Quantification of dialkylphosphate metabolites of organophosphorus insecticides in human urine using 96-well plate sample preparation and high-performance liquid chromatography–electrospray ionization-tandem mass spectrometry☆

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ABSTRACT

Organophosphorus (OP) pesticides kill by disrupting a targeted pest's brain and nervous systems. But if humans and other animals are sufficiently exposed, OP pesticides can have the same effect on them. We developed a fast and accurate high-performance liquid chromatography–tandem mass spectrometry method for the quantitative measurement of the following six common dialkylphosphate (DAP) metabolites of organophosphorus insecticides: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate, (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). The general sample preparation included 96-well plate solid phase extraction using weak anion exchange cartridges. The analytical separation was performed by high-performance liquid chromatography with a HILIC column. Detection involved a triple quadrupole mass spectrometer with an ESI probe in negative ion mode using multiple reaction monitoring. Repeated analyses of urine samples spiked at 150, 90 and 32 ng/mL with the analytes gave relative standard deviations of less than 22%. The extraction efficiency ranged from 40% to 98%. The limits of detection were in the range of 0.04–1.5 ng/mL. The throughput is 1152 samples per week, effectively quadrupling our previous throughput. The method is safe, quick, and sensitive enough to be used in environmental and emergency biological monitoring of occupational and nonoccupational exposure to organophosphates.

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1. Introduction

Organophosphorus (OP) insecticides are potent and effective anti-cholinergic insecticides, and remain the largest class of insecticides sold worldwide. They are widely and effectively used in agriculture, and to a lesser extent, in domestic pest control [1,2]. Through their inhibition of acetylcholinesterase, the enzyme responsible for catalyzing the breakdown of the neurotransmitter acetylcholine, OP insecticides are highly toxic. Enzyme inhibition causes the accumulation of acetylcholine and leads to symptoms related to the autonomic nervous system and the central nervous system [3,4]. Also, OP insecticides may act on targets other than acetylcholinesterase; but the extent of these effects is just beginning to emerge [5].

OP insecticides are hydrolyzed environmentally and biologically to dialkylphosphates (DAPs) and to more specific metabolites. The determination of these DAPs in urine has been reported as a sensitive indicator for assessment of OP exposure, especially in acute exposure events such as occupational exposure and intentional poisonings [6]. Fig. 1 shows the structures of the six most commonly measured DAP metabolites: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate, (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). These metabolites are common to the majority of OP insecticides but they do not retain any of the structure unique to the pesticides from which they were derived; thus, it is impossible to identify individual pesticides from these metabolites. Nevertheless, because the metabolites are common to the majority of OP insecticides, they can provide information about exposure to the OP insecticide class. About 75% of registered OP insecticides metabolize to DAPs excreted in urine. Especially in acute exposure incidents, quantification of these metabolites provides an estimate of cumulative exposure to the class of OP insecticides, despite the inability to associate directly any metabolite with a given OP insecticide [6,7].

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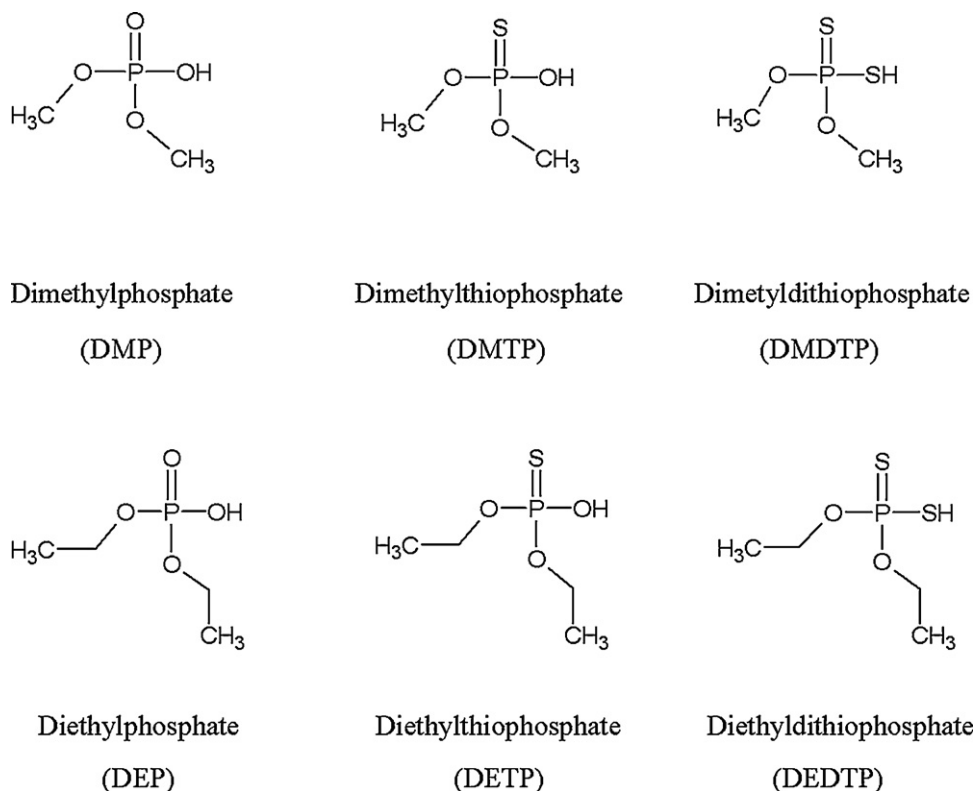*M.S. Odetokun et al. / J. Chromatogr. B 878 (2010) 2567–2574*

Fig. 1. Chemical structures of the DAP metabolites.

Several methods have been reported for the measurement of DAPs in urine using gas chromatography (GC) with mass spectrometry (MS) or tandem mass spectrometry (MS/MS) detection [8–17]. In fact, our laboratory reported a sensitive GC–MS/MS method for measuring internal dose DAPs in urine [13]. This method has been used on samples collected from a U.S. general population survey (National Health and Nutrition Examination Survey, NHANES). The results are presented in CDC's National Report on Human Exposure to Environmental Chemicals (www.cdc.gov/exposurereport), which provides an ongoing assessment of the U.S. population's exposure to environmental chemicals. But in addition to its use in the NHANES survey, our laboratory's GC–MS/MS method has also been used in several epidemiologic studies to assess exposure to OP insecticides [18–21]. Because of the low urinary concentration of these metabolites in urine from nonacutely exposed persons and the necessary sensitivity required for detection, the procedure traditionally used in our laboratory is complex and time-consuming. It involves several steps including lyophilization, derivatization, and extraction of the analytes. Yet for emergency response purposes, when time is critical and concentrations are apt to be much higher, this method is not practical. In addition, older technology precluded our using quicker high performance liquid chromatography (HPLC)-MS/MS methods for analysis because of the inadequate separation of several of the target compounds.

For this reason, we developed a new method that is rapid, efficient, less labor-intensive, and more time-efficient. It can be used in routine and in emergency biological monitoring of OP insecticides. The newer method allows not only a more rapid throughput, but also because of the separation of critical analytes, it also offers more sensitivity for some analytes. Our method employs solid phase extraction using weak anion exchange sorbent on a 96-well plate followed by a highly selective and sensitive analysis using isotope dilution, HPLC with electrospray ionization-tandem mass spectrometry (HPLC/ESI-MS/MS).

2. Experimental

2.1. Chemicals

DMP and DEP (98.9% 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 (98%) were purchased from Aldrich Chemicals Co. Isotopically labeled analogues of the analytes, D_6-DMP (dimethyl-d_6), D_{10}-DEP (diethyl-d_{10}), D_6-DMTP (dimethyl-d_6), D_6-DMDTP (dimethyl-d_6), D_{10}-DETP (diethyl-d_{10}), and $^{13}C_4$-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%. Acetonitrile and methanol were purchased from Tedia Company, Inc. (Fairfield, OH). Deionized water was purified with an Aqua Solutions, Inc. water system (Jasper, GA). Triethylamine (TEA) was purchased from Fisher Scientific (Pittsburgh, PA). Formic acid was purchased from Acros Organics (Morris Plains, NJ). Ammonium acetate was purchased from Sigma–Aldrich (St. Louis. MO).

2.2. Standard and internal standard preparation

Individual stock solutions of the native DMP, DMTP, DMDTP, DEP, DETP, and DEDTP were prepared by weighing out and dissolving 1 mg of each analyte in 100 mL of acetonitrile. Stock solutions were stored at −70 °C. Eleven working standard stock solutions, each a mixture of an equal concentration of all the analytes and covering a range of 0.010–16.0 μg/mL, were prepared by diluting with acetonitrile appropriate volumes of the individual stock solutions in 100 mL volumetric flasks. The working standard solutions were stored at −20 °C. Eleven calibration standards, made by adding the working stock solutions to blank urine, covered a range from 0.125

to 200 ng/mL. Before each analytical run, the calibration standards were made fresh.

The labeled internal standard stock solutions D_6-DMP, D_{10}-DEP, D_6-DMTP, D_6-DMDTP, D_{10}-DETP, and $^{13}C_4$-DEDTP were prepared by weighing approximately 1.0 mg of each isotopically labeled analyte into a 100 mL volumetric flask and dissolving with acetonitrile. These were stored at $-70\,^{\circ}$C. A stock solution mixture of all the labeled analytes was prepared at a concentration 1.0 μg/mL in acetonitrile and stored at $-20\,^{\circ}$C.

2.3. Quality control (QC) materials

Urine samples were collected from multiple (>30) donors, combined together, diluted with water (1:1 v/v) to reduce endogenous levels of the analytes of interest, and mixed overnight at 20 °C. The urine pool was pressure filtered with a 0.2 μm filter capsule and divided into four pools. The first pool QCL (QC low), the second pool QCM (QC medium) and the third pool QCH (QC high) were spiked with the native standard stock solution to yield concentrations of 32, 90 and 150 μg/mL, respectively. The fourth pool was not spiked. After screening for possible endogenous analytes, the fourth pool was used as matrix material for calibration standards and blanks.

2.4. Sample preparation

Samples were thawed and vortex-mixed to ensure homogeneity. Urine samples (600 μL) were pipetted into a rectangular 96-well plate, (Varian, Inc., Palo Alto, CA). Forty μL of formic acid were added to acidify the samples. The samples were spiked with 12.5 μL of the labeled internal standard working solution to give a urinary concentration of 20.83 ng/mL. The extraction procedure was performed automatically using a TOMTEC Quadra 3 96-well plate extraction unit (Hamden, CT) to improve sample throughput and to minimize human intervention in the extraction process. The system was programmed to perform the clean-up process using a 96-well Strata X-AW (weak anion exchange) solid phase extraction cartridge plate (Phenomenex, Torrance, CA, USA). The station positions for the pipette tips, 1% formic acid in methanol, 1% formic acid in water, and 20% TEA in acetonitrile solvent reservoirs, samples, shucking the used tips and the vacuum box for the filtration process were specified in the program. Samples were placed on a plate at the specified station. The 96-well cartridge plate was conditioned with 0.9 mL of 1% formic acid in methanol, followed by 0.9 mL of 1% formic acid in water, and then the vacuum was applied. The samples were aspirated from the storage wells and dispensed onto the 96-well cartridge plate. The 96-well cartridge plate was washed with 0.9 mL of water, followed by 0.9 mL of methanol. The analytes were eluted with 1.350 mL of 20% TEA in acetonitrile into a square 96-well plate. The extracts were concentrated to dryness using a TurboVap® 96-well plate concentration workstation (Caliper Life Sciences, Hopkinton, MA) at 50 °C and 30–90 flow of nitrogen. The residues were reconstituted with 50 μL of acetonitrile and transferred to auto injection vials.

2.5. Chromatography and mass spectrometry conditions

The analytes were separated by HPLC using the Agilent 1100 Series autosampler and pump (Agilent, Santa Clara, CA). The column was a Luna 5 μm HILIC 200A, 100 × 2.00 mm (Phenomenex, Torrance, CA, USA). The analytes were separated with isocratic elution by using 93% of acetonitrile and 7% of 100 mM ammonium acetate in deionized water. The total run time was 10 min. The flow rate was 250 μL/min, and the injection volume was 1 μL. The maximum pressure was set at 400 bar for the HPLC pump.

For the MS/MS analysis we used a TSQ Quantum triple quadrupole mass spectrometer from ThermoFisher Scientific (San Jose, CA, USA). The instrument was operated with an ESI source, in negative ion mode and with selective reaction monitoring (SRM). The capillary temperature was set at 350 °C, the spray voltage was 4500 V, the sheath and auxiliary nitrogen gas pressures were set to 17 and 5 psi, respectively, and the collision gas pressure was at 1.5 mTorr.

2.6. Method validation

2.6.1. Extraction efficiency

We determined the method's extraction recovery at two concentrations of 10 and 50 ng/mL. We spiked blank urine samples with the appropriate standard concentration and extracted the analytes according to the method. Additional blank urine samples (unspiked) were extracted concurrently. To correct for instrument variation, before the evaporation steps all of the samples were spiked with a known amount of labeled internal standards. After evaporating and reconstitution, the samples were analyzed. The recovery was calculated by comparing the responses of blank urine samples spiked before extraction to the responses of the blank urine samples spiked after extraction.

2.6.2. Limits of detection

The LOD was defined as three times the standard deviation of the noise at zero concentration ($3S_0$), where S_0 was estimated as the *y*-intercept of a linear regression analysis of a plot of the standard deviation of the three lowest standards versus the expected concentration from 10 runs [22]. Furthermore, the LOD was compared with the results of the calibration standard samples and low-level spiked samples to ensure that the calculated values agreed with the peak observed and that a minimum signal-to-noise ratio of 3 was present at these low levels.

2.6.3. Precision

The method precision was determined by calculating the relative standard deviations (RSDs) of repeat measurements of the QC materials at three different concentrations (32, 90, and 150 ng/mL). To evaluate the within- and between-day variation, at least 20 repeat measurements of QC materials were analyzed in 20 different runs.

2.6.4. Accuracy

The accuracies, sometimes called relative recoveries, were calculated by spiking blank working matrix material samples at different concentrations and calculating the concentration by this method. A linear regression analysis was performed on a plot of the measured concentrations versus the expected concentrations. A slope of 1.00 was considered 100% accuracy.

2.6.5. Matrix effects

We evaluated urine matrix effects using our previous published protocols [23,24]. Ten urine samples collected from different donors were analyzed individual. We then compared the results with those obtained by analyzing a spiked urine pool sample formed by combining urine from the same ten donors. Urine samples were spiked to yield a concentration of 50 ng/mL. The urine samples were prepared for analysis according to the procedure already described. Five replicates were analyzed from each urine sample. An aliquot of each urine matrix was screened for possible endogenous analytes.

2.6.6. Stability test over analysis time

For the extracted samples we further evaluated post-preparative stability and short-term temperature stability. For post-preparative stability, extracted samples were placed in the autosampler at room temperature (RT) for 24, 48 and 72 h. For

Table 1
The precursor and product ions, the collision energy, the tube lens offset, and the expected retention time for the native analytes and their labeled internal standard.

Analyte	Precursor → Product	Collision energy (V)	Tube lens offset	Expected retention time (min)
DMP – Q	125 → 79	39	61	6.87
DMP – C	125 → 63	19	61	6.87
DMP – label	131 → 79	42	61	6.89
DEP – Q	153 → 79	40	47	5.08
DEP – C	153 → 125	14	47	5.08
DEP – label	163 → 131	15	47	5.11
DMTP – Q	141 → 126	17	59	1.97
DMTP – C	141 → 95	26	59	1.97
DMTP – label	147 → 129	17	59	1.99
DMDTP – Q	157 → 142	18	43	1.12
DMDTP – C	157 → 112	23	43	1.12
DMDTP – label	163 → 145	20	43	1.14
DETP – Q	169 → 95	22	49	1.77
DETP – C	169 → 141	14	49	1.77
DETP – label	179 → 95	23	49	1.78
DEDTP – Q	185 → 111	21	49	1.07
DEDTP – C	185 → 157	15	49	1.07
DEDTP – label	189 → 111	27	49	1.09

"Q" Quantification ion.
"C" Confirmation ion.

short-term stability, extracted samples were stored at −70 °C for 24, 48, and 72 h. For each condition of storage, concentrations were calculated for the three quality control extracted samples.

2.7. Quantification and quality control of analytical runs

Before each analytical run, calibration standards were prepared by diluting the working standard stock solutions in blank urine. The concentrations of the 11 calibration standards ranged from 0.125 to 200 ng/mL for each of the analytes. To each run was added the 11 calibration samples, 2 sets of 3 quality control samples (2*QCL,2* QCM, and 2*QCH), and 2 blank urine samples; these were extracted and analyzed in parallel with 72 unknown samples. (Note: the remaining 5 wells were used for continued validation testing.) The area of the analyte divided by the area of the internal standard was plotted against the concentration of the sample to derive a calibration plot. The best-fit line of a linear regression analysis of the plot was used to derive an equation from which unknown sample concentrations could be calculated.

By consecutively analyzing at least 20 samples from each QC pool, all QC pools were characterized before use to determine the mean and 99th and 95th control limits. QC samples were analyzed in 20 runs over 20 days. After establishing the control limits of the pools, individual QC samples contained within each analytical run were evaluated for validity by use of the Westgard multirules [25].

2.8. Cross-comparison of analytical results

To ensure both the accuracy and robustness of our analytical methods for determining the DAP metabolites concentrations, we conducted a cross-comparison study using the HPLC–MS/MS method described here and using the GC–MS/MS method [13] currently employed in our laboratory. The percentage of agreement between the two methods was calculated as the slope of a linear regression analysis of a plot of the values obtained from each method.

3. Results and discussion

Many incidences involving OP insecticide poisonings, both accidental and intentional, have been reported [26–29]. In these situations, an effective emergency response is necessary; but this depends upon rapid and accurate confirmation of the exposure. The goal of this study was to develop a highly selective, sensitive analyt-ical method with simple sample preparation procedure that could be used in emergency response cases.

The optimized precursor/product ion pairs as well as the collision offset energy and tube lens offset for the target compounds and internal standards and the expected retention times are summarized in Table 1. All transitions were based on the [M−H]⁻ precursor ions. To improve selectivity of the analysis, we used the most abundant product ion as a quantification ion and the second most abundant as a confirmation ion. We tentatively chose the most abundant product ion as the quantification ion and the second most abundant product ion as the confirmation ion, knowing that we might encounter a high background that would, in practice, cause the less abundant product ion to give a better LOD. We found that the choice of the most abundant product ion to be the quantification ion was correct, since in actual analytical runs in almost all cases this product ion had better S/N than the product ion chosen to be the confirmation ion. Typical chromatograms of the quantification ions of the six DAPs in urine extract spiked with 100 ng/mL analytes are shown in Fig. 2. The chromatographic analyze of a blank urine sample demonstrating no interference of the six DAPs.

The analytical separation of the six analytes was done using a HILIC column. The HILIC column allows the separation of polar compounds by partitioning and is extremely effective for polar compounds which are particularly difficult to separate by standard reversed phase columns. In the past, this was the primary reason that HPLC–MS/MS could not be used effectively to measure the DAP metabolites—the two most polar DAPs, DMP and DEP could not obtain either chromatographic or mass separation (as a results of in-source fragmentation, DEP forms the same ions as DMP) [30]. More recently, one paper was published showing separation of DMP and DEP but a gradient elution was used with a total run time 2.2 times longer than ours, preventing the rapid response we desired [31]. However, they also appeared to see the in-source fragmentation to which we refer. Preliminary experiments were conducted to optimize conditions for separation and retention for all analytes. Using a 93% organic phase and 7% aqueous phase, under isocratic conditions we achieve optimal separation of the analytes (Fig. 2). Because HPLC eliminates the need for the derivatization step required in the old GC–MS/MS method, significant time savings in sample preparation are obtainable [8–17].

One of the most important steps in analyzing DAP metabolites is their extraction from the polar urine matrix. A variety of methods reported in the literature have used azeotropic distil-
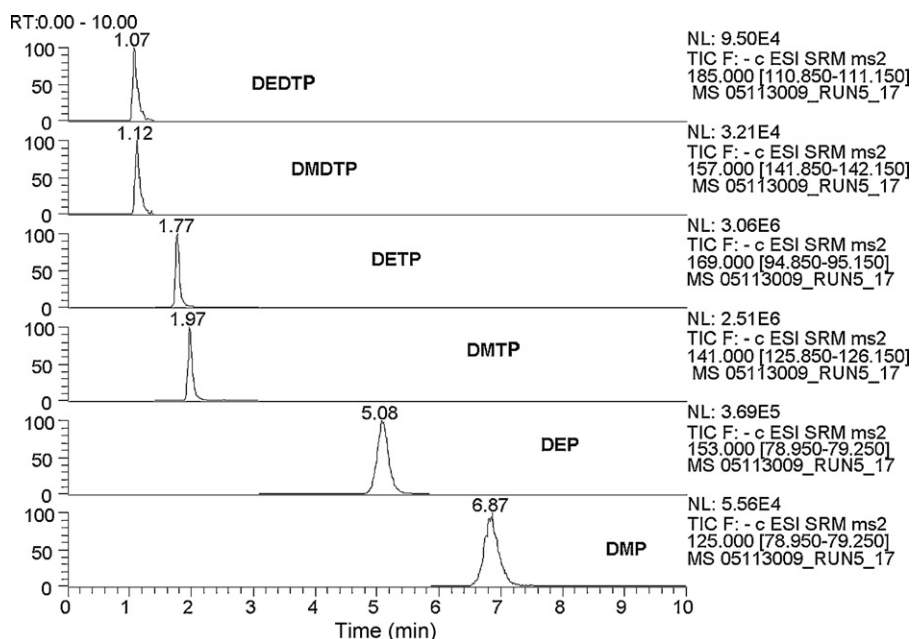
Fig. 2. A typical ion chromatogram of native in urine spiked with 100 ng/mL.

lation [12], liquid–liquid extraction (LLE) [9,30] or lyophilization [10,11,13,16,17] for extracting DAPs from the urine matrix. The sample preparation that we propose is simple, efficient, and reproducible. DAP metabolites were extracted in a procedure using anion exchange solid phase extraction (SPE) in an automated system. We tried several approaches to find the best sample preparation procedure and to optimize recovery efficiency for the weak anion exchange cartridge 96-well plate system. The advantages of using a weak anion exchange cartridge are that the negatively charged compounds [H$^-$] are retained more strongly and non-polar matrix contaminants are eliminated completely when an organic solvent wash is used. By allowing matrix components to be eliminated during the SPE process, it results in cleaner extracted samples and better separation efficiency. Our extraction recoveries obtained are better than those that previously reported using reversed-phase SPE cartridge [17]. For most of the analytes, the recoveries were greater than 70% except for DMP and DEDTP, which were somewhat lower (Fig. 3).

The LODs for this method ranged from 0.04 to 1.549 ng/mL for all analytes (Table 2). These LODs are lower in magnitude than the LODs previously published by Dulaurent et al. in their HPLC–MS/MS procedure for determination of DAPs in urine using LLE extraction and reversed-phase separation [30]. The LODs in the HPLC–MS/MS method described in this paper are similar to the
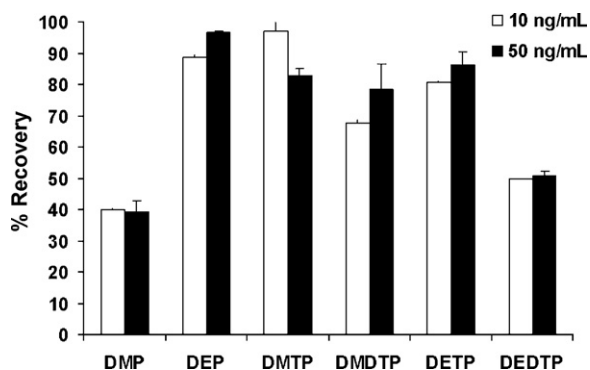
LODs of our existing GC–MS/MS method [13], except for DMP and DEDTP, both of which exhibited higher LODs on the HPLC–MS/MS method. Nevertheless, the advantage of the HPLC method is the rapid turn-around time, making this method appropriate for emergency response.

For all analytes, a slope average of a linear regression analysis of 11 calibration standards of 10 runs of calibration curves was calculated. The R^2 values were greater than 0.997 for all analytes (Table 2). In 10 urine matrices, calibration curves were linear over the entire range. Fig. 4 shows a typical calibration curve for the metabolite DEDTP in urine matrix. The limit of quantification was 0.125 ng/mL (lowest standard point). The method's accuracy was 100% (Table 2). The calculation was based on a slope average of linear regression analyses of plots of calculated concentrations of spiked samples versus the expected concentration of the same samples from 10 runs.

The QC values were calculated as an average of 20 runs with one at each level in each run. In most instances, the precision of
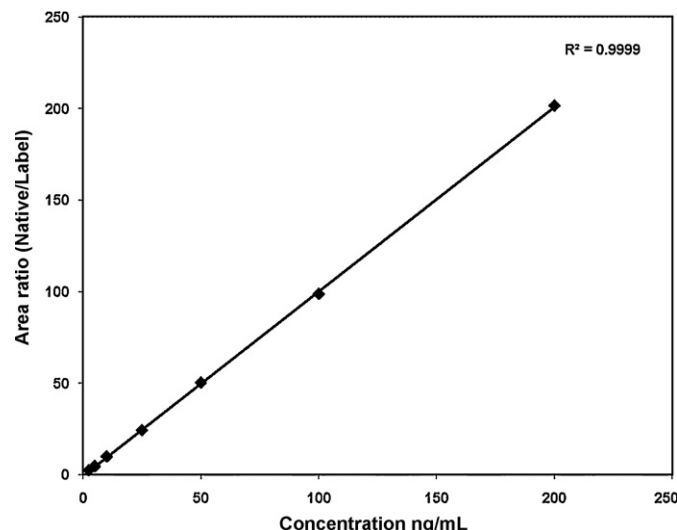

Fig. 3. Percentage of recovery of the analytes in two concentrations of spiked urine after the standard clean-up procedure.

Fig. 4. A typical calibration curve for DEDTP quantification ion.

Table 2
Summary of method specifications for DAPs metabolites.

Analyte	LOD (ng/mL)	Standard curve R^2	Accuracy (%)	QC values (ng/mL)			% RSD		
				QCL	QCM	QCH	QCL	QCM	QCH
DMP	0.468	0.9997	99.99	28.35	85.43	132.96	19.72	12.97	17.23
DEP	0.044	0.9998	100	29.78	89.26	136.62	18.29	9.81	19.81
DMTP	0.066	0.9999	100	28.90	88.82	131.71	19.68	10.82	15.68
DMDTP	0.073	0.9999	99.99	24.99	87.01	133.48	21.37	12.29	11.27
DETP	0.110	0.9998	100	28.47	89.31	126.18	17.85	10.79	15.51
DEDTP	1.549	0.9999	99.99	28.50	97.36	146.17	19.13	8.13	17.13

LOD: calculated as $3S_0$. Standard deviation at zero concentration (S_0) was estimated as the y-intercept of a plot of the standard deviation of the three lowest calibration standards from ten runs versus the expected concentration.
Standard curve: slope average of a linear regression analysis of eleven calibration standards from 10 runs.
Accuracy: expressed as the percentage of the expected concentration that was quantified from 10 runs.
QC (quality control) values: average of QCL (low), QCM (medium) and QCH (high) from twenty runs. Blank urine pools were spiked with the native standard stock solution to yield a concentration of 32 ng/mL (QCL), 90 ng/mL (QCM) and 150 ng/mL (QCH).
RSD: relative standard deviation of the QC values from nine runs.

our method is reasonable, with RSD of repeated analyses of the QC materials averaging around 22% or less (Table 2).

Experiments to assess urine matrix effects causing ion suppression in the mass spectrometer are mandatory for HPLC–MS methods. Ion suppression, caused by salts and biomolecules in the sample, plays an important role in method sensitivity. The potential for ion suppression varies among urine samples because of differences in salt and biomolecule concentration and because of differences of extraction efficiency of these. Urine samples from 10 different donors were collected, and the specific gravity (compara-
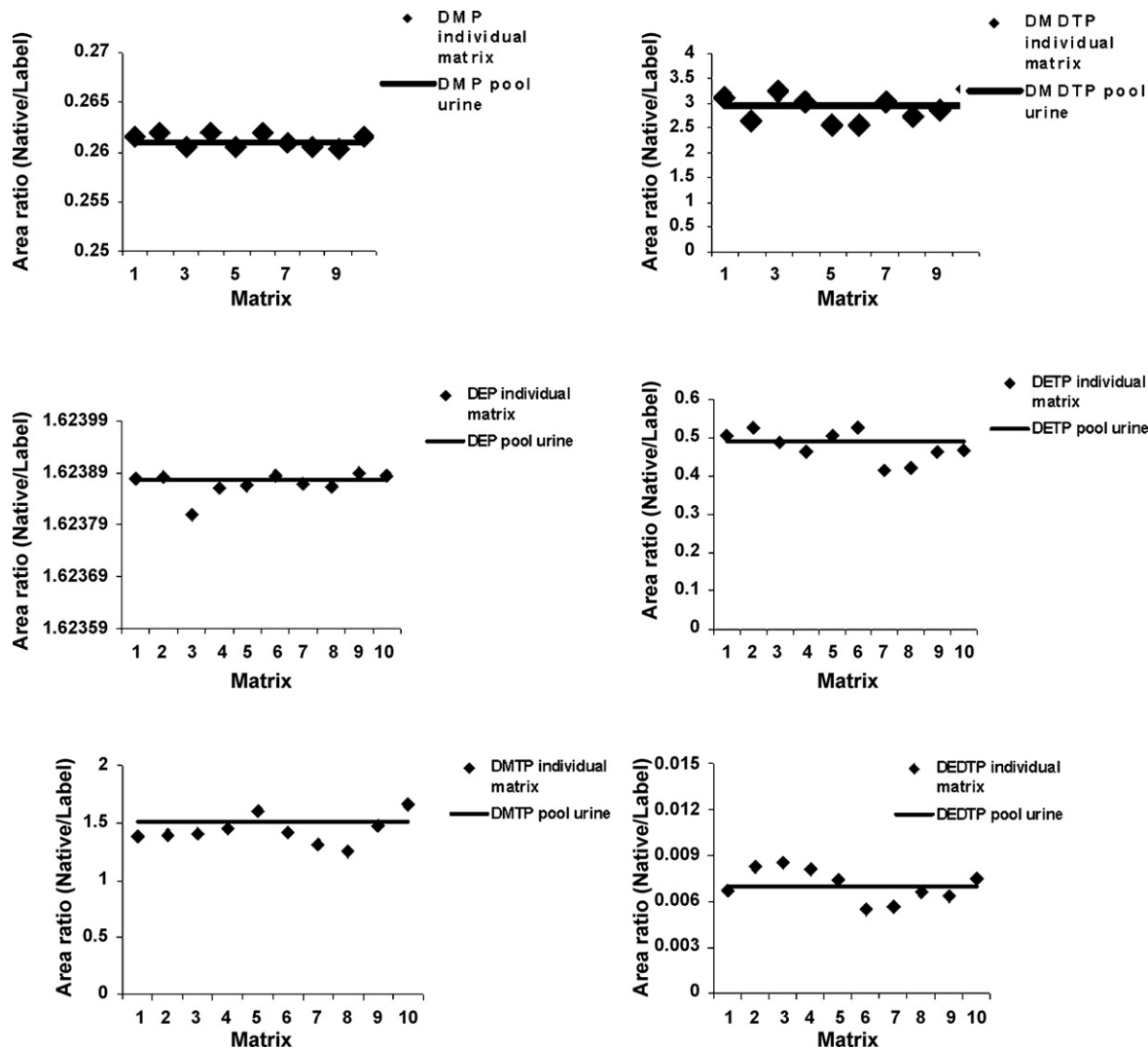

Fig. 5. Matrix effects. Urine samples from eight different individual donors and a combined urine sample pool were spiked with the analytes and quantified in five analytical runs. The percentage of variation was calculated as: $\left(1 - \frac{\text{Obtained}}{\text{Expected}}\right) \times 100$. Expected = area ratio for the combined urine sample pool. Obtained = area ratio for the individual urine sample.

Table 3
Temperature stability test (% of variation).

Analyte	Post-preparative									Short-term								
	24 h/RT			48 h/RT			72 h/RT			24 h/−70 °C			48 h/−70 °C			72 h/−70 °C		
	QCL	QCM	QCH	QCL	QCM	QCH	QCL	QCM	QCH	QCL	QCM	QCH	QCL	QCM	QCH	QCL	QCM	QCH
DMP	0	0	2.4	0	0	1.0	0	0	0.3	1.8	0	0.3	0	0	0	0	0	0
DEP	0	0	0.7	0	0	2.5	0	0	0.7	0.7	0	0	0	0	0	0.6	0	0
DMTP	0	0	0.7	0	0	0.6	0	0	0.7	0	0	0	0	0	0	0	0	0
DMDTP	0	0	0.3	0	0	0.3	0	0	0.3	0.8	0	0	0.6	0	0	0	0	0
DETP	0	0	0.3	0	0	0.2	0	0	0.3	0	0	0.7	0	1.0	0.8	0	0	0
DEDTP	0	0	0	0	0	0	0	0	0	0	0	0.3	1.0	0.2	0.9	0.8	0	0

Variation (%) of temperature stability was calculated as a ratio of the area ratio (native/label) at different conditions and times of storage versus the area count at storage, $t = 0$.

ble to urine creatinine) was calculated from each individual sample. The specific gravity ranged from 1.003 to 1.029 ρ/ρ_{H_2O} (data not shown). After spiking with 50 ng/mL of the analytes, each individual urine matrix was analyzed and compared with a urine pool combining urine from the same 10 donors. The variation of matrix effects was calculated, with the data shown in Fig. 5. The variation of matrix effects ranged between 1 and 10%, suggesting that

individual differences in matrix composition did not affect method sensitivity.

We investigated post-preparative and short-term temperature stability on extracted samples. We chose the storage conditions based on situations that could occur during analysis. If a run has a large number of samples, they might sit in the autosampler for relative long periods at room temperature; or, for example, samples
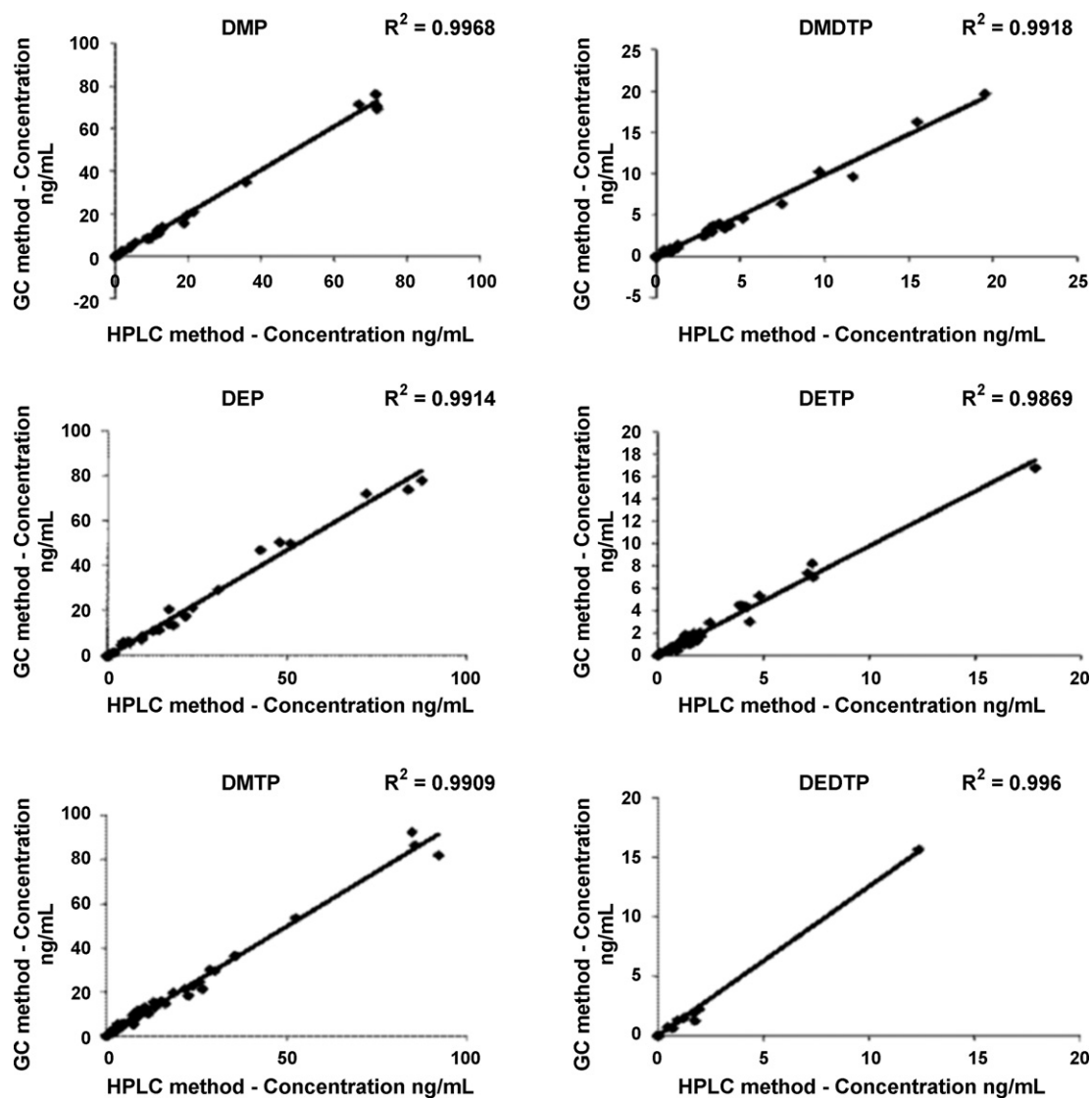

Fig. 6. Comparison of measurements of the six DAPS metabolites in 100 urine samples by two methods: HPLC and GC. The slope of 0.98 indicates excellent agreement between the two methods.

might sit in storage at $-70\,°C$ waiting for a technical repair on the mass spectrometer. Regardless, the temperature stability variation was less than 2.5% over 72 h for both the room temperature and the $-70\,°C$ storage condition (Table 3). These data suggest that the analytes in extracted samples are essentially stable over 72 h in the two relevant storage conditions—but the method performance is not significantly affected.

Finally, a cross validation test was done applying this method and the existing GC–MS/MS method [13] on 100 urine samples (Fig. 6). For all analytes, a slope of a linear regression analysis was calculated considering the calculated concentration of the analyte in the two analytical methods. The R^2 values were greater than 0.9869 for all analytes. The quality of the method was tested, and practice proved that it can be used in emergency response involving exposed human populations.

4. Conclusions

We present a high-throughput method for measuring OP metabolites in human urine using isotope dilution HPLC–MS/MS. After performing the validation study and using the method in a cross validation test, we conclude that the method is an accurate, sensitive, and robust means to measure the six dialkylphosphate metabolites and thereby determine OP pesticide exposure in an emergency response environment.

Disclaimer

The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the Centers for Disease Control and Prevention. The authors have no competing financial interests to declare.

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