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Efficiency of pretreatment of aqueous samples using a macroporous strong anion-exchange resin on the determination of nerve gas hydrolysis products by gas chromatography–mass spectrometry after *tert*.-butyldimethylsilylation

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Abstract

A pretreatment procedure, using a macroporous strong anion-exchange resin (MSA) has been established for the determination of nerve gas hydrolysis products by gas chromatography–mass spectrometry (GC–MS) after *tert*.-butyldimethylsilyl (TBDMS) derivatization. Aqueous solutions of methylphosphonic acid (MPA) and three alkyl methylphosphonic acids (AMPAs) (ethyl, isopropyl and pinacolyl methylphosphonic acid), were retained on the MSA column, and then quantitatively eluted with 0.1 *M* hydrochloric acid. The neutralized column eluate was dried, and MPA and AMPAs were derivatized with *N*-methyl-*N*-(*tert*.-butyldimethylsilyl)-trifluoroacetamide and analyzed by GC–MS. The column eluate was also analyzed in order to determine the exact hydrolysis product levels by capillary electrophoresis using borate and benzoate buffer (pH 6). The MSA pretreatment was examined for the clean-up of aqueous extracts of three types of soils and an aqueous solution containing 10% sucrose, which is regarded as model for a typical soft drink, after spiking with MPA and AMPAs. MPA and AMPAs were quantitatively recovered in the MSA eluate fraction from those samples, except for MPA from volcanic acid and alluvial soils. The yields of TBDMS derivatives were remarkably improved, compared with for which no pretreatment was used and also for those in which a strong cation-exchange resin was used. The achieved detection limits of MPA and AMPAs ranged from 0.12 to 0.18 μg/g of soil ($S/N=3$). The established MSA method was applied to the pretreatment of spiked sea water, two types of beverages, Pepsi Cola and canned coffee. Although the yields of TBDMS derivatives of MPA and AMPAs in sea water (in a range between 44 and 96%) and AMPAs in Pepsi Cola (in a range between 58 and 92%) were rather high, those for MPA in the Pepsi Cola (27%) and those for MPA and AMPAs in the canned coffee (in a range between 5 and 17%) were low.

Keywords: Warfare agents; Forensic analysis; Sample treatment; Alkyl methylphosphonic acids; Methylphosphonic acid

1. Introduction

An act of terrorism involving mass murder occurred in Matsumoto City in 1994 and in the Tokyo Subway System in 1995 [1]. Allegedly, these two incidents were committed by Aum Shinrikyo, a Japanese Cult, and involved the use of sarin, a chemical warfare agent (CWA), which had been manufactured in the cult's facilities. In 1998, a series

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of poisoning cases occurred all over Japan, where various types of toxic substances were added to food and beverages which were then used in the crimes [2]. There is a need to detect such toxic agents and to identify them in suspected evidence samples by forensic science laboratories. Forensic samples are compositionally quite variable, ranging from environmental to biological samples. Among toxic substances, nerve gases such as sarin, soman, tabun and VX, are characterized by their extremely high toxicity and instability. Such compounds easily hydrolyze to produce alkyl methylphosphonates (AMPAs) [3]. Due to hydrolysis, it may be difficult to directly detect nerve gases in their active form from crime scenes or CWA verification sites. However, indirect evidence can be obtained via the identification of the characteristic AMPAs [4]. Determination of methylphosphonic acid (MPA) is also important because it is a stable hydrolysis product of AMPAs and also derived from dimethyl methylphosphonate, methylphosphonyl dichloride or methylphosphonyl difluoride, which are, in turn, intermediates in sarin production.

In terms of the analysis of MPA and AMPAs in a complex matrix, liquid chromatography–tandem mass spectrometry (LC–MS–MS), holds considerable promise [5]. Capillary electrophoresis (CE) and ion chromatography (IC) are also possible candidates for detecting these anions, but they often suffer from severe interference by other molecules in the sample matrix. The use of gas chromatography (GC)–MS for the analysis of AMPAs and MPA, as volatile derivatives, is well established, and the selectivity of GC–MS gives the analyst a distinct advantage over CE and IC. Methylation [6], trimethylsilylation [7], *tert*.-butyldimethylsilyl (TBDMS) [8] or pentafluorobenzylation [9] have been used as derivatization agents in the research areas of chemical warfare verification. We have chosen TBDMS because of its ease of use and high efficiency of derivatization.

Our laboratory, which has been engaged in forensic investigation of the sarin incidents, has used GC–MS exclusively for the analysis of nerve gas hydrolysis products [10,11]. In the course of our forensic investigation, we have observed a low level of detectability of MPA and AMPAs from evidence samples, such as soil and wipe samples taken from a variety of crime scenes. In addition, beverage and food samples also fall under the category in poisoning cases. In order to detect MPA and AMPAs from such a wide diversity of forensic samples, all types of possible interference need to be considered, and clean-up techniques must be adopted for the removal of these interfering substances. The low level of detectability can be derived from both chromatographic interference on GC–MS detection and the suppression of formation of TBDMS derivatives. With respect to the latter issue, we have indicated that divalent cations, Ca^{2+} and Mg^{2+}, severely disturbed the detection of MPA and AMPAs, and developed strong cation-exchange (SCX) pretreatment methods to remove cations from soil extracts. This procedure significantly improved detectability levels [12]. However, even using SCX pretreatment, raising the yields were limited by the possible existence of the other interfering substances. Neutral and anionic compounds which pass through the SCX resin may interfere with the formation of TBDMS derivatives [12].

Anion-exchange is the best candidate for purifying MPA and AMPAs in complex matrix. Solid-phase extraction using the commercially available silica-based strong anion-exchange (SAX) cartridge has been recommended as a pretreatment method for derivatization GC–MS of nerve gas hydrolysis products [13], but resulted in insufficient yields [14]. Other types of cartridges, such as aminopropyl, have also been reported [15], and satisfactory recovery could not be achieved. Considering the extremely high levels of compounds such as salts, carbohydrates and oils in forensic samples, commercially available SAX cartridges, which possess a rather low ion-exchange capacity, do not seem enough to offer sufficient retaining capacity. A polystyrene–divinylbenzene copolymer type strong anion-exchange resin, such as Dowex 1, has a high anion-exchange capacity. It has, however, a drawback in terms of a low column flow-rate. Macroporous strong anion-exchange resin (MSA), however, fulfills both requirements of high anion-exchange capacity and high flow-rate.

We wish to report here an investigation of the efficiency of MSA pretreatment on GC–MS detection, in conjugation with TBDMS derivatization for nerve gas hydrolysis products, and a comparison with the previously reported method using a SCX

resin. The CE method was also adopted for measuring the exact levels of MPA and AMPAs in the MSA clean-up fraction. The MSA pretreatment method has been examined for clean-up of MPA and AMPAs from soils, sea water, a beverage-imitated model which contains a high sucrose concentration solution, and two types of actual beverages, Pepsi Cola and canned coffee.

2. Experimental

2.1. Reagents

Dowex 50W-X8 (50–100 mesh), Muromac MSA-1 (50–100 mesh) were obtained from Muromachi (Tokyo, Japan). *N*-Methyl-*N*-(*tert*.-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) was obtained from Pierce (Rockford, IL, USA). MPA, ethyl methylphosphonic acid (EMPA) and pinacolyl methylphosphonic acid (PMPA) were obtained from Aldrich (Milwaukee, WI, USA). Isopropyl methylphosphonic acid (IMPA) was prepared as previously reported [12]. 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 (400 ppm) and stored at −20°C for use as stock solutions. A working solution was prepared by diluting the stock solution with acetonitrile or distilled water.

2.2. Aqueous extraction of soil samples

The following soil samples were used as previously reported [12]. Soil sample No. 1 (sand) was collected from a seashore in the Okayama prefecture (Japan). Soil sample No.2 (alluvial soil) was collected from a garden in the Kyoto prefecture (Japan). Soil sample No. 3 (a volcanic ash soil) was collected from a garden in our previous Institute (Chiyoda-ku, Tokyo, Japan). These soil samples were filtered through a 2-mm sieve and dried at room temperature for several days. Chemical and physical characteristics of the soil samples Nos. 1 and 2 can be found in a previous paper [12].

A 50-μl volume of acetonitrile solution containing MPA and AMPAs was spiked to 2 g of the soil samples and allowed to stand at room temperature for 3 h. A 4-ml volume of distilled water was added and the resulting suspension vortex-mixed for 1 min and then sonicated for 10 min. Samples were centrifuged at 1500 *g* for 5 min, and the resultant supernatant was filtered through a 0.45-μm cellulose acetate membrane. Aliquots (0.5–2.0 ml) were analyzed directly, and after pretreatment using SCX or MSA, and then subjected to TBDMS derivatization and subsequent GC–MS or CE.

2.3. Other sample pretreatment

''Sea water'' was collected from a seashore in Kasai seaside park which is situated on Tokyo bay. The potassium, sodium and magnesium contents were 179, 5440 and 619 ppm, respectively, determined by CE and reported previously [12], and chloride and sulfate concentrations were 7630 and 968 ppm, respectively, also determined by CE using a 5 m*M* sodium chromate buffer (pH 8.0) and 0.5 m*M* CIA-Pak OFM Anion-BT (Waters) and indirect absorbance at 254 nm, respectively. Two types of beverages, ''Pepsi Cola'', a caffeinated and carbonated drink containing sugar (Suntory, Osaka, Japan) and ''Monte Alban'', a canned coffee drink containing milk (Nestle Japan, Kobe, Japan) were purchased commercially. A 10% sucrose solution was prepared by dissolving sucrose in distilled water.

An 800-μl volume of a solution containing MPA and AMPAs was spiked to 5 ml of each sample, which had been filtered through a 0.45-μm cellulose acetate membrane, and directly, or after pretreatment using SCX or MSA, subjected to TBDMS derivatization and subsequently analyzed by GC–MS or CE.

2.4. Ion-exchange pretreatment

Dowex 50W-X8 was activated according to the manufacturer's protocol, converted to the H^+ form and equilibrated with distilled water. A 2-ml volume of aqueous soil extract or spiked sample was applied to the column (1.53 cm×1.2 cm I.D., 2 ml resin) at a flow-rate of about 0.5 ml/min and eluted with distilled water at an ambient flow-rate. Both effluent (2 ml) and eluate (3 ml) were combined as the SCX elution fraction.

MSA (Muromac MSA-1) was activated according to the manufacturer's protocol, converted to the OH^- form and equilibrated with distilled water. A 2-ml volume of an aqueous soil extract or a spiked sample was applied to the column (2.3 cm×0.5 cm I.D., 0.5 ml resin) at a flow-rate of about 0.3 ml/min and washed with 10 ml of distilled water at an ambient flow-rate, followed by 3.5 ml of 0.1 *M* HCl solution. Analytes were eluted with an additional 3.0 ml of 0.1 *M* HCl solution, and combined as the MSA elution fraction. For sea water, after sample application and water washing, the analytes were eluted with 6.5 ml of a 0.1 *M* HCl solution, and combined as the MSA elution fraction. The elution flow-rate was manually maintained between 1 and 2 ml/min.

2.5. tert.-Butyldimethylsilylation and gas chromatography–mass spectrometry of methylphosphonic acid and alkyl methylphosphonates

The column eluate fraction, spiked sample or aqueous soil extract was neutralized with sodium hydrogencarbonate (pH≈7), if pH of the solution was not already neutral, and concentrated under reduced pressure at 50°C on a rotary evaporator. The concentrated solution or the working solution was transferred to a 1-ml glass vial stoppered with a PTFE screwed cap (Nichiden Rika Garasu, type MV-07, Tokyo, Japan), and dried on a Model VC-360 centrifugal concentrator (Taitec, Saitama, Japan) under reduced pressure at 50°C. A 50-μl volume of MTBSTFA and 50 μl of acetonitrile, which also contained 25 ppm of anthracene (internal standard, I.S.) were added, homogenized with sonication for 5 min and incubated at 60°C for 1 h. A 1-μl volume of the mixture was applied to the GC system described below.

The GC–MS system consisted of an HP 5890 series II gas chromatograph combined with an HP 5989B quadrupole mass spectrometer (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, Folsom, CA, USA). Carrier-gas (helium) flow-rate and splitter ratio were adjusted at 0.64 ml/min and 42, respectively. The injection port, transfer line and ion source were maintained at 250°C, 280°C and 250°C, respectively. 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 at 90°C (1 min hold), then to 290°C at 20°C/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 I.S.

2.6. Capillary electrophoretic determination of nerve gas hydrolysis products in aqueous soil extracts

Nerve gas hydrolysis products in a spiked sample, an aqueous soil extract or the column elution fraction were determined according to Pianetti et al. [16] using a Quanta 4000E CE system (Waters, Milford, MA, USA). The capillary column used was fused-silica (60 cm×75 μm I.D.), and electrophoresis buffer was 100 m*M* boric acid containing 10 m*M* 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 and the column temperature was maintained at 25°C. Samples were applied hydrostatically for 30 s.

2.7. Determination of carbohydrates

Sucrose was determined colorimetrically by the phenol–sulfuric acid method [17].

3. Results

3.1. Capillary electrophoresis of methylphosphonic acid and alkyl methylphosphonic acids

In a previous paper [18], we compared various CE methods for the detection of MPA and AMPAs from the standpoint of sensitivity, resolution and interference by chloride and carbonate ions. In this paper, we adopted the CE conditions reported by Pianetti et al. [16], which showed good separation of alkylphosphonates. As shown in Fig. 1, the CE conditions

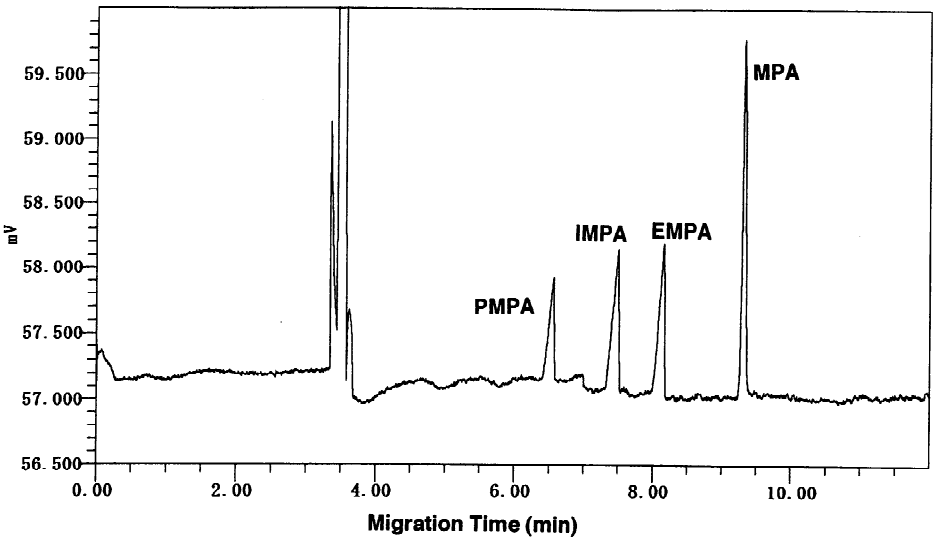

Fig. 1. Electropherogram of nerve gas hydrolysis products. A standard solution containing 49 ppm of MPA, 47 ppm of EMPA, 58 ppm of IMPA, 47 ppm of PMPA was hydrostatically applied for 30 s to a capillary electrophoretic system maintained at 25°C using a capillary fused-silica column (60 cm×75 μm I.D.) and an electrophoresis buffer composed of 100 m*M* boric acid containing 10 m*M* benzoate (pH 6.0). Voltage was set at 30 kV with a positive power supply. Detection was indirect ultraviolet absorption at 254 nm.

provide for a distinctive separation of MPA and AMPAs with detection limits of about 2.5 ppm, along with high numbers of theoretical plates (21 000–190 000) and with no interference by high concentrations of chloride and carbonate [18]. The recovery of MPA and AMPAs was nearly 100%.

3.2. Anion-exchange behavior of nerve gas hydrolysis products

In the neutral pH range, MPA and AMPAs exist as strong anions [3]. They were efficiently trapped on the Muromac MSA-1 resin (OH^- form), and can be eluted with HCl solution. A preliminary experiment indicated that MPA and AMPAs were not eluted from the resin with a reasonable volume of 0.05 *M* HCl. A 0.1 *M* HCl solution was sufficient to entirely elute MPA and AMPAs. MPA and AMPAs showed almost the same elution profile. A column volume of 0.5 ml was chosen, in consideration of the sufficient anion-exchange capacity and the minimum elution volume. Accordingly, an MSA column of 0.5 ml resin volume was eluted with 0.1 *M* HCl and the fractions eluting between 4.0 and 6.0 ml of the acidic eluate were collected. The recovery was reproducible: 93.9% for MPA, 97.1% for EMPA, 95.3% for IMPA and 90.4% for PMPA (average of three determinations).

3.3. Effect of strong anion-exchange pretreatment on tert.-butyldimethylsilyl derivatization for an aqueous soil extract

The aqueous extract of the soil sample which had been spiked with MPA and AMPAs (4–10 μg/g) was pretreated with MSA. An aliquot of the resulting eluate was analyzed by TBDMS derivatization followed by GC–MS and CE. As shown in Table 1, except for MPA in the No. 2 and No. 3 soils, yields in excess of 35% were achieved. The yields of TBDMS derivatives were considerably higher than those achieved without MSA treatment (in the range from 0 to 41%) [12]. Compared to SCX pretreatment [12], the yields of TBDMS derivatives for MPA and AMPAs in the No. 2 soil sample (alluvial soil) were significantly improved (in the range from 17 to 44% vs. from 0 to 16%), although the yields in No. 1 or No. 3 soils were not so much improved. TBDMS derivatization yields with or without MSA pretreatment for the other spiked samples (20 μg AMPAs

Table 1
Recoveries of methylphosphonic acid and alkyl methylphosphonates from soil samples

Analyte	Analysis	Recovery (%)		
		Soil 1 (sand)	Soil 2 (alluvial soil)	Soil 3 (volcanic acid soil)
MPA	GC–MS[a]	44.5±9.5[c]	16.7±1.6[d]	2.3
	CE[b]	72.7±2.7	59.9±3.2	7.9
EMPA	GC–MS	54.2±10.5[c]	35.1±4.3[c,d]	44.3
	CE	96.0±3.6	93.4±9.6	91.1
IMPA	GC–MS	61.5±11.6[c]	42.9±4.3[c,d]	50.5
	CE	95.6±1.1	98.5±12.2	94.1
PMPA	GC–MS	65.3±6.4[c]	43.6±9.2[c,d]	48.3
	CE	92.4±2.4	97.6±14.4	91.5

[a] A 50-μl volume of an acetonitrile solution containing from 4 to 10 μg each MPA, EMPA, IMPA and PMPA was spiked to 2 g of a soil, and the mixture was extracted with 4 ml of water. A 2-ml aliquot of the extract was treated with MSA, *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 yield value represents an average (five determinations for No. 1 soil and three for No. 2 soil samples)±SD.

[b] A 50-μl volume of an acetonitrile solution containing each 120 μg of MPA, EMPA, IMPA and PMPA was spiked to 1 g of soil, and the mixture was extracted with 2 ml of water. An aliquot of the resultant supernatant was analyzed by capillary electrophoresis. The recovery value represents an average (five determinations for No. 1 soil, four for No. 2 soil and two for No. 3 soil samples except for IMPA)±SD.

[c] Significantly increased ($P<0.01$) compared to the value for the untreated samples (Table 2 in Ref. [8]).

[d] Significantly increased ($P<0.01$) compared to the value for samples treated with strong cation-exchange (Table 4 in Ref. [8]).

and MPA per 1 g of soil) were almost the same as those in Table 1.

The recovery values were also analyzed for soil extracts by CE. Higher spiked levels (120 μg AMPAs and MPA per 1 g of soil) were needed because of low CE sensitivity. As shown in Table 1, AMPAs were nearly completely extracted from all the soil samples. In contrast, MPA was extracted from No. 1 and No. 2 soil samples less efficiently, and from No. 3 soil with a very low recovery. By comparing the yield values of TBDMS derivatives, as determined by GC–MS with the recovery values measured by CE, the reaction efficiency of TBDMS derivatization in the purified MSA fraction can be evaluated. The ratios are in the range from 28% to 71%, indicating that MSA pretreatment is an efficient method for elimination of matrix components which inhibit or interfere with TBDMS derivatization. The recovery values for the other spiked samples (50 μg AMPAs and MPA per 1 g of soil) were almost the same as those in Table 1.

The accuracy and precision of GC–MS determination with the MSA pretreatment was examined by spiking known amounts of MPA and AMPAs in the No. 1 soil sample. The calibration curves of the peak area ratios of TBDMS derivatives to I.S. were linear for spiked amounts ranging from 0.2 to 5 μg/g for all analytes with correlation coefficients of higher than 0.946, and a within-day repeatability (3 μg/g soil, $n=5$) were 21.3% (RSD) for MPA, 19.3% for EMPA, 18.9% for IMPA and 9.7% for PMPA. Detection limits ($S/N=3$, signal: extracted ion of m/z 153) are given 0.12 μg for EMPA and IMPA and 0.18 μg for MPA (m/z 267) and PMPA (m/z 153) per g of soil.

3.4. Effect of strong anion-exchange pretreatment on tert.-butyldimethylsilyl derivatization for solutions which contain high concentration of sucrose

The issue of whether or not MSA pretreatment method permitted the efficient detection of nerve gas hydrolysis products from aqueous solution of high sucrose concentration was examined. A 10% sucrose solution was chosen as a model for a soft drink beverage because such beverages all contain similar sugar content. The sucrose solution, which was

spiked with MPA and AMPAs (each 0.8–1.2 ppm), was analyzed by GC–MS with TBDMS derivatization, directly, after SCX pretreatment or after MSA pretreatment. As shown in Table 2, the yields of TBDMS derivatives in the absence of pretreatment were very low (in the range from 3 to 12%). Preliminary experiments indicated that even lower levels (0.1% or less) of sucrose in a sample solution suppressed the detection of TBDMS derivatives of MPA and AMPAs. SCX pretreatment improved the yields of AMPAs (ranging from 35 to 64%), but not so much of MPA (6%). In contrast, MSA pretreatment significantly improved the yields for all analytes (in the range from 37 to 94%). In the course of MSA pretreatment, sucrose was recovered in the column pass-through fraction (94%), but was still widely distributed throughout the elution fractions. A small part of sucrose (1.8%) was eluted at the position which overlapped with MPA and AMPAs.

3.5. Application of strong anion-exchange pretreatment to the clean-up of sea water and beverages

To confirm that the established MSA pretreatment method is applicable to environmental and beverage samples, Pepsi Cola, canned coffee drink and sea water which had been spiked with MPA and AMPAs (1.4–2.0 ppm each) were analyzed by GC–MS in conjugation with TBDMS derivatization, directly, after MSA pretreatment. As shown in Table 3, for beverage samples, without MSA pretreatment the yields of TBDMS derivatization were very low (<4.1%), which can be explained by interference by sucrose. The sucrose concentration was 5.08% in the Pepsi Cola and 8.77% in the canned coffee samples. As shown in Fig. 2, MPA and AMPAs were quantitatively recovered from the spiked Pepsi Cola by MSA pretreatment, but small part of the sucrose was coeluted with MPA and AMPAs, and, as a result, the yields of TBDMS derivatives from Pepsi Cola were in the range from 27 to 92%, and compatible with those for a 10% sucrose solution (Table 2). MPA and AMPAs in the sucrose solution were eluted almost at the same fraction as those in the control solution.

For canned coffee, although the spiked MPA and AMPAs were quantitatively recovered by MSA pretreatment (except for PMPA which was subject to interference by unknown substances), the yields of TBDMS derivatives was low in the range from 5 to 17%.

For sea water, in the absence of MSA pretreatment, the yields of TBDMS derivatives were very low, even with a four-fold dilution of the sample. This can be attributed to the high level of magnesium ions in sea water (619 ppm). A preliminary experiment concerning MSA pretreatment indicated that, when the sample was applied without sample dilution, MPA and AMPAs were eluted in the water wash fraction. Even when the sample was diluted

Table 2
Yields of *tert*.-butyldimethylsilyl derivatives of methylphosphonic acid and alkyl methylphosphonates from a solution of high sucrose concentration

	Pretreatment			
	None GC–MS[a]	SCX GC–MS[b]	SAX GC–MS[c]	SAX CE[d]
MPA	2.9	5.3	36.7	93.9
EMPA	7.7	36.9	41.0	98.9
IMPA	6.4	35.4	41.9	93.1
PMPA	12.2	63.7	93.9	86.3

[a] A 2-ml volume of a 10% sucrose solution containing 2 μg each of MPA, EMPA, IMPA and PMPA was left untreated, *tert*.-butyldimethylsilylated and analyzed by GC–MS.

[b] A 2-ml volume of a 10% sucrose solution containing 2 μg each of MPA, EMPA, IMPA and PMPA was treated with cation-exchange using Dowex 50W, *tert*.-butyldimethylsilylated and analyzed by GC–MS.

[c] A 2-ml volume of a 10% sucrose solution containing 2 μg each of MPA, EMPA, IMPA and PMPA was treated with MSA[c], *tert*.-butyldimethylsilylated and analyzed by GC–MS.

[d] A 2-ml volume of 10% sucrose solution containing each 120 μg of MPA, EMPA, IMPA and PMPA was treated with anion-exchange using Muromac MSA-1, and analyzed by capillary electrophoresis. The value was represented as average of two determinations or that of single determination.

Table 3
Recoveries of methylphosphonic acid and alkyl methylphosphonates from sea water and beverage samples

Analyte	Analysis	Recovery (%)			
		Pepsi Cola	Canned coffee	Sea water (diluted ×2)	Sea water (diluted ×4)
MPA	None GC–MS[a]	3.3	1.3	0.9	1.1
	SAX GC–MS[b]	26.6	5.2	64.2	62.7
	CE[c]	88.4	108.5	90.4	95.8
EMPA	None GC–MS	3.7	3.3	3.0	7.7
	SAX GC–MS	57.8	7.8	23.8	43.9
	CE	106.5	107.5	88.9	95.1
IMPA	None GC–MS	4.1	2.8	5.6	11.2
	SAX GC–MS	64.3	8.2	56.8	71.2
	CE	91.5	108.7	104.9	102.5
PMPA	None GC–MS	3.9	2.8	24.6	29.3
	SAX GC–MS	91.5	16.9	97.2	95.9
	CE	88.1	N.D.[d]	91.0	96.7

[a] A 2-ml sample (Pepsi Cola and canned coffee, sea water two-fold or four-fold diluted with water) spiked with each 2 μg of MPA, EMPA, IMPA and PMPA was directly *tert*.-butyldimethylsilylated and analyzed by GC–MS.

[b] A 2-ml sample (Pepsi Cola and canned coffee, sea water two-fold or four-fold diluted with water) spiked with each 2 μg of MPA, EMPA, IMPA and PMPA was after MSA treatment, *tert*.-butyldimethylsilylated and analyzed by GC–MS.

[c] A 2-ml sample, spiked with 120 μg each of MPA, EMPA, IMPA and PMPA was analyzed by capillary electrophoresis. The value was an average of two determinations or that of single determination.

[d] Not determined.

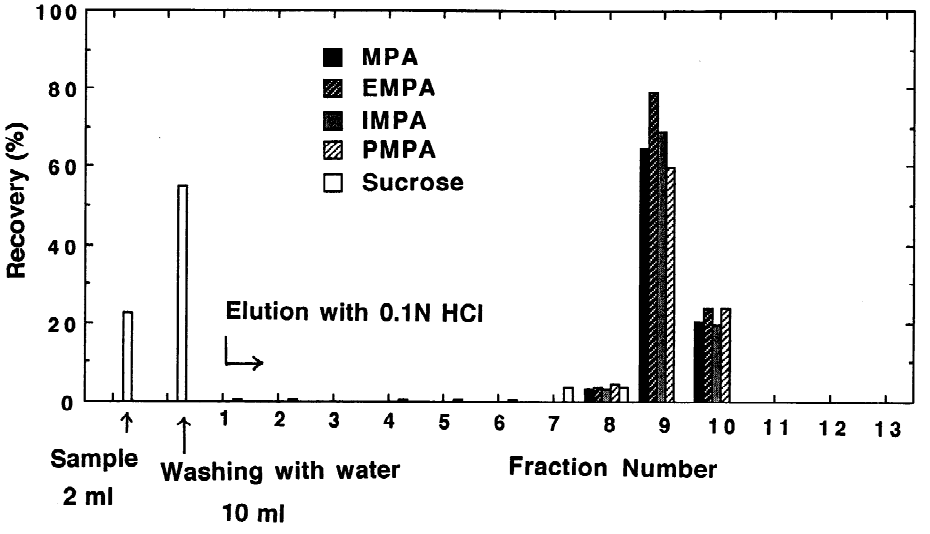

Fig. 2. Elution profile of nerve gas hydrolysis products and sucrose from a strong anion-exchange resin. A 2 ml Pepsi Cola solution containing 100 ppm MPA, EMPA, IMPA and PMPA was applied to a Muromac MSA-1 column (0.5 ml, 2.3 cm×0.5 cm I.D., OH^- form) equilibrated with distilled water, and the column was washed with 10 ml of distilled water. Nerve gas hydrolysis products were eluted with a 0.1 *M* HCl solution, and the sequentially collected 0.5 ml column eluate fractions were analyzed for MPA and AMPAs by capillary electrophoresis. The horizontal line depicts the fraction number and vertical line depicts the percentage recovery of MPA and AMPAs against applied quantity to the resin.

two- or four-fold with water and applied, MPA and AMPAs were eluted in earlier fractions of the 0.1 *M* HCl eluent. High levels of sodium chloride in sea water sample should deteriorate the retention of MPA and AMPAs on the MSA column. By alteration of the MSA pretreatment procedure for collecting all fractions of 0.1 *M* HCl elution (3.5 ml+3.0 ml), MPA and AMPAs were quantitatively recovered in MSA elution fraction (Table 3). The yields of TBDMS derivatives after the modified pretreatment reached levels in excess of 24 and 44% for two-fold and four-fold diluted sea water samples, respectively.

4. Discussion

In terms of clean-up of AMPAs from serum, organic solvent extraction using acetonitrile has been reported to isolate EMPA for analysis by GC–MS with TBDMS [19] or IMPA using isobutanol–toluene for LC–MS [20], but these methods are not effective for the quantitative extraction of polar MPA. Fredriksson et al. [21] developed a GC–MS method with pentafluoro-benzylation after pretreatment using a SCX resin and a SAX cartridge, and achieved good recoveries of AMPAs from water, serum, urine and soil. However, the recovery of MPA was very low. The other reference paper reported low recoveries of AMPAs and MPA [14]. The advantage of using a macroporous type strong anion-exchange resin is its high ion-exchange capacity (1.2 mequiv./ml resin) and high column flow-rate. MPA and AMPAs could be quantitatively recovered from the resin by elution with 0.1 *M* HCl. Only 20 min from sample application to analyte elution were required, and, thus, the sample pretreatment performance compares well with solid-phase extraction.

The recoveries of AMPAs and MPA from soil samples measured by CE were compared with the TBDMS derivatization yields measured by GC–MS, in the different spiking conditions of higher levels for CE and lower levels for GC–MS (Table 1). Because the sensitivity of GC–MS is superior to that of CE, it could not be done under the same spiking conditions. The recovery of the aqueous extraction from soil samples may show different values for different spiked analyte levels, which is based on concentration dependence of adsorption and desorption of spiked phosphonates on the soil. However, the deference of the spiked levels was not so large (4–20 μg/g vs. 50–120 μg/g), and the extraction recoveries from soil samples can be regarded similar for both GC–MS and CE. The recovery of the spiked AMPAs and MPA from aqueous samples (sucrose solution, sea water, beverages) is supposed not to change in principle, so even though the spiked levels differed to large extent the comparison of the recovery values for CE and GC–MS can be reasonably possible (Tables 2 and 3).

The CE result for soil extracts indicated that MPA and AMPAs were extracted into water from soils except for the case of MPA from volcanic acid and alluvial soils. These types of soils have a high phosphorus absorption coefficient [12], and which would also strongly absorbed other chemically similar phosphorus compounds, such as MPA, resulting in a resistance to water extraction. By removing the interfering compounds by MSA pretreatment, reasonable yields of TBDMS derivatives could be obtained. The reason for why full derivatization reaction efficiency was still not attained after the MSA treatment may be due to either the high concentration of sodium chloride, derived from the neutralized MSA elution solvent, or to other interfering compounds extractable from soil.

Sea water resembles the aqueous extract of No. 1 soil (sand collected from seashore) in inorganic content. Although absolute concentrations of such inorganic salts differ in sea water and the No. 1 soil extract, MSA pretreatment gave similar yields of TBDMS derivatives. Saturation of chloride ion over MSA resin may weaken the binding of MPA and AMPAs. However, under the regulated conditions, where the column application content did not exceed the column anion-exchange capacity, MSA pretreatment provides a suitable clean-up for any type of water sample.

Beverages which contain high concentration of carbohydrates are ubiquitous, and it is probable that nerve gases could be absorbed into beverages which are left in some crime scenes. The sucrose molecule, which has numerous hydroxyl functions and reacts with silylating reagents, could interfere with the detection of TBDMS derivatives of MPA and AMPAs. In addition, it is difficult to remove water absolutely from a solution containing high concen-

trations of sugar. In this study, we were particularly interested in the effect of carbohydrates on TBDMS derivatization. A preliminary experiment indicated that IMPA was quantitatively produced from sarin in a 10% sucrose solution and that no conjugate of sucrose and sarin could be detected, suggesting that hydrolysis products can be detected in forensic investigations of beverage adulteration with nerve gases. The established MSA pretreatment method considerably improved the yields. Even using MSA pretreatment, a minute volume of sugar could not be removed from the fraction of MPA and AMPAs, and as a result, full yields were not obtained. However, generally, we were able to detect MPA and AMPAs efficiently in Pepsi Cola. In contrast, the yields were below 17% for the spiked canned coffee. Although MSA pretreatment is capable of circumventing the interfering effects of sugar, the other compounds in milk-blended coffee beverages, such as organic acids, may seriously interfere with the derivatization reaction.

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