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Determination of chemical warfare agents in water samples by solid phase microextraction and GC-FID

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English summary

The Norwegian Defence Research Establishment (FFI) is presently developing a mobile laboratory for the identification of chemical warfare agents (CWA) and related compounds. The laboratory will be used in the field, and it is therefore advantageous that the analytical methods give a high priority to low solvent consumption and minimal need for sample preparation. Solid phase microextraction (SPME) meets these requirements, since the technique is based on automatic extraction and concentration of the analyte from the sample without the use of solvents.

The aim of this study was thus to develop a fast screening method for selected CWA in water by the use of SPME coupled to a gas chromatograph (GC) with a flame ionisation detector (FID). Parameters such as fiber selection, extraction time, desorption temperature and desorption time were investigated by using spiked distilled and de-ionised water samples. Thereafter, the developed method for SPME was compared with the existing analytical procedure, which is based on liquid-liquid extraction (LLE).

The work clearly shows that a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber is the best choice for the extraction of sarin (GB), soman (GD), sulphur mustard (HD) and cyclohexyl sarin (GF) from distilled and de-ionised water. Optimal conditions for SPME of the selected CWA were obtained when the fiber was immersed for 30 min in water saturated with sodium chloride, and subsequently desorbed for 2 min at 250 ºC in the inlet of the GC. Freshly spiked water samples gave method limit of detection (MLOD) in the range from 0.2 to 2 µg/L, which is 14 - 42 times better than by LLE. Interestingly, the greatest improvement in the MLOD was in the case of GF, which has not been determined by SPME in previous reported work. The presently obtained linear ranges were in the interval from 0.54 ng/mL to 4.9 µg/mL, depending on the analyte, with squared regression coefficients from 0.96 to 0.997. The precisions (%) measured as the relative standard deviations at the concentrations of 0.01 µg/mL (n = 7) and 1 µg/mL (n = 6) CWA were 2 - 8% and 4 - 10%, respectively. The developed method was also applied successfully for determination of CWA in spiked natural water samples.

The developed SPME method is less time-consuming than the standard LLE technique, as the total time for the sample preparation and the analysis is approximately one hour compared to 4 - 5 hours for the LLE technique. In addition, the developed method does not involve the use of carcinogenic solvents. Therefore, the developed SPME method is a significant contribution towards a faster and a more user-friendly determination of CWA, which is suitable for a mobile laboratory in the field.

Contents

Preface

The present study has been performed at The Norwegian Defence Research Establishment (FFI), Protection Division, in the period from February 2006 to August 2007.

The main aim of the present work was to develop a solid phase microextraction (SPME) method for chemical warfare agents (CWA) in water. However, SPME had not been previously used at FFI. Therefore, it was initially necessary to perform some preliminary experiments and evaluate the SPME technique. Hence, the chapter “results and discussion” in this thesis is sub-divided into several sections: preliminary work, evaluation of the SPME GC-FID instrumentation, development and validation of a new SPME method, investigation of the existing procedure based on liquid-liquid extraction (LLE), and the sample preparation of natural water with SPME and LLE. The raw data for each section are given in the appendix with the same titles as in the “results and discussion” part of the report. Note that some of the results are presented as plots and figures, and that these were made in Excel. In these figures, Excel used the Norwegian comma (,) instead of the English comma (.).

It may be noted that initially it was attempted to develop a method using the headspace GC-MS technique. Due to problems with this instrument, however, and the delay caused by the unexpected reconstruction of the “Høytoks” laboratory at FFI, this approach was abandoned.

First of all, I would like to thank FFI for giving me the opportunity to follow a Master of Science study program (MSc). FFI was interested in defining a thesis which was relevant for their ongoing research, and challenging and progressing to me. Working with the thesis, has undoubtedly given me a lot of valuable practical and theoretical experience.

I will give a huge hug to my supervisor Egil Bakken who continued to guide me, even though he left FFI and started to work for SINTEF. Thank you for inspiring me and giving me instructive guidance through the whole period. Thereafter, I am grateful for the supervision by John Aasulf Tørnes despite his stressful days at FFI. Additionally, my supervisor at UiO, Elsa Lundanes, has given me useful comments on the thesis.

I will also give great thanks to Bent Tore Røen for giving me CWA from the "Store Room". I have also appreciated his interest and critical questions concerning the master thesis. Leif Haldor Bjerkeseth; thank you for all your computer and software assistance. Additionally, sincerely thanks to "INFOenheten" at FFI for their support in taking photos, and for making the poster and the video.

Last but not least, a big thank you to myself! ☺

Kjeller, August 2007

Fatima Hussain

Abbreviations

Says	**Denotes**
BMA/OH-TSO	co-poly(butyl methacrylate/hydroxy-terminated silicone oil)
b.p.	boiling point
BSP 3	phenol-based polymer
BZ	3-quinuclidinyl benzilate
CAP	split/splitless capillary injection port
CAR	Carboxen
Carbotrap C	graphitized carbon black resin
CE	capillary electrophoresis
COD	chemical oxygen demand
CS	*ortho*-chlorobenzylidenemalononitrile
CW	Carbowax
CWA	chemical warfare agent(s)
CWC	Chemical Weapons Convention
DNA	deoxyribose nucleic acid
DVB	divinylbenzene
FID	flame ionisation detector
FFI	Norwegian Defence Research Establishment (Forsvarets forskningsinstitutt)
GA	*O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate (tabun)
GB	*O*-isopropyl methylphosphonofluoridate (sarin)

GC	gas chromatography
GD	*O*-pinacolyl methylphosphonofluoridate (soman)
GD 1	diastereoisomer 1 of soman
GD 2	diastereoisomer 2 of soman
GF	*O*-cyclohexyl methylphosphonofluoridate (cyclohexyl sarin)
HD	bis(2-chloroethyl)sulphide (sulphur mustard)
HS	headspace
LC	liquid chromatography
LCt_{50}	lethal concentration that kills 50% of a population during an exposure time (mg min/m^3)
LLE	liquid–liquid extraction
LOQ	limit of quantification
MS	mass spectrometry
MLOD	method limit of detection
NMR	nuclear magnetic resonance
NPD	nitrogen phosphorus detector
PA	polyacrylate
PDMS	polydimethylsiloxane
PR	pressure regulator
PSS	programmable split/splitless capillary injection port
ROP	recommended operating procedure
rpm	rounds per minute
RSD	relative standard deviation
SPME	solid phase microextraction

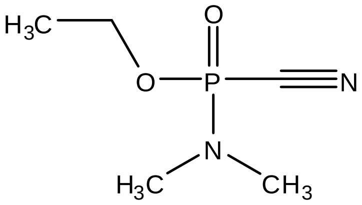
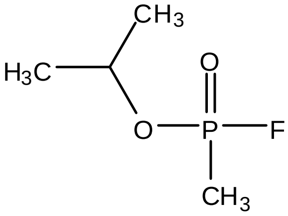
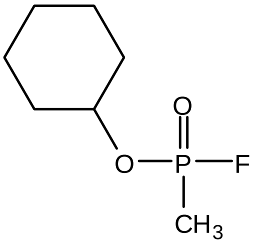
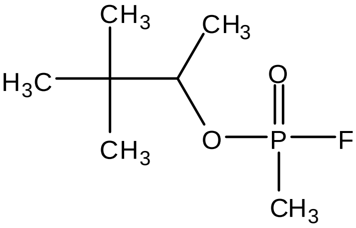
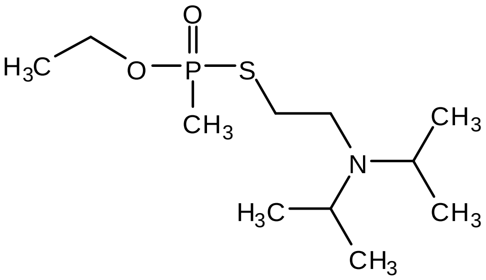
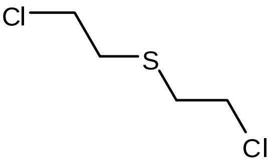
Tenax	2,6-diphenylene-oxide polymer resin
TLC	thin layer chromatography
VX	*O*-ethyl *S*-2-(diisopropylamino)ethyl methylphosphonothiolate

1 Introduction

The determination of chemical warfare agents (CWA) has experienced increased attention during the last years because of the Chemical Weapons Convention (CWC) coming into force on 29. of April 1997. This treaty prohibits the development, production, stockpiling and use of CWA. The CWC involves detection and identification of CWA and their precursors and degradation products (so-called markers) in samples collected from production, storage and suspected sites of chemical warfare activity. The samples are sent to designated laboratories for identification of CWA and their markers. The most frequently used methods to identify these compounds are based on gas chromatography (GC) and liquid chromatography (LC) in combination with mass spectrometry (MS), and on nuclear magnetic resonance (NMR) spectrometry. Thin layer chromatography (TLC) and capillary electrophoresis (CE) have also been employed. Comprehensive reviews of sample preparation and analyses concerning CWA and their related compounds in an off-site laboratory can be found in the literature (1-9).

CWA are divided into several groups with respect to their effect on the organism, and the nerve agents constitute the most lethal group. The nerve agents belong chemically to the group of organophosphorus compounds, see Table 1.1 (10). Due to different chemical and physical properties, the nerve agents are normally divided into two classes, referred to as G and V agents (11, 12). The most common G agents are *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate (GA, tabun), *O*-isopropyl methylphosphonofluoridate (GB, sarin), *O*-cyclohexyl methylphosphonofluoridate (cyclohexyl sarin, GF) and *O*-pinacolyl methylphosphonofluoridate (soman, GD). The primary V-agent is *O*-ethyl *S*-2-(diisopropylamino)ethyl methylphosphonothiolate (VX). The nerve agents react irreversibly in the nervous system by attacking the enzyme acetylcholinesterase, thereby inhibiting the enzyme and paralysing nerve transmission (10). Lack of nerve transmission affects the respiratory muscles and thus causes death by suffocation.

Table 1.1 Names, structures, molecular mass and boiling points (b.p.) of some selected chemical warfare agents (4, 10, 13).

Common name	Abbreviation	Structure	Molecular mass (g/mol)	b.p. (ºC)
Tabun	GA		162.1	230[a]
Sarin	GB		140.1	151.5[a]
Cyclo-hexyl sarin	GF		180.2	239
Soman	GD		182.1	167[a]
VX	VX		267.3	>300[a]
Sulphur mustard	HD		159.1	217[a]

[a] Decomposition of compound.

Inhalation is the most important route for intoxication by G-agents. The toxicity of nerve agents on man has been extrapolated from animal data. Therefore, the apparent toxicity of chemical warfare agents vary to a great extent (10, 11, 14-17). The toxicity values are mainly given as a product of vapour or aerosol concentration and duration of exposure to kill 50% of an exposed population (LCt_{50}). Newmark (17) has reported the following inhalation LCt_{50} data: 400 mg min/m^3, 100 mg min/m^3 and 50 mg min/m^3, for GA, GB and GD, respectively. Signs of mild poisoning after inhalation are typically contraction of pupils (miosis) with dimming of vision, feeling of tightness in the chest, running nose, salivation, hallucinations and nausea (10).

The vesicants, which are another class of CWA, affect the eyes and lungs and blister the skin, see Figure 1.1. Bis(2-chloroethyl)sulphide (HD, sulphur mustard) is the most frequently occurring vesicant, see Table 1.1. HD reacts with proteins and deoxyribose nucleic acid (DNA), giving disturbed metabolism, cell damage, cell death and increased risk of cancer (10, 18). The mortality due to exposure to the vesicant is low. The inhaled dose of sulphur mustard required to cause death is estimated to be 1500 mg min/m^3 (19).

Figure 1.1 Injuries on Iranians caused by Iraq's use of sulphur mustard during the Iran - Iraq war (1980-1988). (Photo: Iranian News Agency)

Normally, death is caused by complications resulting from lung damage due to the inhalation of sulphur mustard.

Three other groups of chemical warfare agents are classified as blood, incapacitation and pulmonary agents. Blood agents (for example hydrogen cyanide) reduce the oxygen transport and may cause death by suffocation (10). Incapacitating agents like the tear gas *ortho*-chlorobenzylidenemalononitrile (CS) and the psychochemical 3-quinuclidinyl benzilate (BZ), cause great discomfort, vomiting or mental effects, thereby preventing the victims to perform their tasks (10). The pulmonary or choking agents include chlorine gas, phosgene, diphosgene and chloropicrin. These compounds cause reduced ability to breathe which results in suffocation of the victim (20).

Rapid and reliable detection methods are needed to verify CWA in both military and civil settings. Detection of CWA in the field is done by commercially available chemical warfare agent monitors, e.g. the Chemical Agent Monitor (CAM) from Smiths Detection (21). They indicate the presence of CWA but may give false positive readings. Additionally, they do not provide high sensitivity (22). Hence, confirmatory analysis is performed in an off-site laboratory using for example GC or LC separation followed by mass spectrometry, as mentioned above.

Reliable analytical data in the field is of great advantage for military units and civilian emergency response organizations, and technological advances have made it possible to use GC-MS in the field. However, traditional sample preparation can take more time than the GC-MS analysis (6). In addition, traditional sample preparation in the field requires the transportation, storage, use and disposal of hazardous solvents. The relatively new technique, solid phase microextraction (SPME) simplifies and speeds up the sample preparation, reduces the need for solvents and shortens the analysis time compared to conventional methods (23).

The SPME technique uses a fused silica fiber coated with a polymer (24). Figure 1.2 shows a schematic diagram where the fiber is exposed to a gas or a liquid sample and the analytes are distributed between the sample and the polymer coating (25, 26). If the fiber is exposed for a sufficient period, equilibrium will be reached where further fiber exposure does not result in additional sorption of the analyte. After sampling the fiber is retracted into a protective syringe-type needle. Usually, the retracted fiber is inserted into a heated gas chromatographic injection port, and then the fiber is exposed and the analytes are thermally desorbed.

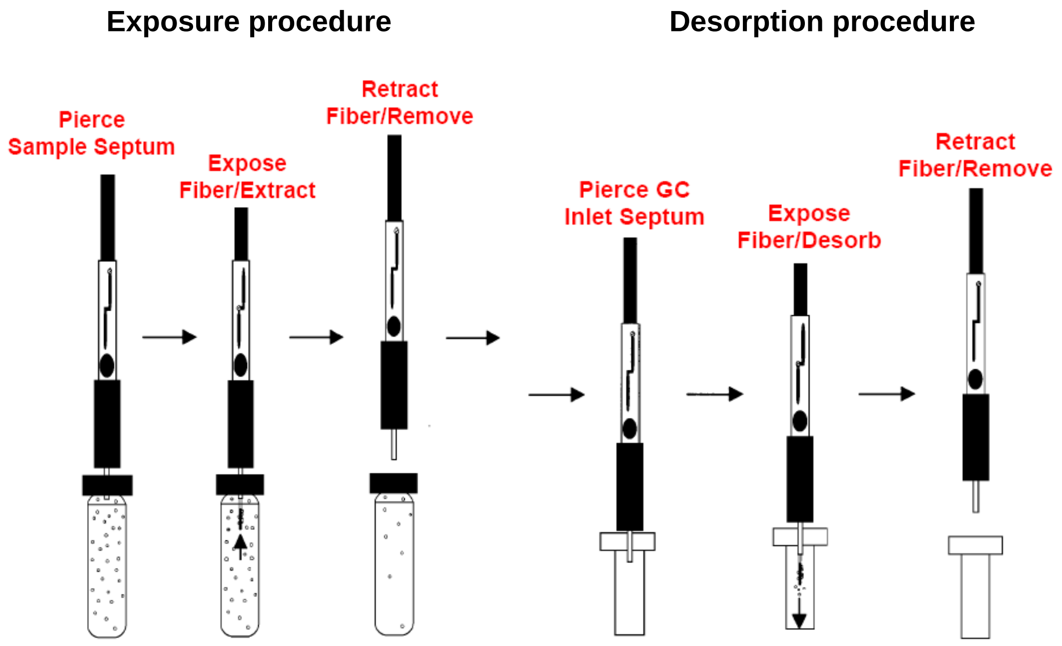


Figure 1.2 Exposure and desorption procedures in an SPME GC device. (Illustration: Supelco)

Only a limited number of polymer coatings are commercially available for SPME, and they roughly cover a wide range of polarity. In particular these coatings are polydimethylsiloxane (PDMS), divinylbenzene (DVB), polyacrylate (PA), Carboxen (CAR; a molecular sieve) or Carbowax (CW; polyethylene glycol). To improve the extraction efficiency of chemical warfare related compounds, some non-commercial selective stationary phases have also been developed. A phenol-based polymer (BSP 3) (27) and co-poly(butyl methacrylate/hydroxy-terminated silicone oil) (BMA/OH-TSO) (3:1) (28) coatings have been prepared and used to determine GB in air and an HD simulant in soil, respectively.

The commercially available coatings for SPME have been applied to detect one or several of the following compounds: GA, GB, GD, GF, HD and VX in air (22, 27, 29-31), water

(22, 23, 31-33), soil (32, 34, 35), hydraulic fluid (36), clothing and gasket materials (32, 37) and decontamination solutions (32, 38). In addition, SPME has also been used to analyse simulants, starting materials, precursors, by-products and degradation products of CWA in a number of matrices (28, 32, 33, 39-47).

Determination of CWA in water by SPME GC-MS has been investigated thoroughly by Lakso and Ng (23). They tested different fibers with varying polarities by immersing them in natural and de-ionised waters spiked with CWA. A 65 µm combined polymer coating of PDMS/DVB was found optimal for extraction of GA, GB, GD and VX (23). Several others have employed the same polymer coating to verify the presence of CWA in water (22, 32, 33). Smith et al. (31) have instead applied 85 µm PA for the determination of high concentrations of GA, GB, GD, HD and VX. Furthermore, previous studies have shown that an increase in the ionic strength of water by adding sodium chloride (NaCl) or sodium sulphate (Na_2SO_4) decrease the water solubility of the nerve agents (23). Hence, the extraction efficiencies of the agents increased compared to water samples with no addition of salt ("salting-out effect").

The SPME method developed by Lakso and Ng (23) was compared with the recommended operating procedure (ROP) based on LLE (6). With the exception of VX, the SPME method gave higher recoveries of all the investigated compounds than the ROP method (23). The use of SPME sampling gave detection limits of approximately 0.05 µg/L for GA, GB and GD, and 0.5 µg/L for VX in de-ionised water saturated with salt. For natural water samples, 60 µg/L of the mentioned compounds were detected a few days to a week after spiking. Schneider et al. (22) and Palit et al. (33) have used concentrations of 12 mg/L of GB and 1 mg/L of GB and HD in de-ionised water samples, respectively, and no validation of the methods have been presented. It should be emphasized that determination of GF in water by SPME has so far not been described in the literature.

Headspace (HS) gas chromatography is another sampling technique which gives benefits compared to conventional methods. HS does not consume solvents because of minimum or non-existing sample preparation, and it is also little time-consuming. In static HS the sample, which is either a liquid or a solid, is placed in a sealed vial and heated for a certain period of time. An aliquot of the gas phase surrounding the sample (HS) is subsequently introduced into a GC (48). In dynamic HS an inert gas like helium or nitrogen is bubbled through the sample and the analytes are transferred to an adsorbent trap. The trap is then heated to desorb and transfer the analytes to the GC. Dynamic HS is more sensitive than static HS because the former can theoretically remove all of the analytes from the sample, whereas the latter just removes a limited aliquot of the gas phase. However, dynamic HS requires complex instrumentation with labor-intensive maintenance, and the technique itself may cause foaming of the sample. These problems can be avoided by combining static HS with an adsorbent trap, see Figure 1.3. With this technique, several aliquots of the gas phase are in repetitive cycles concentrated on the trap prior to injection into the GC. Adsorbent materials like 2,6-diphenylene-oxide polymer (Tenax) and graphitized carbon black resins (Carbotrap C) are commercially available for this purpose.

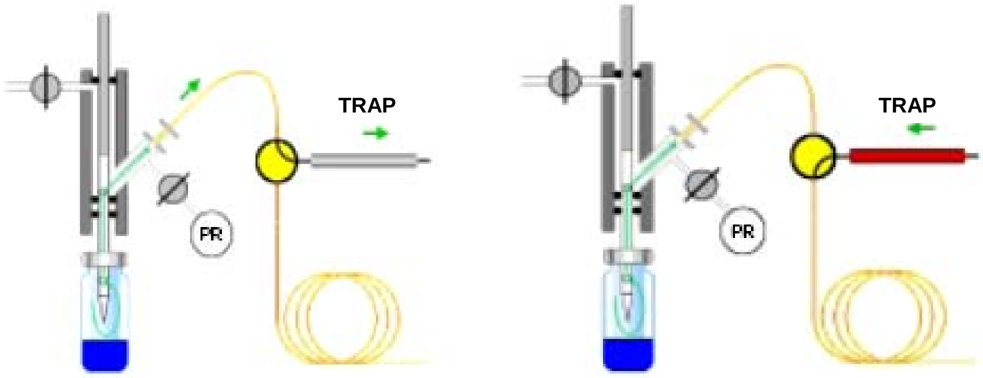


Figure 1.3 Schematic presentation of a static HS with trap device produced by Perkin Elmer. The analytes in the gas phase are transferred from the sample vial onto a trap in repetitive cycles (left). The trap is heated to desorb the analytes and a gas flow carries the analytes to the GC column (right). PR denotes a pressure regulator.

There is limited work reported on determination of CWA with the HS technique. However, the Norwegian Defence Research Establishment (FFI) has earlier applied HS in static modus without trap to determine GA, GB, GD and HD in water, grass, soil, sand, paper, silicone, neoprene, butyl rubber, polyurethane foam with activated charcoal, polyester and cotton (49). Static HS coupled to a GC-flame ionisation detector (FID) gave detection limits ranging from 0.01 µg to 10 µg in the mentioned matrices, and these values are given as the total amount of agent in the sample matrix. Moreover, application of dynamic HS for the determination of HD in rubber has been reported by Wils et al. (50). HD was purged out from the sample by a helium flow (15 mL/min) and trapped in deactivated fused silica at -120 ºC. Afterwards, HD was purged from the trap at 220 ºC to the GC column. The application of static HS with trap to determine CWA in environmental samples and polymer materials has not yet been described.

FFI is presently developing a mobile laboratory for the determination of CWA and their markers in the field. This work should be completed by the end of 2008. Analytical methods based on low solvent consumption and little sample preparation are advantageous for this purpose. SPME and the static HS GC-MS with trap methods meet these requirements since they automatically perform sampling, extraction and sample concentration in one step without the use of solvents.

The aim of the present study was thus to develop fast screening methods for selected CWA such as GB, GD, GF and HD in water by the use of the newly purchased SPME GC-FID instrument and the static HS GC-MS with trap instrument. However, due to problems with the HS GC-MS, further investigation of this technique was excluded from this study. Therefore, the work has been focused on comparison of the performance of the developed SPME GC-FID method with the existing analytical procedure, which is based on liquid-liquid extraction (LLE) (6).

2 Experimental

All the practical work related to the master thesis was performed at FFI, Protection Division at Kjeller in Norway.

2.1 Chemicals and equipment

2.1.1 Chemicals and gases

O-ethyl *N*, *N*-dimethylphosphoramidocyanidate (GA, tabun), *O*-isopropyl methylphosphonofluoridate (GB, sarin), *O*-cyclohexyl methylphosphonofluoridate (cyclohexyl sarin, GF) and *O*-pinacolyl methylphosphonofluoridate (soman, GD) were purchased from Netherlands Organisation for Applied Scientific Research (TNO, Delft, The Netherlands). Bis(2-chloroethyl)sulphide (HD, sulphur mustard) and deuterated diisopropyl methylphosphonate (DIPMP-d_3) were synthesized in-house at FFI (Kjeller, Norway).

Ultra resi-analysed acetone (> 99.4%), acetonitrile (> 99.8%) and dichloromethane (> 99.8%) were obtained from J.T. Baker (Deventer, The Netherlands). Methanol of HPLC grade was purchased from Rathburn Chemicals Ltd (Walkerburn, UK). Distilled and de-ionised water was delivered in-house by maxima ultra pure water system from ELGA Labwater (Marlow, UK).

Analytical grade sodium chloride (≥ 99.5%) and anhydrous sodium sulfate (> 99.0%) were purchased from Merck (Darmstadt, Germany). Helium, hydrogen, nitrogen and synthetic air were obtained from AGA (Oslo, Norway) with a purity of 99.9999% (6.0), 99.999% (5.0), 99.999% (5.0) and 99.999% (5.0), respectively.

2.1.2 Equipment

Three different fibers were applied for SPME: 85 µm polyacrylate (PA), 65 µm polydimethylsiloxane/ divinylbenzene (PDMS/DVB) and 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) were all supplied by Supelco (Bellefonte, PA, USA). The two last fibers were of StableFlex type.

The two balances AT 200 and AE 260 DeltaRange were delivered by Mettler Toledo Gmbh (Greifense, Switzerland). The whirlmixer called Super-Mixer was obtained from Lab-Line Instruments, Inc. (Melrose Park, IL, U.S.A.). Pippettes (Finnpipette) of 0.5 – 10 µL, 5 – 40 µL, 40 – 200 µL, 200 – 1000 µL, 1 – 5 mL and 2 – 10 mL were delivered from Labsystems (Helsinki, Finland). The plunger-in-needle syringes of 1 µL and 5 µL were bought from Hamilton (Bonaduz, Switzerland) and SGE Analytical Science (Victoria, Australia), respectively, and the 10 µL gas tight microliter syringe was purchased from Hamilton (Reno, NV, USA).

For filtration, a syringe driven filter unit with a pore size of 0.45 µm of Millex-HV type, SLHV 025, was purchased from Millipore (Bedford, MA, USA). The filter units were coupled to Omnifix single-use plastic syringes of 10 mL, which were purchased from Braun (Melsungen, Germany).

Glassware and belonging parts, such as a 20 mL vial with a magnetic screw cap with silicone blue transparent septum, and a 2 mL vial with a magnetic crimp cap with PTFE red/silicone/PTFE septum red were delivered by Gerstel (Mülheim an der Ruhr, Germany). A 60 mL separating funnel with PTFE stop-cock was supplied by Kimax (Vineland, NJ, USA).

2.2 Samples

The natural sea water samples were collected at “Aker Brygge” situated in Oslo harbour, and the natural fresh water samples were taken from Aker River close to “Nybrua” (Oslo, Norway). These water samples were taken the 4th of March 2007. The snow samples were collected inside the fenced area of FFI the 2nd of March 2007 (Kjeller, Norway). The snow and water samples were collected some centimeters below the surface to avoid contaminations from the surface of the matrices. The samples were kept in borosilicate glass 3.3 (DURAN) bottles having screw caps with teflon gaskets (Schott, Mainz, Germany). All the samples were stored in a refrigerator at 2 – 6 ºC.

2.3 Instrumentation

2.3.1 SPME GC-FID

The automated SPME experiments were carried out by the multipurpose autosampler (MPS2) from Gerstel (Munich, Germany) which was mounted on an AutoSystem XL GC-FID from Perkin Elmer, see Figure 2.1. The SPME was programmed by the GERSTEL MASter version 1.86.17.8 software. The TotalChrom version 6.2.1 was used both to control the GC-FID and to perform data analysis. In the following some details of the instrumental setup will be given.

During SPME analysis, a fiber was installed in the robotic head unit (Figure 2.1, unit B). The robotic head of the autosampler is used for the extraction and subsequent injection of the sample. It also transports the sample vial from the sample tray to the heating and agitation unit, with the aid of a magnetic holder on the robotic head and a magnetic metal screw cap on the vial (Figure 2.1, unit C and E). The vial containing the sample was simultaneously heated and agitated in this unit (Figure 2.1, unit E), and the fiber was subsequently immersed directly into the sample solution for extraction of the analytes.

After extraction of the analytes from the matrix, it is possible to automatically perform derivatisation. The fiber with the sorbed analytes is then dipped in a derivatisation solution. In the present case, it was not necessary to derivatise the selected analytes, and the option of derivatisation was instead used to rinse the fiber free from possible salt particles (23). The rinsing of the fiber was done by dipping it in a vial containing distilled and de-ionised water, which was placed in the agitation and heating device
(Figure 2.1, unit E).

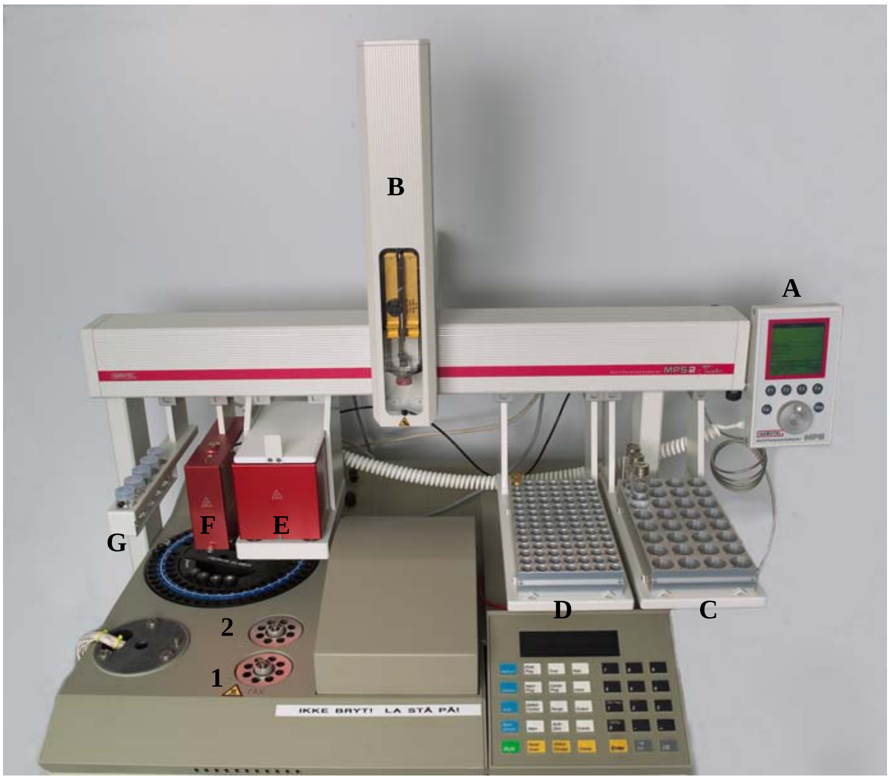


Figure 2.1 Setup of the multipurpose autosampler for SPME and liquid injections: (A) hand-held-programming unit, (B) robotic head unit, (C) vial tray for SPME at room temperature, (D) vial tray for liquid injection at room temperature, (E) vial heater and agitation unit, (F) SPME fiber heater unit, (G) solvents for cleaning of liquid injection syringe, (1) PSS injector, (2) CAP injector. (Photo: FFI)

After the fiber was rinsed, the fiber was injected in the GC split/splitless injection port (CAP) for thermal desorption of the analytes, see Figure 2.1, unit 2. Subsequently, the robotic head unit moved the sample vial from the agitation and heating unit to the sample tray (Figure 2.1, from unit E to C). Thereafter, the robotic head unit was moved to the fiber heater unit, where the fiber was thermally cleaned in a purge gas flow of nitrogen, see Figure 2.1, unit F. After the thermal conditioning of the fiber, the robotic head was initiated to perform an extraction of a new sample.

The inner diameter of the injector insert was 0.75 mm, and a thermogreen LB-2 predrilled 11 mm septum was used in the split/splitless injection port (capillary injector, CAP), see Figure 2.1, unit 2. The use of predrilled septum has been recommended due to less probability of septum pieces entering the injector insert and thereby blocking the exit of the fiber during injection (23). Both the injector insert and the septa were purchased from Supelco (Bellafonte, PA, USA). For the chromatographic separation, a DB-5 MS fused silica capillary column (5% phenyl, methylpolysiloxane) with a dimension of 30 m x 0.25 mm inner diameter and a film thickness of 0.25 µm was used (J&W Scientific, Folsom, CA, USA).

2.3.2 GC-FID

Liquid injections were performed on the same instrument as described in section 2.3.1. A 10 µL fixed needle syringe with a steel plunger was bought from Gerstel (Mülheim an der Ruhr, Germany), and mounted in the robotic head unit of the multipurpose autosampler. The insert (Perkin Elmer, Waltham, MA, USA) in the programmable split/splitless capillary injecton port (PSS) was 2 mm in inner diameter, see Figure 2.1, unit 1. The same type of septum was used in the PSS injection port as in the case of SPME technique, except the septum was not predrilled. The DB-5 MS column used for SPME GC-FID analysis was also applied for liquid injection GC-FID analyses.

During liquid injections, the robotic head unit of the multipurpose autosampler was first moved to the unit with vials containing different solvents, see Figure 2.1, unit G. There the syringe was washed and subsequently moved to the vial tray and filled with sample (Figure 2.1, unit D). Then the robotic head was moved to the PSS injector where the sample was injected. After injection, the syringe was cleaned once again with solvents.

2.4 Procedures

The amount of CWA accepted for laboratory work without a protective mask and a protection suit is quite limited. For practical purposes, the use of full body protection in the present work with CWA was avoided. Therefore, no stock solution with relatively high concentration was prepared. Instead, several stock solutions were made to obtain the desired concentrations. The use of gloves, laboratory coat, goggles and fume hood were considered as sufficient protection for the preparation of the present stock solutions with limited amount of the agents. The same protective equipment was also used for other types of practical work which involved CWA.

For simplification, the concentration of the stock and the working solutions will in the following text be specified only for GB, GD and GF. The nerve agents have a density of approximately 1 g/cm^3. Therefore, the concentrations of HD may be obtained by multiplying the concentrations of the stock and the working solutions with the density of HD (1.27 g/cm^3). This procedure gives the concentrations of HD in the same unit as for the nerve agents. However, in the case of the validation solutions, the concentrations of both HD and the three nerve agents will be given.

Additionally, the preparation of the solutions and samples were done in weighed amount of either acetone or water. These amounts were recalculated to volume (mL) in order to give the concentrations of the CWA as weight per volume. The solutions descried below were made in either 2 mL or 20 mL glass vials with screw caps (see chapter 2.1.2).

It is important to notice that the spiking of water with the selected CWA, as described below, was done 5 – 10 min prior to the extraction of each sample. This was done in order to avoid degradation of the CWA (13, 51). In addition, both the distilled and de–ionised water samples and natural water samples were spiked with all of the selected agents: GB, GD, GF and HD. This was done to ensure that the samples were as similar as possible to the samples made for the method development of SPME.

2.4.1 Preparation of validation solutions

Validation solutions for SPME

Stock solutions were made by diluting 0.8 - 5 µL of concentrated GB, GD, HD and GF with 0.75 - 5 mL of acetone. The amount of acetone was weighed and the selected CWA were added by the use of either a 1 µL or a 5 µL syringe (Hamilton, Bonaduz, Switzerland). Working solutions were made by diluting aliquots (µL) of the stock solutions in 15.5 mL of weighed amount of acetone. Subsequently, validation solutions were prepared by spiking 15.5 mL of weighed amount of distilled and de-ionised water with 10 µL of each of the prepared working solutions. The concentrations of the prepared stock, working and validation solutions are listed in Table 2.1. In the case of HD, two additional validation solutions were made by diluting the stock solutions of 3.40 mg/mL

Table 2.1 Preparation of validation solutions for SPME from different working and stock solutions. The sign (-) marks that no working solution was prepared from the corresponding stock solution.

Concentration			
Nerve agents in stock solution (mg/mL)	**Nerve agents in working solution (µg/mL)**	**Nerve agents in validation solution (ng/mL)**	**HD in validation solution (ng/mL)**
0.161	0.85	0.54	0.69
	2.1	1.3	1.7
	5.0	3.3	4.2
2.50	10.1	6.55	8.32
	20.2	13.0	16.5
	143	91.8	117
	425	272	345
	804	518	658
	$1.54\cdot10^3$	991	$1.26\cdot10^3$
3.40	-	$2.20\cdot10^3$	$2.79\cdot10^3$
5.96	-	$3.87\cdot10^3$	$4.91\cdot10^3$

and 5.96 mg/mL with 15.5 mL of weighed amount of distilled and de-ionised water. The nerve agents were also present in these solutions.

Table 2.2 shows the validation solutions used for the determination of the detection limits and the repeatability of SPME. The stock, working and validation solutions were prepared in the same manner as described above, and the concentrations of the solutions are given in Table 2.2. The four validation solutions made from the stock solution of 0.161 mg/mL, were used for determination of the detection limits of the selected CWA. The stock solution of 2.50 mg/mL was used to make solutions to investigate the repeatability.

All the prepared validation solutions described above were extracted and analysed by SPME GC-FID, see chapter 2.4.4 and 2.4.5.

Table 2.2 Preparation of validation solutions from different working and stock solutions. The validation solutions were used for the determination of the detection limits and the repeatability of CWA by SPME.

Concentration			
Nerve agents in stock solution (mg/mL)	**Nerve agents in working solution (µg/mL)**	**Nerve agents in validation solution (ng/mL)**	**HD in validation solution (ng/mL)**
0.161	0.32	0.20	0.25
	1.1	0.67	0.85
	2.1	1.3	1.7
	3.2	2.0	2.5
2.50	20.2	10.4	13.2
	$1.55 \cdot 10^3$	$1.00 \cdot 10^3$	$1.27 \cdot 10^3$

Validation solutions for LLE

To determine the method limit of detection for the selected CWA by LLE, solutions were made in the same manner as described above. The concentrations of the solutions are given in Table 2.3. The validation solutions were extracted and analysed by GC-FID, as described in chapter 2.4.4 and 2.4.5.

Table 2.3 Preparation of validation solutions from different working and stock solutions. The validation solutions were used for the determination of the detection limits of CWA by LLE.

Concentration			
Nerve agents in stock solution (mg/mL)	**Nerve agents in working solution (µg/mL)**	**Nerve agents in validation solution (ng/mL)**	**HD in validation solution (ng/mL)**
0.161	3.1	3.1	3.9
2.33	27	28	34

2.4.2 Preparation of natural water samples

The natural water samples described in chapter 2.2, were spiked with the selected CWA for comparison of SPME and LLE techniques. The pH of the collected river water, sea water and melted snow samples were measured prior to storage at 2 - 6 ºC in the refrigerator. The natural water samples were tempered to ambient temperature, prior to the sample preparation. In the case of SPME, 15.5 mL of water was weighed in a vial with screw neck, whereas the amount of water was 10 mL for LLE. Thereafter, the water samples intended for both SPME and LLE, were spiked with an amount of CWA corresponding to twice the limit of quantification (LOQ) for LLE. Note that the LOQ is defined as 3 times the method limit of detection. The LOQ for CWA by LLE corresponds to concentrations of 18 ng/mL for GD and approximately 180 ng/mL for GB, GF and HD.

To obtain the relevant concentrations, a stock solution was prepared by diluting 3 µL of GB, GF and HD in 15.5 mL of weighed amount of acetone. Furthermore, 122 µL of a solution of 5 µL of GD diltuted in 2 mL of weighed amount of acetone, was added to the stock solution. Thereafter, 10 µL of the stock solution was added to 10 mL and 15.5 mL of water samples. The concentrations of the stock solution and the water samples are given in Table 2.4, in addition to the total amounts of each CWA in the samples. Finally, the 10 mL and 15.5 mL spiked water samples were extracted by LLE and SPME, respectively, and the extraction procedures for the two techniques are given in chapter 2.4.4.

In addition, water samples were also made for the SPME analyses with concentrations of CWA corresponding to twice the LOQ for SPME. The LOQ corresponds to concentrations of 12 ng/mL (GB), 1.2 ng/mL (GD), 4.0 ng/mL (GF) and 7.8 ng/mL (HD). In this case, a stock solution was made by adding 10 µL of GB, 1 µL of GD, 4 µL of GF and 5 µL of HD in 2 mL of weighed amount of acetone. Next, 20 µL of the stock solution was diluted in 5 mL of weighed amount of acetone to make a working solution. 10 µL of the working solution was diluted in 15.5 mL of water. The concentrations of the solutions and water samples are given in Table 2.4, in addition to the total amounts of each CWA in water. These samples were only extracted by the SPME technique, see chapter 2.4.4.

Table 2.4 Total amounts and concentrations of CWA in natural water samples prepared for LLE and SPME. The concentrations of the stock and working solutions used for preparing the water samples are also given.

Method		**GB**	**GD**	**GF**	**HD**
LLE	Stock solution (mg/mL)	0.195	0.0165	0.194	0.248
	Concentration in water (ng/mL)*	195	16.5	194	248
	Total amount in water (µg)	1.95	0.165	1.94	2.48
SPME	Stock solution (mg/mL)	0.195	0.0165	0.194	0.248
	Concentration in water (ng/mL)*	126	10.6	125	160
	Total amount in water (µg)	1.95	0.165	1.94	2.48
SPME	Stock solution (mg/mL)	4.78	0.48	3.04	1.91
	Working solution (µg/mL)	19.5	1.95	12.4	7.78
	Concentration in water (ng/mL)**	12.6	1.26	5.02	7.97
	Total amount in water (µg)	0.195	0.0195	0.0778	0.124

*2 times limit of quantification of LLE.

**2 times limit of quantification of SPME.

2.4.3 Preparation of blank water samples

Blank water samples were analysed prior to the spiked water samples. The matrix of the blank water samples was equal to the matrix of the spiked water samples, and 10 µL of acetone was thus added to the weighed amount of water used for LLE and SPME. The samples were thereafter prepared as described by the flow chart given in Figure 2.2 in chapter 2.4.4.

2.4.4 Extraction procedures

In the following, the final extraction procedure for SPME and LLE are described. The SPME procedure is partially based on the work published by Lakso and Ng (23). The extraction procedure for LLE is mainly taken from ROP, chapter SP 7.4 a (6).

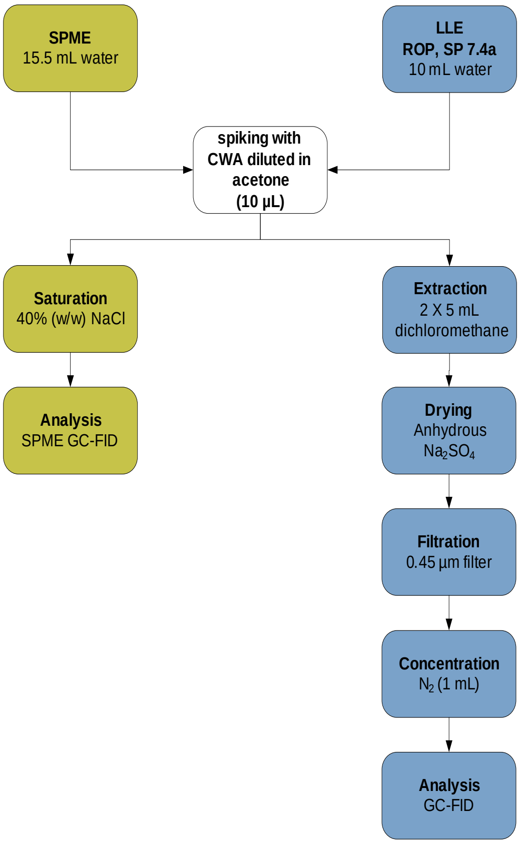


Figure 2.2 Flow chart for the analyses of spiked water samples by SPME and LLE.

SPME

The spiked 15.5 mL water samples which were prepared according to the details given in chapter 2.4.1-2.4.3 were saturated with NaCl (40%, w/w). The solutions were mixed on the whirlmixer, prior to SPME GC-FID analyses, see Figure 2.2. The sample volumes were 15.5 mL in order to ensure sufficient immersion of the SPME fiber in the water.

LLE

The extraction of water samples were performed without neutralizing the pH of the samples. The samples were extracted as described by the flow chart given in Figure 2.2. The CWA were extracted twice from the water by use of 5 mL of dichloromethane each time. The extracts were combined and thereafter dried with anhydrous sodium sulphate. Then the extract was filtered for removal of particulate material and concentrated to approximately 1 mL under a gentle flow of nitrogen. The amount of extract was weighed and dichloromethane was added to a total amount of 1.3 g corresponding to 1 mL. Finally, the sample was analysed by GC-FID.

2.4.5 Instrumental analyses

The final SPME GC-FID and liquid injection GC-FID methods are described below in detail.

SPME GC-FID

A DVB/CAR/PDMS fiber was installed in the robotic head unit of the multipurpose autosampler, see Figure 2.1. Prior to SPME analysis, a newly installed fiber was thermally conditioned in the fiber heater device, according to the manufacturer's recommendation. For the DVB/CAR/PDMS fiber the manufacturer advises to condition the fiber for 1 hour at 270 ºC. With these recommendations, a newly installed fiber was conditioned consecutively five times. Thereafter, 20 blank water samples were analysed to achieve a clean fiber, before analyses of real samples were performed. In addition, prior to every sample analysis, the fiber was thermally cleaned in the fiber heater unit at 250 ºC for 30 min. This last step was done to desorb compounds which may have been sorbed to the fiber since last time used. After this conditioning, the fiber was analysed to verify that no extraneous compounds or carry-over of the analytes from previous extractions were present. Next, 2 - 3 blank water samples were also analysed before real samples were extracted by the fiber, see chapter 2.4.3. The purpose of the blank water sample analyses was to condition the fiber with the matrix which was used in the subsequent analyses. The fiber was also conditioned for 10 min between extractions of two real samples (Table 2.5).

The prepared water sample was placed in the vial tray for SPME, see Figure 2.1, unit C. The extraction was performed according to the parameters given in Table 2.5.

Table 2.5 Parameters for the SPME of water by the multipurpose autosampler.

Heating and agitation:		**Sampling and desorption:**	
Vial temperature (°C)	35	Vial penetration (mm)	31
Vial heating time (s)	6	Extraction time (min)	30
Agitation speed (rpm)	750	Injection penetration (mm)	50
Agitation on time (s)	10	Desorption time (s)	120
Agitation off time (s)	1	Cycle time (min)	45
Conditioning of fiber:		**Rinsing of fiber:**	
Conditioning time (min)	10	Distilled and de-ionised water (mL)	18
Conditioning penetration (mm)	55	Rinsing time (s)	3
Purge gas flow of nitrogen (bar)	0.5	Vial penetration (mm)	31
Fiber heater temperature (°C)	250		

The carrier gas for GC-FID was helium with a gas pressure of 12.5 psi that corresponds to a flow rate of approximately 1 mL/min. During desorption of the fiber in the injection port (CAP) of the GC, the inlet was held in splitless mode and the initial oven temperature was kept at 40 ºC. When the fiber was retracted from the injection port (after 120 s), the split valve was opened with a gas flow of 25 mL/min and the oven was ramped at 10 ºC/min to 250 °C and held at this temperature for 2 min. The FID detector temperature was kept at 280 ºC with a range of 1 and an attenuation of -5. Hydrogen (50 mL/min) and synthetic air (450 mL/min) were used as detector gases.

GC-FID

Liquid injections were performed on the same chromatographic system as above. A sample volume of 1 µL was injected in the PSS injector. The DB-5 MS column used previously for the SPME GC-FID analyses was installed in the PSS injector port. The chromatographic method was the same as in the case of SPME GC-FID, except that the split valve was opened 1 min after injection.

Prior to injection into the GC, the syringe was cleaned twice with acetone, three times with dichloromethane and finally once with the sample solution, see Figure 2.1, unit D and G. After the injection of the sample, the syringe was cleaned with acetone and subsequently with dichloromethane.

3 Results and discussion

FFI is presently developing a mobile laboratory for the identification of CWA and related compounds. This laboratory will be used in the field and it is therefore advantageous that the analytical methods are based on low solvent consumption and little sample preparation. SPME meets these requirements since the technique simplifies and speeds up the sample preparation, reduces the need for solvents and shortens the analysis time compared to the existing analytical procedure, which is based on LLE (26). Therefore, the motivation of the present study was to develop a fast method for GB, GD, GF and HD in water, by the use of SPME coupled to a GC-FID. Analyses were performed by GC-FID since it was the only available instrument.

This chapter will thus focus on presenting the work done on the evaluation of the SPME GC-FID instrumentation, the devolpment of an SPME GC-FID method, and the comparison of the SPME technique with LLE.

3.1 Preliminary work

The multipurpose autosampler for performing SPME was purchased and mounted on a GC-FID in the spring 2006. The GC-FID had not been used since 2002, and therefore, there was an essential need for evaluating the GC-FID prior to applying the SPME technique. The instrument was evaluated by studying the linearity and the response areas of some selected CWA by liquid injections of validation solutions. Subsequently, a test solution delivered with the autosampler was analysed in order to verify that the installation of the SPME unit was in agreement with the manufacturer's specifications. Additionally, GC-MS analyses of GB, GD, HD and GF diluted in acetone, acetonitrile or methanol were performed. Finally, the stability of the agents diluted in acetone and stored for 7 days in a freezer were investigated. A brief description of the preliminary work is given in appendix A.

Based on experiences made from this initial work; acetone was chosen as the solvent for making stock and working solutions of CWA. It was also found that these prepared solutions could be used for 7 days, if the solutions were stored in a freezer at < -18 ºC.

3.2 Evaluation of SPME GC-FID instrumentation

The SPME technique had not previously been used at FFI, and the need for validating the performance of the newly installed instrument was thus obvious. An article published by Lakso and Ng (23) was used as a basis for the evaluation of the SPME GC-FID system. The experiments done at FFI were carried out using methods as similar as possible to the methods described by Lakso and Ng. The results obtained were compared with the reported observations. Experiments revealing the repeatability, detection limits and linear ranges for some selected CWA in distilled and de-ionised water samples are described below. Lakso and Ng investigated GA, GB, GD and VX in water. The nerve agent VX was excluded from the present work due to its high toxicity.

3.2.1 Repeatability

The SPME experiments done by Lakso and Ng are based on manual injections of the fiber in the inlet of a GC-MS (23). This is different from the SPME experiments performed in the present study, which were done by the use of a MPS 2 autosampler coupled to a GC-FID (chapter 2.3.1), but the conditions for the experiments were set as similar as possible to the values given by Lakso and Ng (23).

The investigation of the repeatability was done by the immersion of a 65 µm PDMS/DVB fiber in 1.00 µg/mL freshly spiked water saturated with salt (40%, w/w). The sample was prepared by spiking 15.5 mL of distilled and de-ionised water with 15.5 µL of a solution containing 1.00 mg/mL of the agents diluted in acetone. The water sample was spiked 5-10 min before the SPME procedure was started. This was done to avoid possible degradation of the CWA, which would cause large variations in the response. Thereafter, the spiked water sample was extracted using the conditions given in Table 2.5, except for the cycle time, which was 55 min during these experiments. After the extraction, the fiber was injected into the GC-FID, which was operated with the conditions described in chapter 2.4.5. Figure 3.1a shows a chromatogram for one of the analyses. As seen in the the figure, GD has two chromatographic peaks due to diastereomerism, and the peaks are therefore marked with GD 1 and GD 2.

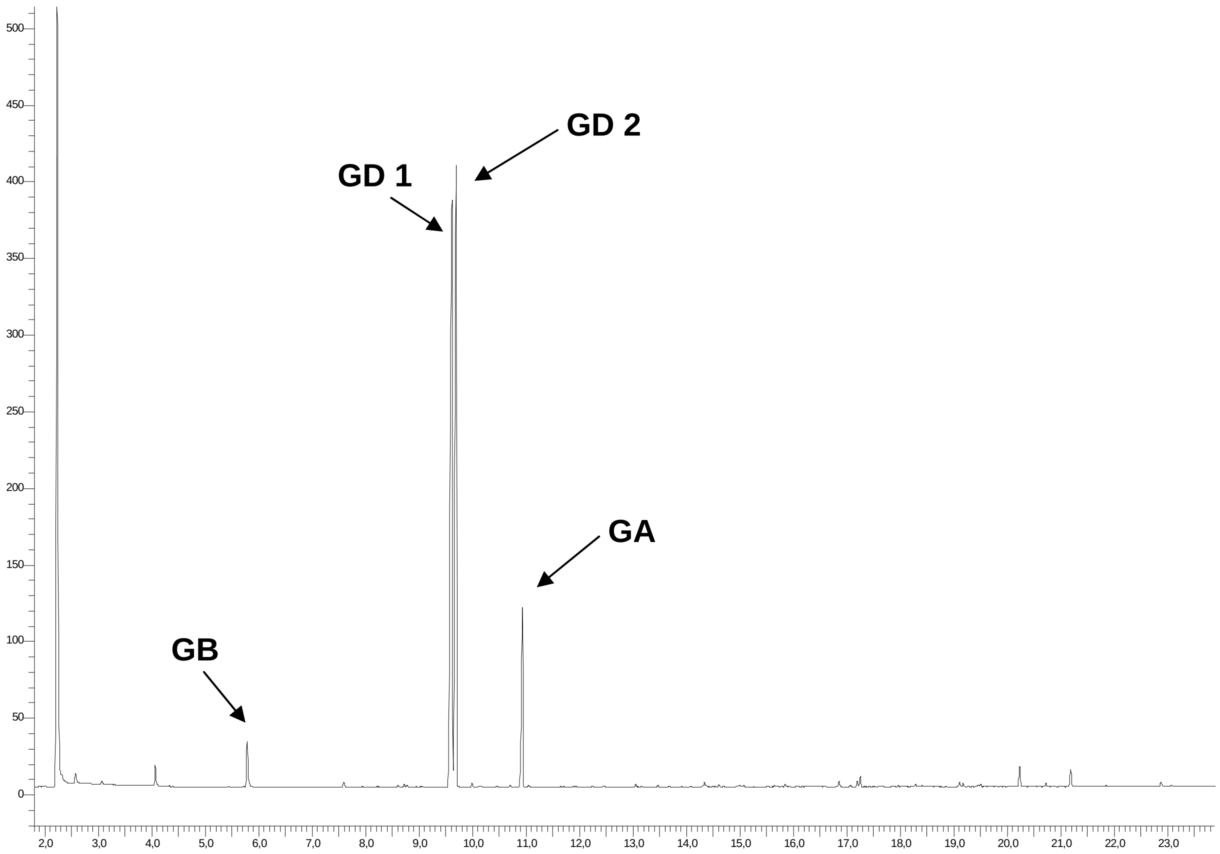


Figure 3.1a Chromatogram of GA, GB and GD in distilled and de-ionised water saturated with salt. The concentrations of the nerve agents were 1.00 µg/mL. A 65 µm PDMS/DVB fiber with GC-FID was used. GD 1 and GD 2 are two diastereoisomers of GD.

With seven successive analyses a precision (RSD) of 4.9% for GB, 5.3% for GD and 4.7% for GA was obtained (see also appendix B.1.1). A precision at the same concentration level equal to 4.5%, 16.8% and 3.3%, respectively, is reported in the literature (23). The precision of GA and GB obtained by FFI are thus in good agreement with the reported values. Note also that the precision achieved for GD is considerably improved compared with the reported value (23). The improved precision obtained for GD may be due to the use of an autosampler when performing SPME, instead of manual injection.

In another experimental serie, 6 samples were prepared simultaneously and analysed consecutively in a sequence. The delay-time between subsequent analyses was 55 min (cycle time), resulting in a difference in starting time between first and sixth sample of 275 min (4 hours and 35 min). A repeatability of 6.1% for GB, 5.5% for GD and 7.0% for GA were obtained (see appendix B.1.2), and these values are quite similar to the repeatability obtained for freshly spiked samples (see above). This indicates that a clear degradation of the investigated CWA does not take place during 275 min. For the preparation of these samples, a volumetric pipette with a range from 5 to 40 µL was used to spike the water sample with 15.5 µL of 1.00 mg/mL GA, GB and GD in acetone. The same volume of acetone (15.5 µL) was pipetted and weighed (n = 15) and these measurements had a relative standard deviation of 6.1%, which is the same magnitude as the RSD of the mentioned analyte responses. Water samples were therefore spiked with the use of both a 5 µL Hamilton and a 10 µL gas tight syringe to see if the RSD of the analyte responses could be reduced. The RSD achieved with the Hamilton and the gas tight syringe were 3.6-7.0% (n = 6) and 5.2-11% (n = 6), respectively (see also appendices B.1.3 - B.1.4). The RSD for the experiments with the Hamilton syringe are in the same range as obtained with the volumetric pipette. However, RSD for the experiments with the gas tight syringe are somewhat higher than what was achieved with the volumetric pipette. The volumetric pipette was thus used in the following investigations since it is easy to use and also gives a relatively low RSD.

Further investigations were made to see if the repeatability was influenced by the degradation of CWA in water samples stored for longer periods of time. Six samples prepared simultaneously were analysed immediately after preparation, as well as 11 hours and 20 hours after preparation. Figure 3.1b (see also appendices B.1.2, B.1.5 - B.1.6) shows that the response areas of the analytes are reduced when the samples are analysed 11 hours after preparation. However, only a small reduction in the response areas is seen upon further storage (20 hours). In addition, the replicates for the experiment after 11 hours have larger standard deviation than the replicates analysed after 20 hours. This is probably due to the declining responses of the analytes for the six consecutive replicates. Decreasing response areas may indicate an ongoing degradation of the CWA around 11 hours which is slower from 11 hours to 20 hours. GA is the only analyte which shows reduced response area 20 hours after sample preparation. The response area of GA after 20 hours is approximately one third of the initial response area. Note that the response areas of the analytes were obtained by integrating their chromatographic peaks.

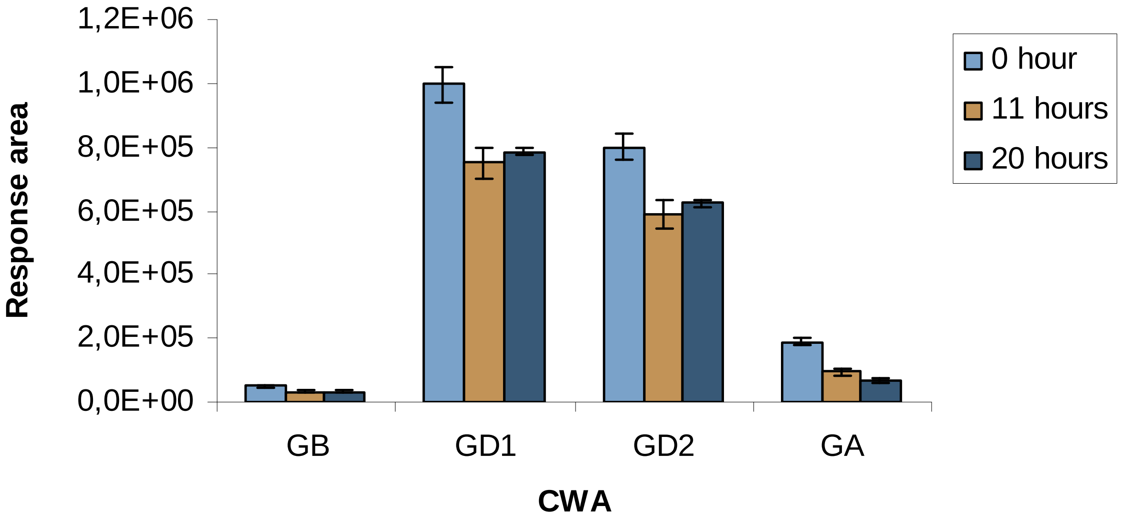


Figure 3.1b Mean values of response areas for GB, GD and GA (n = 6). 1.00 µg/mL of the agents in distilled and de-ionised water samples were analysed immediately, 11 hours and 20 hours after preparation. The error bars indicate the standard deviations of the mean values.

It is important to emphasize that the future work will include the determination of HD and GF in water, in addition to GB and GD. Therefore, experiments were also performed to investigate if degradations of HD and GF influenced the repeatability for these agents. 15.5 mL of distilled and de-ionised water was spiked with 10 µL of 0.3 mg/mL of GB, GD, HD and GF diluted in acetone. Hence, the concentrations of the nerve agents and HD in the water sample were 0.16 µg/mL and 0.20 µg/mL, respectively. Thereafter, the spiked water sample was saturated with NaCl (40%, w/w). The water samples were prepared simultaneously and analysed consecutively in a sequence (n = 12). Each sample was extracted for only 10 min and the time between subsequent analyses was 42 min (cycle time). Otherwise, the SPME GC-FID conditions were similar to the experiments described above. The response areas for the CWA for each of the 12 analyses are shown in Figure 3.2 (see also appendix B.1.7). The response area of HD is in sample 3 reduced to half of its initial value in sample 2. However, the response areas for all the CWA are reduced for sample 3, although not to the same extent as for HD. Sample 3 was analysed approximately 126 min after the sample preparation.

In general, the response areas for GB, GD and GF had a declining profile as the analyses of the samples proceeded. Interestingly, these observations are in accordance with the varying half-life times for the hydrolysis of the CWA in distilled water: HD has a shorter half-life time for hydrolysis than the nerve agents. The half-life times for hydrolysis at a pH of about 7 (20-25 ºC) for GA, GB, GD and GF are 8.5 hours, 80 hours, 45 hours and 42 hours, respectively (13, 52). However, the half-life time for HD is just 8.5 min in distilled water and increases to 60 min in salt water (51, 52). The hydrolysis rates of the nerve agents in salt water are not described in the open literature. Due to the results given in Figure 3.2, all samples in the following work were prepared 5 – 10 min prior to the extraction.

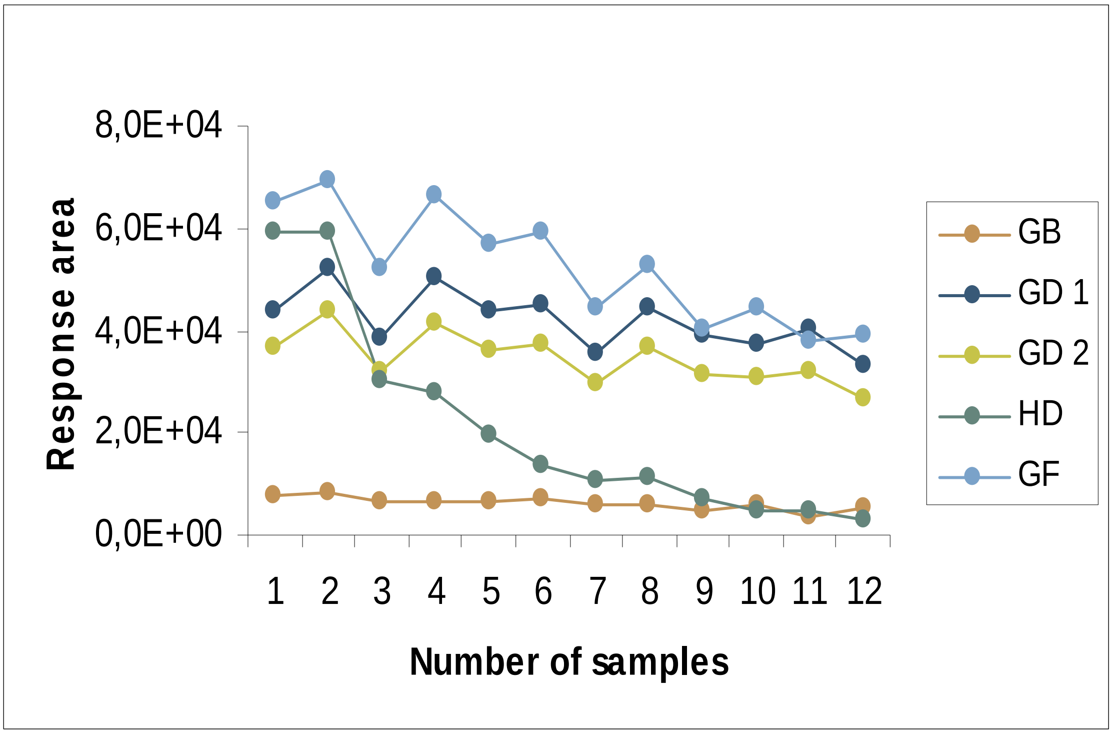


Figure 3.2 Response areas of CWA in distilled and de-ionised water samples prepared simultaneously and analysed consecutively in a sequence. The concentrations of the nerve agents and HD were 0.16 µg/mL and 0.20 µg/mL, respectively.

During the repeatability experiments with the 65 µm PDMS/DVB fiber, it was observed that a newly installed fiber did not give repeatable responses. However, the repeatability was improved by aging the fiber by numerous injections prior to the real analyses, see Table 3.1. The raw data for Table 3.1 are given in appendices B.1.8 - B.1.10. Table 3.1 shows that the RSD for the analyte responses is larger when the fiber has been used only 6 times (see fiber 1) prior to analyses, compared to 17 times.

An even further increase in the number of injections prior to analyses (see fiber 2) did not decrease the RSD significantly. Note also that after 17 injections with fiber 1, the responses of the analytes increase compared to the responses achieved after just 6 injections. This can probably be explained by a decreased amount of softeners in the fiber after multiple injections. The softeners may occupy sites where the analytes can be sorbed and result in lower uptake of the analytes in the fiber. Hence, in all further studies a newly installed fiber was used up to 20 times in blank water samples in addition to being conditioned with the suppliers recommended procedures. Also, prior to any sample analysis, the fiber was conditioned 1 - 2 times in the fiber heater unit, before it was additionally conditioned with 2 - 3 blank water samples, see chapter 2.4.5.

Table 3.1 Response areas of the analytes (1.00 µg/mL) given as a mean value of six replicates with their standard deviations (std. dev.) and relative standard deviations (RSD, %) Fiber 1 and 2 are two 65 µm PDMS/DVB fibers.

	Injections performed		**GB**	**GD 1**	**GD 2**	**GA**
Fiber 1	**6**	**Mean value**	$4.3\cdot10^{4}$	$9.1\cdot10^{5}$	$7.3\cdot10^{5}$	$1.8\cdot10^{5}$
		Std. dev.	$0.7\cdot10^{4}$	$1.0\cdot10^{5}$	$0.9\cdot10^{5}$	$0.4\cdot10^{5}$
		RSD (%)	16	12	12	21
Fiber 1	**17**	**Mean value**	$5.0\cdot10^{4}$	$9.9\cdot10^{5}$	$8.0\cdot10^{5}$	$1.9\cdot10^{5}$
		Std. dev.	$0.3\cdot10^{4}$	$0.6\cdot10^{5}$	$0.4\cdot10^{5}$	$0.1\cdot10^{5}$
		RSD (%)	6.1	5.7	5.5	7.0
Fiber 2	**51**	**Mean value**	$5.9\cdot10^{4}$	$9.6\cdot10^{5}$	$7.7\cdot10^{5}$	$1.9\cdot10^{5}$
		Std. dev.	$0.4\cdot10^{4}$	$0.5\cdot10^{5}$	$0.4\cdot10^{5}$	$0.2\cdot10^{5}$
		RSD (%)	6.1	5.1	5.1	8.7

Figure 3.3 chromatogram A shows results from a newly installed non-conditioned 65 µm PDMS/DVB fiber that liberates compounds, which may overlap with the analytes and cause problems with the reliable identification of CWA. Even after 5 consecutive conditionings of the fiber in the fiber heater unit, the first analysis of a blank distilled and de-ionised water sample, shows interferences (Figure 3.3, chromatogram B). These

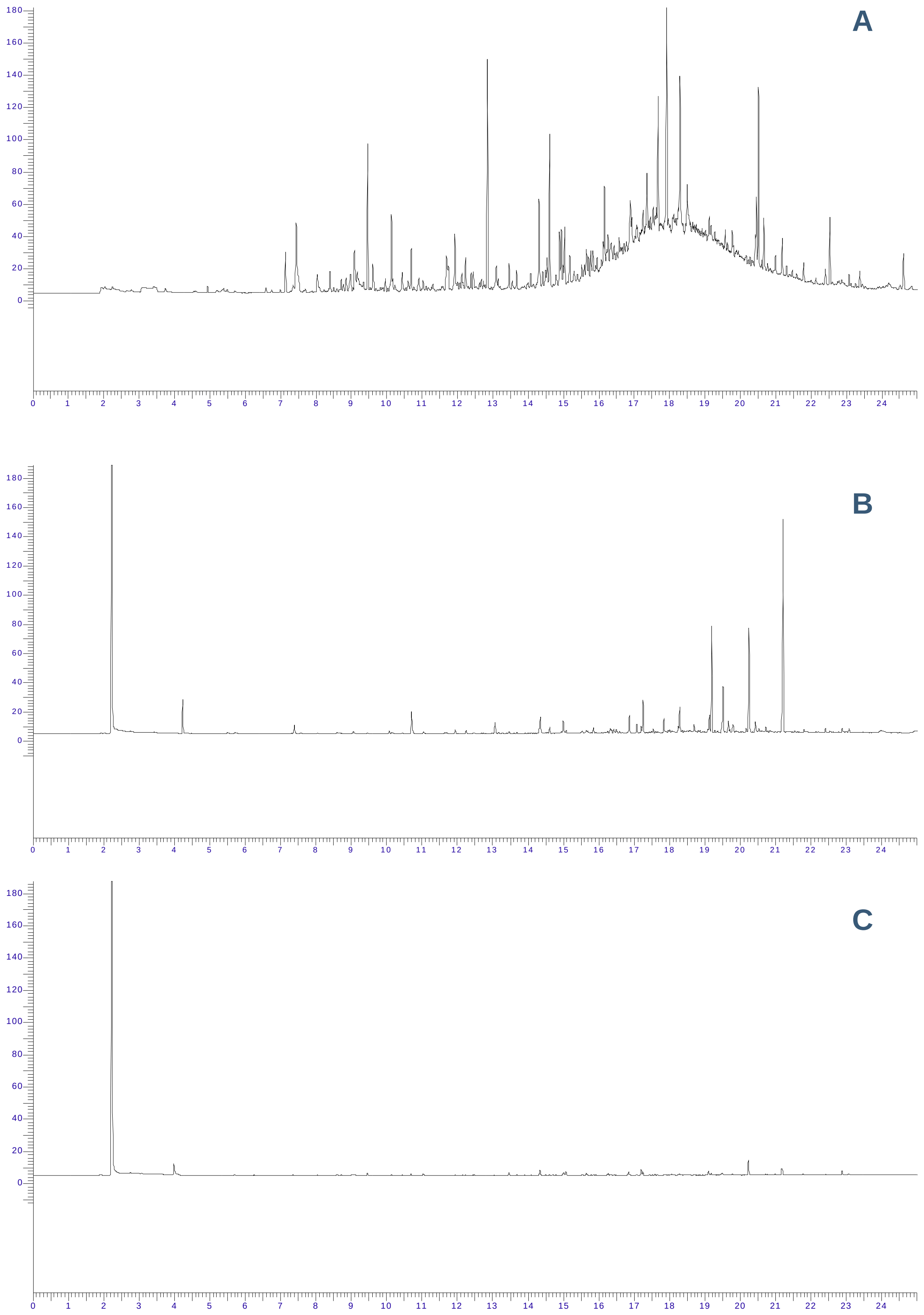


Figure 3.3 Newly installed 65 µm PDMS/DVB fiber before conditioning of the fiber in the fiber heater unit (chromatogram A). Analyses of the first (chromatogram B) and the twentieth (chromatogram C) blank water sample. The analyses were performed after 5 consecutive conditionings of the fiber in the fiber heater unit.

interferences can be due to the ability of the sample matrix to wash out components from the fiber. After 20 analyses of different blank water samples, the interferences are reduced significantly, see Figure 3.3, chromatogram C.

In the present work, newly installed fibers gave compounds interfering with the analytes. This was also reported by Lakso and Ng (23). They performed several blank runs of the fiber prior to analysis, to ensure the absence of compounds that would interfere with the analytes (23). Sng and Leow have performed experiments to reduce the background of already conditioned fibers (53). They studied different storage environments for conditioned 65 µm PDMS/DVB and 75 µm CAR/PDMS fibers. The storage of the fibers in carbon bags in the refrigerator for 28 days gave less background than the storage of the fiber without carbon bags at room temperature. This indicates that a conditioned fiber which is not used for a while should be de-installed from the autosampler and stored in carbon bags in the refrigerator. The storage of the fiber in a cold and protective environment will probably decrease the required number of conditionings of the fiber needed before it can be used for the extraction of real samples. It is important to emphasize that Sng and Leow reported the background of a fiber stored in the refrigerator for 28 days to be greater than a newly conditioned fiber. Further investigation on this subject should be performed once an appropriate fiber for the extraction of CWA has been selected.

3.2.2 Method limit of detection

The lowest concentrations used during the determination of the linearity for GA, GB and GD were used to investigate the signal to noise ratio (Table 3.2, see also appendix B.2.1). The samples were prepared by spiking 15.5 mL of distilled and de-ionised water with 10 µL of a solution of CWA diluted in acetone. After spiking, the water samples were saturated with NaCl (40%, w/w) before a 65 µm PDMS/DVB fiber was immersed in the sample. The SPME was performed with the conditions given in Table 2.5. Next, the fiber was injected into the inlet of the GC-FID and analysed according to the conditions given in chapter 2.4.5.

Table 3.2 Signal to noise ratios (S/N) for GA, GB and GD in distilled and de-ionised water for estimation of method limit of detection. A 65 µm PDMS/DVB fiber and GC-FID was used.

Analyte	Concentration (ng/mL)	S/N
GA	0.34	4
GB	3.2	13
GD	0.19	8

Table 3.2 shows that in the case of GB and GD, a concentration of one third to a half of the analysed concentrations could have been investigated. This would theoretically give a limit of detection of 1 ng/mL for GB and 0.1 ng/mL for GD. Except for GB, the obtained values are close to the reported values by Lakso and Ng (23). They obtained limits of detection of approximately 0.05 ng/mL for GA, GB and GD by using GC MS-SIM and GC-nitrogen phosphorus detector (NPD) (23). The method limits of detection (MLOD) determined in this work by GC-FID are 4, 7 and 65 times higher than the reported (23) values for GD, GA and GB, respectively. The rather poor MLOD for GB can be related to the co-elution of GB with a compound present in the water. Otherwise, the small differences in MLOD for GA and GD are mainly due to the use of more sensitive and selective detector systems by Lakso and Ng.

3.2.3 Linearity

The linearity of GA, GB and GD using the SPME GC-FID system was investigated by spiking water samples with 10 µL of a solution of the agents diluted in acetone. Water samples with concentrations ranging from 0.19 ng/mL to 8.60 µg/mL were prepared (see also appendix B.2.1), and all the samples were prepared just prior to the analysis. The samples were saturated with NaCl (40%, w/w) and the 65 µm PDMS/DVB fiber was immersed for 30 min in each sample for the extraction of the CWA. The SPME and GC-FID analysis conditions are given in Table 2.5 and chapter 2.4.5. One injection at each concentration level was made, except for the concentration of 0.516 µg/mL that was analysed twice.

Figure 3.4 (see also appendix B.2.1) shows the obtained linearity for GA and GD, and the linear ranges were found to be 0.34 ng/mL - 2.58 µg/mL (R^2 = 0.991) and 0.19 ng/mL - 2.58 µg/mL (R^2 = 0.99), respectively. For GB, the linear range was from 3.2 ng/mL to 1.03 µg/mL (R^2 = 0.9896) (Figure 3.5). When the linearity was investigated in the whole concentration range from 0.19 ng/mL to 8.60 µg/mL, the regression coefficients were as following: 0.92 (GA), 0.95 (GD 1), 0.88 (GD 2) and 0.92 (GB). However, the concentrations included in the obtained linear ranges are far higher than the concentrations reported by Lakso and Ng (23). Their values are given in Table 3.3 and their determinations of the linear ranges were based on the use of both GC-MS in selected ion monitoring (SIM) mode and GC-NPD. These detectors are more sensitive than the FID and are therefore able to give responses at ppt level.

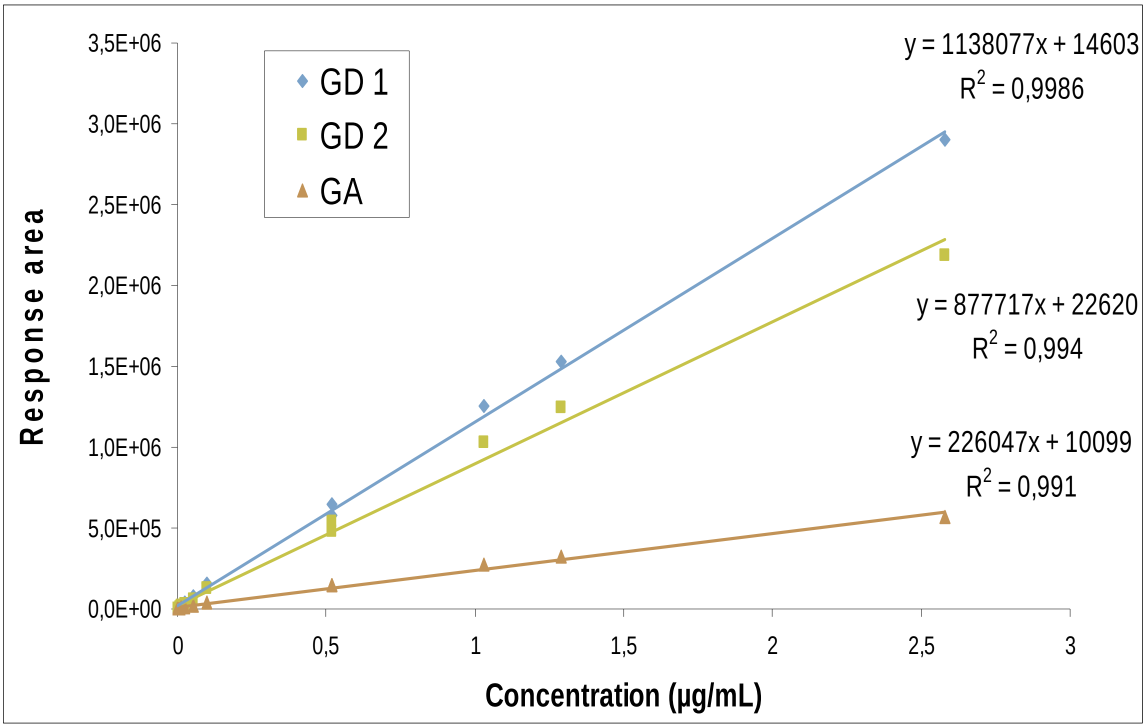


Figure 3.4 Response area as a function of concentration for GA and GD in distilled and de-ionised water. The concentration ranges were 0.34 ng/mL - 2.58 µg/mL and 0.19 ng/mL - 2.58 µg/mL, respectively. A 65 µm PDMS/DVB fiber and GC-FID was used.

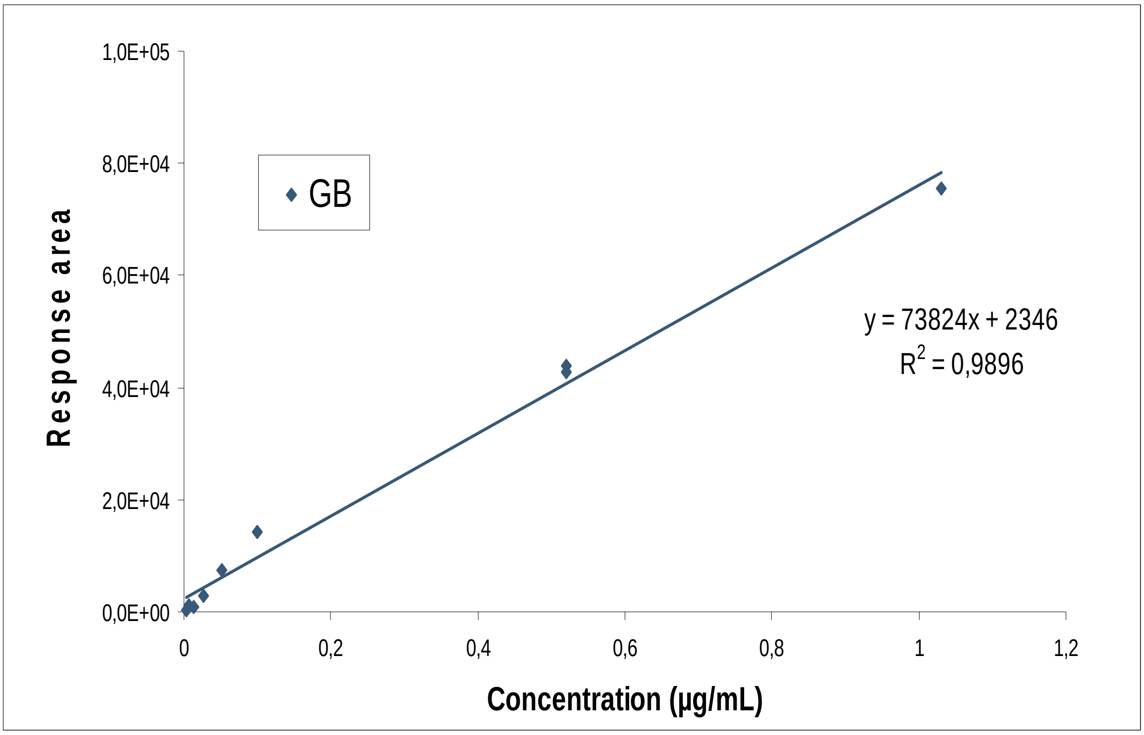


Figure 3.5 Response area as a function of concentration for GB in distilled and de-ionised water. The concentration range is from 3.2 ng/mL to 1.03 µg/mL. A 65 µm PDMS/DVB fiber and GC-FID was used.

In the present work, the lowest concentration levels for GA, GB and GD are 3, 32 and 2 times higher, respectively, than the reported values obtained by the GC-MS SIM by Lakso and Ng (Table 3.3). The highest concentration levels for GA, GB, and GD are 26, 10 and 52 times higher, respectively, than the upper concentration level reported by Lakso and Ng. (23). The MS has a limitation in the upper concentration range due to saturation of the electron multiplier detector. The FID does not have this limitation and can detect higher concentrations without being saturated. Therefore, the linear ranges for GA and GD achieved by GC-FID cover almost 4 decades, compared to 2 – 3 decades for the GC-MS SIM (Table 3.3).

The regression coefficients (R^2) for the linear plots obtained with GC-MS SIM are closer to unity than in the case of the GC-FID. This can be due to the extraction of ions in SIM mode which enables the differentiation of the analytes from the matrix (increased selectivity), making it easier to integrate the responses of the analytes.

Note also that the range of quantification by GC-NPD is even more limited than by GC-MS SIM. However, the lowest quantification level for GC-NPD is half of the concentration of the lowest quantification level for GC-MS SIM. The NPD is a selective detector giving lower detection limits than MS in SIM mode.

Table 3.3 Linearity for GA, GB and GD reported by Lakso and Ng (23). A 65 µm PDMS/DVB fiber was immersed for 30 min in spiked de-ionised water saturated with NaCl. The fiber was analysed by GC MS-SIM and GC-NPD.

	GC-MS SIM		GC-NPD	
Analyte	**Range of quantification (ng/mL)**	**Coefficient of linearity (R^2)**	**Range of quantification (ng/mL)**	**Coefficient of linearity (R^2)**
GB	0.1 - 100	0.997	0.05 - 25	0.994
GD	0.1 - 50	0.993	0.05 - 25	0.999
GA	0.1 - 100	0.998	0.05 - 10	0.999

It is important to emphasize that the present results described above are based on 10 µL constant spiking volume of a solution of CWA. However, Lakso and Ng have not stressed the use of same volume of solution for spiking of the water samples. From their description of how the solutions were made, it is not obvious if the volume used for spiking was kept constant or not. In order to investigate how the linear range changes when the spiking volume varied, solutions were made from a stock solution of 1.00 mg/mL. Spiking volumes in the range from 0.2 µL to 38 µL of the stock solution were added to distilled and de-ionised water, giving a concentration range from 12.9 ng/mL to 2.45 µg/mL. These solutions were subsequently saturated with NaCl (40%, w/w). Figure 3.6 and 3.7 (see also appendix B.2.2) show that the linear range is reduced compared to previous results (Figure 3.4 and 3.5). When the solution with a concentration of 2.45 µg/mL is excluded from the linear plots for GA and GD, the regression coefficients are 0.991 and 0.999, respectively. The upper concentration limit for their linear ranges is reduced to 1.00 µg/mL. This is half of the upper concentration limit as determined when the solutions were spiked with a constant volume of 10 µL of a solution with CWA. In the case of GB, when the upper concentration limit was reduced from 1.00 µg/mL to 0.516 µg/mL, the regression coefficient increased from 0.93 to 0.991.

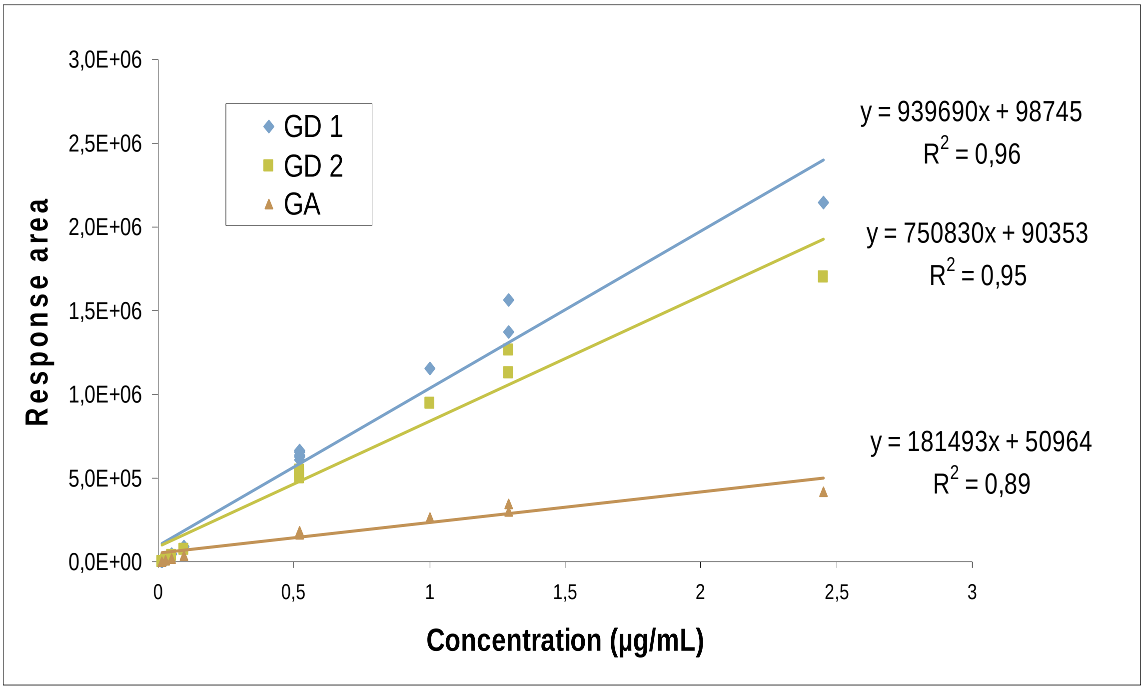


Figure 3.6 Response area as a function of concentration for GA and GD in the range from 12.9 ng/mL to 2.45 µg/mL. Distilled and de-ionised water samples were spiked with 0.2 - 38 µL of 1.00 mg/mL solution of CWA in acetone.

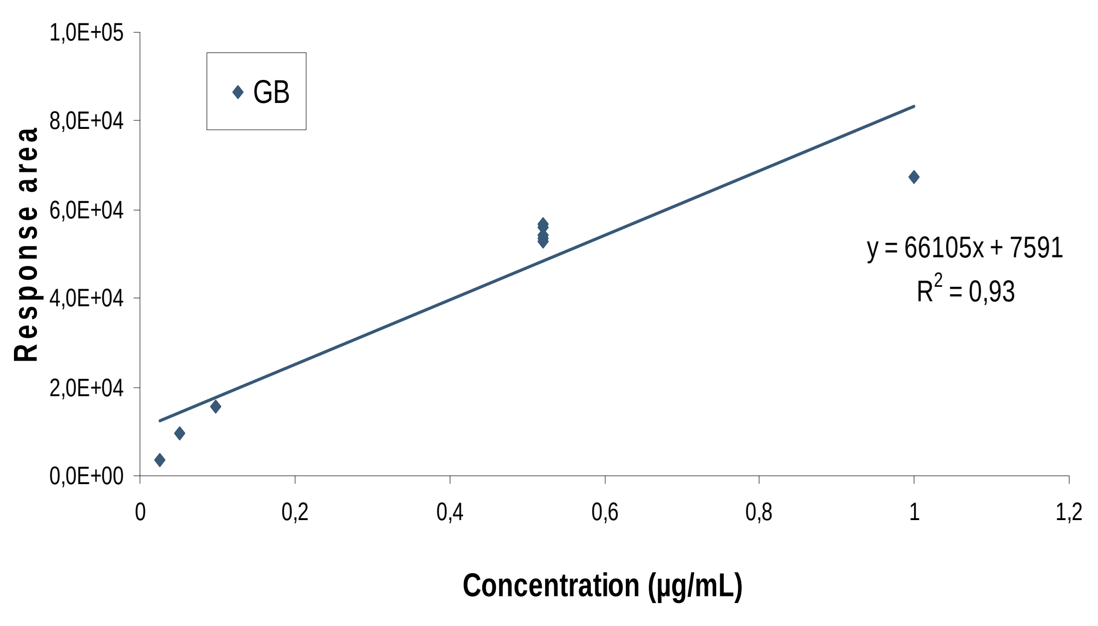


Figure 3.7 Response area as a function of concentration for GB in the range from 25.8 ng/mL to 1.00 µg/mL. Distilled and de-ionised water samples were spiked with 0.4-15.5 µL of 1.00 mg/mL solution of CWA in acetone.

An increase of spiking volume when making the solutions gave reduced response areas for the CWA. This may be due to an increased uptake of acetone in the fiber, having a negative influence on the uptake of CWA. This phenomenon may explain the reduced linear range when using varying spiking volumes. Therefore, in further investigations, the water samples were spiked with a constant volume of 10 µL of a solution of CWA in acetone.

Note also that the linear plot for GB with an upper concentration level of either 0.516 µg/mL or 1.00 µg/mL have a rather logarithmic shape (Figure 3.5 and 3.7). This has also previously been observed with liquid injection on the same instrument, but further investigations are needed in order to understand this observation.

3.3 Development of SPME GC-FID method

The aim of this study was to develop an SPME GC-FID method for the determination of GB, GD, GF and HD in water samples. The work focused on the optimization of the parameters for the SPME. The parameters for the GC-FID method were kept according to those reported by Lakso and Ng, and FFI's previous work (23, 54). The development of a new SPME method requires optimization of the following parameters: type of fiber coating, extraction mode (headspace, immersion), agitation speed, desorption conditions, sample volume, extraction conditions (pH, salt, temperature) and extraction time (26). The optimization of the extraction mode, extraction conditions, sample volume and agitation speed are described in the literature, and was not optimized in the present study. A brief discussion of these parameters is included below.

Extraction of the agents from water has previously been performed by immersion into the sample solution rather than by exposure of the fiber to the headspace of the sample (22, 23, 31-33). In this study the fiber was immersed in the water sample, and the vial was filled to the neck with water to minimize the headspace above the sample. The sample was kept at 35 ºC during the extraction. This temperature was chosen to ensure a low concentration of the analytes in the headspace, and also to minimize degradation of the agents (13, 51). An even lower temperature during extraction would have been preferable from a molecular stability-perspective, but was judged to be too time-consuming, since the heater and agitation unit needed considerable time to obtain a stable temperature of 30 ºC (chapter 2.3.1).

Adjusting the pH of the water samples was not considered necessary as the pH of the different water samples was in the range of 5 - 8. In this pH-range the hydrolysis rate of GB, GD, HD and GF is considered to be at the lowest (13, 51). Increased hydrolysis is observed when the conditions are more acidic or alkaline.

The water samples were saturated with NaCl since an increase of the ionic strength has been reported to give larger amounts of the nerve agents in both PDMS and PA based fibers, due to the “salting-out” effect (23). Interestingly, the hydrolysis of HD is reduced in salt water compared to distilled water. At a temperature of 25 ºC, the half-life time of HD is 60 min in salt water and only 8.5 min in distilled water (51).

During immersion of the fiber in the water solution, the fiber and the vial were agitated with a speed of 250 rpm. The agitation of the sample reduces the equilibrium time and thus increases the extraction efficiencies. In most cases agitation is required for aqueous samples to facilitate the mass transport between the bulk of the aqueous sample and the fiber (26). In previous work, teflon-coated magnetic stirring bars were used to gain a stirring speed of 800 rpm for the extraction of GB and GD from water (23). With the use of the MPS 2 autosampler from Gerstel, the fiber and the vial were moved instead of using a magnethic stirring bar. With this technique, it was not possible to use an agitation speed higher than 250 rpm during the extraction, and this value was therefore kept in the method. However, prior to the immersion of the fiber into the sample, the vial was agitated by the MPS 2 autosampler at 750 rpm for 6 s to homogenize the sample, see Table 2.5.

In chapter 3.3.1 - 3.3.4, the optimization of other SPME parameters as fiber coating, extraction time and desorption conditions will be described. The influence of these parameters on the recovery of the agents from distilled and de-ionised water will be explained.

3.3.1 Selection of fiber

The selection of the SPME fiber is very important because it determines the affinity of the analytes to the fiber. Therefore, three commercially available fibers which were compatible with the polarity of the agents were chosen for further investigation: 65 µm PDMS/DVB, 50/30 µm DVB/CAR/PDMS and 85 µm PA fibers. The PA and PDMS/DVB fibers have been reported for extraction of GB, GD and HD by the immersion of the fibers in water samples (23, 31-33). The use of DVB/CAR/PDMS for extraction of the CWA from water has not yet been reported. The selection of the three fibers is based on their ability to retain polar, semi volatile and volatile compounds, with molecular masses in the range from 50 to 300 g/mol. The PA fiber is specially suited to extract polar and semi-volatile compounds, whereas the mixed phase coating fibers are developed for extraction of semi-volatiles and volatiles, such as amines and flavors. Fibers with relatively unpolar coatings were not investigated, since earlier publications have shown that unpolar coating like polydimethylsiloxane (PDMS) is not as suitable as PDMS/DVB to extract GA, GB, GD, and VX from water (23).

The selected fibers were conditioned by following the recommendations given by the manufacturers. The 65 µm PDMS/DVB, 50/30 µm DVB/CAR/PDMS and 85 µm PA were conditioned for 0.5 hour at 250 ºC, 1 hour at 270 ºC and 2 hours at 300 ºC, respectively. Thereafter, the fibers were conditioned further by analyses of blank water samples before real samples were analysed, as described in chapter 2.4.3. Between two consecutive sample extractions, the fiber was conditioned in the fiber heater unit for 10 min, and the conditioning temperatures for PDMS/DVB, DVB/CAR/PDMS and PA were 250 ºC, 250 ºC and 280 ºC, respectively. The three fibers were evaluated by using the same SPME GC-FID parameters as described in chapter 2.4.3, except that the extraction time was reduced from 30 min to 10 min. A shorter extraction time was considered as sufficient for the evaluation of the fibers.

Selection of the most suitable fiber for the extraction of GB, GD, HD and GF was done by investigating the uptake of CWA using a certain concentration range. By using a concentration range, a profile of the uptake including low, intermediate and high concentrations was obtained. The chosen concentration range was mainly the same as the linear range for the PDMS/DVB fiber, see chapter 3.2.3. To obtain the desired concentration range, a stock solution of 2.50 mg/mL of the analytes was prepared. The stock solution was used to make working solutions in the range from 10.0 µg/mL to 1.55 mg/mL. Several working solutions were made to ensure a spiking volume of 10 µL to the distilled and de-ionised water samples. The concentration of the nerve agents in the water samples were: 6.45 ng/mL, 12.9 ng/mL, 0.0968 µg/mL, 0.300 µg/mL, 0.538 µg/mL and 1.00 µg/mL. All the different concentrations were analysed within the same day and each level was analysed only once, except the concentration of 0.0968 µg/mL. The number of replicates at 0.0968 µg/mL for the different fibers were: PDMS/DVB (n = 6), PA (n = 5) and DVB/CAR/PDMS (n = 1). Due to breakage of the DVB/CAR/PDMS fiber, only one analysis was performed at 0.0968 µg/mL. However, the repeatability (n = 7) at this concentration was performed with the use of another DVB/CAR/PDMS fiber, and will be discussed in another section below.

The uptake (measured as response area) of GB, GD, HD and GF at different concentration levels obtained by the three fibers are shown in Figure 3.8. The raw data are given in appendices C.1.1 – C.1.5. Figure 3.8 clearly shows that the sorption of the nerve agents by the PA fiber is considerably lower than by the DVB/CAR/PDMS and the PDMS/DVB fibers. The sorption of GD and GF by the DVB/CAR/PDMS fiber are approximately 1.5 times the sorption by the PDMS/DVB fiber. For GB the sorption by the DVB/CAR/PDMS is even larger compared to PDMS/DVB fiber, and it is increased approximately by a factor of 2.5. It is important to emphasize that the sorption of the nerve agents over the whole concentration range is larger with the DVB/CAR/PDMS fiber than by the PDMS/DVB fiber. Figure 3.8 also shows that the response area for GB is the smallest compared to the other nerve agents. However, the uptake of HD by the PDMS/DVB fiber is superior in the whole concentration range compared to PA and DVB/CAR/PDMS fibers. The sorption of HD by the PDMS/DVB fiber is 1.7 times larger than the sorption by the PA fiber, and increases with a factor of 2.6 with the DVB/CAR/PDMS fiber (see Figure 3.8). Hence, the DVB/CAR/PDMS fiber is not the optimal fiber for the extraction of HD from water samples. The extraction of water samples contaminated with HD should thus be done by the use of PDMS/DVB fiber. Even so, in the further investigations, the DVB/CAR/PDMS fiber was used since the purpose was to develop a method for all the CWA including HD. Additionally, it is important to emphasize that in general the response area of GB is the lowest of the

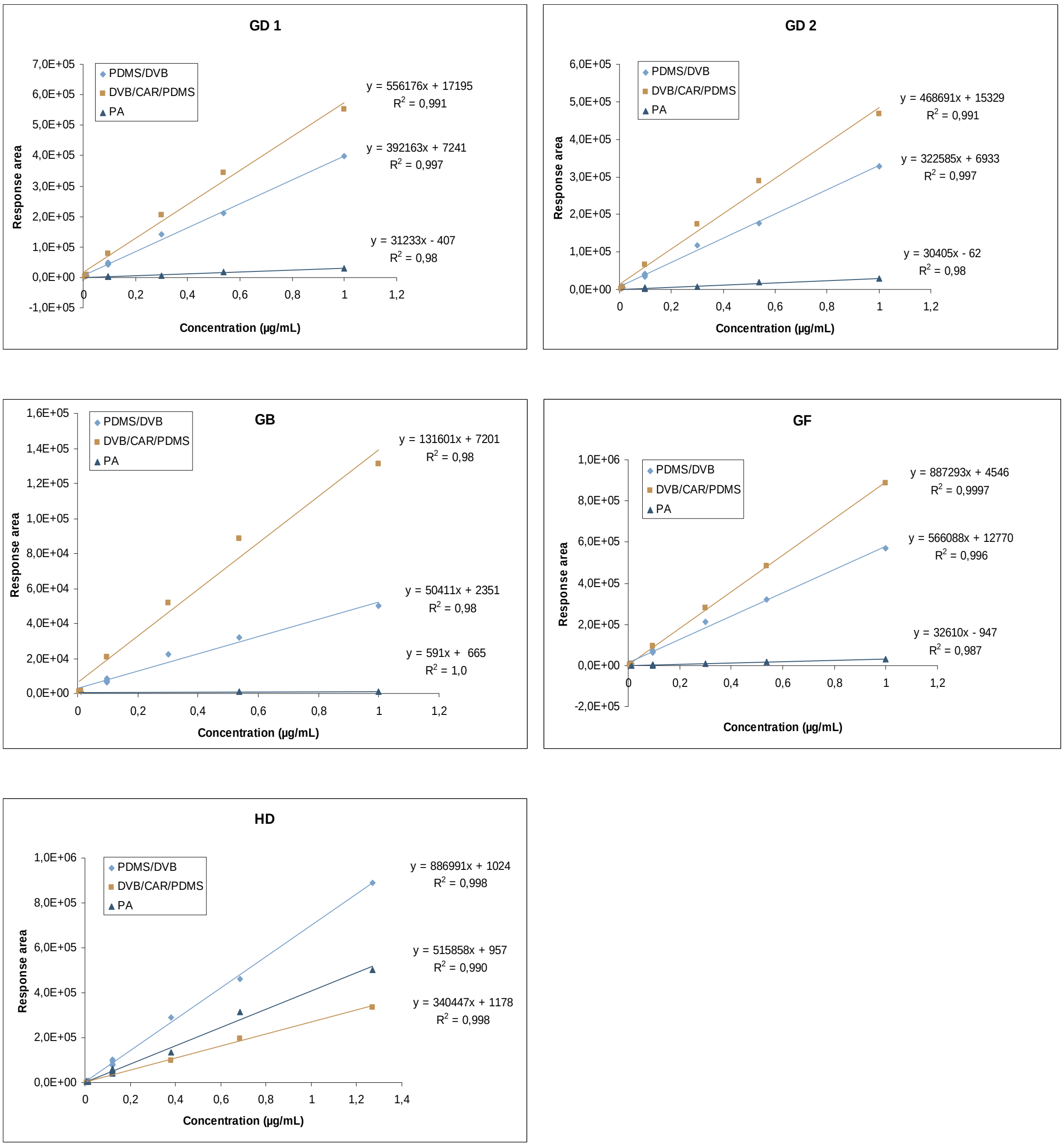


Figure 3.8 Response area as a function of concentration for CWA in distilled and de-ionised water, with the use of 65 µm PDMS/DVB, 50/30 µm DVB/CAR/PDMS and 85 µm PA fibers. The investigated concentration ranges for the nerve agents and HD were 6.45 ng/mL - 1.00 µg/mL and 8.19 ng/mL - 1.27 µg/mL, respectively.

investigated CWA. Taking this into consideration, the DVB/CAR/PDMS fiber should be chosen instead of the PDMS/DVB fiber.

The PA fiber was least suitable for extraction of the investigated CWA from water samples. This can be explained by the fact that PA is a solid material at room temperature, which results in slow uptake and desorption of the analytes (55). Thus, it takes longer time to extract the analytes with the PA fiber than with the other conventional fibers. Additionally, higher temperatures are required to desorb the analytes from this fiber. This may indicate that the fiber should be evaluated at a longer extraction time than 10 min and probably with a higher desorption temperature than 250 ºC.

In addition to study of the sorption capacity of the fibers at different concentrations, the repeatability was investigated, see Table 3.4. The raw data for Table 3.4 are given in appendices C.1.1 - C.1.6. The repeatability was measured at a concentration of 0.0968 µg/mL for the nerve agents and 0.123 µg/mL for HD in distilled and de-ionised water. The DVB/CAR/PDMS fiber which has the highest sorption capacity for the nerve agents, had a relative standard deviation of the response areas of 3.2-10%. HD had the largest RSD, which was also the case with the use of the PDMS/DVB fiber. In general, the RSDs for both PDMS/DVB and DVB/CAR/PDMS were acceptable, considering that each of the replicates were prepared separately. It should also be mentioned that HD degrades more rapidly than the other CWA (13, 51). In contrary to the mixed fibers, the PA fiber gave relatively high RSD for GD and GF. This may be due to the greater uncertainty in the integration of the relatively small response areas of GD and GF in comparison to the integration of the large response area for HD.

Table 3.4 Relative standard deviation (RSD, %) in percent for the response areas of the CWA. The fibers were immersed in distilled and de-ionised water spiked with nerve agents (0.0968 µg/mL) and HD (0.123 µg/mL). n.d. indicates that the concentration was below the limit of detection.

		RSD of response area (%)				
Type of fiber	**Number of replicates**	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
PDMS/DVB	**6**	9.3	6.3	6.2	12	6.5
DVB/CAR/PDMS	**7**	3.2	5.6	4.1	10	4.0
PA	**5**	n.d.	30	52	16	30

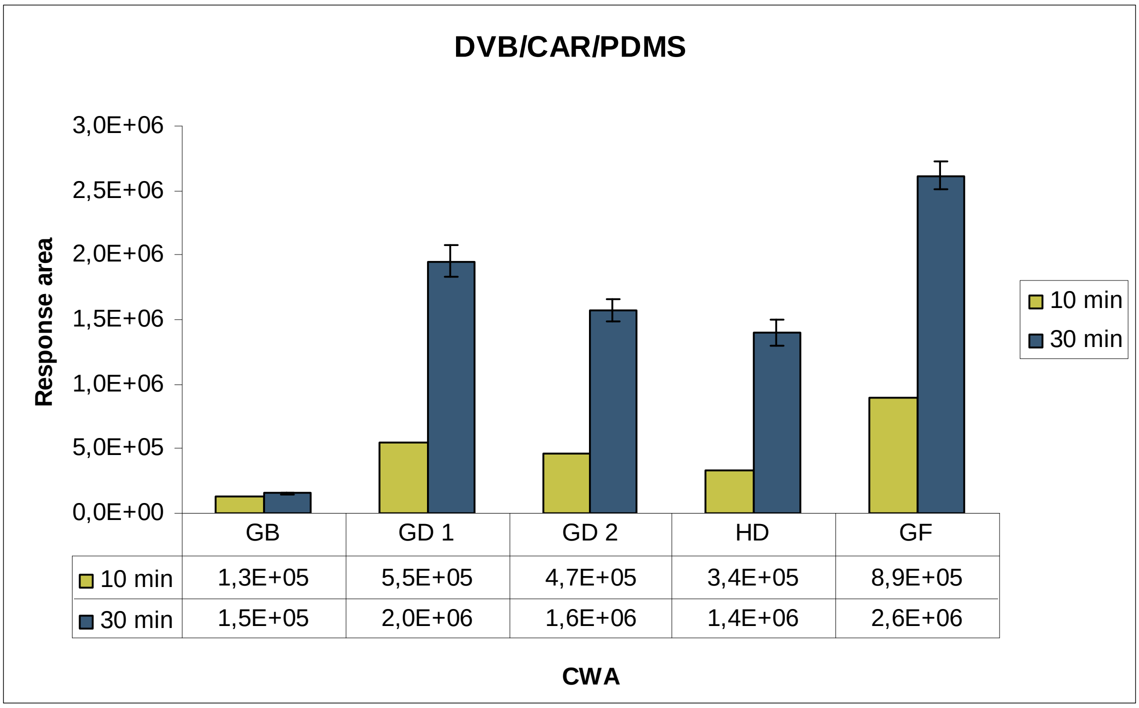
3.3.2 Extraction time

The extraction time of 10 min that was used in the evaluation of the three fibers (chapter 3.3.1) is not sufficient to establish equilibrium between the analytes and the fiber. Ideally, the extraction time should be the equilibrium time in order to achieve maximum sensitivity. The extraction profiles for GA, GB, GD and VX in saturated salt solution of water have been investigated by using a 65 µm PDMS/DVB fiber (23). GB has the shortest equilibrium time because of its low molecular mass (23), see Table 1.1. However, an extraction time longer than the equilibration time of 30 min for GB, showed lower extraction efficiency, probably due to the degradation of GB and hence lower concentration of GB in saturated salt solutions (23). This may cause desorption of the absorbed GB from the fiber, resulting in a decrease of the extracted amount of GB in the fiber. The equilibrium time for GA, GD and VX was not reached within 150 min. This is probably due to the slow rate of diffusion of the nerve agents into the fiber.

The experiences made by Lakso and Ng indicate that choosing an extraction time close to the equilibrium time for GD, will lead to the unfortunate degradation of GB. At an extraction time which equals the equilibrium time of GB (30 min), the amount of GB and GD extracted by the fiber were approximately 300 ng and 400 ng, respectively (23). Therefore, an extraction time of 30 min will not cause lower amount of GD to be extracted compared to GB.

It is known that the nerve agents degrade in aqueous solution, with a rate determined by the characteristics of the matrix such as pH, type and concentration of cations present, and salinity (23). Considering these facts and the experiences made by Lakso and Ng, an extraction time of 30 min was investigated for the three fibers in the present study. Additionally, a relatively high concentration of the nerve agents (1.00 µg/mL) and HD (1.27 µg/mL) in water saturated with salt was analysed. The high concentrations were used to check if the fibers were saturated or not at the previous extraction time of 10 min.

Figure 3.9 - 3.11 display the uptake of CWA by the three fibers at an extraction time of 30 min (n = 4) (see also appendices B.2.1 and C.2). The results are shown together with the uptake of the CWA by the fibers at an extraction time of 10 min (n = 1)

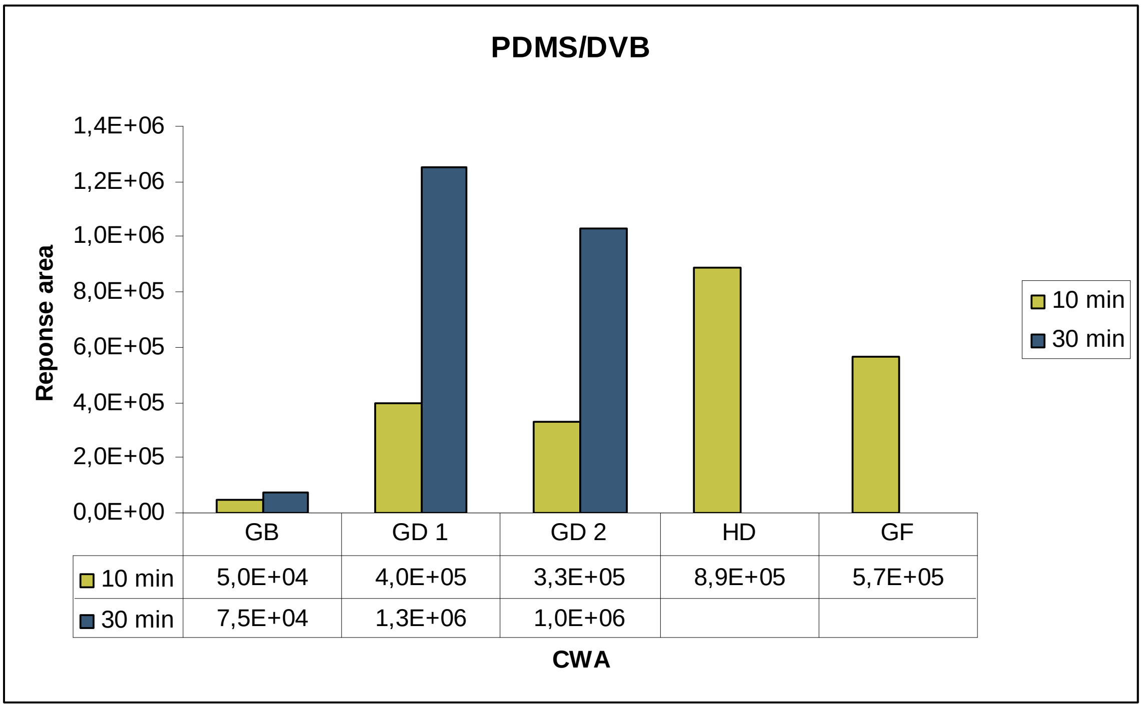


	GB	GD 1	GD 2	HD	GF
10 min	1,3E+05	5,5E+05	4,7E+05	3,4E+05	8,9E+05
30 min	1,5E+05	2,0E+06	1,6E+06	1,4E+06	2,6E+06

Figure 3.9 Sorption (measured as response area) of the selected CWA at an extraction time of 10 min and 30 min. The 50/30 µm DVB/CAR/PDMS fiber was immersed in distilled and de-ionised water containing 1.00 µg/mL of nerve agents and 1.27 µg/mL of HD. The error bars indicates the standard deviations of the mean values (n = 4).

see appendices C.1.1 - C.1.5). Figure 3.9 - 3.10 clearly demonstrate that the sorption of the CWA by the DVB/CAR/PDMS and the PDMS/DVB fibers increases with increased extraction time. For the DVB/CAR/PDMS fiber, the sorption of GD, HD and GF are 3 – 4 times higher at an extraction time of 30 min compared to an extraction time of 10 min. However, the uptake of GB by this fiber is not significantly influenced by increasing the extraction time beyond 10 min. The response area for GD by the PDMS/DVB fiber increases with a factor of three when the extraction time is tripled. For this fiber the response area for GB increases 1.5 times when the extraction time is prolonged from 10 min to 30 min. However, the response area for GB using PDMS/DVB fiber is only half of the area obtained by the DVB/CAR/PDMS fiber at an extraction time of 30 min.

The response areas for HD and GF by the PDMS/DVB at an extraction time of 30 min are not given. The experiments performed using an extraction time of 30 min were done as part of the evaluation of the SPME GC-FID instrumentation, where the study only



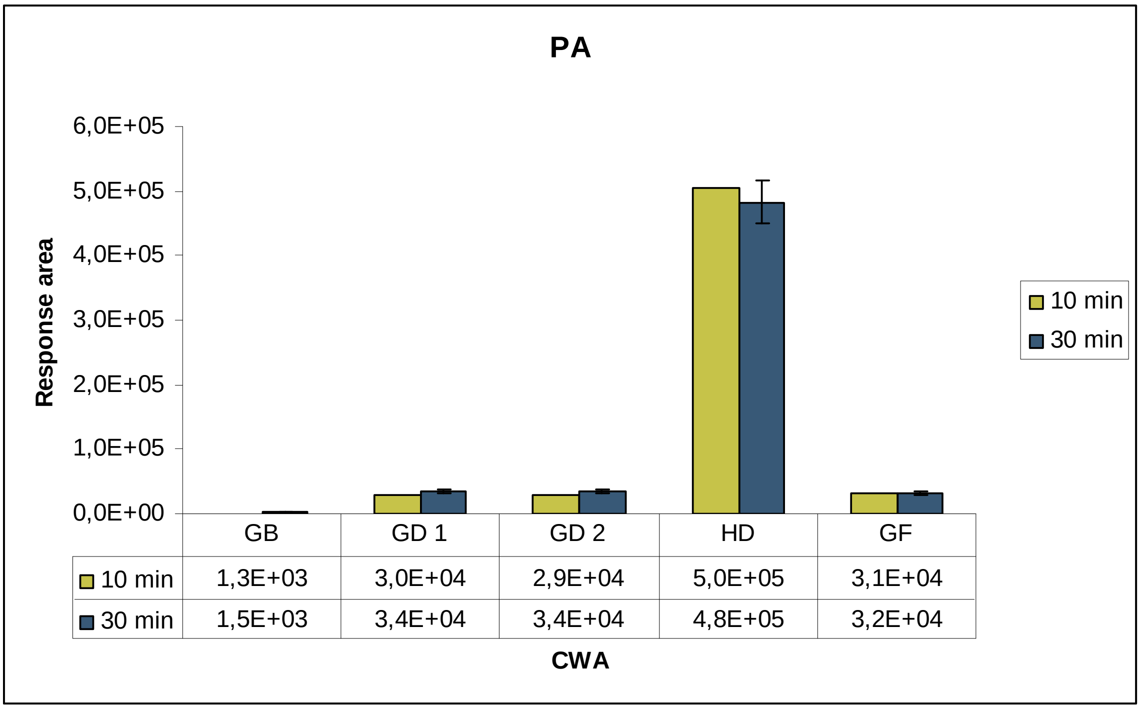
	GB	GD 1	GD 2	HD	GF
🟨 10 min	5,0E+04	4,0E+05	3,3E+05	8,9E+05	5,7E+05
🟦 30 min	7,5E+04	1,3E+06	1,0E+06		

Figure 3.10 Sorption (measured as response area) of the selected CWA at an extraction time of 10 min and 30 min. The 65 µm PDMS/DVB fiber was immersed in distilled and de-ionised water. The concentration of the nerve agents were 1.00 µg/mL and 1.27 µg/mL for HD.

focused on GA, GB and GD (chapter 3.2.3).

The sorption of CWA by the PA fiber is not significantly influenced by increasing the extraction time beyond 10 min, see Figure 3.11. As mentioned earlier, PA is a solid material at room temperature, which could result in a slow uptake and desorption of the analytes. Thus, it takes longer to extract the analytes with the PA fiber than with the other conventional fibers. Another possible explanation for the low uptake of CWA by the PA fiber is that the fiber is too polar for the extraction of the nerve agents.

Based on these results, the DVB/CAR/PDMS fiber was chosen and used with an extraction time of 30 min. 30 min is also the time required for each GC analysis. Hence, the extraction of a sample can be done simultaneously as the GC analyses the previous sample. After the start of a GC cycle, the fiber is conditioned for 10 min prior to the extraction of a new sample. These operations are performed while the GC analyses the sample and the GC oven cools to its initial temperature. Therefore, additional time is not



	GB	GD 1	GD 2	HD	GF
10 min	1,3E+03	3,0E+04	2,9E+04	5,0E+05	3,1E+04
30 min	1,5E+03	3,4E+04	3,4E+04	4,8E+05	3,2E+04

Figure 3.11 Sorption (measured as response area) of the selected CWA at an extraction time of 10 min and 30 min. The 85 µm PA fiber was immersed in distilled and de-ionised water containing 1.00 µg/mL nerve agents and 1.27 µg/mL HD. The error bars indicates the standard deviations of the mean values (n = 4).

required when the extraction time is increased from 10 min to 30 min.

3.3.3 Desorption temperature

After completing the extraction of the analytes, the analytes are thermally desorbed in the injector port of the GC. The desorption temperature used so far has been 250 ºC, which is the same temperature as used by Lakso and Ng. At this temperature the ability of the fiber coating to retain the analytes is very low. Additionally, the flow of carrier gas within the GC injector helps to release the analytes from the polymer material. The released analytes are transferred to a cold column for refocusing.

It is important to select a desorption temperature at which the analytes are not thermally decomposed. The decomposition temperatures for GB, GD and HD are 150 ºC, 130 ºC and 180 ºC, respectively (13, 51). A desorption temperature of 250 ºC is thus relatively high considering the decomposition temperatures of the agents. GF is considered to be very stable, but detailed data for the decomposition temperature for GF is not available (13).

The fibers also have recommended operating temperatures. The recommended operating temperatures for the DVB/PDMS/CAR fiber are in the range from 230 to 270 ºC. Taken the decomposition temperatures of the agents into consideration, desorption temperatures lower than the recommended temperature range of the fiber needed to be investigated. Desorption temperatures lower than the recommended temperature range of the fiber, may cause less release of the agents from the fiber, which may require a more thorough conditioning of the fiber between sample analyses.

During optimization of the desorption temperature, the other parameters for the SPME was kept as described in chapter 2.4.5. The desorption temperature was varied from 130 to 270 ºC. At each temperature, only one analysis was performed to establish a profile of the effect of the desorption temperature on the response areas of the CWA. Distilled and de-ionised water samples with a concentration of 1.29 µg/mL of the nerve agents were analysed. The concentration of HD in the same water samples were 1.64 µg/mL. The water samples were saturated with NaCl (40%, w/w). Between two subsequent extractions, the fiber was conditioned at 250 ºC for 20 min in the fiber heater unit, to avoid carry-over from the previous sample. For the same reason, the analyses were performed from high to low desorption temperatures. Figure 3.12 shows the influence of the desorption temperature on the response area of GB, GD, HD and GF (see also appendix C.3.1). The response areas show a declining profile as the desorption temperatures are decreased. Additionally, the peak width for GB increased at temperatures below 220 ºC, and the separation of the two diastereoisomers of GD were poorer at low temperatures compared with high temperatures. The response areas at 130 ºC for GB, GD 1, GD 2, HD and GF, 75%, 23%, 50%, 14% and 15%, respectively, of the response area of the agents at 250 ºC. Hence, a desorption temperature in the upper temperature range is more suitable for the desorption of the agents (see Figure 3.12). The upper temperature range corresponds to the recommended temperature range for the DVB/CAR/PDMS fiber. Therefore, a relatively high desorption temperature seems to be essential for the liberation of the sorbed analytes from the fiber. The undesirable decomposition of the agents are not pronounced at the relatively high temperatures, possibly because of the rather short desorption time of 2 min.

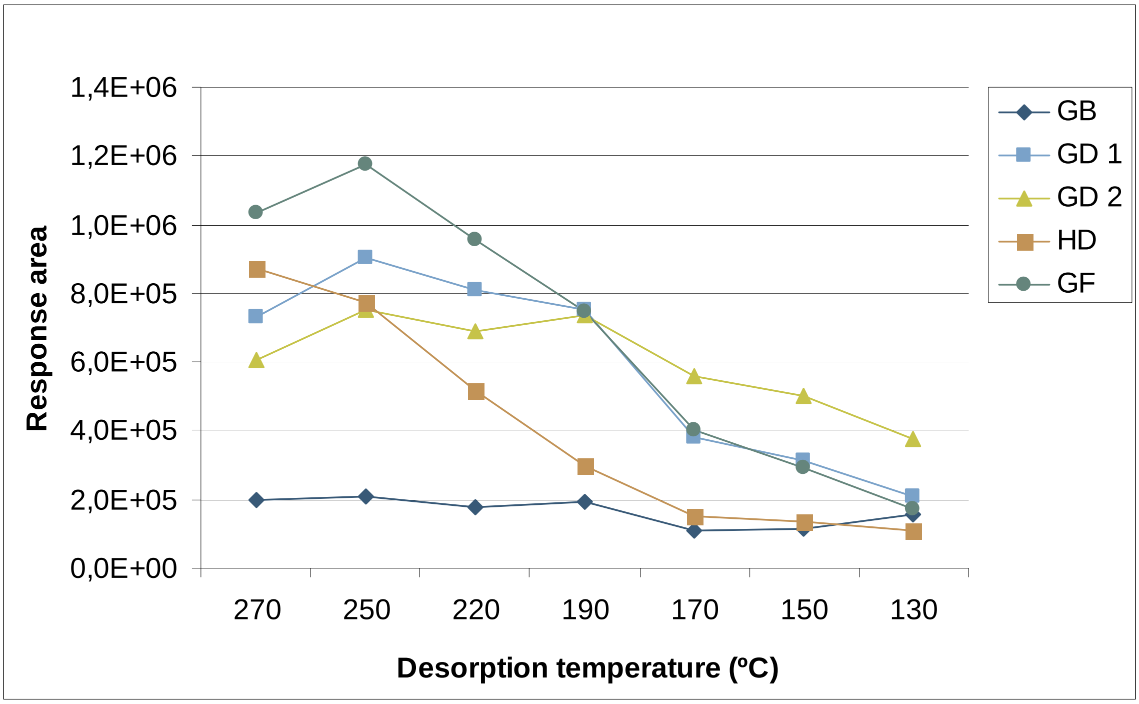


Figure 3.12 The response areas for GB, GD, GF and HD as a function of the desorption temperature(ºC) at the inlet of the GC. The DVB/CAR/PDMS fiber was immersed in distilled and de-ionised water spiked with nerve agents (1.29 µg/mL) and HD (1.64 µg/mL) (n = 1).

A closer investigation of the response areas of the CWA with a desorption temperature in the recommended temperature range of the fiber was performed, see Figure 3.13 (see also appendices C.3.2 - C.3.4). A concentration of 0.645 µg/mL of the nerve agents were analysed at a desorption temperature of 230 ºC (n = 6), 250 ºC (n = 5) and 270 ºC (n = 5). The concentration of HD in the same water samples was 0.819 µg/mL. There are no significant differences in response areas for the nerve agents at the different desorption temperatures. However, the response area of HD is significantly reduced with decreasing desorption temperature.

The use of the fiber at its maximum temperature of 270 ºC will lead to shorter life time of the fiber. The fiber becomes more porous when it is operated at relatively high temperatures. High temperature causes desorption of the softeners in the fiber, which gives a more porous polymer material, and the danger for breakage of the fiber is greater when the fiber is porous. Therefore, a desorption temperature of 250 ºC was used in the following experiments.

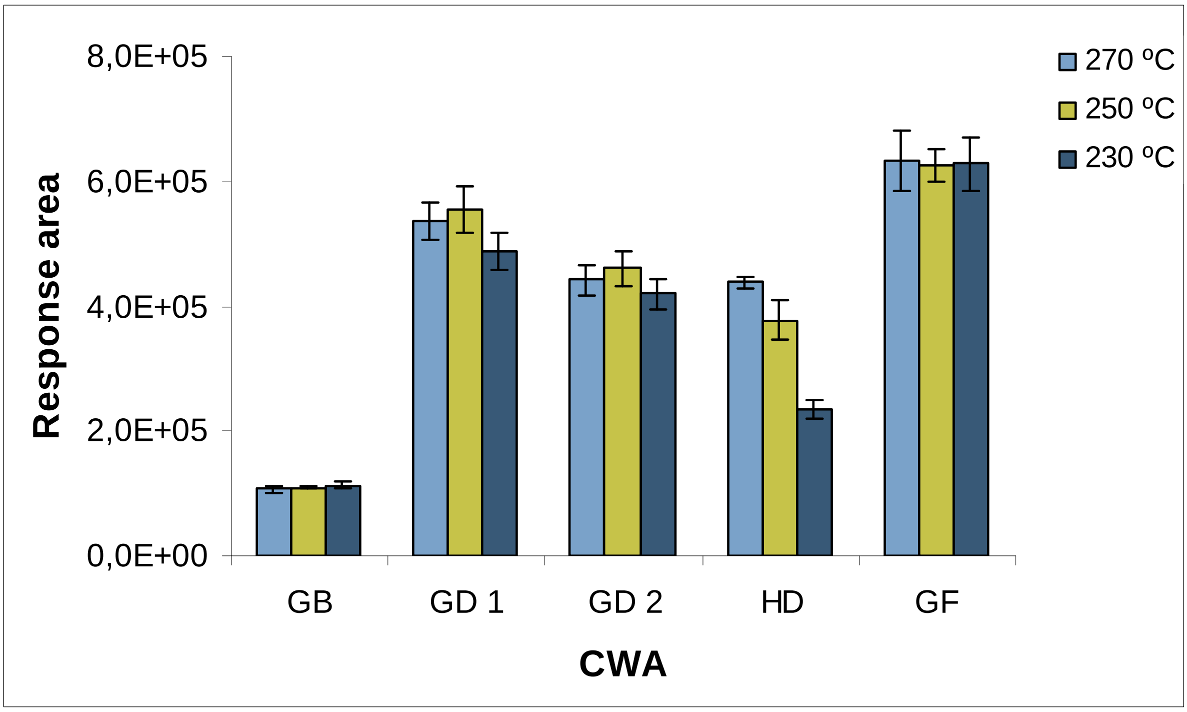


Figure 3.13 The response areas for GB, GD, HD and GF as a function of the desorption temperature (°C) of 230 °C (n = 6), 250 °C (n = 5) and 270 °C (n = 5). The DVB/CAR/PDMS fiber was immersed in distilled and de-ionised water spiked with nerve agents (0.645 µg/mL) and HD (0.819 µg/mL). Error bars indicates the standard deviations of the mean response areas.

3.3.4 Desorption time

A desorption time of two minutes is considered to be satisfactory to thermally release the analytes from the fiber coating (24). Therefore, desorption times of 1 min, 2 min and 4 min were investigated. It is important that the desorption process does not add to the band broadening, in order to obtain good separation efficiency. The few seconds that are required to transfer the analytes onto the front of the column may be too long to give narrow peaks for very volatile analytes. It is therefore necessary to refocus the band after the desorption is completed. Focusing of the band was accomplished by keeping the initial oven temperature constant for the same period of time as the desorption time of the fiber. Correspondingly, the split vent was opened for 1 min, 2 min and 4 min after insertion of the fiber into the inlet of the GC. Otherwise, the parameters for SPME GC-FID were kept as given in chapter 2.4.5. Nerve agents with a concentration of 0.645 µg/mL in distilled and de-ionised water saturated with NaCl were extracted for

30 min by the DVB/CAR/PDMS fiber. The corresponding concentration of HD in the water sample was 0.819 µg/mL.

Chromatograms from these experiments showed tailing of the peaks of GD, HD and GF at all the three desorption times. Response areas of the agents for these experiments are given in the appendices C.4.1 - C.4.3. The tailing of the peaks can be due to analyses of samples with high concentrations of the analytes. Based on these experiments it was therefore difficult to select the most appropriate desorption time for the analytes. Hence, the extraction time was decreased to 10 min to lower the amount of analytes injected into the GC-system. As observed earlier (chapter 3.3.2), the amount of GD, HD and GF sorbed to the fiber is now reduced to ¼ – ⅓ of the amount sorbed at an extraction time of 30 min. The experiments with an extraction time of 10 min were conducted as described above. Table 3.5 shows the response height, response area and asymmetry factor of the agents using desorption times of 1 min, 2 min and 4 min (appendices C.4.4 - C.4.9).

No significant differences in the response heights and areas of the agents as a function of the desorption time was observed (see Table 3.5). This is also the case for the asymmetry factors of the analytes, except for HD. The asymmetry factor of HD increased from 0.89 to 1.3, when the desorption time was increased from 2 min to 4 min. The calculation of the asymmetry factor was done at 10% of the response height by the TotalChrom software. Note also that the asymmetry factor for GB is high for all the three desorption times (see Table 3.5). This indicates poor focusing of the compound, which has also been seen for liquid injection of GB on a GC with a DB-5 MS column. GB is a highly volatile nerve agent and probably requires more efficient focusing of the injection band by either applying cryofocusing or a column with a film thickness of 1 µm or greater (13). These techniques were not pursued in this work.

At a desorption time of 1 min the relative standard deviations of the response areas are 1.3 - 2.8 times higher compared to a desorption time of 2 min (see appendices C.4.7 - C.4.8). This may be due to an insufficient time for focusing of the injection band on the front of the GC column.

In conclusion, a desorption time of 2 min was considered as sufficient to obtain efficient desorption and adequate peak shapes of the analytes.

Table 3.5 The response height, response area and the asymmetry factor for the analytes at a desorption time of 1 min, 2 min and 4 min. Response height and area are given as the mean value ± one standard deviation (n = 6). Asymmetry factor is given as the mean value of six replicates. The DVB/CAR/PDMS fiber was immersed in distilled and de-ionised water containing nerve agents (0.645 µg/mL) and HD (0.819 µg/mL).

Analyte	Desorption time (min)	Response height	Response area	Asymmetry factor
GB	1	$4.5\cdot10^4 \pm 0.7\cdot10^4$	$1.0\cdot10^5 \pm 0.7\cdot10^4$	1.6
	2	$4.7\cdot10^4 \pm 0.3\cdot10^4$	$1.1\cdot10^5 \pm 0.3\cdot10^4$	1.6
	4	$4.1\cdot10^4 \pm 0.2\cdot10^4$	$1.0\cdot10^5 \pm 0.4\cdot10^4$	1.7
GD 1	1	$2.4\cdot10^5 \pm 0.2\cdot10^5$	$4.7\cdot10^5 \pm 0.6\cdot10^5$	0.80
	2	$2.4\cdot10^5 \pm 0.1\cdot10^5$	$4.9\cdot10^5 \pm 0.3\cdot10^5$	0.78
	4	$2.3\cdot10^5 \pm 0.7\cdot10^4$	$4.7\cdot10^5 \pm 0.1\cdot10^5$	0.77
GD 2	1	$2.2\cdot10^5 \pm 0.2\cdot10^5$	$3.9\cdot10^5 \pm 0.5\cdot10^5$	0.80
	2	$2.3\cdot10^5 \pm 0.1\cdot10^5$	$4.1\cdot10^5 \pm 0.2\cdot10^5$	0.80
	4	$2.1\cdot10^5 \pm 0.6\cdot10^4$	$4.0\cdot10^5 \pm 0.7\cdot10^4$	0.80
HD	1	$1.5\cdot10^5 \pm 0.2\cdot10^5$	$2.7\cdot10^5 \pm 0.4\cdot10^5$	0.85
	2	$1.6\cdot10^5 \pm 0.2\cdot10^5$	$3.2\cdot10^5 \pm 0.4\cdot10^5$	0.89
	4	$1.4\cdot10^5 \pm 0.3\cdot10^5$	$3.4\cdot10^5 \pm 0.6\cdot10^5$	1.3
GF	1	$3.0\cdot10^5 \pm 0.2\cdot10^5$	$6.2\cdot10^5 \pm 0.4\cdot10^5$	0.74
	2	$3.1\cdot10^5 \pm 0.8\cdot10^4$	$6.6\cdot10^5 \pm 0.2\cdot10^5$	0.74
	4	$3.1\cdot10^5 \pm 1.0\cdot10^4$	$6.6\cdot10^5 \pm 0.4\cdot10^5$	0.74

3.4 Validation of SPME GC-FID method

3.4.1 Method limit of detection

The method limit of detection (MLOD) of GB, GD, HD and GF by immersion of the DVB/CAR/PDMS fiber in distilled and de-ionised water was investigated. The water samples were prepared as described in chapter 2.4.1. The MLOD for the agents were determined at a signal to noise ratio of 3. The chromatograms for GB, GD, HD and GF are given in Figure 3.14 - 3.17. In each figure, the chromatogram for the blank water sample is also displayed. The figures show that the analytes co-eluted with compounds present in the matrix. These co-eluting compounds were also present in the blank water samples. Hence, the presence of co-eluting peaks had to be taken into consideration when the MLOD of the agents was determined. For instance, GB eluted in the tail of a co-eluting component. Thus, defining the height and the baseline of the signal was complex, and it was not straightforward to determine the MLOD. In this case, the height of the signal was measured from the "valley" between the co-eluting peak and the peak of GB, see Figure 3.14. The noise level was measured at the end of the tail of the co-eluting peak. Also in the case of all the other agents, similar corrections were made by taking the signal of the blank water sample into consideration.

The MLOD obtained with the DVB/CAR/PDMS fiber (see Table 3.6) for GB and GD were similar to the MLOD values obtained with the PDMS/DVB fiber (see Table 3.2). This indicates that the sorption of GB and GD by the two fibers is relatively similar at low concentrations. At higher concentrations, the uptake of GB and GD by the DVB/CAR/PDMS fiber is significantly larger than with the PDMS/DVB fiber (chapter 3.3.1).

Table 3.6 The MLOD of the CWA in distilled and de-ionised water by the use of a DVB/CAR/PDMS fiber and GC-FID.

CWA	**GB**	**GD**	**HD**	**GF**
Concentration (ng/mL)	**2.0**	**0.20**	**1.7**	**0.67**

Even lower MLOD for the analytes than given in Table 3.6 can probably be obtained by using a selective detector or a MS instead of a FID. With the MS detector it is easier to separate the peaks of the analytes from the co-eluting peaks. The possibility to extract specific fragment ions of the analyte (extracted ion chromatogram) after running the sample in SCAN mode, will increase the signal to noise ratio compared to the GC-FID analyses. MS in SCAN mode is normally used for the analyses of CWA, in order to get full scan mass spectra of the analytes. Thus, the collected spectra make it possible to perform a reliable identification of CWA.

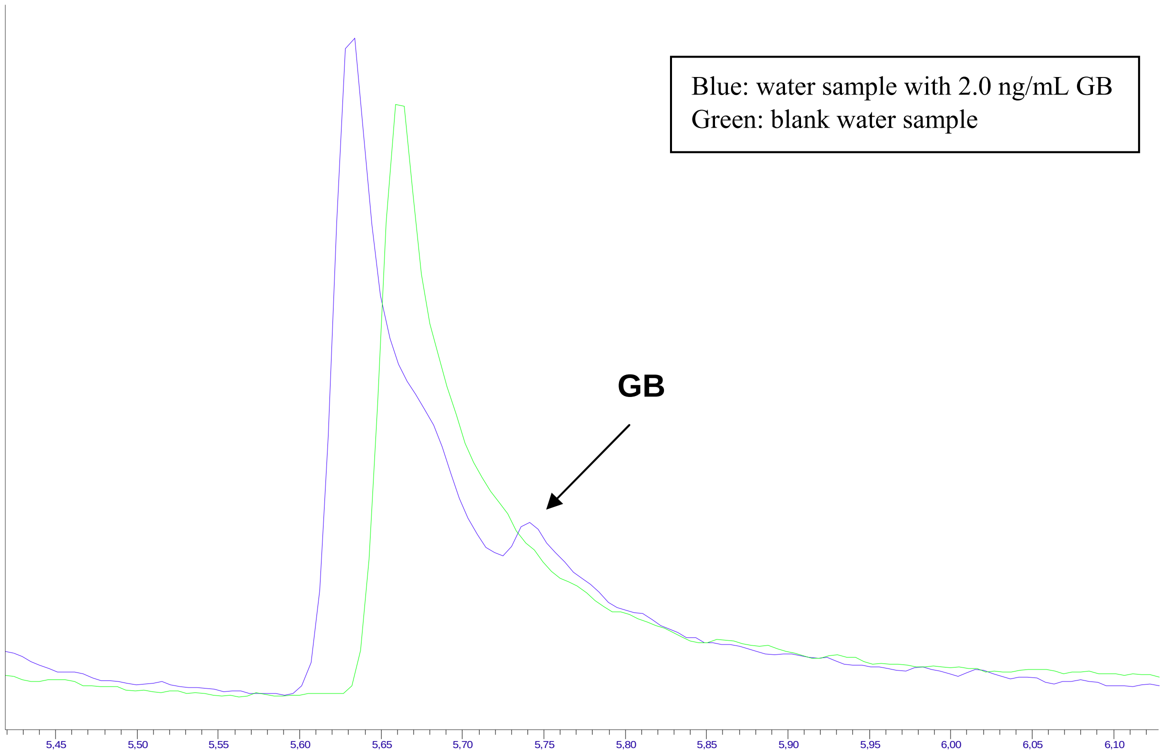


Figure 3.14 Chromatogram of GB at the MLOD with a DVB/CAR/PDMS fiber and GC-FID.

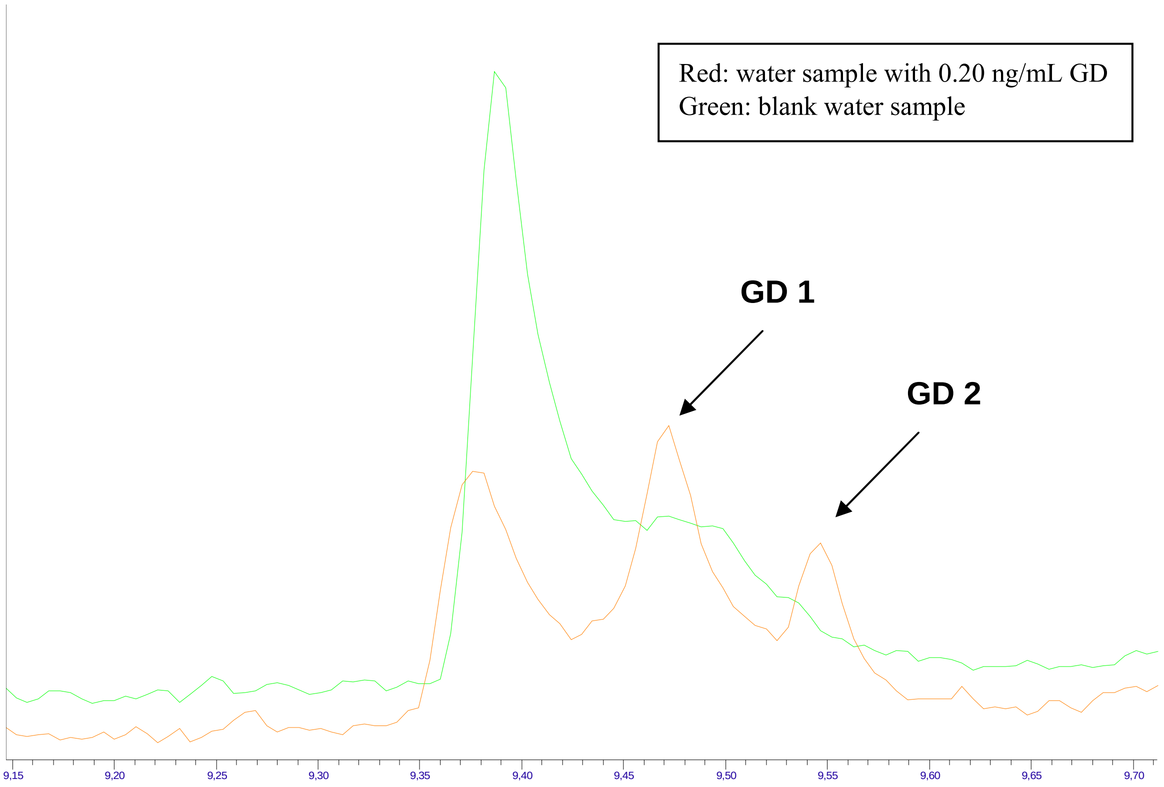


Figure 3.15 Chromatogram of the two diastereoisomers of GD, GD 1 and GD 2 at the MLOD with a DVB/CAR/PDMS fiber and GC-FID.

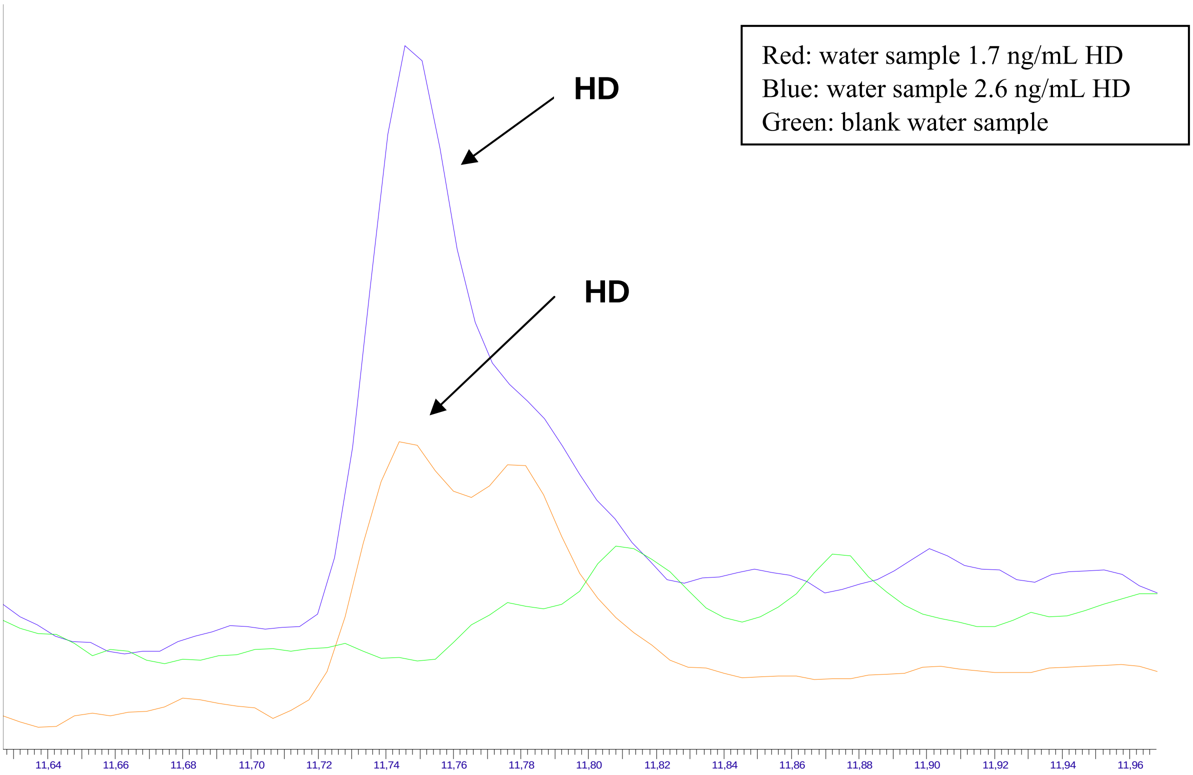


Figure 3.16 Chromatogram of HD at the MLOD with a DVB/CAR/PDMS fiber and GC-FID.

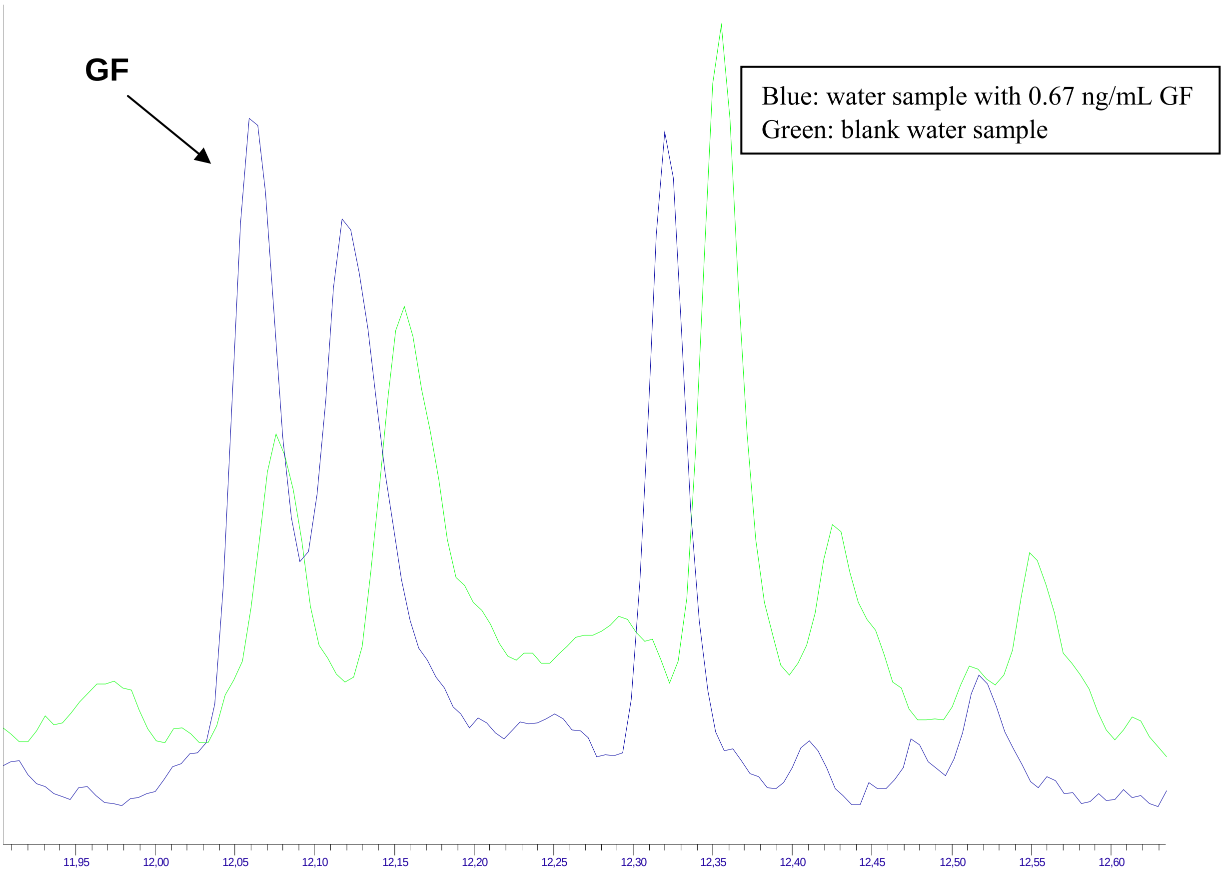


Figure 3.17 Chromatogram of GF at the MLOD with a DVB/CAR/PDMS fiber and GC-FID.

3.4.2 Linearity

Solutions were prepared and extracted as described in chapter 2.4.1 and 2.4.4. Each solution was prepared 5 - 10 min prior to the extraction procedure, to avoid degradation of GB, GD, HD and GF (13, 51). The analyses of the prepared solutions were performed according to the procedure given in chapter 2.4.5. One analysis was performed at each concentration, and all the solutions were analysed during the same day. The response area of the analytes were plotted as a function of the concentration of the analytes, see Figure 3.18. The raw data are given in appendix D.1.

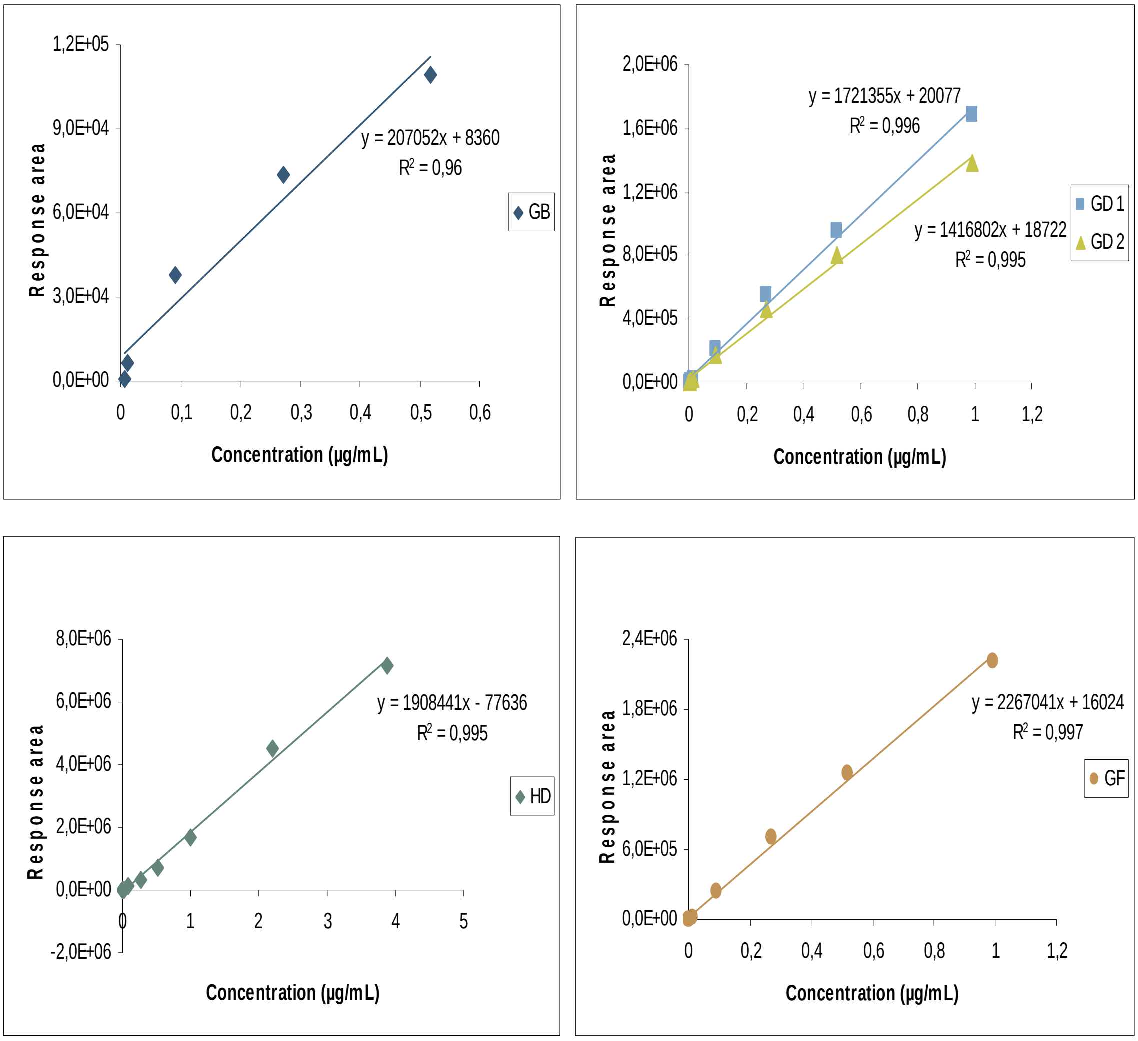


Figure 3.18 Response area as a function of concentration for GB, GD, HD and GF in distilled and de-ionised water. A DVB/CAR/PDMS fiber was used for the extraction.

As Figure 3.18 shows, linearities for GD and GF were obtained in a concentration range from 0.54 ng/mL to 0.991 µg/mL, and the linear plots had a R^2 of 0.995 and 0.997, respectively. GB has a more “logarithmic” than a linear profile in the range from 6.55 ng/mL to 0.518 µg/mL, which is in agreement with previous observations, see chapter 3.3.1. The linear range obtained for HD was from 4.2 ng/mL to 4.91 µg/mL, with a R^2 of 0.995. The obtained R^2 for the plots are acceptable considering the fact that the solutions were prepared individually from different stock and working solutions, see chapter 2.4.1. Note that the investigation of the linearity up to a concentration of 3.89 µg/mL for GB, GD and GF, reduced the squared regression coefficients considerably compared with the values given above. For HD, the linearity was not investigated at a concentration higher than 4.91 µg/mL.

The obtained linearty of GB and GD were reduced in the upper concentration ranges with the use of the DVB/CAR/PDMS fiber compared to the PDMS/DVB fiber, see chapter 3.2.3. As Figure 3.4 and 3.5 show, the upper concentrations of the linear ranges with the PDMS/DVB fiber were 1.03 µg/mL (GB) and 2.58 µg/mL (GD). For the DVB/CAR/PDMS fiber, the corresponding concentrations were 0.518 µg/mL and 0.991 µg/mL. The reduced linearity in the upper concentration range for the DVB/CAR/PDMS fiber may be related to the fiber’s increased ability to sorb CWA compared with the PDMS/DVB fiber, see Figure 3.8. As observed earlier the sorption of GB and GD by the DVB/CAR/PDMS fiber is approximately 1.5 – 2.5 times higher than obtained by the PDMS/DVB fiber, see chapter 3.3.1. The high uptake of the CWA by the DVB/CAR/PDMS fiber may lead to saturation of the fiber at a lower concentration than by using the PDMS/DVB fiber. This is a possible explanation to the reduced linearity in the upper concentration range for the DVB/CAR/PDMS fiber.

In conclusion, the linear ranges obtained by the DVB/CAR/PDMS fiber cover three decades for GD, HD and GF, and almost two decades for GB.

3.4.3 Repeatability

The repeatability of the developed SPME method was studied at two different concentrations for GB, GD, HD and GF. The two concentrations chosen were a relatively low and a relatively high value within the linear ranges of the analytes. In case of the nerve agents, the concentrations 10.4 ng/mL (n = 7) and 1.00 µg/mL (n = 6) were analysed. The corresponding concentrations of HD in the same water solutions were 13.2 ng/mL (n = 7) and 1.27 µg/mL (n = 5). These validation solutions were prepared according to the procedure given in chapter 2.4.1. Prior to the immersion of the DVB/CAR/PDMS fiber in the validation solution, the solution was saturated with NaCl. Thereafter, the solutions were analysed within one day as described in chapter 2.4.5. The within-assay repeatability of the analytes at the two concentrations are given in Table 3.7, and the calculations are based on the response areas of the analytes, see appendix D.2.

Table 3.7 Within-assay repeatabilities at two concentration levels for selected CWA. A DVB/CAR/PDMS fiber was immersed in distilled and de-ionised water spiked with the agents.

Analyte concentration (µg/mL)	GB 0.0104	GD 1 0.0104	GD 2 0.0104	HD 0.0132	GF 0.0104
Mean value	$6.4 \cdot 10^3$	$2.7 \cdot 10^4$	$2.3 \cdot 10^4$	$1.18 \cdot 10^4$	$2.24 \cdot 10^4$
Standard deviation	$0.1 \cdot 10^3$	$0.2 \cdot 10^4$	$0.1 \cdot 10^4$	$0.06 \cdot 10^4$	$0.09 \cdot 10^4$
RSD (%)	1.6	8.2	4.5	5.2	4.4
Analyte concentration (µg/mL)	**GB 1.00**	**GD 1 1.00**	**GD 2 1.00**	**HD 1.27**	**GF 1.00**
Mean value	$1.60 \cdot 10^5$	$1.8 \cdot 10^6$	$1.46 \cdot 10^6$	$1.2 \cdot 10^6$	$2.38 \cdot 10^6$
Standard deviation	$0.06 \cdot 10^5$	$0.1 \cdot 10^6$	$0.07 \cdot 10^6$	$0.1 \cdot 10^6$	$0.09 \cdot 10^6$
RSD (%)	3.6	6.1	5.1	10	3.6

Table 3.7 shows that the RSD of the response areas for the analytes at the two concentrations are in the range from 1.6 to 10%. Interestingly, the precision of GD with the DVB/CAR/PDMS fiber at a concentration of 1.00 µg/mL is 3 times better than obtained by Lakso and Ng by using a PDMS/DVB fiber (23). However, the obtained precision of GB at 1.00 µg/mL is quite similar to the reported value of 4.5% (23). The precision of GF at both concentrations are below 5%, and the precision of HD is between

5.2-10%. Note that the RSD for HD is doubled when the concentration is increased from 0.0132 µg/mL to 1.27 µg/mL. In this study the precision of the response area of HD has been the poorest; and can possibly be related to a faster degradation of HD compared to the nerve agents (13, 51). The half-life time of HD in salt water is 60 min at 25 ºC (51), whereas for example the half life time of GB is a few hours (23). The precision may probably be improved by employing internal standards. Further optimization of the method should involve the presence of internal standards, which will at least correct for variations in the spiking and the injections within the replicates. Deuterated internal standards are considered to be the most appropriate choice, in order to obtain similar sorption of the analytes and the internal standards by the SPME fiber. For the moment, FFI do not have deuterated CWA, and therefore the experiments in the present study were done without the use of internal standards. Note also that the future use of deuterated components will make it necessary to employ a MS detector instead of a FID, in order to separate the internal standard from the analyte.

In addition, due to time limitations, the between assay repeatability of CWA by performing analyses over several consecutive days were not performed. This investigation should be conducted in the future work.

3.5 Validation of the standard method

The developed SPME GC-FID method was compared with the standard method for extraction of CWA from water samples (6). The standard method is based on LLE of the water sample with dichloromethane, and the procedure is explained in detail in chapter 2.4.4. The prepared liquid extracts were analysed on the same GC-FID instrument as used for SPME. The existing MLODs when using the standard method are based on analysis by a GC-MS. To be able to compare the developed SPME GC-FID method with the standard method, it was therefore necessary to determine the MLODs for the standard method by using a GC-FID.

3.5.1 Method limit of detection for LLE GC-FID

The MLODs for GB, GD, HD and GF in distilled and de-ionised water by LLE were determined. The validation solutions at the MLODs for the analytes were prepared as described in chapter 2.4.1, and thereafter extracted and analysed according to the descriptions given in chapter 2.4.4 and 2.4.5. The MLODs of the analytes by LLE and GC-FID are given in Table 3.8.

Table 3.8 The MLODs of the CWA in distilled and de-ionised water by LLE and GC-FID.

CWA	**GB**	**GD**	**HD**	**GF**
Concentration (ng/mL)	**28**	**3.1**	**34**	**28**

Analysis of a blank water sample which was prepared in the same way as the validation solutions (chapter 2.4.3) were used to correct for matrix interferences, see appendix E. The presence of the matrix interferences had a significant influence on the MLOD when using the standard method. The MLODs of the selected analytes by LLE are far higher than the MLODs obtained by SPME, see Table 3.6 and 3.8. The MLODs with LLE for GB, GD, HD and GF are 14, 16, 20 and 42 times higher than MLODs with SPME, respectively. Note also that it is GF that shows the greatest improvement in the MLOD by SPME compared to LLE. It should also be mentioned that there has not been published any work on analysis of GF by SPME previously. The pronounced improvement in the MLODs for the analytes with the use of SPME encourages further studies of other CWA by the SPME technique. GA and VX are interesting candidate analytes. Possible reasons for the relatively large difference in MLODs for the analytes with the two methods will be discussed in the next chapter.

It is important to emphasize that real water samples that may be contaminated with CWA, are normally analysed several hours or days after sampling. The time delay is caused by the transportation of the sample from the contaminated site to the laboratory. During the transportation the CWA in the water sample will degrade to for example phosphonic acids and thiodiglycol, and the remaining amount of CWA will be at trace levels.

Considering the obtained MLOD, the SPME method will be more suitable for the detection of trace levels of GB, GD, HD and GF from water than LLE.

Another disadvantage with the LLE method is that it is more time-consuming, as the total time for the sample preparation and the analysis is approximately 4 - 5 hours compared to one hour with the SPME technique. Furthermore, the LLE of CWA from a water sample requires the use of dichloromethane, which is considered to be a carcinogenic solvent. In conclusion, the developed SPME method is considered to be a more user-friendly technique for a mobile laboratory in the field.

3.6 SPME and LLE of natural water samples

Finally, the developed SPME method and the standard method based on LLE were used for the extraction of CWA from natural water samples. Sea water, river water and melted snow samples were investigated. The natural water samples prepared for SPME and LLE were spiked with equal amounts of CWA, see chapter 2.4.2. The concentrations of the CWA in the samples prepared for LLE were approximately two times the LOQ for the standard method.

The instrumental analysis for the two methods were performed as described in chapter 2.4.5. Additionally, the amounts recovered by the two extraction techniques were estimated by the following approach: In the case of LLE, a one point calibration where the origin was included in the plot was used for the quantification of the extracts. The concentrations of the calibration solutions were 1.94 μg/mL (GB and GF), 2.46 μg/mL (HD) and 0.165 μg/mL (GD), and the agents were diluted in acetone. All the agents were present in the same solution. These concentrations corresponded to 100% extraction efficiency of the CWA. With the aid of the calibration equations of each analyte (see appendix F.1), the recovery (%) of the analytes, and also the injected amount of CWA compared with the total available amount (%) in the water sample were calculated, see Table 3.9 - 3.11. A calculation example is given in appendix F.1. In the case of SPME, the amounts of CWA recovered (%) and injected (%) by the DVB/CAR/PDMS fiber were also quantified by liquid injections (1 μL) of the following calibration solutions of the nerve agents: 3.21 μg/mL, 8.59 μg/mL, 25.4 μg/mL and 43.0 μg/mL. The corresponding concentrations of HD in the solutions were 4.10 µg/mL, 10.9 µg/mL, 32.3 µg/mL and 54.6 µg/mL. The equations describing the calibration curves are given in appendix F.2. These equations were used to calculate the recovered and the injected amount of CWA by SPME. These two parameters have the same value in the case of SPME, since it was assumed that all the CWA that was recovered by the fiber, was injected on the GC-FID.

The comparison of the response area, RSD (%), recovery (%) and the amount injected (%) on the GC-FID for natural water samples using the LLE and SPME techniques are given in Table 3.9 – 3.11 (see also appendices F.1 and F.2.1 - F.2.3). Table 3.9 shows that the recovery of the agents by LLE of melted snow is 32% - 108%, whereas the recovery of CWA by SPME is 0.4 – 4.1%. This indicates that the extraction efficiency of SPME is low compared to LLE. However, the percentage of CWA injected for the melted snow sample, is greater with SPME than with LLE. The percentage amount injected of CWA by SPME are 5 times (GB), 40 times (GD), 57 times (HD) and 25 times (GF) higher than by preparing the samples with LLE. Similar significant

Table 3.9 LLE and SPME of CWA from spiked melted snow samples. The amount of CWA in the sample was as following: 1.95 µg (GB), 0.165 µg (GD), 2.48 µg (HD) and 1.94 µg (GF).

Melted snow		**GB**	**GD 1**	**GD 2**	**HD**	**GF**
LLE (n = 5)	Mean response area	$6.6 \cdot 10^3$	$4.1 \cdot 10^2$	$3.8 \cdot 10^2$	$3.1 \cdot 10^3$	$1.1 \cdot 10^4$
	Standard deviation	$0.5 \cdot 10^3$	$0.4 \cdot 10^2$	$0.3 \cdot 10^2$	$0.5 \cdot 10^3$	$0.09 \cdot 10^4$
	RSD (%)	7.6	9.1	8.6	15	7.9
	Recovery (%)	79	94	94	32	108
	Amount injected (%)	0.08	0.1	0.1	0.03	0.1
SPME (n = 6)	Mean response area	$3.5 \cdot 10^4$	$2.3 \cdot 10^4$	$1.9 \cdot 10^4$	$2.1 \cdot 10^5$	$3.5 \cdot 10^5$
	Standard deviation	$0.2 \cdot 10^4$	$0.2 \cdot 10^4$	$0.2 \cdot 10^4$	$0.3 \cdot 10^5$	$0.3 \cdot 10^5$
	RSD (%)	4.7	11	11	17	8.3
	Recovery (%)	0.4	4.1	3.9	1.7	2.5
	Amount injected (%)	0.4	4.1	3.9	1.7	2.5

Table 3.10 LLE and SPME of CWA from spiked sea water samples. The amount of CWA in the samples was as following: 1.95 µg (GB), 0.165 µg (GD), 2.48 µg (HD) and 1.94 µg (GF).

Sea water		**GB**	**GD 1**	**GD 2**	**HD**	**GF**
LLE (n = 5)	Mean response area	$5.2\cdot10^3$	$3.9\cdot10^2$	$3.7\cdot10^2$	$6.7\cdot10^3$	$8.4\cdot10^3$
	Standard deviation	$0.3\cdot10^3$	$0.4\cdot10^2$	$0.4\cdot10^2$	$0.4\cdot10^3$	$1.2\cdot10^3$
	RSD (%)	6.0	10	12	5.3	14
	Recovery (%)	61	88	94	70	81
	Amount injected (%)	0.06	0.09	0.1	0.07	0.08
SPME (n = 5)	Mean response area	$2.1\cdot10^4$	$1.72\cdot10^4$	$1.24\cdot10^4$	$2.2\cdot10^5$	$1.8\cdot10^5$
	Standard deviation	$0.3\cdot10^4$	$0.09\cdot10^4$	$0.07\cdot10^4$	$0.2\cdot10^5$	$0.1\cdot10^5$
	RSD (%)	14	5.5	5.9	10	7.7
	Recovery (%)	0.2	3.1	2.5	1.7	1.3
	Amount injected (%)	0.2	3.1	2.5	1.7	1.3

Table 3.11 LLE and SPME of CWA from spiked river water samples. The amount of CWA in the samples was as following: 1.95 µg (GB), 0.165 µg (GD), 2.48 µg (HD) and 1.94 µg (GF).

River water		**GB**	**GD 1**	**GD 2**	**HD**	**GF**
LLE (n = 5)	Mean response area	$5.2\cdot10^3$	$3.6\cdot10^2$	$3.1\cdot10^2$	$3.7\cdot10^3$	$9.1\cdot10^3$
	Standard deviation	$0.5\cdot10^3$	$0.3\cdot10^2$	$0.3\cdot10^2$	$0.4\cdot10^3$	$0.6\cdot10^3$
	RSD (%)	9.2	8.3	11	12	6.4
	Recovery (%)	62	82	77	38	88
	Amount injected (%)	0.06	0.08	0.08	0.04	0.09
SPME (n = 5)	Mean response area	$5.4\cdot10^4$	$2.5\cdot10^4$	$2.1\cdot10^4$	$2.14\cdot10^5$	$3.9\cdot10^5$
	Standard deviation	$0.1\cdot10^4$	$0.2\cdot10^4$	$0.1\cdot10^4$	$0.03\cdot10^5$	$0.3\cdot10^5$
	RSD (%)	1.9	6.1	6.4	1.4	7.1
	Recovery (%)	0.6	4.4	4.4	1.7	2.7
	Amount injected (%)	0.6	4.4	4.4	1.7	2.7

differences in the injected amount by LLE and SPME are also seen in the case of the sea water and the river water samples, see Table 3.10 and 3.11. The SPME has the ability to inject larger amounts of CWA than by the injection of 1 µL of the extract prepared by LLE. Therefore, lower MLOD are obtained with SPME than with LLE, see chapter 3.4.1 and 3.5.1.

The difference in the detected amount of CWA by SPME and LLE is also clearly seen in the chromatograms shown in Figure 3.19 and 3.20. As emphasized earlier, the samples prepared by SPME and LLE were spiked with the same amounts of CWA. However, the response areas for CWA by SPME are significantly larger than the response areas obtained by LLE.

The chromatogram for LLE of a sea water sample shows some intense peaks at the end of the chromatogram (see Figure 3.20). These peaks were found in all the chromatograms of

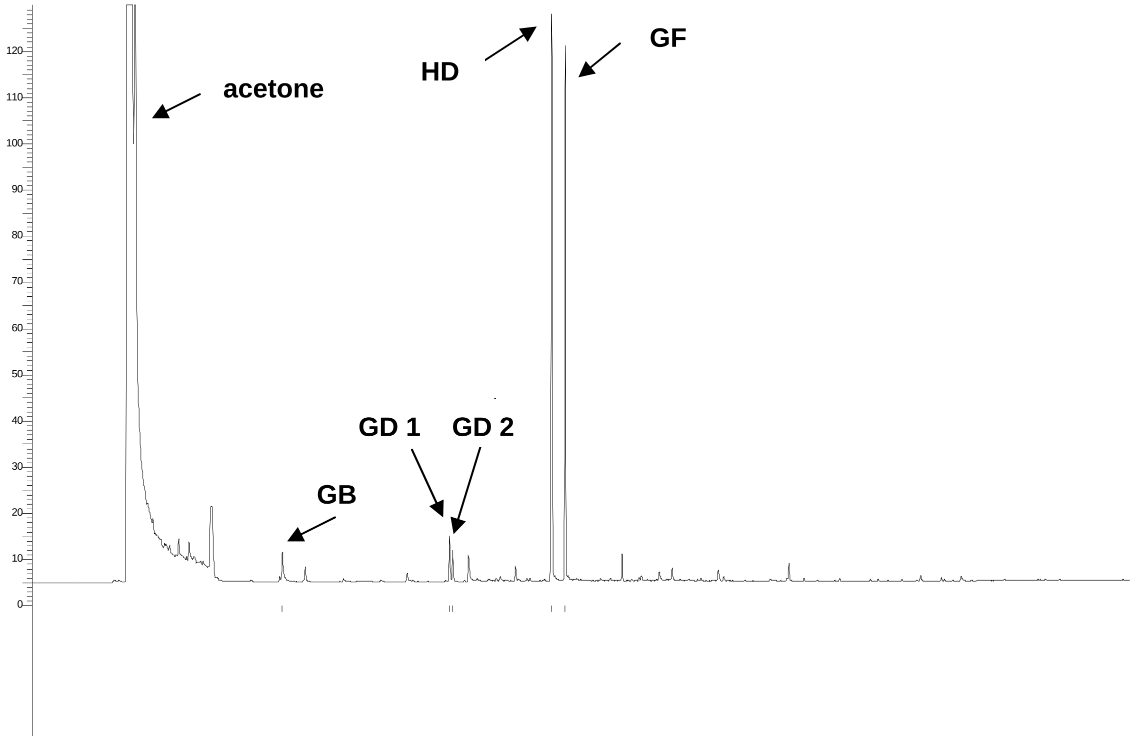


Figure 3.19 Chromatogram of selected CWA by SPME of a spiked sea water sample. The amount of CWA in the sample was as following: 1.95 µg (GB), 0.165 µg (GD), 2.48 µg (HD) and 1.94 µg (GF).

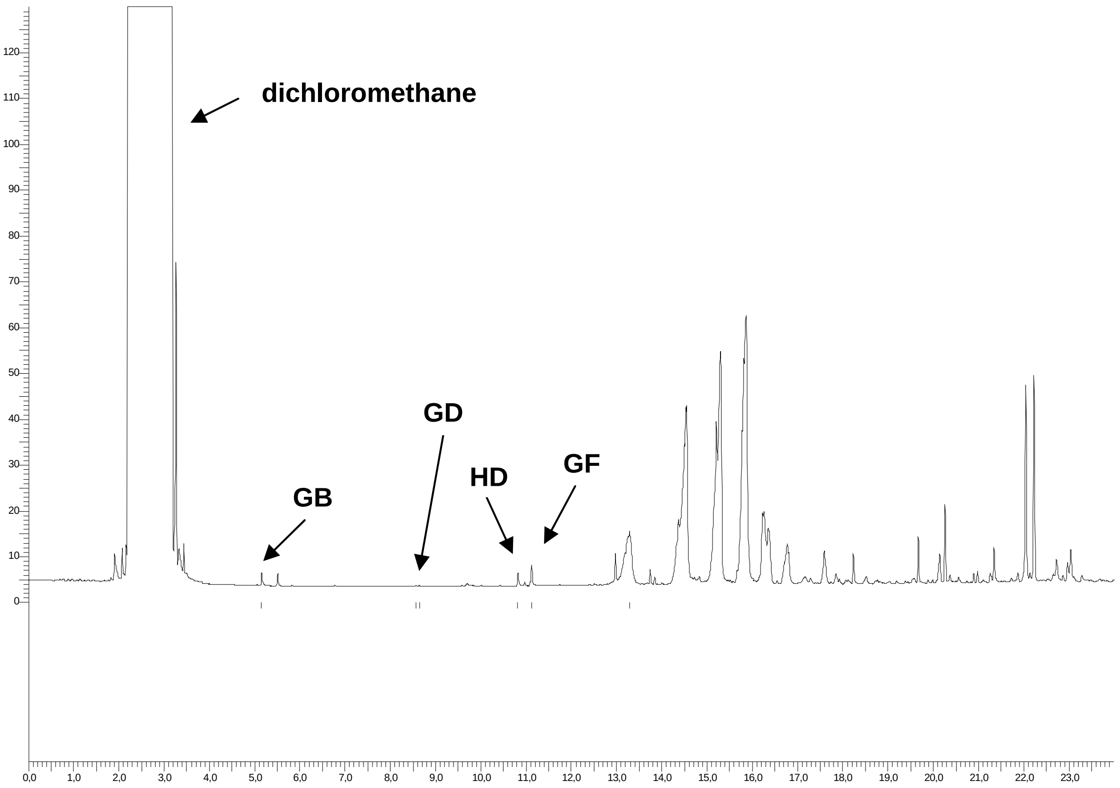


Figure 3.20 Chromatogram of selected CWA by LLE of a spiked sea water sample. The amount of CWA in the sample was as following: 1.95 µg (GB), 0.165 µg (GD), 2.48 µg (HD) and 1.94 µg (GF).

sea water spiked with CWA and prepared by LLE. Interestingly, these peaks were not observed in the blank sea water sample prepared by LLE, which may indicate a relation between the extraneous peaks and the CWA in the samples. Similar profiles of unknown peaks were also seen in the chromatograms of the river water and the melted snow samples. In the case of SPME, the extraneous peaks were significantly smaller. The analysis of these peaks by for example MS, may help to explain the chemical structure of these unknown compounds. The investigation of these unknown peaks in the extracts by LLE will be interesting for future studies of new extraction techniques for CWA in water samples.

Figure 3.19 and 3.20 also show the great difference in the response areas of the solvent injected by the two extraction techniques. A relatively narrow peak of acetone is seen with SPME, whereas a broad dichloromethane peak is obtained due to the injection of a large amount of solvent when the LLE extracts are analysed. It is beneficial to have a narrow solvent peak considering the determination of compounds with short retention times.

Table 3.9 - 3.11 show that the recovery of HD by LLE of sea water is almost the double of the recovery by LLE of river water and melted snow samples. However, the recovery of HD by SPME is the same for the three water types. In the case of the nerve agents, the recovery in sea water by SPME is lower than by SPME of melted snow and river water samples. In order to explain the different recoveries of CWA from the three water matrices, it is necessary to characterize the natural water samples by for example performing measurements of the pH, turbidity, chemical oxygen demand (COD), hardness, conductivity, salinity, type and concentration of the cations present. Next, multivariate data analysis could be performed to see which of the measured parameters influence the recovery of the analytes. In the present work, the only parameter which was measured was pH. The pH for the natural water samples were measured before and after spiking of the water with CWA. No significant change in pH was measured after the addition of CWA to the natural water samples. The pH of melted snow and river water were 5.0 - 5.5, whereas the pH of the sea water was 7.0 - 7.5. The other parameters which are mentioned above should be investigated in future work.

Subsequently, the natural water samples were also spiked with lower concentrations of the CWA, which corresponded to concentrations of twice the LOQ for SPME (Table 3.12). The samples were prepared as described in chapter 2.4.2, and were extracted only by the SPME technique (n = 6), see chapter 2.4.4. The concentration of the CWA in the water samples were: 12.6 ng/mL (GB), 1.26 ng/mL (GD), 7.97 ng/mL (HD) and 5.02 ng/mL (GF), corresponding to total amounts of 0.195 µg, 0.0195 µg, 0.0778 µg and 0.124 µg, respectively. The mean value of the response areas of the CWA and their standard deviations, RSD, recovery (%) and the injected amount (%) are given in Table 3.12 (see also appendices F.2.4 - F.2.6). For the calculation of the recovery and the amount of CWA injected, it was necessary to quantify the amount of CWA sorbed by the DVB/CAR/PDMS fiber. The quantification was performed by liquid injection (1 µL) of calibration solutions as described above (see appendix F.2). The response areas for the analytes in the three different natural water matrices with low concentration vary in the same manner as observed earlier (Table 3.9 - 3.11).

Table 3.12 shows that identification of the analytes in natural water samples is possible even though the concentrations of the analytes are only approximately twice the LOQ of distilled and de-ionised water samples. Distilled and de-ionised water is a less complex matrix than natural water. Therefore, the matrices of the natural water samples will influence the extraction efficiency of the CWA differently than distilled and de-ionised water.

Table 3.12 SPME of CWA from natural water samples at twice the LOQ of SPME. The amount of CWA in the samples was as following: 0.195 µg (GB), 0.0195 µg (GD), 0.124 µg (HD) and 0.0778 µg (GF).

Type of water		**GB**	**GD 1**	**GD 2**	**HD**	**GF**
Snow	Mean response area	$2.7\cdot10^3$	$2.7\cdot10^3$	$2.4\cdot10^3$	$3.8\cdot10^3$	$1.0\cdot10^4$
	Standard deviation	$0.2\cdot10^3$	$0.2\cdot10^3$	$0.2\cdot10^3$	$0.4\cdot10^3$	$0.2\cdot10^4$
	RSD (%)	5.6	8.0	6.4	10	18
	Recovery (%)	0.3	4.2	4.4	0.6	1.7
	Amount injected (%)	0.3	4.2	4.4	0.6	1.7
Sea	Mean response area	$4.2\cdot10^2$	$1.0\cdot10^3$	$6.2\cdot10^2$	$8.6\cdot10^3$	$2.2\cdot10^3$
	Standard deviation	$0.5\cdot10^2$	$0.5\cdot10^2$	$0.6\cdot10^2$	$1.3\cdot10^3$	$0.2\cdot10^3$
	RSD (%)	12	4.7	9.3	16	11
	Recovery (%)	0.05	1.5	1.0	1.4	0.4
	Amount injected (%)	0.05	1.5	1.0	1.4	0.4
River	Mean response area	$5.7\cdot10^3$	$1.7\cdot10^3$	$1.2\cdot10^3$	$7.3\cdot10^3$	$4.9\cdot10^3$
	Standard deviation	$0.6\cdot10^3$	$0.2\cdot10^3$	$0.2\cdot10^3$	$0.9\cdot10^3$	$0.3\cdot10^3$
	RSD (%)	9.9	14	20	13	6.9
	Recovery (%)	0.62	2.6	2.1	1.1	1.0
	Amount injected (%)	0.62	2.6	2.1	1.1	1.0

The RSDs for CWA in Table 3.12 are in some cases higher than the RSDs obtained at twice the LOQ for LLE (see Table 3.9 - 3.11). An increase in RSD is possibly due to the relatively low concentrations of the CWA in the samples. The influence of the matrix composition on the RSD of CWA is more evident at a low concentration than at a high concentration of the analytes. Additionally, the recoveries (%) and injected amounts (%) of CWA at a low concentration are approximately in the same range as for CWA at a high concentration (see Table 3.9 - 3.12).

4 Conclusion and future work

In the present study, an SPME GC-FID method was developed for the determination of GB, GD, GF and HD in water samples. The obtained MLOD for the CWA by SPME is 0.2 - 2 µg/L, which is 14 - 42 times better than by the standard method based on LLE (6). This improvement in MLOD is mainly due to the ability of the SPME technique to inject larger amounts of CWA into the GC-FID, as compared to the amount injected by liquid injection of 1 µL of the extract prepared by LLE.

Furthermore, the SPME is a less solvent and time-consuming technique than LLE. The total time for the sample preparation and the analysis with SPME is approximately one hour, compared to 4 – 5 hours with the LLE technique. Therefore, the SPME procedure is more suitable for use in a mobile field laboratory. For the range of CWA in this study, the SPME technique shows a large potential to extract the agents from water, and SPME should be used as a supplement to LLE.

As mentioned, CWA hydrolyses relatively fast in water (13, 51). Therefore, the identification of the degradation products in water could be more important than the identification of CWA. The existing method for the determination of these degradation products is based on a tedious procedure of evaporating the aqueous extract to dryness, followed by derivatisation prior to GC-MS analysis (6). The work presented here does not include the analysis of degradation products. Such analysis should be considered in future studies. For example, Sng and Ng investigated in-situ derivatisation of degradation products of CWA by SPME and GC-MS (45). Detection limits in the ppb (µg/L) and sub-ppb range were obtained for these compounds when the MS was operated in full scan mode. An alternative approach is to use hollow fiber-protected liquid phase microextraction (HF-LPME) for the determination of degradation products of CWA in water (56). With this technique, limits of detection from 0.01 to 0.2 µg/L may be obtained. The use of HF-LPME was reported to give a significant improvement in the limits of detection compared with SPME. HF-LPME is also considered to be a fast and an almost solvent free extraction technique. Thus, HF-LPME is also a suitable method for sample preparation in the field.

Future studies may therefore focus on the use of SPME and HF-LPME for the identification of CWA and degradation products in water. Additionally, the potential of the purchased HS GC-MS with trap should be investigated. Future work should also extend the evaluation of SPME for a wider range of CWA than in the present work, e.g. including agents such as GA and VX. Since these compounds have chemical and physical properties very similar to the presently investigated CWA, the MLODs of GA and VX will most likely be at a ppb level (µg/L). Finally, new studies using a more selective and sensitive detector will possibly further enhance the MLOD for the CWA by SPME.

The results and conclusions of the present study have been presented at international symposia (57, 58) (see appendix G).

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Appendix A Preliminary work

A.1 Evaluation of GC-FID instrumentation

The GC-FID was evaluated by analyzing solutions of GB, GD and HD. The solutions were made from a stock solution of 1 mg/mL of the CWA in dichloromethane. Following validation solutions were made: 5 µg/mL, 10 µg/mL, 50 µg/mL and 100 µg/mL. 10 µL of 2 µL/mL of tridecane diluted in dichloromethane was added to the solutions above. 1 µL of each solution was injected into the GC-FID by the MPS 2 autosampler, and each validation solution was analysed consecutively three times. A DB 5-MS column was used and the instrumental parameters were as given below:

Ovn program: 40 ºC (1 min) – 10 ºC/min – 280 ºC (10 min)
Column flow: 1.0 mL/min
Inlet temperature: 280 ºC
Split vent flow: 25 mL/min at 1.0 min
FID temperature: 350 ºC
FID gas flows: 450 mL/min (synthetic air) and 45 mL/min (H_2)
FID range: 1
FID attenuation: -5

Calibration equations for the investigated CWA when the plots were forced through the origin:

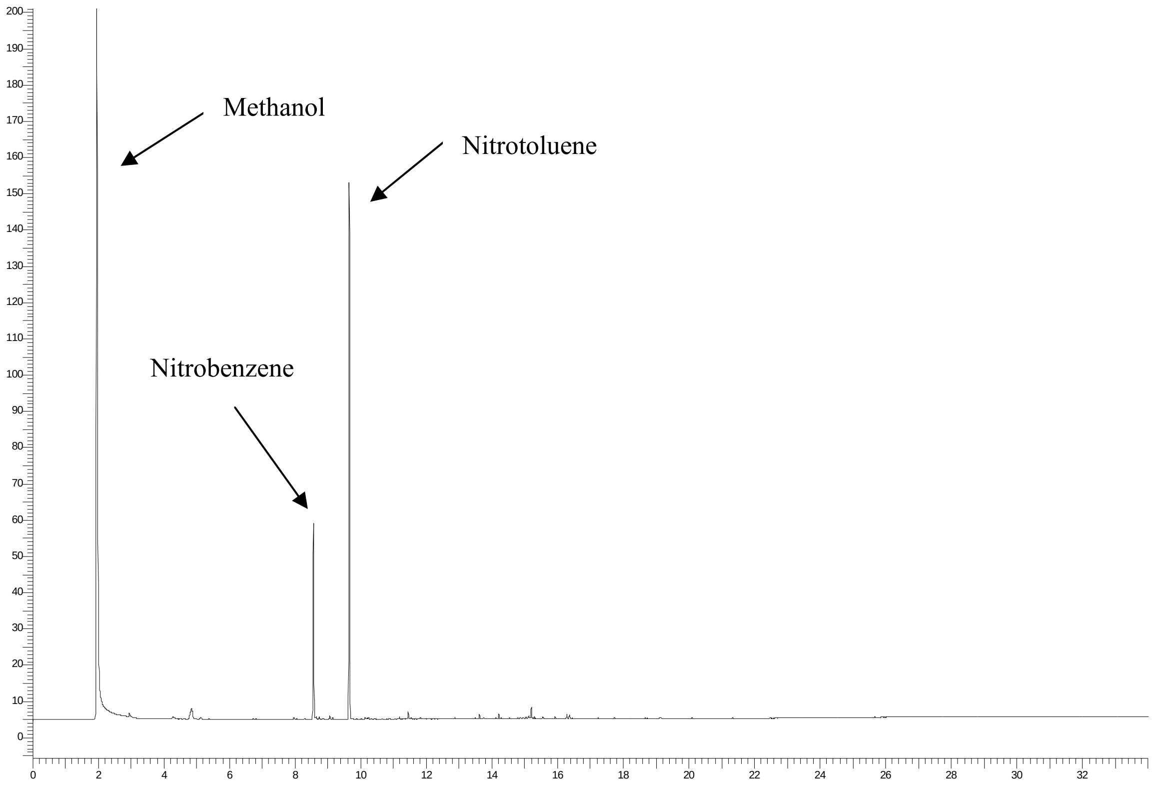
GB: $y = 0.021 \cdot x$ $R^2 = 0.998$ GD 1: $y = 0.015 \cdot x$ $R^2 = 0.9987$
GD 2: $y = 0.013 \cdot x$ $R^2 = 0.9987$ HD: $y = 0.025 \cdot x$ $R^2 = 0.9987$

The obtained linearity, regression coefficients and the response areas for GB, GD and HD were in good agreement with similar experiments performed in 2002.

A.2 Test solution for SPME autosampler

The “SPME Sensitivity Test Sample” delivered with the MPS 2 autosampler was composed of 1 ng/µL of nitrobenzene and nitrotoulene in water. A 100 µm PDMS fiber was kept in the headspace of the test sample and injected into the GC-FID with a DB–5 MS column installed. The test sample was analysed as described by the procedure given by the manufacturer, but the oven temperature was as following:
50 ºC (1 min) – 10 ºC/min – 280 ºC (10 min). The chromatogram for the analysis of the test sample is shown in A.2.1. As A.2.1 shows, the response height of nitrotoluene is almost three times the response height of nitrobenzene.

A.2.1 Chromatogram of the analysis of “SPME Sensitivity Test Sample”



A.3 GC-MS analyses of CWA in different solvents

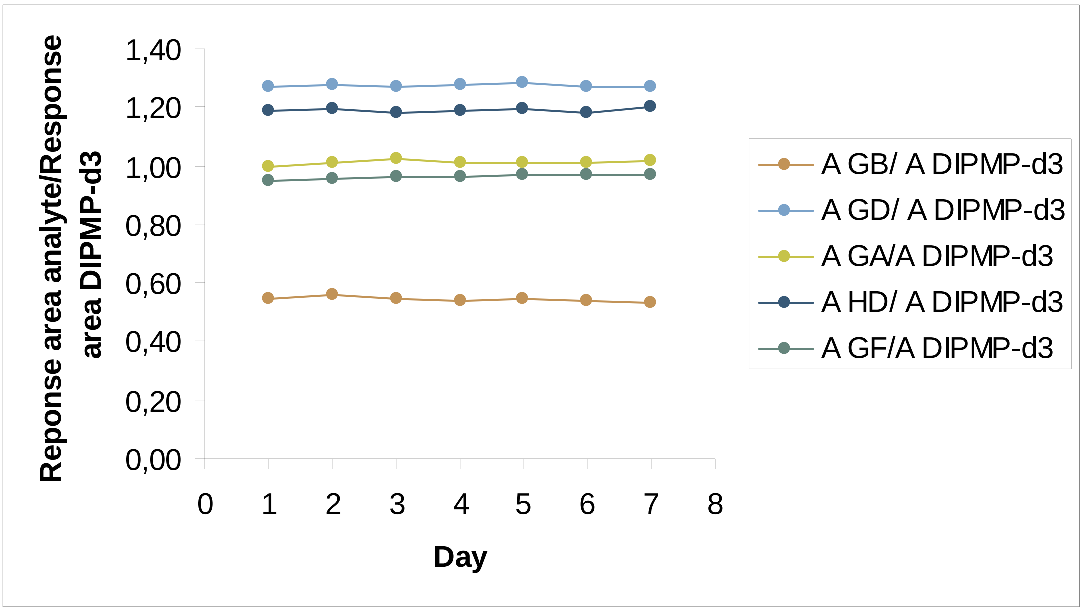
The article written by Lakso and Ng (23) was used as a basis for the evaluation of the SPME GC-FID system. Therefore, during the evaluation, an effort was made to keep the experimental parameters as close as possible to the description given in the article. Lakso and Ng prepared a stock solution of CWA (GA, GB, GD and VX) in acetonitrile, and the solution was used for spiking of water samples. At FFI, GC-MS analyses of GB, GD, HD and GF in acetonitrile was performed and the analytes were observed as several peaks in the chromatogram. The “ffi EI Full Scan CWA” method was used for GC-MS analysis. Different temperatures at the inlet of the column were employed to focus the injection band in the start of the column. However, no change was seen in the number of peaks for each analyte.

Thereafter, the CWA were diluted in two other water soluble solvents: methanol and acetone. CWA diluted in methanol showed similar behavior as acetonitrile. Interestingly, CWA diluted in acetone gave a single peak for each of the investigated analytes, which was also accomplished with the analysis of the solution on GC-FID. Hence, in this study acetone was used to make stock and working solutions of CWA.

A.4 Stability of CWA in acetone

The stability of the CWA in acetone was investigated. A solution of 10 µg/mL of GA, GB, GD, GF and HD in acetone prepared and stored in the freezer at < -18 ºC. An aliquot of the solution was analysed over 7 consecutive days by GC-MS with the “ffi EI Full Scan CWA” method, and each day, 5 injections from the same aliquot were made. Deuterated diisopropyl methylphosphonate (DIPMP-d_3) was used as an internal standard and its concentration was similar to the concentration of the CWA in the solution. The mean values of the ratio of the response area of the analyte/response area of DIPMP-d_3 for each of the seven consecutive days are given in A.4.1. As A.4.1 shows the mean values of the area response ratios are relatively the same for the seven following days. This indicates that the investigated CWA are stable in acetone when the solution is stored in the freezer for 7 days. Therefore, the prepared stock and working solutions could be used for up to a week, which was advantageous concerning the laboratory work.

A.4.1 Mean values of the response area ratios for analyte/DIPMP-d_3 for 7 consecutive days



Appendix B Evaluation of SPME GC-FID instrumentation

The PDMS/DVB fiber was immersed in distilled and de-ionised water which was saturated with NaCl. For appendices B.1.1 - B.1.6 and B.1.8 - B.1.10, the concentrations of GA, GB and GD were 1.00 µg/mL.

B.1 Repeatability

B.1.1 Repeatability of freshly spiked water samples

	Response area			
Replicate	**GB**	**GD 1**	**GD 2**	**GA**
1	60009	1046154	854385	236186
2	61791	1088248	889674	239674
3	62490	1056263	867592	241885
4	61620	1164233	949555	257484
5	61347	1057421	867965	247553
6	67552	1187023	972282	266056
7	67690	1149523	943581	262120
Mean value	**$6.3 \cdot 10^4$**	**$1.10 \cdot 10^6$**	**$9.1 \cdot 10^5$**	**$2.5 \cdot 10^5$**
Std. dev.	**$0.3 \cdot 10^4$**	**$0.06 \cdot 10^6$**	**$0.5 \cdot 10^5$**	**$0.1 \cdot 10^5$**
RSD (%)	**4.9**	**5.3**	**5.2**	**4.7**

B.1.2 Repeatability of water samples prepared simultaneously and analysed consecutively in a sequence

	Response area			
Replicate	**GB**	**GD 1**	**GD 2**	**GA**
1	47101	907455	733063	186513
2	47239	985394	787825	191226
3	52438	1055013	848094	213561
4	47998	972490	778841	175417
5	54581	1003780	809414	186183
6	49748	1058251	848915	180107
Mean value	**$5.0 \cdot 10^4$**	**$1.00 \cdot 10^6$**	**$8.0 \cdot 10^5$**	**$1.9 \cdot 10^5$**
Std. dev.	**$0.3 \cdot 10^4$**	**$0.06 \cdot 10^6$**	**$0.4 \cdot 10^5$**	**$0.1 \cdot 10^5$**
RSD (%)	**6.1**	**5.7**	**5.5**	**7.0**

B.1.3 Repeatability of water samples prepared simultaneously and analysed consecutively in a sequence, spiked with the aid of a Hamilton syringe

Hamilton syringe	**Response area**			
Replicate	**GB**	**GD 1**	**GD 2**	**GA**
1	48868	893441	724005	175617
2	47980	958698	768264	179579
3	44733	891284	713745	159777
4	49712	915470	736510	167834
5	52252	972168	784517	168108
6	46224	926555	740643	147002
Mean value	**$4.8 \cdot 10^4$**	**$9.3 \cdot 10^5$**	**$7.4 \cdot 10^5$**	**$1.7 \cdot 10^5$**
Std. dev.	**$0.3 \cdot 10^4$**	**$0.3 \cdot 10^5$**	**$0.3 \cdot 10^5$**	**$0.1 \cdot 10^5$**
RSD (%)	**5.5**	**3.6**	**3.6**	**7.0**

B.1.4 Repeatability of water samples prepared simultaneously and analysed consecutively in a sequence, spiked with the aid of a gas tight syringe

Gas tight syringe	Response area			
Replicate	**GB**	**GD 1**	**GD 2**	**GA**
1	55898	1018827	825406	213475
2	52233	1035922	828877	204621
3	48814	1003520	801808	190568
4	46041	930826	740099	181238
5	44504	909155	723249	163820
6	47033	964458	765452	166563
Mean value	**$4.9 \cdot 10^4$**	**$9.8 \cdot 10^5$**	**$7.8 \cdot 10^5$**	**$1.9 \cdot 10^5$**
Std. dev.	**$0.4 \cdot 10^4$**	**$0.5 \cdot 10^5$**	**$0.4 \cdot 10^5$**	**$0.2 \cdot 10^5$**
RSD (%)	**8.7**	**5.2**	**5.7**	**11**

B.1.5 Repeatability of spiked distilled and de-ionised water samples prepared simultaneously and analysed consecutively in a sequence, where the first analysis was initiated 11 hours after sample preparation

	Response area			
Replicate	**GB**	**GD 1**	**GD 2**	**GA**
1	36289	839417	663650	116433
2	31524	699403	550193	97609
3	28469	756850	593493	92221
4	29548	711617	555645	84772
5	28797	733073	572477	85394
6	30271	758425	596390	83996
Mean value	**$3.1 \cdot 10^4$**	**$7.5 \cdot 10^5$**	**$5.9 \cdot 10^5$**	**$9.3 \cdot 10^4$**
Std. dev.	**$0.3 \cdot 10^4$**	**$0.5 \cdot 10^5$**	**$0.5 \cdot 10^5$**	**$1.2 \cdot 10^4$**
RSD (%)	**9.4**	**6.7**	**7.0**	**13**

B.1.6 Repeatability of spiked distilled and de-ionised water samples prepared simultaneously and analysed consecutively in a sequence, where the first analysis was initiated 20 hours after sample preparation

	Response area			
Replicate	**GB**	**GD 1**	**GD 2**	**GA**
1	32895	766393	606886	72148
2	31827	796540	629251	71094
3	35860	799086	636225	67863
4	32036	785386	621553	63515
5	33194	780225	617813	61919
6	34138	782194	624685	54501
Mean value	$3.3\cdot10^4$	$7.8\cdot10^5$	$6.2\cdot10^5$	$6.5\cdot10^4$
Std. dev.	$0.2\cdot10^4$	$0.2\cdot10^5$	$0.1\cdot10^5$	$0.7\cdot10^4$
RSD (%)	**4.5**	**1.5**	**1.6**	**10**

B.1.7 Response areas of CWA for spiked distilled and de-ionised water samples prepared simultaneously and analysed consecutively in a sequence

Sample nr.	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	7708	43640	36865	59393	65175
2	8098	52225	43848	59100	69280
3	6514	38661	32286	30033	51904
4	6465	50368	41773	28038	66326
5	6500	43934	36439	19738	57138
6	7064	45065	37567	13545	59122
7	5679	35624	29766	10503	44460
8	5647	44430	36593	11096	52650
9	4768	38980	31551	6856	40337
10	6056	37110	30618	4729	44233
11	3828	40294	31947	5027	38075
12	5096	32984	26922	2853	38935

B.1.8 Repeatability of CWA with fiber 1 after 6 injections of the fiber

	Response area			
Replicate	**GB**	**GD 1**	**GD 2**	**GA**
1	47832	939538	764329	205217
2	48576	942249	763432	212518
3	52106	1083932	874480	210474
4	38284	835056	666814	15514
5	33965	797173	635489	125591
6	39596	847701	680325	149844
Mean value	**$4.3 \cdot 10^4$**	**$9.1 \cdot 10^5$**	**$7.3 \cdot 10^5$**	**$1.8 \cdot 10^5$**
Std. dev.	**$0.7 \cdot 10^4$**	**$1.0 \cdot 10^5$**	**$0.9 \cdot 10^5$**	**$0.4 \cdot 10^5$**
RSD (%)	**16**	**12**	**12**	**21**

B.1.9 Repeatability of CWA with fiber 1 after 17 injections of the fiber

	Response area			
Replicate	**GB**	**GD 1**	**GD 2**	**GA**
1	47101	907455	733063	186513
2	47239	985394	787825	191226
3	52438	1055013	848094	213561
4	47998	972490	778841	175417
5	54581	1003780	809414	186183
6	49748	1058251	848915	180107
Mean value	**$5.0 \cdot 10^4$**	**$9.9 \cdot 10^5$**	**$8.0 \cdot 10^5$**	**$1.9 \cdot 10^5$**
Std. dev.	**$0.3 \cdot 10^4$**	**$0.6 \cdot 10^5$**	**$0.4 \cdot 10^5$**	**$0.1 \cdot 10^5$**
RSD (%)	**6.1**	**5.7**	**5.5**	**7.0**

B.1.10 Repeatability of CWA with fiber 2 after 51 injections of the fiber

	Response area			
Replicate	**GB**	**GD 1**	**GD 2**	**GA**
1	62851	953544	776787	211827
2	60850	936360	759398	199438
3	63116	1028084	827989	192697
4	54160	910341	730799	181380
5	59490	1008275	806788	181801
6	56112	916130	733414	164329
Mean value	**$5.9 \cdot 10^4$**	**$9.6 \cdot 10^5$**	**$7.7 \cdot 10^5$**	**$1.9 \cdot 10^5$**
Std. dev.	**$0.4 \cdot 10^4$**	**$0.5 \cdot 10^5$**	**$0.4 \cdot 10^5$**	**$0.2 \cdot 10^5$**
RSD (%)	**6.1**	**5.1**	**5.1**	**8.7**

B.2 Linearity

B.2.1 Response areas of CWA with a spiking volume of 10 µL

Concentration of CWA (µg/mL)	GB	GD 1	GD 2	GA
0.00019		451	222	
0.00034		596	496	104
0.00040		753	535	133
0.0011		2198	1844	455
0.0032	285	5356	4372	1561
0.00645	1151	10091	8479	2877
0.0129	983	18920	15695	5257
0.0259	2781	37811	31143	10226
0.0516	7366	74154	61487	18954
0.103	14212	154397	126803	37929
0.516	43941	648408	535114	150842
0.516	42629	577280	479807	145974
1.03	75400	1254731	1032045	272029
1.29	83783	1526285	1243946	318785
2.58	142003	2898511	2188898	564762
4.30	185803	3752074	2651499	696285
8.60	262967	5687304	3277938	990395

B.2.2 Response areas of CWA with a spiking volume of 0.2 - 38 µL

Concentration of CWA (µg/mL)	GB	GD 1	GD 2	GA
0.0129	249	2448	2304	2317
0.0258	3411	13229	11228	7982
0.0516	9527	41523	34722	21317
0.0968	15433	88034	73710	38621
0.516	54210	630908	519574	164537
0.516	56792	667566	549309	181132
0.516	52878	608489	504076	173425
0.516	53508	635298	525437	174790
0.516	56053	650119	535740	169658
1.00	67421	1154351	948105	266015
1.29	71126	1376428	1125935	302756
1.29	79551	1559178	1264054	346193
2.45	83427	2142298	1701208	414201
5.01	103936	3121219	2337854	538313

Appendix C Development of SPME GC-FID method

C.1 Selection of fiber

For Table C.1.1 – C.1.5 the mean value, standard deviation and relative standard deviation (RSD) are calculated for the replicates in the table. The sign (-) in the tables indicates that no measurement of the response area was performed. The abbreviation of n.d. indicates that the component was not detected, whereas n.c. is used in the tables to describe that no calculation were done in order to obtain the mean value, standard deviation or the relative standard deviation.

C.1.1 Response area for GB in a concentration range with the use of three fibers

	Response area		
Concentration of GB (µg/mL)	**PDMS/DVB**	**DVB/CAR/ PDMS**	**PA**
0.00645	n.d.	916	n.d.
0.0129	849	1848	n.d.
0.0968	6443	20958	n.d.
0.0968	7544	-	n.d.
0.0968	7236	-	n.d.
0.0968	7106	-	n.d.
0.0968	8362	-	n.d.
0.0968	6736	-	n.d.
0.300	22314	51989	n.d.
0.538	310906	88421	983
1.00	50039	131150	1256
Mean value	**$7.2\cdot10^3$**	**n.c.**	**n.c.**
Std. dev.	**$0.7\cdot10^3$**	**n.c.**	**n.c.**
RSD (%)	**9.3**	**n.c.**	**n.c.**

C.1.2 Response area for GD 1 in a concentration range with the use of three fibers

	Response area		
Concentration of GD 1 (µg/mL)	**PDMS/DVB**	**DVB/CAR/ PDMS**	**PA**
0.00645	3130	3656	n.d.
0.0129	6176	8535	n.d.
0.0968	41190	77510	3780
0.0968	48147	-	3060
0.0968	45779	-	2224
0.0968	49230	-	1816
0.0968	47017	-	2262
0.0968	44496	-	-
0.300	140851	203569	6812
0.538	212327	344839	19155
1.00	397461	551911	29978
Mean value	**$4.6 \cdot 10^4$**	**n.c.**	**$2.7 \cdot 10^3$**
Std. dev.	**$0.3 \cdot 10^4$**	**n.c.**	**$0.8 \cdot 10^3$**
RSD (%)	**6.3**	**n.c.**	**30**

C.1.3 Response area for GD 2 in a concentration range with the use of three fibers

	Response area		
Concentration of GD 2 (µg/mL)	**PDMS/DVB**	**DVB/CAR/ PDMS**	**PA**
0.00645	2776	3619	n.d.
0.0129	5790	7939	n.d.
0.0968	34783	67132	4770
0.0968	40452	-	4166
0.0968	38745	-	1762
0.0968	41730	-	1519
0.0968	39769	-	2137
0.0968	37852	-	-
0.300	117881	172688	7560
0.538	175200	289918	18765
1.00	327794	466571	29470
Mean value	**$3.9 \cdot 10^4$**	**n.c.**	**$2.9 \cdot 10^3$**
Std. dev.	**$0.2 \cdot 10^4$**	**n.c.**	**$1.5 \cdot 10^3$**
RSD (%)	**6.2**	**n.c.**	**52**

C.1.4 Response area for HD in a concentration range with the use of three fibers

	Response area		
Concentration of HD (µg/mL)	**PDMS/DVB**	**DVB/CAR/ PDMS**	**PA**
0.00819	6918	1208	2536
0.0164	9895	3402	5703
0.123	79225	35911	62345
0.123	82529	-	55851
0.123	77095	-	49201
0.123	94997	-	40618
0.123	101204	-	48435
0.123	79463	-	-
0.381	289496	99594	134073
0.683	462725	195784	315231
1.27	890331	336452	503384
Mean value	**$8.6\cdot10^4$**	**n.c.**	**$5.1\cdot10^4$**
Std. dev.	**$0.9\cdot10^4$**	**n.c.**	**$0.8\cdot10^4$**
RSD (%)	**12**	**n.c.**	**16**

C.1.5 Response area for GF in a concentration range with the use of three fibers

	Response area		
Concentration of GF (µg/mL)	**PDMS/DVB**	**DVB/CAR/ PDMS**	**PA**
0.00645	3986	5071	n.d.
0.0129	9997	9603	n.d.
0.0968	60729	95260	2946
0.0968	72096	-	2410
0.0968	68744	-	1796
0.0968	72152	-	1389
0.0968	64926	-	1743
0.0968	67578	-	-
0.300	210230	280237	7237
0.538	322126	482913	19086
1.00	568117	888098	30868
Mean value	**$6.8 \cdot 10^4$**	**n.c.**	**$2.1 \cdot 10^3$**
Std. dev.	**$0.4 \cdot 10^4$**	**n.c.**	**$0.6 \cdot 10^3$**
RSD (%)	**6.5**	**n.c.**	**30**

C.1.6 Response areas for CWA with the use of the DVB/CAR/PDMS fiber

The concentration of the nerve agents and HD in distilled and de-ionised water are 0.0968 µg/mL and 0.123 µg/mL, respectively.

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	21700	72570	63504	36800	96521
2	22715	77691	67892	33779	101928
3	23757	77497	67492	36852	108068
4	22555	84266	71846	40398	99515
5	21796	76831	66302	39943	99128
6	21849	74188	65196	46636	105734
7	22435	71310	64646	40980	103594
Mean value	**$2.24 \cdot 10^4$**	**$7.6 \cdot 10^4$**	**$6.7 \cdot 10^4$**	**$3.9 \cdot 10^4$**	**$1.02 \cdot 10^5$**
Std. dev.	**$0.07 \cdot 10^4$**	**$0.4 \cdot 10^4$**	**$0.3 \cdot 10^4$**	**$0.4 \cdot 10^4$**	**$0.04 \cdot 10^5$**
RSD (%)	**3.2**	**5.6**	**4.1**	**10**	**4.0**

C.2 Extraction time

C.2.1 Response area for CWA by the DVB/CAR/PDMS fiber

The concentration of the nerve agents are 1.00 µg/mL and 1.27 µg/mL for HD. The extraction time was 30 min.

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	154893	1951440	1568128	1400172	2613699
2	158502	1787086	1436181	1407583	2501468
3	167390	1654863	1361628	1221271	2374776
4	152734	1856031	1496020	1806421	2582476
Mean value	**$1.58 \cdot 10^5$**	**$1.8 \cdot 10^6$**	**$1.47 \cdot 10^6$**	**$1.3 \cdot 10^6$**	**$2.5 \cdot 10^6$**
Std. dev.	**$0.06 \cdot 10^5$**	**$0.1 \cdot 10^6$**	**$0.09 \cdot 10^6$**	**$0.1 \cdot 10^6$**	**$0.1 \cdot 10^6$**
RSD (%)	**4.1**	**6.9**	**6.0**	**7.9**	**4.2**

C.2.2 Response area for CWA by the PA fiber

The concentration of the nerve agents are 1.00 µg/mL and 1.27 µg/mL for HD. The extraction time was 30 min.

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	1538	32060	31613	463928	30541
2	1540	33474	33551	502226	30399
3	1661	38225	37654	517651	37167
4	1397	32661	32157	445407	31656
Mean value	**$1.5 \cdot 10^3$**	**$3.4 \cdot 10^4$**	**$3.4 \cdot 10^4$**	**$4.8 \cdot 10^5$**	**$3.2 \cdot 10^4$**
Std. dev.	**$0.1 \cdot 10^3$**	**$0.3 \cdot 10^4$**	**$0.3 \cdot 10^4$**	**$0.3 \cdot 10^5$**	**$0.3 \cdot 10^4$**
RSD (%)	**7.0**	**8.2**	**8.1**	**6.9**	**9.9**

C.3 Desorption temperature

The DVB/CAR/PDMS fiber was immersed in distilled and de-ionised water which was saturated with NaCl. For appendix C.3.1, the concentration of the nerve agents were 1.29 µg/mL and the concentration of HD was 1.64 µg/mL. For appendices C.3.2 - C.3.4, the concentrations were 0.645 µg/mL and 0.819 µg/mL, respectively.

C.3.1 Response area for CWA at varying desorption temperatures

	Response area				
Temperature (ºC)	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
270	197160	729686	607904	874235	1034882
250	207124	905876	751126	770988	1176050
220	179421	810925	689070	516041	954518
190	193240	754131	738406	298912	749563
170	111011	381795	560965	152006	403231
150	116149	311571	500059	135753	291088
130	155982	209427	376818	107924	173605

C.3.2 Response area for CWA at a desorption temperature of 270 ºC

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	114816	557862	462179	418364	661191
2	107441	570181	466438	442784	639720
3	104169	505198	412390	444229	552849
4	107527	540059	446848	438233	636081
5	101458	506320	421497	442808	671570
Mean value	**$1.07 \cdot 10^5$**	**$5.4 \cdot 10^5$**	**$4.4 \cdot 10^5$**	**$4.4 \cdot 10^5$**	**$6.3 \cdot 10^5$**
Std. dev.	**$0.05 \cdot 10^5$**	**$0.3 \cdot 10^5$**	**$0.2 \cdot 10^5$**	**$0.1 \cdot 10^5$**	**$0.5 \cdot 10^5$**
RSD (%)	**4.7**	**5.5**	**5.5**	**2.5**	**7.4**

C.3.3 Response area for CWA at a desorption temperature of 250 ºC

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	114825	599562	494400	412704	626992
2	110366	560451	463169	391415	581744
3	109681	576991	478475	393816	640418
4	106865	525246	438128	345685	620940
5	106660	508777	426740	343865	655396
Mean value	**$1.10 \cdot 10^5$**	**$5.5 \cdot 10^5$**	**$4.6 \cdot 10^5$**	**$3.8 \cdot 10^5$**	**$6.3 \cdot 10^5$**
Std. dev.	**$0.03 \cdot 10^5$**	**$0.4 \cdot 10^5$**	**$0.3 \cdot 10^5$**	**$0.3 \cdot 10^5$**	**$0.3 \cdot 10^5$**
RSD (%)	**3.0**	**6.7**	**6.1**	**8.2**	**4.4**

C.3.4 Response area for CWA at a desorption temperature of 230 ºC

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	104079	457779	396288	232211	583024
2	119858	530520	454710	255979	691096
3	116528	493276	423865	232065	632476
4	112426	450859	387792	206422	579027
5	119693	510817	437701	241213	657794
6	107884	485541	415946	240043	622442
Mean value	**$1.13 \cdot 10^5$**	**$4.9 \cdot 10^5$**	**$4.2 \cdot 10^5$**	**$2.3 \cdot 10^5$**	**$6.3 \cdot 10^5$**
Std. dev.	**$0.06 \cdot 10^5$**	**$0.3 \cdot 10^5$**	**$0.3 \cdot 10^5$**	**$0.2 \cdot 10^5$**	**$0.4 \cdot 10^5$**
RSD (%)	**5.7**	**6.3**	**6.0**	**7.0**	**6.9**

C.4 Desorption time

The DVB/CAR/PDMS fiber was immersed in distilled and de-ionised water which was saturated with NaCl. The concentration of the nerve agents were 0.645 µg/mL and the concentration of HD was 0.819 µg/mL.

C.4.1 Response areas for CWA at a desorption time of 1 min and an extraction time of 30 min

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	121682	1251798	1036107	773847	1620509
2	126411	1287592	1066021	874237	1709343
3	123708	1231753	1021647	778952	1647771
4	122680	1295673	1081932	807562	1721130
5	126377	1272028	1057333	872425	1697542
6	131246	1293152	1072901	780132	1767119
Mean value	**$1.3 \cdot 10^5$**	**$1.3 \cdot 10^6$**	**$1.1 \cdot 10^6$**	**$8.1 \cdot 10^5$**	**$1.7 \cdot 10^6$**
Std. dev.	**$0.3 \cdot 10^4$**	**$0.3 \cdot 10^5$**	**$0.2 \cdot 10^5$**	**$0.5 \cdot 10^5$**	**$0.5 \cdot 10^5$**
RSD (%)	**2.8**	**2.0**	**2.2**	**5.8**	**3.1**

C.4.2 Response areas for CWA at a desorption time of 2 min and an extraction time of 30 min

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	117478	1233050	1016295	1023293	1621196
2	125818	1193880	989425	958785	1609411
3	121390	1296787	1071431	1028515	1740073
4	124852	1269863	1056892	990434	1744529
5	117594	1324652	1098106	1135159	1804142
Mean value	**$1.2\cdot10^5$**	**$1.3\cdot10^6$**	**$1.0\cdot10^6$**	**$1.0\cdot10^6$**	**$1.7\cdot10^6$**
Std. dev.	**$0.4\cdot10^4$**	**$0.5\cdot10^5$**	**$0.4\cdot10^5$**	**$0.7\cdot10^5$**	**$0.8\cdot10^5$**
RSD (%)	**3.2**	**4.1**	**4.2**	**6.5**	**5.0**

C.4.3 Response areas for CWA at a desorption time of 4 min and an extraction time of 30 min

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	109367	1195613	998124	1320221	1593806
2	115564	1284668	1067199	1426820	1743423
3	122612	1417344	1180807	1565244	1943853
4	128034	1214720	1019232	1039203	1681830
5	130747	1221068	1024045	1039303	1712342
Mean value	**$1.2\cdot10^5$**	**$1.3\cdot10^6$**	**$1.0\cdot10^6$**	**$1.3\cdot10^6$**	**$1.7\cdot10^6$**
Std. dev.	**$0.9\cdot10^4$**	**$0.9\cdot10^5$**	**$0.7\cdot10^5$**	**$0.2\cdot10^6$**	**$0.1\cdot10^6$**
RSD (%)	**7.3**	**7.2**	**6.9**	**18**	**7.5**

C.4.4 Response height for CWA at a desorption time of 1 min and an extraction time of 10 min

	Response height				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	39580	251127	237492	168929	303554
2	50175	264636	250064	179111	327782
3	33848	223138	205157	154659	286482
4	47689	220884	210831	128394	290383
5	50381	225806	220451	126245	296392
6	47123	233343	221516	157427	287412
Mean value	**$4.5\cdot10^4$**	**$2.4\cdot10^5$**	**$2.2\cdot10^5$**	**$1.5\cdot10^5$**	**$3.0\cdot10^5$**
Std. dev.	**$0.7\cdot10^4$**	**$0.2\cdot10^5$**	**$0.2\cdot10^5$**	**$0.2\cdot10^5$**	**$0.2\cdot10^5$**
RSD (%)	**15**	**7.5**	**7.5**	**14**	**5.2**

C.4.5 Response height for CWA at a desorption time of 2 min and an extraction time of 10 min

	Response height				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	46631	235830	224610	143525	322782
2	48496	238921	222552	145212	310273
3	45948	238802	228365	164683	313145
4	45037	256368	249947	185639	323963
5	50535	248034	241584	177518	307044
6	42883	225919	210992	147233	303495
Mean value	**$4.7\cdot10^4$**	**$2.4\cdot10^5$**	**$2.3\cdot10^5$**	**$1.6\cdot10^5$**	**$3.1\cdot10^5$**
Std. dev.	**$0.3\cdot10^4$**	**$0.1\cdot10^5$**	**$0.1\cdot10^5$**	**$0.2\cdot10^5$**	**$0.8\cdot10^4$**
RSD (%)	**5.7**	**4.4**	**6.1**	**11**	**2.7**

C.4.6 Response height for CWA at a desorption time of 4 min and an extraction time of 10 min

	Response height				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	39514	230784	213203	107489	303990
2	37650	242623	218728	150715	291933
3	43953	230919	218850	172699	296768
4	40902	234408	212169	144276	317868
5	41219	235830	217899	162342	310741
6	43293	221659	202315	119163	310332
Mean value	$4.1 \cdot 10^4$	$2.3 \cdot 10^5$	$2.1 \cdot 10^5$	$1.4 \cdot 10^5$	$3.1 \cdot 10^5$
Std. dev.	$0.2 \cdot 10^4$	$0.7 \cdot 10^4$	$0.6 \cdot 10^4$	$0.3 \cdot 10^5$	$1.0 \cdot 10^4$
RSD (%)	**5.7**	**3.0**	**3.0**	**18**	**3.2**

C.4.7 Response areas for CWA at a desorption time of 1 min and an extraction time of 10 min

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	94589	431213	358553	277470	572121
2	106931	516084	426971	318298	624513
3	108587	569704	470484	321130	695169
4	110375	435433	364098	222615	603384
5	106945	439464	367425	223006	615610
6	95791	451627	376091	283799	588831
Mean value	$1.04 \cdot 10^5$	$4.7 \cdot 10^5$	$3.9 \cdot 10^5$	$2.7 \cdot 10^5$	$6.2 \cdot 10^5$
Std. dev.	$0.07 \cdot 10^5$	$0.6 \cdot 10^5$	$0.4 \cdot 10^5$	$0.4 \cdot 10^5$	$0.4 \cdot 10^5$
RSD (%)	**6.6**	**12**	**11**	**16**	**6.9**

C.4.8 Response areas for CWA at a desorption time of 2 min and an extraction time of 10 min

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	109413	481064	405588	281478	670108
2	107623	463533	388695	289584	646651
3	103863	488100	409944	325634	667785
4	106882	508183	425671	356163	674047
5	107332	452993	383199	294869	632615
6	102434	524622	439759	374739	693819
Mean value	$1.06\cdot10^5$	$4.9\cdot10^5$	$4.1\cdot10^5$	$3.2\cdot10^5$	$6.6\cdot10^5$
Std. dev.	$0.03\cdot10^5$	$0.3\cdot10^5$	$0.2\cdot10^5$	$0.4\cdot10^5$	$0.2\cdot10^5$
RSD (%)	**2.4**	**5.5**	**5.3**	**12**	**3.3**

C.4.9 Response areas for CWA at a desorption time of 4 min and an extraction time of 10 min

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	108778	462424	393576	247158	669813
2	102364	480414	402406	363349	603190
3	108175	478435	401684	411451	633133
4	105958	467042	398079	333038	696266
5	103127	478954	407698	363708	689737
6	97658	454488	387599	294537	681730
Mean value	$1.04\cdot10^5$	$4.7\cdot10^5$	$3.99\cdot10^5$	$3.4\cdot10^5$	$6.6\cdot10^5$
Std. dev.	$0.04\cdot10^5$	$0.1\cdot10^5$	$0.07\cdot10^5$	$0.6\cdot10^5$	$0.4\cdot10^5$
RSD (%)	**4.0**	**2.3**	**1.8**	**17**	**5.5**

Appendix D Validation of SPME GC-FID method

D.1 Linearity

Solutions were prepared and extracted by the DVB/PDMS/CAR fiber as described in chapter 2.4.1 and 2.4.4. The solutions were analysed by GC-FID and the reponse areas for GB, GD, HD and GF are given below.

D.1.1 Response areas for GB, GD and GF in a concentration range

	Response area			
Concentration (µg/mL)	**GB**	**GD 1**	**GD 2**	**GF**
0.00054		793	454	281
0.0013		3650	2478	1304
0.0033		6196	5266	4234
0.00655	983	12136	10101	8501
0.0130	6433	24471	21077	20571
0.0918	37979	210504	177367	247826
0.272	73906	548583	459595	703234
0.518	109148	957949	800012	1248961
0.991	135012	1682829	1380653	2211204
2.20	134199	2904112	2205180	3743799
3.89	122957	3621705	2617884	4722981

D.1.2 Response areas for HD in a concentration range

	Response area
Concentration (µg/mL)	HD
0.0042	3047
0.00832	5175
0.0165	10857
0.117	106929
0.345	333333
0.658	705069
1.26	1661766
2.79	4523998
4.91	7191340

D.2 Repeatability

The repeatability of the DVB/CAR/PDMS fiber was measured at two concentrations. For appendix D.2.1, the concentration of the nerve agents were 0.0104 µg/mL and the concentration of HD was 0.0132 µg/mL. For appendix D.2.2, the concentrations were 1.00 µg/mL and 1.27 µg/mL, respectively.

D.2.1 Response areas for CWA at a low concentration level

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	6593	28367	23905	12079	21851
2	6368	26815	23167	11993	23938
3	6262	25694	22511	11212	22251
4	6431	24163	21037	10967	20966
5	6337	31182	23305	11962	22221
6	6427	28612	24077	12862	23373
7	6429	27147	23495	11849	22422
Mean value	**$6.4 \cdot 10^3$**	**$2.7 \cdot 10^4$**	**$2.3 \cdot 10^4$**	**$1.18 \cdot 10^4$**	**$2.24 \cdot 10^4$**
Std. dev.	**$0.1 \cdot 10^3$**	**$0.2 \cdot 10^4$**	**$0.1 \cdot 10^4$**	**$0.06 \cdot 10^4$**	**$0.09 \cdot 10^4$**
RSD (%)	**1.6**	**8.2**	**4.5**	**5.2**	**4.4**

D.2.2 Response areas for CWA at an high concentration level

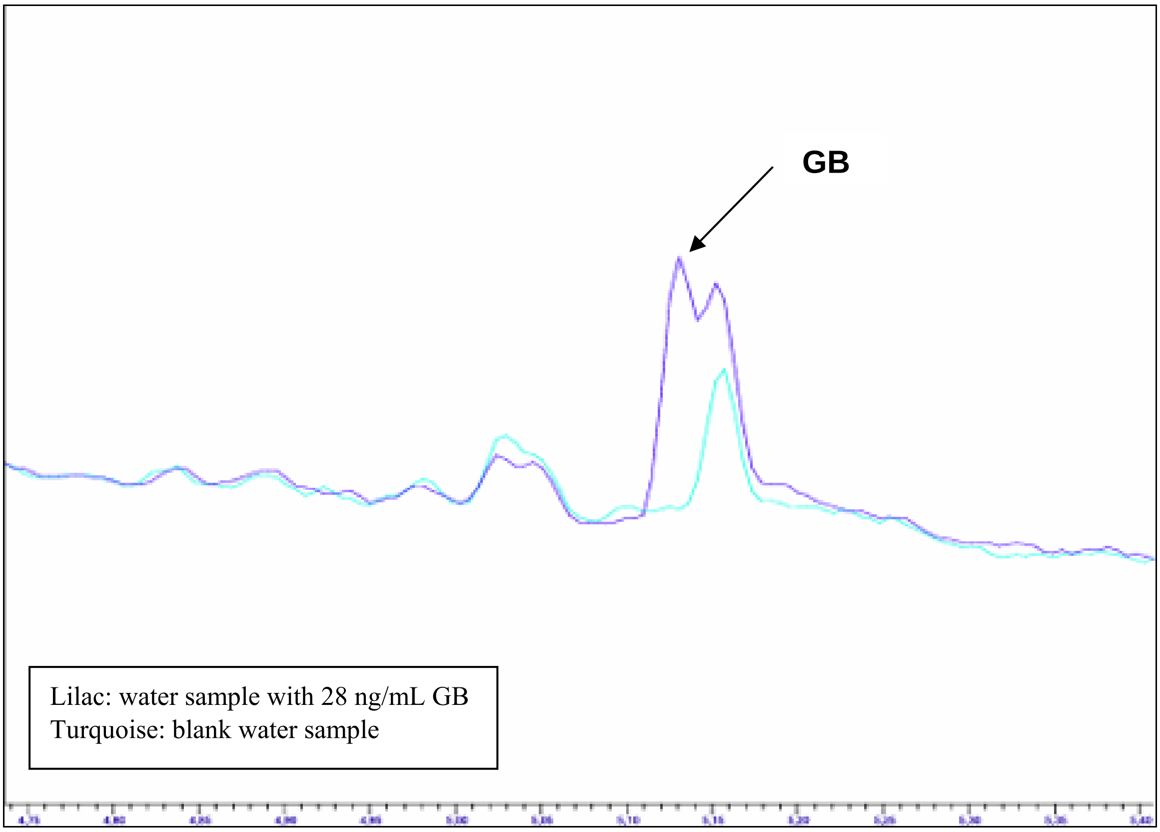
	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	169914	1766020	1443732	993740	2294184
2	161088	1891381	1528372	1243634	2380041
3	155030	1881413	1521468	1252864	2464078
4	161085	1702419	1396137	1298634	2288881
5	158581	1642756	1351825	1139240	2339686
6	153653	1893338	1511765	1492424*	2493972
Mean value	**$1.60 \cdot 10^5$**	**$1.8 \cdot 10^6$**	**$1.46 \cdot 10^6$**	**$1.2 \cdot 10^6$**	**$2.38 \cdot 10^6$**
Std. dev.	**$0.06 \cdot 10^5$**	**$0.1 \cdot 10^6$**	**$0.07 \cdot 10^6$**	**$0.1 \cdot 10^6$**	**$0.09 \cdot 10^6$**
RSD (%)	**3.6**	**6.1**	**5.1**	**10**	**3.6**

*the value is an outlier according to Dixon's test.

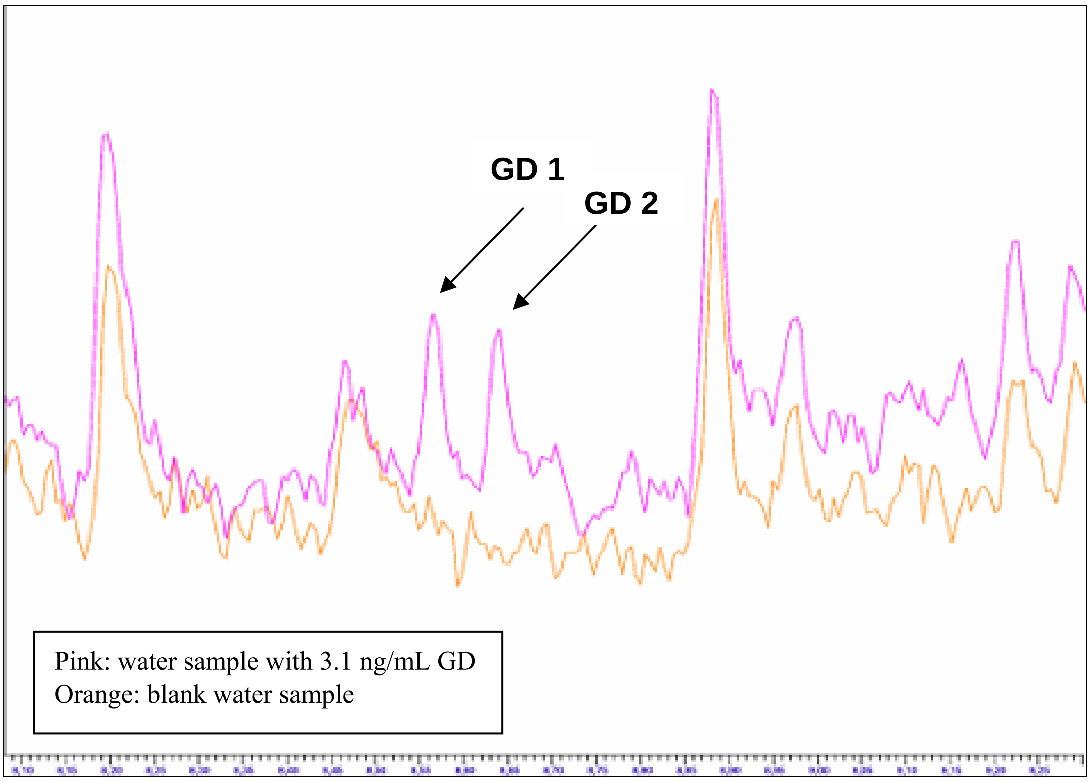
Appendix E Validation of the standard method

The MLOD for GB, GD, HD and GF were determined by LLE. The extracts were analysed on GC-FID. The chromatograms at the MLOD of the analytes are shown in appendices E.1.1 - E.1.3.

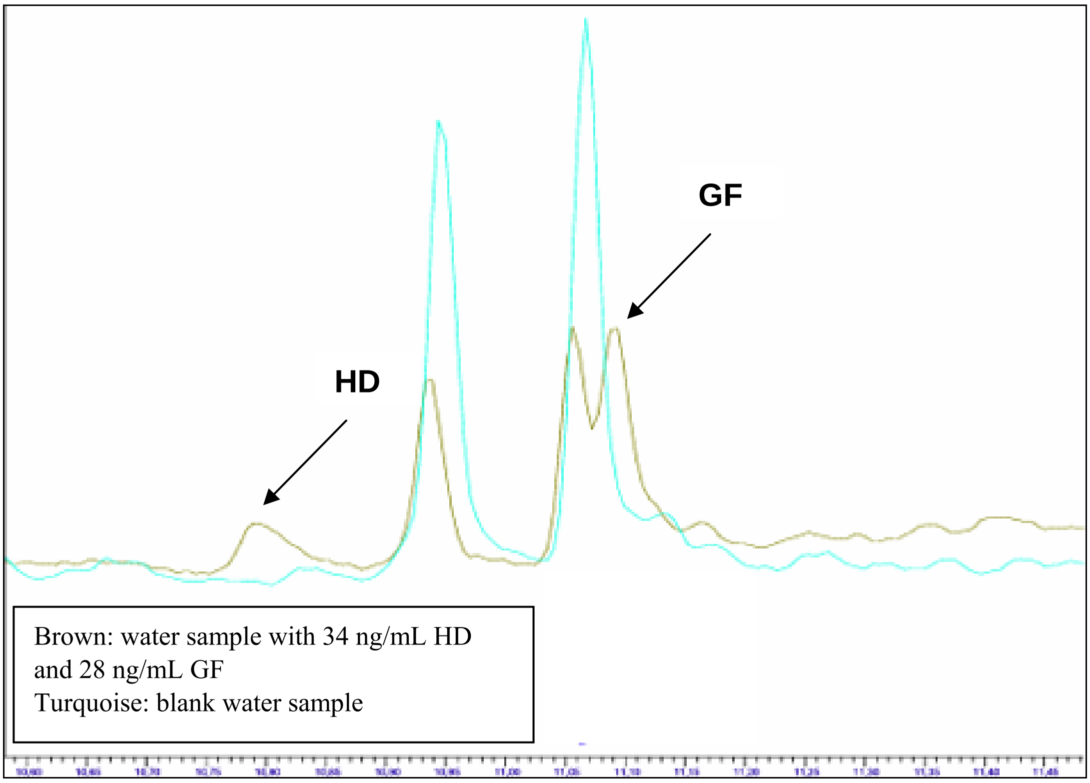
E.1.1 Chromatogram of the MLOD for GB with LLE



E.1.2 Chromatogram of the MLOD for GD with LLE



E.1.3 Chromatogram of the MLODs for HD and GF with LLE



Appendix F SPME and LLE of natural water samples

F.1 LLE

The extracts of natural water samples prepared by LLE were quantified with one point calibration where the origin was included as a point.

The equations for each analyte are given below:

GB: $y = 4399 \cdot x$ GD 1: $y = 2619 \cdot x$ GD 2: $y = 2383 \cdot x$
HD: $y = 3936 \cdot x$ GF: $5336 \cdot x$

With the aid of these equations and the response areas of the analytes given in F.1.1–F.1.3, the recovery and the injected amount (%) for the analytes were calculated.

An example is given below for the calculation of the recovery and the injected amount of GB for the extract of melted snow prepared by LLE (see appendix F.1.1, replicate 1):

Amount of GB in 1 mL extract: 6782/4399 = $\underline{1.54\ \mu g}$
Amount of GB added to melted snow: $\underline{1.95\ \mu g}$
Recovery (%): $(1.54\ \mu g/1.95\ \mu g) \cdot 100\% = \underline{79.0\%}$
Amount injected of the total available amount (%):
$((1.54\ \mu g/mL \cdot 1\ \mu L \cdot 10^{-3}\ \mu L/mL)/1.95\ \mu g) \cdot 100 = \underline{0.079\%}$

F.1.1 LLE of melted snow samples spiked with CWA

Blank snow sample gave signal at the retention time of GB, HD and GF. The response areas were 398, 117 and 739, respectively. The response areas for the analytes in the spiked snow samples were corrected for the responses in the blank melted snow sample. The corrected response areas for the analytes are given below in the table.

	Corrected response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	6782	435	418	2796	11149
2	6388	397	367	2531	10402
3	6471	393	347	3263	10353
4	5953	356	368	3783	10026
5	7290	449	420	3137	12167
Mean value	**$6.6 \cdot 10^3$**	**$4.1 \cdot 10^2$**	**$3.8 \cdot 10^2$**	**$3.1 \cdot 10^3$**	**$1.1 \cdot 10^4$**
Std. dev.	**$0.5 \cdot 10^3$**	**$0.4 \cdot 10^2$**	**$0.3 \cdot 10^2$**	**$0.5 \cdot 10^3$**	**$0.09 \cdot 10^4$**
RSD (%)	**7.6**	**9.1**	**8.6**	**15**	**7.9**

F.1.2 LLE of sea water samples spiked with CWA

Blank sea water sample gave signal at the retention time of GB, HD and GF. The response areas were 936, 121 and 3056, respectively. The response areas for the analytes in the spiked sea water samples were corrected for the responses in the blank sea water sample. The corrected response areas for the analytes are given below in the table.

	Corrected response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	5019	331	300	6329	7113
2	5544	414	384	6916	9731
3	5426	423	416	7141	9248
4	5377	406	384	6862	8517
5	4788	365	387	6364	7143
Mean value	**$5.2 \cdot 10^3$**	**$3.9 \cdot 10^2$**	**$3.7 \cdot 10^2$**	**$6.7 \cdot 10^3$**	**$8.4 \cdot 10^3$**
Std. dev.	**$0.3 \cdot 10^3$**	**$0.4 \cdot 10^2$**	**$0.4 \cdot 10^2$**	**$0.4 \cdot 10^3$**	**$1.2 \cdot 10^3$**
RSD (%)	**6.0**	**10**	**12**	**5.3**	**14**

F.1.3 LLE of river water samples spiked with CWA

Blank river water sample gave signal at the retention time of GB and GF. The response areas were 837 and 1174, respectively. The response areas for the analytes in the spiked river water samples were corrected for the responses in the blank river water sample. The corrected response areas for the analytes are given below in the table.

	Corrected response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	5321	341	286	3993	8561
2	5439	311	267	3821	9906
3	4420	383	353	3084	8495
4	5638	362	303	4080	9354
5	5107	378	329	3401	9118
Mean value	$5.2 \cdot 10^3$	$3.6 \cdot 10^2$	$3.1 \cdot 10^2$	$3.7 \cdot 10^3$	$9.1 \cdot 10^3$
Std. dev.	$0.5 \cdot 10^3$	$0.3 \cdot 10^2$	$0.3 \cdot 10^2$	$0.4 \cdot 10^3$	$0.6 \cdot 10^3$
RSD (%)	9.2	8.3	11	12	6.4

F.2 SPME

The extracts of natural water samples prepared by SPME were quantified by liquid injections of calibration solutions in a concentration range of 3 µg/mL to 55 µg/mL. The curves were “forced” through the origin. The equations for each analyte are given below:

GB: $y = 4733 \cdot x$ $R^2 = 0.998$

GD 1: $y = 3335 \cdot x$ $R^2 = 0.996$

GD 2: $y = 2877 \cdot x$ $R^2 = 0.997$

HD: $y = 5075 \cdot x$ $R^2 = 0.996$

GF: $y = 7367 \cdot x$ $R^2 = 0.995$

With the aid of these equations and the response areas of the analytes given in F.2.1–F.2.6, the recovery and the injected amount of the analytes with the DVB/CAR/PDMS fiber were calculated.

An example is given below for the calculation of the recovery and the injected amount of GB when the fiber has been immersed in a melted snow sample (see appendix F.2.1, replicate 1):

Amount of GB injected by the fiber for replicate 1: 34171/4733 = 7.2 ng = <u>0.0072 µg</u>

Amount of GB added to melted snow: <u>1.95 µg</u>

Recovery and amount injected (%): (0.0072 µg/1.95 µg) · 100 = <u>0.4%</u>

F.2.1 SPME of melted snow samples spiked with CWA

Blank snow sample gave signal at the retention time of GD 1 and GD 2. The response areas were 1613 and 1480, respectively. The response areas for the analytes in the spiked snow samples were corrected for the responses in the blank water sample. The corrected response areas for the analytes are given below in the table.

	Corrected response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	34171	20956	17239	177277	320487
2	33770	19316	15967	171957	310806
3	36511	24313	20288	221849	352189
4	37986	22729	18896	179610	384408
5	35428	25546	21578	246784	356116
6	34006	25207	20848	243697	373268
Mean value	**$3.5 \cdot 10^4$**	**$2.3 \cdot 10^4$**	**$1.9 \cdot 10^4$**	**$2.1 \cdot 10^5$**	**$3.5 \cdot 10^5$**
Std. dev.	**$0.2 \cdot 10^4$**	**$0.2 \cdot 10^4$**	**$0.2 \cdot 10^4$**	**$0.3 \cdot 10^5$**	**$0.3 \cdot 10^5$**
RSD (%)	**4.7**	**11**	**11**	**17**	**8.3**

F.2.2 SPME of sea water samples spiked with CWA

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	20538	18170	13270	227304	184781
2	17035	15864	11431	200028	159071
3	18928	17636	12529	228716	180161
4	21526	17703	12737	237184	193002
5	24717	16596	11868	185311	193002
Mean value	$2.1 \cdot 10^4$	$1.72 \cdot 10^4$	$1.24 \cdot 10^4$	$2.2 \cdot 10^5$	$1.8 \cdot 10^5$
Std. dev.	$0.3 \cdot 10^4$	$0.09 \cdot 10^4$	$0.07 \cdot 10^4$	$0.2 \cdot 10^5$	$0.1 \cdot 10^5$
RSD (%)	**14**	**5.5**	**5.9**	**10**	**7.7**

F.2.3 SPME of river water samples spiked with CWA

	Response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	54013	27234	23202	215469	426350
2	53159	25140	21122	214767	385907
3	53364	24015	20335	212607	369961
4	55606	24617	20611	217400	392865
5	53225	23183	19692	209633	353309
Mean value	$5.4 \cdot 10^4$	$2.5 \cdot 10^4$	$2.1 \cdot 10^4$	$2.14 \cdot 10^5$	$3.9 \cdot 10^5$
Std. dev.	$0.1 \cdot 10^4$	$0.2 \cdot 10^4$	$0.1 \cdot 10^4$	$0.03 \cdot 10^5$	$0.3 \cdot 10^5$
RSD (%)	**1.9**	**6.1**	**6.4**	**1.4**	**7.1**

F.2.4 SPME of melted snow samples spiked with CWA at low concentration

Blank snow sample gave signal at the retention time of GB, HD and GF. The response areas were 218, 468 and 2191, respectively. The response areas for the analytes in the spiked snow water samples were corrected for the responses in the blank snow sample. The corrected response areas for the analytes are given below in the table.

	Corrected response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	2631	2722	2445	3499	11312
2	2592	2935	3379*	4495	11557
3	2700	2680	2341	3790	7961
4	2795	5422*	2625	3599	7961
5	2977	2551	2201	3549	9625
6	1665*	2362	2397	4043	11659
Mean value	**$2.7 \cdot 10^3$**	**$2.7 \cdot 10^3$**	**$2.4 \cdot 10^3$**	**$3.8 \cdot 10^3$**	**$1.0 \cdot 10^4$**
Std. dev.	**$0.2 \cdot 10^3$**	**$0.2 \cdot 10^3$**	**$0.2 \cdot 10^3$**	**$0.4 \cdot 10^3$**	**$0.2 \cdot 10^4$**
RSD (%)	**5.6**	**8.0**	**6.4**	**10**	**18**

*the value is an outlier according to Dixon’s test.

F.2.5 SPME of sea water samples spiked with CWA at low concentration

Blank sea water sample gave signal at the retention time of HD and GF. The response areas were 359 and 362, respectively. The response areas for the analytes in the spiked sea water samples were corrected for the responses in the blank sea water sample. The corrected response areas for the analytes are given below in the table.

	Corrected response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	429	970	671	7368	2499
2	458	1053	676	8547	2313
3	434	964	542	7196	1959
4	492	1594*	608	8643	2267
5	357	1045	584	8670	52294*
6	372	3298*	959*	10944	1958
Mean value	**$4.2\cdot10^2$**	**$1.0\cdot10^3$**	**$6.2\cdot10^2$**	**$8.6\cdot10^3$**	**$2.2\cdot10^3$**
Std. dev.	**$0.5\cdot10^2$**	**$0.5\cdot10^2$**	**$0.6\cdot10^2$**	**$1.3\cdot10^3$**	**$0.2\cdot10^3$**
RSD (%)	**12**	**4.7**	**9.3**	**16**	**11**

*the value is an outlier according to Dixon’s test.

F.2.6 SPME of river water samples spiked with CWA at low concentration

Blank river water sample gave signal at the retention time of HD and GF. The response areas were519 and 535, respectively. The response areas for the analytes in the spiked river water samples were corrected for the responses in the blank water sample. The corrected response areas for the analytes are given below in the table.

	Corrected response area				
Replicate	**GB**	**GD 1**	**GD 2**	**HD**	**GF**
1	6119	2032	1485	8355	4980
2	6392	1740	1389	6476	4922
3	5595	1794	1475	7649	5307
4	4754	1321	869	6231	4292
5	5786	1527	1101	6670	5055
6	5589	1650	1155	8318	4979
Mean value	**$5.7 \cdot 10^3$**	**$1.7 \cdot 10^3$**	**$1.2 \cdot 10^3$**	**$7.3 \cdot 10^3$**	**$4.9 \cdot 10^3$**
Std. dev.	**$0.6 \cdot 10^3$**	**$0.2 \cdot 10^3$**	**$0.2 \cdot 10^3$**	**$0.9 \cdot 10^3$**	**$0.3 \cdot 10^3$**
RSD (%)	**9.9**	**14**	**20**	**13**	**6.9**

Appendix G Presentation of the master thesis in international symposia

An abstract and a poster of the master thesis were accepted for presentation at the following symposia:

- Ninth International Symposium on Protection against Chemical and Biological Warfare Agents, Gothenburg Sweden, 22-25 May 2007.
- Ninth International Symposium on Advances in Extraction Technologies, Ålesund Norway, 3-6 June 2007.

G.1.1 Abstract

DETERMINATION OF CHEMICAL WARFARE AGENTS BY SOLID PHASE MICROEXTRACTION AND HEADSPACE EXTRACTION TECHNIQUE

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Norwegian Defence Research Establishment (FFI) is presently developing a mobile laboratory for determination of chemical warfare agents (CWA) and related compounds. The laboratory will be used in the field and it is therefore beneficial that the analytical methods are based on low solvent consumption and little sample preparation. Solid phase microextraction (SPME) and static headspace (HS) extraction with trap meet these requirements. Both techniques extract and concentrate the analyte from the sample automatically without using solvents (26, 48).

The aim of this study is to develop fast screening methods for selected CWA in water and polymer materials by use of SPME and static HS extraction with trap, coupled to a gas chromatograph with a flame ionisation detector (FID). The choice of SPME fibers, trap adsorbents and instrumental parameters will be determined from a set of initial experiments. The developed methods for SPME and HS extraction will be compared with the existing analytical procedures (6).

1. Pawliszyn, J. Solid phase microextraction. In *Comprehensive Analytical Chemistry: Sampling and Sample Preparation for Field and Laboratory*, Pawliszyn, J.; Elsevier: Amsterdam, **2002**; 389-477.
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3. Rautio, M. *Recommended Operating Procedures for Sampling and Analysis in the Verification of Chemical Disarmament;* 1994 ed.; The Ministry for Foreign Affairs of Finland: Helsinki, **1994**.

G.1.2 Poster

Determination of Chemical Warfare Agents in Water by Solid Phase Microextraction

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Introduction

The Norwegian Defence Research Establishment (FFI) is developing methods for determination of chemical warfare agents (CWA) in the field by a deployable laboratory.

Analytical methods based on low solvent consumption and little sample preparation are beneficial. Solid phase microextraction (SPME) meets these requirements (1).

The aim of this study was to develop a method to determine CWA in water by the use of SPME, coupled to a gas chromatograph (GC) with a flame ionisation detector (FID). The analysis by the SPME technique was compared with the existing procedure (ROP) based on liquid-liquid extraction (2).

Theory of SPME

A fused silica fiber coated with a polymer material is exposed to a liquid sample in a closed vial, and the analytes are distributed between the sample and the polymer coating (Fig. 1 and 2) (1). The fiber is inserted into the injection port of a GC to thermally desorb the analytes from the polymer coating.

Fig. 1 SPME fiber coated with a polymer material (white needle tip). (Photo: Fremstad, FFI)

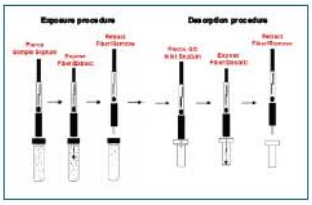
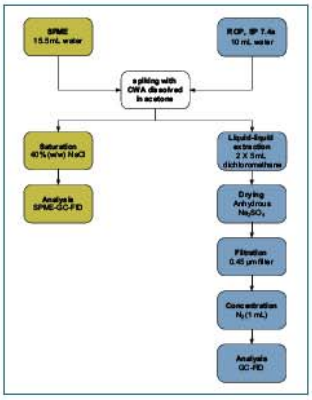


Fig. 2 Principles of SPME. (Illustration: Supelco)

Analyses by SPME and liquid – liquid extraction



SPME
15.5mL water

ROP, SP 7.4a
10 mL water

spiking with CWA dissolved in acetone

Saturation
40 % (w/w) NaCl

Analysis
SPME-GC-FID

Liquid-liquid extraction
2 X 5mL dichloromethane

Drying
Anhydrous Na_2SO_4

Filtration
0.45 µm filter

Concentration
N_2 (1 mL)

Analysis
GC-FID

Fig. 3 Flow chart for analyses of spiked water samples by SPME as compared to liquid – liquid extraction.

Instrumentation

A MPS2 multipurpose autosampler (Gerstel, Munich, Germany) was mounted on an AutoSystem XL GC-FID (Perkin Elmer, CT, USA) (Fig. 4).

Fig. 4 Setup of the multipurpose autosampler for SPME and liquid injections (ROP): (A) hand-held-programming unit, (B) robotic head unit, (C) vial tray for SPME extraction, (D) liquid injection vial tray, (E) vial heater and agitation unit, (F) SPME fiber heater unit and (G) solvents for cleaning of liquid injection syringe.
(Photo: Fremstad, FFI)

Column: DB-5 MS, 30 m x 0.25 mm with 0.25 µm film thickness (J&W Scientific, Folsom, CA, USA).
Oven program: 40 °C (1 min) – 10 °C/min - 250 °C (2 min).
Carrier gas: 1 mL/min of helium (6.0, AGA, Norway).
Injector: 250 °C, splitless for 1 min for injection of 1 µL solution and splitless for 2 min for SPME injection.
Detector temperature: 280 °C
Detector gases: 50 mL/min hydrogen and 450 mL/min synthetic air (grade 5.0).
Instrument control and data handling: GERSTEL MASter version 1.86.17.8 and TotalChrom version 6.2.1 (Perkin Elmer).

Table 1 Parameters for SPME analysis by MPS2 autosampler.

Heating and agitation:		Sampling and desorption:	
Vial temperature (°C)	35	Vial penetration (mm)	31
Vial heating time (s)	6	Extraction time (min)	30
Agitation speed (rpm)	750	Injection penetration (mm)	50
Agitation on time (s)	10	Desorption time (s)	120
Agitation off time (s)	1	Cycle time (min)	45
Conditioning of fiber:		**Rinsing of fiber:**	
Conditioning time (min)	10	Distilled and de-ionized water (mL)	18
Conditioning penetration (mm)	55	Rinsing time (s)	3
Purge gas flow of nitrogen (bar)	0.5	Vial penetration (mm)	31
Fiber heater temperature (°C)	250		

Results

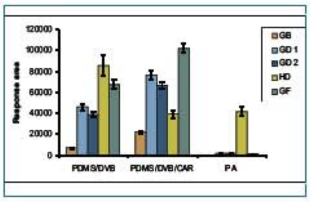


Fig. 5 Evaluation of three different types of SPME fibers. The fibers were immersed for 10 min. in distilled and de-ionized water spiked with sarin (GB), soman (GD), sulphur mustard (HD) and cyclosarin (GF) (n = 6). GD 1 and GD 2 are the two diastereoisomers of GD.

Table 2 Method limits of detection (ng/mL) for distilled and de-ionized water spiked with selected CWA.

Method	GB	GD	HD	GF
SPME, PDMS/DVB/CAR	2	0.2	1	0.7
Liquid-liquid extraction	28	3	34	28

Table 3 Repeatability of PDMS/DVB/CAR fiber and the range of linearity. RSD is the relative standard deviation.

Compound	Repeatability (RSD, %) 0.01 µg/mL	Repeatability (RSD, %) 1.0 µg/mL	Range of linearity (µg/mL)	Regression coefficient (R^2)
GB	1.6	3.6	$7*10^{-3}-0.5$	0.98
GD	6.4	5.8	$6*10^{-4}-1$	0.996
HD	5.2	13	$3*10^{-2}-4$	0.995
GF	4.4	3.6	$6*10^{-4}-1$	0.997

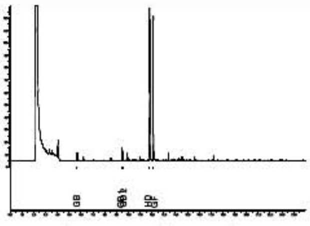


Fig. 6 Chromatogram showing extraction of selected CWA from sea water with SPME.

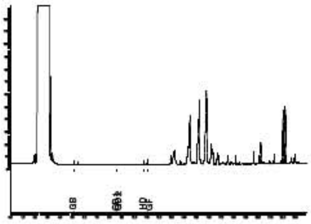


Fig. 7 Chromatogram showing extraction of selected CWA from sea water with liquid - liquid extraction.

Conclusions

- The uptake of nerve agents by the PDMS/DVB/CAR fiber is 1.5 - 3 times greater than with the PDMS/DVB fiber (Fig. 5). In contrary, the uptake of sulphur mustard is reduced to the half.
- Method limits of detection for selected CWA are from 15 to 50 times lower with SPME (PDMS/DVB/CAR fiber) than with liquid - liquid extraction (Table 2).
- The area responses for CWA are from 4 to 44 times greater when the extraction of sea water is performed with SPME compared with liquid - liquid extraction. In addition, the chromatogram achieved for sea water with SPME is more distinct compared to the chromatogram obtained with liquid - liquid extraction (Fig. 6 and 7).

References

1. Pawliszyn, J. Solid phase microextraction. In Comprehensive Analytical Chemistry: Sampling and Sample Preparation for Field and Laboratory, Pawliszyn, J.; Elsevier: Amsterdam, 2002; 389-477.
2. Rautio, M. Recommended Operating Procedures for Sampling and Analysis in the Verification of Chemical Disarmament; 1994 ed.; The Ministry for Foreign Affairs of Finland: Helsinki, 1994.

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