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Simultaneous determination of urinary dialkylphosphate metabolites of organophosphorus pesticides using gas chromatography–mass spectrometry

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Abstract

In this study, we developed a safe and sensitive method for the simultaneous determination of urinary dialkylphosphates (DAPs), metabolites of organophosphorus insecticides (OPs), including dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), and diethylthiophosphate (DETP), using a pentafluorobenzylbromide (PFBBr) derivatization and gas chromatography–mass spectrometry (GC–MS). Several parameters were investigated: pH on evaporation, reaction temperature and time for the derivatization, the use of an antioxidant for preventing oxidation, and a clean-up step. The pH was set at 6, adjusted with K_2CO_3, and the reaction temperature and time of derivatization were 80 °C and 30 min, respectively. Sodium disulfite was chosen as an antioxidant. The clean-up step was performed with a Florisil/PSE mini-column to remove the unreacted PFBBr and sample matrix. This established procedure markedly shortened the sample preparation time to only about 3 h, and completely inhibited the unwanted oxidization of dialkylthiophosphates. The limits of determination (LOD) were approximately 0.3 μg/L for DMP, and 0.1 μg/L for DEP, DMTP, and DETP in 5 mL of human urine. Within-series and between-day imprecision for the present method using pooled urine spiked with DAPs was less than 20.6% in the calibration range of 1–300 μg/L, and the mean recovery was 56.7–60.5% for DMP, 78.5–82.7% for DEP, 88.3–103.9% for DMTP, and 84.2–92.4% for DETP. This method detected geometric mean values of the urinary DAPs in Japanese with and without occupational exposure to OPs, 16.6 or 27.4 for DMP, 1.0 or 0.7 for DEP, 1.3 or 2.3 for DMTP, and 1.0 or 1.1 μg/L for DETP, respectively. The present method, which does not require special equipment except for GC–MS, is quick, safe, and sensitive enough to be adopted in routine biological monitoring of non-occupational as well as occupational exposure to OPs.

Keywords: Organophosphorus insecticide; Urinary metabolite; Dialkylphosphates; GC–MS; Biological monitoring

1. Introduction

Organophosphorus compounds have been widely and effectively used as insecticides with applications in agriculture and pest control [1]. However, it is well known that severe exposure to organophosphorus insecticides (OPs) in human and animal

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causes an acute cholinergic syndrome such as miosis, salivation, seizures, paralysis, neuromuscular and cardiac conduction disorders [2,3] through the inhibition of acethylcholinesterase [4,5]. Moreover, it has been reported that occupational long-term exposure could cause a decrease in sensory nerve conduction velocity [6] and enduring extrapyramidal symptoms as well as transient motor and psychiatric symptoms [7].

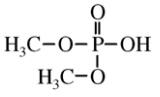
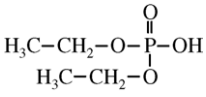
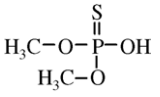
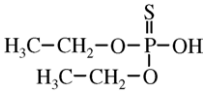
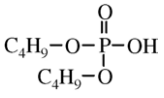
Occupational human exposure to OPs is usually evaluated by determination of the decrease of cholinesterase activity in blood between pre- and post-exposure, since it has large inter-individual variability [8,9]. However, this indicator is not sensitive enough to monitor the body burden of OPs in the general population, and blood collection from many people is not always feasible. Therefore, it is necessary to develop a biological monitoring method using urine that is sensitive enough to detect low exposure levels to OPs.

OPs are mainly metabolized into any of the following compounds and excreted in urine: dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), and diethylthiophosphate (DETP) (Table 1) [11]. The determination of these dialkylphosphates (DAPs) in human urine has been reported as a sensitive indicator for non-occupational background exposure levels [10–13]. Consequently, many biological monitoring studies using DAPs in human urine have been reported [10,12,14–16]. However, it is difficult to analyze OP metabolites due to their low urinary concentration and the complicated procedure needed to extract and derivatize metabolites from urine.

Previously reported methods for the extraction of DAPs were liquid phase extraction [15,17], column clean-up [14,18], lyophilization [10], and azeotropic distillation [11,19]. Unfortunately, urinary DAP extraction using the lyophilization or azeotropic distillation technique is extremely time consuming (over 16–24 h) and is labor intensive [10]. Pentafluorobenzylbromide (PFBBr), which is able to yield a single reaction product [20], is widely used for the determination of trace amounts of urinary DAPs in the general population [10,14,15,17,21]. However, the handling of PFBBr is troublesome due to its highly irritating nature.

In this paper, using gas chromatography–mass spectrometry equipped with electron ionization system (GC–MS-EI), we improved some conditions of evaporation, derivatization, and clean-up for removing PFBBr to facilitate the determination of urinary DAPs. The present method was then applied to measure DAP concentrations in 48 urine samples collected from a Japanese population in order to evaluate non-occupational as well as occupational OP exposure levels. We focused on four DAPs (DMP, DEP, DMTP, and DETP) since the pest control operators, our study population, seldom used pesticides metabolized to dimethyldithiophosphate (DMDTP) or diethyldithiophosphate (DEDTP), and the reported urinary levels of DMDTP and DEDTP are very low in the general population [10,16].

Table 1
Chemical structures, fragment ions, and retention time of dialkylphosphates and internal standard dibutylphosphate

Compound	Structure	*m/z*		Retention time (min)
		C-ion	Q-ion	
DMP		306, 194	110	16.1
DEP		334, 197	258	18.6
DMTP		322, 211	322	20.0
DETP		350, 213	274	22.2
DBP		335	335	25.9

Abbreviations: DMP, dimethylphosphate; DEP, diethylphosphate; DMTP, dimethylthiophosphate; DETP, diethylthiophosphate; DBP, dibutylphosphate; C-ion, selected ions for confirmation; Q-ion, selected ions for quantification.

2. Experimental

2.1. Reagents

DMP tetramethylammonium salt (99.9% purity), DMTP ammonium salt (98.9%), DEP (98.2%), and DETP ammonium salt (95.2%) were purchased from Hayashi Pure Chemical Ind. (Osaka, Japan), and dibutylphosphate (DBP), used for an internal standard (I.S.), was from Tokyo Kasei Kogyo (Tokyo, Japan). Diethyl ether, acetonitrile, *n*-hexane, acetone, and toluene, which are pesticide residue grade, and sodium sulfate anhydrous, sodium chloride (NaCl), sodium disulfite ($Na_2S_2O_5$), L-ascorbic acid, potassium carbonate (K_2CO_3), and 6 mol/L hydrochloric acid were obtained from Kanto Chemicals (Tokyo, Japan). Pyrogallol was purchased from Yoneyama Yakuhin (Kyoto, Japan), PFBBr from Sigma-Aldrich (St. Louis, MO, USA), Florisil (60–100 mesh) and tributyl phosphate from Wako Pure Chemicals (Osaka, Japan), and Bondesil-Primary/Secondary Amine (PSA) 40 μm from Varian, Inc. (Palo Alto, CA, USA). Water used throughout the experiments was distilled and deionized to 18 MΩ with a Millipore Milli-Q System (Millipore Co., Bedford, MA, USA). All other reagents were of analytical grade purity.

2.2. Apparatus and GC–MS conditions

A GC–MS-EI (PerkinElmer TurboMass Systems equipped with an auto sampler (Wellesley, MA, USA)) was used. The GC operating conditions were as follows: GC column, DB-5MS (J&W Scientific, Folsom, CA, USA), 30 m × 0.25 mm i.d., 1.0-μm film thickness; column temperatures, 70 °C (1 min)–5 °C/min–220 °C (0 min)–15 °C/min–280 °C (5 min); injection port temperature, 250 °C; carrier gas, helium (99.999%

purity); flow rate, 1 mL/min; injection pressure, 83 kPa. The injection volume was 1 μL splitless changing to split 20:1 at 1.5 min. The MS operating conditions were as follows: ionization source temperature, 250 °C; electron ionization, 70 eV; interface temperature, 300 °C. Chromatogram peak was identified by target and qualify ions for each pentafluorobenzyl (PFB)-DAP as shown in Table 1. The monitored ions for quantitation and confirmation were shown in Table 1. Clean-up columns were composed of 0.3 g of Florisil (lower), 0.1 g of Bondesil-PSA (middle), and 0.5 g of sodium sulfate anhydrous (upper) in a Pasteur pipette.

2.3. Sample collection

Spot urine samples were collected between June 2000 and December 2001 with written informed consent from 23 healthy volunteers and 25 pest control operators aged 36.5 ± 11.1 years (mean ± S.D.). The samples were transferred into 10 mL polyethylene test tubes and stored without any pre-treatment at −80 °C prior to analysis. All urinary creatinine analysis was performed according to the Jaffé reaction. This study was conducted in accordance with the Declaration of Helsinki. Signed informed consent was obtained from all the subjects, and the study protocols were approved by the Medical Ethics Committee of the Nagoya University Graduate School of Medicine.

2.4. Standard preparation and analytical procedure

Each standard (DMP, DEP, DMTP, and DETP) was prepared at a concentration of 1000 mg/L in methanol, and diluted with methanol to each working standard solution at concentrations ranging from 1 to 100 mg/L. The standard solutions were stored in the dark at 4 °C, and were used within 1 month. Healthy volunteer urine, which was neither treated with any drugs nor exposed to chemicals before collection, was used for the basic methodological study in this paper.

A flow chart of the urinary DAP determination procedure is shown in Fig. 1. Five milliliters of urine was pipetted into a 15-mL screw-top glass test tube, and 25 μL of I.S. solution (100 mg/L DBP), 5 g of NaCl, 1 mL of 6 mol/L HCl, 50 mg of $Na_2S_2O_5$, and 5 mL of diethylether–acetonitrile (1:1, v/v) were added. After vigorous mechanical shaking for 5 min, the test tube was centrifuged (2000 × g for 5 min at 4 °C). The organic phase (upper layer) containing DAPs was transferred into a new screw-top glass test tube containing 15 mg of K_2CO_3. The residuals were re-extracted with 5 mL of diethylether–acetonitrile (1:1, v/v) and then centrifuged. The supernatant obtained from the second extraction was combined with the first extract. The resulting extract was evaporated at 45 °C (heat block) to dryness with a gentle nitrogen stream. To the dried extracts, 15 mg of K_2CO_3, 1 mL of acetonitrile, and 50 μL of PFBBr were added and incubated in a water bath at 80 °C for 30 min with occasional swirling. Afterwards, 4.5 mL of water and 4.5 mL of *n*-hexane were added, and the mixture was shaken vigorously for 5 min and centrifuged for 5 min. The upper layer containing PFB-DAPs was transferred to new test-glass tubes. The extraction was then repeated with 4.5 mL of *n*-hexane, and the supernatant obtained from the second extraction was combined with the first extract. The combined extract was loaded into a three-layer column of 0.3 g of Florisil, 0.1 g of Bondesil-PSA, and 0.5 g of anhydrous sodium sulfate, followed by washing with 5 mL of acetone–*n*-hexane (2:98, v/v) for removing unreacted PFBBr. The PFB-DAPs were then eluted with 5 mL of acetone–*n*-hexane (15:85, v/v), and the eluate was evaporated at 45 °C to dryness with a gentle nitrogen stream. The residue was dissolved in 200 μL of toluene and injected into GC–MS.

2.5. Assay validation

Using this proposed method, calibration curves were prepared with a spiked concentration of 50 μL of DAP solution (ranging from 0.1 to 50 mg/L) mixed into 5 mL of urine. Eventually, the final concentrations of urinary DAPs were designed ranging from 1 to 500 μg/L of urine (seven points). Calibration curves were represented by the analyte/I.S. peaks area ratio versus the concentrations of the calibration samples. Extraction recovery was evaluated by analyzing urine spiked with DAP standards in triplicate at each concentration level of 1, 50, and 300 μg/L.

The within-series imprecision for our proposed method was examined by assaying the pooled urine spiked with DAP concentrations of 1, 5, 50, and 300 μg/L ($n = 3$–8). Moreover, the between-day imprecision was examined by a duplicate assay of the pooled urine spiked with DAPs at a concentration of 50 μg/L for 5 consecutive days ($n = 5$). The limits of determination (LOD) and quantitation (LOQ) were calculated based on the signal-to-noise ratio 3 and 10, respectively.

2.6. Statistical analysis

Non-parametric statistics (Mann–Whitney *U*-test) were used to determine the statistical significance of differences between experimental groups. Undetectable urinary DAP concentrations were recorded as half of the LOD values [22,23]. The 0.05 level of probability was used as the criterion of significance. These statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2000 (Microsoft Corporation, Seattle, WA, USA).

3. Results and discussion

3.1. Optimum conditions for method

To optimize the conditions for the determination of DAPs, we examined the reaction time and temperature for derivatization, pH and stability on evaporation and derivatization, and separation of unreacted PFBBr. Five milliliters of human urine spiked with a known amount of DAPs (final concentration 10 mg/L) was used except for the optimization study of the derivatization condition. There was no interfering peak for internal standard DBP.

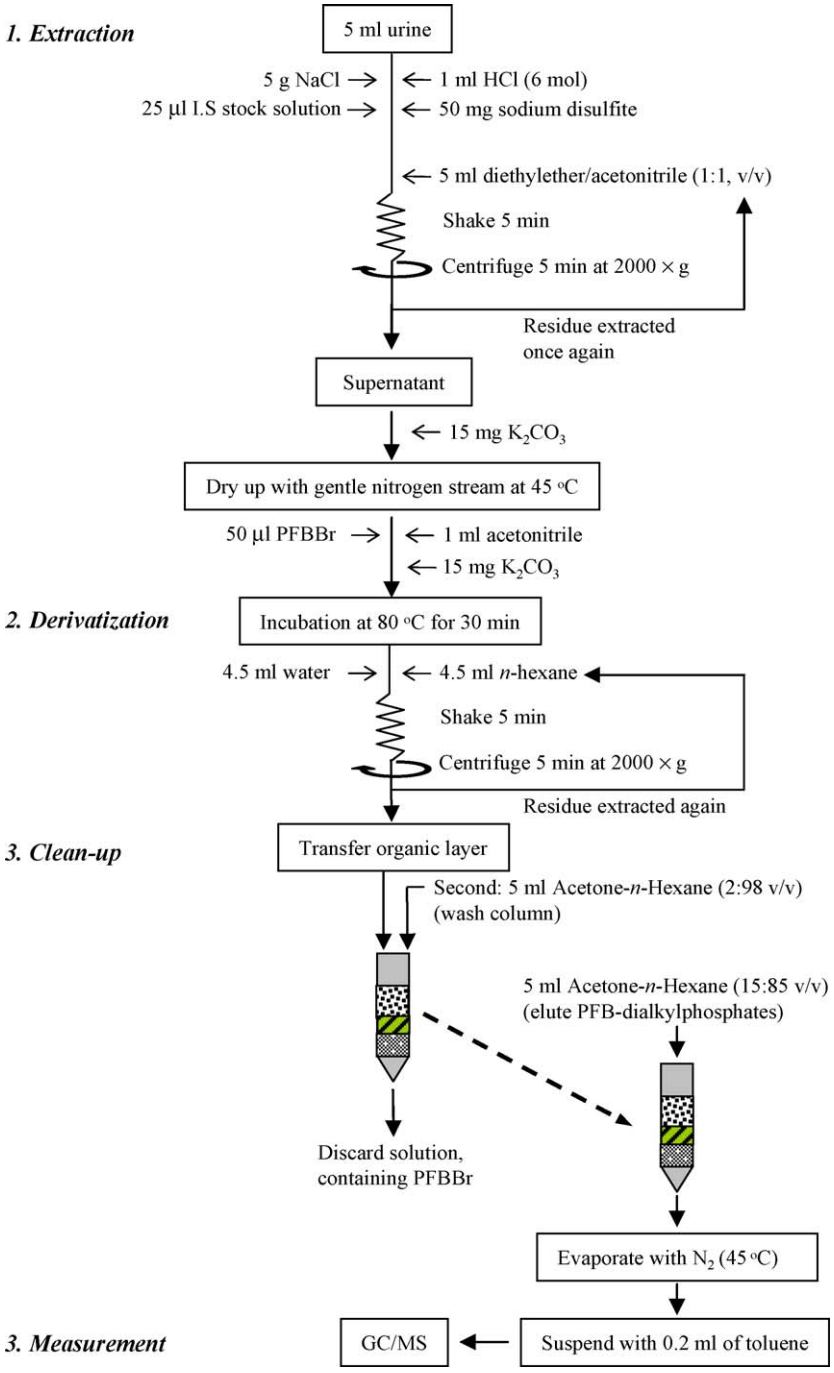


Fig. 1. Analytical procedure for urinary DAPs.

3.1.1. Derivatization using PFBBr

Reid and Watts [21] and Moate et al. [17] investigated the condition of PFB-derivatization of DAPs at two different temperatures (ambient and 90 °C) [17,21]. Hardt and Angerer [15] demonstrated a method of derivatization under low temperature (40 °C) for a prolonged reaction time (15 h) to avoid the oxidation of DMTP and DETP [15]. Although these methods gave good results, they required a complex, labor-intensive technique and long reaction time.

The derivatization of DAPs and I.S. (absolute contents were 10 and 20 μg, respectively) using 50 μL PFBBr, 1 mL acetonitrile, and 30 mg K_2CO_3 in a screw-top glass tube was carried out in an 80 °C water bath with shaking. This temperature was determined in consideration of the high yield of PFB-DAP under stable conditions and the acetonitrile boiling point of 82 °C. The PFB-DAPs were extracted with *n*-hexane, cleaned up by Florisil/PSA column and dried up with a nitrogen stream. After the residue was dissolved in 200 μL of toluene, the solution was injected into GC–MS.

The effect of reaction time on derivatization of DAPs is illustrated in Fig. 2. A reaction time < 15 min was less efficient for derivatization, while the PFB-DAP production levels in the

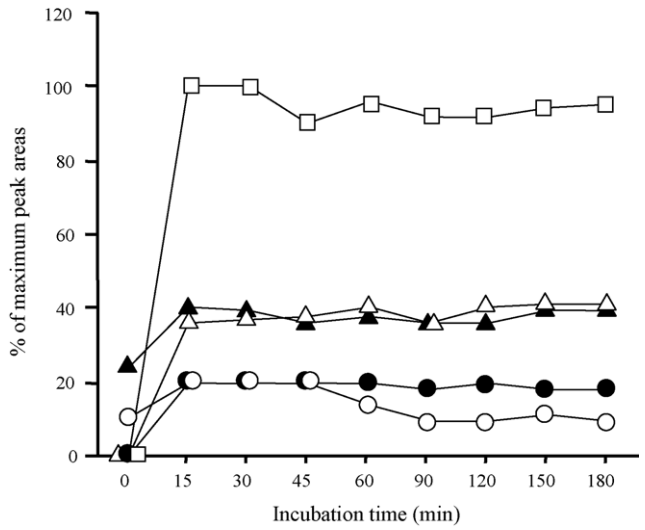


Fig. 2. Effect of incubation time on derivatization of four DAPs, DMP (◯), DEP (△), DMTP (●), DETP (▲), and internal standard DBP (□), using PFBBr at 80 °C. Each result represents the mean of duplicate experiments.

range of 15–45 min reached maximum for all the metabolites. After considering possible slight changes of the reaction time to achieve maximum production levels according to various factors such as reagent lots, suppliers, and operators, we determined the optimum derivatization condition to be a 30-min incubation at 80 °C.

A simultaneous and efficient derivatization of DAPs was difficult for the following reason. While DMTP and DETP were derivatized sufficiently with PFBBr at room temperature, the derivatization of DMP and DEP was only achieved at a high temperature. However, the derivatization of DMTP and DETP at a high temperature caused slight oxidation to produce DMP and DEP, respectively. The derivatization condition (at 80 °C for 30 min) in this study ensured a simultaneous and effective derivatization in the shorter time, but the unwanted production of false DMP from DMTP, and false DEP from DETP could not be completely prevented.

3.1.2. Effect of antioxidants on oxidization of DMTP and DETP during derivatization procedure

Hardt and Angerer [15] reported that the production of about 10% of DMP occurred from DMTP under PFBBr derivatization at 40 °C for 15 h [15]. Consequently, an optimum derivatization condition was explored at 80 °C for 30 min. To avoid the oxidation of dialkylthiophosphate, we used an antioxidant (L-ascorbic acid, SOD or pyrogallol) which might effectively prevent oxidization of the DMTP and DETP during derivatization.

The pooled human urine spiked with DMTP and DETP was assayed using the sample preparation procedure as shown in Fig. 1. The level of unwanted DMP and DEP produced from DMTP and DETP, respectively, by oxidation during derivatization was measured in the presence/absence of each antioxidant (10 mg/mL of urine) added to 5 mL of human urine. As shown in Fig. 3A, in the absence of antioxidants, the peak-areas of artificial DMP and DEP, accounted for approximately 25% of the DMTP and DETP levels. By contrast, all antioxidants except pyrogallol effectively prevented the unwanted production of DMP and DEP (Fig. 3B). However, in our experimental condition, unknown peaks interfered with the expected PFB-DAP peaks when using ascorbic acid, which is often used as a potent antioxidant in various settings. Therefore, we used 10 mg SOD/mL of urine afterward, which reduced the production of DMP and DEP so as to be <1% of the DMTP and DETP, respectively.

The above method needs only 3 h for all the sample preparation, which markedly reduces the time required in the previous method (16–24 h for lyophilization and additional 4 h for derivatization) [10]. In addition, the present method can make it possible to determine precise profiles of personal exposure to OPs, and would even give a scientific basis to estimate individual activities of metabolic enzymes including paraoxonase families from the urine samples.

3.1.3. Effect of solution pH during evaporation on recovery of DAPs

To minimize the loss of DAPs during sample preparation, the optimum solution pH during heated evaporation using

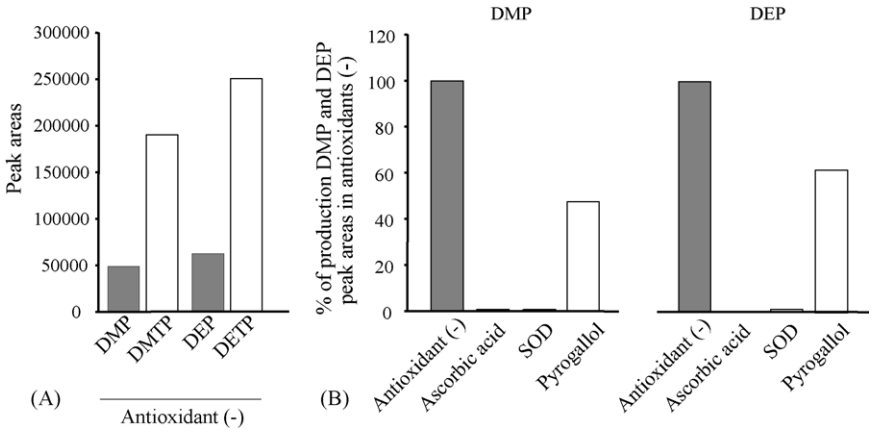


Fig. 3. Production of DMP and DEP from DMTP and DETP by oxidation during derivatization procedure using 5 mL of urine spiked with DMTP and DETP (10 mg/L) (A) and the effects of antioxidant ascorbic acid, sodium disulfite (SOD) and pyrogallol for prevention of DMTP and DETP oxidation (B). Data represent the mean of duplicate experiments.

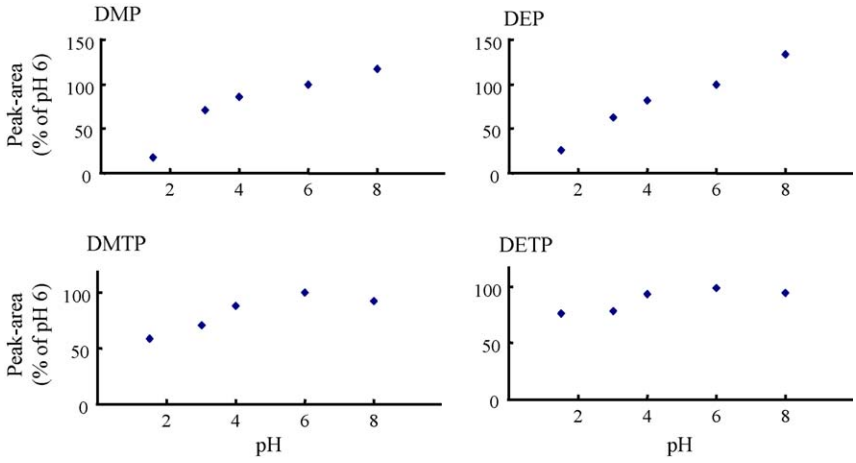


Fig. 4. Effect of pH during evaporation-step using K_2CO_3 on the recovery of DAPs. Each plot represents the mean percent recovery ($n = 3$, 100% at pH 6).

nitrogen stream was examined. We prepared solutions with different pH conditions (approximately pH 1.5, 3, 4, 6, and 8, adjusted by using 0, 5, 10, 15, and 20 mg K_2CO_3, respectively). After evaporation by a nitrogen stream, K_2CO_3 (30, 25, 20, 15, and 10 mg) was added to the residues, respectively, to maintain the same pH 11 during the derivatization. As shown in Fig. 4, the recoveries of DMP and DEP at acid conditions were in the range of approximately 15–80% of the recovery at pH 6. The recoveries of DMTP and DETP showed similar results, but the standard deviation of the recoveries in DMTP and DETP at pH 8 was larger than those at other pHs. Therefore, the pH 6 condition achieved by adding 15 mg of K_2CO_3 was considered to be best.

3.1.4. Column clean-up using Florisil/PSA column

Excess PFBBr in injected samples would damage GC–MS systems including the column and the detector [20]. In fact, *n*-hexane extracts after derivatization contained unreacted PFBBr, fat-soluble compounds, and pigments in urine. Therefore, a clean-up step after derivatization was studied using a Florisil/SPE column for removing unreacted PFBBr, the fat-soluble compounds, and pigments. Thus, the hexane extract was passed through the clean-up column, and then washed with 5 mL of acetone–*n*-hexane (2:98, v/v) for removing PFFBr and eluted with 5 mL of acetone–*n*-hexane (15:85, v/v). That clean-up procedure effectively removed the very irritant PFBBr, drastically reduced the unknown peaks in total ion chromatogram (TIC) and SIM chromatograms, and stabilized the baseline.

Urinary DAPs stored at <−20 °C remain stable for up to 12 months [19]. However, to our knowledge, there are few reports about the stability of PFB-DAPs in an injection sample at room temperature. This is an important issue especially when numerous samples are measured simultaneously at room temperature. The peak-areas of PFB-DAPs in injection samples at room temperature (approximately 25 °C) were virtually similar within 36 h after the sample preparation (CV%, 2–5%), indicating that PFB-DAPs are stable by means of the present column clean-up procedure.

3.2. Calibration curve and reproducibility

Since the recovery from urine was not the same as from water (data not shown), we used urinary calibration standards in all experiments. Five milliliters pooled urine was mixed with various concentrations of DAPs. Calibration curves was then obtained by subtracting DAP concentrations in the pooled urine from those of the spiked urine in order to cancel out the background concentrations of urinary DAPs. The calibration curves ($n = 3$) were linear for DMP and DEP in the range of 1–300 μg/L, and for DMTP and DETP in the range of 1–500 μg/L with higher correlation coefficients of more than 0.953 for DMP, 0.997 for DEP, 0.989 for DMTP, and 0.984 for DETP, respectively. LOD and LOQ values, defined as a signal-to-noise ratio 3 and 10, were about 0.3 and 1.0 μg/L for DMP, 0.1 and 0.3 μg/L for DEP, 0.1 and 0.3 μg/L for DMTP, and 0.1 and 0.3 μg/L for DETP, respectively. These values are less than or similar to LOD levels reported by Hardt and Angerer [15] using GC–MS-EI or by Oglobline et al. [10] using GC–MS–MS. This improvement in the LOD levels can allow the routine biological monitoring of urinary DAPs in the general population with a less time- and labor-consuming procedure than in the previous methods [14–16].

Within-series imprecision was estimated by assaying pooled urine spiked with 1–300 μg/L DAPs; the coefficient of variations (CV%) ranged from 4.9 to 20.6%, for each DAP. Moreover, between-day imprecision was estimated by duplicate assay during 5 consecutive days ($n = 5$); the CV% was between 9.2 and 14.2% (Table 2). In this method, the within-series imprecision (CV%) of I.S. DBP was 19.9% ($n = 7$). Reproducibility deteriorated without using the analyte/I.S. peak area ratio (data not shown). These imprecision values were almost the equivalent of, or lower than, those of the previous methods [15,17]. The mean absolute recoveries were 56.7–67.7% for DMP, 78.5–85.7% for DEP, 88.3–103.9% for DMTP, and 84.2–92.4% for DETP with pooled urine spiked with DAPs (50 μg/L) (Table 2).

Table 2
Imprecision, recovery, LOD, and LOQ data of analytical procedure

	Pooled urine spiked concentration (μg/L of urine)	*N*	DMP	DEP	DMTP	DETP
Within-series						
Imprecision (%)	1	3	18.4	20.2	4.9	15.6
	5	3	16.1	20.6	12.9	19.1
	50	8	10.5	13.8	10.0	8.4
	300	3	13.1	12.6	17.5	12.3
Mean recovery[a] (%)	5	3	67.7	78.5	88.3	84.2
	50	3	60.5	82.7	91.4	92.4
	300	3	56.7	85.7	103.9	90.0
Between-day						
Imprecision (%)	50	5	12.2	13.3	9.2	14.2
LOD (μg/L) (signal-to-noise ratio = 3)			0.3	0.1	0.1	0.1
LOQ (μg/L) (signal-to-noise ratio = 10)			1.0	0.5	0.3	0.3

Abbreviations: *N*, number of observations; DMP, dimethylphosphate; DEP, diethylphosphate; DMTP, dimethylthiophosphate; DETP, diethylthiophosphate; LOD, limit of detection; LOQ, limit of quantitation.
[a] Recovery given by adding the standards on derivatization step.

3.3. Biological monitoring

Table 3 shows urinary concentrations (μg/L) of DMP, DEP, DMTP, and DETP from 23 healthy volunteers without occupational exposure to OPs and 25 pest control operators. Fig. 5 shows the typical GC–MS chromatogram in the SIM mode of pooled urine spiked with DAPs, or of volunteer urine with detectable peaks. The retention times of the PFB-DAPs were as follows: 16.1 min for DMP; 18.6 min for DEP; 20.0 min for DMTP; 22.2 min for DETP; and 25.9 min for DBP (I.S.). The urine samples in which the DAP level exceeded the calibration range were diluted to appropriate concentrations before quantitation. Urinary concentrations of the four DAPs were not significantly different between the control and occupationally exposed group (Table 3), even after the levels were adjusted by the urinary creatinine level (data not shown). However, the levels of the 95th percentile of urinary DAPs in sprayers were obviously higher than those in the controls except for urinary DEP.

There have been some reports in which the sensitivity was sufficiently improved to measure urinary DAPs in the general population [10,14–16,24]. These studies detected DAPs in urine among a high percentage of the population. However, the level of urinary DAPs varied between studies. For example, Barr et al. [16] reported the median value of DMP as 0.74 μg/L of spot urine, whereas Hardt and Angerer [15] reported it as 30 μg/L of spot urine. Saieva et al. [25] indicated that the consumption of food containing pesticide residues might represent a potential source of widespread low-level human exposure. Therefore, differences in dietary habits and/or residential area (urban or rural) and DAP determination method would be possible reasons why these studies found such a wide range of urinary DAP levels. The above-mentioned results confirm that the analytical method established in the present study is sensitive enough to measure low levels of OP exposure.

Table 3
The level of dialkylphosphates in spot urine from healthy volunteers ($n = 23$) not occupationally exposed to OPs and pest control operators exposed to OPs ($n = 25$)

Dialkylphosphates	Detected (%)	Geometric mean (μg/L of urine)	Median (μg/L of urine)	95th percentile (μg/L of urine)	Maximum (μg/L of urine)
DMP					
Controls	96	16.6	17.7	117.4	127.0
Sprayers	100	27.4	22.7	527.0	880.6
DEP					
Controls	83	1.0	0.7	22.8	30.6
Sprayers	80	0.7	0.4	14.7	112.0
DMTP					
Controls	78	1.3	1.1	83.3	191.0
Sprayers	92	2.3	4.1	177.9	216.8
DETP					
Controls	91	1.0	1.0	10.8	32.7
Sprayers	80	1.1	1.5	18.5	22.2

Abbreviations: DMP, dimethylphosphate; DEP, diethylphosphate; DMTP, dimethylthiophosphate; DETP, diethylthiophosphate.

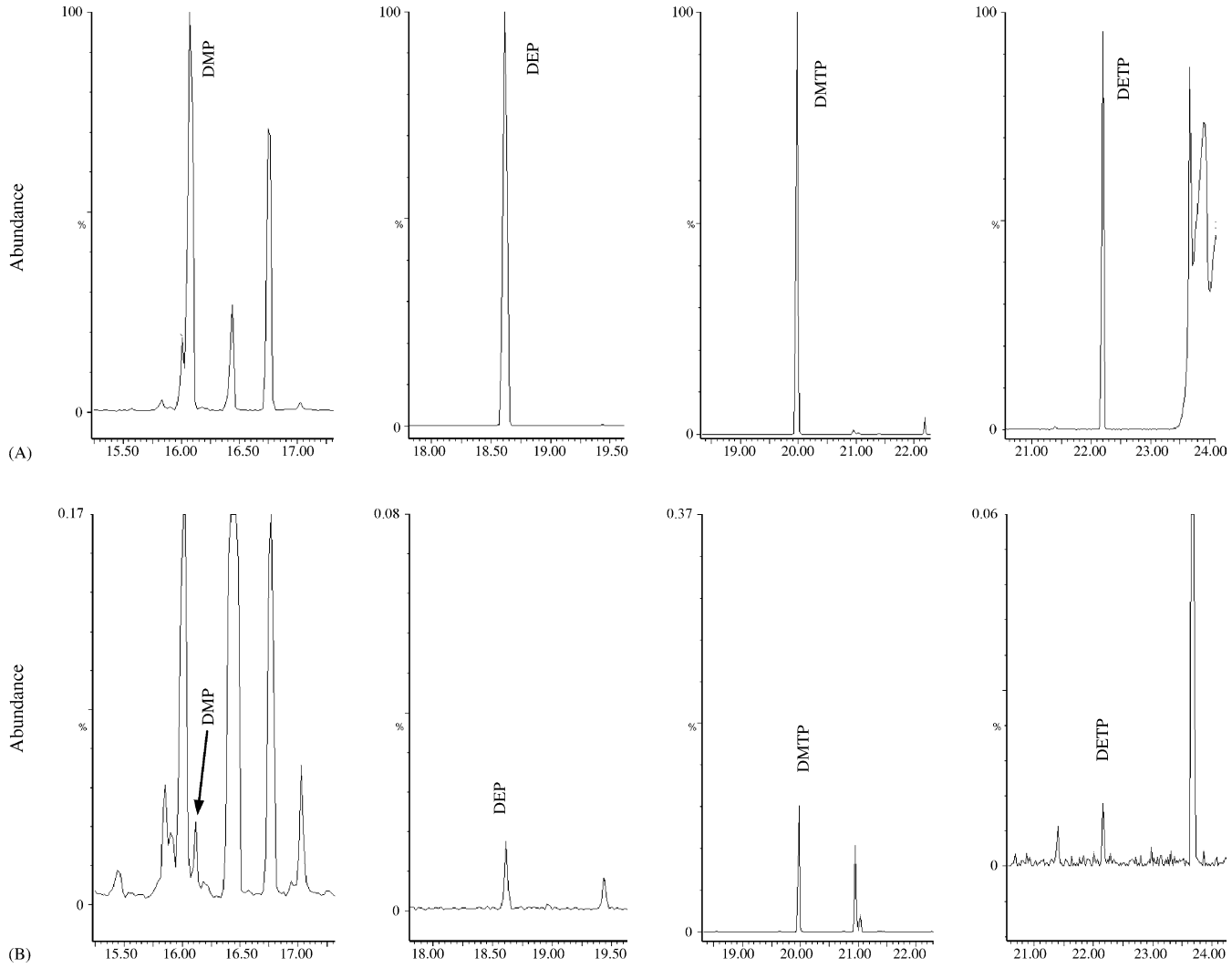


Fig. 5. Typical SIM GC–MS chromatograms in pooled urine sample spiked with 50 μg/L of each DAP (A) and in volunteer urine containing DAPs at concentrations around the limit of detection (DMP 0.8, DEP 0.5, DMTP 4.4, and DETP 0.1 μg/L) (B). Detected masses for quantitation were *m/z* 110 for DMP, 258 for DEP, 322 for DMTP, and 274 for DETP. The identification of the designated peak in panel B was confirmed by typical fragment ions for each DAP (Table 1) and full spectrum.

4. Conclusion

The present method allows us to determine four urinary DAPs simultaneously, quickly, safely, and with high sensitivity. It does not require special equipment except for GC–MS, needs only 3 h for the sample preparation, and completely inhibits the unwanted oxidization of dialkylthiophosphates. The PFB-DAPs in injection samples remain stable for over 36 h after preparation. A single operator will be able to determine about 100 urine samples within 3 or 4 working days. Thus, this method can be applied to routine biological monitoring not only for occupational OP exposure but also for environmental background levels in the general population.

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