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Identification of acidic degradation products of chemical warfare agents by methylation with trimethylsilyldiazomethane and gas chromatography–mass spectrometry

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Abstract

Sensitive and reliable analysis of alkylphosphonic acids (APAs) and 2-(*N,N*-dialkylamino)ethanesulfonic acids (SAs), the degradation products of chemical warfare agents (CWAs), is one of the most important tasks for verification of the Chemical Weapons Convention (CWC). Unambiguous identification of these chemicals is required in a variety of environmental matrices, including soil and water. These acids with low volatility are very polar, and efficient and reliable methylation methods for their derivatization are needed for analysis with gas chromatography–mass spectrometry (GC–MS). In this study, the derivatization conditions for trimethylsilyldiazomethane (TMSDAM) methylation were optimized for rapid GC–MS screening. Optimized methylation of APAs and SAs with TMSDAM was compared with methylation with diazomethane. The TMSDAM methylation of SAs and benzilic acid was further compared with silylation with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide. The significance and necessity of cation exchange prior to derivatization and analysis were tested on samples with a high inorganic background. A recommendation to use the method for methylation of water samples and aqueous extracts using TMSDAM is given. The robustness of the method was illustrated by the successful identification of APAs and SAs in aqueous samples from proficiency tests organized by the Organisation for the Prohibition of Chemical Weapons.

Keywords: Methylation, Trimethylsilyldiazomethane, Alkylphosphonic acids, 2-(*N,N*-dialkylamino)ethanesulfonic acids, Chemical warfare agents, Chemical Weapons Convention, Water samples, Water extracts of soil samples

Introduction

The Chemical Weapons Convention (CWC) (CWC 2021) entered into force in 1997, but the threat of the use of chemical warfare agents (CWAs) has regularly proved to be a source of news especially in the last decade. The Organisation for the Prohibition of Chemical Weapons (OPCW) (OPCW 2021) implements the CWC internationally. Designated laboratories nominated by the Director General of the OPCW uphold the analytical capability to verify scheduled chemicals such as CWAs, their precursors and degradation products in various matrices. Identification of a CWC-related chemical in samples requires confirmation with at least two analytical, preferably spectrometric, methods. Therefore, a number of instrumental methods are used for the analysis. Nonpolar, volatile chemicals are detected with chromatography–mass spectrometry (GC–MS). The degradation products of CWAs are typically polar and nonvolatile in character and readily soluble in an aqueous environment. In general, hydrolysis and degradation products of CWAs are more stable and persistent in the

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environment than their corresponding intact CWA. For this reason, the detection and identification of degradation and hydrolysis products of CWC-related chemicals play an important role in the verification of the CWC, as these chemicals provide an indirect indication of the previous presence of an intact CWA. Direct analysis of polar CWC-related chemicals by liquid chromatography–mass spectrometry (LC–MS) or nuclear magnetic resonance (NMR) spectroscopy is straightforward. However, the analysis is usually confirmed with GC–MS and other GC-based techniques as it provides additional required analytical evidence.

Organophosphorus nerve agents are the most toxic chemicals related to the CWC. The nerve agents are easily hydrolyzed in the environment to their corresponding alkylphosphonic (APAs) and alkylthiophosphonic acids (ATPAs). In addition, VX (O-ethyl S-2-(*N,N*-diisopropylaminoethyl) methylphosphonothiolate) degrades under oxidative conditions to the corresponding sulfonic acid, 2-(*N,N*-diisopropylamino)ethanesulfonic acid (DIPAESA). For GC–MS analysis of these acids, derivatization is needed.

Sample preparation procedures and analytical techniques used widely by the designated laboratories of the OPCW have been published since 1993 as monographic recommended operation procedures (ROPs), the latest of which was in 2017 (Vanninen 2017). These ROPs have been compiled by an international collaboration of expert laboratories working in the field. Analysis focuses on qualitative analysis for unambiguous identification at a reasonably low level (e.g., < 1 mg/kg; mg/l) in environmental samples (Vanninen and Söderström 2017).

Derivatization of the CWC-related chemicals has regularly been reviewed (Black and Muir 2003; Witkiewicz et al. 2016, 2018; Valdez et al. 2018a), and in 2017, the ROP for derivatization was updated (Black et al. 2017). APAs and ATPAs are mostly silylated or alkylated. Environmental samples are usually alkylated using methylation or pentafluorobenzylation, although the latter is more often used for biomedical samples (Fredriksson et al. 1995; Miki et al. 1999; Palit et al. 2004; Riches et al. 2005; Subramaniam et al. 2013). Pentafluorobenzylation has not been efficient enough for derivatization of the most polar free APAs such as methylphosphonic acid (MPA) and ethylphosphonic acid (Fredriksson et al. 1995; Palit et al. 2004; Riches et al. 2005).

2-(*N,N*-dialkylamino)ethanesulfonic acids (SAs) are also methylated and silylated, but *tert*-butyldimethylsilyl derivatives have to be used because trimethylsilyl derivatives are not stable (Kuitunen 2010). In addition, the derivatization of benzilic acid (BA) was also tested. BA is the acidic degradation product of BZ (3-quinuclidinyl benzilate), a hallucinogenic CWA. BA is usually silylated or methylated like APAs (Sng and Ng 1999; Black and Muir 2003; Amphaisri et al. 2011; Park et al. 2013; Black et al. 2017; Valdez et al. 2018a).

Many OPCW designated laboratories routinely use methylation with diazomethane (DAM) for derivatization of the APAs and ATPAs. The first reports, in which APAs were isolated from plant, soil or water samples and diazomethylated for GC analysis, were published in the 1970s (Hambrook et al. 1971; Howells et al. 1973; Verweij and Boter 1976; Verweij et al. 1979). Since then, only a few publications on the diazomethylation of CWC-related chemicals have appeared in the open literature (Kuitunen et al. 1991; Driskell et al. 2002; Barr et al. 2004; Qi et al. 2012). A reason for this could be that methylation using DAM is regularly considered too complex, dangerous and time-consuming. DAM itself is both highly toxic, spontaneously explosive and is probably carcinogenic (Black et al. 2017; Schoental 1960; Diazomethane EPA Document 2016). Additional file 1: Section 1 provides essential information on diazomethylation that is relevant to know when using the method.

The development of methylation and alkylation reagents that are more straightforward to produce, store and/or handle than DAM has been an active area of research. Online methylation in a hot injection port using tetraalkylammonium, trialkylarylammonium or trialkylsulfonium hydroxides as alkylation reagents is a commonly used method for the derivatization of acidic substances for GC analysis (Wells 1999; Rompa et al. 2004). Trimethyl phenylammonium hydroxide (TMPAH), which is commercially available as MethElut™ reagent, was used by Tørnes and Johnsen for derivatization of APAs (Tørnes and Johnsen 1989). Modifications of this method have been published (Sega et al. 1997; Vijaya Saradhi et al. 2007; Amphaisri et al. 2011). It should be pointed out that online alkylation reagents such as TMPAH are effective and efficient, but they are all also highly caustic and therefore likely to cause rapid GC column deterioration (Black and Muir 2003). This might be one of the reasons why there is no widespread use of these reagents in CWC analysis.

A third methylation reagent, trimethyloxonium tetrafluoroborate, was used for the methylation of alkyl alkylphosphonic acids, APAs and SAs at room temperature (Valdez et al. 2016, 2018b). Aqueous samples or extracts were not derivatized, however. In addition to methylation, other alkylation reactions have been used to produce larger O-alkyl esters of APAs (see Additional file 1: Section 2). There is no widespread use of these methods.

Trimethylsilyldiazomethane (TMSDAM) has regularly been suggested as a substitutive methylation reagent for DAM. TMSDAM has been used for derivatization in

numerous applications using both GC–MS and LC–MS for analysis. TMSDAM was suggested already in 1998 for methylation of CWC-related alkyl APAs, DIPAESA, 2-(*N,N*-diethylamino)ethanesulfonic acid (DEAESA) and BA (Crenshaw and Cummings 1998, 2004). In 2011, derivatization solvent, temperature and time were optimized and methylation with TMSDAM was compared with methylation with DAM, TMPAH and trimethylsulfonium hydroxide for on-site analysis of CWA. However, the authors did not find TMSDAM superior to the other reagents and prioritized thermally assisted methylation with TMPAH and subsequent silylation for derivatization in the field laboratory (Amphaisri et al. 2011).

The derivatization properties of TMSDAM should be approximately equivalent to DAM, but the chemical background in chromatograms from samples derivatized with TMSDAM is more pronounced than with DAM. The addition of 10–20% of a short-chain alcohol (e.g., methanol) to the reaction solution is required for efficient methyl ester formation (see Additional file 1: Section 3) (Hashimoto et al. 1981). In addition, the commercially available reagent is more stable, but unlike DAM, it is not reported to be explosive or carcinogenic. However, as a very reactive chemical, it should be handled with great care (NCBI 2021).

There have been quite a few published efforts to investigate methylation of acidic CWC-related chemicals with TMSDAM in environmental matrices. In the recent ROP (Black et al. 2017), derivatization of CWC-related chemicals by TMSDAM is based on those few publications of Crenshaw and Cummings and Amphaisri et al. mentioned previously (Crenshaw and Cummings 1998, 2004; Amphaisri et al. 2011). The evaporation residue is dissolved in acetonitrile, which is not the optimal dissolution solvent for methylation of acidic chemicals in evaporation residues of environmental samples. In addition, the 80 °C temperature is quite high in relation to the boiling points of the solvents used. Apparently, these drawbacks decrease the efficiency of the methylation reaction. However, it is important to be able to derivatize these chemicals sufficiently reliably, quickly and efficiently. Herein, TMSDAM methylation was optimized for rapid GC–MS screening and identification of APAs, SAs and BA in various environmental aqueous matrices. Methylation of APAs and SAs with TMSDAM and DAM were compared to find out possible differences in performance. Further, methylation of SAs and BA was compared with silylation with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) to find the best method for derivatization. The necessity of cation exchange prior to derivatization and analysis was tested on samples with a high inorganic background. Based on the optimization, a recommended derivatization procedure is proposed for the derivatization of APAs and SAs in aqueous samples and extracts to identify them in OPCW proficiency tests (PTs) and real samples. Finally, the operation of the method was tested in official OPCW PTs.

Materials and methods

Chemicals and reagents

Residue analysis grade methanol was purchased from BDH (Poole, UK) and Fluka (Buchs, Switzerland). Dichloromethane (CH_2Cl_2) used in derivatization was residue analysis grade:SupraSolv® from Fluka and from Merck (Darmstadt, Germany). Residue analysis grade acetonitrile for silylation was from BDH. Deionized water was produced using a Millipore Direct-Q® 3UV water purification system (Millipore, Billerica, MA, USA). Acidic methanol (AcMeOH) solutions were prepared from methanol and gaseous hydrogen chloride (HCl) (99.5%, AGA, Espoo, Finland) in the laboratory.

The derivatization reagents TMSDAM (as 2 M solution in *n*-hexane) and MTBSTFA (silylation grade in 1 ml ampoules) were from Aldrich (Milwaukee, WI, USA). To produce gaseous DAM as described (Hazai and Alexander 1982; Wrolstad et al. 2004) methylation reagent, Diazald® (99%, Fluka) was dissolved in HPLC-grade methyl *tert*-butyl ether (MTBE, Rathburn, Walkerburn, Scotland). A saturated potassium hydroxide solution in methanol was prepared from pellets (85%) supplied by J.T. Baker (Deventer, the Netherlands). The apparatus is described in Additional file 1: Section 1 Figure S1.

All analytes were of 95% or higher purity determined by NMR. MPA (Fluka), ethyl methylphosphonic acid (EMPA, Aldrich), pinacolyl methylphosphonic acid (PMPA, Aldrich) and BA (Merck) were from commercial sources, whereas propyl propylphosphonic acid (PPPA), isopropyl methylphosphonic acid (IPMPA), methyl ethylphosphonic acid (MEPA), 2-(*N,N*-dimethylamino) ethanesulfonic acid (DMAESA), DEAESA and DIPAES were synthezed in-house (Spiez Laboratory). The internal standard hexachlorobenzene (HCB, Aldrich) was dissolved in CH_2Cl_2 (residue analysis grade, Fluka).

GC–MS analysis

The GC–MS analyses were performed with an Agilent 6890N GC (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 5973 mass selective detector (MSD) or an Agilent 7890GC coupled to an Agilent 5975C inert XL MSD. Injector temperature was 250 °C and injection volume 1 µl in splitless mode with 1 min splitless time. Helium was used as the carrier gas (AGA) at a constant flow rate of 1.0 ml/min. A DBWax^{etr} column (polyethylene glycol phase, 30 m, 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA) was

used for analysis of methylated samples with the following temperature program: 40 °C (1 min)–10 °C/min–260 °C/10 min. Silylated samples were analyzed with a DB-5 ms UI column (phenyl arylene polymer phase, 30 m, 0.25 mm i.d., 0.25 µm film thickness, Agilent Technologies, Folsom, CA, USA) with temperature program: 40 °C (1 min)–10 °C/min–280 °C/15 min). The MS source was operated in electron impact ionization (EI) mode (70 eV), EI source temperature was 230 °C and the scan range at m/z 40–500.

Matrices

Laboratory tap, spring, artificial seawater and a water sample from an OPCW PT containing an inorganic ion background were used as aqueous matrices. Spring water was collected from Ruotsinkylä spring (Tuusula, Finland). The composition of the spring water was determined by the Geological Survey of Finland and is presented in Additional file 1: Section 4 Table S1. Artificial seawater was prepared by diluting 3.8 g of Instant Ocean® Sea Salt (Instant Ocean, Blacksburg, VA, USA) in 100 ml of deionized water. The water sample matrix containing inorganic background ions ('PT water') was prepared by diluting 200 mg of both calcium chloride (94%, J.T. Baker) and sodium sulfate (SupraPur, Merck) in 100 ml of deionized water to simulate a PT water sample.

Fine sand, clay, humus and sand soil were used as soil matrices. Before use, the soil samples were dried in air and sieved through a 2 mm screen to remove debris. Fine sand, humus and clay soil were collected from the experimental farm of the Agricultural Research Centre of Finland in Jokioinen and sand was obtained from Soil Analysis Service Ltd. (Helsinki, Finland). The suppliers provided the details presented in Additional file 1: Section 4 Table S2.

Solutions, extraction and cation exchange

Two sets of separate stock solutions of spiking chemicals were used: the first set containing 200 µg/ml of APAs and 200 µg/ml of DEAESA each separately in deionized water and the other set containing 1 mg/ml of SAs and 1 mg/ml BA each separately in deionized water. Stock solution of HCB (1 mg/ml) was prepared in CH_2Cl_2.

For preparation of the linear calibration plots for each set of experiments, 6.5, 7.5, 8.5 and 10 µg/ml of the spiking chemicals were added into matrix blank samples. However, when SAs and BA were silylated and methylated, calibration standards contained 5, 10, 15 or 20 µg/ml of each analyte and the solutions were prepared in deionized water for water samples and in tap water for aqueous extracts of soil samples. The standard addition method for calculating the responses of the spiking chemicals was used.

Usually, 10 µg/ml of each analyte was spiked into an aqueous sample or soil extract and HCB was added in 10 µl to each sample before analysis. However, when methylation of the SAs and BA with TMSDAM was compared with silylation, a spiking level of 20 µg/ml was used to ensure reproducible results. The sample volume was 0.5 ml.

The aqueous extract of each soil was prepared by shaking a portion of 5 g of soil in a glass vial with 5 ml of deionized water for 10 min. The sample was centrifuged at 2000 G (Hettich Universal 16 centrifuge, Hettich, Tuttlingen, Germany) for 5 min and the extract was filtered through Whatman 4 filter paper (Ø 90 cm, Whatman, Maidstone, UK). The extraction was repeated with another 5 ml of water, and the centrifuged and filtered extracts were combined.

10 ml of the water sample or the prepared aqueous extract of soil was cation-exchanged with a 500 mg SCX cartridge (LRC, 3 ml, 40 µm, Varian, Harbor City, CA, USA), which was conditioned with 3 ml of methanol and 6 ml of deionized water.

Evaporation and derivatization

All experiments were done in triplicate. 0.5 ml of the water samples and aqueous extracts was placed in a 1.5 ml screw cap vial and evaporated to dryness with a TurboVap evaporator (TurboVap® LV Concentration Workstation, Caliper Life Sciences, Hopkinton, MA, USA) at 45 °C and 7 psi (for approximately 55 min).

For methylation with TMSDAM, the residue was dissolved in 0.1 ml of 0.2 M dry AcMeOH and vortexed (IKA MS1 minishaker, IKA, Wilmington, NC, USA). 0.85 ml of CH_2Cl_2 was added followed by 0.1 ml of TMSDAM drop by drop without mixing. The vial was capped immediately and heated at 60 °C for 30 min shaking the vial occasionally after every 5 min. To increase the recoveries, the molarity of AcMeOH was increased to 1 M. When the yellow color of the TMSDAM in the solution disappeared, another portion of 0.5 ml of the sample was processed and the derivatization procedure repeated using 0.7 ml of CH_2Cl_2 and 0.2 ml of TMSDAM.

For methylation with DAM, the residue was dissolved in 0.1 ml of 0.2 M dry AcMeOH and vortexed. Approximately 2 g of Diazald® was dissolved in a screw-capped storage bottle (100 ml) in ca. 30 ml of MTBE. The bottle was capped with a three-hole, three-valve screw cap, and 2 ml of saturated KOH/methanol solution (4 g/100 ml) was added to start the formation of DAM. Nitrogen was used as the DAM carrier for the sample methylation. The reaction was continued until the sample color changed to yellow, which marked the end of the reaction and indicated an excess of DAM (see Additional file 1: Section 1). Especially with soil extracts, the color change is often

difficult to detect, and therefore, it was always checked that the pH of the sample was not acidic after methylation to be sure that all HCl had reacted with DAM and that methylation had continued for long enough.

For silylation with MTBSTFA, the evaporation residue was dissolved in 0.25 ml of MTBSTFA and vortexed. 0.25 ml of acetonitrile was added, and the vial was capped and heated at 60 °C for 30 min.

Results and discussion

Dissolution solvent and derivatization conditions

The structures of the tested chemicals are presented in Fig. 1. First pretests of the methylation with TMSDAM were made as presented by Crenshaw and Cummings (Crenshaw and Cummings 2004) using a methanol/toluene mixture as derivatization solvent without real success (see Additional file 1: Section 5). Derivatization of the acids in the evaporation residue was tested because, based on our experience, the composition of the residue and the dissolution solvent used have a major effect on the success of the reaction. In the recent ROP (Black et al. 2017), the evaporation residue is dissolved in 0.04 ml of acetonitrile, 0.03 ml of methanol and 0.08 ml of *n*-hexane, which are added with 0.03 ml of the TMSDAM solution, and the mixture is heated at 80 °C for 20 min. The method was modified. Methylation with TMSDAM was tested for selected APAs, SAs and BA in the water samples. A sample volume of 0.5 ml was used to speed up the whole procedure because evaporation of an aqueous sample to dryness is the most time-consuming step. AcMeOH was used instead of acetonitrile as the dissolution solvent of the evaporation residue, based on our knowledge, is the best dissolution solvent for acidic CWC-related chemicals. 0.5 ml of water was evaporated to dryness and 0.01 ml of 0.2 M AcMeOH was added. Instead of *n*-hexane, 0.8 ml of CH_2Cl_2 was used for dilution, because it is a more polar solvent and is well suited to GC–MS analysis of these polar analytes. 0.1 ml of TMSDAM was used, and the sample was heated at 60 °C for 30 min.

Figure 2 shows a comparison of the methylation with TMSDAM and the methylation using gaseous DAM. In addition to MPA, also EMPA, PMPA and IPMPA were spiked into tap water and aqueous extracts of three different soil samples. The composition of the soil has a major influence on the extraction efficiency of the analyte as well as on how well the analyte is derivatized from the evaporation residue. The analytes were spiked into aqueous extracts of soil in order to focus on testing the derivatization efficiency. For the same reason, the soil extract was cation-exchanged before spiking. Acidic degradation products, especially divalent APAs, formed salts with cations (e.g., Na^+, K^+, Mg^{2+}, Ca^{2+}) present in environmental aqueous matrices. Therefore, cation exchange of aqueous samples and extracts containing an inorganic background before chromatographic analysis is preferred to

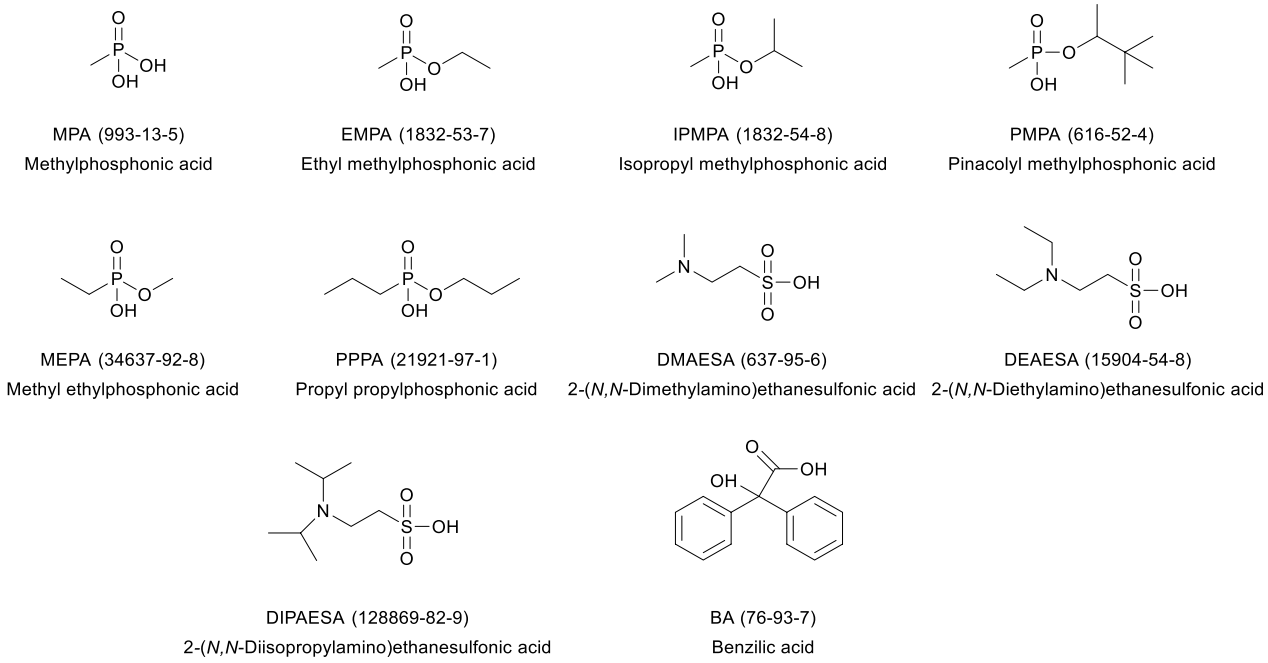

Fig. 1 Studied chemicals, their abbreviations and CAS numbers (in brackets)

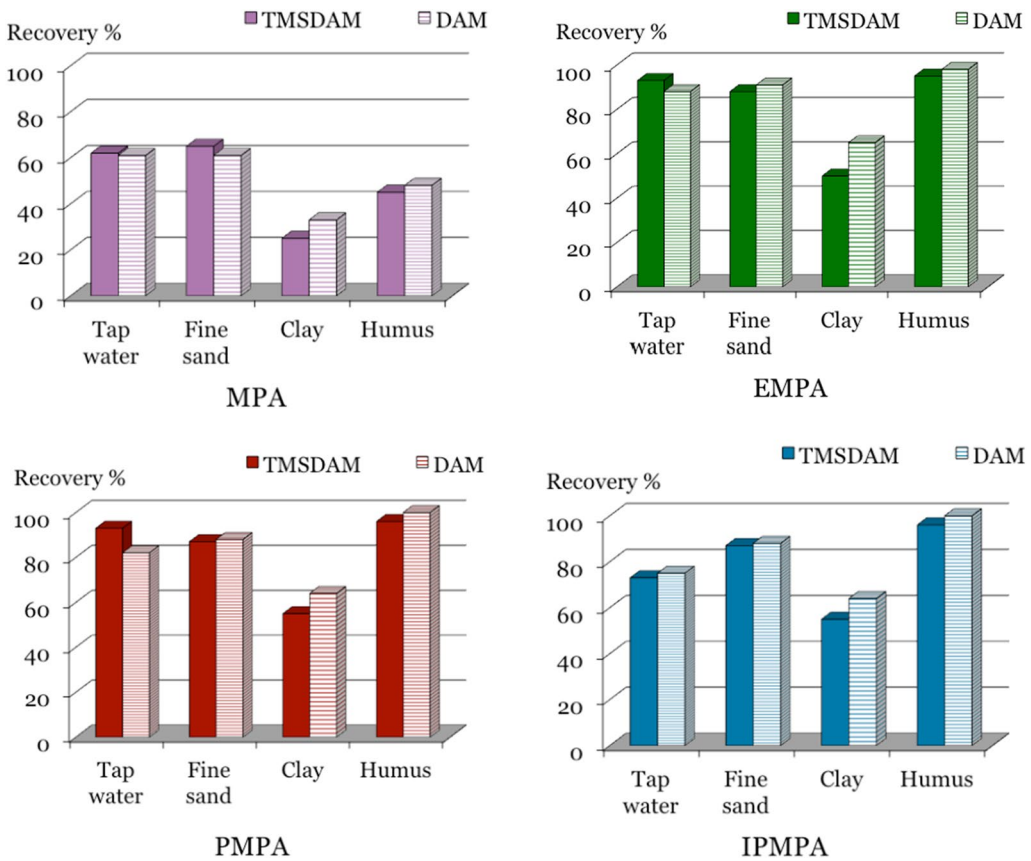

Fig. 2 Recovery (%) of APAs (10 µg/ml) in tap water and aqueous extracts of different soil samples. The aqueous extract was cation-exchanged and evaporated to dryness, and the residue was dissolved in AcMeOH before TMSDAM methylation

increase the recovery of these acids (see Additional file 1: Section 5 and Table S3) (Kuitunen 2005, 2010; Wils 2005; Vanninen 2017).

Comparable recoveries were obtained with both methylation methods, and there was no significant difference in the recoveries of the tested acids. As expected, methylations of MPA and clay extracts were the most challenging. Clay soil contains water-soluble particles, which decrease the recoveries of APAs in methylation. Methylation with TMSDAM was clearly easier to perform than with DAM, but as was known, there were more background signals in total ion chromatograms (TICs) from TMSDAM reaction products (see Additional file 1: Section 6 Figure S2), especially when the matrix contained interfering components. This is also reflected in the reproducibility of the recovery values. Relative standard deviation (RSD) was usually from 2 to 8%, except in some cases it was over 10% (11, 15 and 17%, respectively, $n=3$) for clay extract and PMPA.

The derivatization conditions were modified using 1 M AcMeOH instead of 0.2 M resulting in a twofold increase of the recovery in methyl derivative of MPA both using DAM and TMSDAM. This means that the solvent must be sufficiently acidic to dissolve the acids. Figure 3 shows the recoveries of methylated MPA in clay soil extract. When the solvent was more acidic, 0.1 ml of TMSDAM had to be used to increase the recovery of MPA to the same level as with DAM. RSDs were ca. 5% ($n=3$).

The effect of water on the recoveries in TMSDAM methylation was studied without a sample matrix by spiking MPA, MEPA and PPPA directly into 0.1 ml of 1 M AcMeOH and 10, 25, 50 or 100 µl of deionized water before addition of 0.8 ml of CH_2Cl_2 and 0.1 ml of TMSDAM. When 10 µl or 25 µl of water was added, the samples remained yellow during heating, and the recoveries were ca. 100%. Adding 50 µl or 100 µl of water decreased the recoveries to ca. 20–30% and the yellow color of these samples disappeared during methylation. When 50 µl of water was added, the direct addition of 200 µl of TMSDAM instead of 100 µl increased the recoveries to nearly 90%. The yellow color of these samples remained during the methylation process. When 100 µl of water was added and the amount of TMSDAM was increased to 200 µl, the yellow color disappeared during the methylation.

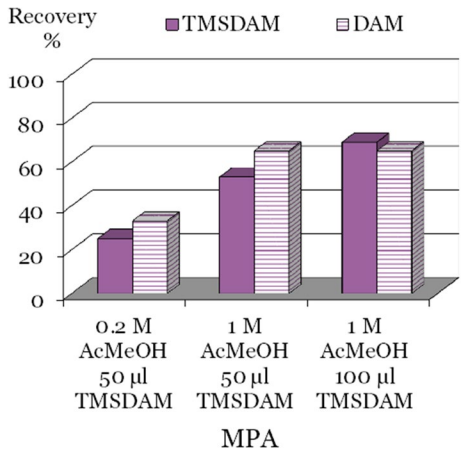

Fig. 3 Recovery (%) of MPA (10 µg/ml) in aqueous extracts of clay soil. The solution was cation-exchanged and evaporated to dryness, and the residue was dissolved in 0.2 M or 1 M AcMeOH before TMSDAM methylation (0.05 ml or 0.1 ml of TMSDAM). The recovery of MPA derivatized by DAM is shown for comparison

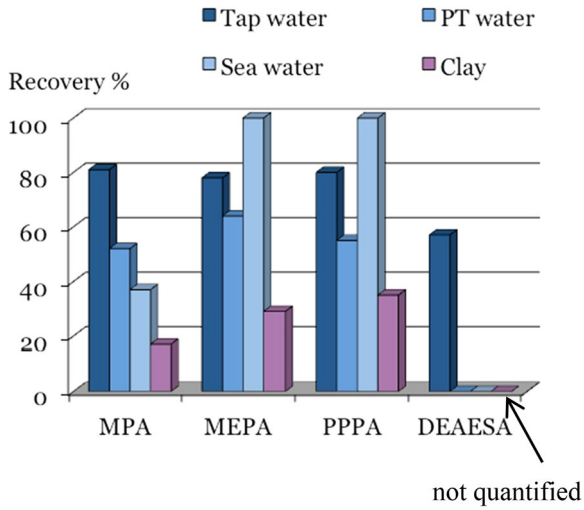

Fig. 4 Recovery (%) of APAs (10 µg/ml) and DEAESA in different water samples and in a clay extract. The solution was evaporated to dryness, and the residue was dissolved in AcMeOH before TMSDAM methylation

The recoveries of the spiking chemicals did not clearly increase either. It can be concluded that the methylation reaction tolerated some water in the samples. When the amount of water increased to 50 µl, the recoveries decreased, but the acids were still recovered when the reaction solution contained 10%–20% water. However, it is recommended to methylate dry solutions because the recoveries are less reproducible when the sample contains water. The yellow color of TMSDAM should remain during methylation. If the color disappears, it is recommended to add another 100 µl of TMSDAM. However, more reliable results are obtained if a new portion of the sample is taken and 200 µl of TMSDAM is used for methylation.

Necessity of cation exchange

The influence of cation exchange on methylation with TMSDAM for acidic CWC-related chemicals was further examined. MPA, MEPA, PPPA and DEAESA were methylated. In addition to tap water and clay soil extract, seawater and simulated PT water were used as matrices. The PT water contained 2 mg/ml $CaCl_2$ and Na_2SO_4. A water sample of the 25th OPCW PT contained these chemicals at a concentration of 1 mg/ml. Based on our knowledge, it has been the largest amount of inorganic salts in an aqueous sample in PTs so far. Figure 4 summarizes the results of these experiments.

In all samples, all APAs were detected with GC–MS in full-scan mode in the extracted ion chromatograms. Further, the quality of the obtained spectra met the OPCW identification criteria (OPCW PT04 2017). The MS spectra of the identified methyl derivatives are presented in Additional file 1: Section 7 Figure S3. Depending on the sample matrix, it was not possible to quantify DEAESA because the response was not linear. In addition, the quantitative results of MEPA and PPPA were not reproducible in the seawater samples. Other RSDs of the recovery values were usually below 10% and for recovery values of PT water samples 15%. Figure 5 shows the TICs of a tap water sample and a clay soil extract. In addition, the retention time window during which the DEAESA derivative elutes is shown and those for a seawater sample and a clay soil extract.

Usually in GC–MS analysis of CWC-related chemicals, a nonpolar SE-54-type column is used (Häkkinen et al. 2017). The peaks of the polar, derivatized chemicals, especially peaks of APAs, tend to tail, and for this reason, during the development of the sample preparation methods, we have generally used a polar Carbowax-type column to improve the peak shape and reproducibility in quantitation especially for samples with complex matrices.

In addition, tap water samples and clay soil extracts were also analyzed with a nonpolar DB-5 ms column. In scan mode runs, all the acids were readily detected with selected ion monitoring. All the spiking chemicals were recovered in both samples. From MPA and MEPA in tap water, it was difficult to obtain a GC–MS measurement that would meet the criteria for an 'OPCW acceptable identification.' As expected, DEAESA was

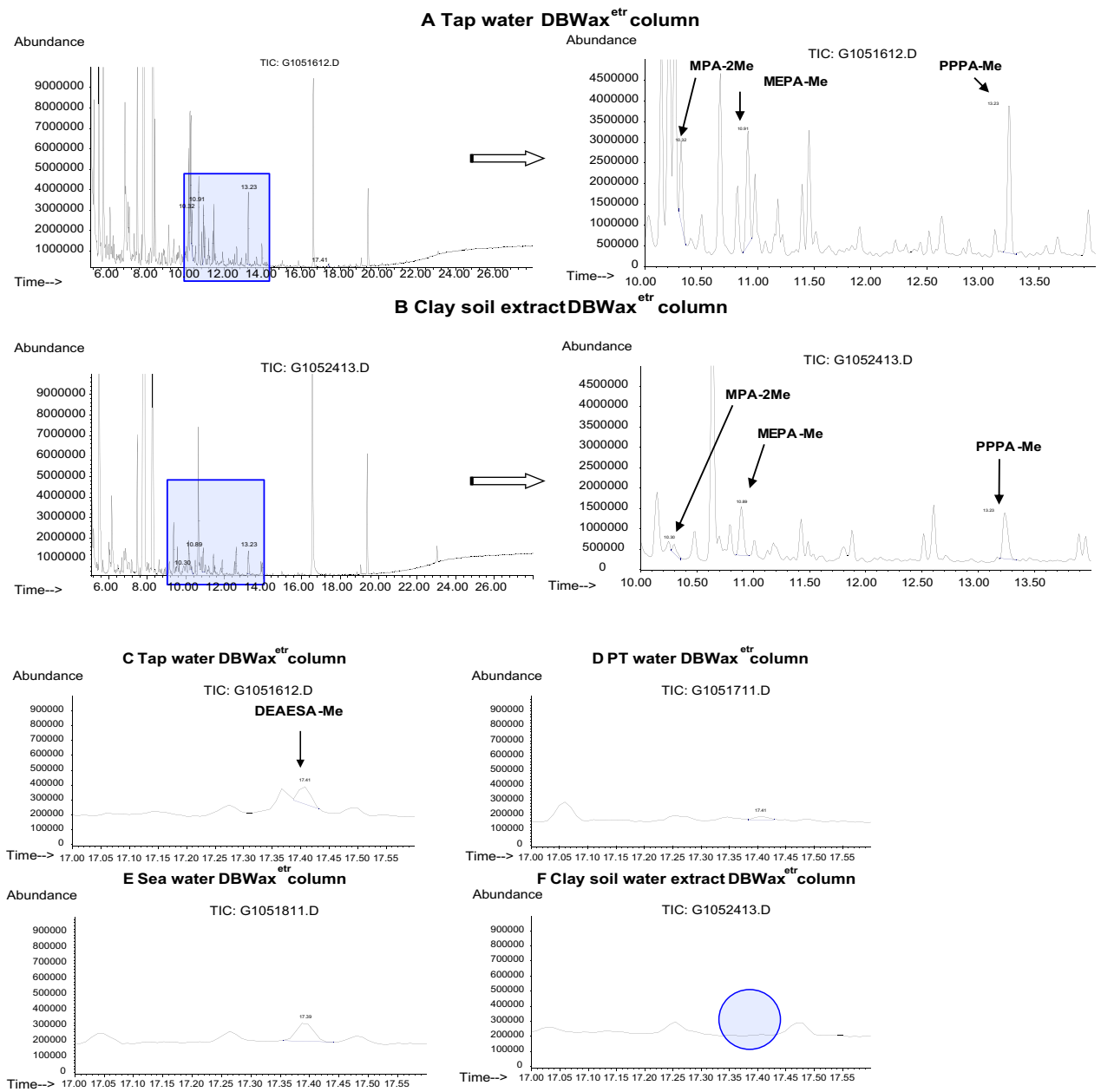

Fig. 5 GC–MS TICs of non-cation-exchanged water samples and clay extract. TMSDAM was used for methylation of a tap water sample (**A**) and a clay soil extract (**B**). Retention time window during which the DEAESA derivative elutes for a tap water sample (**C**), a PT water sample (**D**), a seawater sample (**E**) and a clay soil extract (**F**). A DBWaxetr column was used

readily recovered with the nonpolar column, but its response was not linear. For comparison, TICs of the samples with the DB-5 ms column are shown in Fig. 6.

Derivatization of SAs

The derivatization of SAs was investigated further. As shown above, even the relatively high spiking level of 10 µg/ml caused problems for quantitation. The responses were no more linear if the concentration was below 2.5 µg/ml. In order to get more reliable quantitative results from the more difficult matrices and to improve the comparison of the results of different derivatization reactions with and without cation exchange, the spiking level was increased to 20 µg/ml.

Figure 7 shows recoveries of SAs DMAESA, DEAESA and DIPAESA after methylation with TMSDAM and

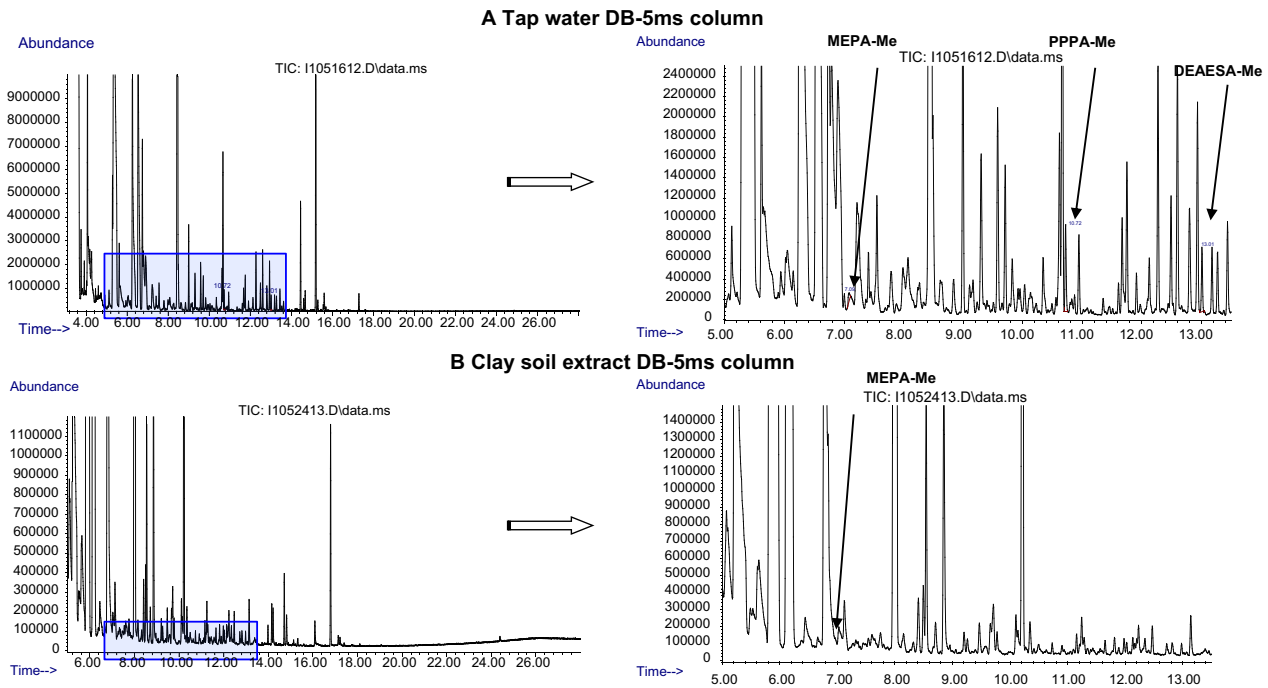

Fig. 6 GC–MS TICs of non-cation-exchanged tap water sample (**A**) and a clay soil extract (**B**). TMSDAM was used for methylation and a DB-5 ms column for analysis

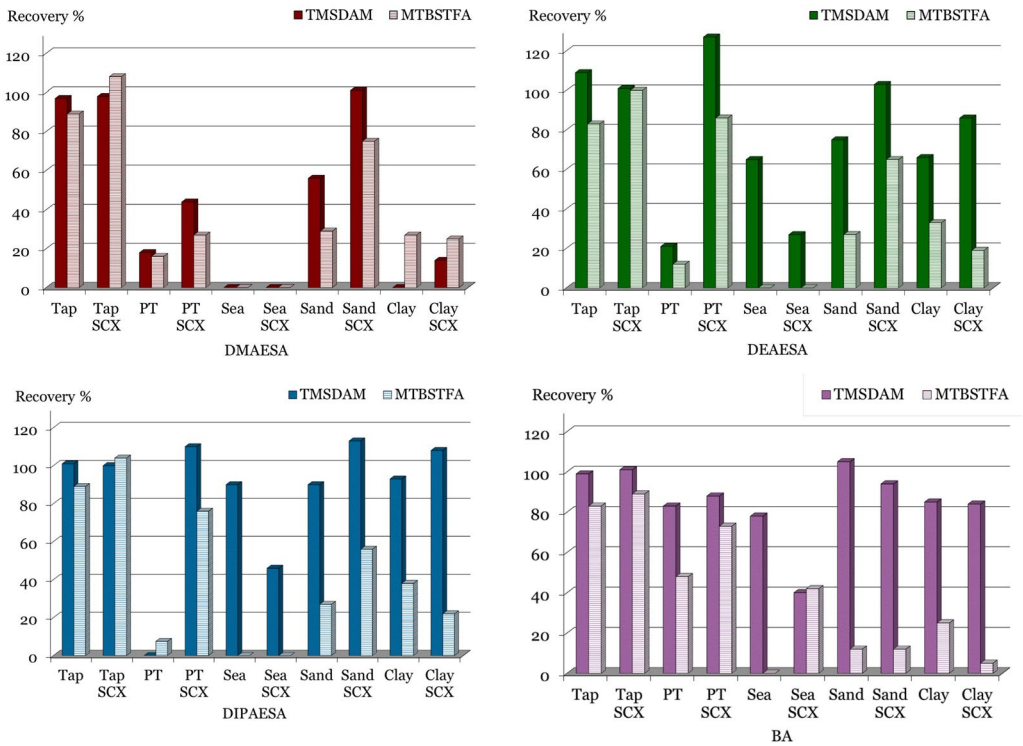

Fig. 7 Recovery (%) of SAs and BA (20 µg/ml) in different water samples and soil extracts without and with cation exchange. The samples were silylated with MTBSTFA or methylated with TMSDAM

silylation with MTBSTFA both without and with cation exchange (SCX cartridge) using tap water, PT water, seawater, sand soil extract and clay soil extract as sample matrices. In addition, BA was derivatized.

RSDs of the recovery values were mostly very reasonable (usually below 10%), especially when the recoveries were high. From tap water, the recoveries of both methyl and silyl derivatives of DMAESA, DEAESA, DIPAESA and BA were high both without and with cation exchange. The recoveries of silylated acids were a little lower than those of methylated acids. Cation exchange did not necessarily increase the recoveries, however. From the non-cation-exchanged PT water sample, only the recovery of methylated BA was high. DIPAESA was not even recovered from this sample. The recoveries of all acids were higher as methyl derivatives compared with silylation and when PT water was cation-exchanged. As expected, seawater was the most problematic matrix to recover the acids with both derivatives but especially using silylation. SAs were not recovered as silyl derivatives and DMAESA was not even recovered as a methyl derivative. The recoveries of the two other SAs and BA as methyl derivatives were quite high from the non-cation-exchanged sample and substantially higher than from the cation-exchanged sample. BA was the only acid recovered with silylation after cation exchange from seawater.

The recoveries of methylated SAs were clearly higher than for silylated SAs from aqueous extracts of sand soil. The recoveries of both methylated and silylated SAs even decreased if the extract was cation-exchanged. The recoveries of methylated SAs increased with the length of the dialkylamino chains of the SAs. On the other hand, only 5–10% of BA was recovered as a silyl derivative from both cation-exchanged and non-cation-exchanged sand soil extract, whereas the methylation reaction was nearly quantitative. Obviously, MTBSTFA is not the optimum silylation reagent for BA, and for this reason, BSTFA is usually used (Black et al. 2017).

Methylation gave much higher recoveries than silylation for all other acids except DMAESA from clay soil extracts with or without cation exchange. DMAESA could not be recovered as a methyl derivative without cation exchange. The recoveries of other SAs and BA decreased after cation exchange and also when silylation was used. A recommendation for methylate water samples and aqueous extracts with TMSDAM is given in Additional file 1: Section 8.

Application

Methylation with TMSDAM is in use in our laboratories and has been tested by us in several OPCW PTs using a DB-5 ms column for analysis. Figure 8A presents the TIC of the methylated water sample of the 32nd OPCW PT. The methyl derivative of PMPA at 7 µg/ml concentration level was identified in a water sample containing a polyethylene glycol background (PEG 200, 2 mg/ml) and both nickel chloride and calcium chloride (0.2 mg/ml each). Figure 8B shows the TIC of the methylated water sample of the 34th OPCW PT. Methyl derivatives of isobutyl ethylphosphonic acid (IBEPA) and DEAESA (both spiked with 10 µg/ml) were identified in a sample also containing a PEG background (PEG 300, 1 mg/ml). In both tests, the chemicals were reported according to the OPCW identification criteria. The MS spectra of the identified methyl derivatives are presented in Additional file 1: Section 7 Figure S3.

Conclusions

Methylation of APAs and SAs with the optimized TMSDAM method is comparable to methylation with DAM with regard to recovery. An advantage is that the method is faster than derivatization with DAM. AcMeOH should be used as a solvent for evaporation residues, and TMSDAM has to be added in excess, i.e., the yellow color of the TMSDAM solution must remain in the sample during methylation. If the sample contains high amounts of background chemicals, cation exchange is required. Methylation with TMSDAM is well suited to the qualitative analysis of the examined acids in aqueous matrices with its simplicity, speed and robustness. The method performance was excellent, and the method is considered reliable for rapid GC–MS screening and identification.

For the derivatization of SAs and BA, methylation with TMSDAM was more robust than silylation with MTBSTFA. The higher and more complex the matrix background, the more important the cation exchange before derivatization and analysis. Cation exchange is always recommended for quantitative analysis. During OPCW PTs, it could be demonstrated that the derivatization of APAs and SAs by TMSDAM in environmental samples is a useful tool for identification by GC–MS. Therefore, it can replace DAM derivatization without compromising the analysis.

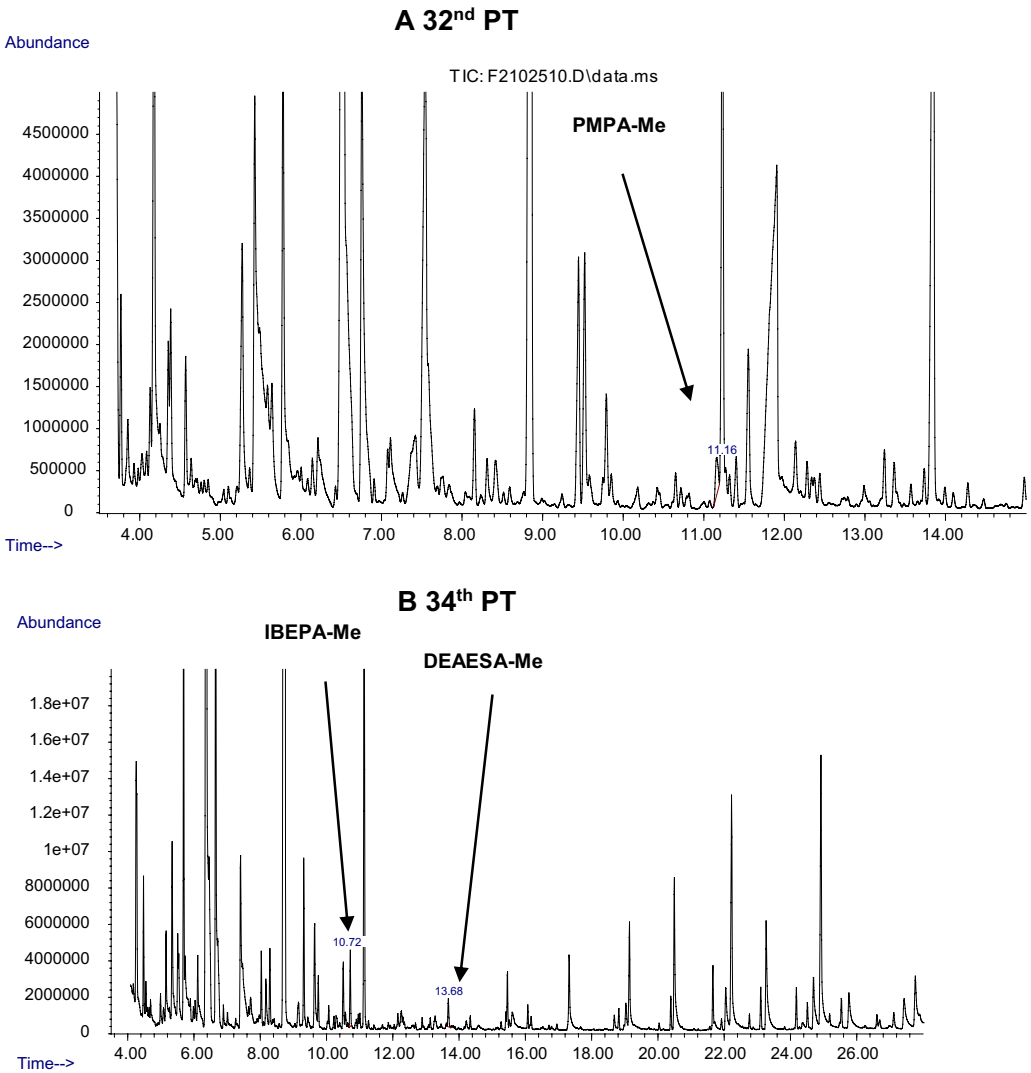

Fig. 8 TICs of GC–MS analysis of water samples of the 32nd (**A**) and 34th (**B**) OPCW PTs. TMSDAM was used for methylation and a DB-5 ms column for analysis. The methyl derivatives of isobutyl ethylphosphonic acid (IBEPA), DEAESA (both spiked with 10 µg/ml) and PMPA (7 µg/ml) could be detected

Abbreviations

AcMeOH: Acidic methanol; APAs: Alkylphosphonic acids; ATPAs: Alkylthiophosphonic acids; BA: Benzilic acid; CWA: Chemical warfare agent; CWC: Chemical Weapons Convention; DAM: Diazomethane; DEAESA: 2-(*N*,*N*-diethylamino) ethanesulfonic acid; DIPAESA: 2-(*N*,*N*-diisopropylamino)ethanesulfonic acid; DMAESA: 2-(*N*,*N*-dimethylamino)ethanesulfonic acid; EI: Electron impact ionization; EMPA: Ethyl methylphosphonic acid; GC–MS: Gas chromatography–mass spectrometry; HCB: Hexachlorobenzene; IBEPA: Isobutyl ethylphosphonic acid; IPMPA: Isopropyl methylphosphonic acid; LC–MS: Liquid chromatography–mass spectrometry; MEPA: Methyl ethylphosphonic acid; MPA: Methylphosphonic acid; MSD: Mass selective detector; MTBE: Methyl *tert*-butyl ether; MTBSTFA: *N*-Methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide; NMR: Nuclear magnetic resonance; OPCW: Organization for the Prohibition of Chemical Weapons; PMPA: Pinacolyl methylphosphonic acid; PPPA: Propyl propylphosphonic acid; PTs: Proficiency tests; ROPs: Recommended operation procedures; RSD: Relative standard deviation; SAs: 2-(*N*,*N*-dialkylamino) ethanesulfonic acids; TICs: Total ion chromatograms; TMPAH: Trimethyl phenylammonium hydroxide; TMSDAM: Trimethylsilyldiazomethane.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-022-00338-1.

Additional file 1. Supplementary information.

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Author contributions
JCD, MLK, MLR and PS designed the study, and MLK and MLR performed the experimental work. MLK and MLR interpreted the results, and MLK drafted the manuscript. JCD, MLK, PS and PSV reviewed the manuscript, and all authors approved the final manuscript.

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Availability of data and materials
Not applicable.

Declarations

Competing interests
There are no competing interests to declare.

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