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Determination of S-2-(N,N-diisopropylaminoethyl)- and S-2-(N,N-diethylaminoethyl) methylphosphonothiolate, nerve agent markers, in water samples using strong anion-exchange disk extraction, *in vial* trimethylsilylation, and gas chromatography–mass spectrometry analysis

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ABSTRACT

Since the establishment of the Chemical Weapons Convention in 1997, the development of analytical methods for unambiguous identification of large numbers of chemicals related to chemical warfare agents has attracted increased interest. The analytically challenging, zwitterionic S-2-(N,N-diisopropylaminoethyl) methylphosphonothiolate (EA-2192), a highly toxic degradation marker of the nerve agent VX, has been reported to resist trimethylsilylation or to result in an unacceptably high limit of detection in GC–MS analysis. In the present study, the problem is demonstrated to be associated with the presence of salt, which hinders trimethysilylation. EA-2192 was extracted from aqueous samples by use of a strong anion-exchange disk, derivatized as a trimethylsilyl derivative via *in vial* solid-phase trimethylsilylation and identified by GC–MS. The limits of detection were 10 ng/mL and 100 ng/mL (in a water sample) for SIM and SCAN mode respectively. The analytical method was found to be repeatable with relative standard deviation <10%. The performance of the method was evaluated using a proficiency test sample and environmental samples (spiked river water and Baltic Bay water) and compared with the commonly used evaporation–silylation method. The disk method displayed good tolerance to the presence of salt and the spiked EA-2192 was conclusively identified in all matrices. In addition, the applicability of the method was further demonstrated for other selected hydrolysis products of VX and Russian VX, namely S-2-(N,N-diethylaminoethyl) methylphosphonothiolate, ethyl methylphosphonic acid, methylphosphonic acid, and isobutyl methylphosphonic acid. For the synthesis of reference compounds, EA-2192 and its analog from degradation of the Russian VX isomer, the present methods were improved by using a polymer-bound base, resulting in >90% purity based on ^{1}H NMR. Based on the current results and earlier work on alkylphosphonic acids using the same method, we conclude that the method is a viable choice for the simultaneous determination of a wide range of degradation products of nerve agents – zwitterionic, monoacid, diacid, and monothioacid chemicals – with excellent performance.

1. Introduction

The establishment of the Chemical Weapons Convention (CWC) [1] in 1997 has accelerated the development of analytical capabilities for the verification of chemical warfare agents (CWAs) and related chemicals [2,3]. Verification requires unambiguous detection and identification of a large number of chemicals, including CWAs, and their precursors and degradation products. The nerve agent O-ethyl S-2-(N,N-diisopropylaminoethyl) methylphosphonothiolate (VX) is an important chemical listed in the CWC. VX is the most toxic nerve agent known and was produced and stockpiled in large amounts, which are currently subject to a destruction plan monitored by the Organisation for the Prohibition of Chemical Weapons (OPCW). It has been used by terrorists, for example, a murder case in Osaka, Japan in 1994 [4] and is now listed as a potential Chemical Threat Agent. Apart from the non-toxic degradation products (alkylphosphonic acids) [5,6], the hydrolysis of VX in neutral and basic conditions will also produce the very toxic degradation product S-2-(N,N-diisopropylaminoethyl) methylphosphonothiolate (EA-2192) (Fig. 1) [4,7,8]. The toxicity of EA-2192 is slightly lower than of VX in mice by intravenous injection and exhibits, as VX, an anticholinesterase effect that affects

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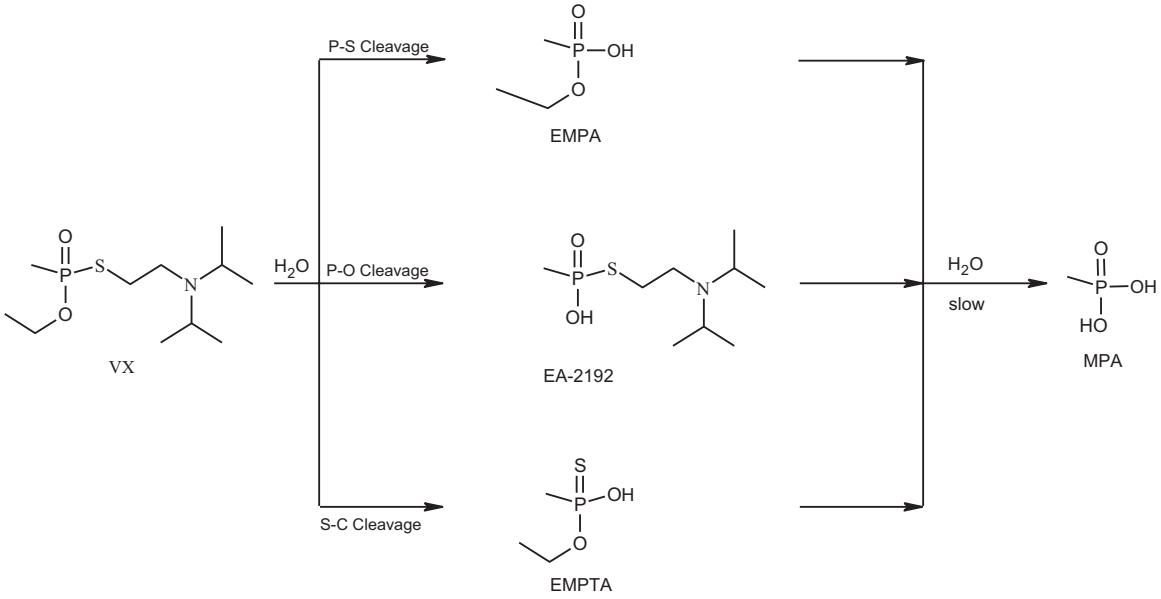

Fig. 1. The hydrolysis pathway of VX into EA-2192 as well as non-toxic degradation products, ethyl methylphosphonic acid (EMPA) and O-ethyl methylphosphonothioic acid (EMPTA) in neutral and weakly basic conditions. Eventually, these chemicals undergo further hydrolysis at slower rate to form the secondary degradation product methylphosphonic acid (MPA). The kinetics and mechanism of the hydrolysis of VX depend on pH, temperature and type of nucleophile present.

central nervous system [9]. This compound is thus both a chemical threat and an important marker for the release of VX. Also, hydrolysis to the final degradation product methylphosphonic acid is slow [10] which makes EA-2192 important as an early marker.

Analysis of EA-2192 is currently performed directly using nuclear magnetic resonance [7] or liquid chromatography–mass spectrometry [11] or identified as its methyl derivative by GC–MS [12]. The methylation is carried out by diazomethane, trimethylsilyl diazomethane, or trimethylphenylammonium hydroxide [5]. Disadvantages of derivatization of EA-2192 by methylation have been reported as poor peak shapes of the methyl derivative, use of carcinogenic and explosive diazomethane [8], and the fact that the methyl derivative itself is listed in the CWC as a VX-related chemical [8]. For verification analysis of CWA and related compounds, GC–MS is the most widespread and the preferred technique owing to the availability of instrumentation in most modern laboratories and the possibility for library search in mass spectra databases [3,13]. Thus, verification analysis by GC–MS would be highly beneficial as an alternative derivatization technique for EA-2192 that is robust and gives high recovery. Trimethylsilylation of EA-2192 would be a preferable option but unfortunately it has been reported to be problematic [8,10,14]. EA-2192 has the property of being a zwitterion (amphoterism), since it has both a phosphonothioic acid group and a tertiary amine group [14,15]. This property is believed to hinder derivatization with trimethylsilyl (TMS) reagent although the actual reason has not been thoroughly investigated. Interestingly, in a recent publication, Pardasani et al. [5] claimed that the condensation and decomposition of the EA-2192 TMS derivative on the GC column led to non-detectability by GC–MS. They proposed an elevated initial column temperature to solve the problem. However, the limit of detection reported for EA-2192 in organic solvent is not sufficient for the OPCW verification criteria and the method was not validated for aqueous samples.

Owing to the contradictory data regarding the trimethylsilylation of EA-2192, we have in this study applied our earlier published method for the determination of alkylphosphonic acids in aqueous samples [16] to the determination of EA-2192. The influence of salt in the sample matrix plays an important role in the analysis of environmental samples and was a major parameter studied. The method includes extraction of the analytes using a strong anion-exchange disk, *in vial* solid-phase trimethylsilylation, and GC–MS analysis. Determination of the analogous zwitterionic degradation product of Russian VX (RVX), S-2-(N,N-diethylaminoethyl) methylphosphonothiolate (REA) [15,17] was validated in parallel. Apart from studying EA-2192 and REA, the applicability of the method was extended to other selected degradation products of VX and RVX; namely ethyl methylphosphonic acid (EMPA), methylphosphonic acid (MPA), and isobutyl methylphosphonic acid (iBMPA) (Fig. 2).

2. Experimental

2.1. Materials and chemical standards

High Performance Anion-Exchange Extraction Disks (model 2252) were purchased from 3M Empore (St. Paul, MN, USA). Sections with a diameter of 13 mm were taken from the disks using a circular punch. The purchased disks had the following chemical and physical properties: 47 mm diameter, 0.5 mm thickness, quaternary amine (propyl-trimethylamine) groups anchored to highly cross-linked inert styrene-divinylbenzene (PS-DVB) copolymers, chloride counter ion, specific surface area 500–1200 m^2/g, anion-exchange capacity of ~0.015 mequiv./g for a 13 mm disk, average particle size 8–12 μm, pore size 6 nm and membrane porosity 0.5–1.5 μm. The 2-piece 13 mm polypropylene disk holder (Swinnex) used was sourced from SKC (Eighty Four, PA, USA). The 13 mm disk was placed in the disk holder. The inlet and outlet of the disk holder were connected to a 5 mL syringe reservoir and vacuum manifold, respectively.

Solvents for disk conditioning and synthesis were of *pro analysi* grade and were purchased either from Merck (Darmstadt, Germany) or Fisher Scientific (Leicestershire, UK). All aqueous solutions were prepared using purified deionized (DI) water from a Millipore Milli-Q water generator (Billerica, MA, USA). The derivatizing reagent, N,O-bis(trimethylsilyltrifluoroacetamide) with 1% trimethylchlorosilane (BSTFA + 1% TMCS) was purchased from Thermo Scientific (Rockford, IL, USA) in 1 mL ampoules. The salts sodium chloride (NaCl) (99.5%), calcium chloride ($CaCl_2$) (96%), sodium hydrogen carbonate ($NaHCO_3$) (99.7%), and magnesium sulfate ($MgSO_4$) (98%) were purchased from Merck.

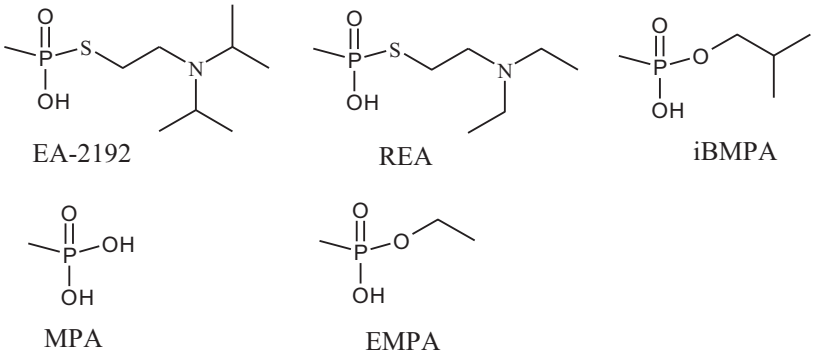

Fig. 2. Structures of the selected degradation products of VX and RVX used in this study. EMPA and iBMPA are the primary degradation products of VX and RVX respectively, while MPA is the secondary degradation product of both VX and RVX.

Phosphonic acids standard solutions (1.0 mg/mL), methylphosphonic acid (MPA) (98%), and ethyl methylphosphonic acid (EMPA) (98%) in water were purchased from Sigma–Aldrich (Milwaukee, WI, USA), while isobutyl methylphosphonic acid (iBMPA) (95%) in water was synthesized in-house [18]. Precursors for synthesis of EA-2192 and REA, N-(2-chloroethyldiisopropyl)ammonium chloride and N-(2-chloroethyldiethyl)ammonium chloride respectively were purchased from Janssen chemical (Beerse, Belgium) and Sigma–Aldrich, while methyl thiophosphosphonic dichloride was synthesized in-house. The precursor for synthesis of d_3-EA-2192 and d_3-REA, deuterated methyl thiophosphosphonic dichloride (100%), was synthesized in-house. Internal standards (1.0 mg/mL) of d_3-EA-2192 and d_3-REA in acetonitrile were synthesized in-house.

The OPCW proficiency test (PT) 19 W1 water sample was used for the robustness test. The sample was fortified with the target compounds at 1.0 and 5.0 μg/mL in order to represent the actual verification criteria required by OPCW. The sample contained the following matrix compounds: magnesium sulfate (100 μg/mL), calcium chloride (250 μg/mL), and sodium bicarbonate (200 μg/mL); four different polyethylene glycols: PEG 200, PEG 300, mPEG 150, and mPEG 250 (150 μg/mL each); 1,3-propanediol (50 μg/mL), and N,N-diisopropylethylamine (50 μg/mL).

2.2. Synthesis of EA-2192, REA, and corresponding deuterated analogs

The synthesis method included a polymer-bound base and resulted in the formation of 90 mol % EA-2192 (99.5% pure, ^{31}P NMR). The whole synthesis pathway is given in Fig. 3.

2.2.1. EA-2192

The precursor methylphosphonothiolic acid was formed from the acidic workup between freshly distilled methylthiophosphonic dichloride in dioxane with four equivalents of 4 M NaOH aqueous [19]. ^{31}P NMR (202 MHz, CD_3OD) δ = 79.05. It is noteworthy that this colorless, oily acid is highly unstable and decomposes gradually over time. The subsequent reaction must be performed without delay, or the compound can be stored as its sodium or dicyclohexylammonium salt [20]. 124 μmol of the synthesized methylphosphonothiolic acid (13.9 mg) was dissolved in 2.0 mL trifluoroethanol in a 7 mL vial with a PTFE-lined screw-cap and an excess amount of dried poly(4-vinylpyridine) (200 mg, four equivalents) was added, together with a small magnetic stirrer-bar. The mixture was slowly stirred for 10 min and then 125 μmol of N-(2-chloroethyldiisopropyl)ammonium chloride (25.0 mg) was added and the vial carefully closed. The reaction vial was heated in an oil-bath at 50 °C for 4 h, still under slow stirring. The mixture was allowed to stay at room temperature for 15 min, then the liquid phase was removed using a plastic syringe and filtered through a Pasteur-pipette equipped with a plug of glass-fiber paper and 0.15 g silica gel 60A. The solution was then used as a stock solution. ^{1}H NMR analysis showed that the solution contained 90 mol% of EA-2192 and 10 mol% of N-(2-chloroethyldiisopropyl)ammonium chloride. ^{31}P NMR analysis of the solution showed less than 0.5% impurities. The isolated yield of EA-2192 was about 34%. ^{1}H NMR (500 MHz, CD_3OD) δ = 1.43 (dd, J_{HH} = 6.6 Hz, 12H), 1.64 (d, J_{HP} = 15.1 Hz, 3H), 3.10 (dt, J_{HH} = 6.0 Hz, J_{HP} = 14.5 Hz, 2H), 3.44 (t, J_{HH} = 6.0 Hz, 2H), 3.74 (m, J_{HH} = 6.6 Hz, 2H). ^{31}P NMR (202 MHz, CD_3OD), δ = 39.92.

2.2.2. REA

REA was synthesized using the same method as described for EA-2192, with an isolated yield of about 40%. ^{1}H NMR (500 MHz, CD_3OD) δ = 1.36 (t, J_{HH} = 7.3 Hz, 6H), 1.63 (d, J_{HP} = 15.1 Hz, 3H), 3.05 (dt, J_{HH} = 5.7 Hz, J_{HP} = 14.2 Hz, 2H), 3.21 (q, J_{HH} = 7.3 Hz, 4H), 3.41 (t, J_{HH} = 5.6 Hz, J_{HP} = 14.2 Hz, 2H). ^{31}P NMR (202 MHz, CD_3OD), δ = 39.88.

2.2.3. Internal standards: S-2-(N,N-diisopropylaminoethyl) deuterated methylphosphonothiolate (d_3-EA-2192) and S-2-(N,N-diethylaminoethyl) deuterated methylphosphonothiolate (d_3-REA)

The deuterated standards were synthesized using the same method as described for EA-2192 and REA using deuterated methylthiophosphonic dichloride as starting material.

Caution: These compounds are extremely toxic and must be prepared and handled by specially trained professionals only. The work must be performed in a laboratory fitted for this purpose with all the necessary protections for the involved personnel and all required permissions.

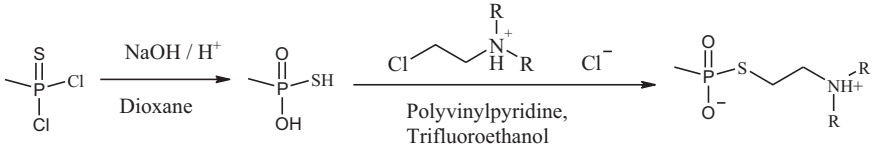

Fig. 3. Synthesis pathway of EA-2192.

2.3. GC–MS analysis

The derivatized samples were analyzed using an Agilent 5890 Series II gas chromatograph coupled to an Agilent 5975C inert XL mass selective detector (MSD) with a Triple Axis Detector. Splitless injections (1 μL) were performed at an injection temperature of 200 °C. The gas chromatograph was fitted with a 2 mm i.d. silanized liner for splitless injections and a DB5-MS capillary column (J&W, 30 m × 0.25 mm i.d., 0.25 μm film thickness). The carrier gas was He at a flow rate of 1 mL/min. The transfer line temperature was 280 °C. The mass spectrometer was operated in the EI mode. The source temperature was 230 °C. The ions were acquired in SCAN mode (*m*/*z* 40–600) and SIM mode (*m*/*z* 114 for EA-2192; *m*/*z* 86 for REA). We applied a standard GC oven temperature program normally employed in OPCW proficiency tests: 40 °C for 1 min, increased by 10 °C/min to 280 °C (held for 5 min), with a total runtime of 30 min. Mass spectral data analysis was performed using an Agilent MSD ChemStation, NIST MS Search and deconvolution software (AMDIS) with the OPCW Central Analytical Database (OCAD) and our own MS libraries.

2.4. Performance of the evaporation–silylation method (reference method)

The direct evaporation–silylation method [21] consists of the evaporation of the water sample fortified with 5.0 μg/mL EA-2192 and REA under nitrogen flow at 40 °C followed by trimethylsilylation using BSTFA + 1% TMCS and acetonitrile (2:1, v/v, 500 μL) at 90 °C for 40 min in a heating block. An aliquot of the liquid was injected and analyzed directly by GC–MS. The experiments were repeated for the PT19 W1 aqueous sample.

2.5. Performance of the disk method

The optimized disk method described in Subramaniam et al. [16] was validated for the silylation of the zwitterionic EA-2192 and REA in DI water. In brief, the anion-exchange disk was activated by conditioning sequentially with acetone, methanol, water and 1 M NaOH, followed by introduction of an aqueous sample (5.0 mL or 0.50 mL) drawn through the disk under reduced pressure. Acetonitrile (1 mL) was then applied to remove non-polar contaminants and moisture from the disk. Vacuum (∼0.6 bar) was applied to the disk for 15 min. The disk was then removed from the holder and placed in an oven at 100 °C for 10 min to complete the removal of moisture. After drying, the disk was placed into a 2 mL GC vial. BSTFA + 1% TMCS and acetonitrile (2:1, v/v, 500 μL) were added to immerse the whole disk. Internal standard was added and the vial was heated at 90 °C for 40 min in a heating block. An aliquot of the liquid was injected and analyzed directly by GC–MS.

The method's limit of detection for each analyte was calculated according to the definition of the signal amplitude being three times that of the background noise when averaged over three replicate runs. To determine the recovery of the EA-2192 and REA using the method, three replicate samples were spiked with a known amount of the target compounds and the peak–area ratios of their TMS derivatives and the corresponding deuterated internal standard were compared with those obtained using the reference method. The repeatability of the method was calculated from recovery data.

2.6. Investigation of influence of salt on disk method

2.6.1. Salt concentration

Loss in recovery due to the presence of salt in the sample was investigated using the disk method. The salt at different concentrations (expressed as a percentage) was spiked into the water sample to correspond to the expected salt concentration in the environmental samples (Table 1). The sample volume used was 5.0 mL, with a concentration of 5.0 μg/mL EA-2192 and REA. The alkylphosphonic acids EMPA, iBMPA, and MPA were included for comparison.

2.6.2. Sample volume

For estimation of the desalting effect using a smaller sample volume, the salt experiments from 2.6.1 were repeated using a sample volume of 0.50 mL.

2.6.3. Influence of extraction and derivatization steps

Experiments were conducted to determine the actual contribution of yield losses of EA-2192 and REA in the extraction (SPE) and derivatization steps due to salt concentration. The procedure in Section 2.6.2 was repeated and 5.0 μg/mL d_3-EA-2192 and d_3-REA were spiked into the GC vial prior to derivatization. The recovery losses from SPE breakthrough were determined by comparing the difference in the peak areas of derivatives with the corresponding deuterated analogs, since these are added to the derivatization and are not influenced by the SPE step. For determination of derivatization losses, the relative peak areas of deuterated standard at the various salt concentrations were compared to the DI water sample.

2.7. Application of the disk method

Identification of EA-2192 and REA in the proficiency test sample was made in order to verify the performance of the method. The W1 sample was fortified with EA-2192 and REA at 1.0 μg/mL and 5.0 μg/mL concentration levels. Apart from W1, the applicability of the method was also evaluated for environmental water samples spiked with EA-2192 and REA at 5.0 μg/mL. The samples were river and Baltic Bay water samples (Sävar, Sweden).

2.8. Storage of samples on dried disks

The stability of underivatized EA-2192 and REA on the dry SPE disks was assessed by applying an aqueous sample, W1 (from OPCW proficiency test, 19th Round), fortified with 5.0 μg/mL EA-2192 and REA, to conditioned SPE disks. The disks were dried and stored in sealed clear GC vials in GC autosampler at room temperature. Compounds retained on the disks were derivatized on days 1 ($n = 3$), 5 ($n = 3$), and 14 ($n = 3$) then analyzed by GC–MS.

3. Results and discussion

3.1. Performance of the direct evaporation–silylation method

There is a significant body of literature on the failure of trimethylsilyation of EA-2192 [5,8,10,14,22]. The sustained effort to achieve trimethylsilyation of this chemical shows the importance of this derivative for identification of EA-2192 [5]. It is evident that an efficient extraction and derivatization would be needed to enable GC–MS identification of EA-2192 TMS within the stipulated detection limit required by CWC [1].

The only earlier published synthesis method of EA-2192 [5] involves reaction of the base pyridine salt of methylphosphonothiolic acid with N-(2-chloroethyldialkyl)ammonium chloride in acetonitrile. The final product contains large amounts of unreacted dialkylammonium chloride and pyridine salt and the EA-2192 was found to be difficult to purify. The salts are assumed to be a contributing cause of the derivatization problems earlier reported in the literature. Thus, we developed an alternative synthesis method that gives pure, salt-free reference compounds. The direct evaporation–silylation method used for phosphonic acids [21] was

Table 1
The range of salt concentrations spiked into the water sample to challenge derivatization (salt concentration in Brackish water I corresponds to Baltic Bay water).

Salt concentration corresponding to:	$MgSO_4$ (μg/mL)	$CaCl_2$ (μg/mL)	$NaHCO_3$ (μg/mL)	NaCl (μg/mL)	Total concentration (%)
DI water	–	–	–	–	0
Tap water	100	200	200	–	0.05
Brackish water I	100	200	200	1500	0.2
Brackish water II	100	200	200	7500	0.8

selected to determine a recovery level of EA-2192 and REA in samples prepared in DI water. The derivatization was successful, as shown by the GC–MS data in Fig. 4a. This result is in contradiction to earlier results in the literature and is likely explained by the use of a pure and salt-free reference compound in our study. Also the earlier reported problems during GC analysis [5] were not noticed. The method was demonstrated to produce good yields (>93% compared to deuterated standards added to the derivatization mixture after evaporation and reconstitution). The derivatized analytes were also subjected to ^{1}H NMR and ^{31}P NMR, from which two important observations were made: firstly, the derivatization was complete, as underivatized EA-2192 and REA were not detected. Secondly, di(trimethylsilyl) methylthiophosphonate was present in the sample. The fact that the corresponding thioic acid was not present in the standard solutions of EA-2192 and REA suggests that its formation was a result of the derivatization and the amount was estimated to be a 5% conversion of the total amount of EA-2192 and REA (by ^{31}P NMR). The result using GC–MS gave an estimate of 10% but NMR is supposed to give a better quantitative determination. The di(trimethylsilyl) methylthiophosphonate was also formed in the disk method. It is important to note that this derivatization by-product is classified as a scheduled compound in the

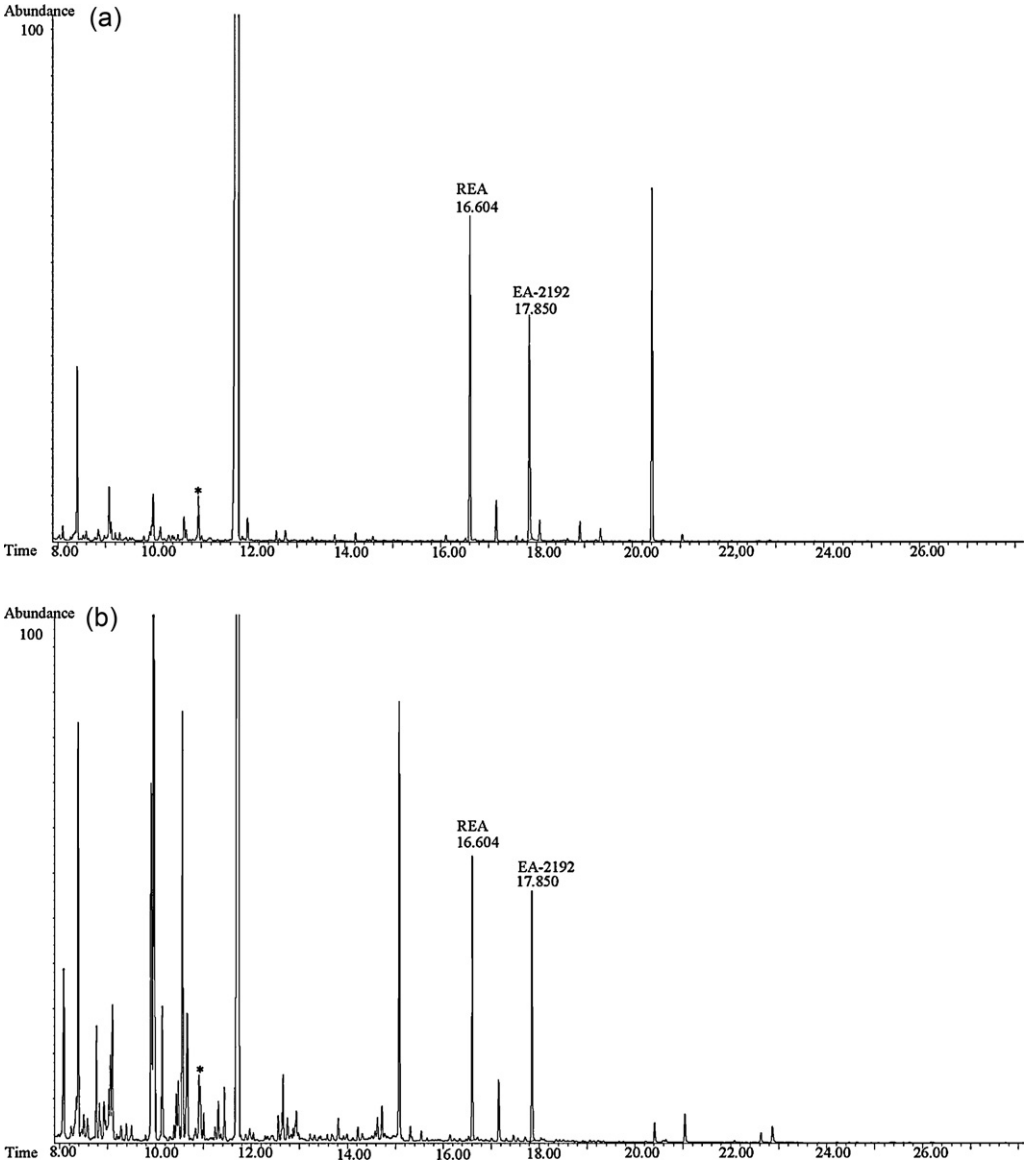

Fig. 4. GC–MS EI analysis of TMS derivatives of EA-2192 and REA in DI water samples. (a and b) Total ion chromatograms showing the comparison of evaporation–silylation method and disk method respectively, (c and d) EI spectra of EA-2192, REA, and their corresponding deuterated analogs (inserts). The mass spectra of both EA-2192 and REA show similar fragmentation patterns. The molecular ions of EA-2192 and REA are *m/z* 311 and *m/z* 283 respectively. Minor silylation byproduct* di(trimethylsilyl) methylthiophosphonate, r.t. 10.939 min, co-eluting with another component. No other chemicals related to the CWC were detected.

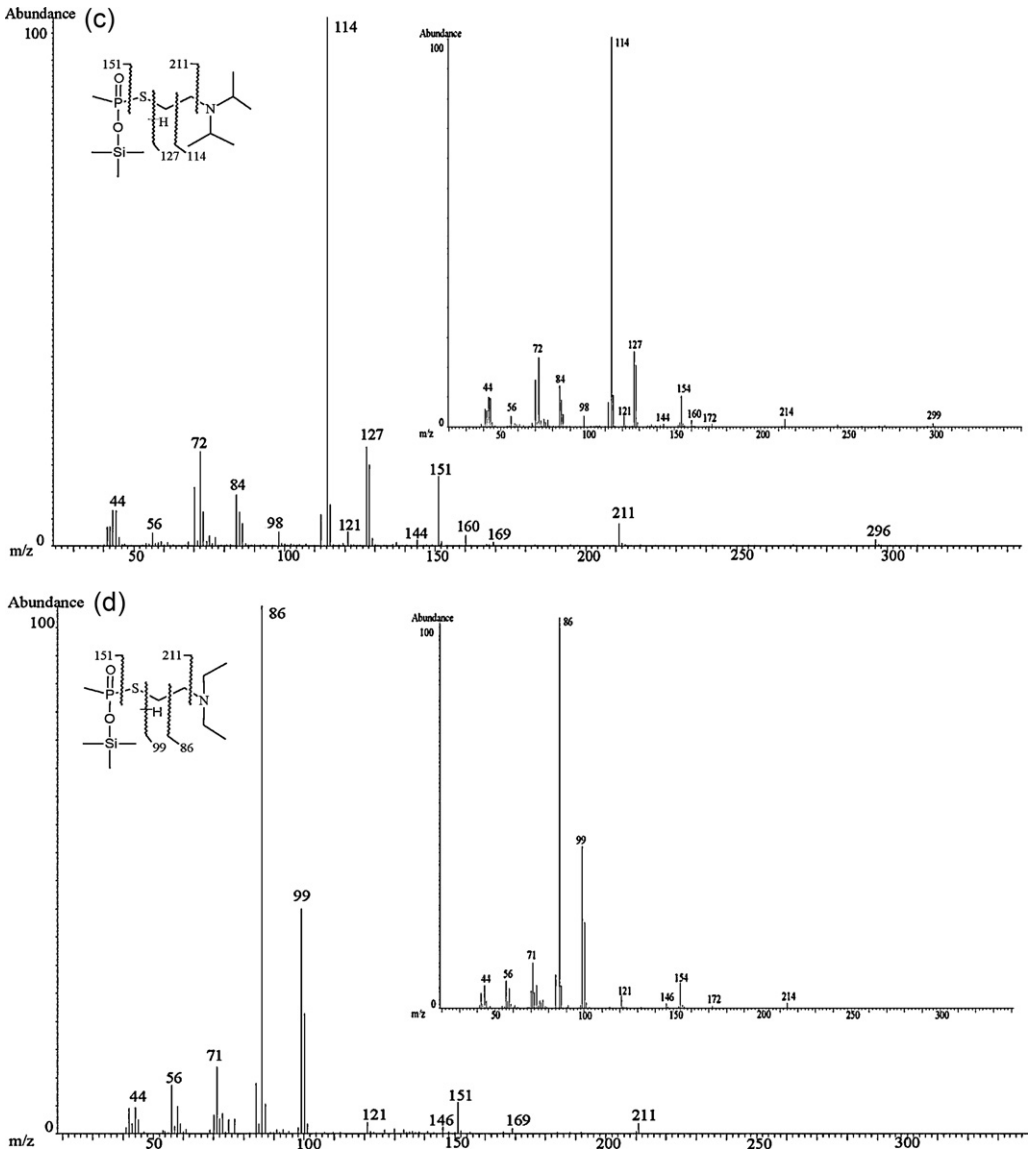

Fig. 4. (*Continued*)

CWC and care should be taken to not cause a false positive identification. However, in the recovery calculations for the disk method, the evaporation–silylation method was chosen as the reference method and its recovery was set to 100%.

In the subsequent experiments, EA-2192 and REA were spiked into PT19 aqueous sample W1 having a salt content of 500 μg/mL (0.05%, equivalent to hard tap-water) and analyzed using the reference method that produced no signal. Further dilution of the

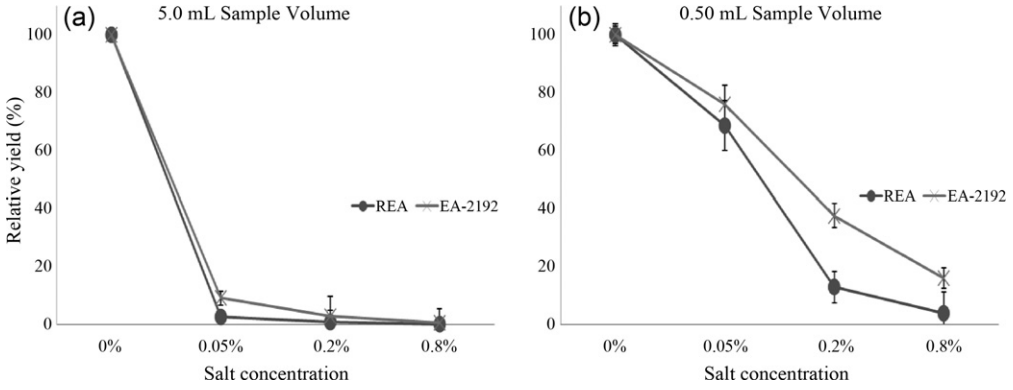

Fig. 5. Salt influence on yield by disk method: (a) 5.0 mL sample volume, (b) 0.50 mL sample volume. The concentration of the spiked target compounds in the samples was 5.0 μg/mL.

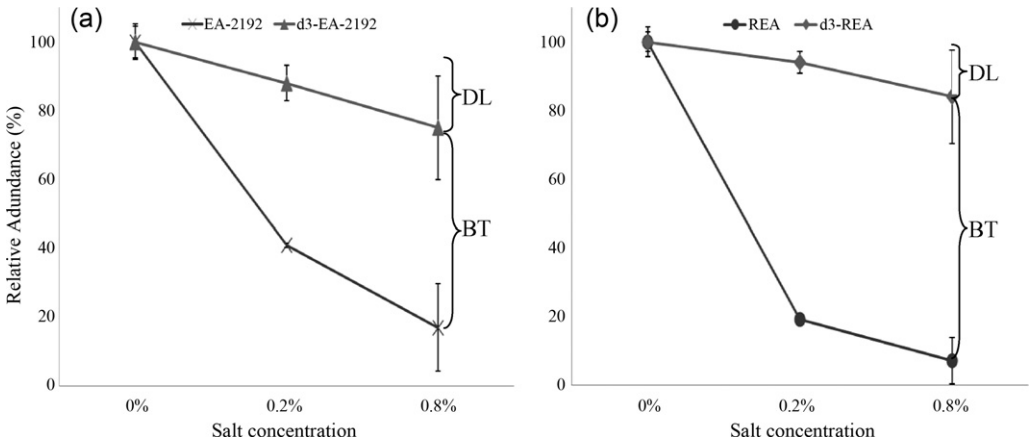

Fig. 6. Relative yield of EA-2192 (a) and REA (b) and their corresponding deuterated standards at different salt concentrations. DL, derivatization loss; BT, breakthrough.

samples to 333 and 167 μg/mL, respectively, using DI water produced the same negative results. Thus the trimethylsilylation of EA-2192 and REA appear to be extremely sensitive to the presence of salt in the sample matrix. In order to verify that the cause of the very low recovery was due to the presence of salt in the sample, additional experiments were made with a range of $MgSO_4$ and $CaCl_2$ concentrations in DI water (0–200 μg/mL). The derivatization results verified the strong negative impact of salt on the derivatization yield (data not shown).

3.2. Performance of the disk method

The disk method, previously evaluated for the analysis of alkyl alkylphosphonic acids [16], was successfully applied for analysis of EA-2192 and REA in DI water samples (Fig. 4b–d) and the recoveries of EA-2192 TMS and REA TMS were 104% and 87% respectively (as compared to the levels found for DI water samples using the reference method) with relative standard deviations of 5–10%. The method detection limits at $S/N \geq 3$ were calculated to be 10 ng/mL and 100 ng/mL using the GC–MS SIM and SCAN mode respectively. Thus, the method is appropriate for use at the 1–10 μg/mL concentration level required by OPCW for verification under the CWC [1].

As can be seen from our initial findings, the trimethylsilylation of EA-2192 and REA is very sensitive to the presence of salt which likely explains the repeated failure of earlier reported sample preparation methods. Also, the salts $MgSO_4$ and $CaCl_2$ have been reported to affect the silylation of alkylphosphonic acids [8]. Taking into consideration that many samples – for example, OPCW proficiency-test water samples and environmental samples – contain various salts, it is critical that the developed method is able to tolerate a reasonable salt content in the samples.

Therefore, a detailed study of the effect of salt on the performance of the disk method was conducted. The results from analysis of 5.0 mL samples using the disk method are shown in Fig. 5a. As expected, the highest yield was obtained for the DI water sample. The yield of EA-2192 and REA drops with the increase in salt concentration. The effect of salt was pronounced in the 0.05% salt sample, with a 3–9% yield relative to DI water, and in the sample containing 0.8% salt, with a yield of less than 0.5%. At 0–0.2% salt, both the spiked chemicals were identified, with average match factors above 80. At 0.8% salt, the match factors were poor for all spiked chemicals. These findings indicate that the 13 mm anion-exchange capacity used in the method is too low to handle high salt concentrations.

Therefore, the sample volume was reduced to 0.50 mL to reduce the amount of salt loaded onto the anion-exchange disk, which was expected to lower the likelihood of saturation of the anion-exchange sites on the disk. This reduced sample volume resulted in improvement of the yield; for example, for the 0.05% salt, to 76% for EA-2192 and to 69% for REA (Fig. 5b). For the 0.50 mL sample volume, the match factors in AMDIS were above 90 for both chemicals at all salt concentrations in spite of the lower total amount of analytes loaded onto the filter. Thus, a sample volume of 0.50 mL would be preferred if a sample has a high salt content. In addition, the salt influences were found to be similar for the alkylphosphonic acids (EMPA, iBMPA and MPA) (data not shown).

To separate the salt impact on the SPE filter breakthrough and on the derivatization efficiency, the deuterated analogs were added directly to the sample vial together with the dried SPE-disk used for sampling [16]. Then the sample was derivatized and analyzed. The results for EA-2192, REA, and their corresponding deuterated analogs are given in Fig. 6a and b. The relative yield for deuterated EA-2192 and deuterated REA dropped by ~20% at 0.8% salt concentration, which corresponds to the loss in derivatization efficiency that could be explained by the presence of anions on the SPE disk. The main contributing factor to yield losses was SPE breakthrough. The relative losses were close to 80% at 0.8% salt concentration. The presence of other anions, such as chloride, could have interfered with the zwitterions for the ion-exchange sites causing breakthrough of zwitterions during sample introduction through SPE. Taking into consideration that the SPE breakthrough represents the biggest yield losses, the sample eluent from SPE could be collected and the disk procedure repeated to improve the yield if low levels are present or when quantitation is the aim of the analysis.

3.3. Application of the disk method for determination of EA-2192 and REA in proficiency test and environmental samples

The OPCW PT19 sample and the spiked environmental water samples were used to evaluate the robustness of the disk method. The target compounds in the PT19, river water, and Baltic Bay water samples were successfully identified with purity >89% and match factors above 80, according to AMDIS deconvolution and the OCAD library. The manual background subtraction and library search in NIST using the OCAD library improved the spectra match factors and, in combination with the retention time of the internal standard (retention times differ by 1.4 s), the identification was sufficient. In addition, the combined spectra of both target compounds and their deuterated analogs (Fig. 7a and b) were observed for 'qualifier' ions (m/z 151/154 and m/z 211/214) to identify the target compounds. The successful analysis of these chemicals demonstrated the robustness of the procedure.

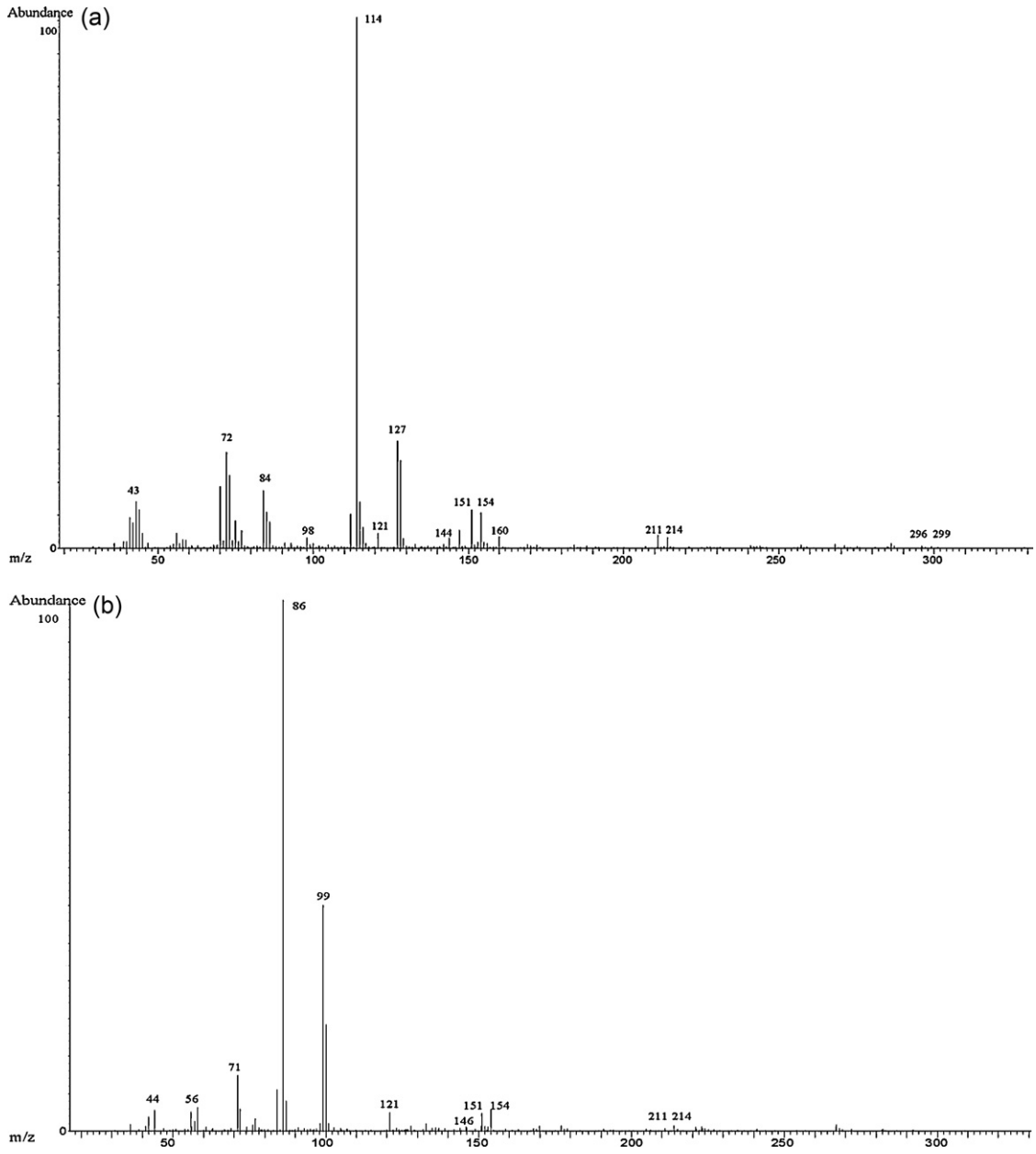

Fig. 7. The combined spectra of (a) EA-2192 and d_3-EA-2192, (b) REA and d_3-REA in the Baltic Bay water sample.

3.4. Application of the disk for storage of EA-2192 and REA

As can be seen from our earlier work [16], SPE disks can be used to store extracts of analytes in dry form before despatch to an off-site laboratory for analysis. The stability of underivatized EA-2192 and REA on the SPE disks was assessed over a 14-day period using portions of the OPCW 19th proficiency test aqueous sample W1 spiked with these chemicals. All recoveries were comparable with the recovery measured on the first day. Considering the toxicity of EA-2192 and logistical issues involved in transporting aqueous samples from the field to a laboratory for analysis, the SPE disk provide a tool to preserve and transport the analytes.

4. Conclusions

A straightforward *in vial* solid-phase derivatization method which enables silylation in high yields for identification of EA-2192 and selected VX degradation products in aqueous samples was evaluated. The results proved that the method meets the requirements for unambiguous identification according to the CWC.

The problems associated with silylation of EA-2192 previously reported in the literature, anticipated as being due to its zwitterionic property, thermal degradation or decomposition on the GC were not observed. Instead, the compounds EA-2192 and REA both gave similar experimental results showing that silylation of the compounds was affected by the sample salt content present in the sample matrix.

In addition, the anion-exchange disk method was demonstrated to cover the determination of the whole range of VX degradation products in aqueous samples which include EMPA, MPA, and EMPTA [16]. The method is likely to also have potential for the determination of other compounds of similar nature.

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