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Analysis of methylphosphonic acid, ethyl methylphosphonic acid and isopropyl methylphosphonic acid at low microgram per liter levels in groundwater

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Abstract

A method is described for determining methylphosphonic acid, ethyl methylphosphonic acid and isopropyl methylphosphonic acid, which are hydrolysis products of the nerve agents VX (S-2-diisopropylaminoethyl O-ethyl methylphosphonothiolate) and GB (sarin, isopropylmethyl phosphonofluoridate). The analytes are extracted 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 derivatized with methanolic trimethylphenylammonium hydroxide. Separation and quantitation are achieved using a capillary column gas chromatograph equipped with a flame photometric detector operated in its phosphorus-selective mode. Two independent statistically-unbiased procedures were employed to determine the detection limits, which ranged between 3 and 9 μg/l, for the three analytes. Published by Elsevier Science B.V.

Keywords: Warfare agents; Methylphosphonic acids

1. Introduction

Alkyl-substituted organophosphorus acids are the primary hydrolysis products of biologically-active organophosphorus compounds such as nerve agents [1] and pesticides. In particular, ethyl methylphosphonic acid (EMPA) and isopropyl methylphosphonic acid (IMPA) are hydrolysis products of the nerve agents VX (S-2-diisopropylaminoethyl O-ethyl methylphosphonothiolate) and GB (sarin, isopropylmethyl phosphonofluoridate), respectively. Both EMPA and IMPA may be further hydrolyzed to methylphosphonic acid (MPA), as shown in Fig. 1. All three phosphonic acids are very water soluble, and their presence can be expected in groundwaters from regions contaminated with VX and/or sarin. Monitoring for these chemicals can indicate the previous presence of those agents, and can guide clean-up operations or monitor for agent production and use. In order for such strategies to be effective, analytical methods must be available which can detect the three aforementioned acids at low μg/l concentrations in groundwater.

Interest in the ultratrace-level detection of the alkylphosphonic acids has increased sharply in the last few years in response to monitoring efforts needed for anti-terrorist activities and to those defined by the Chemical Weapons Convention [2].

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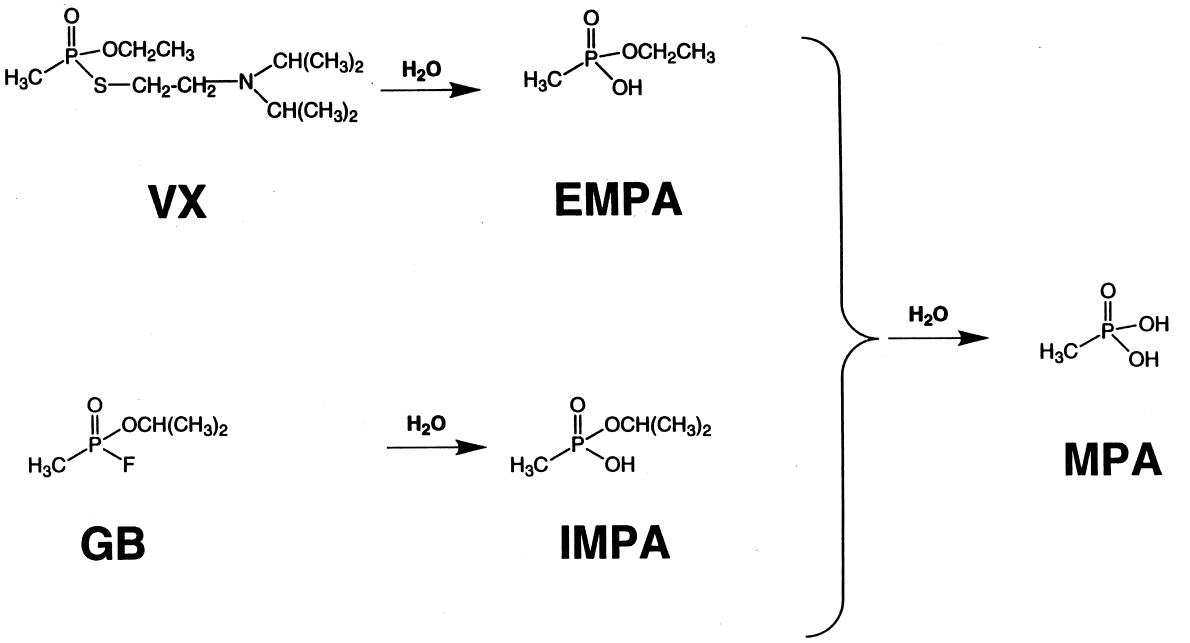

Fig. 1. Hydrolysis mechanism of the nerve agents VX and GB (sarin) into EMPA, IMPA and MPA.

State-of-the-art analytical procedures were employed to demonstrate the deployment of chemical agents during the Iraq–Iran conflict [3]. Ultratrace detection of the alkylphosphonic acids presents unique challenges for the analytical chemist. These species are poor UV absorbers, are nonvolatile and highly polar, and are readily soluble in water. Based on these general considerations, high-performance liquid chromatography (HPLC) would appear to be a suitable method for analysis [4,5]. HPLC detection can employ UV absorbance [4], which requires derivatization of the acids to introduce a chromophore, or conductivity [5]. Sensitivities for IMPA and EMPA range from 4 mg/l to 43 μg/l. Capillary electrophoresis (CE) [6–8] provides high-resolution of the analytes; however, sensitivity is presently in the mg/l range. Creasy et al. [8] described CE of six alkylphosphonic acids, including IMPA and EMPA, in which the UV detection capabilities were improved by adding an absorbing compound (here, phenylphosphonic acid) to the buffer solution. The alkylphosphonic acids were detected when an analyte displaced the absorber from the capillary column. The authors demonstrated baseline resolution, rapid separation (less than 10 min) and ready detection of the analytes at 20 μg/ml; the detection limit was not more than a factor of ten lower. Ion chromatography with conductivity detection [9] is claimed to have sensitivity of ca. 1 μg/l for EMPA and IMPA, but chromatograms of groundwater samples spiked at 50 μg/l showed barely detectable chromatographic peaks. Kientz et al. [10] coupled microcolumn liquid chromatography (LC) with online flame photometric or thermionic detection for the detection of organophosphorus acids isolated from various sample matrices. Black and Read [11] described an application of liquid chromatography–atmospheric chemical ionization mass spectrometry and tandem mass spectrometry which enabled qualitative screening of samples for various nerve agent products. The authors estimated detection limits for MPA, EMPA and IMPA of 200, 10 and 10 μg/l, respectively, using a criterion of S/N~3. Wils and Hulst [12] discussed the determination of organophosphonic and organophosphoric acids using thermospray liquid chromatography–mass spectrometry (LC–MS). Detection limits typically ranged between 10 and 100 μg/l. The authors described the difficulty in quantitating methylphosphonic acid at some length. Very broad peaks were obtained for this analyte in the eluent pH

range tested (3.0–6.0). Eluents with the lower pH values needed to obtain reasonable peak shape would also have destroyed the reversed-phase (C_{18}) column employed. The authors also tested a preconcentration procedure employing Sep-Pak C_{18} cartridges using O-pinacolyl methyphosphonic acid, the primary hydrolysis product of the nerve agent soman, as a model. Because this particular analyte was the largest and least polar of all the analytes tested, it is not clear how well the method would have performed with the smaller more polar analytes discussed in the present paper.

Gas chromatography (GC) using flame photometric detection (FPD) in its phosphorus-selective mode or MS detection is a standard technique for quantitating many phosphorus compounds; however, chemical derivatization of the alkylphosphonic acids to more volatile and thermally stable species is required. Derivatization of the phosphonic acids to their corresponding methyl esters has been performed using diazomethane [13], which is both toxic and explosive. Trimethylsilyl esters [14] or *tert*.-butyldimethyl-silyl esters [15] have been formed from the acids, but the resulting products, particularly the former, may be unstable and sensitive to moisture [16]. Creasy et al. [8] discussed GC–MS techniques using trimethylsilyl derivatives of many types of organic acids, including alkylphosphonic acids. O-Pinacolyl methylphosphonic acid could be detected and positively identified at 100 ppb; the matrix is assumed to be soil. Both Fredricksson et al. [17] and Shih et al. [16] discussed GC–MS procedures which employed an initial derivatization of the alkylphosphonic acids to form pentafluorobenzyl (PFB) esters. Solid-phase extraction (SPE) cartridges employing a C_{18} sorbent were used to collect O-pinacolyl methylphosphonic acid and IMPA because neither aminopropyl nor quaternary amine cartridges would retain these acids from the urine samples. When negative-ion chemical ionization procedures (reagent gas is isobutane) were employed, linear calibration data could be obtained from urine samples fortified to concentrations ranging between 10–200 μg/l. The estimated detection limit for IMPA was 10 μg/l using the criterion S/N~5. Tørnes and Johnsen [18] developed a procedure using SPE on amine ion-exchange columns and methanolic trimethylphenyl ammonium hydroxide (TMPAH) to both elute the acids from the SPE column and to derivatize the acids in the injection port of the GC. Sensitivities of 2 μg/l were achieved for IMPA and EMPA in pure water, but the method performance was not evaluated using groundwater. The authors applied this method to sarin and soman which were spiked into water, grass, sand and soil, then aged for four weeks under summer conditions. Neither agent was detected in the water samples; however, IMPA was one of two degradation products detected in all four matrices [19].

We report modifications to the Tørnes and Johnsen procedure [18] which permit the quantitation of MPA, EMPA and IMPA at μg/l concentrations in groundwater samples containing typical concentrations of dissolved salts. In addition, we have studied the effect of TMPAH concentration on derivatization efficiency to optimize detection sensitivity to these analytes.

2. Experimental

2.1. Chemicals

MPA was purchased from the Aldrich Chemical Co. (Milwaukee, WI, USA) (CAS No. 993-13-5). EMPA (CAS No. 1832-53-7) and IMPA (CAS No. 1832-54-8) were obtained from the US Army Toxic and Hazardous Materials Agency (now the Army Environmental Center) Standard Analytical Reference Materials Repository (Research Triangle Park, NC, USA). The reported purity of EMPA and IMPA as mol% were 97.6% and 99.0%, respectively. Diisopropyl methylphosphonate (DIMP) (CAS No. 1445-75-6) was obtained from Lancaster Synthesis (Windham, NH, USA). TMPAH, 0.1 *M* in methanol (CAS No. 1899-02-1) was purchased from the Eastman Kodak Co. (Rochester, NY, USA) and from Fluka (Ronkonkoma, NY, USA). HPLC-grade water (J.T. Baker, Phillipsburg, NJ, USA) was used for American Society of Testing and Materials (ASTM) Type-II water in sample preparation.

2.2. Sorbents

BakerBond Quaternary Amine (N^+) 3 ml, 500 mg SPE columns, were obtained from J.T. Baker.

Aminopropyl (NH_2) 1 ml, 100 mg SPE columns were purchased from Varian Separations Products Division (Sunnyvale, CA, USA).

2.3. Preparation of standard groundwater surrogate

A standard groundwater surrogate matrix was made by dissolving 165 mg of sodium chloride and 148 mg of sodium sulfate in 1 l of HPLC-grade water. Final chloride and sulfate concentrations were each 100 mg/l. This is the standard groundwater surrogate required for method performance evaluation [20].

2.4. Chromatographic equipment and conditions

A Varian Model 3400 capillary column GC system equipped with FPD in its phosphorus-selective mode and a septum-programmable injector (SPI) was used for all measurements. Automatic plotting and integration of the chromatograms were performed initially using the onboard printer/plotter of the Varian 3400. Later, an external PE/Nelson Model 1020 Personal Integrator (Perkin-Elmer, Norwalk, CT, USA) was used because of its greater control over integration parameters and the ability to make post-run changes in start/stop peak integration points. A Dynatech Model GC 411V (Baton Rouge, LA, USA) or a Varian 8200 CX autosampler was used for automated injections into the GC system.

Thermogreen LB-2 septa (Supelco, Bellefonte, PA, USA) were used in the GC injection port. These septa are designed for low bleed at temperatures up to 350°C. A deactivated glass inlet SPI sleeve with buffer (54 mm×4.5 mm O.D.) was used in the injection port (Restek, Bellefonte, PA, USA). The sleeve was packed in our laboratory with a bottom layer (ca. 0.5 cm) of deactivated fused-silica wool, followed by a layer (ca. 0.8 cm) of deactivated fused-silica beads and a top layer (ca. 0.3 cm) of additional deactivated fused-silica wool. Both the deactivated fused-silica wool and the deactivated fused-silica beads were purchased from Restek.

A capillary Rtx-5 fused-silica column (crossbond 5% diphenyl–95% dimethylpolysiloxane), 30 m× 0.53 mm I.D., 1.5 μm film (Restek) was used for separations. The column oven temperature was programmed linearly from 70°C to 100°C at 5°C/min (hold for 6 min). The injector temperature was 350°C and the detector temperature was set at 200°C. Gas flows were as follows: (a) carrier gas: helium, ultra-high purity, 8.4 ml/min; (b) make-up gas: nitrogen, ultra-high purity, 30 ml/min; (c) FPD gases: hydrogen, ultra-high purity, 140 ml/min; ''air-1,'' breathing air, 80 ml/min; and ''air-2,'' 250 ml/min.

Injection volumes were fixed at 1 μl. Samples were injected after a needle dwell time of 4 s in the injection port. The analyte concentrations were determined using the method of external standards.

2.5. Solid-phase extraction procedure

The SPE columns (500 mg, quaternary amine) were attached to a vacuum manifold (Alltech Associates, Deerfield, IL, USA, 12-port) and left dry because pre-conditioning the columns did not improve either the analyte recoveries or the ''cleanliness'' of the gas chromatograms. Each SPE column was loaded with a sample aliquot to ~80% of its full volume capacity (ca. 2 ml). A 75 ml reservoir was then attached to the column, and the remaining water sample was poured into the reservoir. When each set of samples had been set up on the vacuum manifold, the vacuum was turned on and the flow through each column was set to ~2.5 to 3 ml/min. After all water (50 ml) had drained through the columns, the 75 ml reservoirs were removed and the SPE columns were rinsed with 1 ml of methanol. Vacuum (at −500 mm Hg; 1 mm Hg=133.322 Pa) was applied to the SPE columns for 1 h to be certain they were thoroughly dry. After drying, the vacuum was released and a rack holding collection vessels was placed in the chamber. Analytes bound to the SPE columns were eluted using a single 1.4 ml aliquot of 0.3 *M* TMPAH and collected directly into autosampler vials. The volume of TMPAH recovered was nominally 1 ml, with ~0.3 ml being retained on the SPE column. Exact volumes recovered for each sample were determined by weighing. (The density of 0.3 *M* TMPAH in methanol at 25°C was determined gravimetrically to be 0.885 g/ml).

In the evaluation of the effect of salt on the recoveries of the analytes (see immediately below), other SPE phases were tested, and a different elution procedure was used. Calibrated Kuderna–Danish

receivers (10 ml capacity) were positioned below the SPE columns (aminopropyl or quaternary amine). Analytes bound to the SPE columns were eluted using three 1-ml aliquots of 0.1 *M* TMPAH and collected in the Kuderna–Danish tubes. (Each 1 ml aliquot was drawn through the SPE column in ~15 s). The combined eluates were then evaporated in place, in the chamber of the SPE manifold, to exactly 1 ml. With the three-fold concentration of the eluate, the final concentration of TMPAH was 0.3 *M* in methanol. Samples were transferred to autosampler vials with disposable glass pipettes and analyzed as described.

2.6. Effect of dissolved salt on recovery of the analytes from an aqueous matrix

50-ml aliquots of either ASTM Type-II water or surrogate groundwater (100 mg/l in both chloride and sulfate) were spiked with a methanol solution of EMPA, IMPA and MPA to a concentration of 50 μg/l in each analyte (total methanol added: 100 μl). The samples were subjected to SPE and elution as described.

2.7. Effect of TMPAH molarity on peak response

Solutions of 0.1 *M* TMPAH in methanol were concentrated to 0.2, 0.3 and 0.4 *M* using a gentle stream of dry flowing nitrogen. For each TMPAH concentration, a 50-μl aliquot of MPA, EMPA and IMPA in methanol was added to 940 μl of methanolic TMPAH solution. 10 μl of DIMP (125 μg/ml in methanol) was added to each sample as an internal standard. The final concentration of each analyte was 2.5 μg/ml (1 ml final volume). Three sets of analyte standards were prepared for each concentration of TMPAH.

3. Results and discussion

3.1. GC of MPA, EMPA and IMPA using FPD

Typical chromatograms of MPA, EMPA and IMPA, shown in Fig. 2, were obtained using the GC conditions described in Section 2. FPD was used with these analytes to reduce possible interferences

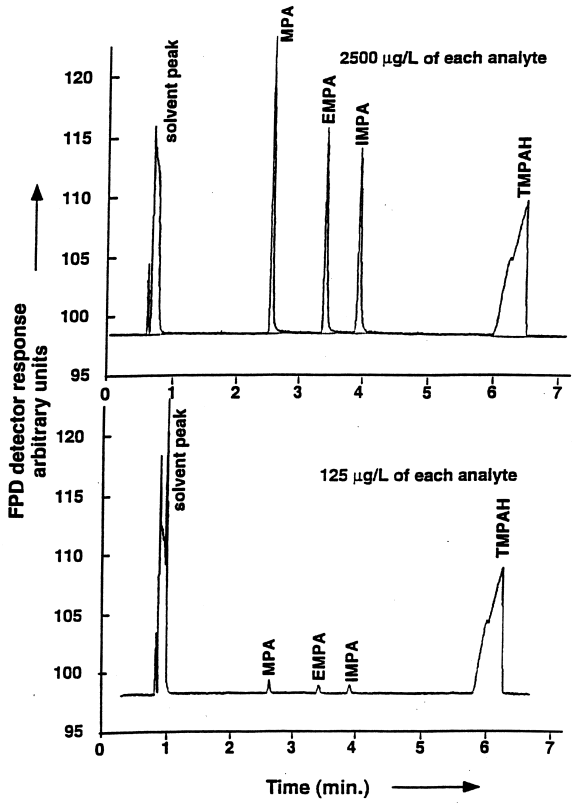

Fig. 2. Typical chromatograms of MPA, EMPA and IMPA obtained under conditions described in Section 2 using 0.3 *M* TMPAH.

from other non-phosphorus containing components present in extracted groundwater samples. Each chromatographic separation was completed in 7 min (total run time was 12 min) and all three analytes were well resolved both from each other and from dimethyl sulfate and trimethyl phosphate (not shown), which are potential methylation products of inorganic sulfate and phosphate in groundwater. The peak shapes were sharp, narrow and symmetrical, with little tailing even at the highest concentrations injected. Standard analyte concentrations as low as 125 μg/l were measurable in the derivatized SPE extracts, as shown in Fig. 2. Since the procedures reported here involve extraction of the analytes from 50 ml of groundwater, analyte detectability would be expected to be about 50-fold lower or about 2 μg/l of each analyte in the groundwater.

Calibration data for standards covering the forty-

fold concentration range from 125 μg/l to 5000 μg/l were compiled for all three analytes and analyzed statistically using both linear regression and ''lack of fit'' calculations. The linear regression analysis demonstrated that the calibration data for each analyte exhibited a linear fit with $r^2>0.995$, while the ''lack of fit'' tests showed that the same data were well-described by a linear model with a zero intercept.

3.2. Effect of TMPAH concentration on derivatization efficiency

The analytes were converted to their methyl esters by reaction with TMPAH in the GC inlet. The derivatization efficiency was improved by packing the inlet liner with deactivated fused-silica wool and beads to enhance gas phase mixing and to provide additional surface area which might promote reaction. The effect of the TMPAH concentration on the formation of the methyl esters, as indicated by their peak areas, is shown in Fig. 3. As the concentration of TMPAH increased from 0.1 *M* to 0.3 *M*, there was an increase in the peak areas of all three analytes. At 0.4 *M* TMPAH, the average peak area for MPA began to drop while the peak areas for EMPA and IMPA both continued to increase slightly. In view of the drop in peak area for MPA when 0.4 *M* TMPAH was used, further experiments were all conducted using 0.3 *M* TMPAH. Concentrating the commercially available 0.1 *M* TMPAH with dry flowing nitrogen produced a cleaner chromatogram than concentrating with dry flowing air.

The data in Fig. 3 indicate that concentrating the TMPAH from 0.1 *M* to 0.3 *M* resulted in almost an eight-fold increase in the peak area for MPA, and about a 60% increase in the peak areas for both EMPA and IMPA. While the peak areas of all three analytes increased very significantly when using 0.3 *M* TMPAH instead of the 0.1 *M* (standard commercial preparation), there was no increase in noise in the GC baseline. Furthermore, using the higher molarity of TMPAH for both derivatization and blank injections reduced the number of blanks that had to be run between sample injections in order to remove ghost peaks from the baseline. The ghosting was attributed to traces of underivatized analytes left in the GC inlet liner or the front section of the column after an injection. Instead of making several blank injections of 0.1 *M* TMPAH, only one blank was needed to restore the baseline when using 0.3 *M* TMPAH for derivatization and as the blank.

It is interesting to note the ratio of derivatizing agent molecules (TMPAH) to analyte molecules for the samples shown in Fig. 3. There was already a nearly 1000-fold excess of TMPAH molecules over the total ionizable protons present on the three analytes when 0.1 *M* TMPAH was used with 2.5 μg of each analyte (equivalent to SPE of 50 ml of water having 50 μg/l of each analyte). Yet, when the TMPAH concentration was increased to 0.2 and 0.3

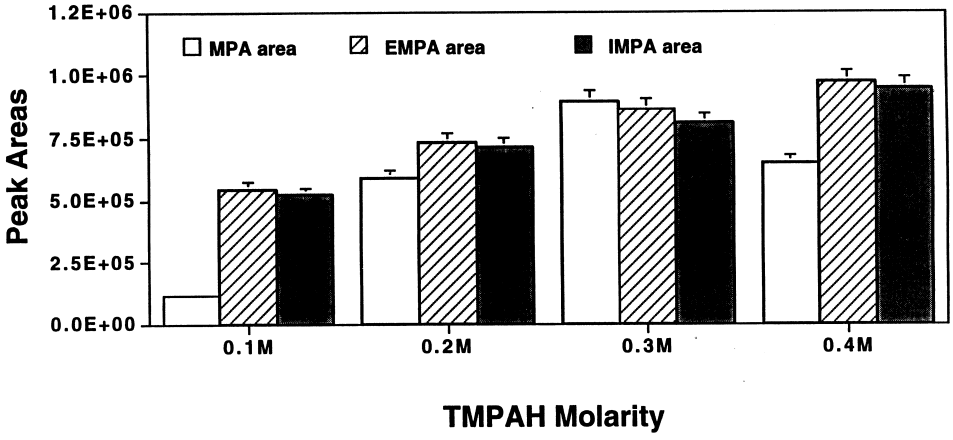

Fig. 3. GC peak areas obtained for 2.5 μg/ml standards of MPA, EMPA and IMPA as a function of TMPAH concentration. Triplicate analyses performed for each TMPAH concentration. Error bars represent ±1 standard error.

M, there were significant increases in the number of derivatized molecules formed, as measured by increases in the analyte peak areas. The increase in the efficiency of the derivatization was especially apparent for MPA, where the peak areas increased roughly as the square of the TMPAH concentration in the range of 0.1 *M* to 0.3 *M*. (It is not known why the derivatized MPA concentration decreased when using 0.4 *M* TMPAH). Since MPA has two ionizable protons, each requiring one TMPAH molecule for derivatization, it was expected that the concentration of the doubly-methylated MPA should increase as the square of the TMPAH concentration.

The high ratio of TMPAH molecules to analyte molecules needed for methylated derivatives to be formed, even at 350°C (the GC injection port temperature), shows that TMPAH-derivatization of all three analytes was not easily achieved. Reactions in the gas phase or catalyzed on surfaces in the GC inlet must be less efficient than those conducted in solution. Nonetheless, high concentrations of TMPAH coupled with a high reaction temperature (350°C inlet) and additional surface area in the GC inlet liner (from the glass wool and beads) were able to drive the derivatizations to a reasonable completion. The FPD temperature was initially set at 350°C but was later reduced to 200°C to prolong the life of components such as the Pyrex window and high-temperature rubber gaskets. The lower detector temperature had no noticeable effect on the GC peak areas.

3.3. Effect of the presence of chloride and sulfate on the recovery of MPA, EMPA and IMPA from amino- and quaternary amine SPE columns

In an earlier work, Tørnes and Johnsen [18] used aminopropyl (NH_2) SPE columns to recover IMPA and 1,2,2-trimethylpropyl methylphosphonic acid (TMPA) from water samples. Their best reported recoveries were 88% and 75% for IMPA and TMPA, respectively. We likewise had good success in using 100 mg NH_2 SPE columns to recover MPA, EMPA and IMPA that were dissolved in ASTM Type-II water using elution with 0.1 *M* TMPAH, as described in Section 2 (see Fig. 4A) However, when the three analytes were dissolved in standard groundwater surrogate (100 mg/l each of chloride and sulfate)

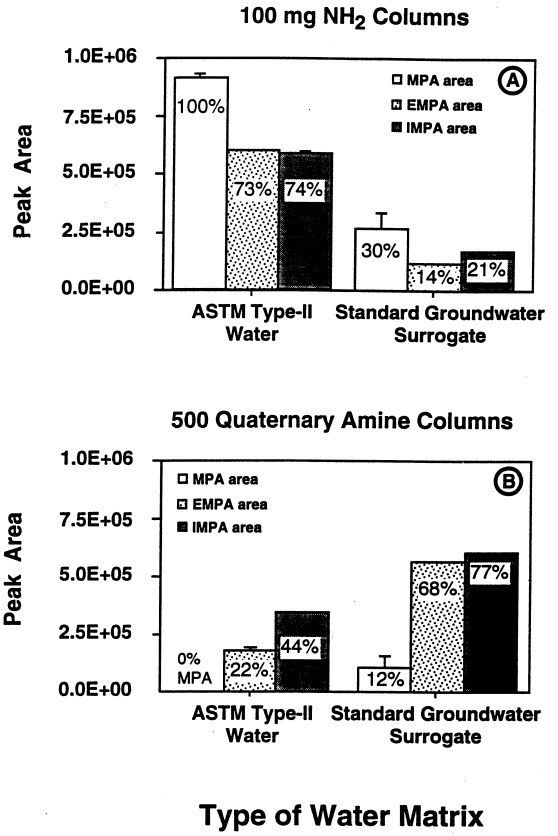

Fig. 4. Recoveries of MPA, EMPA and IMPA from 50 ml aliquots of ASTM Type-II water or ASTM synthetic groundwater. (A) Analytes recovered using aminopropyl SPE columns. (B) Analytes recovered using quaternary amine SPE columns. Three independent samples were extracted under each condition, and eluted using 0.1 *M* TMPAH. Error bars represent ±1 standard error.

designed to mimic typical groundwater, recoveries were drastically reduced, as shown in Fig. 4A. Subsequently, several other types of SPE columns were tested, including cyano-, mixed primary and secondary amino- and quaternary amine SPE columns. The best recoveries were obtained using 500 mg quaternary amine columns.

The results of one experiment using the quaternary amine SPE columns are shown in Fig. 4B. For direct comparison with Fig. 4A, the same 0.1 *M* TMPAH method (as described in Section 2) was used for elution of the analytes. Recoveries of IMPA and EMPA from the standard groundwater surrogate were particularly improved when the quaternary amine

SPE columns were used. The recovery of MPA from this experiment was only about 12%. Later experiments used 0.3 *M* TMPAH to improve the recoveries of the analytes from the quaternary amine columns further (see below).

The differences in analyte recoveries from the two types of ion-exchange SPE columns studied in this paper may be explained as follows: aminopropyl groups formed weaker ionic interactions with the analytes than the more strongly positively charged quaternary amine groups. When the only ions present in the water were the negatively charged MPA, EMPA and IMPA analytes, the aminopropyl groups were able to form sufficiently strong ionic bonds with the analytes to extract them out of the water and retain them until they were eluted with the TMPAH. Chloride and sulfate ions, which were present in the groundwaters at concentrations much greater than those of the alkyl phosphonic acids, successfully competed for the binding sites of the aminopropyl SPE phase, resulting in poor analyte recoveries (Fig. 4A). When more basic quaternary amine groups bound the analytes, the ionic interaction was stronger. Our data (Fig. 4B) show that this stronger ionic interaction was sufficient to retain the analytes in the presence of chloride and sulfate, at least at concentrations up to 100 mg/l of each competing anion.

Fig. 4 also shows that when the analytes were present in ASTM Type-II water, the aminopropyl columns yielded higher recoveries of the analytes than did the quaternary amine columns. This result again reflects the tighter binding of the analytes to the quaternary amine groups. In the absence of chloride and sulfate, which otherwise would have occupied the excess binding sites, the TMPAH was not able to elute the analytes as effectively from the quaternary amine groups as from the weaker-binding aminopropyl groups. The effect was most notable for the MPA (with two ionizable protons). The MPA was apparently bound so strongly to the quaternary amine functionalities that TMPAH was unable to elute it.

3.4. Determination of the Method Reporting Limit and Method Detection Limit

The method performance was evaluated using the standard groundwater surrogate and two statistically-unbiased protocols: the US Army Method Reporting Limit (MRL) protocol [20,21] and the US Environmental Protection Agency Method Detection Limit (MDL) protocol [21,22]. The former is equivalent to determining a ''found'' concentration so that both the false positive and the false negative errors are 5% [20], while the latter is the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero [22].

The MRL was evaluated using the protocol established by the US Army [20], which is discussed in detail elsewhere [21]. Briefly, the standard groundwater surrogate is spiked at six concentrations ranging from 2.5 to 100 μg/l, or 0.5 to 20-times the target reporting limit (TRL) of 5 μg/l. Samples were prepared using the procedure with 0.3 *M* TMPAH, as described in Section 2, and analyzed on two consecutive days. The spiked (''true'') and analyzed (''found'') concentrations of the analytes are listed in Table 1. The MRL values were calculated using software supplied by the sponsor. When all of the available data were evaluated, i.e., six concentration levels and the related blanks from both certification days, the calculated MRL values for MPA, EMPA and IMPA were 9.4, 3.7 and 4.9 μg/l, respectively. Somewhat lower MRL values for two of the analytes were obtained by truncating the range of the ''found'' versus the ''true'' values, as described in Ref. [20], until the slope of the regression line changed by greater than 10%. Under these restrictions, the regression line was truncated to four

Table 1
Comparison of ''found'' versus ''true'' concentrations of MPA, EMPA and IMPA in Method Reporting Limit (MRL) certification samples

''True'' concentration (μg/l)	Average ''found'' concentration[a] (μg/l)		
	MPA	EMPA	IMPA
0.0	3.4	0.9	0.9
2.5	3.1	5.1	3.3
5.0	4.0	8.6	6.8
10.0	5.5	15.6	11.6
25.0	13.6	31.0	25.1
50.0	26.9	59.3	46.5
100.0	54.8	118.4	92.5

[a] Values not corrected for recovery.

Table 2
Determination of the Method Detection Limit (MDL) for MPA, EMPA and IMPA from synthetic groundwater

Sample	"Found" concentrations, μg/l		
	MPA	EMPA	IMPA
1	23.35	36.82	26.19
2	25.89	33.26	29.00
3	21.92	29.30	24.60
4	27.10	32.80	30.39
5	23.71	31.71	26.60
6	25.23	33.21	28.30
7	24.25	31.04	27.21
Mean	24.49	32.59	27.47
Experimental S.D.	1.72	2.34	1.93
MDL	5.41	7.34	6.05
% Recovery±S.D.	98.0±6.9	130±9.4	110±7.7

S.D.=Standard deviation.

concentration levels (2.5 to 25 μg/l) and the related blanks. The final MRL values for MPA, EMPA and IMPA were 8.5, 4.3 and 2.9 μg/l, respectively. The recoveries were 53%, 116% and 91% for MPA, EMPA and IMPA, respectively.

MDL evaluation [21,22] also was performed for all three analytes, each spiked at 25 μg/l, or five-times the TRL, in standard groundwater surrogate. Seven replicates were prepared using the procedure with 0.3 *M* TMPAH and analyzed. The experimental standard deviation (S.D.) was calculated for each analyte, then multiplied by 3.143, the one-tailed Student's *t*-value corresponding to 99% confidence and 6 degrees of freedom (appropriate for seven samples) to calculate the MDL [22]. The results are shown in Table 2. The MDL values were 5.4, 7.3 and 6.1 μg/l for MPA, EMPA and IMPA, respectively. The MRL and MDL values agree within a factor of two, with the latter consistently greater for EMPA and IMPA. As Table 2 shows, there was complete recovery of MPA and IMPA, and a slight high bias to the recovery of EMPA. The reason for this bias is not known.

4. Conclusions

The combination of SPE on 500 mg quaternary amine columns, elution/derivatization to the methyl ester using 0.3 *M* TMPAH, and detection by GC–FPD allows low μg/l concentrations of MPA, EMPA and IMPA in 50 ml volumes of groundwater to be determined confidently.

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