		**1,4-dithiane**	
	Replicates	**(10^6)**	**a (10^6)**	**b**	**a (10^6)**	**b**	**a (10^6)**	**b**
Slurry	1	1.68	2.29	1.22	5.37	2.94	2.95	1.64
	2	1.71	2.23	1.19	5.31	2.91	2.95	1.64
	3	1.49	1.95	1.21	4.74	3.02	2.65	1.71
	4	1.65	1.87	1.06	4.52	2.62	2.58	1.52
Mean		1.63	2.1	1.17	5.0	2.9	2.8	1.63
SD		0.10	0.2	0.08	0.4	0.2	0.2	0.08
Soil A	1	1.98	1.05	0.561	2.38	1.31	1.21	0.675
	2	1.81	1.07	0.596	2.47	1.42	1.24	0.725
	3	1.70	1.06	0.640	2.44	1.52	1.21	0.762
	4	1.74	1.08	0.662	2.52	1.58	1.27	0.808
Mean		1.81	1.06	0.62	2.45	1.46	1.23	0.74
SD		0.12	0.02	0.05	0.06	0.12	0.03	0.06
% recovery			51 ± 1	53 ± 4	49 ± 1	51 ± 4	44 ± 1	46 ± 3
Soil B	1	2.71	1.18	0.454	3.17	1.25	1.89	0.754
	2	2.68	1.41	0.548	3.79	1.51	2.18	0.884
	3	2.74	1.30	0.479	3.44	1.30	2.08	0.799
	4	2.39	1.19	0.518	3.23	1.44	1.91	0.862
Mean		2.6	1.27	0.50	3.4	1.38	2.01	0.82
SD		0.2	0.10	0.04	0.3	0.12	0.14	0.06
			61 ± 5	43 ± 4	68 ± 6	48 ± 4	72 ± 5	51 ± 4

C.4 Method validation, sulphur mustard

Table C.9 Linearity test of the method for trace determination of HD in soil. Sample matrix was soil A, and concentration range was from LOQ to 100 times LOQ, with two replicates at each concentration level. Regression calculations are performed with each replicate as a single point.

Concentration level	ng/g	peak area (10^5)
1a	8.75	0.53
1b	8.81	0.44
2a	90.1	6.2
2b	90.4	6.0
3a	266	25
3b	268	17
4a	435	47
4b	446	34
5a	680	57
5b	681	61
6a	850	102
6b	859	79
R^2, linear regression		0.95
R^2, polynomial regression		0.96

Table C.10 Robustness test of the method for trace determination of HD in soil A. Factorial design experiment at two levels was set up for the thermostatting temperature, water to soil ratio and percentage salt saturation of the added water. Two replicates were analysed at each level (a and b).

Therm. temperature °C	Water to soil ratio ml:g	Salt saturation %	Peak area (10^6) a	Peak area (10^6) b
68	1.80:2.00	89	3.4	4.2
72	1.80:2.00	89	2.5	3.2
68	2.20:1.80	91	2.6	2.3
72	2.20:1.80	91	3.0	3.3
68	1.80:2.00	100	6.0	5.1
72	1.80:2.00	100	4.0	6.2
68	2.20:1.80	100	2.1	3.7
72	2.20:1.80	100	3.6	3.0

Table C.11 Recovery test of HD in soil A and soil B at concentrations of 50 times LOQ.

		Peak area (10^6)	
Replicates	**In slurry**	**Soil A**	**Soil B**
1	6.2	4.9	4.4
2	11.6	3.5	4.8
3	7.1	4.2	4.9
4	5.8	5.1	4.4
Mean	7.7	4.4	4.6
SD	2.7	0.7	0.2
% recovery		58 ± 9	60 ± 3