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In-situ derivatisation of degradation products of chemical warfare agents in water by solid-phase microextraction and gas chromatographic–mass spectrometric analysis

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Abstract

A new analytical procedure was developed for the extraction of degradation products of chemical warfare agents from water and for in-situ derivatisation prior to analysis by gas chromatography–mass spectrometry (GC–MS). With this new procedure, degradation products of the chemical warfare agents can be analysed and identified without going through laborious sample preparation. Parameters such as fiber selection, pH, salt content, derivatisation temperature, extraction and derivatisation periods, and sequence of adsorption/derivatisation were experimented with, to optimise the efficiency of this method. The detection limit is in the ppb to sub-ppb range with GC–MS in the full scan mode. Based on six injections of the devised procedure, a relative standard deviation from 10–35% can be achieved, depending on the compound. This method was demonstrated during the 4th International Interlaboratory Proficiency Test organised by the Organisation for the Prohibition of Chemical Weapons to be comparable to existing recommended operating procedures for verification of degradation products of chemical warfare agents.

Keywords: Water analysis; Warfare agents; Derivatization, LC; Extraction methods

1. Introduction

With the Chemical Weapons Convention in force on 29 April 1997, there is a need to develop a fast and sensitive method for detecting chemical warfare agents in the environment during on-site verification. These compounds undergo hydrolysis rather rapidly in water with G-agents forming the alkyl methylphosphonic acids as primary hydrolysis products whereas thiodiglycol is the main degradation product of sulphur mustard. Extraction of these compounds from water normally entails evaporating the aqueous extract to dryness and derivatising the hydrolysis products to a form suitable for GC analysis [1]. The evaporation of water is a tedious process and may result in loss of analytes while solid-phase extraction (SPE), the alternative sample preparation technique, has poor recovery for these degradation products. The developed method based on solid-phase microextraction (SPME) can overcome some of these difficulties as illustrated below.

SPME, a fast and simple extraction method, has found wide acceptance in the analytical arena with applications ranging from environmental to biological matrices. However, its application has mostly

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been for compounds that can be analysed directly by GC. In-situ derivatisation using SPME was introduced in 1995 by Pan et al. on fatty acids that are amenable to direct GC analyses albeit with poor sensitivity and reproducibility [2]. Since then, this technique has also been employed in steroid analysis [3].

In this study, we have extended the application of in-situ derivatisation on SPME to the analysis of degradation products of chemical warfare agents and related compounds in water. The technique we have developed has some advantages over the recommended operating procedures (ROPs) with respect to on-site analysis. SPME is non-destructive unlike rotary evaporation. Water samples that have undergone SPME still preserve their integrity. Multiple SPME analyses can therefore be performed on the same sample, analysing for volatiles, semi-volatile agents and their degradation products. This would give the sample preparation more flexibility and allow the analyst to adopt a strategy for the extraction with minimal sample depletion. As such, this technique would be particularly suited for on-site verification analysis. In addition to the merits of this technique mentioned above, the technique is also favourable because of the minimum logistics support required. The derivatisation is undertaken at room temperature with a very small amount of derivatisation reagent needed. With our earlier work on the use of SPME for analysis of nerve agents in water [4], SPME can now cover a wider range of chemicals, which is of interest for the verification of chemical weapons.

The compounds studied were ethyl-2-hydroxyethyl sulphide (EHES), thiodiglycol (TDG) which represent the class of sulphur mustard hydrolysis degradation products; ethyl methylphosphonic acid (EMPA), methylphosphonic acid (MPA), *n*-propylphosphonic acid (nPPA) are the degradation products of nerve agents and benzilic acid (BA) is a precursor and a degradation product of Bz [5]. The structures of these analytes are depicted in Fig. 1. The derivatisation reagent *N*-methyl-*N*-(*tert*.-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) with 1% tert.-butyldimethylsilyl chloride (TBDMSCl) was selected for this study. This derivatisation reagent was selected because the *tert*.-butyldimethylsilyl (TBDMS) derivatives formed are hydrolytically stable.

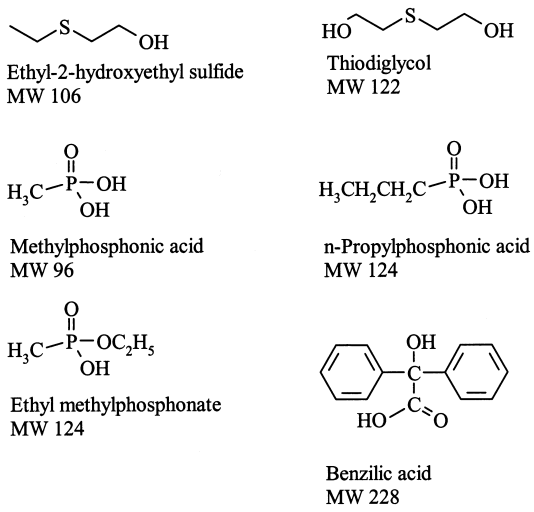

Fig. 1. Structures of the degradation products used in this study. MW=Molecular mass.

2. Experimental section

2.1. Reagents and materials

EHES (97%), MPA (98%), EMPA (98%), nPPA (95%) and MTBSTFA (97%) were purchased from Aldrich. TDG (98%) and BA (>98%) were obtained from Merck. Water was obtained from a Labconco WaterPro system. Hydrochloric acid (HCl, 10 *M*), ammonium hydroxide (NH_4OH) and sodium chloride were obtained from J.T. Baker.

The SPME device was from Supelco. Fibers coated with 100 μm polydimethylsiloxane (PDMS), 85 μm polyacrylate (PA), 65 μm PDMS–divinylbenzene (PDMS–DVB) and 75 μm Carboxen–polydimethylsiloxane (Carboxen) were used for the study. All the fibers were conditioned as recommended by the supplier before use.

2.2. Instruments

All analyses were performed on a Finnigan GCQ system. The GCQ transfer line was at 275°C. Separation was carried out using a HP-5MS column, 25 m×0.25 mm, 0.25 μm. Splitless injections were performed. The temperature program for the analysis of TBDMS derivatives was: 40°C, hold for 2 min ramp at 20°C/min to 280°C hold for 4 min. The

carrier gas was helium at 35 cm/s. The split/splitless injector was maintained at 250°C. All analyses were performed in full scan mode. This method optimises the separation of derivatised analytes and the analysis time.

2.3. Procedure

2.3.1. Fiber selection

Stock solutions of 10 000 ppm of the analytes: EHES, TDG, EMPA, MPA, nPPA and BA were individually prepared in methanol (HPLC grade, Fisher). Appropriate amounts of these stocks were then spiked into 100 ml of deionised water to make up a 20 ppm solution containing all the analytes.

Four different fibers were evaluated to determine their extraction efficiencies for the degradation products used in this study. The four fibers were 100 μm PDMS, 85 μm PA, 65 μm PDMS–DVB and 75 μm Carboxen. The fiber was first exposed to the headspace of a vial containing MTBSTFA for 5 min. This fiber was then inserted into 3 ml of an aqueous solution containing 20 ppm of the spiked chemicals. The solution was continuously stirred during the 15 min of extraction. The fiber was then withdrawn and then exposed again to MTBSTFA vapour for a further 15 min. This fiber was then injected into the GC–MS for analysis. This procedure was repeated for all fibers, using fresh spiked solutions. Duplicate analyses were performed for each fiber. This sequence of procedure for extraction/derivatisation was adopted as it was found to be feasible from our preliminary studies. The best fiber was used for all subsequent analyses.

2.3.2. Effects of pH and salting

The effects of pH and salt on the adsorption efficiency were examined next. Spiked solutions of 20 ppm were prepared as before. The pH was then adjusted to pH 1.5 using 10 *M* HCl. Adopting the above sequence of extraction/derivatisation procedure, the experiments were performed on the spiked solution (pH 1.5) and then on a fresh lot of the acidified spiked solution saturated with NaCl.

A fresh lot of spiked solution was prepared and adjusted to pH 7 using ammonium hydroxide. Again two sets of experiments were performed: one unsalted and the second with saturated salt solution. Duplicates were performed for each of the above experiments.

Salt saturated acidified samples were found to be optimal for SPME extraction and derivatisation. All samples for subsequent analyses were therefore acidified and saturated with salt.

2.3.3. Effect of temperature

The effect of temperature on the derivatisation was studied. Three sets of experiments were performed at the following temperatures: room temperature (25°C), 40°C and 60°C. Both 5-min headspace adsorption of MTBSTFA and 15-min extraction of the spiked solution were carried out at room temperature. Only the final derivatisation step of 15-min headspace MTBSTFA was carried out at the three defined temperatures.

2.3.4. Sequence of adsorption/derivatisation procedure

Three different adsorption/derivatisation procedures were experimented with. In the first procedure, MTBSTFA was adsorbed onto the fiber by exposing the fiber in the headspace of a vial containing neat MTBSTFA for 15 min. This fiber was then inserted into the water sample for another 15 min of extraction. In the second procedure, this sequence was reversed with the adsorption preceding the derivatisation. The third procedure is similar to the second but with the fiber being exposed to MTBSTFA for 5 min before the adsorption step. Duplicate analyses were performed.

The third procedure was found to be most effective and therefore adopted for later experiments.

2.3.5. Equilibration studies

The equilibration studies were conducted in two approaches. Firstly the extraction time was kept constant while the derivatisation period was varied and in the second, the derivatisation period was kept constant while the extraction time was varied. The time periods for the extraction and derivatisation processes were 5, 10, 15, 30 and 45 min. Duplicate analyses were performed.

2.3.6. Reproducibility

Using the optimised parameters established in previous experiments, this procedure was repeated

six times at two different concentrations to yield the percentage relative standard deviation (RSD) of this method. The concentrations studied were 1 ppm and 20 ppm. The optimised parameters were: use of Carboxen fiber; extraction from acidified (pH 1.5) salt saturated samples; derivatisation at room temperature; extraction/derivatisation procedure of 5 min pre-adsorption with MTBSTFA followed by 30 min extraction and then 15 min of derivatisation.

Calibration standards of concentrations 0.1, 0.5, 1, 5 and 10 ppm were prepared. The calibration curves established were used to assess the amount extracted and derivatised by the SPME procedure from a 1 ppm spiked solution.

2.3.7. Detection limit

The experiments were performed in duplicates with the developed procedure for each of the spiked solutions of varying concentrations at ppb to ppm levels. The spiked concentrations were 1 ppb, 10 ppb, 50 ppb, 100 ppb, 500 ppb and 1 ppm. Blank injections were performed in between each injection to ensure that carry over, if present, was negligible.

3. Results and discussion

3.1. Fiber selection

The results are compiled in Fig. 2. The fibers coated with Carboxen were found to be most efficient for all compounds except for TDG. PDMS–DVB extracts TDG better than Carboxen. The Carboxen fibers were found to be more rugged, withstanding more extraction/injection per fiber compared to PDMS–DVB fibers and were hence used for subsequent studies.

3.2. Effect of pH and salting

From the graph compiled in Fig. 3, both acidifying the solution and saturating it with NaCl clearly improves the adsorption process. The salting effect was more pronounced and this increase was as much as ten times for EHES. This compound is the most hydrophobic among the compounds studied and by increasing the ionic strength of the sample, the adsorption of this compound is greatly enhanced.

The analytes were better extracted at pH 1.5 than

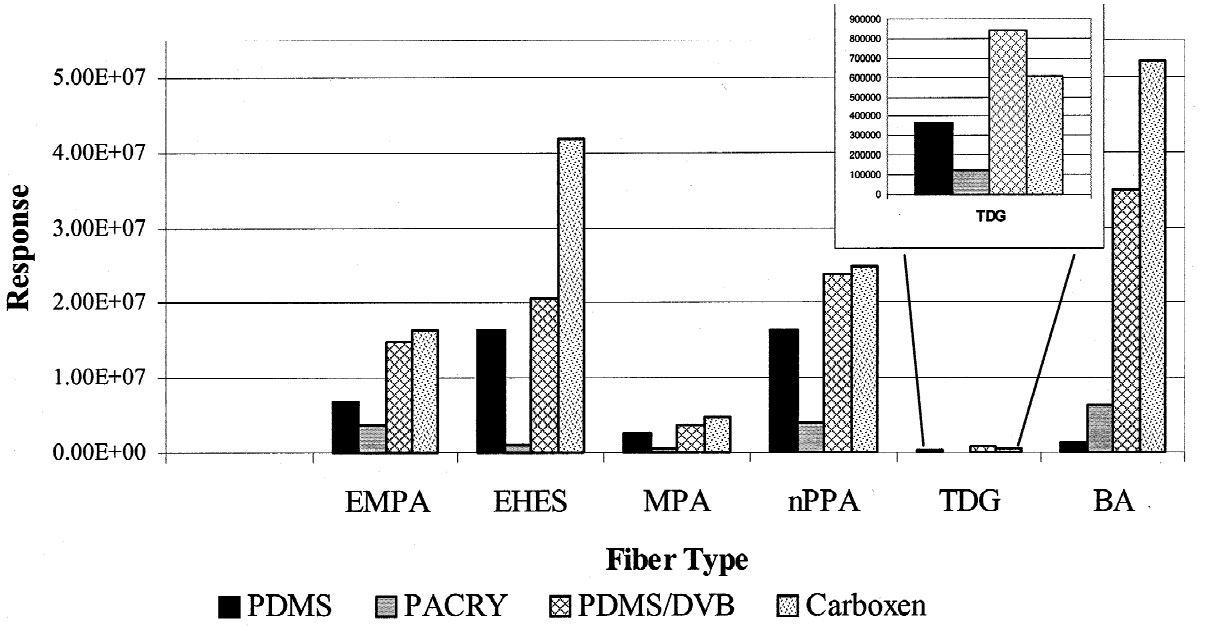

Fig. 2. A comparison of the efficiencies of the different fibers.

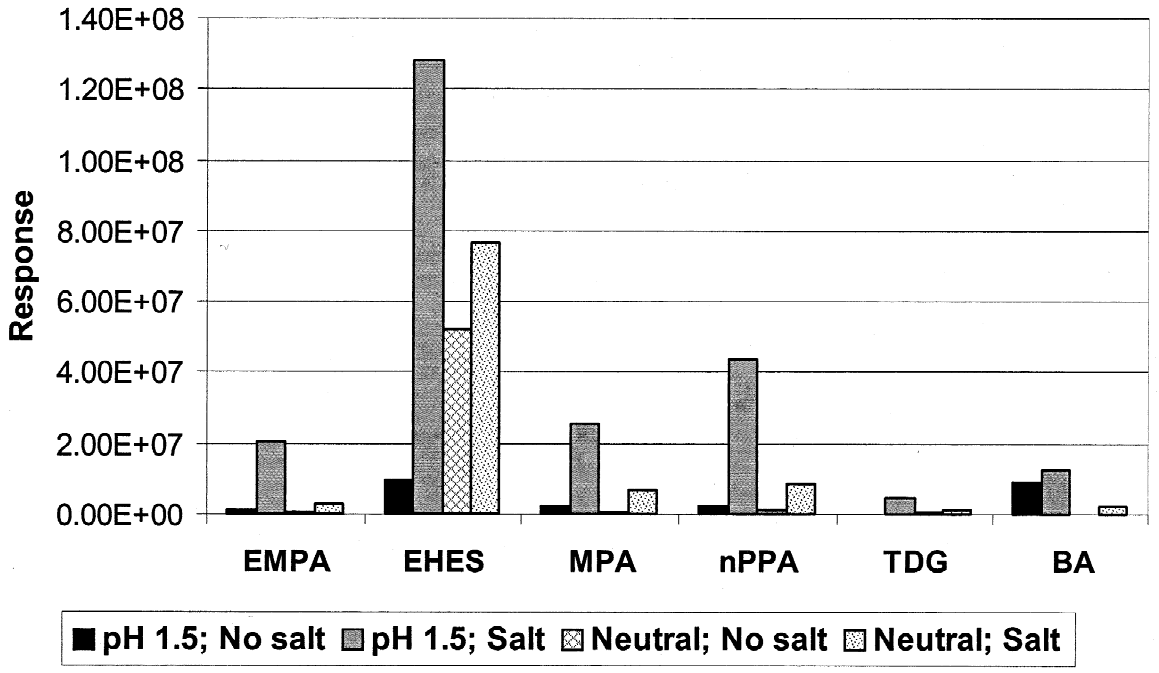

Fig. 3. The effect of pH and salt on extraction of analytes.

at pH 7. The effects were more pronounced for MPA, nPPA, BA and EMPA. These compounds showed a 3.8 to 7.6 times improvement at acidic pH compared to neutral pH under salted conditions. These acids are mostly un-ionised at pH 1.5 and are hence extracted more efficiently.

3.3. Effect of temperature

As is evident from Fig. 4, the yield of derivatisation at room temperature is higher than that carried out at 40°C which is in turn higher than that carried out at 60°C. The higher temperature probably assisted in the desorption of the derivatives from the fiber, resulting in poorer yields.

3.4. Sequence of adsorption/derivatisation procedure

By performing the adsorption before the derivatisation, the yield of the derivatised products was found to be higher (Fig. 5). This is understandable as the derivatisation agent, MTBSTFA is susceptible to hydrolysis and by inserting the SPME fiber coated with this agent into the water sample, the derivatisation agent will be destroyed before a reaction can occur. The yield could be further increased when the fiber is exposed to MTBSTFA for 5 min prior to the extraction. This step probably helps to remove residual acidic protons from the fiber hence increasing the efficiency of derivatisation, subsequently.

3.5. Equilibration studies

Increasing the derivatisation period while keeping the headspace pre-adsorption (5 min) of MTBSTFA and adsorption (15 min) of analytes from water constant, does not affect the yield significantly except for BA (Fig. 6). The extraction profile for BA showed a sharp increase at the start and reached a plateau after 10 min. The other compounds displayed a rather flat profile. Hence it was deduced that 15 min of derivatisation is sufficient for the conversion of the adsorbed analytes to MTSBTFA derivatives.

When the water extraction time was increased while keeping the headspace adsorption of MTBSTFA constant, the extracted amount was found to increase except for BA (Fig. 7). This is

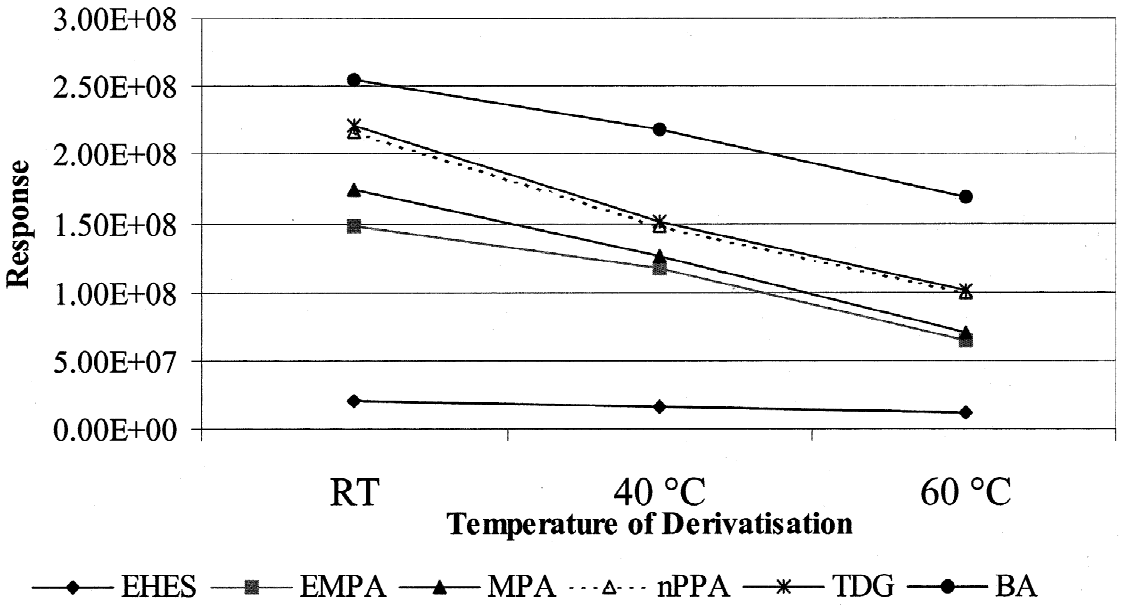

Fig. 4. The effect of temperature on the derivatisation step. RT=Room temperature.

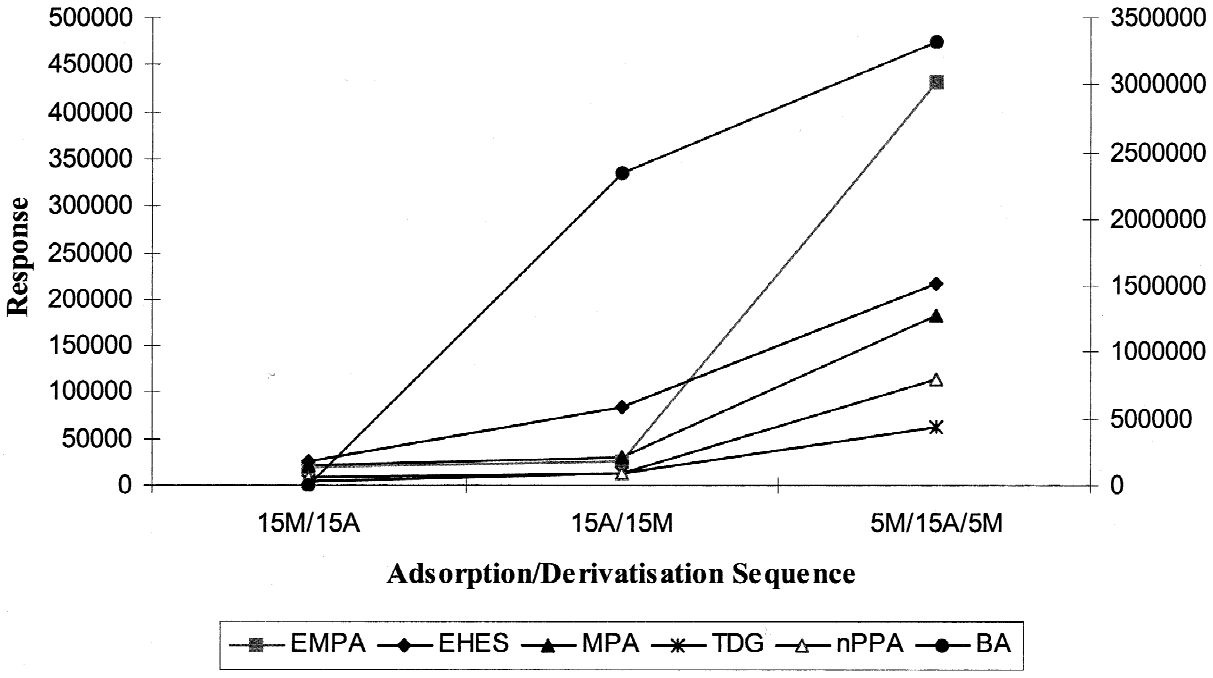

Fig. 5. The effect of adsorption/derivatisation sequence on the yield of derivatisation.

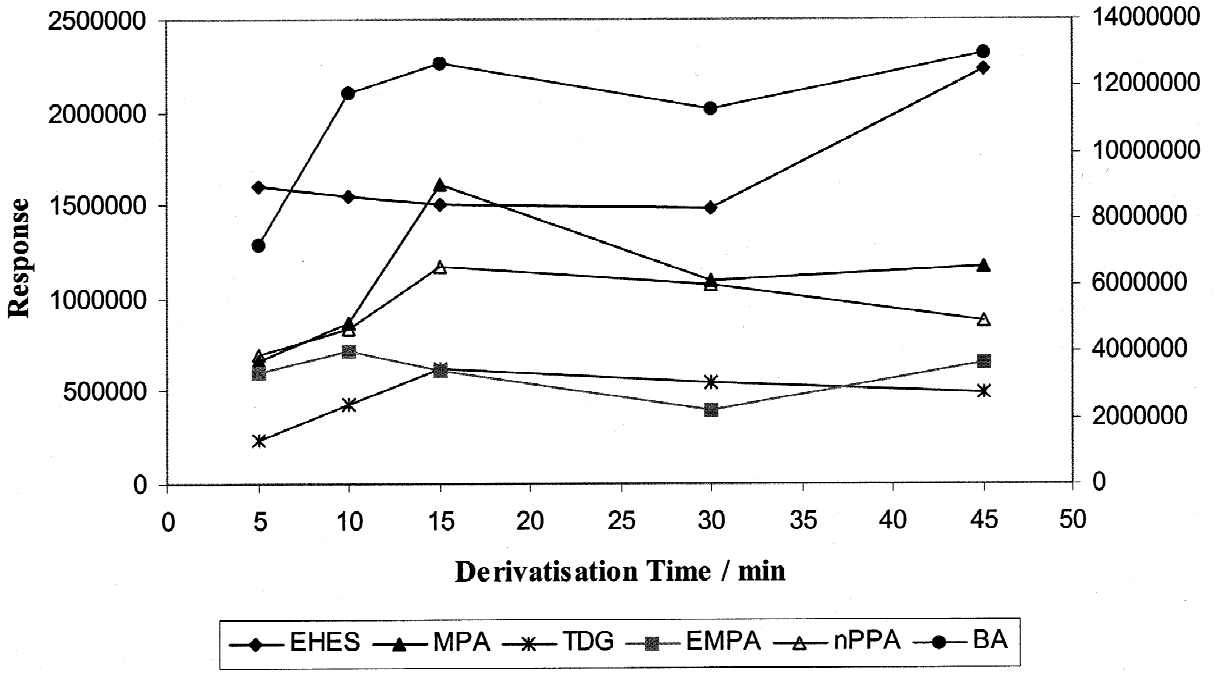

Fig. 6. The effect of post-adsorption derivatisation time on the derivatised product yield.

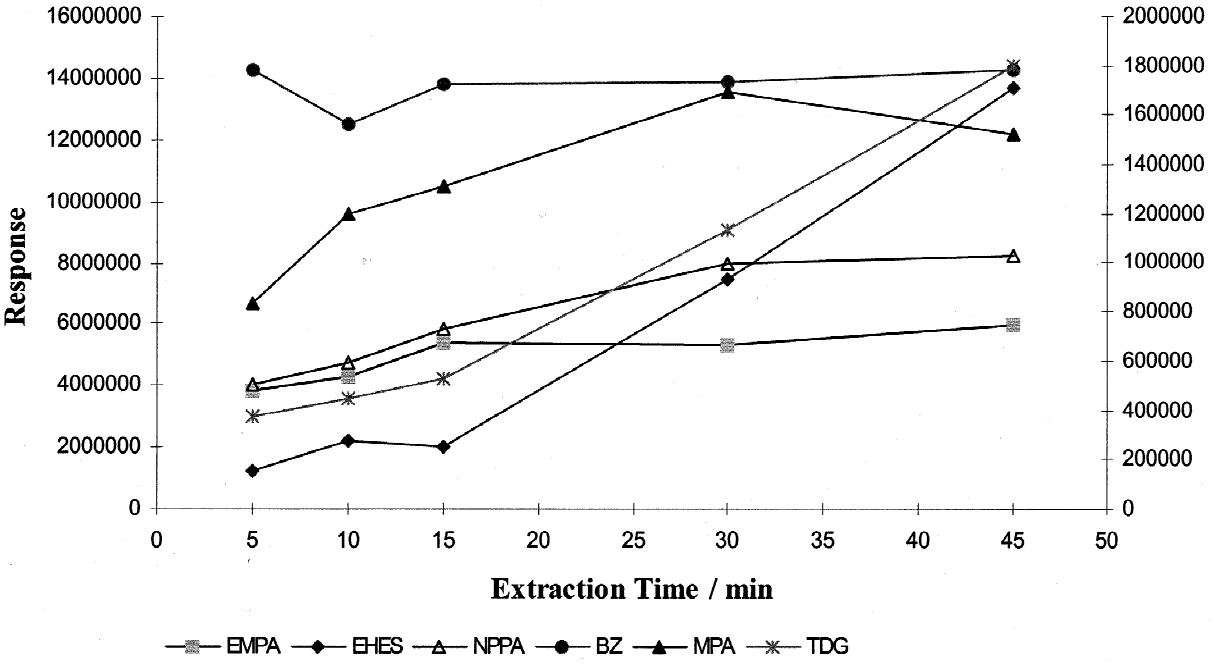

Fig. 7. The effect of extraction time on the derivatised product yield.

Table 1
Reproducibility of the optimised procedure

Concentration	RSD (%) from six injections					
Level	EMPA	EHES	MPA	nPPA	TDG	BA
1 ppm	17.7	35.4	21.9	15.2	18.1	10.4
20 ppm	14.1	21.1	15.0	12.3	23.2	11.3
Amount recovered per SPME extraction and derivatisation from a 1 ppm spiked solution	2.5	8	0.3	1.4	0.6	6

because BA has a short equilibration period and hence its yield did not improve with the increased adsorption. This demonstrated that the limiting step in the previous experiment was the adsorption step and not the derivatisation step. By increasing the adsorption time, the yield of derivatives produced can be improved. The increase was marked for EHES and TDG, as is evident from Fig. 7. The extraction period of 30 min was hence selected as optimal. The total sample preparation time using the optimised procedure is 50 min. This time period is suitable to achieve the required sensitivity as shown in the subsequent results.

3.6. Reproducibility

The relative standard deviations based on six SPME injections of the derivatised compounds using the established procedures were found to vary from 10.4% to 35.4% for 1 ppm spiked solutions and 11.2% to 23.2% for 20 ppm spiked solutions. The results are tabulated in Table 1.

There are several factors determining the consistency of the results. Many of the compounds such as TDG, MPA and nPPA have two acidic hydrogens to be substituted. Since the derivatisation reaction is incomplete, the products yield consists of a mixture of the singly derivatised and doubly derivatised species. The uncertainty in the yield is factored in the relatively large % RSD. Another factor is the amount of water absorbed by the fiber. The amount of water absorbed by the fiber is found to be critical to the efficiency of derivatisation. However, in our procedure, despite the pre-derivatisation step of coating of the fiber with the derivatisation agent to minimise the amount of water absorbed by the fiber, it is difficult to control the amount of water absorbed, which will lead to some variations in the amount of absorbed analytes derivatised. In addition to these factors, the absorption of the analytes is under non-equilibrium conditions and this will result in a larger standard deviation for analysis. BA is the only analyte that is extracted under equilibrium conditions, which explains why its standard deviation is the lowest.

Of the analytes studied, the extractions of EHES

Table 2
Main mass fragments of the compounds

Compound name	*m/z*	Relative intensity
Ethyl-2-hydroxyethyl	163	100
sulphide–TBDMS	89	83.3
derivative	75	51.9
	61	27.2
Thiodiglycol–	149	100
TBDMS derivative	293	48.3
	233	46.1
	191	23.3
Methylphosphonic acid	267	100
–TBDMS derivative	225	30.4
	153	9.5
	195	7.2
n-Propylphosphonic	295	100
acid–TBDMS	253	23
derivative	296	22.2
	337	6.7
Benzilic acid–	371	100
TBDMS derivative	149	53.6
	297	48.2
	399	36.6

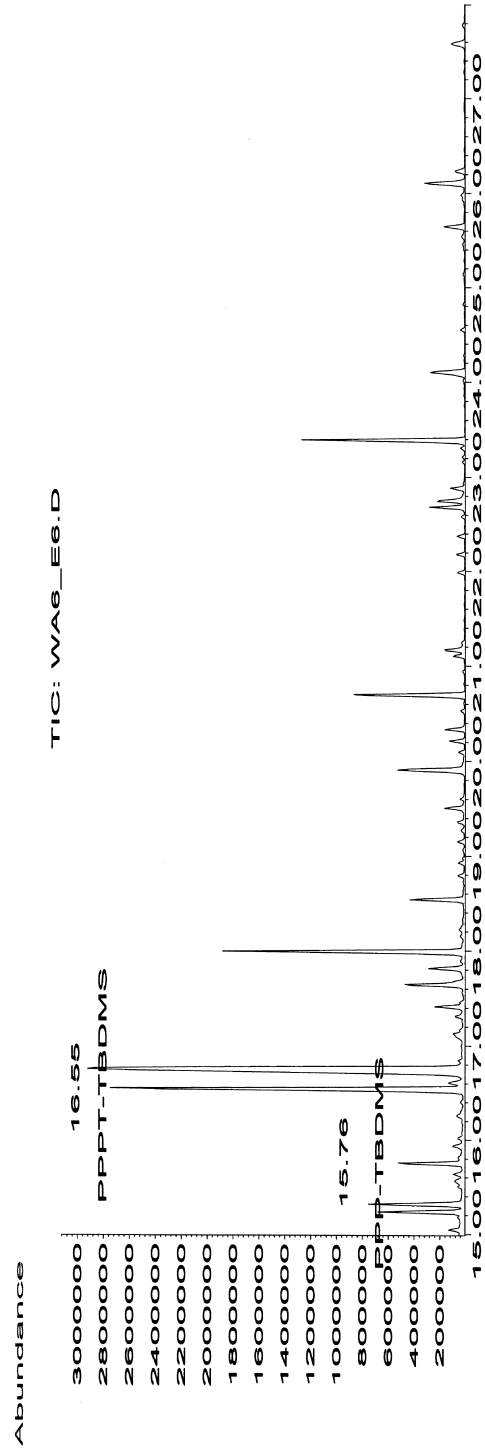

Fig. 8. TIC of the SPME in-situ derivatised water sample from the 4th Proficiency Test. Time in min.

and BA were more efficient owing to their hydrophobicity. The yields of the extraction and derivatisation per SPME analysis from a 3-ml solution with a spiked concentration of 1 ppm is shown in the last row of Table 1.

3.7. Detection limits

The detection limits for the analytes using the devised procedure was defined as producing a chromatographic peak with signal-to-noise ratio of greater than 5 using full scan GC–MS. The selected ion for EHES is m/z 163; for EMPA, m/z 153; for MPA, m/z 267; for nPPA, m/z 295; for TDG m/z 293 and for BA, m/z 371 and m/z 399. The main mass fragments of these compounds are compiled in Table 2. The limits were determined to be 1 ppb for BA, 10 ppb for MPA and nPPA, 100 ppb for EMPA and EHES, and 200 ppb for TDG.

3.8. Applications

To demonstrate the successful application of the developed method to verification analyses, the total ion chromatogram (TIC) of a water sample SPME extract is shown in Fig. 8. This water sample was from the recent 4th Proficiency Test organised by the Organisation for the Prohibition of Chemical Weapons (OPCW). The analysis revealed the presence of the TBDMS derivatives of two acids at 15.76 and 16.55 min. The acids were subsequently identified as *O*-*n*-propyl *n*-propylphosphonate and *O*-*n*-propyl *n*-propylphosphonothiolate respectively. From the chromatogram, the spiked compounds can be distinctly distinguished from the background, despite the large number of interfering peaks. These interfering peaks were due to glycols which were deliberately added as background.

4. Conclusion

Degradation products of chemical warfare agents were shown to be successfully extracted from water using SPME with derivatisation and followed by GC–MS analysis. This method is rapid compared to traditional liquid–liquid extraction methodology and rotary evaporation of water. With very little sample preparation involved, it can easily be extended to field sampling and analysis. However, it may not be ideal for quantification of such compounds. This is because the derivatisation reaction may be rate-limited by the concentration of analyte when its concentration is low or by the derivatisation process at high concentration of the analyte. Nevertheless, if time is a determining factor, this analytical procedure is viable, as a wide range of analytes can be detected in a single analysis.

The applicability of the technique to verification analyses was demonstrated during the recent proficiency test. The spiked compounds were distinct from the myriad of background peaks contributed by glycols.

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