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Gas chromatographic determination and gas chromatographic-mass spectrometric determination of dialkyl phosphates via extractive pentafluorobenzoylation using a polymeric phase-transfer catalyst

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Abstract

A GC and GC-MS procedure was established for dialkyl phosphates (DAP), including dialkyl thiophosphates and dialkyl dithiophosphates (alkyl = Me, Et) via extractive pentafluorobenzoylation using a polymeric phase-transfer catalyst (tri-phase catalyst). The abilities of five tri-phase catalysts were compared and among them tri-*n*-butylmethylphosphonium bromide, polymer bound [0.75 mequiv. Br⁻/g, 200–400 mesh (TB-0.75)], was found to be the most effective. The dialkyl thiophosphates and dialkyl dithiophosphates in an aqueous sample were readily extracted in the form of their pentafluorobenzyl (PFB) derivatives when the reaction mixture consisting of buffered aqueous sample, toluene, pentafluorobenzyl bromide and TB-0.75 was stirred at 45°C. The extractive pentafluorobenzoylation of dimethyl phosphate and diethyl phosphate was effected with an additional reaction at 90°C. It was also found that DAP, even in a relatively large volume of aqueous sample, could be captured efficiently by this catalyst.

1. Introduction

Dialkyl phosphates (DAP), including dialkyl thiophosphates and dialkyl dithiophosphates, are the primary hydrolysis products of organophosphorus pesticides (OP) in the environmental degradation or metabolic pathways [1–4]. The determination of DAP is useful from the viewpoint of the evaluation of both environmental and occupational hazards associated with the use of OP. In the forensic field, the determination of

DAP in urine, suspected foods, etc., is important for obtaining evidence of OP intake or addition to foods, etc., and for estimating the type of OP owing to their degradable properties [5].

In recent years, various procedures for the determination of DAP have been developed [6–9]. Generally, these procedures are based on the isolation of DAP from the aqueous phase followed by derivatization and determination by GC or GC-MS. All of these methods have several disadvantages, such as the unfavourable partitioning of highly polar DAP into the extraction solvent, and the samples required exten-

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sive clean-up, which tends to accompany further losses of the phosphates.

In previous papers [10,11], GC methods were described for the determination of DAP by extractive pentafluorobenzoylation in a two-phase system using quaternary ammonium salt ion-pair extraction reagents as a phase-transfer catalyst (PTC), and pentafluorobenzyl bromide (PFB-Br) as a derivatization reagent. The disadvantages of using quaternary ammonium salt PTC without additional treatment are the formation of an emulsion and its unavoidable introduction on to the separation column. This could result in baseline drift and, in the worse case, contamination of the highly sensitive detector.

To improve the extractive pentafluorobenzoylation as an important step in the GC determination of highly polar anions, we adopted polymer-bound phosphonium salt PTC, which has recently become well known in organic synthesis [12,13]. This led to emulsion-free reactions, easy layer separation and injection of the sample into the GC system without an extraction reagent because the catalyst is in the form of a bonded solid particle. We applied this tri-phase procedure to the GC and GC-MS determination of dialkyl phosphates, dialkyl thiophosphates and dialkyl dithiophosphates (alkyl = Me, Et), and their simultaneous determination was developed.

2. Experimental

2.1. Chemicals and reagents

Dimethyl phosphate (DMP) and diethyl phosphate (DEP) were prepared from trimethyl phosphate and triethyl phosphate (Tokyo Kasei, Tokyo, Japan), respectively, according to standard procedures [14], and purified in the form of their sodium salts (>96% purity on titration and proton NMR spectroscopy). The potassium salts of dimethyl thiophosphate (DMTP) and diethyl thiophosphate (DETP) and the sodium salt of dimethyl dithiophosphate (DMDTP) were supplied by courtesy of Takeda Chemical Industries (Osaka, Japan). Diethyl dithiophosphate (DEDTP), Benzyltri-*n*-butylphosphonium bromide (BTBPB), tetra-*n*-butylammonium bro-

midate (TBAB) and tetra-*n*-hexylammonium bromide (THAB) were obtained from Tokyo Kasei. PFB-Br (99+%) was obtained from Aldrich (Milwaukee, WI, USA). Tri-*n*-butylmethylphosphonium chloride, polymer bound [0.78 mequiv. Cl⁻/g (TC-0.78)] was obtained from Fluka (Buchs, Switzerland). Tri-*n*-butylmethylphosphonium chloride, polymer bound [2.26 mequiv. Cl⁻/g (TC-2.26)] was prepared from SX-1 Chloromethylated Bio-Beads [4.15 mequiv./g; Bio-Rad Labs. (Richmond, VA, USA)] and tri-*n*-butylphosphine (97%; Tokyo Kasei) according to the method of Cinouni et al. [15]. The catalyst TB-0.75 and tri-*n*-butylmethylphosphonium bromide, polymer bound [2.05 mequiv. Br⁻/g (TB-2.05)] were prepared from TC-0.78 and TC-2.26, respectively, as follows: TC-0.78 or TC-2.26 (1.0 g) was stirred three times with 5% HBr (3 × 20 ml) for 30 min at room temperature, filtered and successively washed with water (5 × 20 ml), ethanol (5 × 20 ml), methylene chloride (3 × 20 ml) and diethyl ether (3 × 20 ml), and dried under reduced pressure overnight. Kryptofix 222B polymer [0.25 mequiv./g, resin-bound cryptand [2.2.2B] (K-222B)] was obtained from Merck (Darmstadt, Germany). Phosphate buffers (0.1 M) were prepared by mixing 0.1 M potassium dihydrogenphosphate and 0.1 M disodium hydrogenphosphate (Wako, Osaka, Japan). All organic solvents and inorganic reagents were of pesticide or analytical-reagent grade, and all of them were obtained from Wako. Deionized, distilled water was used throughout.

2.2. Preparation of DAP standards

Stock standard solutions of the six DAP (1 mg/ml aqueous solutions, adjusted to neutral pH) were prepared weekly, and stored at 0–5°C under an argon atmosphere. Dilutions were made as needed.

2.3. Preparation of standards of PFB derivatives

In order to be used for the identification of the derivatization products and the calibration of the GC response factors, the PFB derivatives of six

DAP were synthesized as follows: the reaction mixture consisting of the appropriate DAP or its salt (5.0 mmol), 20 ml of acetone (for sulfur-containing DAP) or 15 ml of acetonitrile (for DMP and DEP), PFB-Br (5.0 mmol) and potassium carbonate (5.0 g) was refluxed for 0.5–3 h under an argon atmosphere and then filtered. After the solvent had been evaporated, the PFB derivative was extracted with *n*-hexane and the extract was washed with NaCl-saturated water. The extract was flushed through a silica gel column with *n*-hexane and methylene chloride to remove the remaining DAP and PFB-Br. The PFB derivatives obtained were checked for purity and identified by using GC-MS and proton NMR spectroscopy.

2.4. Gas chromatography

2.4.1. Flame-ionization detection

A GC-9A gas chromatograph (Shimadzu, Kyoto, Japan) fitted with a flame-ionization detector was used for quantitative analysis in combination with a DB-17 megabore column (15 m × 0.53 mm I.D., film thickness of 1 μm) (J & W Scientific, Folsom, CA, USA) in the splitless mode. The column temperature was programmed from 50 to 250°C at 10°C/min. The detector and injector temperatures were both 280°C. Nitrogen was used as the carrier gas at a flow-rate of 6.5 ml/min.

2.4.2. Electron-capture detection

A Shimadzu GC-14A gas chromatograph fitted with an electron-capture detector was used in combination with a DB-17 capillary column (30 m × 0.32 mm I.D., film thickness of 0.25 μm) (J & W Scientific) with a splitting ratio of 30:1. The column temperature was programmed from 70 to 270°C at 10°C/min. The detector and injector temperatures were both 300°C. Helium was used as the carrier gas at a flow-rate of 2.0 ml/min.

2.5. GC-MS

A Shimadzu GCMS-QP2000A mass spectrometer equipped with a Shimadzu GC-14A gas

chromatograph was operated with electron impact ionization (70 eV). The GC conditions were the same as those for GC with electron-capture detection (ECD).

2.6 Procedure for extractive pentafluorobenzoylation

A 15 mg amount of tri-phase catalyst was placed in a 15-ml two-necked, flat-bottomed cylindrical glass reactor (15 mm I.D.) fitted with a Dimroth condenser, and the reactor was purged with argon. Next, an aqueous sample (1.0 ml for exploring the optimum conditions for the procedure, 0.5–12.0 ml for studying the influence of dilution of the aqueous phase), an appropriate phosphate buffer (mainly pH 6.5; usually 20% of the volume of the sample), an organic solvent (mainly toluene; 0.4 ml) and PFB-Br (3.0 μl for sulfur-containing phosphates, 6.0 μl for non-sulfur-containing phosphates) were injected successively into the reactor and the reaction mixture was stirred vigorously with a magnetic stirrer. After an appropriate reaction time (mainly 20 min at 45°C for sulfur-containing phosphates and 120 min at 90°C for non-sulfur-containing phosphates), the reaction was inhibited by addition of 1.0 M phosphoric acid (1.0 ml), and when quantitative analysis was required 500 μl of naphthalene [internal standard (I.S.)] in *n*-hexane (50–500 μg/ml) were added. The organic layer was sucked up and filtered by passage through a disposable Pasteur glass pipette packed with a piece of cotton and anhydrous Na₂SO₄ (0.2 g), and then 1.0 μl was subjected to GC-MS or GC. In the preliminary examinations for optimization of the procedure, we focused on the derivatization of DMP and DEP, which are not as easily subjected to the procedure as are other DAP.

2.7. Simultaneous determination of six DAP

Fortified samples of urine and river water were prepared by diluting the stock standard solutions of six DAP stepwise with urine from a healthy volunteer who had not taken any drugs or with water from the Neyagawa river (Chuo-ku, Osaka, Japan) to the concentration range 0.005–

10 $\mu\text{g/ml}$. First, a fortified sample of urine (4 ml) or of river water (8 ml), pH 6.5 phosphate buffer (1.0 ml) and toluene (0.4 ml) were injected into the reactor in which 15 mg of tri-phase catalyst TB-0.75 had been placed and purged with argon. Next, PFB-Br (3 μl) was injected into the reactor, followed by stirring vigorously at 45°C for 20 min.

The organic layer was then sucked up completely using the pipette packed with a piece of cotton in its tip, and the remainder in the reactor was washed with toluene (0.5 ml) three times. The organic layer and the washings were combined to give extract A. Second, the tri-phase catalyst drawn out with the pipette was returned to the original reactor, and then toluene (0.4 ml) and PFB-Br (6 μl) were added. The mixture was stirred for 120 min at 90°C, after which, 1 ml of n-hexane was added and the organic layer was sucked up to give extract B. With a urine sample, these extracts were washed quickly with 4 ml of 10% sulphuric acid as needed. After the extracts A and B had been combined, the mixture was

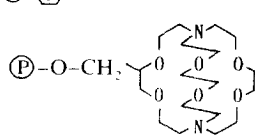
passed through the pipette packed with a piece of cotton and anhydrous Na_2SO_4 . This extract was concentrated to 0.5 ml under a gentle stream of nitrogen, and 1.0 μl was subjected to GC-MS or GC-ECD. The separate analysis of extracts A and B was carried out as needed.

3. Results and discussion

3.1. Optimum conditions for extractive pentafluorobenzylation

Preliminary experiments and other fundamental studies [16] allowed us to determine the optimum conditions for the extractive pentafluorobenzylation as described under Experimental. We tested various solvents, and among them methyl isobutyl ketone (MIBK) was found to be the most effective, followed by toluene. Both of them produced a sufficiently high reaction temperature for DMP [7] and gave a good layer separation, but toluene gave much cleaner chro-

Table 1
Comparison of catalysts in the extractive pentafluorobenzylation of DMP and DEP

Catalyst	Amount [mg ($\mu\text{equiv.}$)]	Conversion (%)		
		DMP	DEP	
TB-0.75	$\text{P}(\text{C}_6\text{H}_5)_2\text{-CH}_2\text{P}^+\text{Bu}_3 \cdot \text{Br}^-$ (0.75 mequiv. Br ⁻ /g) ^a	15 (11)	35	74
TC-0.78	$\text{P}(\text{C}_6\text{H}_5)_2\text{-CH}_2\text{P}^+\text{Bu}_3 \cdot \text{Cl}^-$ (0.78 mequiv. Cl ⁻ /g) ^a	15 (12)	22	49
TB-2.05	$\text{P}(\text{C}_6\text{H}_5)_2\text{-CH}_2\text{P}^+\text{Bu}_3 \cdot \text{Br}^-$ (2.05 mequiv. Br ⁻ /g) ^a	15 (31)	21	48
TC-2.26	$\text{P}(\text{C}_6\text{H}_5)_2\text{-CH}_2\text{P}^+\text{Bu}_3 \cdot \text{Cl}^-$ (2.26 mequiv. Cl ⁻ /g) ^a	15 (34)	6	14
K-222B	 (0.25 mequiv./g) ^a	15 (4)	2	7
		50 (13)	3	15
BTBPB	$\text{P}(\text{C}_6\text{H}_5)_2\text{-CH}_2\text{P}^+\text{Bu}_3 \cdot \text{Br}^-$	15 (40)	5	8
THAB	$(n\text{-C}_6\text{H}_{13})_4\text{N}^+ \cdot \text{Br}^-$	15 (35)	40	88
TBAB	$(n\text{-C}_4\text{H}_9)_4\text{N}^+ \cdot \text{Br}^-$	15 (47)	3	10

Derivatization was performed in a reaction mixture consisting of a standard solution of DMP and DEP (100 $\mu\text{g/ml}$ each, 1.0 ml), 0.1 M phosphate buffer (pH 6.5, 0.2 ml), toluene (0.4 ml), PFB-Br (6 μl) and a catalyst (15–50 mg) for 90 min at 90°C.

^a P refers to polystyrene resin.

matograms than did MIBK. The optimum pH was found to be between 6 and 7. It is assumed that the low yields in acidic and basic media were caused by the protonation of the phosphate anions and by the formation of pentafluorobenzyl alcohol and dipentafluorobenzyl ether [17] as by-products, respectively. Table 1 shows the efficiency of some tri-phase catalysts and ordinary ion-pair reagents for comparison, and among them TB-0.75 was found to be the most effective tri-phase catalyst.

This proposed PFB-Br alkylation for DMTP and DETP selectively yielded only their S-alkylated esters, although both the diazoalkane [18] and the triazene [19] alkylations produce a mixture of the O- and S-alkylated esters. As PFB-Br alkylation occurs with the ionized species [20], the more nucleophilic sulfur atom would be expected to react with the electrophilic reagent to form preferentially the S-alkylated ester.

The derivatization of every member of the four sulfur-containing phosphates was quantitatively effected within 20 min at 45°C, and no side-reactions were observed. However, the resulting PFB derivatives decomposed on prolonged reaction. This decomposition was greater at elevated temperature. Trace amounts (≈ 20 nmol) of the desulfurization products were generated when the derivatization of sulfur-containing phosphates was carried out at 90°C for 120 min. On the other hand, no derivatizations of DMP and DEP were observed at 45°C. Although DEP was quantitatively derivatized after 120 min at 90°C, the derivatization yield of DMP reached a plateau at 43% after 120 min.

3.2. Influence of dilution of the aqueous phase

When the ordinary ion-pair reagent THAB was used, a decrease in the yields was observed in inverse proportion to the stepwise dilution of the aqueous phase. In contrast, the tri-phase catalyst TB-0.75, which has the structure of an ion-exchange resin, gave only a slight decrease in the yields even when the aqueous sample was diluted to a volume of 6 ml (aq./org. = 18). The

greatest advantage of this tri-phase procedure is that the extraction, derivatization and concentration of the phosphates are simultaneously achieved in a single step. This concentration effect is particularly important for the determination of trace levels of DAP.

3.3. Simultaneous determination of DAP

The general procedure for the simultaneous determination of six DAP was established as described under Experimental. Extract A containing the derivatives of sulfur-containing phosphates should be isolated before the second derivatization. Fig. 1A illustrates the efficiency of GC for the separation of PFB-Br and PFB derivatives of the six phosphates. Trace amounts of desulfurization products could be formed from

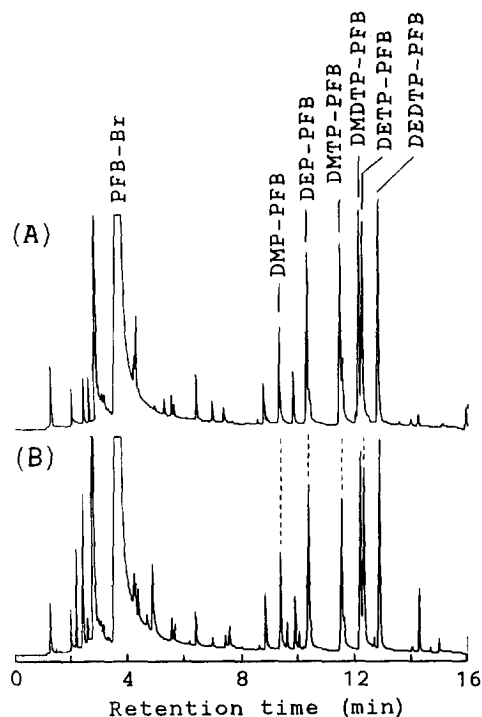


Fig. 1. Gas chromatograms of PFB derivatives obtained (A) from the diluted standard solution and (B) from the fortified river water. The concentration of each of the six DAP was 1.0 $\mu\text{g}/\text{ml}$ and the sample volume was 8 ml. Derivatization and GC-ECD were performed as described under Experimental.

Table 2
Detection limits of dialkyl phosphates with simultaneous determination

Dialkyl phosphate	Detection limit ($\mu\text{g/ml}$)					
	River water		Urine		Diluted standard	
	GC-MS ^a	GC-ECD	GC-MS ^a	GC-ECD	GC-MS ^a	GC-ECD
DMP ^b	0.25	0.013	0.50	0.050	0.20	0.010
DEP ^b	0.10	0.013	0.33	0.033	0.10	0.010
DMTP	0.13	0.005	0.25	0.013	0.10	0.005
DETP	0.10	0.005	0.25	0.020	0.10	0.005
DMDTP	0.10	0.005	0.25	0.020	0.10	0.005
DEDTP	0.10	0.005	0.33	0.013	0.10	0.005

Fortified river water (8 ml) or urine (4 ml) with the six dialkyl phosphates (0.005–0.50 $\mu\text{g/ml}$ each), 0.1 M phosphate buffer (pH 6.5, 1.0 ml), toluene (0.4 ml), catalyst TB-0.75 (15 mg) and PFB-Br (3 μl) were used.

^a Detection limits of the five major peaks involving M⁺ in the scan mode.

^b 6 μl of PFB-Br were used in the second derivatization.

the corresponding sulfur-containing phosphates when large amounts of them are present in a sample. In such a case, individual analyses of extracts A and B are required. A blank test of the organic layer before the second derivatiza-

tion also seems to be effective for ensuring reliability.

3.4. Application to urine and river water samples

The gas chromatogram obtained from the river water sample is shown in Fig. 1B. In this procedure for the urine sample, PFB cation was partially consumed by the chloride anion in urine to form PFB-Cl, but no significant disturbance was found. Acid washing of the extracts was effective for reducing undesirable peaks and noise derived from urinary components. The gas chromatograms of extracts A and B obtained from the urine sample with and without acid washing are shown in Fig. 2. The detection limits of six DAP in river water, urine and diluted standard solution with this GC-MS and GC-ECD procedure are listed in Table 2. These detection limits in the urine sample were lower than those obtained by previous GC methods [6–9].

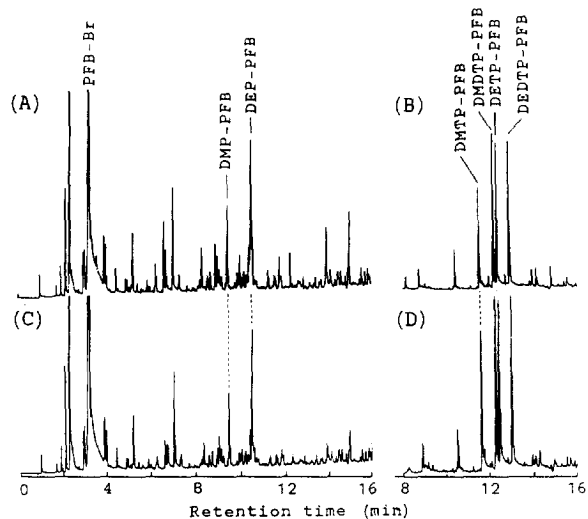


Fig. 2. GC-ECD analysis of the fortified urine sample (A and B) before acid washing and (C and D) after acid washing. (A and C) chromatograms of extract B; (B) and (D) partial chromatograms of extract A. The concentration of each of the six DAP was 1.0 $\mu\text{g/ml}$ and the sample volume was 4 ml. Derivatization and GC-ECD were performed as described under Experimental.

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