*Anal. Chem.* **2002,** *74,* 4709−4715

# Improved *tert*-Butyldimethylsilylation Gas Chromatographic/Mass Spectrometric Detection of Nerve Gas Hydrolysis Products from Soils by Pretreatment of Aqueous Alkaline Extraction and Strong Anion-Exchange Solid-Phase Extraction

Masaaki Noami,[†] Mieko Kataoka,[‡] and Yasuo Seto*,[‡]

*Forensic Science Laboratory, Wakayama Prefectural Police H.Q., 1, Nishi, Wakayama, Wakayama 640-8313, Japan, and National Research Institute of Police Science, 6-3-1, Kashiwanoha, Kashiwa, Chiba 277-0882, Japan*

**In the analysis of *tert*-butyldimethylsilyl derivatives (TBDMS) of alkyl methylphosphonic acids (RMPA) and methylphosphonic acid (MPA), from soils by gas chromatography/mass spectrometry (GC/MS), the detection yields are generally low, due to the suppression of TBDMS derivatization by the soil matrix components and the adsorption of RMPA and MPA to the soils. An ion-exchange pretreatment of the aqueous soil extract can be used to overcome the former factor by removing interfering compounds. A pretreatment method is described for improving the detection yields due to the latter factor, using an alkaline extraction procedure. The recovery was estimated quantitatively using capillary electrophoresis. The soil samples tested included volcanogenous immature soils and showed a low aqueous extraction recovery and GC/MS detection yields. The inclusion of sodium hydroxide in the extraction solvent dramatically increased the recovery. Using a 0.1 M sodium hydroxide solution, the recovery was in excess of 68%. Interfering components were removed from the alkaline soil extract by solid-phase extraction of the acids on a silica-based strong anion exchanger. The alkaline soil extract was neutralized with hydrofluoric acid and applied to the cartridge in the fluoride form. After washing with water, MPA and RMPA could be eluted with methanolic ammonia nearly quantitatively. Using the established pretreatment method, MPA and RMPA were detected from all the soil samples in more than 67% yield.**

Alkyl methylphosphonic acids (RMPA) are hydrolysis products of nerve gases. Isopropyl methylphosphonic acid (IMPA), pinacolyl methylphosphonic acid (PMPA), and ethyl methylphosphonic acid (EMPA) are derived from sarin, soman, and VX, respectively. Under more drastic conditions, they are further converted to methylphosphonic acid (MPA).[1] MPA is also found as a residue at sites where nerve gas is or has been produced (Figure 1).[2] Therefore, the determination and identification of MPA and RMPA in evidence samples is important in order to verify the usage or production of a chemical warfare agent, nerve gas, from the standpoints of both chemical verification[3] and forensic aspects.[4] Typically, gas chromatography/mass spectrometry (GC/MS) is used for this,[3] and *tert*-butyldimethysilylation (TBDMS) is used to for the derivatization of polar compounds, such as MPA and RMPA.[5] Liquid chromatography (LC)/MS has recently been applied for the analysis of RMPA.[6,7]

Among the samples used in such analysis, soil is one of the most complex matrixes from which chemical warfare agents and related compounds are determined.[8] There is, however, a serious problem of the low detectability of MPA and RMPA that is encountered in such procedures.[9−12] The low detectability for soil samples in TBDMS GC/MS analysis can be attributed mainly to interference in the TBDMS derivatization and the adsorption of RMPA and MPA to soils. With respect to the former mechanism, we previously reported that divalent cations, $Ca^{2+}$ and $Mg^{2+}$ and carbohydrates suppress the TBDMS derivatization of RMPA and MPA, and we developed simple ion-exchange pretreatment methods to remove interferences from aqueous soil extracts.[13,14]

* Corresponding author. Tel: (81)-4-7135-8001. Fax: (81)-4-7133-9159. E-mail: seto@nrips.go.jp.

[†] Wakayama Prefectural Police H.Q.

[‡] National Research Institute of Police Science.

(1) Kingery, A. F.; Allen, H. E. *Toxicol. Environ. Chem.* **1995**, *47*, 155−184.

(2) Munro, N. B.; Talmaga, S. S.; Griffin, G. D.; Waters, L. C.; Watson, A. P.; King, J. F.; Hauschild, V. *Environ. Health Perspect.* **1999**, *107*, 933−974.

(3) Rautio, M. *Recommended operating procedures for sampling and analysis in the verification of chemical disarmament*; The Ministry for Foreign Affairs of Finland, Helsinki, 1994.

(4) Tsunoda, N.; Seto, Y. *Rep. Natl. Res. Inst. Police Sci.* **1997**, *50*, 58−80.

(5) Purdon, J. G.; Pagotto, J. G.; Miller, R. K. *J. Chromatogr.* **1989**, *475*, 261−272.

(6) D'Agostino, P. A.; Hancock, J. R.; Provost, L. R. *J. Chromatogr., A* **2001**, *912*, 291−299.

(7) Read, R. W.; Black, R. M. *J. Chromatogr., A* **1999**, *862*, 69−177.

(8) Black, R. M.; Clarke, R. J.; Read, R. W.; Reid, T. J. *J. Chromatogr., A* **1994**, *662*, 301−321.

(9) D'Agostino, P. A.; Provost, L. R. *J. Chromatogr.* **1992**, *589*, 287−294.

(10) Creasy, W. R.; Rodriguez, A. A.; Stuff, J. R.; Warren, R. W. *J. Chromatogr., A* **1995**, *709*, 333−344.

(11) Fredriksson, S.-A.; Hammarstrom, L.-G.; Henriksson, L.; Lakso, H.-A. *J. Mass Spectrom.* **1995**, *30*, 1133−1143.

(12) Vermillion, W. D.; Crenshaw, M. D. *J. Chromatogr., A* **1997**, *770*, 253−260.

(13) Kataoka, M.; Tsunoda, N.; Ohta, H.; Tsuge, K.; Takesako, H.; Seto, Y. *J. Chromatogr., A* **1998**, *824*, 211−221.

(14) Kataoka, M.; Tsuge, K.; Seto, Y. *J. Chromatogr., A* **2000**, *891*, 295−304.

10.1021/ac020200o CCC: $22.00 
Published on Web 08/14/2002

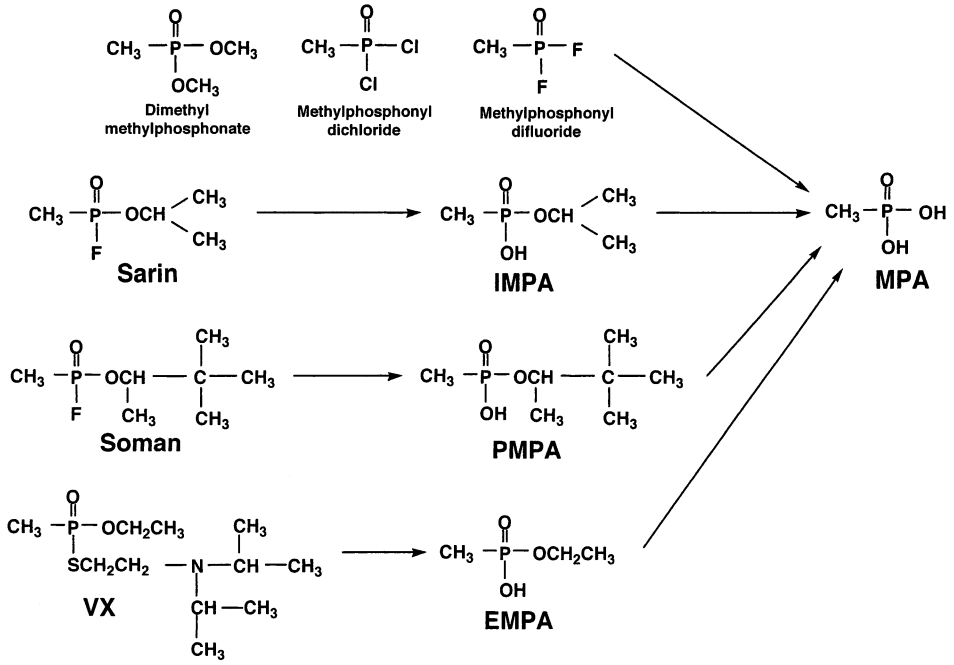


Figure 1. Hydrolysis products of nerve gases and synthetic intermediates.

However, the TBDMS derivatization yields were not significantly improved by these pretreatments for certain types of soils, such as volcanic acid soils. Therefore, with respect to the latter mechanism of low detectability, we investigated the relationship between pedological characteristics and the aqueous extraction recoveries of RMPA and MPA, using various types of soil samples, and concluded that the aqueous extraction recoveries for all phosphonates were inversely correlated with the phosphate absorption coefficient (PAC).[15]

The adsorption mechanism[16] indicates that the binding of MPA and RMPA to soils is weak under conditions of elevated pH. Thus, increasing the pH of the aqueous soil extraction solution could possibly improve the extraction recovery. However, treatment of soil with an alkaline solution may also result in the release of the high levels of soil matrix components into the aqueous extract, and this could affect the TBDMS derivatization procedure, as well as the GC separation. The interfering compounds can be removed from the soil extracts by solid-phase extraction. In this paper, we describe a pretreatment method for the GC/MS detection of MPA and RMPA from soils after TBDMS derivatization, using alkaline extraction followed by a cleanup using strong anion-exchange (SAX) solid-phase extraction.

## EXPERIMENTAL SECTION

**Reagents.** MPA, EMPA, and PMPA were obtained from Aldrich Chemicals (Milwaukee, WI). *N*-Methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) was obtained from Pierce (Rockford, IL). A Bond Elut SAX cartridge (regular type, 500 mg/3 mL), a strong anion-exchanger solid-phase extraction cartridge, was obtained from Varian (Harbor City, CA). IMPA was prepared as previously reported.[11] All other chemicals used were of analytical grade. All aqueous solutions were prepared with distilled, deionized water.

MPA, EMPA, IMPA, and PMPA were dissolved in acetonitrile or water (1.6 mg/mL) and stored at −20 °C as stock solutions. A working solution was prepared by diluting the stock solution with additional acetonitrile or water.

**Soil Samples.** Five soil samples were collected near Sakurajima, an active volcano, and in Kagoshima city, which is located ∼10 km from Sakurazima. The no. 1 soil sample was obtained from a park associated with a housing complex. The no. 2 sample was obtained from another park in the city. The no. 3 sample was from a parking lot, no. 4 from a field in Sakurazima, and no. 5 was composed of ashes from the housetop of a building of the Kagoshima Prefectural Police H.Q. These five soils were passed through a 2-mm sieve and dried at room temperature for several days. The percentage of water and organic matter, granulation, pH, and PAC were examined according to a standard method,[17] and the results are shown in Table 1. These soils were volcanogenous immature soils, and according to FAO-Unesco,[18] are classified as "gleyic, vitric andosols". Soil no. 6, an alluvial soil, obtained from a shrine park in Kyoto City, was also used, as previously reported.[14]

**Alkaline Aqueous Extraction of Soil Samples.** For the determination of extraction recovery by capillary electrophoresis (CE), 500 μL of an acetonitrile solution containing 136−175 μg of RMPA and MPA was spiked to 2.0 g of each soil sample and allowed to stand at room temperature for 4−5 h. For the determination of yields by GC/MS, 25 μL of an acetonitrile solution containing 27−35 μg of RMPA and MPA was spiked to 2.0 g of each soil sample. Four milliliters of a known concentration of NaOH solution was added, and the resulting suspension was

(15) Kataoka, M.; Tsuge, K.; Takesako, H.; Hamazaki, T.; Seto, Y. *Environ. Sci. Technol.* **2001**, *35*, 1823−1829.
(16) Nowack, B.; Stone, A. T. *J. Colloid Interface Sci.* **1999**, *214*, 20−30.
(17) Japanese Society of Soil Science and Plant Nutrition. *Standard Method of Soil Analysis*, Hakuyusha: Tokyo, Japan, 1986.
(18) FAO-Unesco. *Soil Map of the World. Revised Legend, World Soil Resources Report 60*, FAO, Rome, 1990.

Table 1. Chemical and Physical Characteristics of the Soil Samples Used

| | soil number | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| particle distribution (%) | | | | | | |
| clay <2 mm | 0.3 | 0.0 | 0.7 | 0.4 | 0.5 | 14.0 |
| silt 2−20 mm | 6.2 | 1.5 | 2.4 | 5.4 | 5.5 | 13.3 |
| sand >20 mm | 93.5 | 98.5 | 96.9 | 94.2 | 94.0 | 72.6 |
| water (%) | 1.7 | 0.2 | 0.1 | 0.7 | 0.3 | 4.8 |
| organic matter (%) | 13.1 | 1.4 | 1.5 | 6.5 | 3.8 | 3.6 |
| pH($H_2O$) | 6.8 | 5.7 | 5.2 | 5.4 | 7.5 | 6.4 |
| pH(KCl) | 5.3 | 6.6 | 5.2 | 5.4 | 6.0 | 5.7 |
| phosphate absorptn coeff[a] | 1095 | 417 | 113 | 473 | 325 | 548 |

[a] $P_2O_5$ mg/100 g of soil.

vortexed for 1 min and then sonicated for 10 min at room temperature. Samples were centrifuged at 1500*g* for 5 min, and the resultant supernatant was filtered through a 0.45-$\mu$m cellulose acetate membrane. After dilution with distilled water or neutralization with concentrated HCl or HF, aliquots (0.3 mL) were analyzed by CE, and also directly or after SAX treatment, they were subjected to TBDMS derivatization and subsequent GC/MS analysis.

**Capillary Electrophoretic Determination of Nerve Gas Hydrolysis Products.** RMPA and MPA were quantified as previously described[19] with minor modifications using a HP $^{3D}$CE capillary electrophoresis system (Yokowaga Analytical Systems, Tokyo, Japan). The capillary column was fused silica (50 $\mu$m i.d. × 52 cm), and the electrophoresis buffer was 100 mM boric acid, which contained 10 mM benzoate (pH 6.0). The voltage was set at 30 kV with a positive power supply. Detection was by indirect ultraviolet absorption at 254 nm (reference at 200 nm), and the column temperature was maintained at 25 °C. Samples were applied hydrodynamically at 50 mbar for 4 s. Prior to each analysis, the column was washed with a 0.1 M potassium hydroxide solution (1 min), water (1 min), and electrolyte buffer (1 min). The buffer bottles were replaced after each three analyses.

**Strong Anion-Exchange Pretreatment.** RMPA and MPA were purified as previously described[18] with slight modifications. All solid-phase extraction procedures were performed using a Waters extraction manifold (Waters, Milford, MA). The flow rate of sample charge and elution was maintained at ∼0.5 mL/min, and that of the washing at about 1 mL/min. A 2-mL sample of each of the alkaline aqueous soil extracts was neutralized with concentrated HF and applied to the Bond Elut SAX cartridge, which was activated with 2 mL of methanol, 2 mL of water, 12 mL of 1.0 M sodium fluoride solution, and 5 mL of water. The cartridge was washed with 5 mL of water, and the MPA and RMPA were eluted with 5 mL of 3% (v/v) methanolic ammonia. The eluted fraction was concentrated under reduced pressure at 50 °C on a rotary evaporator.

***tert*-Butyldimethylsilylation and Gas Chromatography/Mass Spectrometry.** The standard solution, the aqueous soil extract, or the SAX elution fraction was subjected to TBDMS derivatization followed by GC/MS detection, as previously described.[13] A 1-mL sample of the aqueous soil extract or the SAX eluate fraction was concentrated under reduced pressure at 50 °C on a rotary evaporator and dried on a model VC-360 centrifugal concentrator (Taitec, Saitama, Japan) under reduced pressure at 50 °C in a 1-mL glass vial (Nichiden Rika Garasu, type MV-07, Tokyo, Japan). Fifty microliters of MTBSTFA and 50 $\mu$L of acetonitrile, which also contained 12 ppm anthracene (internal standard, IS) were added, and the vial was then closed with a Teflon screw cap, homogenized by sonication for 5 min, and incubated at 60 °C for 1 h. A 1-$\mu$L aliquot of the mixture was applied to the GC/MS system, which consisted of an HP 6890 gas chromatograph combined with an HP 5973 quadrupole mass spectrometer (Yokowaga Analytical Systems, Tokyo, Japan). The stationary phase was a capillary column HP-5MS (30 m × 0.25 mm i.d., 0.25-$\mu$m thickness, J&W Scientific, Folson, CA). The carrier gas (helium) flow rate and splitter ratio were adjusted to 0.8 mL/min and 50, respectively. The injection port, transfer line, and ion source were maintained at 250, 280, and 230 °C, respectively. Electron impact ionization (ionization energy 70 eV, ionization current 60 $\mu$A) was used as the ionization mode. The oven temperature was controlled by a program (initial temperature, 90 °C (1-min hold) and then a ramp to 290 °C at 20 °C per min (5-min hold)). The acquisition mass range was 50−550, and sampling was 0.8 scan/s. Acquisition was started 4 min after sample injection. The extracted ion chromatograms were obtained at *m*/*z* 153 for the EMPA, IMPA, and PMPA derivatives, *m*/*z* 267 for the MPA derivative, and *m*/*z* 178 for IS.

**Safety Considerations.** HF is toxic by inhalation, in contact with skin. This compound should be handled with special care.

## RESULTS

**Aqueous Extraction of Nerve Gas Hydrolysis Products from Soils and Subsequent Gas Chromatographic/Mass Spectrometric Analysis.** We examined the detectability of MPA and RMPA by GC/MS analysis, using several of the soils collected in Kagoshima. In addition to these five volcanogenous immature soils, one soil sample (alluvial soil, no. 6), which had been examined previously, was also included for this investigation.[13−15] The soil samples (2 g) were spiked with RMPA and MPA (150 or 3 $\mu$g each) and extracted with water (4 mL). The aqueous extracts were analyzed by CE (extraction recovery), and also, either directly or after SAX treatment, they were subjected to TBDMS derivatization followed by GC/MS analysis (detection yield). As shown in Table 2, the detection yields of RMPA from all the soil samples were low (<30%), and those of MPA were particularly low (<3.1%). Cleanup of the solid-phase extraction with an anion-exchange cartridge improved the yields to a remarkable extent, and these values approached the extraction recoveries. However, the yields in samples no. 1−5 were lower than 51%, and the yields of MPA were less than 11%.

**Improvement of the Capillary Electrophoretic Method for the Determination of Nerve Gas Hydrolysis Products in Alkaline Solution.** To evaluate the efficiency of an alkaline extraction, it is necessary to quantify MPA and RMPA in the alkaline soil extracts. The CE method using benzoate−borate buffer permits the accurate determination of RMPA and MPA in aqueous soil extracts without interference by chloride and bicarbonate ions and with full recovery.[19] However, in the presence of a strongly alkaline solution (>0.01 M NaOH), MPA and RMPA

(19) Kataoka, M.; Tsuge, K.; Seto, Y. *Jpn. J. Assoc. Sci. Technol. Ident.* **2000**, *4*, 67−75.

Table 2. Aqueous Extraction Recoveries and Detection Yields by Gas Chromatography/Mass Spectrometry after *tert*-Butyldimethysilylation of RMPA and MPA from Soil Samples

| | | soil number | | | | | |
|---|---|---|---|---|---|---|---|
| analyte | analysis | 1 | 2 | 3 | 4 | 5 | 6 |
| MPA | CE[a] | 8.7 ± 1.4 | 10.1 ± 2.1 | 8.7 ± 1.6 | 6.9 ± 1.1 | 11.9 ± 2.1 | 56.6 ± 3.6 |
| | direct GC/MS[b] | 1.5 ± 0.1 | 1.9 ± 0.1 | 1.1 ± 0.1 | 3.1 ± 0.5 | 2.1 ± 0.2 | 1.7 ± 0.5 |
| | SPE GC/MS[c] | 7.5 ± 1.1 | 9.7 ± 2.1 | 6.9 ± 1.2 | 6.2 ± 1.1 | 10.9 ± 1.4 | 49.6 ± 5.0 |
| EMPA | CE | 25.3 ± 3.2 | 47.5 ± 4.6 | 18.8 ± 3.4 | 16.3 ± 2.7 | 49.0 ± 4.4 | 93.2 ± 9.5 |
| | direct GC/MS | 9.9 ± 1.5 | 23.4 ± 2.4 | 10.8 ± 2.0 | 8.3 ± 1.2 | 9.8 ± 0.4 | 15.4 ± 3.7 |
| | SPE GC/MS | 22.8 ± 4.3 | 34.1 ± 3.8 | 18.0 ± 3.4 | 15.8 ± 2.6 | 43.8 ± 3.5 | 82.7 ± 5.8 |
| IMPA | CE | 38.1 ± 5.3 | 30.1 ± 4.2 | 38.6 ± 6.1 | 28.9 ± 3.8 | 48.6 ± 5.7 | 93.4 ± 11.5 |
| | direct GC/MS | 16.9 ± 2.2 | 29.6 ± 3.2 | 18.8 ± 3.5 | 12.9 ± 2.3 | 29.6 ± 3.5 | 15.0 ± 3.9 |
| | SPE GC/MS | 34.2 ± 4.2 | 29.9 ± 3.2 | 28.6 ± 2.5 | 23.1 ± 2.1 | 39.1 ± 4.1 | 68.5 ± 5.1 |
| PMPA | CE | 51.4 ± 4.7 | 22.6 ± 7.3 | 46.4 ± 5.7 | 36.6 ± 5.2 | 54.5 ± 3.8 | 89.8 ± 15.2 |
| | direct GC/MS | 19.5 ± 3.1 | 18.4 ± 2.8 | 5.9 ± 1.1 | 5.9 ± 1.0 | 19.3 ± 3.1 | 22.9 ± 4.6 |
| | SPE GC/MS | 35.2 ± 1.6 | 20.5 ± 3.8 | 40.2 ± 2.8 | 33.7 ± 3.6 | 50.8 ± 10.1 | 73.5 ± 5.9 |

[a] A 2-g soil sample spiked with 136−175 $\mu$g of MPA and RMPA was extracted with 4 mL of water, and the extract was analyzed by CE. [b] A 2-g soil sample spiked with 27−35 $\mu$g of MPA and RMPA was extracted with 4 mL of water, and the extract was directly *tert*-butyldimethylsilylated and analyzed by GC/MS. The yield is defined as the percentage value of the peak area ratio of TBDMS derivatives to internal standard, compared to the value for acetonitrile solution containing the same concentrations of phosphonates. [c] A 2-g soil sample spiked with 27−35 $\mu$g of MPA and RMPA was extracted with 4 mL of water, and the extract was treated by SAX solid-phase extraction, *tert*-butyldimethylsilylated, and analyzed by GC/MS. The values was an average of three determinations ± standard deviation.

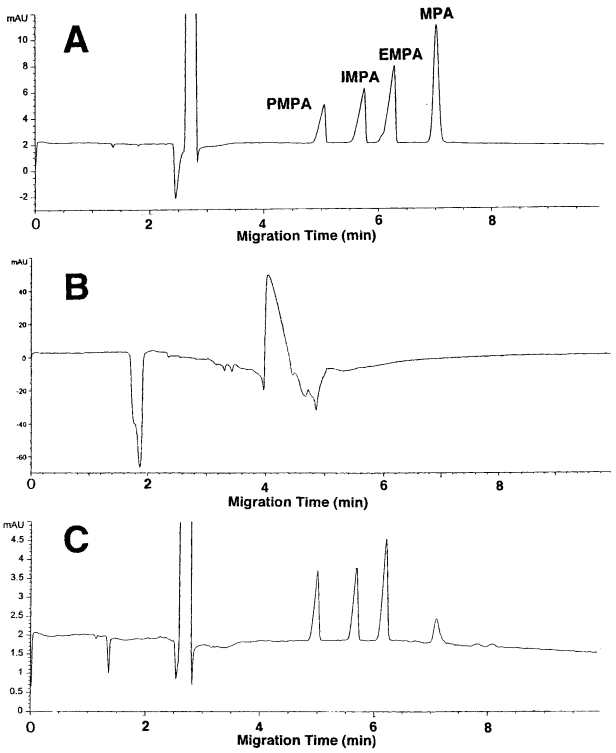


Figure 2. Electropherograms of RMPA and MPA. (A) Standard solution (MPA 70.0 $\mu$g/mL, EMPA 69.2 $\mu$g/mL, IMPA 54.4 $\mu$g/mL, PMPA 65.6 $\mu$g/mL), (B) the extract of the no. 1 soil (soil 2 g, spiked with 175 $\mu$g of MPA, 173 $\mu$g of EMPA, 136 $\mu$g of IMPA, and 164 $\mu$g of PMPA with 0.1 M NaOH solution (4 mL), and (C) the soil extract neutralized with HCl.

could not be detected, because of the severely distorted electropherogram (Figure 2B). Dilution or neutralization of the alkaline solution enabled the detection of MPA and RMPA. As shown in Figure 2C, the electropherogram of the alkaline soil extract that had been neutralized with HCl was identical to that of the standard sample, with no distortion. The compounds extracted from the soil matrix with the alkaline solution (up to 0.1 M NaOH), and also chloride ion used as neutralizer, did not interfere with the determination of MPA and RMPA.

**Extraction of Nerve Gas Hydrolysis Products from Soils with an Alkaline Solution.** The soil samples (2 g) were spiked with RMPA and MPA (~150 $\mu$g each) and extracted with 4 mL of NaOH solution. The alkaline extract was neutralized with HCl and analyzed by CE. Increasing the concentration of NaOH raised the pH of the soil extract. Using a 0.1 M NaOH solution, the pH of the soil extract was in the range of 13.3 (no. 5 soil) to 13.9 (no. 2 soil) except for the no. 1 soil (pH, 10.6). Because the latter sample contained a rather high organic matter content (Table 1), the extract from such a soil would show a remarkable buffering effect.

We examined the effect of an alkaline solution on extraction efficiency, using no. 1 soil, which showed a lower extraction recovery for MPA and RMPA and had the highest PAC and organic matter content of all the soil samples examined. As shown in Figure 3, increasing the concentration of NaOH increased the recovery of both MPA and RMPA considerably. With a 0.02 M NaOH solution, more than 50% recovery was obtained. An alkaline solution of more than 0.1 M NaOH was not examined, because MPA and RMPA could not be quantified accurately by CE. The extraction recovery reached a plateau at around the 0.1 M concentration range. With a 0.1 M NaOH solution, the approximate recoveries were 90 (RMPA) and 68% (MPA). In the case of the other soil samples, recoveries in excess of 68 (RMPA) and 72% (MPA) were achieved (Table 3).

**Cleanup by Solid-Phase Extraction.** The alkaline treatment would extract not only MPA and RMPA but high levels of matrix components from soils as well. These extracted components would be expected to interfere with TBDMS GC/MS analysis. We were not able to detect MPA and RMPA from alkaline soil extracts using the TBDMS GC/MS procedure. Therefore, even though MPA and RMPA were extracted with reasonable recovery, the simultaneously extracted soil matrix components interfered with the TBDMS derivatization and GC separation.

We adopted our previously reported solid-phase extraction method using a SAX cartridge[20] to clean up the MPA and RMPA.

(20) Kataoka, m.; Seto, Y. *Jpn. J. Assoc. Sci. Technol. Ident.* **2002**, *6*, 117−127.

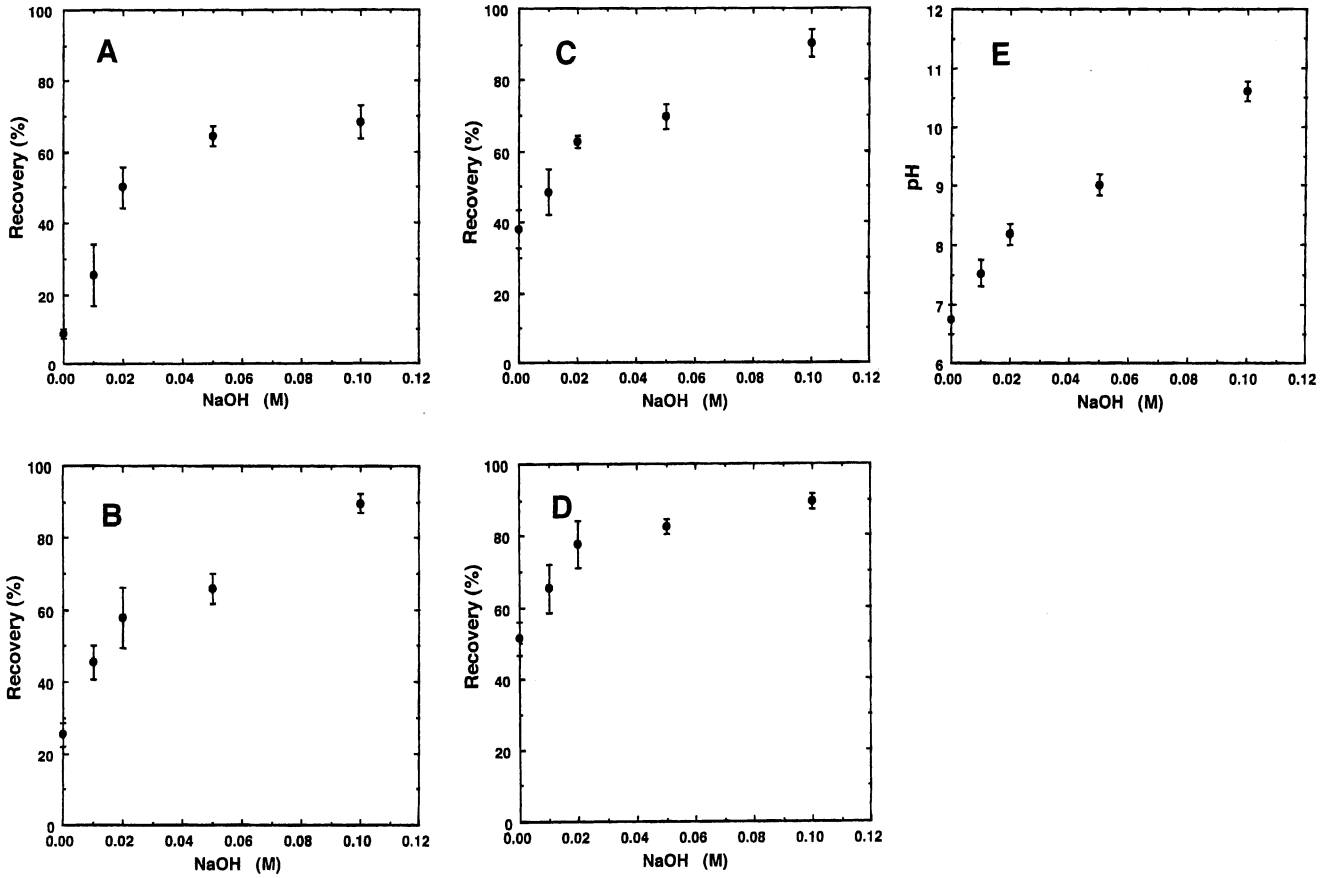


Figure 3. Effect of an alkaline solution on the extraction of RMPA and MPA from soil (no. 1). (A) MPA, (B) EMPA, (C) IMPA, (D) PMPA, and (E) pH. The recovery values were obtained by direct capillary electrophoretic analysis. Each value represents an average (three determinations) ± standard deviation.

Table 3. Alkaline Extraction Recoveries and Detection Yields by Gas Chromatography/Mass Spectrometry after *tert*-Butyldimethysilylation of RMPA and MPA from Soil Samples

| | | soil number | | | | | |
|---|---|---|---|---|---|---|---|
| analyte | analysis | 1 | 2 | 3 | 4 | 5 | 6 |
| MPA | CE[a] | 68.4 ± 4.8 | 82.9 ± 9.1 | 73.3 ± 3.6 | 82.7 ± 5.3 | 82.4 ± 7.3 | 72.8 ± 5.3 |
| | SPE CE[b] | 69.5 ± 3.6 | 82.3 ± 3.6 | 72.6 ± 4.4 | 79.2 ± 3.1 | 81.9 ± 2.3 | 72.2 ± 2.7 |
| | SPE GC/MS[c] | 67.3 ± 12.1 | 75.8 ± 8.2 | 70.8 ± 4.0 | 74.6 ± 9.7 | 75.8 ± 10.4 | 70.1 ± 4.2 |
| EMPA | CE | 89.7 ± 2.7 | 84.3 ± 7.6 | 91.3 ± 8.3 | 85.7 ± 1.2 | 99.7 ± 3.5 | 94.6 ± 2.8 |
| | SPE CE | 88.3 ± 3.9 | 83.9 ± 2.2 | 91.5 ± 3.2 | 85.4 ± 2.6 | 98.1 ± 6.2 | 86.9 ± 5.6 |
| | SPE GC/MS | 78.4 ± 6.1 | 80.6 ± 4.2 | 90.4 ± 3.9 | 83.6 ± 3.7 | 88.6 ± 5.9 | 88.4 ± 4.2 |
| IMPA | CE | 90.2 ± 3.8 | 95.6 ± 5.1 | 88.5 ± 5.2 | 87.8 ± 2.6 | 68.9 ± 5.1 | 88.5 ± 5.3 |
| | SPE CE | 84.1 ± 2.8 | 94.2 ± 3.1 | 88.9 ± 5.2 | 87.4 ± 2.5 | 68.4 ± 2.2 | 78.4 ± 3.2 |
| | SPE GC/MS | 82.4 ± 2.5 | 90.6 ± 4.4 | 84.6 ± 3.1 | 85.4 ± 2.9 | 65.2 ± 3.4 | 79.4 ± 4.7 |
| PMPA | CE | 89.4 ± 2.1 | 89.6 ± 10.5 | 97.4 ± 5.7 | 88.0 ± 1.4 | 80.6 ± 5.1 | 92.7 ± 5.3 |
| | SPE CE | 86.3 ± 4.6 | 89.5 ± 2.1 | 96.5 ± 3.7 | 87.8 ± 2.1 | 81.4 ± 4.2 | 92.8 ± 3.4 |
| | SPE GC/MS | 80.3 ± 6.5 | 82.3 ± 3.2 | 90.3 ± 6.1 | 84.6 ± 2.7 | 72.3 ± 3.6 | 85.7 ± 5.7 |

[a] A 2-g soil sample spiked with 136−175 μg of MPA and RMPA was extracted with 4 mL of 0.1 M NaOH solution, and the extract was neutralized with HCl and analyzed by CE. [b] A 2-g soil sample spiked with 136−175 μg of MPA and RMPA was extracted with 4 mL of 0.1 M NaOH solution, and the extract was treated by SAX solid-phase extraction and analyzed by CE. [c] A 2-g soil sample spiked with 27−35 μg of MPA and RMPA was extracted with 4 mL of 0.1 M NaOH solution, and the extract was treated by SAX solid-phase extraction, *tert*-butyldimethylsilylated, and analyzed by GC/MS. The yield is defined as the percentage value of the peak area ratio of TBDMS derivatives to internal standard, compared to the value for acetonitrile solution containing the same concentrations of phosphonates. The values was an average of three determinations ± standard deviation.

We initially attempted to apply the alkaline sample solution directly to the cartridge. As shown in Figure 4A, part of the applied RMPA (15−25%) appeared in the wash fraction, although MPA was quantitatively recovered in the final purification fraction. The alkalinity interferes with the adsorption of RMPA onto the anion-exchange cartridge. Neutralization of the alkaline sample solution with HF improved the extraction recovery. As shown in Figure 4B, MPA and RMPA were quantitatively recovered in the final elution fraction. HF was used as a neutralizer, because fluoride ion does not compete with MPA and RMPA in the anion exchange.

We examined the efficiency of the solid-phase extraction cleanup method with the alkaline soil extracts. The soil samples

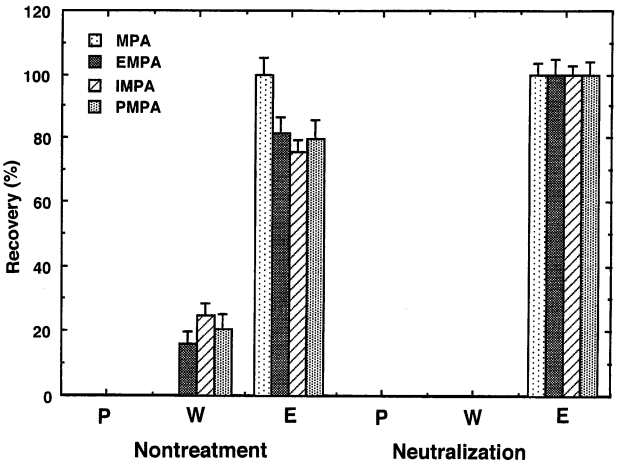


Figure 4. Elution profile of RMPA and MPA in the solid-phase extraction using a strong anion exchanger. A 2-mL sample of 0.1 M NaOH solution containing 100 $\mu$g of MPA, EMPA, IMPA, and PMPA without (nontreatment) and with (neutralization) neutralization by HF was applied to a Bond Elut SAX cartridge (fluoride form) and washed with 5 mL of water. The analytes were eluted with 5 mL of 3% ammonia in methanol. The pass-through fraction (P), the wash fraction (W), and the elution fraction (E) were assayed for RMPA and MPA by capillary electrophoresis after sample neutralization if the solution was alkaline. Each value represents an average recovery (three determinations) ± standard deviation.

(2 g) were spiked with RMPA and MPA (each ~150 $\mu$g) and extracted with 4 mL of 0.1 M NaOH solution. The alkaline soil extract was neutralized with HF and subjected to the solid-phase extraction, and the resulting extracts were analyzed by CE. As shown in Table 3, for PMPA and IMPA in the no. 1 soil sample, MPA in the no. 4 soil, and IMPA and EMPA in the no. 6 soil, a small part of analyte was eluted in the washing fraction, although the recoveries for the other cases were almost quantitative. The organic matter content of soil samples no. 1, 4, and 6 was rather high, and the organic matter extracted from such soils would be expected to interfere with the binding of MPA and RMPA to the SAX cartridge.

**Application to Soil Samples.** The established method for the TBDMS GC/MS determination of MPA and RMPA using aqueous alkaline extraction combined with solid-phase extraction was applied to the soil samples. As shown in Table 3, the detection yields were in the range of 80%, except for the somewhat low values for MPA in all the soil samples and IMPA and PMPA in no. 5 soil. The ratios of the detection yields to the extraction recoveries were, however, in excess of 0.89, indicating a fairly good removal of interfering compounds from the alkaline extracts by the solid-phase extraction.

## DISCUSSION

With respect to the chemical nature of the phosphonates analyzed, in contrast to more hydrophobic PMPA, MPA is very polar and is never extracted completely with an organic solvent. EMPA is intermediate in nature. Therefore, it is rather difficult to extract and determine MPA and RMPA simultaneously from complex matrixes. Some research groups have developed methods for the determination of RMPA but not for MPA, because MPA is not considered to be a primary degradation product of nerve gas.[11,21] However, MPA is also formed from precursors and intermediates in nerve gas production, such as methyphosphonyl dichloridate, methyphosphonyl difluoride, and dimethyl methylphosphonate (Figure 1).[2] Therefore, the detection of MPA is important from the standpoint of chemical verification.

The low detectability of anionic phosphonates, MPA and RMPA, from soils in silylation GC analysis is mainly attributed to the following two mechanisms: interference in the derivatization reaction and the adsorption of RMPA and MPA to soils. As for the silylation interference, two categories of interferences can be considered. The first is metal cations, such as $Ca^{2+}$ and $Mg^{2+}$, which form insoluble complexes with anionic phosphonates, leading to the suppression of derivatization. The second is compounds that react with silylating reagent, such as carbohydrates, which compete with phosphonates in the silylation reaction. Another possible factor is compounds that interfere with GC separation and the detection of derivatized MPA and RMPA. These interferences can be present in aqueous soil extracts but can be removed by strong cation- or anion-exchange pretreatment.[13,14] The Finnish recommended operating procedures[3] also raise the pretreatment method of chemical warfare agent degradation products from soils for the derivatization GC/MS analysis, using the aqueous soil extraction followed by the strong cation-exchange solid-phase extraction. If possible, LC/MS offers direct determination for the aqueous extracts.

Even though such an ion-exchange pretreatment is performed, MPA and RMPA are not well detected from some types of soils. Anionic phosphonates are strongly adsorbed onto soils and cannot be extracted with water. This can be attributed to ionic interactions. RMPA are acidic with a p$K_a$ value of 2−3, and the p$K_a$ of MPA are 2.2 and 7.7.[1] These negatively charged molecules are fixed in the soil matrix, adsorbing to the same sites via the same mechanism as phosphate. Positively charged polyvalent cations, adsorbed on clays such as aluminum and ferric, are typical phosphate adsorption sites in mineral matter.[22] It is also thought that phosphonates are adsorbed to soils via the formation of a ternary surface complex when $Ca^{2+}$ levels are high.[16] Nowack and Stone reported that the adsorption of phosphonates to goethite was via a ligand-exchange reaction with decreasing adsorption at increasing pH values.[23] Organic matter, such as humic material, should be considered to be alternate adsorption sites, and the binding mechanism has been reported to involve hydrogen bond formation in the case of the phosphonate herbicide, glyphosate.[24]

To improve the extraction recovery of phosphonates from soils, we attempted to increase the ionic strength in the soil extraction solvent, but this did not have a remarkable effect.[13] Successive supercritical fluid and pressurized liquid extraction has been examined to extract hydrocarbons and phosphonate derivatives from soils,[25] and 68−83% of the spiked EMPA was recovered from soils. However, these investigators did not consider a variety of soil types and did not investigate the effect on MPA extraction. Vermillion and Crenshaw developed a bicarbonate/carbonate

(21) Noort, D.; Hulst, A. G.; Platenburg, D. H. J. M.; Polhuijs, M.; Benschop, N. P. *Arch. Toxicol.* **1998**, *72*, 671−675.
(22) Foth, H. D. *Fundamentals of Soil Science*, 8th ed.; John Wiley and Sons: New York, 1990.
(23) Nowack, B.; Stone, A. T. *Environ. Sci. Technol.* **1999**, *33*, 3627−3633.
(24) Piccolo, A.; Celano, G.; Conte, P. *J. Agric. Food Chem.* **1996**, *44*, 2442−2446.
(25) Chaudot, X.; Tambute, A.; Caude, M. *J. Chromatogr., A* **2000**, *866*, 231−240.

extraction method,[12] where the recoveries of IMPA and MPA from silt loam were 100 and 60%, but the issue of whether their developed methods work for both MPA and RMPA from any type of soil sample is unclear.

The above-mentioned adsorption mechanisms suggest that the binding of MPA and RMPA onto soils can be weak under conditions of elevated pH. As shown in Figure 2, increasing the alkaline concentration in the extraction solution increased the recovery of MPA and RMPA from one of volcanogenous immature soils. Using a 0.1 M NaOH solution, more than an 80% recovery of RMPA was obtained. The reason that only a 68% recovery was obtained for MPA may not be due to the intrinsic mechanism of MPA binding in the soil structure but rather merely due to the inaccuracy in CE quantification in the presence of high levels of hydroxide ion. The Finnish recommended operation procedure[3] raises the alkaline methanol extraction (including triethylamine or potassium hydroxide), but it is assumed that ionized MPA is not perfectly solubilized from soils under methanolic alkaline conditions and the extraction recovery of MPA is not so high.

Treatment of soils with an alkaline solution would be expected to result in the drastic extraction of a variety of soil matrix components. The mineral structure of soils may be destroyed in part, and organic compounds, such as humic materials, may be released from the organic matter. In this study, the organic matter in the soil extract was not chemically measured, but the extent of ultraviolet absorption of the soil extract increased significantly with an increase in alkalinity (absorbance at 254 nm: 2 in water, 7 in 0.01 M NaOH, 12 in 0.02 M NaOH, 50 in 0.05 M NaOH, and 180 in 0.2 M NaOH). The extracted matrix components, which would be expected to interfere with the TBDMS derivatization and GC separation, must be removed from the alkaline extracts.

We investigated the efficiency of solid-phase extraction using a SAX cartridge[20] for the cleanup of MPA and RMPA. In the solid-phase extraction, the ion exchanger was conditioned in the fluoride form, which has a weak anion-exchange capacity, and MPA and RMPA in the applied sample were retained on the cartridge under these conditions. Neutral and cationic compounds were washed out with water, and the target compounds were eluted with a high concentration of ammonia in methanol. The solvent in the eluate could be easily removed by evaporation. However, under alkaline sample conditions, RMPA did not completely adsorb on the cartridge, and part of the analytes applied were eluted in the process of washing. This decreased recovery is not due to the deterioration of the silica cartridge, such as partial destruction of silica solid-phase backbone, because no significant increase in silica level was observed (8 ppm silica in water extract vs 10 ppm silica in alkaline (0.1−0.1 M NaOH) extracts) in the effluent or the wash fraction in the solid-phase extraction of the alkaline solution, as measured by inductively coupled plasma atomic emission spectrometry. Neutralization of the alkaline extract with HF (having weak anion-exchange capacity) permitted the quantitative recovery of RMPA and MPA. For the soil samples, even though various types of interfering compounds were extracted with the alkaline solution, the solid-phase extraction permitted a recovery in excess of 89% (obtained as the ratio of the analytes before and after solid-phase extraction).

The established method using an alkaline soil extraction and solid-phase extraction pretreatment led to detection yields ranging from 67 to 90% in the TBDMS GC/MS analysis of MPA and RMPA from the soil samples tested. This significant efficiency of the established pretreatment method can be contrasted with those of the original aqueous extraction method where the detection yields were in the range from 1.1 (MPA, no. 3 soil) to 29.6% (IMPA in no. 2 and 5 soils) and, in addition, those of the aqueous extraction combined with solid-phase extraction method where the yields were in the range from 6 (MPA, no. 4 soil) to 83% (EMPA, no. 6 soil). Only 4 h is required for all the procedures from soil extraction to GC/MS analysis. Our method offers a convenient, structure-confirming, and quantitative determination of nerve gas hydrolysis products in soil samples and should be useful in chemical verification and forensic fields. LC/MS[6,7] or CE/MS[26] is another choice for the determination of MPA and RMPA in the aqueous alkaline extracts of soils, saving us some sample-handling steps.

## CONCLUSIONS

We have presented in this study the development of the aqueous alkaline extraction followed by solid-phase extraction for the *tert*-butyldimethylsilyl derivatization GC/MS of nerve gas hydrolysis products from soils. More than 68% recoveries of methylphosphonic acid and alkyl methylphosphonic acids were achieved by the extraction of some volcanogenous immature soils with 0.1 M sodium hydroxide solution. Strong anion-exchange solid-phase extraction enabled the elimination of the interfering compounds from the aqueous alkaline extracts, providing the quantitative determination in GC/MS.

## ACKNOWLEDGMENT

We greatly acknowledge Mr. Kiyohide Nagahama (Forensic Science Laboratory of Kagoshima Prefectural Police Headquarters) for providing the soil samples no. 1−5.

Received for review March 26, 2002. Accepted July 7, 2002.

AC020200O

(26) Mercier, J.-P.; Chaimbault, P.; Morin Ph.; Dreux, M.; Tambute, A. *J. Chromatogr., A* **1998**, *825*, 71−80.