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Cross validation of gas chromatography-flame photometric detection and gas chromatography–mass spectrometry methods for measuring dialkylphosphate metabolites of organophosphate pesticides in human urine☆

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ABSTRACT

We report two analytical methods for the measurement of dialkylphosphate (DAP) metabolites of organophosphate pesticides in human urine. These methods were independently developed/modified and implemented in two separate laboratories and cross validated. The aim was to develop simple, cost effective, and reliable methods that could use available resources and sample matrices in Thailand and the United States. While several methods already exist, we found that direct application of these methods required modification of sample preparation and chromatographic conditions to render accurate, reliable data. The problems encountered with existing methods were attributable to urinary matrix interferences, and differences in the pH of urine samples and reagents used during the extraction and derivatization processes. Thus, we provide information on key parameters that require attention during method modification and execution that affect the ruggedness of the methods. The methods presented here employ gas chromatography (GC) coupled with either flame photometric detection (FPD) or electron impact ionization-mass spectrometry (EI-MS) with isotopic dilution quantification. The limits of detection were reported from 0.10 ng/mL urine to 2.5 ng/mL urine (for GC-FPD), while the limits of quantification were reported from 0.25 ng/mL urine to 2.5 ng/mL urine (for GC–MS), for all six common DAP metabolites (i.e., dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate). Each method showed a relative recovery range of 94–119% (for GC-FPD) and 92–103% (for GC–MS), and relative standard deviations (RSD) of less than 20%. Cross-validation was performed on the same set of urine samples ($n = 46$) collected from pregnant women residing in the agricultural areas of northern Thailand. The results from split sample analysis from both laboratories agreed well for each metabolite, suggesting that each method can produce comparable data. In addition, results from analyses of specimens from the German External Quality Assessment Scheme (G-EQUAS) suggested that the GC-FPD method produced accurate results that can be reasonably compared to other studies.

Introduction

Most organophosphate (OP) pesticides are composed of a phosphate, phosphorothioate, or phosphorodithioate moiety that, in most cases, is *O*,*O*-dialkyl substituted, where the alkyl groups are usually methyl or ethyl, and an additional organic group bound to the phosphorus atom, accounting for differential toxicity. Once entering the body, OP pesticides can be enzymatically converted to their oxon form, which then inhibits the enzyme acetylcholinesterase (AChE) responsible for the breakdown of the neurotransmitter acetylcholine. The oxon also can be enzymatically

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Table 1
OP pesticides and their potential dialkyl phosphate metabolites.

Pesticides	DMP methylated	DMTP	DMDTP	DEP ethylated	DETP	DEDTP
Azinphos methyl	√	√	√			
Chlorethoxyphos				√	√	
Chlorpyrifos				√	√	
Chlorpyrifos methyl	√	√				
Coumaphos				√	√	
Dichlorvos	√					
Diazinon				√	√	
Dicrotophos	√					
Dimethoate	√	√	√			
Disulfoton				√	√	√
Ethion				√	√	√
Fenitrothion	√	√				
Fenthion	√	√				
Isazaphos-methyl	√	√				
Malathion	√	√	√			
Monocrotophos	√					
Methamidophos	√	√	√			
Methidathion	√	√	√			
Methyl parathion	√	√				
Naled	√					
Oxydemeton-methyl	√	√				
Parathion				√	√	
Phorate				√	√	√
Phosmet	√	√	√			
Pirimiphos-ethyl				√	√	
Pirimiphos-methyl	√	√				
Sulfotepp				√	√	
Temephos	√	√				
Terbufos				√	√	√
Tetrachlorviphos	√					
Triasophos				√	√	
Trichlorfon	√					

or spontaneously hydrolyzed to form a dialkyl phosphate (DAP) metabolite and an organic molecule specific to the pesticide. If the pesticide is not converted to its oxon form, it can hydrolyze similarly to form its specific and dialkylthionate metabolites (i.e., dialkylthiophosphate and/or dialkyldithiophosphate) (Barr et al., 2004). The DAP metabolites, common to about 75% of the United States Environmental Protection Agency registered OP pesticides, are dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), and diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP). The majority of these metabolites are excreted in urine (Barr et al., 2004). Table 1 shows the list of OP pesticides and their respective DAP metabolites (Bravo et al., 2004).

Since the 1970s, urinary DAP metabolites have been quantified in human urine as non-specific biomarkers of exposure to OP pesticides (Barr et al., 2004, 2011; Bradway and Shafik, 1977; Bravo et al., 2004). Quantification of these metabolites offers information on cumulative exposure to this class of pesticides and the data are used in several epidemiological studies for health outcome assessment (Bouchard et al., 2011; Engel et al., 2011; Harley et al., 2011; Perry et al., 2011; Quiros-Alcala et al., 2011; Rauh et al., 2011; Sudakin and Stone, 2011). In order to quantify these metabolites, several analytical methods have been developed, and usually consist of three parts: extraction, derivatization, and analysis (Alwis et al., 2006, 2008, 2009; Aprea et al., 1996; Bravo et al., 2004; Hardt and Angerer, 2000; Moate et al., 1999; Oglobline et al., 2001a,b; Ueyama et al., 2006, 2010; Wu et al., 2010).

Although a few high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) methods have been developed to avoid the derivatization process (Hernandez et al., 2002; Odetokun et al., 2010), this technology is often not available in laboratories or is labor intensive, so alternative methods must be employed. The extraction of these highly polar metabolites from urine samples is difficult and often relies on techniques such as liquid–liquid extraction (LLE) (Aprea et al., 1996; Hardt and Angerer, 2000; Ueyama et al., 2006, 2010; Wu et al., 2010), traditional solid-phase extraction (SPE) (Alwis et al., 2006, 2008, 2009; Odetokun et al., 2010), molecularly imprinted SPE (Santos et al., 2012), and azeotropic co-distillation (Moate et al., 1999). Lyophilization was introduced with the expectation that the extraction recovery would be nearly quantitative because the water was simply removed and the residues were re-dissolved in solvent (Bravo et al., 2004; Oglobline et al., 2001a,b; Petchuay et al., 2008; Yucra et al., 2006). Similarly, azeotropic co-distillation was used to remove water residue from the sample (Moate et al., 1999). While all of the metabolites were essentially retained, both techniques provided very dirty, sometimes gummy samples that were difficult to process further. Molecularly imprinted SPE was efficient but expensive and required the use of materials that are difficult to obtain. Although extraction recoveries were lower for LLE and SPE, the process was simpler, involved less time, and provided cleaner extracts.

In recent years, 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr) has commonly been used as a derivatizing agent (Alwis et al., 2006, 2008, 2009; Aprea et al., 1996; Moate et al., 1999; Oglobline et al., 2001a,b; Petchuay et al., 2008; Ueyama et al., 2006, 2010; Wu et al., 2010). Other agents such as 1-chloro-3-iodopropane (Bravo et al., 2004) and benzyltolytriazine (Yucra et al., 2006) were also used but to a lesser extent. Although PFBBr is a lachrymator, its use has continued because of its effectiveness in producing a single reaction product and because of the quickness and ease of the reaction process (Hardt and Angerer, 2000). After derivatization, analysis of these derivatives has been done using gas chromatography (GC) coupled with flame photometric detection (FPD) (Aprea et al., 1996; Moate et al., 1999; Oglobline et al., 2001b; Petchuay et al., 2008; Wu et al., 2010; Yucra et al., 2006), mass spectrometry (MS) (Alwis et al., 2008, 2009; Hardt and Angerer, 2000; Ueyama et al., 2006, 2010), and tandem mass spectrometry MS/MS