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Thermally assisted methylation and subsequent silylation of scheduled acids of chemical weapon convention for on-site analysis and its comparison with the other methods of methylation☆

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ABSTRACT

On-site verification of the chemical weapon convention (CWC) requires provision for the detection and identification of alkyl phosphonic acids as well as some organic acids that are amenable to GC–MS only after derivatisation. Various derivatisation methods have been used for the identification of these acids and for many cases the methyl derivatives are less prone to artifacts possibly leading to false positive identification. Methylation with diazomethane is widely used but, especially for on-site analysis it has limitation due to the potential explosive and health hazards. Other methylation procedures like trimethylsilyldiazomethane (TMSD), thermally assisted methylation (TAM) by trimethylphenylammonium hydroxide (TMPAH) and trimethylsulfonium hydroxide (TMSH) are evaluated. Data for methylation for the alkyl alkylphosphonic acids, alkylphosphonic acids and benzilic acid are reported. In addition, TAM followed by the silylation in the same sample without any additional sample preparation is also reported. Several parameters such as solvent, temperature, amount of reagents, time, etc. were studied. The two commercially available reagents namely, TMPAH and TMSH for TAM and subsequent silylation were evaluated. The LOD with TMPAH was below 0.5 ng per injection since all of the acids were detected by GC–MS with the S/N of >3 in full scan mode by AMDIS and their inter day relative standard deviation was from 4.7% to 10.8%.

1. Introduction

The chemical weapon convention (CWC) prohibits the development, production, stockpiling and use of chemical weapons [1]. The Organisation for the Prohibition of Chemical Weapons (OPCW), The Hague, The Netherlands ensures implementation of CWC by a verification program [2]. For verification, "sampling and analysis" is one of the important activities and it is accomplished by using on-site analysis—primarily with gas chromatography–mass spectrometry (GC–MS) analysis [3].

Particularly in investigations of alleged use of chemical weapons samples of soil and water contaminated during deliberate or inadvertent spread of chemical warfare agents (CWA) [4,5] are important. The nerve agent CWA typically undergo hydrolysis in the environment resulting in alkyl alkylphosphonic acids (AAPAs) and alkylphosphonic acids (APAs) [6,7]. The detection and identification of these acids indicate the probable prior presence of the parent compound (for example; nerve agents) in a given sample and is an important aspect of verification analysis of CWC [5,8].

Although many different instrumental techniques have been used successfully to detect these phosphonic acids [9–13], GC–MS is the most preferred one because of its adequate sensitivity and selectivity. In addition, only a very limited range of equipment is approved for on-site analysis by the OPCW inspectors. The phosphonic acids are amenable to GC–MS only after derivatisation and have been reviewed in the recent articles [14–18]. The most important and common derivatisation methods are silylation, methylation and pentafluorobenzylation [19–21].

The analyses of these acids are cumbersome, since either they are present in the aqueous matrices or water is required to extract them from any other matrix and the water must be subsequently removed prior to derivatisation [14]. The use of strong anion exchange (SAX) for on-site sample preparation procedure had reduced the time for the evaporation of water [22] but further improvements are needed. In the on-site sample preparation protocol of OPCW, trimethylsilyl is the only derivative currently used for alkyl phosphonic acids. Trimethylsilylation is a quite effective derivatisation but identification of alkyl alkylphosphonic acids due to spectral similarity of trimethylsilyl derivatives of O-alkyl alkylphosphonic acids when O-alkyl is higher than

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C4 and especially for O-cyclic alkyls is ambiguous. The corresponding methyl derivatives give additional information about the type of O-alkyl groups and thus it offers an alternate method of identification.

Methylations with diazomethane is widely used and although, the reaction is fast and the yield of the products is high, alternative methylation methods are preferred due to the potential hazards, difficulty in the preparation of diazomethane and especially transportation for on-site analysis. Trimethylsilyldiazomethane (TMSD) is a methylation reagent that is commercially available in standardised solution, it does not require cumbersome preparation steps and is neither mutagenic nor explosive. TMSD has been suggested as an efficient alternative to diazomethane [23–25]. Similarly thermally assisted or pyrolytic alkylation reactions are also well documented in the literature for various kinds of organic chemicals and have been reviewed in some recent reviews [26–30]. The pyrolytic alkylation reactions are generally conducted with tetra-alkylammonium salts. The analytes which are acidic in nature are mixed with the tetra-alkylammonium salts/hydroxides and injected into the GC injection port operated at 250–300 °C, where the analytes are derivatised.

Methylation of the scheduled acids gives additional information for the unambiguous identification of these acids. To include the methylation in the on-site analysis protocol, all of these methylation procedures were evaluated. Here the comparison of these methylation procedures in context with the alkyl alkylphosphonic acids, alkylphosphonic acids and benzilic acid are reported. There is a limited time for the on-site analysis so a procedure that can give information on both methyl and trimethylsilyl derivatives without additional sample preparation time is valuable. Prior to this study trimethylsilyldiazomethane is referred as synthetic procedure for methylation of various phosphonic acid [25], in this study the evaluation of TMSD as an analytical derivatisation method has been reported. In this study, thermally assisted methylation followed by the silylation in the same sample without any additional sample preparation is also reported. The two commercially available reagents namely, trimethylphenylammonium hydroxide (TMPAH) and trimethylsulfonium hydroxide (TMSH) for thermally assisted methylation (TAM) and subsequent silylation were also evaluated. Finally, comparison of all the above stated methylation processes for the scheduled acids, application of TAM and subsequent silylation on OPCW organised proficiency test is reported.

2. Experimental

2.1. Chemicals and materials

For this study, O-ethyl methylphosphonic acid (EMPA), methylphosphonic acid (MPA), ethylphosphonic acid (EPA), O-pinacolyl ethylphosphonic acid (PinEPA) and benzilic acid (BA) were used as a model compounds. Standards of these acids were purchased as neat commercial chemicals from Aldrich (Seelze, Germany) with purity higher than 95%.

The analytical grade solvents hexane, methanol (MeOH), 1-propanol (1-PrOH), 2-propanol (isopropanol; 2-PrOH) and acetonitrile were from J.T. Baker, (Deventer, The Netherlands), ACS grade ethanol (EtOH) from Riedel De Haen (Germany), dichloromethane from Fluka (Buchs, Switzerland), ACS grade benzene and tetrahydrofuran (THF) 99% from Aldrich (Steinheim, Germany), Ultra residue analysed grade toluene, ethyl acetate and chloroform from J.T. Baker (Phillipsburg, USA), concentrated 37% hydrochloric acid (HCl) from Aldrich (Seelze, Germany). The derivatising agent *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and Diazald were purchased from SUPELCO (Bellefonte, PA, USA) and 2.0 M solution of trimethylsilyldiazomethane in hexane from Aldrich (Steinheim, Germany). Thermally assisted methylating agents 0.5 M solution of trimethylphenylammonium hydroxide in methanol and 0.25 M trimethylsulfonium hydroxide were purchased from Fluka (Switzerland). The internal standard tri-*n*-butyl phosphate (TBP) was purchased from Aldrich (Seelze, Germany). Millipore water (18 MΩ cm) was used as deionized water. The standard water for spiking was prepared by spiking magnesium sulfate heptahydrate and calcium chloride dihydrate procured from Aldrich (Seelze, Germany), sodium carbonate procured from J.T. Baker (Deventer, The Netherlands), sodium sulfate procured from Fluka (Buchs, Switzerland) at concentration of 250 μg/mL.

The Accubond II SAX cartridges (silica, 200 mg, 3 mL) were obtained from Agilent Technologies (Milwaukee, WI, USA).

2.2. Standard solutions

Stock standard solutions (5 mg/mL) for each of the acids as described earlier were prepared from the neat commercial chemicals without further purification by separately weighing 20 mg of the chemical into a 4 mL vial and diluting with 4 mL of acetonitrile. Stock solutions of the TBP (5 mg/mL and 1 mg/mL) were prepared by weighing 20.0 mg and 4.0 mg, respectively, into a 4 mL vial and diluting with 4 mL of acetonitrile. A working solution was prepared from the stock solutions of MPA, EPA, PinEPA, EMPA and BA with a concentration of 100 μg/mL (100 ppm) of each of the acids.

2.3. Instrumentation

The samples were analysed by GC–MS in electron ionization mode and dual flame photometric detector (dual-FPD) in phosphorus {P} and sulfur {S} channel, using an Agilent 6890N gas chromatograph equipped with a 5975 inert XL mass selective detector (Agilent Technologies, Milwaukee, WI, USA). A Restek Rxi-5ms capillary column, 30 m × 0.25 mm × 0.25 μm, was used. The column oven temperature was programmed from 40 °C (hold for 2 min) to 280 °C at 10 °C/min, and hold at 280 °C for 6 min. Helium (99.999%) at a constant flow rate of 0.9 mL/min was used as a carrier gas. The samples were analysed in the splitless mode at an injection temperature of 250 °C. Injected volume was 1 μL using a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a 10 μL Hamilton syringe. GC interface temperature was set at 280 °C. Mass spectra were obtained with electron energy of 70 eV, and mass spectral data were acquired over a mass range of 40–450 amu. EI source temperature and the quadrupole temperature were set at 230 °C and 150 °C, respectively.

2.4. Analytical procedures

During this study each and every experiment was repeated three times and the data reported here are the averages of these data.

2.4.1. Strong anion exchange extraction procedure

The analytes were extracted from aqueous samples using strong anion exchange SAX [22]. The SAX cartridge was conditioned by passing 1 mL of methanol followed by 1 mL of Milli Q water, 2 mL of aqueous sample was loaded on the cartridge and washed with 4 mL of water and 4 mL of methanol. The acids were extracted from the cartridge by eluting with 2 mL of 0.1 N hydrochloric acid in methanol. This eluent was evaporated to dryness and analysed after derivatisation.

2.4.2. Methylation by diazomethane

Diazomethane was freshly prepared by slowly adding potassium hydroxide into *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide ethereal solution. Diazomethane was condensed with diethyl ether and stored at −20 °C.

For each experiment, 40 μL of the 100 ppm stock solution or acetone blank was added to 100 μL of diazomethane and shaken for 10 min. If the solution did not show a yellow colour, indicating excess diazomethane, another 100 μL of diazomethane was added. Gentle nitrogen flow was applied to the reaction vial until the solution was colourless typically resulting in substantial solvent loss. The volume of the solution was adjusted after adding 20 μL of TBP internal standard solution and then with hexane to bring the volume up to 200 μL. The concentration of methyl derivatives in the solution was derived from initial acid concentrations of 20 μg/mL

The same procedure was carried with 2 μL and 4 μL of the 100 μg/mL stock solution resulting in final concentrations of methyl derivatives in the solution derived from initial acid concentrations of 1 and 2 μg/mL.

2.4.3. Methylation by TMSD

(A) Evaluation of solvents: For each experiment, 40 μL of the 100 ppm stock solution or acetone blank was added to 30 μL of methanol, 30 μL of TMSD and 80 μL of the solvents being studied: methanol, ethanol, isopropanol, toluene, benzene, hexane, ethyl acetate, acetonitrile, dichloromethane, chloroform and tetrahydrofuran. The capped vial with the solution was placed into the heating block at 80 °C for 20 min. The capped vial was cooled down to room temperature; 20 μL of TBP internal standard was added prior to analysis with additional solvent being added to bring the total volume to 200 μL.

The corresponding procedure was carried out with starting volumes of 2 μL and 4 μL of the stock solution resulting in 10 and 20-fold decreases in the acid concentration.

(B) Evaluation of reaction temperatures: Replicate sets of samples as indicated in (A) were prepared for a series of temperatures. Data were taken for temperatures of 25, 60, 80, 100 and 120 °C.

(C) Evaluation of reaction times: Replicate sets of samples as indicated in (A) were prepared with a reaction time of 10, 20, 30 and 120 min.

2.4.4. Methylation by TMPAH

(A) Evaluation of solvents: For each experiment, 40 μL of the 100 ppm stock solution or acetone for blank was mixed with 10 μL of TMPAH solution. For evaluation of solvents the concentration was kept at 0.025 M by adding 130 μL of the various solvents. For this series the solvents used were methanol, ethanol, n-propanol, isopropanol, toluene, benzene, hexane, ethyl acetate, acetonitrile, dichloromethane, chloroform, and tetrahydrofuran, 1:1 mixture of hexane with tetrahydrofuran (Hex–THF) and 1:1 mixture of dichloromethane with tetrahydrofuran (DCM–THF). 20 μL of working TBP internal standard was added. The vials were mixed thoroughly prior to analysis.

(B) Evaluation of GC inlet temperature: Replicate sets of samples as indicated in (A) were prepared but only five single solvents and two mixed solvent combination were selected namely methanol, isopropanol, hexane, dichloromethane, tetrahydrofuran, Hex–THF and DCM–THF for the temperature study. The GC inlet temperatures used were 250, 280, 300 and 320 °C.

(C) Evaluation of TMPAH quantity: Replicate sets of samples as indicated in (A) were prepared with Hex–THF as solvent and 12 TMPAH concentrations were varied from 0.3×10^{-6} M to 75×10^{-3} M.

(D) Study on detection limit: Replicate sets of samples as indicated in (A) were prepared except that only 2 μL and 4 μL of the stock solution were used with tetrahydrofuran as solvent and 10 μL of TMPAH.

(E) Silylation after methylation: Replicate sets of samples as indicated in (A) were prepared using five single solvents and two mixed solvent combination: ethyl acetate, hexane, dichloromethane, tetrahydrofuran, chloroform, DCM–THF and Hex–THF mixture. Analysis was done on the resulting reaction mixture for the methyl derivatives, with GC–MS–FPD. Subsequently, 50 μL of BSTFA was added in the vial and heated at 70 °C for 30 min and then reanalysed using GC–MS–FPD.

2.4.5. Methylation by TMSH

(A) Evaluation of solvent and GC inlet temperature: For each experiment, 40 μL of the 100 ppm stock solution or acetone blank was added to 20 μL of TMSH solution, 120 μL of solvents and 20 μL of working TBP internal standard. The vials were mixed thoroughly and then the solutions were subjected to GC–MS–FPD analysis. For this set of studies, five single solvents with two mixed solvent combination were used: methanol, isopropanol, hexane, dichloromethane, tetrahydrofuran, Hex–THF and Hex–DCM. Experiments were performed at GC inlet temperatures of 250 °C, 280 °C, 300 °C and 320 °C.

(B) Study on detection limit: Replicate sets of samples as indicated in (A) except only 2 μL and 4 μL of the stock solution were used with tetrahydrofuran as solvent.

(C) Silylation derivatisation after methylation: Replicate sets of samples as indicated in Section 2.4.5 (A) were prepared using five single solvents and two mixed solvent combination: ethyl acetate, hexane, dichloromethane, tetrahydrofuran, chloroform, DCM–THF and Hex–THF mixture. Analysis was done on the resulting reaction mixture for the methyl derivatives, via GC–MS–FPD. Subsequently, 50 μL of BSTFA was added in the vial and heated at 70 °C for 30 min and then reanalysed using GC–MS–FPD.

3. Results and discussion

Two monobasic phosphonic acids (EMPA and PinEPA), two dibasic phosphonic acids (MPA and EPA) and benzilic acid all of which are scheduled chemicals under the CWC were used in this study as a model compounds.

3.1. Conventional methylation by diazomethane (DM)

Methylation by diazomethane derivatisation is widely used because the reaction is rapid, the yields are high and there are minimal side reactions. In the GC–MS–FPD analysis the acids were detected as their methyl derivative [MPA as dimethyl methylphosphonate (DMMP), EPA as dimethyl ethylphosphonate (DMEP), PinEPA as methyl pinacolyl ethylphosphonate (MPinEP), EMPA as methyl ethyl methylphosphonate (MEMP) and BA as methylbenzilate (BA-Me)] using mass spectrometric detection for all derivatives and FPD{P} for all except BA-Me. The response was linear on both the detectors for the methyl derivatives formed from DM between initial acid concentrations of 0.5 ng/μL to 10 ng/μL.

3.2. Methylation by trimethylsilyldiazomethane

Crenshaw and Cummings [25] have reported methylation of straight chain, branched chain and cyclic alkyl methylphosphonic acids for preparation of the corresponding methyl esters by TMSD. To use this method as an analytical tool it was desirable to optimize the various parameters associated with this method. Specifically, solvents, derivatisation temperature and time were evaluated.

3.2.1. Evaluation of solvents

Crenshaw and Cummings [25] used benzene as a solvent; however usage restrictions on benzene have necessitated an alternative solvent. In this study, polar, aprotic polar, aromatic and non-polar solvents for TMSD methylation were evaluated. Polar solvents such as methanol, ethanol and isopropanol provide lower yields than the less polar solvents and the difference between the yields was greatest for the most ionizable acids (such as MPA). Thus for

methylphosphonic acid there was a 7-fold increase in product yield in going from methanol to benzene, while for benzilic acid, there was roughly a 2-fold increase. While benzene provided the best yields other low polarity solvents such as hexane, ethyl acetate, THF and toluene gave satisfactory yields. For the remainder of the study, hexane was adopted as a solvent of a choice for methylation using TMSD because of its availability in the OPCW on-site sample preparation kit.

3.2.2. Evaluation of derivatisation temperature and time

Crenshaw and Cummings [25] had reported that the completion of the methylation by TMSD for the mixture of alkyl methylphosphonic acids required less than 30 min at ambient temperature. However, since for analytical procedures a shorter time would be advantageous, the effects of temperature and time on TMSD methylation were evaluated. For evaluating the derivatisation temperature, the temperature was varied from room temperature (25 °C) to 120 °C. The ratio of peak areas of extracted ion chromatogram obtained for methyl esters to those of the internal standard were plotted against the derivatisation temperature. On analysing these plots, increasing the temperature from room temperature to 80 °C gave either a small increase (20–40%) or essentially no change. Further increase in the temperature appear to reduce the yield for EPA-Me, but in fact the side reactions at higher temperature are creating co-eluting peaks so that the total ion current can no longer be extracted automatically. For further study the derivatisation temperature for methylation by TMSD was 80 °C.

For evaluating the derivatisation time, the time was varied from 10 to 120 min at a derivatisation temperature of 80 °C and plotted the ratio of peak areas of methyl esters to those of the internal standard. For EPA and PinEPA increasing the derivatisation time from 10 to 20 min produced a significant increase in the yield of methyl derivatives. For the other analytes, there was no effect. For times longer than 20 min the yields were either constant or decreased. Based on these results the optimized conditions for the methylation by TMSD methylation of these scheduled acids were set at 80 °C for maximum of 20 min.

3.3. Thermally assisted methylation by trimethylphenylammonium hydroxide

Trimethylphenylammonium hydroxide reacts as a methylating reagent in the GC injection port. In line with the usage of thermally assisted hydrolysis methylation (TAHM) for the analysis of higher triglycerides or fatty acids in which hydrolysis of esters followed by methylation occurred simultaneously in the hot injection port of GC [31], we have termed this method as Thermally Assisted Methylation. TMPAH has been used as an ion pairing reagent to create hydrophobic ion pairs from alkylphosphonic acids in aqueous samples in order to bind the acid to activated carbon SPE prior to elution with methanol. The eluents were concentrated and analysed by GC–MS as their methyl derivatives [32]. Sega et al. [33] reported the extraction of methylphosphonic acid and alkyl methylphosphonic acid from 50 mL groundwater using a solid-phase extraction column packed with 500 mg of silica with a bonded quaternary amine phase, and are eluted and derivatised with methanolic trimethylphenylammonium hydroxide and analysed by GC–FPD {P}. Sutherland [34] used 1:1 mixture of benzene and methanolic TMPAH for the analysis of samples from artworks followed by the analysis by GC–MS. TMPAH at 0.03 mM (300 μL of 0.1 M TMPAH in MeOH) was used with 1000 μg/mL of alkyl phosphonic acid by Tornes and Johnson [29]. Amijee et al. [35] had reported methylation with the higher concentrations of a wide variety of analytes at 70–100 mg/kg with 10 mM of TMPAH. TMPAH in 20-time stoichiometric excess over analytes was preferred and

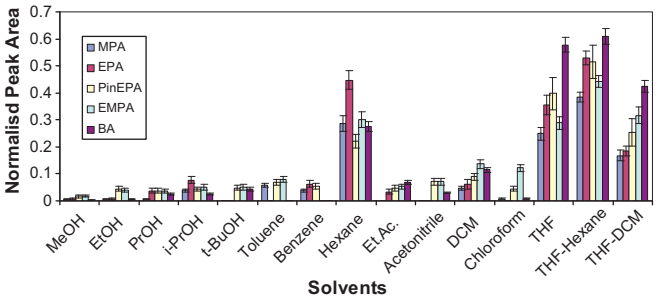

Fig. 1. The effect of solvents on the normalized response of methyl esters of scheduled acids with TMPAH.

recommended by Rompa et al. [36] to achieve highest yield of methylation. The literature generally indicated that the solvent, injection port temperature and the amount of derivatising agent are critical parameters for the optimization of the derivatisation; hence all these parameters were evaluated.

3.3.1. Evaluation of solvents

A wide variety of solvents of differing polarity and chemical composition were selected to study solvent effects on the TAM by TMPAH. The results (Fig. 1) show that polar alcohols (such as EtOH and MeOH) provided the poorest reaction efficiency for TMPAH methylations while the less polar alcohols (such as n-propanol, isopropanol) gave higher levels of the methyl esters. In t-BuOH dibasic acids were not detected. Experiments with hexane and THF as solvents had the highest levels of methyl esters. Mixtures of THF with hexane or DCM at 1:1 ratios provided better results, with the Hex–THF mixtures giving the best results and the responses for all the acids studied were similar.

3.3.2. Evaluation of injection port temperature

The influence of injection temperature on the methylation by TMPAH was studied for most of the solvents and mixtures (Hex–THF and DCM–THF) at 250 °C, 280 °C, 300 °C and 320 °C. When MeOH was employed as solvent at the higher injection temperatures methylation was achieved for every compound but with lower abundance of products in comparison to other solvents. It is clear that with TMPAH, less polar solvent such as Hexane and THF produce methyl derivatives in higher yield than the polar solvents such as MeOH or iPrOH. This result is in agreement with the observations of Tornes and Johnsen [29]. The increase in the injection port temperature does not have an effect on the methylation yield in the THF–Hex solvent and hence for TAM with TMPAH can be achieved with the OPCW normal chromatographic condition. Thus, the combination of THF–Hex is the best solvent system for the TMPAH methylation for these scheduled acids at the injection port temperature of 250 °C.

3.3.3. Effect of TMPAH concentration

The concentration of TMPAH relative to the analyte has varied considerably [32,33,35]. The concentration ratio of TMPAH to analyte has been recommended to be at least 20 times [36].

Here the TMPAH concentration was varied from 0.3×10^{-6} M (0.0003 mM) to 75 mM using the stock solution of 5 acids each at 10 μg/mL. At the lowest concentration, 0.0003 mM of TMPAH, the ratio of TMPAH to acids is about 5 on a molar ratio. At these low levels no methylation was detected. BA-Me is first to appear at 0.3 mM (1000-fold molar excess) and once the concentration of TMPAH was 2.5 mM (or a 40,000-fold molar excess) than the methyl derivatives of all the acids were seen with no significant increase for higher concentration ratios (Fig. 2). Thus, the minimum amount of TMPAH for methylation must be more than 1000 M excess over the acids.

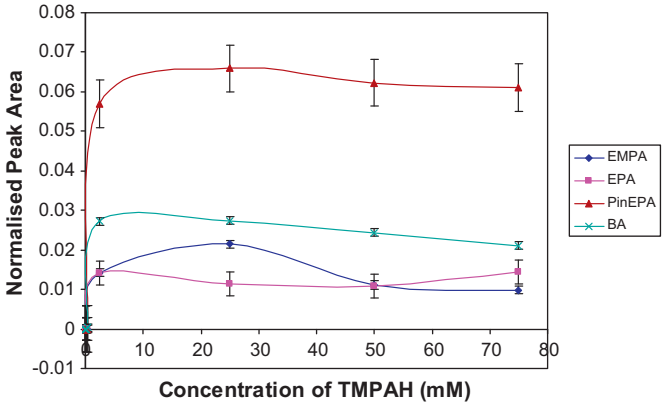

Fig. 2. The effect of amount of TMPAH on methylation of scheduled acids with respect to the internal standard in Hex:THF as solvent shown in the form of normalized peak area.

3.3.4. Subsequent silylation after TAM by TMPAH

As noted earlier, the use of trimethylsilyl derivatives for on-site analysis is the OPCW standard method. With TMPAH, methylation of these acids occurs in the GC injection port, whereas in solution these acids do not react with the TMPAH. As a result, it is possible to analyse a sample (containing TMPAH and scheduled acids) by silylation using BSTFA at 70 °C for 30 min. Such a procedure allows for observation of both the TMS and the methyl derivative of the acids from the same sample, thus giving more confidence to the identification. During the optimization of subsequent silylation, THF was found to be the best solvent. However, the silylation in the Hex–THF mixture was also comparable. In general, the presence of TMPAH solution did not shown any significant effect on the silylation process either in terms of response or linearity. The GC–MS response of the silyl derivatives is higher than that of the methyl derivatives for the same acid concentration.

3.3.5. Limit of detection, linearity of response and reproducibility of methylation by TMPAH

The limit of detection (LOD) and linearity of response were investigated by injecting 1 μg/mL, 2 μg/mL and 20 μg/mL standard solutions – corresponding to 0.5, 1 and 10 ng of the analytes using both GC–MS and GC–FPD. In addition, the same solutions were processed by methylation with diazomethane. The TMPAH response appeared to fall off relative to that of DM. The LOD with TMPAH was below 0.5 ng since all of the acids were detected by GC–MS with the S/N of >3 in full scan mode by AMDIS.

The reproducibility of methylation by TMPAH was studied by injecting 1 μL of 10 μg/mL concentrations of scheduled acids in Hex–THF with 25 mM of TMPAH and three injections per day for 3 days. The inter day relative standard deviation was from 4.7% to 10.8%, the maximum relative standard deviation was observed for PinEPA when peak area for both the stero-isomeric peaks were summed.

3.4. Thermally assisted methylation by trimethylsulfonium hydroxide

TMSH was also used as a reagent for TAM for the analysis of macromolecules or high boiling compounds. Most of the literature related to the TMSH is associated with the determination of lipids, fatty acids or triglycerides including waxes and their related compounds. TMPAH and/or TMSH were used for methylation of organic materials in artworks [34,37], determination carbamate pesticides [38], for O-methyl derivatives from lipids containing hydroxyl groups [39] and for methylation of lipids [40].

The same ranges of parameters studied for TMPAH were studied for TMSH. As with TMSH also, polar solvents such as MeOH provided poor methylation environment except for BA which gave moderate reaction yield at the inlet temperature of 250 °C. The inlet temperature had a slightly greater effect on methylation by TMSH than for TMPAH. Inlet temperatures had dominant effect when DCM was the solvent, higher injection temperature favored the reaction progress for all the target acids leading to 320 °C as the optimum temperature in this case. Hexane, DCM, THF and the other two mixed solvents THF–Hex and THF–DCM gave satisfactory methylated product yields. Thus, an inlet temperature of 320 °C is recommended for the TMSH methylation.

In comparison to TMPAH, with TMSH the response is linear in the same concentration range. This might be attributed to the greater reactivity of TMSH. However, in contrast to TMPAH, silylation of the targeted acids was not achieved in the presence of TMSH. This can be attributed to the higher basicity of trimethylsulphonium ion in comparison to the trimethylphenylammonium ion. Since, TMSH interferes in the process of subsequent silylation, hence TMSH was not used further in this study and data were also not presented.

3.5. Comparison of methylation methods

All the four types of methylation methods studied here i.e., diazomethane, trimethylsilyldiazomethane, trimethylphenylammonium hydroxide and trimethylsulfonium hydroxide were compared. The evaluation was done based on the analysis of some acids that are relevant to the chemical weapons convention using GC–MS. In case of three methylation methods (DM, TMPAH and TMSH), with GC–FPD in phosphorus mode all the phosphonic acids were identified. In case of TMSD, a significant shift in the retention time for methyl derivative of MPA, EMPA and EPA were observed; hence it cannot be identified based on retention time. With MS detector, AMDIS was able to identify all the spiked compounds using three methods of methylation (DM, TMPAH and TMSH), for the TMSD method the early eluting chemicals were not identified due to the very high background from the TMSD method as shown in Fig. 3. In addition to the high background levels from this reagent, the signal for the methylated chemicals is reduced by also producing silyl derivatives with this reagent.

Fig. 3 also shows the methylation using DM produces significantly lower background signals and higher yield of methyl derivatives. Methylation yield by using thermally assisted methylation with both TMPHA and TMSH are significantly nosier than DM, but still provide reasonable signal to noise characteristics and can be fully analysed with AMDIS at levels down to 0.5 ppm (0.5 μg/mL). Given the added safety of these reagents and ease of its use they are valuable tools for use in field analysis.

3.6. Application of TAM by TMPAH and subsequent silylation

As noted in the introduction, the use of TMS derivatives is the principal method used for on-site analysis of the acids discussed here. Adding the analysis of methyl derivatives increases the confidence of the identification. Since the TAM methods only react with the acids in injection port, there is no interference with the analysis by conventional TMS derivatisation. Thus the same sample can be prepared with TAM reagents, injected and the remaining sample can be analysed with silylation by BSTFA. This has been successfully employed for the analysis of the aqueous sample and water extract of the soil samples from the 21st and 20th OPCW official proficiency tests [41], respectively. The aqueous sample contained the inorganic salts magnesium sulfate, sodium hydrogen carbonate and calcium chloride and poly(ethyleneglycol) monomethyl ester as interference along with the spiked chemicals. Poly(ethylene glycol)s are commonly added as interferences

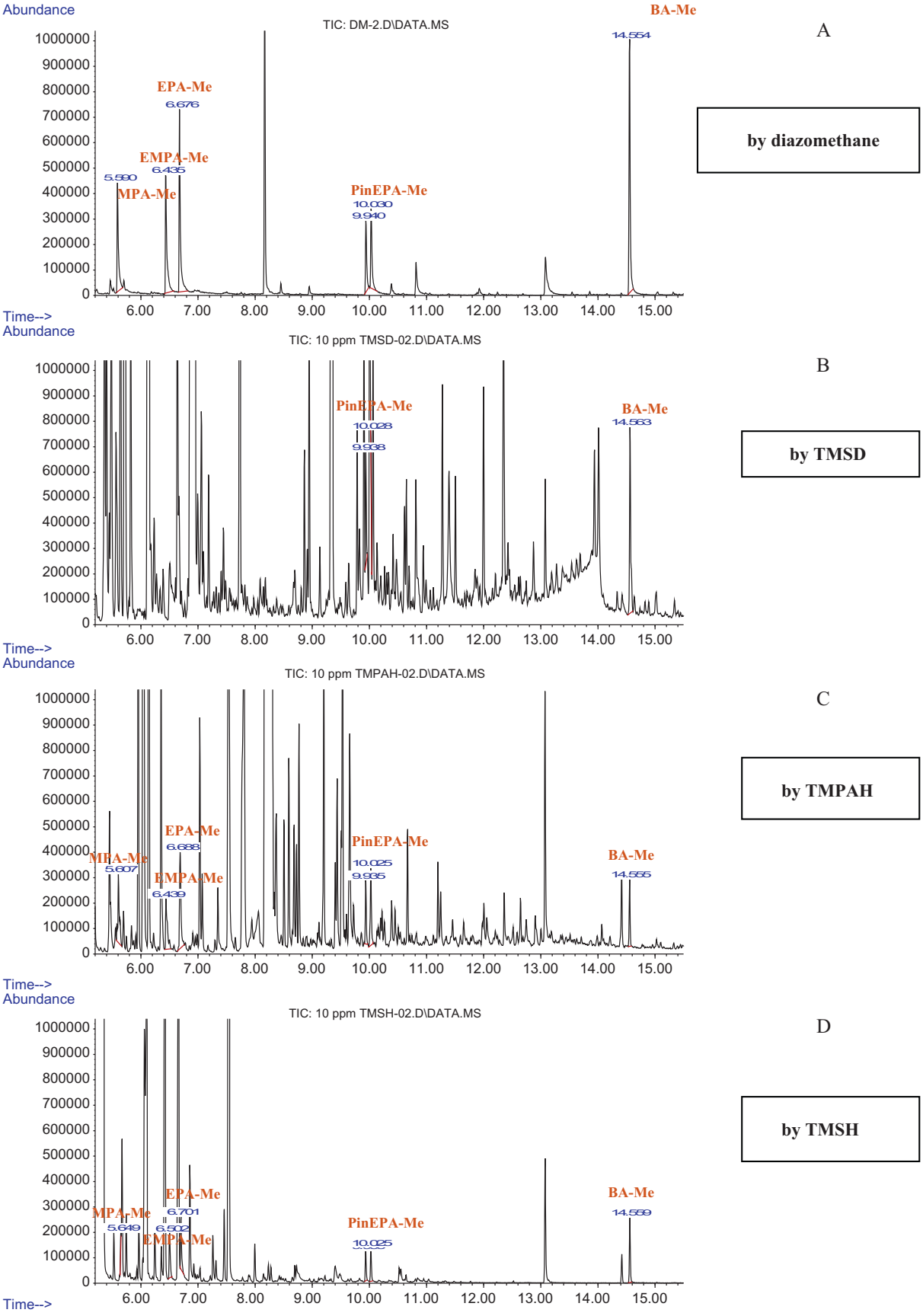

Fig. 3. Total ion chromatogram (TIC) showing the comparison of all the methylation methods for the methylation of model scheduled acids. Total ion chromatogram (TIC) for the methylation of model scheduled acids by diazomethane. TIC for the methylation of model scheduled acids by trimethylsilyldiazomethane. TIC for the methylation of model scheduled acids by trimethylphenylammonium hydroxide. TIC for the methylation of model scheduled acids by trimethylsulfonium hydroxide.

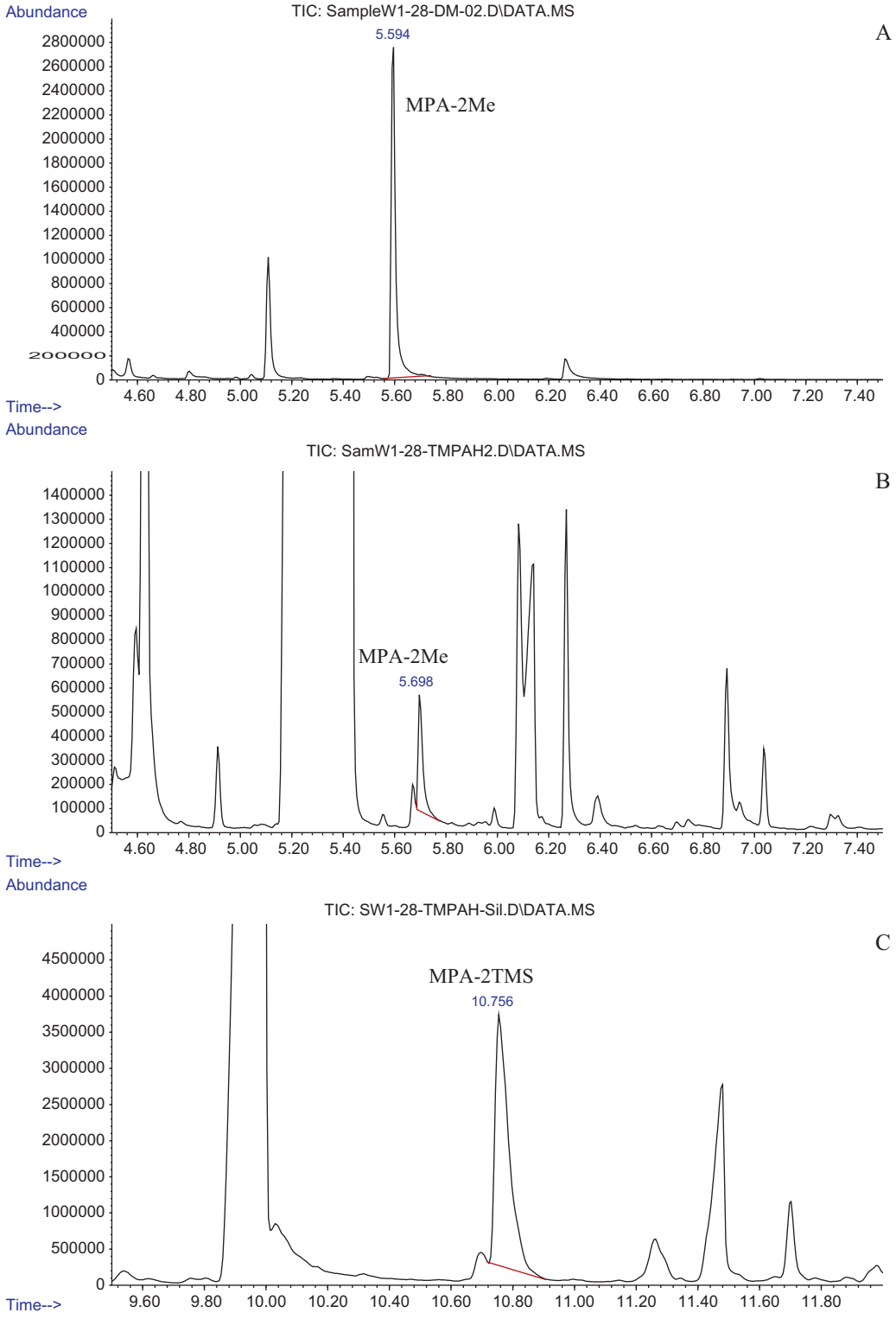

Fig. 4. TIC of methyl and silyl derivative of methylphosphonic acid in water (W1) sample from the 21st proficiency test by (A) diazomethane, (B) TMPAH and (C) subsequent silylation after TAM analysis.

as their presence always masks the presence of spiked chemicals and the inorganic salts mask the acidic compounds by transforming them to their corresponding salts. The soil sample is always considered as a complex matrix due to the presence of various cations, anions and with the high adsorption capability. All the spiked chemicals were detected by this method from both the samples.

The soil sample was extracted once with the 2 mL of deionized water and it is further referred here as aqueous sample W while the aqueous sample from the 21st PT is referred as W1. 2 mL each of aqueous samples (W1 and W) were extracted by SAX method as described in Section 2.4.1 prior to methylation. Each sample was made in duplicate for comparison with DM methylation. One fraction was analysed by diazomethane and another fraction was

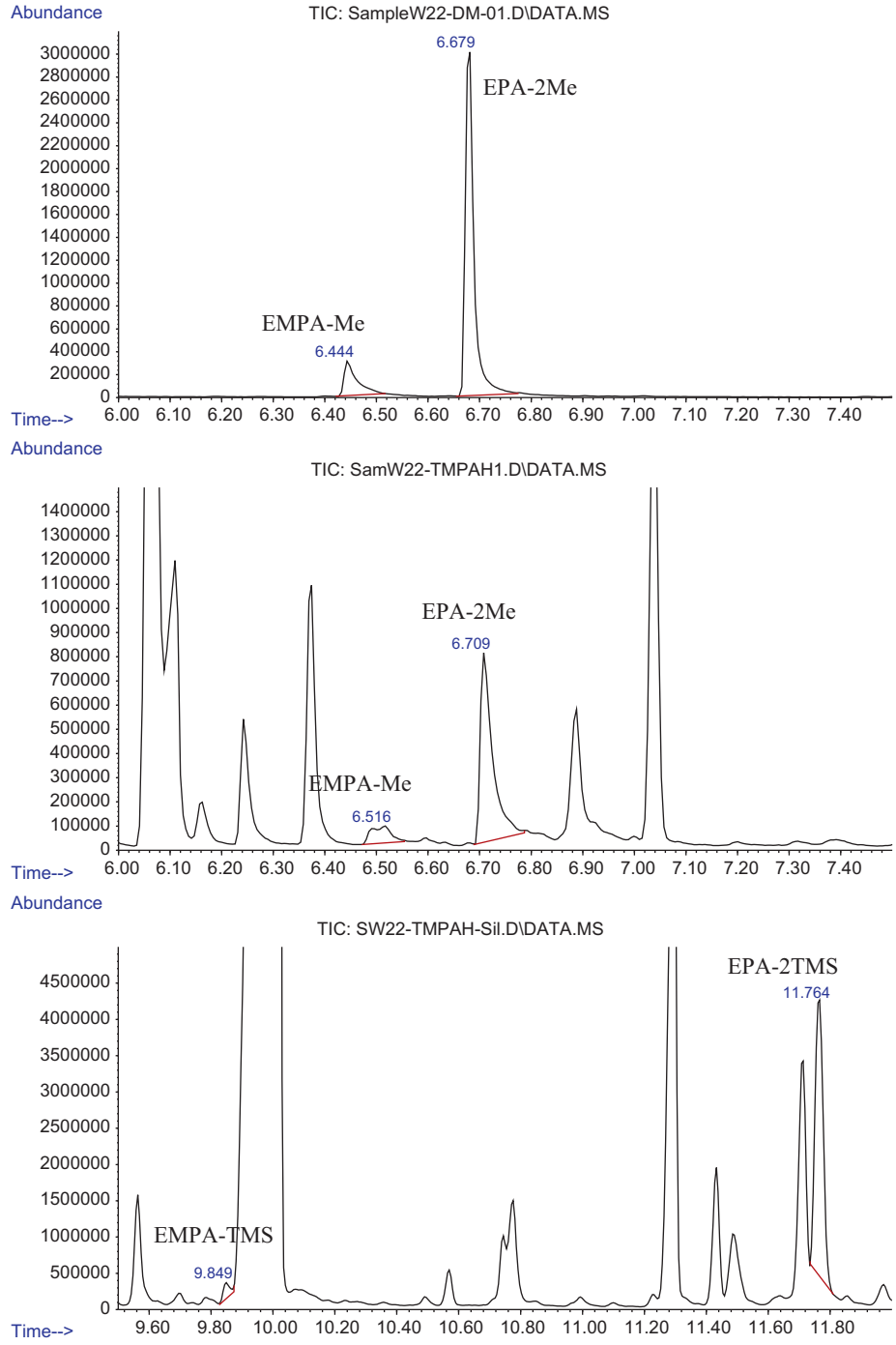

Fig. 5. TIC of methyl and silyl derivative of ethyl methylphosphonic acid and ethylphosphonic acid in the water extract (W) of soil sample from the 20th proficiency test by (A) diazomethane, (B) TMPAH and (C) subsequent silylation after TAM analysis.

analysed by this method (TAM by TMPAH) and subsequent silylation by BSTFA.

The TICs obtained from the GC–MS analysis of the W1 and W after methylation by DM and TMPAH showed the methyl esters of all the spiked phosphonic acids namely, methylphosphonic acid, ethyl methylphosphonic acid and ethylphosphonic acid respectively. These chemicals were also identified as their trimethylsilyl derivative on subsequent silylation after TAM by TMPAH. The TICs of these compounds after methylation by DM and TMPAH, and subsequent silylation showing the presence of all spiked chemicals as methyl and trimethylsilyl esters in the W1 sample are presented in the Fig. 4 while TIC's for W sample is presented in Fig. 5.

4. Conclusion

The parameters for the use of trimethylsilyldiazomethane as an analytical methylation procedure were studied and an optimized set was developed. In addition, the use of trimethylphenylammonium hydroxide via thermally assisted methylation with subsequent silylation by BSTFA has been shown to provide good sensitivity for acids scheduled under the CWC. Significant solvent effects were observed in the TMPAH analysis as well as the need for large (1000×) concentration excesses of the reagent over the target compounds and a requirement for high injector temperature (250 °C). Both of these reagents provide a safer

method to use methylation for the analysis of acids in portable laboratories.

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