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Measurement of human urinary organophosphate pesticide metabolites by automated solid-phase extraction, post extraction derivatization, and gas chromatography–tandem mass spectrometry

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Abstract

Organophosphorus (OP) pesticides are among the most widely used pesticides in the United States. Human exposure to these pesticides may occur from their use on crops in agriculture and for pest control in residential settings. Most of the OP pesticides used in the United States are metabolized to up to three of six common urinary dialkyl phosphate metabolites. Quantification of these metabolites provides information on cumulative exposure to most OP pesticides. To accurately quantify OP pesticide metabolites in human urine, we developed a simple, highly sensitive, analytic method involving automated solid-phase extraction (SPE) of human urine, followed by post-extraction derivatization of the organophosphorus metabolites with 1-chloro-3-iodopropane, and analysis by isotope dilution gas–chromatography–tandem mass spectrometry. The styrene-divinyl benzene polymer-based SPE cartridges yielded good SPE recoveries of the metabolites because of their enhanced non-polar interactions. This method is less labor-intensive, more time-efficient, and reproducible than previously reported methods. Automation of the SPE allowed unattended extraction of urine samples, and hence, increased the sample throughput and reduced the inter- and intra-day variations. The method limits of detection were excellent for all analytes ranging from 50 pg/ml to 170 pg/ml. Relative standard deviations ranged from 2% to 12%.

Keywords: Organophosphorous pesticides; Dialkyl phosphate metabolites

1. Introduction

About 70% (by kg of active ingredients) of all the insecticides used in the United States are organophosphorous (OP) pesticides [1]. They are used primarily on crops in agriculture but are also used for pest control in residential settings and to control vector-borne diseases for public health programs. Approximately 33 million kg of OP pesticides are used annually in all market sectors [1]. Nonagricultural uses account for about 8 million kg per year [2]. OP pesticides remain popular agricultural insecticides because they have a broad spectrum of applications, are highly toxic to pests, and are relatively inexpensive. Acute toxicologic effects of OP pesticides are a result of the inhibition of acetyl cholinesterase in the nervous system, which can cause respiratory, myocardial, and neuromuscular transmission impairment. Chronic effects of OP exposures are not well documented; however, several recent reports indicate certain birth outcomes (e.g., decreased gestational age, decreased birth length) and abnormal reflex functions in infants may be associated with low-level environmental exposures to OP pesticides [3–6].

Human exposure to OP pesticides is often assessed by measuring general dialkyl phosphate (DAP) metabolites of OPs in urine [7–10]. After human exposure, most of the OP pesticides used in the United States are metabolized to form up to three of six common dialkyl phosphate metabolites, namely, dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), diethyl thiophosphate (DETP), dimethyl dithiophosphate (DMDTP), and diethyl dithiophosphate (DEDTP) (Fig. 1), which are excreted in urine [7,11]. Quantification of these urinary metabolites provides information on cumulative exposure to OP pesticides [12].

Over the past three decades many analytic methods have been developed to measure urinary DAPs. Most of these meth-

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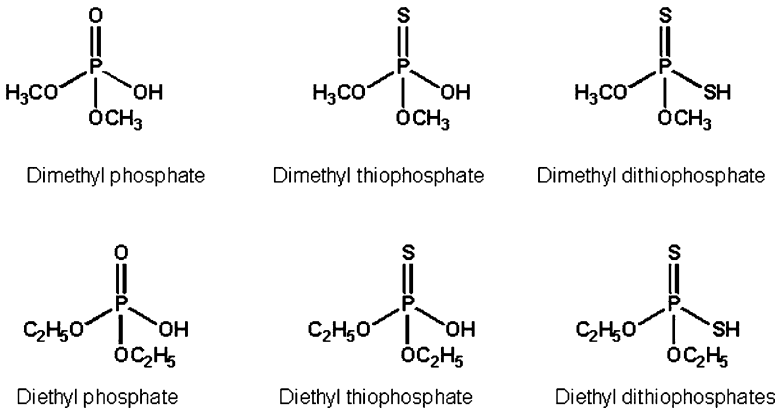

Fig. 1. Structures of dialkyl phosphate metabolites.

ods involve extraction of the highly polar alkyl phosphates from the urine matrix followed by derivatization and quantification. Separation of the metabolites from urine was achieved using liquid–liquid extraction [13–17], solid-phase extraction (SPE) [18–20], anion exchange [18,21], extractive derivatization [22], azeotropic distillation [23–28], or lyophilization (freeze–drying) [29–32]. Many reagents have been used to derivatize the analytes. Most of the earlier work involved conversion of the OP metabolites to volatile esters with diazoalkanes [13,15,16,18,21,30,33–35]. However, several limitations were associated with use of these reagents [12,23,25]. Many other reagents such as quaternary ammonium salts [36–38], silylating agents [19], arylalkyltriazenes [39], 1-chloro-3-iodopropane [28,32] and pentafluorobenzylbromide [17,22,24,27,31,40,41] have also been used with the latter being used more frequently in the recent past. The derivatives are analyzed using gas chromatography coupled with flame photometric detection, flame ionization detection, mass spectrometry, or tandem mass spectrometry. In addition, one high performance liquid chromatography–tandem mass spectrometry method has been reported without using derivatization [42].

These methods offer some advantages such as high sensitivity or inexpensive instrumentation; however, most of the published methods have several limitations. For example, the methods that are not mass spectrometry-based lack the selectivity to confirm low-level exposures. The extraction methods reported result in dirty matrices which may require extensive instrumentation maintenance or poor extraction yields resulting in poorer sensitivity. The SPE methods that have been published have been largely unrepeatable by several research groups which is the reason most groups have focused on liquid–liquid extraction, azeotropic codistillation, or lyophilization as their means of isolating the DAP metabolites.

In this paper, we addressed these limitations and developed a simple, accurate, high throughput, sensitive and a selective method for measuring urinary OP pesticide metabolites using a highly reliable SPE method for isolation of the DAPs. This novel method involves automated SPE of the OP metabolites in human urine, followed by derivatization with 1-chloro-3-iodopropane, and analysis by isotope dilution–gas chromatography–tandem mass spectrometry. Our method is more sensitive than previously published methods, including those previously published by our laboratory. Furthermore, it is less labor intensive and more rapid. Total sample preparation requires only 4 h, which is five times less than that of our currently used method [24]. Automation of the SPE reduces the variation introduced by human error, thus improving the repeatability of the measurements.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile and toluene were purchased from Tedia Co. Inc. (Fairfield, OH, USA). Concentrated hydrochloric acid, 1-chloro-3-iodopropane (98% purity), and anhydrous potassium carbonate were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA). A 3 M hydrochloric acid (HCl) solution was prepared by diluting about 25 ml of concentrated HCl with 75 ml deionized water. Deionized water was organically and biologically purified with a Barnstead Nanopure Infinity Ultrapure water purification system (Barnstead/Thermolyne, Dubuque, IA, USA). Ultra high purity grade nitrogen was purchased from Airgas Inc. (Radnor, PA, USA). Bond Elut PPL 500 mg/3 ml cartridges used for the solid-phase extraction were obtained from Varian Sample Preparation Products (Harbor City, CA, USA). Organic solvents were all of analytic grade.

DMP and DEP (98% purity) were purchased from Pfaltz and Bauer Inc. (Waterbury, CT) and Acros Chemicals (Fairlawn, NJ), respectively. DMTP (98%) and DMDTP (98%) were purchased from Cambridge Isotope Laboratories (Andover, MA). DETP (98%) and DEDTP (90%) were purchased from Aldrich Chemicals Co. Isotopically labeled analogues of the analytes, D6-DMP (dimethyl-d_6), D10-DEP(diethyl-d_{10}), D6-DMTP(dimethyl-d_6), D6-DMDTP(dimethyl-d_6), D10-DETP(diethyl-d_{10}) and 13C4-DEDTP(diethyl-$^{13}C_4$) were custom synthesized by Cambridge Isotope Laboratories (Andover, MA). All isotopically labeled standards had chemical and isotopic purities of at least 99%.

2.2. Standard preparation

About 1 mg of each DAP analyte was accurately weighed and dissolved in 10 ml acetonitrile in a volumetric flask to make a 100 mg/l stock solution. A set of ten standard solutions

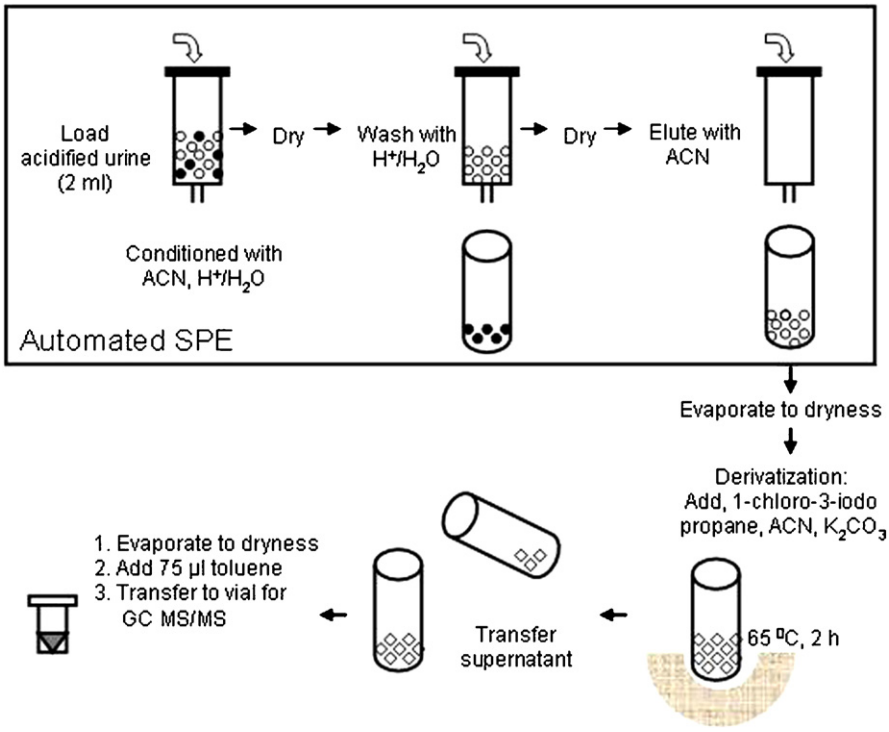

Fig. 2. Sample preparation of DAP metabolites.

with varying concentrations (0.02–8 mg/l) were made from the stock solution by dilution with acetonitrile. A 12.5 μl aliquot of working standard in 2 ml of urine gives the urinary concentration ranging from 0.1 ng/ml to 100.0 ng/ml. A stock solution (100 mg/l) of the labeled analogues was prepared in a similar manner. The working solution of the internal standard (10 ng/ml) was made by diluting the stock solution with acetonitrile. Stock standards were stored at −20 °C, and working standards were stored at 4 °C until use.

2.3. Sample preparation and automated SPE

The procedure used to extract DAP is summarized in Fig. 2. Human urine (2.0 ml) was thawed, vortex mixed, and dispensed into a Pyrex screw-cap tube (16 mm × 100 mm, Corning Inc., Corning, NY, USA). The urine was spiked with the internal standard spiking solution (12.5 μl), acidified with 3 M hydrochloric acid (50 μl), and vortex mixed. Urine samples were placed on a Zymark RapidTrace Station (Zymark Corporation, Hopkinton, MA, USA) for the automated SPE procedure. SPE cartridges were placed on the turret. Solvent lines were purged with water, acetonitrile, and 0.1 M aqueous hydrochloric acid to prime the reagent lines. SPE cartridges were conditioned with acetonitrile (4 ml) followed by 0.1 M HCl (4 ml). The urine sample was loaded onto the SPE cartridge at a rate of 0.38 ml/min. The cartridge was dried by passing nitrogen (∼30 psi) through it for 5 min, washed with 0.1 M HCl (1 ml), and then dried again for 2 min. Elution was accomplished with acetonitrile (7 ml) at a rate of 0.5 ml/min into a 10 ml screw-cap vial containing potassium carbonate (∼25 mg). The eluate was evaporated to dryness in a Turbovap LV evaporator (Zymark Corporation, Hopkinton, MA, USA) at 50 °C with nitrogen (Airgas Inc., Radnor, PA, USA) as the evaporating gas (11 psi) for about 45 min. The dried residue was resuspended in acetonitrile (1 ml). Potassium carbonate (20 mg) and 1-chloro-3-iodopropane (30 μl) were added to the vial, and it was capped and vortex mixed. The sample tube was placed in a preheated dry-bath incubator (Isotemp 145 D, Fisher Scientific, Pittsburgh, PA, USA) and maintained at 65 °C for 2 h with occasional vortex mixing. After the heating step, sample tubes were allowed to come to room temperature. Using a Pasteur pipette, the top layer was carefully transferred to a 10 ml centrifuge tube without disturbing the sediment at the bottom of the vial. Samples were evaporated to dryness in a TurboVap at 30 °C with nitrogen (10 psi) for about 20 min. The residue was reconstituted with 75 μl of toluene and transferred to an autosampler vial for analysis.

2.4. Instrument analysis

Analysis of the chloropropyl derivatives (Fig. 3) was performed using a gas chromatograph (3300 TraceGC, ThermoQuest, San Jose, CA, USA) coupled with a triple-quadrupole

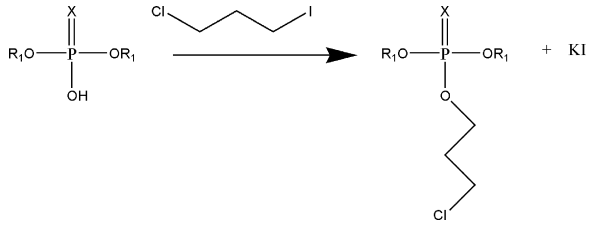

Fig. 3. The derivatization reaction results in the formation of chloropropyl esters of the DAP metabolites where X = S or O and R1 = methyl or ethyl.

Table 1
Chloropropyl derivatives of DAP analytes, their labeled analogues and precursor and product masses corresponding to the quantitation and confirmation ions, collision energies (CE), and retention times (RT)

Analyte	Quantitation ion Precursor/product ions (*m/z*)[a]	Confirmation ion Precursor/product ions (*m/z*)	CE (V)	RT (min)
DMP	203/127	205/127	12	7.14
DMP (dimethyl-d_6)	209/133	211/133	12	7.11
DEP	231/127	233/127	13	8.07
DEP (diethyl-d_{10})	241/133	243/133	13	8.02
DMTP	219/143	221/143	13	8.61
DMTP (dimethyl-d_6)	225/149	227/149	13	8.58
DMDTP	235/125	237/125	10	9.30
DMDTP (dimethyl-d_6)	241/131	243/131	10	9.27
DETP	247/191	249/193	12	9.43
DETP (diethyl-d_{10})	257/193	259/195	12	9.37
DEDTP	263/153	265/153	12	10.00
DEDTP (diethyl-$^{13}C_4$)	267/157	269/157	12	10.00

[a] Mass/charge ratio.

mass spectrometer (TSQ-7000, ThermoFinnigan, San Jose, CA, USA). An aliquot of each sample (1 μl) was injected in the splitless mode onto a J & W (Folsom, CA, USA) DB-5MS ([5%-phenyl]-methylpolysiloxane) capillary column (30 m, 0.25 mm ID, 0.25 μm) using an autosampler (CTC A200s, Carrboro, NC, USA). Helium (Airgas Inc., Radnor, PA) was used as the carrier gas. The injection port and GC/MS interface were set at 250 °C and 280 °C, respectively. Temperature of the column was initially set at 80 °C for 2 min and heated to 235 °C at a rate of 17 °C/min and finally to 270 °C at 50 °C/min. Final temperature was held for 5 min. The total run time was 17.32 min although the analytes eluted in less than 11 min.

Chemical ionization in the positive-ion mode using methane as the reagent gas (2 mT) was used to form positively charged pseudomolecular ions. The instrument was set in multiple reaction monitoring (MRM) mode, and the precursor and product ion combinations specific to the eluting analyte were monitored (Fig. 4, Table 1). Data acquisition and analysis were performed using Xcalibur® software on a PC-based data system and were manually evaluated. The analyte peak was identified by matching the retention time with that of its respective isotope-labeled internal standard (Table 1). Quantification was by the isotope-dilution method [43].

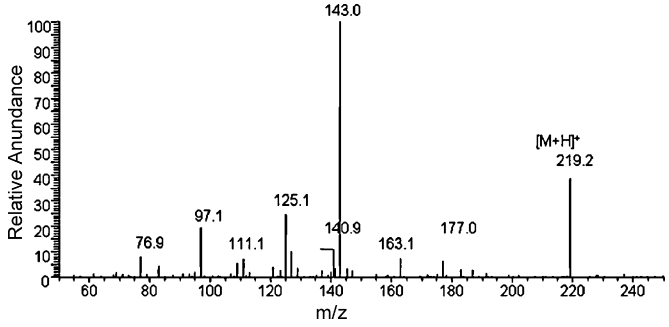

Fig. 4. The product ion spectrum of DMTP-chloropropyl ester (collision energy 13 V).

2.5. Daily operation and quality control procedure

Quality-control (QC) materials were prepared from pooled urine collected from multiple donors. The pooled urine was filtered, diluted with water (1:1), mixed well and then split equally into three pools. Two pools were fortified at low (QCL, 10 ng/ml) and high (QCH, 25 ng/ml) concentrations. The third pool served as the matrix for calibration standards and blanks. Each pool was characterized by a minimum of 30 repeat determinations over a 10-day period to determine the mean and 95th and 99th confidence limits. QC materials were analyzed during each analytic run with a minimum of one QC per sample-preparation module to ensure proper operation of the method and validation of the resulting data. QC data were evaluated using Westgard QC rules [44].

Each analytic run consisted of samples prepared on 10 RapidTrace SPE modules. On each module, one QC material (e.g., QCL, QCM or QCH), one reagent blank, and eight unknown or calibration samples were prepared, thus a full run consisted of 10 QC samples, 10 blank samples, 9 calibration samples, and 71 unknown samples. All samples were extracted simultaneously on the RapidTrace automated SPE system loaded with 100 SPE columns (i.e., 10 per module). After analysis, QC and unknown samples were corrected for the reagent blank although the blank levels were typically negligible, and a QC check was performed. For QC evaluation, each sample preparation module was considered independently. If a QC sample failed in a particular RapidTrace module, all samples within that module were re-extracted.

A calibration plot of peak area ratio of analyte to internal standard versus concentration was constructed daily. Calibration samples were prepared by spiking blank urine (2 ml) with the internal standard (12.5 μl) and the native standard (12.5 μl) and extracting along with the unknowns. Nine standards were used for the plot, and each was corrected for the standard purity of each analyte. Each point in the calibration plot was weighted ($1/X$) to ensure accurate quantification at the low concentration range. An Excel® report was generated with the calibration data, integrated peak areas, and retention times for each analyte, and

exported to a Microsoft Access® database for statistical analysis using SAS statistical software (SAS Institute, Cary, NC).

2.6. Limits of detection

The six lowest standards were each extracted six times. Standard deviation was plotted against their concentrations. Analytic limit of detection (LOD) for each of the analytes was calculated as $3S_0$ where S_0 is the value of the standard deviation as the concentration approaches zero. In addition, LODs were determined as $3S_0$ of the blank concentration. In all instances, LODs determined both ways were comparable.

2.7. Recoveries

Because standards of chloropropyl esters of the analytes were not available, efficiency of the derivatizing reaction could not be determined, and therefore, absolute recovery of the complete sample preparation could not be evaluated. SPE recoveries were calculated using the ratio of the amount of analytes recovered after SPE to amounts originally added. Recoveries were determined at 10 ng/ml and 50 ng/ml. For each level, blank urine samples (1 ml, $N=5$) were spiked with 12.5 μl of the standard and extracted along with five more blank urine samples. Before the derivatization step, all samples were spiked with 12.5 μl of the internal standard. The appropriate standard (12.5 μl) was added to the five samples that were not initially spiked with standard to serve as control samples representing 100% recovery. Samples were processed according to the method and analyzed.

2.8. Relative recovery

Blank urine samples were spiked at three concentration levels, analyzed according to the procedure outlined above, and their concentrations determined. For each concentration level, five replicate samples were used. Relative recovery (sometimes called accuracy) was determined as the slope of a linear regression analysis of a plot of measured concentration versus spiked concentration. A 100% relative recovery is indicated by a slope of 1.00.

2.9. Precision

Quality-control materials at two concentration levels ($n=30$ for each level) were analyzed over a period of 2 weeks. Relative standard deviations (RSD) were determined for both intra-day and inter-day variation.

2.10. Confirmation of detection

Confirmation ions were used to confirm the presense of the target analytes. For a detected peak to be confirmed as the target analyte, it had to coelute with the isotopically labeled internal standard (or within 3–6 s after deuterated standards), have the confirmation ion present, and have a ratio between the quantitation ion and confirmation ion consistent with those derived from standards and QC materials.

2.11. Cross-method validation

Blank urine was spiked with a set of standards and analyzed using the new analytic method and the lyophilization method currently used in this laboratory. The current method used a similar detection method but the sample preparation was based upon lyophilization of urine, resuspension of the residue and derivatization with no automated steps. The data were compared.

3. Results and discussion

Typical chromatograms of a spiked urine sample and a human urine sample are shown in Figs. 5 and 6. Few, if any, matrix interferences were observed in the chromatograms. We obtained linear calibration curves for all our analytes over three orders of magnitude with correlation coefficients exceeding 0.99 (Table 2) with less than 1% error of the slope. LODs were excellent for all the analytes ranging from 0.05 ng/ml to 0.17 ng/ml (Table 2) indicating the high sensitivity of the method.

Although determining of the absolute recovery was not possible because the standards of chloropropyl esters of the analytes were not available, we obtained good SPE recoveries ranging from 56 to 104 depending on the analyte and the spike concentration (Table 2). Relative recoveries ranged from 95% to 98%, indicating a high degree of accuracy (Table 2). Furthermore, relative standard deviations for all DAPs were excellent (Table 2) reflecting the good repeatability of the method. RSDs were <8% for all analytes except DMTP, which was 12%. A typical quality-control Shewart plot is shown in Fig. 7, which

Table 2
Specifications of the analytic method

Analyte	R^2 of calibration line	LOD[a] (ng/ml)	Extraction recovery ± SD[b] (%; $n=6$)		Relative recovery ($n=5$) %	Relative standard deviation (RSD%)	
			10 ng/ml	50 ng/ml		Low pool (10 ng/ml)	High pool (25 ng/ml)
DMP	0.996	0.06	56 ± 11	60 ± 8	105	7.7	7.4
DEP	0.998	0.05	104 ± 11	97 ± 11	105	5.0	6.4
DMTP	0.997	0.17	86 ± 3	82 ± 5	99	12.2	12.0
DMDTP	0.999	0.05	58 ± 7	62 ± 6	98	5.1	4.1
DETP	0.999	0.05	82 ± 4	78 ± 8	102	2.2	7.6
DEDTP	0.998	0.07	57 ± 9	58 ± 6	99	5.6	5.3

[a] LOD: limit of detection.
[b] SD: standard deviation.

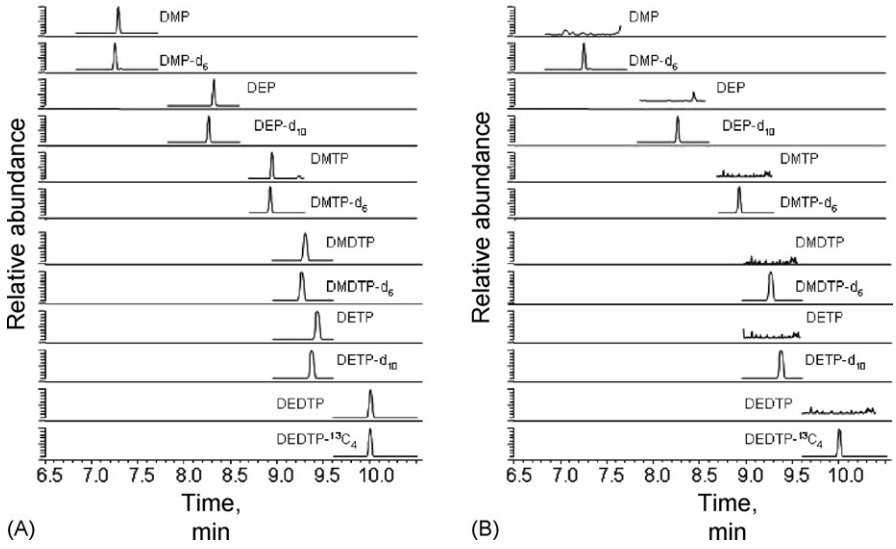

Fig. 5. Sample MS/MS chromatogram of a urine sample spiked (A) with ∼50 ng/ml standard mixture of the DAPs and their labeled internal standards (∼10 ng/ml) on a DB-5MS ([5%-phenyl]-methylpolysiloxane) capillary column. Internal standards generally elute 2–5 s earlier than the native analyte. A blank sample is also shown (B).

reflects both intra-day and inter-day variation. For all analytes, the plot was very similar.

We observed excellent agreement between calculated concentrations derived from this method and our current method (slope 1.00; $R^2 = 0.996$) (Fig. 8). However, area counts from the SPE method were much higher – five to six times higher for DMP and DEP and twice as much for DMTP and DETP – than those seen in the lyophilization method. Higher area counts are likely a result of the added cleanup the SPE method provides, minimizing interfering coextracted components, and likely improving the efficiency of the derivatization reaction.

In determining OP metabolites, the most challenging exercise has been extracting the highly polar and highly water-soluble DAPs, especially DMP, from a highly polar urinary matrix. Many extraction methods, such as liquid/liquid extraction, solid-phase extraction, anion exchange, azeotropic distillation, and lyophilization that offer different levels of sensitivity ranging from mid-ng/mL to low or sub-ng/mL levels [12], have been employed in the past in the quantitative determination of DAPs in occupationally and non-occupationally exposed populations. Solid-phase extraction, in particular, has been tried as an attractive alternative to other extraction methods as it offers many advantages: it generally allows a rapid extraction of analytes, uses less solvent, and is less labor-intensive. Furthermore, solid-

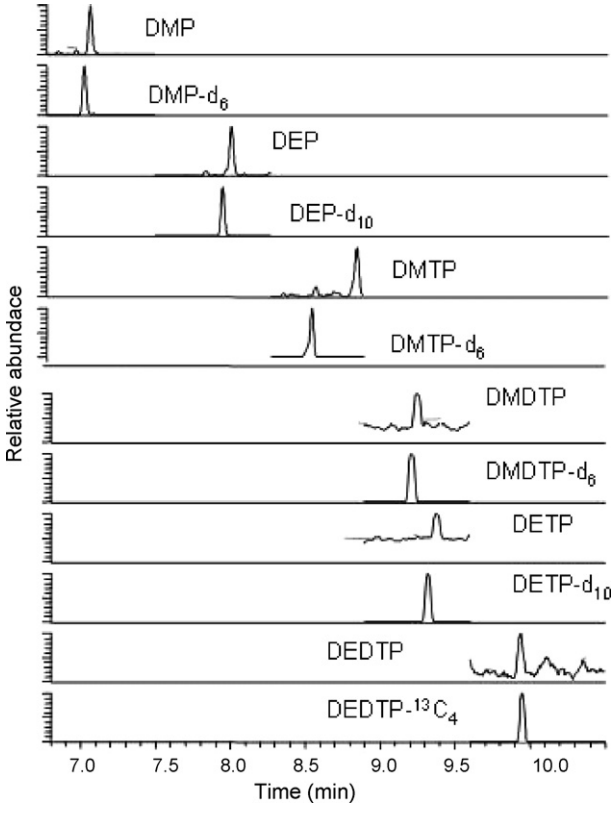

Fig. 6. Sample MS/MS chromatogram for a human urine extract containing 1.0 ng/ml DMP, 1.0 ng/ml DEP, 1.5 ng/ml DMTP, 0.3 ng/ml DMDTP, 0.3 ng/ml DETP, 0.1 ng/ml DEDTP.

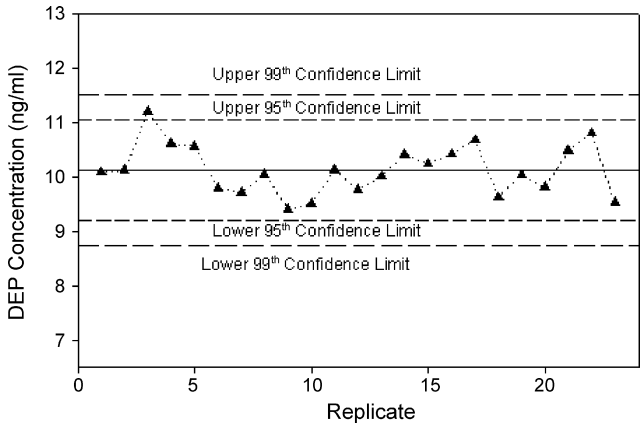

Fig. 7. A plot of DEP concentration of quality-control samples ($n = 23$) over time: (solid line) represents the mean; (dotted lines) represent the 95th and 99th confidence limits.

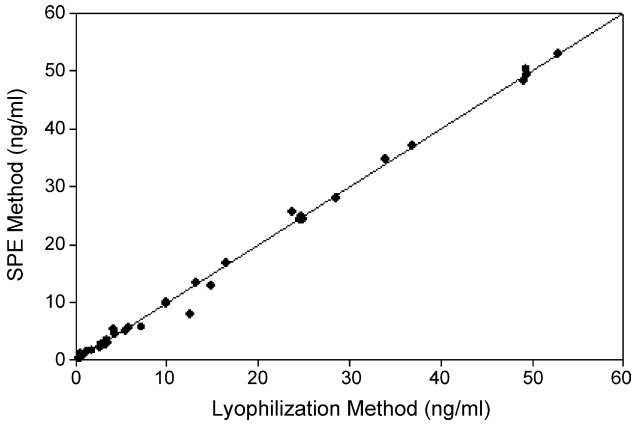

Fig. 8. Concentrations of DAPs from analyses of a series of standards using both the new SPE method and the lyophilization method. The gradient of 1.00 and R^2 value of 0.996 obtained indicate a close match between the two extraction methods.

phase extraction is amenable to automation. Several workers have explored SPE for the extraction of urinary DAP metabolites in the past. Weisskopf and Seiber [20] reported a method using cyclohexyl SPE columns. However, DMP could not be measured using this method, and DEP gave low and variable recoveries. Furthermore, this method produced high LODs, 10 ng/ml for DEP and 2 ng/ml for the thio compounds. Park et al. [19] have also used cyclohexyl columns and experienced similar results. LODs for the metabolites, DMP, DEP, DMDTP and DETP, are reported to be in the range 50–100 ng/ml. A solid-phase extraction method with ion-exchange resin was reported by Lores and Bradway [18]. However, extraction of the compounds from urine was often incomplete and inconsistent [14], again resulting in high LODs.

Our laboratory has evaluated several sample-preparation techniques and found that lyophilization is superior to many other extraction techniques for concentrating DAP analytes from the urine matrix. In fact, in recent times lyophilization has become more or less the extraction method of choice [29–32]. It offered better recoveries, easily handled samples, and better LOD compared with other published methods [32]. However, the lyophilization method has two main short comings. No cleanup of the sample is employed, which is crucial for derivatization as well as GC–MS/MS analysis. Sample cleanup enhances the efficiency of derivatization and reduces interferences during GC analysis. Without sample cleanup, maintenance of the analytic system must be performed frequently. Furthermore, lyophilization is not time-efficient because the overall process takes about 20 h, although much of this time involved unattended overnight operation.

To address these limitations, we looked for a SPE method that is fast and simple, but still sensitive enough to detect trace levels of urinary DAPs. Considering the highly polar nature of DAPs, we selected styrene-divinylbenzene (SDVB) polymer-based sorbent material because of its enhanced non-polar interactions [45]. Another advantage of this polymer sorbent is its ability to withstand pH extremes that are not achievable with silica-based sorbents. We evaluated several commercial SDVB polymers and found that Bond Elut PPL columns performed the best. Bond Elut PPL has been derivatized to create a non-polar surface with extreme hydrophobicity and a high surface area of 600 m^2/g to ensure that it retains even the most polar classes of compounds through non-polar interactions.

The compounds were protonated by lowering the pH to <1.25 (pK_a for DMP = 1.25) [17,46,47] with HCl for efficient retention of DAPs in the SPE column. Although in previous studies [19,20] low recoveries were observed with HCl as the acidifying agent compared with acetic acid, our observation was in complete contrast. To improve the extraction of metabolites, we tried salting out of urine with sodium chloride. However, salt addition had an adverse effect on the overall efficiency of the method and was therefore abandoned. In contrast to our observations, enhancement of the extraction or adsorption processes of DAP with salt addition was reported in some earlier work [17,19,20,46]. With the automated SPE, we achieved much lower flow rates (0.4 ml/min) of urine through the column bed than were possible with manual SPE. This lower flow rate allows the analytes to be in contact with the sorbent bed longer and enhances the adsorption process thus improving recoveries. Furthermore, pre-concentration of the analytes on the cartridge and an acid wash produced a cleaner eluate and enhanced the derivatization efficiency and chromatographic response, resulting in less required maintenance of the GC–MS/MS instrument.

Of the solvent systems tested (e.g., acetonitrile, acetone, methanol, ethyl acetate, diethyl ether, hexane and mixtures) to optimize the DAP elution efficiency, acetonitrile performed the best. However, the less-polar thio compounds were difficult to elute and required a large volume of solvent to accomplish complete elution. An interesting observation we made in trying different solvents is that use of diethyl ether led to decomposition of DMTP and DMDTP. Presumably, the oxidative impurities that may be present in diethyl ether may have caused this degradation.

The derivatization reaction with pentafluorobenzyl bromide requires anhydrous conditions [25]. For the reaction with 1-chloro-3-iodopropane we made a similar observation. Best results were obtained when the SPE eluate was allowed to dry longer in the TurboVap even after all solvent had evaporated. Furthermore, we observed that derivatization occurred best at 65 °C for 2 h, which is slightly different from the optimum conditions (i.e., 60 °C for 3 h) reported [28] for the lyophilization method. After being subjected to two different extraction methods, lyophilization and SPE, the resulting matrix may have slight differences that could explain the differences in derivatization.

4. Conclusions

We developed an automated solid-phase extraction post-extraction derivatization and GC–MS/MS method for accurately quantifying OP metabolites in human urine. This method is characterized by its simplicity, rapidity, high reproducibility, high sensitivity, and high selectivity. It allows for trace-level determination of urinary OP metabolites and enhances the efficiency of exposure studies to determine relevant health effects. Overall sample preparation time is only 4 h including the 2 h derivatization time that represents a significant time savings over our

previous method. Automation of the SPE procedure allowed less labor-intensive, unattended extraction of urine samples. Minimal system maintenance was required through the course of this study whereas our other method required daily or weekly maintenance. Thus, we anticipate that we will be able to analyze hundreds of samples before any significant system maintenance is required.

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