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Effect of cation-exchange pretreatment of aqueous soil extracts on the gas chromatographic–mass spectrometric determination of nerve agent hydrolysis products after *tert*.-butyldimethylsilylation

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Abstract

The efficency of pretreatment of aqueous soil extracts using a cation-exchange resin has been investigated by gas chromatographic–mass spectrometric (GC–MS) determination of nerve agent hydrolysis products after *tert*.-butyldimethylsilyl (TBDMS) derivatization. An aqueous solution containing methylphosphonic acid (MPA) and its monoalkyl esters, ethyl methylphosphonic acid, isopropyl methylphosphonic acid and pinacolyl methylphosphonic acid, was dried, and these phosphonic acids were derivatized with *N*-methyl-*N*-(*tert*.-butyldimethylsilyl)trifluoro-acetamide and analyzed by GC–MS. The yields of TBDMS derivatives were significantly decreased by the addition of calcium and magnesium ions to an aqueous solution (≈0.5 m*M*) before derivatization. The extent of lowered yields was related to the hydrophilicity of phosphonic acids. MPA and its monoalkyl esters were spiked into soil samples (sand, alluvial soil and volcanic ash soil), extracted with distilled water, dried, silylated and applied to GC–MS. The yields of TBDMS derivatives of monoalkyl esters from soil samples were low (3–42%) and MPA derivative was scarcely detected (yield: <0.7%). By desalting the aqueous soil extract by passage through a strong cation-exchange resin, the yields of TBDMS derivatives of monoalkyl esters were significantly improved (12–69%) and MPA derivative was detected (yield: 2–36%). The extent of improved yields was related to the concentrations of divalent metal cations in aqueous soil extracts. In combination with desalting by the cation-exchange resin, GC–MS after TBDMS derivatization enables detection of nerve agent hydrolysis products in soils at sub-ppm (0.2 μg/g) concentrations.

Keywords: Sample handling; Soil; Warfare agents; Methylphosphonic acids; Alkylphosphonic acids

1. Introduction

Lethal nerve gas attacks in Matsumoto city in 1994 and in the Tokyo Subway System in 1995 have killed 19 persons and injured many people [1] and gave us an extraordinarily great shock about illegal usage of the chemical warfare agent (CWA) ‘sarin’. The Matsumoto Sarin incident is the first crime case in which a nerve agent was used for mass terrorism against defenceless people. These terrorism incidents were allegedly caused by the Aum Shinrikyo, Japanese Cult. On the other hand, the Chemical Weapons Convention, which entered into force in April 1997,

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bans the production, stockpiling and use of chemical weapons. In this context, it is important to verify the existence of CWA in sites suspected of CWA production. As one of the primary analytical methods, mass spectrometry is essential to provide convincing evidence for identification of CWA [2].

The nerve agents sarin, soman, tabun and VX, are representatives of organophosphorus compounds used as CWA's. They are rather volatile and easy to degrade. In water, nerve agents are easily hydrolyzed to produce characteristic compounds containing the methyl-phosphorus bond which never exists in nature (Fig. 1). These alkyl methylphosphonates are specific for the original nerve agents. Isopropyl methylphosphonic acid (IMPA) is specific for sarin, ethyl methylphosphonic acid (EMPA) is specific for VX and pinacolyl methylphosphonic acid (PMPA) is specific for soman. These are finally hydrolyzed to methylphosphonic acid (MPA). Tabun has a different degradation process. It is expected that their concentrations in evidence samples are low. Even though nerve agents may not be detected, it is important to detect their hydrolysis products [3].

As for determination of MPA and its monoalkyl esters, gas chromatographic–mass spectrometric (GC–MS) techniques combined with *tert*.-butyldimethylsilylation (TBDMS) [4] seem to be the most useful method for verifying them. One of the authors has already developed a GC–MS method for determination of phosphorus-containing herbicides such as glyphosate using TBDMS derivatization [5]. Our laboratory has been engaged in forensic investigation on both the above-mentioned gas attack incidents and the other Aum-related cases [6]. We have analyzed many samples including victim's blood and on-site samples by GC–MS after TBDMS derivatization. In the course of our forensic investigation, we have observed low detectability of nerve agent hydrolysis products from evidence samples, especially from soil samples. Soils are typical environmental samples, and it is reported that soil analysis by GC–MS after derivatization suffers from serious problems in detectability of low levels of nerve agent hydrolysis products [7]. Another paper also reported the low recovery of MPA and its monoalkyl esters from soil samples in the detection by GC with atomic emission detection after trimethylsilylation [8]. It is suggested that low detectability may be due to interference by divalent metal ions [9], and the usage of an ion-exchange cartridge to remove cations was recommended for pretreatment of soil [10] and water samples [11,12]. However, there was no report quantitatively examining the efficacy of ion-exchange pretreatment. In this

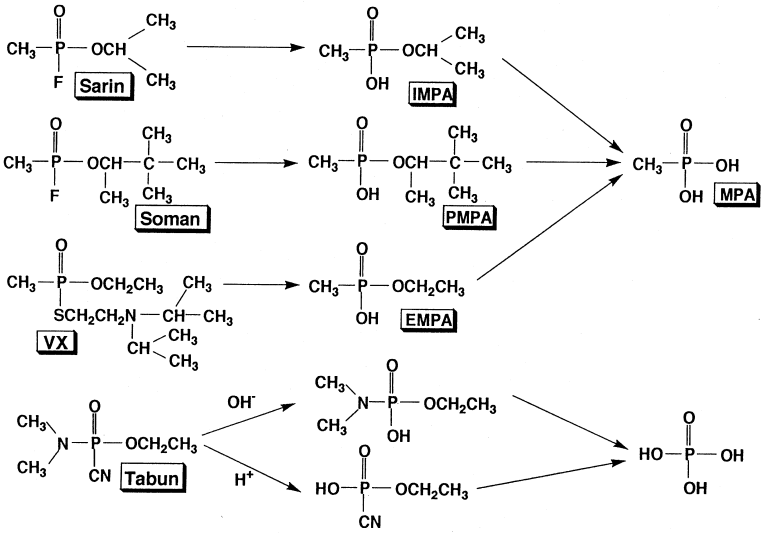

Fig. 1. Hydrolysis routes of nerve agents. EMPA: ethyl methylphosphonic acid; IMPA: isopropyl methylphosphonic acid; PMPA: pinacolyl methylphosphonic acid; MPA: methylphosphonic acid.

paper, we have investigated the efficiency of desalting of aqueous soil extracts using a cation-exchange resin by GC–MS determination of TBDMS derivatives of nerve agent hydrolysis products.

2. Experimental

2.1. Reagents

The strong cation-exchange resin, Dowex 50W-X8 (50–100 mesh) was obtained from Muromachi Kagaku Kogyo (Tokyo, Japan). *N*-Methyl-*N*-(*tert*.-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) was obtained from Pierce (Rockford, IL, USA). MPA, EMPA and PMPA were obtained from Aldrich (Milwaukee, WI, USA). Methylphosphonic dichloride was from Tokyo Chemical Industry (Tokyo, Japan). The other chemicals used were of analytical reagent grade.

2.2. Preparation of isopropyl methylphosphonic acid

Isopropyl methylphosphonic acid (IMPA) was prepared as follows. Into a closed and stirred vial containing 1.28 ml of methylphosphonyl dichloride (14.4 mmol) in 1 ml of dichloromethane, 1.22 ml of isopropanol (15.9 mmol) was added slowly over 30 min, and the mixture was stirred for more than 8 h with cooling. Then, the solution was allowed to reach room temperature and stirred for 12 h. After that, 1 ml of water was added to the mixture with cooling to convert the isopropyl methylphosphonochloridate produced to IMPA and the mixture was stirred for 5 min. After complete evaporation of the reaction mixture to dryness, the residue was chromatographed on silica gel 60 (Merck, Darmstadt, Germany) using *n*-hexane–ethyl acetate–methanol to give IMPA as an oil. IMPA was further purified with reduced distillation (109°C, 0.9 mmHg; 1 mmHg= 133.322 Pa) to give a colorless oil (1.09 g, yield 56%) and confirmed by ^{1}H-NMR in C^{2}HCl$_3$ [δ1.36(d, 6H, C-CH_3), δ1.49 (d, 3H, P-CH_3), δ4.66 (m, 1H, O-C*H*-C), δ6.60 (s, 1H, *H*O-P)].

2.3. tert.-Butyldimethylsilylation and gas chromatographic–mass spectrometry of methylphosphonic acid and its monoalkyl esters

MPA, EMPA, IMPA and PMPA were dissolved in acetonitrile (400 ppm) and stored at −20° as stock solution. The working solution was prepared by dissolving it with distilled water. An aliquot of the working solution was transferred to a 1-ml glass vial stoppered with a PTFE screw cap (Nichiden Rika Garasu, type MV-07, Tokyo, Japan), and dried by a model VC-360 centrifugal concentrator (Taitec, Saitama, Japan) with reduced pressure at 50°C. Fifty μl of MTBSTFA and 50 μl of acetonitrile including 100 ppm *n*-nonadecane [internal standard (I.S.)] were added, homogenized with sonication for 5 min and incubated at 60°C for 1 h. Two μl of the resultant mixture was applied to the following gas chromatograph.

The GC–MS system consisted of a HP 5890 series II gas chromatograph combined with a HP 5989B quadrupole mass spectrometer (obtained from Yokowaga Analytical Systems, Tokyo, Japan). The stationary phase was a capillary (5% phenyl)methylpolysiloxane fused-silica column DB-5 MS (30 m×0.25 mm I.D., 0.25 μm thickness, J&W Scientific, Folson, CA, USA). Carrier-gas (helium) flow-rate was 0.64 ml/min. Injection port, transfer line and ion source were maintained at 250°C, 280°C and 250°C, respectively. The splitter ratio was adjusted to 42. Electron impact ionization (ionization energy 70 eV, ionization current 60 μA) was used as ionization mode. The oven temperature was controlled by a temperature program [starting from 90°C (1 min held), then to 290°C by 20°C per min (5 min held)]. The acquisition mass range was 50–550, and sampling was at 0.8 scan/s. Acquisition was started 4 min after sample injection. Extracted ion chromatograms were obtained at m/z 153 for EMPA, IMPA and PMPA derivatives, m/z 267 for MPA derivative and m/z 85 for I.S..

2.4. Sample treatment for soils and extraction with water

The following soil samples were used. Soil sample No. 1 (sand) was collected from a seashore in Okayama prefecture (Japan). Soil sample No. 2

(alluvial soil) was collected from the garden in Kyoto prefecture (Japan). Soil sample No. 3 (volcanic ash soil) was collected from the garden in our Institute. These soil samples were filtered through a 2-mm sieve and dried at room temperature for several days. Soil samples No. 1 and No. 2 were characterized [13] as shown in Table 1. Because of limited volume, soil sample No. 3 was not characterized.

MPA and its monoalkyl esters in acetonitrile solution were spiked to 2 g of the soil samples and stood at room temperature for 3 h. Four ml of distilled water was added and vortexed for 1 min and sonicated for 10 min. After centrifugation at 1500×*g* for 5 min, the resultant supernatant was filtered through a 0.45-μm cellulose acetate membrane, and an aliquot (0.5–1.0 ml) was applied to the above mentioned TBDMS derivatization and GC–MS.

2.5. Desalting using cation-exchange resin

A commercial strong cation-exchange resin (Dowex 50 W) was activated according to the manufacturer's protocol, converted to the H^+ form and equilibrated with distilled water. Two ml of aqueous soil extract was applied to the column packed with 2 ml of resin and eluted with distilled water. Combined elution fraction of both effluent (2 ml) and eluate (3 ml) was neutralized with sodium hydrogencarbonate (pH≈7) and concentrated with reduced pressure at 50°C by a rotary evaporator and a centrifugal concentrator, and applied to the above mentioned TBDMS derivatization and GC–MS.

Table 1
Chemical and physical characteristics of the soil samples used

		Soil No. 1 (Sand)	Soil No. 2 (Alluvial soil)
Organic matter (g/100 g)		0.0	3.6
Moisture (%)		0.3	8.4
Cation-exchange capacity (mequiv/100 g)		1.1	20.5
Phosphorus absorption coefficient		118	548
Granulation (%)	Clay <2 μm	0.9	14.0
	Silt 2–20 μm	0.7	13.3
	Sand >20 μm	98.4	72.7

2.6. Determination of metal cations in aqueous soil extract

Concentrations of sodium, potassium, calcium and magnesium ions in an aqueous soil extract were measured by capillary electrophoresis on a Quanta 4000E instrument (Waters, Milford, MA, USA). The capillary column used was fused-silica (60 cm×75 μm I.D.), and the electrophoresis buffer was Ion Select Low Mobility Cation Electrolyte. Voltage was set at 20 kV with a positive power supply. Detection was indirect ultraviolet absorption at 214 nm. Column temperature was maintained at 25°C. Samples were applied hydrostatically for 30 s.

3. Results

3.1. GC–MS of tert.-butyldimethylsilylated nerve agent hydrolysis products

As shown in Fig. 2A, TBDMS derivatives of nerve agent hydrolysis products were well separated from each other on a slightly polar DB-5 capillary column. The mass spectra of the derivatives showed typical fragmentation patterns. For the MPA derivative, the peak of *m*/*z* 267 corresponding to the ion $[M-57(tert.\text{-butyl})+H]^+$ was found as base peak. For alkyl methylphosphonic acid derivatives, peak of *m*/*z* 153 corresponding to the ion $[M-57(tert.\text{-butyl})\text{-}(alkyl)+H]^+$ was found as base peak. In the analytical system, manual GC injection was performed because the total sample volume of the derivatizing vial was low (0.1 ml), and so the volume injected could not be controlled correctly. Therefore, we have adopted the I.S. method using *n*-nonadecane for quantitative analysis. On extracted ion chromatograms of *m*/*z* 153, *m*/*z* 267 and *m*/*z* 85, baseline separation of all TBDMS derivative peaks and the I.S. peak could be achieved (Fig. 2). Calibration curves for peak area ratios of TBDMS derivatives of phosphonic acids to I.S. were linear for the amounts in the reaction vial, ranging from 0.15 to 4.5 μg with correlation coefficients better than 0.996 and the repeatability ($n=7$) in the determination of 0.8 μg phosphonic acids in the vial ranged from 9.8% (PMPA) to 12.0% (IMPA) expressed as the relative standard deviation (R.S.D.).

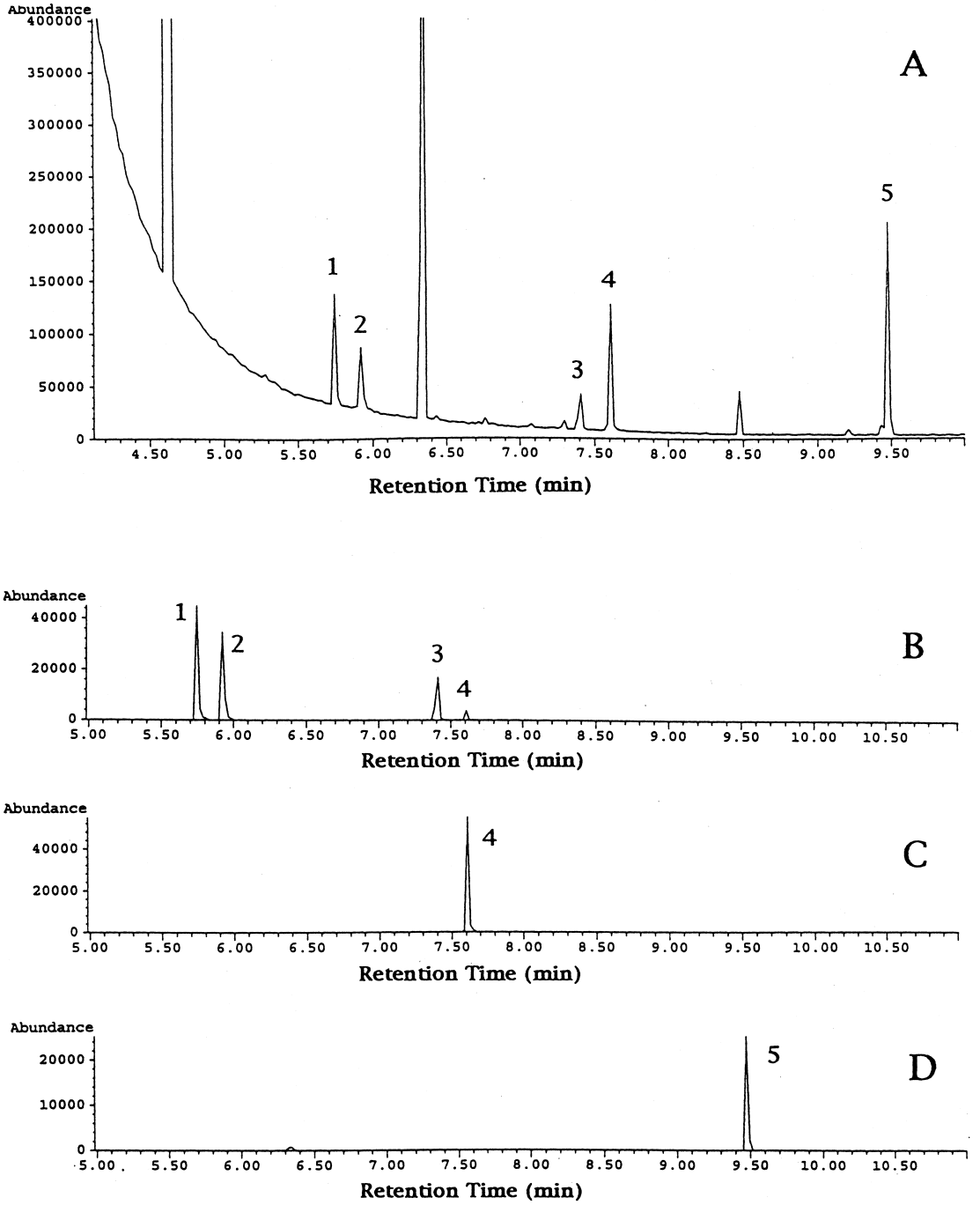

Fig. 2. Gas chromatograms of *tert.*-butyldimethylsilyl (TBDMS) derivatives of nerve agent hydrolysis products. A 50-μl acetonitrile solution containing 43 ppm (w/v) of methylphosphonic acid (MPA), 34 ppm of ethyl methylphosphonic acid (EMPA), 32 ppm of isopropyl methylphosphonic acid (IMPA), 29 ppm of pinacolyl methylphosphonic acid (PMPA) and 100 ppm of nonadecane was reacted with 50 μl of *N*-methyl-*N*-(*tert.*-butyldimethylsilyl)trifluoroacetamide at 60°C for 1 h. The resulting reaction mixture was applied to GC–MS operated as described in the Section 2. (A) Total ion chromatogram, (B) extracted ion chromatogram at *m/z* 153, (C) extracted ion chromatogram at *m/z* 267, (D) extracted ion chromatogram at *m/z* 85. Peaks: 1=TBDMS derivative of EMPA; 2=TBDMS derivative of IMPA; 3=TBDMS derivative of PMPA; 4=TBDMS derivative of MPA; 5=nonadecane.

Detection limits for MPA derivatives were 50 ng in the reaction vial ($S/N=3$ on extracted ion chromatograms).

3.2. tert.-Butyldimethylsilyl derivatization of nerve agent hydrolysis products from aqueous samples

Prior to TBDMS derivatization, the aqueous extract sample should be subjected to absolute dryness and this evaporation of water may introduce loss of analyte. When acetonitrile solution containing known amounts of MPA and its monoalkyl esters was incubated with MTBSTFA, analyzed by GC–MS, and the obtained peak area ratio of TBDMS derivatives to I.S. was taken as 100% reference, these values for an aqueous solution spiked with the same amounts of phosphonic acids decreased under acidic condition during evaporation. pK_a values of MPA and its monoalkyl esters are reported to be 2–3 [14], and phosphonic acids exist as protonated forms below this pK_a range. Alkyl methylphosphonic acids with hydrophobic character may disappear during water evaporation. In order to suppress this kind of loss, the pH of the aqueous solution subject to evaporation was adjusted to neutral pH with sodium hydrogencarbonate in order to change all acids to the deprotonated forms. This pH adjustment provided almost perfect recovery of alkyl methylphosphonic acids for TBDMS derivatization. In contrast, there appeared to be no loss of MPA under acidic aqueous condition, but TBDMS derivatization of MPA was suppressed to a small degree, depending on the amount of neutralizing salt. This type of phenomenon concerning salt interference against TBDMS derivatization was also observed for the effect of metal cations, as described later.

3.3. Recovery of methylphosphonic acids and its monoalkyl esters from soil samples

Soil samples Nos. 1, 2 and 3 were spiked with known amounts of MPA and its monoalkyl esters and extracted with water. After neutralization, aqueous soil extracts were dried up, incubated with MTBSTFA and analyzed by GC–MS. As shown in Table 2, the yields of TBDMS derivatization varied significantly with species of both soil types and acid types. More hydrophobic phosphonic acids showed better yields. The derivative of MPA could scarcely be detected from any soil sample. The soil sample No. 1 (sand) showed rather good yields of more than 24% except for MPA (0.7%). The soil sample No. 2 (alluvial soil) and No. 3 (volcanic ash soil) showed poor yields of less than 17% and 8%, respectively.

3.4. Effect of metal cations on yields of tert.-butyldimethylsilyl derivatization

Some kinds of substances extracted from soil samples should interfere with the determination of MPA and its monoalkyl esters in GC–MS after derivatization. Especially, metal cations are known to influence the yield [9]. Therefore, the effect of alkaline metals and alkaline earth metals, which are expected to be extracted from soil, were examined on TBDMS derivatization. Chloride salts of sodium, potassium, calcium or magnesium were added to the aqueous solution containing phosphonic acids, dried,

Table 2
Yields of derivatives of methylphosphonic acid and its monoalkyl esters from soil samples

	Soil No. 1 (Sand)	Soil No. 2 (Alluvial soil)	Soil No. 3 (Volcanic acid soil)
Methylphosphonic acid	0.7±0.5	ND[a]	ND
Ethyl methylphosphonic acid	24.0±7.8	12.9±0.9	3.5±0.1
Isopropyl methylphosphonic acid	26.9±4.3	13.5±1.5	5.1±2.7
Pinacolyl methylphosphonic acid	41.3±5.9	16.3±6.6	7.9±1.0

Two grams of soil was spiked with each of 12 μg of methylphosphonic acid and its monoalkyl esters, extracted with 4 ml of water. An 0.5-ml aliquot of the resultant extract was dried, *tert*.-butyldimethylsilylated and analyzed by GC–MS. The yield is defined as the percentage value of the peak area ratio of *tert*.-butyldimethylsilylated derivatives to internal standard (*n*-nonadecane), compared to the value for acetonitrile solution containing the same concentrations of phosphonic acids. The yield value was an average of three determinations±standard deviation.

[a] Not detected.

reacted with MTBSTFA and analyzed by GC–MS. As shown in Fig. 3, even at low concentrations (≈0.5 m*M* in aqueous solution), calcium and magnesium ions significantly decreased the yields of TBDMS derivatization of MPA (below 10%). The yields of MPA monoalkyl esters were decreased moderately. The tendency of the decreased yields were pronounced with the order of hydrophilicity. In contrast, monovalent metal cations, sodium and potassium, did not decrease the yields severely. Under the conditions showing the decreased yields, the poor repeatability of yield value for TBDMS derivatization were also observed.

The metal cation concentrations in aqueous soil extracts were measured by capillary electrophoresis. As shown in Table 3, concentrations of sodium in

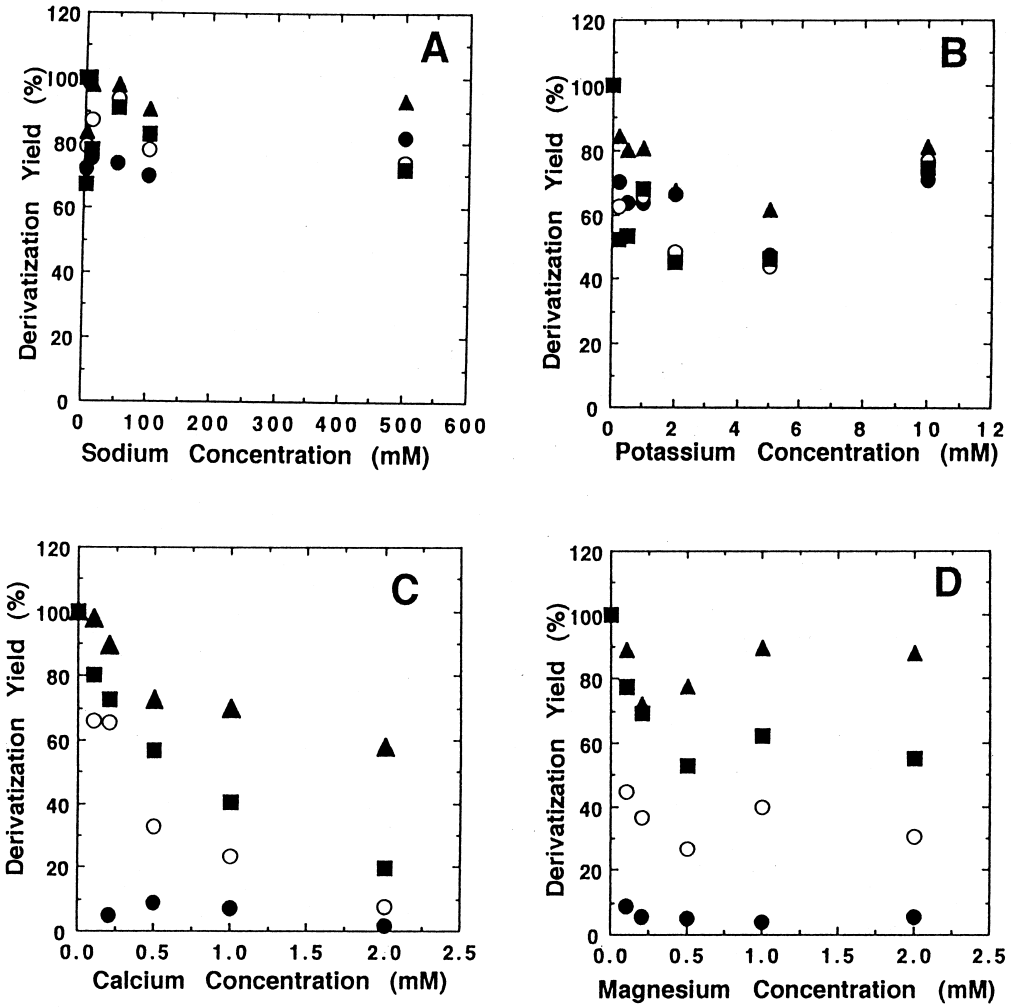

Fig. 3. Effects of alkaline metals and alkaline earth metals on the yields of *tert.*-butyldimethylsilyl derivatives of methylphosphonic acid and its monoalkyl esters. A 500-μl aqueous solution containing 4.5 ppm of methylphosphonic acid (●), ethyl methylphosphonic acid (○), isopropyl methylphosphonic acid (■), pinacolyl methylphosphonic acid (▲), known concentration (m*M*, depicted in figure) of cation (sodium chloride, potassium chloride, calcium chloride or magnesium chloride) was dried, reacted with the combined solution of 50 μl of *N*-methyl-*N*-(*tert.*-butyldimethylsilyl)trifluoroacetamide and 50 μl of acetonitrile containing 100 ppm nonadecane and a 2-μl aliquot was applied to GC–MS. The peak area ratio of derivatives to internal standard (*n*-nonadecane) was calculated and the percentage yields compared to that for the standard acetonitrile solution containing the same concentrations of phosphonic acids and *n*-nonadecane without salt were plotted against the metal cation concentrations.

Table 3
Metal cation concentrations in aqueous soil extracts (m*M*)

	Soil No. 1 (Sand)	Soil No. 2 (Alluvial soil)	Soil No. 3 (Volcanic acid soil)
Sodium	10.6	0.15	6.66
Potassium	0.22	0.65	1.64
Calcium	1.24	0.34	12.8
Magnesium	2.03	0.11	4.90

Two grams of soil sample was extracted with 4 ml of water, and the resultant supernatant was analyzed for cation levels by capillary electrophoresis.

the soil sample No. 1 (sand) were high because of its origin (seashore). The metal cation concentrations were not so high in the soil sample No. 2 (alluvial soil). In the soil sample No. 3 (volcanic ash soil), extremely high calcium concentrations were found. This may be due to contamination with calcium from the environment where the soil samples were collected: the garden of our Institute; there is a considerable chance of the release of inorganic components from reinforced concrete in buildings.

3.5. Effect of desalting using cation-exchange resin on tert.-butyldimetylsilyl derivatization

The aqueous extracts of soil samples spiked with MPA and its monoalkyl esters were passed through a strong cation-exchange resin Dowex 50W. The phosphonic acids were recovered in the through-flow fraction quantitatively. The aliquot of the resulting eluate fraction was neutralized with sodium bicarbonate, dried, reacted with MTBSTFA and analyzed by GC–MS. As shown in Table 4, the yields of TBDMS derivatization were improved for all alkyl methylphosphonic acids in all soil samples tested (12–69%), compared to those without desalting shown in Table 2. The derivative of MPA was detected from all soil samples (yield: 3–36%). In the soil sample No. 1 (sand), derivatives of phosphonic acids were obtained about half of the time (36–69%). In the soil sample No. 3 (volcanic ash soil), extremely raised yields of derivatives were observed even though that of MPA was not so high (2.8%). In the soil sample No. 2 (alluvial soil), the yields were not significantly improved.

The accuracy of determination of TBDMS derivatives by the above desalting method was examined by spiking known amounts of MPA and its monoalkyl esters in the soil sample No. 1. Calibration curves of the peak area ratios of TBDMS derivatives to I.S. were linear for spiked amounts ranging from 0.2 to 3.5 μg of all phosphonic acids with correlation coefficients of more than 0.979, and the repeatability ($n=5$) were 24% (R.S.D.) for MPA, 11% for EMPA, 8% for IMPA and 21% for PMPA. Detection limits were 0.1 μg for EMPA and IMPA and 0.2 μg for MPA and PMPA per g of soil.

3.6. Effect of extraction of soils with high ionic strength solution on tert.-butyldimethylsilyl derivatization

For the soil sample No. 2, which could not be significantly improved for the yields of TBDMS derivatives by desalting using a cation-exchange resin (Table 4), the effect of extraction with a high ionic strength solution was examined. The soil sample No. 2 spiked with MPA and its monoalkyl esters was extracted with water containing 0.2 *M* NaCl, and the resultant aqueous extract was passed

Table 4
Yields of derivatives of methylphosphonic acid and its monoalkyl esters from soil samples after cation-exchange desalting

	Soil No. 1 (Sand)	Soil No. 2 (Alluvial soil)	Soil No. 3 (Volcanic acid soil)
Methylphosphonic acid	35.9±8.6[a]	2.7±0.1	2.8±2.1
Ethyl methylphosphonic acid	56.1±6.1[a]	12.8±2.2	34.2±14.3[a]
Isopropyl methylphosphonic acid	68.8±5.4[a]	15.4±3.1	28.7±9.9[a]
Pinacolyl methylphosphonic acid	62.1±13.0[a]	24.2±7.0	29.9±5.8[a]

Two grams of soil was spiked with each of 12 μg of methylphosphonic acid and monoalkyl esters, extracted with 4 ml of water, and passed through a cation-exchange resin Dowex 50W. An aliquot of the resultant effluent was dried, *tert*.-butyldimethylsilylated and analyzed by gas chromatography/mass spectrometry. The yield value was an average of three determinations±standard deviation.
[a] Significantly increased ($P<0.01$) compared to the value for the untreated sample (Table 2).

Table 5
Yields of derivatives of methylphosphonic acid and its monoalkyl esters from soil sample No. 2 after high salt solution extraction and cation-exchange desalting

Acid	Yield
Methylphosphonic acid	13.9±1.1[a]
Ethyl methylphosphonic acid	33.3±3.9[ab]
Isopropyl methylphosphonic acid	37.4±6.6[ab]
Pinacolyl methylphosphonic acid	29.0±4.9[b]

Two grams of the soil sample No. 2 was spiked with each of 5 μg of methylphosphonic acid and its monoalkyl esters, extracted with 4 ml of 0.2 *M* sodium chloride solution, passed through cation-exchange resin Dowex 50W and neutralized. An aliquot of the resultant effluent was dried, *tert*.-butyldimethylsilylated and analyzed by GC–MS. The yield value was an average of three determinations±standard deviation.

[a] Significantly increased ($P<0.01$) compared to the value for sample treated with cation-exchange after aqueous extraction (Table 4).

[b] Significantly increased ($P<0.01$) compared to the value for untreated sample (Table 2).

through a cation-exchange resin, neutralized, dried, reacted with MTBSTFA and analyzed by GC–MS. Compared to the yields of derivatives for untreated samples (Table 2) and those for samples treated by desalting with cation-exchange resin after aqueous extraction (Table 4), the yields were significantly improved (Table 5), giving more than 29% for alkyl methylphosphonic acids and 14% for MPA, except for PMPA, whose yield was not increased compared to that for samples treated with desalting after aqueous extraction.

4. Discussion

The possibility of detecting nerve agents themselves from crime scenes of the nerve gas attack or CWA verification sites may be low, and therefore detecting the characteristic hydrolysis products can give indirect proof on nerve agent existence. Determination of MPA is also important because MPA is a final and stable hydrolysis product of nerve agents. It is also derived from methylphosphonyl dichloride or methylphosphonyl difluoride, a synthetic intermediate or precursor of sarin production. There are many papers dealing with the determination of nerve agent hydrolysis products for the purpose of CWA verification [2]. Our laboratory has adopted TBDMS derivatization in GC–MS for the determination of nerve agent hydrolysis products. By using the I.S. method, quantitative estimation of TBDMS derivatization becomes possible. As for the GC injection system, on-column injection [7] seems ideal because of its good injection accuracy without heat degradation of derivatives in the injector port, but when ca. 1 μl of MTBSTFA is introduced to the GC and MS systems, such a high amount of reagent may have harmful effects on the surfaces. Splitless injection [8,9] gives good sensitivity, but suffers from severe memory effect and fluctuation of detection because the splitless injector has much wider surface sites. TBDMS derivatives or their acids themselves are retained in portions of the splitless injectors including lines and pipes, and this introduces a memory effect. The derivatives may degrade at active sites on the injector surface. Our laboratory has adopted a split-injection system, and there has been only a small memory effect. If large peaks of TBDMS derivatives appear on GC, the blank injection is performed to ensure no memory effect. The glass liner and septum are replaced with a new one, once a week or more. One capillary column works for about one or two thousand injections till the peak resolutions drop. The detection limits for phosphonic acids were obtained for about 50 ng per vial. This level corresponds to 100 ng/ml solution sample, and is sufficient enough to detect at sub-ppm level. If the evidence sample is water, it is possible to raise more sensitivity by concentrating samples by water evaporation.

We have experienced perturbation of yield values, especially in GC–MS analysis after injection of the TBDMS reaction mixture from rather crude samples or an aqueous sample spiked with a high concentration of metal cation (as shown below). In such cases, even standard solution containing MPA and its monoalkyl esters showed large variation (within-day repeatability >20%). The reason for raised fluctuation of TBDMS derivatization is not elucidated at this point, but it may be due to interference by substances included in the injected derivatization reaction mixture for stable vaporization of TBDMS derivatives. In a routine test, before analysis of an unknown sample, we have checked the linearity of response of TBDMS derivatization using a standard solution. As for the yield data from soil samples

(Tables 2,4 and 5), experiments have been performed for a soil sample once per day, and R.S.D. values depicted in these tables corresponded to inter-day reproducibility. Therefore, from the standpoint of the above mentioned data fluctuation, R.S.D. values of the yields became rather high as shown in tables.

Among evidence samples, soil is one of the most difficult samples from which CWA and their related compounds should be determined, because of its complex matrix nature. Phosphonic acids are polar compounds soluble in water, but their protonated forms show a hydrophobic character, making it possible to apply solid-phase extraction of various kinds of adsorption modes as a pretreatment method for environmental samples [10–12]. Although solid-phase extraction using the ion-exchange mode was successfully adopted for GC detection of alkyl methylphosphonic acids in soils after derivatization [10,11], the recovery of MPA was reported to be very low [8]. The retention of this polar compound on a silica-based solid-phase of the ion-exchange mode may be insufficient and so the extraction of MPA is not expected to get full recovery. In this respect, it is difficult to recover both alkyl methylphosphonic acids and MPA simultaneously from soil samples for GC–MS analysis after derivatization.

Among the substances extracted from soils, metal cations, especially calcium ion, should disturb the detection of MPA and its monoalkyl esters by GC–MS after derivatization [9]. Ionic association of phosphonates with metal cations should lead to insolubility in the derivatization reaction mixture of acetonitrile and MTBSTFA, leading to deteriorated derivatization efficiency [15,16]. The solubility of the complex in the reaction mixture should be related to its TBDMS derivatization yield, which is compatible with the experimental result of Table 4. Complexes of hydrophobic PMPA with even calcium may dissolve in the reaction mixture, giving good derivatization yields. In contrast, complexes of the most polar divalent anion MPA with divalent cations may not dissolve in the reaction mixture, giving poor derivatization yields. Alkaline earth metal concentrations in aqueous soil extracts shown in Table 3 can explain how poor yields of MPA and its monoalkyl esters were observed for soil samples (Table 2). Removal of metal cations from the soil extract has improved the derivatization yields (Table 2 vs. Table 4). The degree of the improvement of the yields is significant for soil samples containing high calcium and magnesium contents (Table 3). Our result concerning a high yield of MPA derivatization is in contrast to the other reporters who adopt a silica-based ion-exchange cartridge [8]. This may be due to the high ionic exchange capacity of our adopted resin, compared to the rather low capacity of silica cartridge. In addition, the calibration curve of the determination of MPA and its monoalkyl esters in the soil sample was given as linear, which indicates our cation-exchange pretreatment combined with GC–MS after derivatization should give a satisfactory method for determining nerve agent hydrolysis products in environmental samples.

Even though metal cations were removed from the aqueous soil extracts, complete yields of the TBDMS derivative could not be obtained (Table 4). The yield of MPA derivative was found to be poor, and this can be attributed to the essential low recovery of MPA from soil samples [17–19]. It may be due to insufficient elution from the soil matrix or other factors interfering with TBDMS derivatization. Soil has a high capacity for binding cations and anions. As shown in Table 1, soil sample No. 1 (sand) showed a low phosphate absorption coefficient, which means a low level of binding between soil and anions, corresponding to the result of a high yield of derivatives. In contrast, the soil sample No. 2 (alluvial soil) showed a high phosphate absorption coefficient, which means high levels of binding between soil and anions, corresponding to the result of a low yield of derivatives. As shown in Table 5, extraction with a high ionic strength solution for such a soil, sample No. 2 (alluvial soil) gave a more improved yield. High salt conditions should elute alkyl methylphosphonic acids from soils. The only exception is PMPA, whose yield was not improved significantly. Under high ionic strength solution conditions, the ionic binding force between the soil matrix and PMPA is weakened, and the hydrophobic interaction is strengthened. As a result of the respective contributions from both binding forces, the yield of PMPA derivatization may not be improved. There is still a rather low yield of MPA derivatization. It is conceivable that high ionic strength extraction is insufficient to remove MPA, which is so polar, from

the soil matrix perfectly. In contrast to the positive effect of a high ionic strength solution extract, salt solution resulted in considerable production of salt residue in the dried sample, and this interferes with the accuracy and yield of TBDMS derivatization. Selection of the extraction medium (water or high ionic strength solution) depends on which is more necessary for detecting MPA or monoalkyl esters.

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