UNIVERSIDADE DA BEIRA INTERIOR
Ciências

Application of dried blood spots for the determination of organophosphorus pesticides by GC-MS/MS

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Dissertação para obtenção do Grau de Mestre em
Bioquímica
(2º ciclo de estudos)

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Covilhã, junho de 2018

Agradecimentos

A realização de uma dissertação de mestrado é o culminar de muitas horas de estudo, reflexão e trabalho pessoal, mas representa também uma etapa que não seria possível sem o contributo fundamental de várias pessoas.

À Doutora Eugénia Gallardo e ao Doutor Mário Barroso, como excelentes mentores que são, quero agradecer toda a orientação, motivação, incentivo e confiança. Toda a ajuda e partilha de experiência e conhecimento durante todo este percurso.

Ao Tiago Rosado, pela imprescindível ajuda e disponibilidade constante, bem como pela amizade. À Joana Gonçalves e ao Ângelo Luís um agradecimento especial pela amizade e carinho demonstrado ao longo deste ano. A todos, quero agradecer pelo bom ambiente proporcionado.

Aos meus pais Ana Soares e José Soares, a quem dedico este feito, por me darem a possibilidade de concretizar conquistas como esta, pelo apoio incondicional, por sempre terem acreditado em mim e por fazerem parte deste momento. Aos meus avós e restante família, por todo o apoio e preocupação.

Um agradecimento particular à Inês Ramos, à Rita Marques, à Maria Cunha e ao Pedro Batista pelo companheirismo, amizade e apoio de há tantos anos.

E por último, mas nunca menos importante, um enorme obrigada ao meu namorado António Fernandes por toda a paciência, pelo incansável apoio, carinho e ajuda. Por me fazeres rir e nunca desistir.

A todos vós, obrigada!

Resumo alargado

A utilização contínua, e na maioria das vezes descontrolada de pesticidas, como por exemplo os produtos da classe dos organofosforados, é uma questão que afeta a sociedade desde a antiguidade até aos dias de hoje. Estes são utilizados para prevenir, controlar ou eliminar pragas e aumentar a produtividade agrícola. No entanto, mesmo que em quantidades vestigiais, causam contaminações de alimentos e/ou águas, mas também danificam gravemente o ecossistema e, devido ao efeito de exposição contínua, representam um grande perigo com crescente risco de segurança para a saúde humana. Para além disso, vão surgindo novos pesticidas cada vez mais eficazes, para um leque de atividades mais abrangente e por sua vez pouco seletivos, cujo mecanismo tóxico não é compreendido. Por se tratar de um assunto que continua a ser atual, o seu acesso ser tão facilitado e atrair cada vez mais preocupação, tornando-se alarmante, foi criada nova legislação com o objetivo de permitir uma gestão adequada e um maior controlo para a utilização destas mesmas substâncias. Estes compostos continuam a apresentar uma estatística significativa em casos de intoxicações e mortes, nomeadamente em Portugal, sendo a sua análise ainda requisitada aos laboratórios, quer do ponto de vista clínico quer forense. Estima-se que ocorrem 3 milhões de casos de intoxicações acidentais por ano, uma incidência de 220.000 mortes em todo o mundo segundo a *World Health Organization* (WHO) e mais de 250.000 mortes por ano de envenenamento por suicídio. Em Portugal, de acordo com o Laboratório de Toxicologia Forense do Instituto Nacional de Medicina Legal e Ciências Forenses da Delegação Sul, os inseticidas presentes neste trabalho estiveram envolvidos em 86 casos de intoxicações entre 2003 e 2006 (dados não publicados).

Por todas as razões apresentadas, a deteção e a quantificação destes compostos em amostras biológicas, particularmente em amostras de sangue, é de grande interesse público e científico. Relativamente aos novos desenvolvimentos na área da toxicologia, recentemente têm surgido novas técnicas de preparação de amostra entre elas os *dried blood spots* (DBS). Esta é uma técnica miniaturizada não invasiva, que requer um baixo volume de sangue, aplicado diretamente a partir da amostra biológica e com secagem no papel antes de ser analisada. Esta abordagem tem sido utilizada na investigação em diversas áreas, aumentando a performance, simplificando questões de logística, reduzindo custos e facilitando o processo de amostragem.

Assim sendo, o objetivo deste trabalho foi desenvolver e validar um método analítico para a deteção e quantificação de cinco pesticidas organofosforados (diazinão, clorpirifos, paratião-etilo, clorfenvinfos e quinalfos), utilizando os DBS como técnica de extração e a cromatografia gasosa acoplada à espectrometria de massa (GC-MS/MS) como instrumento de análise. O padrão interno utilizado foi o etião (ETH) e a amostra biológica empregue foi o sangue. Todos os fatores do processo de extração foram completamente otimizados, sendo estes o solvente ou mistura de solventes utilizados (metanol:acetonitrilo), bem como a proporção aplicada (50:50), o volume utilizado desta mesma mistura de solventes (3 mL), o tempo de extração (10 minutos), a temperatura de extração (temperatura ambiente), o tempo de centrifugação (10 minutos) o

tempo de secagem das manchas de sangue (12 horas) e ainda o volume de amostra a utilizar (50 µL).

O método desenvolvido foi totalmente validado de acordo com as diretrizes aceites internacionalmente, incluindo os princípios orientadores da *Food and Drug Administration* (FDA), da *International Conference on Harmonization* (ICH) e do *Scientific Working Group for Forensic Toxicology* (SWGTOX). Adicionalmente, os parâmetros estudados incluíram seletividade, linearidade, limites de deteção (LOD) e limites de quantificação (LLOQ), precisão e exatidão, estabilidade, fator de diluição e recuperação.

O intervalo de linearidade obtido situou-se entre 0,1-25 µg/mL para todos os compostos, exceto para o diazinão (0,05-25 µg/mL) e para o quinalfos (0,25-25 µg/mL), com coeficientes de determinação superiores a 0,99 em todos os casos. A precisão intradia revelou coeficientes de variação (CVs) tipicamente inferiores a 14%. Em relação à precisão interdia, foi realizado um estudo ao longo de 5 dias e foram obtidos CVs inferiores a 14% para todos os compostos. A estabilidade a curto prazo (24h, temperatura ambiente) e após 3 ciclos de congelação/descongelação, bem como as estabilidades a longo prazo, foram estudadas e revelaram que os compostos estudados são estáveis sob todas as condições testadas. Apesar das baixas recuperações obtidas, que variaram entre 1 e 12%, o método mostrou ser sensível, pois os limites de deteção situaram-se entre 0,05 e 0,1 µg/mL.

Salienta-se que este é o primeiro procedimento descrito sobre a utilização deste método de extração em amostras de sangue aplicado a estes compostos, o que associado à simplicidade, sensibilidade e rapidez do mesmo, permitirá a sua implementação na rotina laboratorial em laboratórios de análises clínicas e forenses para a deteção e/ou quantificação destes pesticidas organofosforados.

Palavras-chave

Pesticidas organofosforados, *Dried blood spots*, GC-MS/MS, Sangue.

Abstract

The uncontrolled use of pesticides, for instance of organophosphorus nature, is an issue that has been affecting societies for a long time. As this is a current subject, and the access to these compounds is so facilitated, legislation has been put in place to monitor their use; furthermore, these compounds still appear in intoxication statistical data, and as such their detection and quantification in biological specimens is still requested to many laboratories, mainly in the clinical and forensic areas.

The objective of this work was to develop and validate an analytical method for the detection and quantification of five organophosphorus pesticides (diazinon, chlorpyrifos, parathion-ethyl, chlorfenvinphos and quinalphos) in blood, using the dried blood spots (DBS) sampling approach for sample preparation and gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). The internal standard used was ethion (ETH), and the entire extraction process was previously optimized. The developed method was fully validated according to internationally accepted guidelines for bioanalytical method validation, and the studied parameters included selectivity, linearity, limits of detection (LOD) and quantification (LLOQ), precision and accuracy, stability, dilution integrity, and recovery. Linearity was obtained in the range of 0.1-25 μg/mL for all compounds, except for diazinon (0.05-25 μg/mL) and quinalphos (0.25-25 μg/mL), with determination coefficients greater than 0.99. Intra- and interday precision revealed coefficients of variation (CVs) typically lower than 14%, while accuracy was within a ±12% interval from the nominal concentrations. Short-term stability (for 24h at room temperature), stability after 3 freeze/thaw cycles and long-term stability were studied, revealing that the analytes were stable under those conditions. Despite the low recoveries obtained (between 1 and 12%), the method was sensitive enough as to present limits of detection between 0.05 and 0.1 μg/mL. This is the first technique described using this extraction approach for these compounds, and its simplicity, sensitivity and speed allow its routine use in the laboratories for the detection and quantification of these organophosphorus pesticides in biological specimens.

Keywords

Organophosphorus pesticides, Dried blood spots, GC-MS/MS, Blood.

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Index of abbreviations

µECD	Microelectron capture detection
APCI	Atmospheric pressure chemical ionization
C18	Octadecylsilane
C8	Octylsilane
CL	Colorimetric
CLF	Chlorfenvinphos
CLP	Chlorpyrifos
CV	Coefficient of variation
CW/DVB	Carbowax™/divinylbenzene
CWC	Chemical Weapons Convention
DAP	Dialkylphosphate
DBS	Dried Blood Spots
DI	Desorption ionization
DZN	Diazinon
ECD	Electron capture detector
EI	Electron ionization
ELISA	Enzyme-linked immunosorbant assay
ESI	Electrospray ionization
ETH	Ethion
EU	European Union
FA-DSDME	Fast agitated directly suspended droplet microextraction
FAO-UN	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FID	Flame ionization detector
FL	Fluorescence
FPD	Flame photometric detector
GC	Gas chromatography

GM	Geometric mean
HESI	Heated electrospray ionization
HF-SPME	Hollow fiber solid phase microextraction
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
HS-SPME	Headspace-solid phase microextraction
ICH	International Conference on Harmonization
IS	Internal standard
LC	Liquid chromatography
LLE	Liquid-liquid extraction
LLME	Liquid-liquid microextraction
LLOQ	Lower limit of quantification
LOD	Limit of detection
LOQ	Limit of quantitation
MEPS	Microextraction by packed sorbent
MIP	Molecularly imprinted polymer
MISPE	Molecularly imprinted solid-phase extraction
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MTBSTFA	N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide
NCI	Negative chemical ionization
NPD	Nitrogen phosphorous detector
PA	Polyacrylate
PDA	Photodiode array detector
PDMS	Polydimethylsiloxane
PFBBr	Pentafluorobenzyl bromide
PFPD	Pulse flame photometric detector
PRT	Parathion-ethyl
PSA	Primary-secondary amine

PTEs	Phosphotriesterases
QLP	Quinalphos
QTOF	Quadrupole Time of Flight
QuEChERS	Quick, easy, cheap, effective, rugged, and safe
R^2	Determination coefficient
RE	Relative error
SA-DSPE	Solvent-assisted dispersive solid-phase extraction
SERS	Surface enhanced Raman scattering
SFODME	Solidified floating organic drop microextraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SPMEM	Solid-phase micro-extraction membrane
SPR	Surface plasmon resonance
SWGTOX	Scientific Working Group for Forensic Toxicology
TCP	Chlorpyrifos metabolite
TIS	Turbo ion spray
TLC	Thin layer chromatography
UHPLC	Ultra-high performance liquid chromatography
UPLC	Ultraperformance liquid chromatography
WHO	World Health Organization

Justification and Objectives

Pesticides, particularly the class of organophosphorus insecticides, are known to play an important role in agriculture and gardening, controlling pests that persist in the environment, preventing or reducing their proliferation. However, even if these chemical compounds are effective against insects and rodents, for example, or in their control in general, they may cause toxic effects on other organisms because they are not species-selective. They can also cause environmental contamination, in water and/or food, which is a problem for fauna, flora, but also for human beings.

For all these reasons, the development of a method for the detection and quantification of organophosphorus pesticides in blood samples is a very useful tool in cases of intoxication or poisoning by such compounds when laboratories are quested by the authorities to evaluate their possible presence, as well as their identification and/or quantification. It is also important that these new methods are less expensive and less time consuming, as well as reliable and easy to perform.

The main objective of this work was the development, optimization and validation of an analytical method for the determination of five organophosphorus pesticides, diazinon (DZN), chlorpyrifos (CLP), parathion-ethyl (PRT), chlorfenvinphos (CLF) and quinalphos (QLP), in blood using the dried blood spots (DBS) sampling approach and gas chromatography/tandem mass spectrometry (GC-MS/MS). In addition, another objective was to evaluate the applicability of this developed methodology to real samples.

These compounds have been chosen because they are the most sold and used of this class in Portugal. The final method uses a low sample volume and low amounts of organic solvents as well; moreover, the extraction time is reduced.

Taking into account that the whole of this dissertation has been submitted for publication, the present work is divided into two chapters, each of which belongs precisely to the submitted articles.

Chapter I corresponds to the work submitted with the title “Toxicological aspects and bioanalytical approaches for the determination of organophosphorus insecticides in human biological samples".

This chapter will not only address the physical-chemical and toxicological aspects of this class of compounds, highlighting published case reports in the literature, but also will perform a comprehensive and critical review on the analytical methods available for the determination of these agents in biological specimens, with special focus on the latest instrumental developments and sample preparation approaches.

Chapter II describes the entire experimental part of this dissertation and corresponds to the submitted article entitled “New analytical approach to determine organophosphorus insecticides in blood by dried matrix spots sampling and GC-MS/MS”.

This article describes a sensitive method for the determination of the above-mentioned organophosphorus insecticides in whole blood using the dried blood spots sampling approach (DBS) and gas chromatography/tandem mass spectrometry (GC-MS/MS).

Chapter I: Introduction and bibliographic review

1. Introduction and history

Pesticides have always been used in its crude form from the earliest times, but their use as synthetic compounds happened early in the middle of the twentieth century (Lock and Wilks 2001).

Highly toxic organophosphorus compounds are a large group of organic phosphorus esters created in the 1930s before the World War II (Holmstedt 1963; Inoue et al. 2007), with the accidental discovery of tabun by Gerhard Schrader in 1937 (Holmstedt 1963; Ballantyne and Marrs 1992). In addition to this finding, the starting point for the development of this class of pesticides was also the synthesis of alkyl phosphorofluoridates by Lange and von Krueger (Worek et al. 2016). Consequently, investigations were initiated to develop other highly toxic compounds subsequently used as chemical warfare nerve agents such as sarin, VX and soman (Holmstedt 1963; Ballantyne and Marrs 1992; Szinicz 2005).

After the war period, the use of these compounds and research on their development with the pesticide function increased (Holmstedt 1963; Eto and Press 1975), due to the lower environmental stability, the high adjacent toxicity and to their high efficiency (Casida and Durkin 2013; Turner 2017). Consequently, these pesticides have become one of the most commonly used classes of substances in the world (Salm et al. 2009; Nshimiyimana et al. 2014). These compounds have their importance and the beneficial effects associated with agriculture as agrochemicals used to minimize crop and post-harvest crop losses, ensuring the effective production and to control pests that affect them, as well as reducing the incidence of diseases transmitted by vectors (Lock and Wilks 2001; Eddleston et al. 2002; Park et al. 2009; Nshimiyimana et al. 2014; Harshit et al. 2017; Turner 2017). These compounds also have much shorter environmental half-lives compared to other classes of pesticides (Bossi et al. 1999; Castillo-Sánchez et al. 2000; Ingerslev and Nyholm 2000).

However, pesticides are manufactured to be toxic to living species and released into the environment, so a large part of the population is exposed to these chemicals in the non-occupational environment or at the workplace, which makes it virtually impossible for exposure to be completely avoided. In addition, since they are ubiquitous in environment and not specific to target organisms, they can be harmful to man, being considered an environmental health problem which means that the associated risks (depending on whether the exposure is acute or chronic) should be properly evaluated (Barr and Needham 2002; Eddleston et al. 2002; Hernández et al. 2002a; Aprea et al. 2002; Multigner 2005; Jaga and Dharmani 2006; Gunnell et al. 2007; Harshit et al. 2017).

Besides their reckless and indiscriminate use, the lack of use of safety devices during manufacture, storage, transport and agricultural application increase the risk of human exposure to these compounds, and as such a high number of accidental and intentional human intoxications occur (Aprea et al. 2002; Jaga and Dharmani 2006; Kumar et al. 2009). Furthermore, the widespread use of these compounds may increase insect resistance (Eddleston et al. 2002).

Although some of these insecticides have been restricted in 2001, their agricultural use is still important (Pérez et al. 2010). Though international organizations such as the World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO-UN) (Bertolote et al. 2006; Vapnek et al. 2007; Rohan et al. 2012) have issued warnings and created strict legislation to prohibit and control the use of these compounds, they are still responsible worldwide for many deaths for accidental ingestion or accidental exposure through the skin and airways (Taira et al. 2006) and poisonings, either suicidal (Casey and Vale 1994; Eddleston et al. 2006) or homicidal (Suzuki et al. 1995; De Letter et al. 2002; Kumar 2004) each year (Eddleston et al. 2002; Gunnell et al. 2007; Kumar et al. 2009; Park et al. 2009). Most cases of suicides or attempts occur in rural areas, where people who use these pesticides usually store them at home, facilitating their ingestion (Kumar et al. 2009; Raposo et al. 2010), since that they are still widely used in commercial and domestic agriculture (Musshoff et al. 2002). Cases of homicidal poisoning involving these compounds are less frequent due to the unpleasant odor and taste conferred by hydrocarbon solvents (Kumar et al. 2009). There is an estimation of 3 million accidental poisoning cases per year, and an incidence of 220,000 deaths worldwide according to the WHO (Konradsen et al. 2003; Chaudhary et al. 2013), and more than 250,000 deaths per year from suicidal poisoning (Sharma et al. 1990; Sogorb et al. 2004; Bertolote et al. 2006; Gunnell et al. 2007; Park et al. 2009; Nshimiyimana et al. 2014).

The morbidity and mortality percentage due to intoxication by these insecticides is high and varies from country to country. Several factors contribute to this rate, such as the level of socioeconomic development, accessibility to these chemicals, importance of the local agricultural sector as well as the delay in diagnosis and inadequate treatment. This reinforces the importance of toxicological screening for the correct diagnosis, since accurate information on the involved substance(s) is often rare. There is also a wide variety of compounds that may eventually be present, making it necessary to develop powerful and versatile analytical methodologies for their identification (Sungur and Güven 2001; Luzardo et al. 2015).

The risk and the potentially increasing threat of terrorist attacks using either these pesticides or the above-mentioned nerve agents continue to be addressed today. This situation encourages the Chemical Weapons Convention (CWC) to ban their development, production and storage, which entered into force in April 1997. Recent episodes and intoxication statistics also reinforce the importance of toxicological aspects (United Nations 1992).

2. Structure and classification

Organophosphorus pesticides are a large group of highly toxic organic phosphorus esters, presenting the following basic structure (Figure 1) (proposed by Gerhard Schrader in 1937 (Holmstedt 1963)).

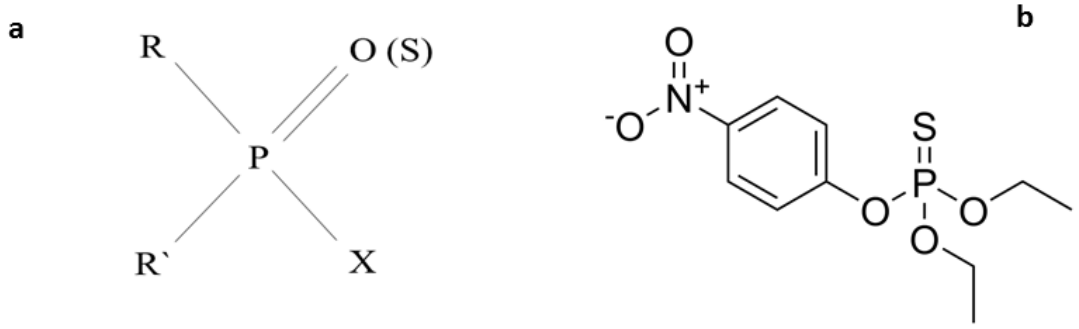

Figure 1. Basic structure of organophosphorus insecticides (a) and example of organophosphorus insecticide-parathion (b).

All compounds are derived from the phosphoric acid molecule (P = O) or from the phosphorothioic acid molecule (P = S). The presence of one of these groups is important for the determination of human toxicity arising from these compounds. The thiophosphates have to be metabolized to the respective oxon, to become effective acetylcholinesterase inhibitors (Casida 1956; Eto and Press 1975), a cytochrome P450-mediated reaction that is susceptible to interindividual variations and consequently individual susceptibility to pesticides with this bond (Mutch and Williams 2006; Foxenberg et al. 2007).

The combination of the possible residues in the R and R' positions and the different groups in the X position allows the synthesis of an indeterminate number of final derivatives. The R and R' radicals may be alkyl, alkoxy, aryloxy or others, while X may be a phenoxy, thiophenoxy, phosphate, carboxylate or other groups (Eto and Press 1975; Moffat et al. 2011).

Therefore, these pesticides can be divided into different subclasses, including phosphates, phosphonates, thiophosphates, phosphoramidates and phosphinates, which originate quite different physicochemical and toxicological properties (Ballantyne and Marrs 1992).

They are usually liquid and nonpolar compounds, which means that most of them are poorly soluble in water and tend to dissolve in fat (Taylor 2006). Many of these compounds evaporate at room temperature. However, their stability depends on the pH of the medium where they are, and they will decompose under strongly alkaline pH values or in the presence of humidity. These properties have an influence on the importance of the routes of entry of these compounds, as well as the conditions that can best destroy them (Saunders and Harper 1994; De la Iglesia Huerta and Cobos 2000).

3. Mechanisms of action

Organophosphorus pesticides affect the central nervous system as they are potent and irreversible inhibitors of cholinesterase activity (in whole blood), inhibit the activity of pseudocholinesterase (in serum) and inhibit the activity of acetylcholinesterase (in red blood cells), and this is known as the cholinergic syndrome (Schenker et al. 1992; Cochran et al. 1995; Ecobichon 2001; Musshoff et al. 2002; Aprea et al. 2002; Sogorb et al. 2004; Inoue et al. 2007; Sullivan and Blose 2008; Park et al. 2009).

The toxicity of these pesticides is determined by this same inhibitory potency, by physicochemical properties, chemical and biological stability and through added additives. These compounds are commercialized as complex formulations, and may therefore contain various organic solvents and emulsifiers which may in turn increase their toxicity. Intoxications can result in a broad spectrum of clinical signs depending on the intrinsic toxicity, the administered dose and the route of exposure (Okumura et al. 1996; Sidell 2008; Eddleston et al. 2012; Peter et al. 2014).

Acetylcholinesterase is an enzyme responsible for the hydrolysis of acetylcholine after its release, and allows the propagation of the action potential as a result. In a normal process, the enzyme hydrolyzes acetylcholine in two steps (Vilanova and Sogorb 1999; Sogorb and Vilanova 2002). Acetylcholine begins by binding to the active site of the enzyme, releasing the choline fraction and forming an acetylated version of it. Then, a water molecule attacks the enzyme releasing acetic acid plus the active enzyme, which is again available for a new catalytic cycle. In the case of organophosphorus pesticide poisoning, acetylcholinesterase is inhibited through a similar mechanism (Vilanova and Sogorb 1999; Sogorb and Vilanova 2002). The pesticide begins by phosphorylating the same active center that also binds to acetylcholine, but in this situation the phosphorus fraction is not released from the protein by hydrolysis (Vilanova and Sogorb 1999; Sogorb and Vilanova 2002) and the phosphorylated enzyme is not capable of hydrolyzing its natural substract, losing its normal function in the catabolism of neurotransmitters (Barr and Needham 2002). Acetylcholinesterase remains inhibited until a new enzyme is generated, or until an enzyme reactivator (oxime) is directed (Barr and Needham 2002; Sogorb et al. 2004). Briefly, the main mechanism of toxic action is the covalent binding to the active site serine OH-group at the base of a deep gorge of the pivotal enzyme acetylcholinesterase. Inhibition of the physiological action of acetylcholinesterase to hydrolyze the neurotransmitter acetylcholine is due to its phosphylation, which includes both phosphorylation and phosphonylation (Aldridge and Reiner 1974).

With this, the altered hydrolysis of acetylcholine leads to its accumulation in the synaptic cleft, with consequent overstimulation of muscarinic and nicotinic receptors at the nerve-nerve and nerve-organs of the cholinergic system (Holmstedt 1959; Ecobichon 2001; Kwong 2002; Sullivan and Blose 2008). This results in an eventual paralysis of nerves or muscles, which can produce neurotoxicity and result in death (Eaton et al. 2008; Eddleston et al. 2008; Kumar et al. 2009; Yu-shan et al. 2013).

Moreover, it is known that this inhibition also involves, in addition to the central and vegetative nervous system, the neuromuscular junctions with production of acute toxicity with high levels of acetylcholine acting on the above-mentioned receptors (Ecobichon 2001; Kwong 2002; Sullivan and Blose 2008).

In addition to acetylcholinesterase, these compounds also exert a potent inhibitory effect over other serine esterases, namely butyrylcholinesterase and carboxylesterase (Maxwell 1992; Moralev and Rozengart 2007; Kumar et al. 2009; Masson et al. 2009). However, this inhibition does not result in additional acute toxic effects, but these enzymes can serve as endogenous source receptors for detoxification of a limited amount of incorporated compound (Maxwell et al. 1987; Lenz et al. 2007).

Consequently, the determination of the activity of the mentioned enzymes can be used in the biological monitoring of the exposure to those compounds that act on their inhibition (Aprea et al. 2002; Sogorb et al. 2004).

4. Toxicokinetics

Exposure to organophosphorus pesticides can occur through several routes, and this is important in the compound's rate of absorption into the systemic circulation. Thus, knowing the physicochemical properties of the compounds is essential for their toxicological evaluation (Benschop and de Jong 2001).

The most common routes are inhalation, dermal contact and ingestion (Ecobichon 2001; Sullivan and Blose 2008; Nshimiyimana et al. 2014), with ocular exposure being a less common route of entry for these toxic compounds (McKeag et al. 2002; Bradberry et al. 2004).

After exposure to these compounds by vapor inhalation or intravenous administration, signs of intoxication (miosis, for instance) may appear early within a few minutes or hours, resulting in a rapid increase in their concentration in circulation resulting in acute toxicity (Ecobichon 2001; Kwong 2002; Sullivan and Blose 2008; Park et al. 2009). However, if poisoning occurs by percutaneous exposure to vapor or liquid, the signs may take hours to appear, and this results in a latency time between exposure and the onset of clinical symptoms, such as local sweating and fasciculations. There is also a delay in hours until they can be detected and quantified in blood. In the case of oral ingestion of these pesticides, gastrointestinal symptoms appear readily, and the risk is much more dominant, followed by the risk of contamination by inhalation (Feldmann and Maibach 1974; Sidell 1974; Wester et al. 1983; Furst et al. 1994; Rengstorff 1994; Okumura et al. 1996; Van Wyk et al. 2001; Van Der Schans et al. 2003; Lee 2003; Hamilton et al. 2004; Thiermann et al. 2007; Goel and Aggarwal 2007; Mumford et al. 2008; Reiter et al. 2008). Systemic exposure through inhalation, dermal contact or ingestion may also lead to symptoms such as lack of vision and burning sensation (Ecobichon 2001; Sullivan and Blose 2008). In addition to the acute toxicity associated to this exposure, in the long term

consequences of chronic toxicity may occur, such as infertility and cancer (Alavanja et al. 2004; Multigner 2005; Rezg et al. 2007; Masri et al. 2011).

These properties are an important factor to take into account for the development of appropriate methods for decontamination and drug treatment (Mikler et al. 2011).

Organophosphorus pesticides easily cross alveolar and dermal membranes due to their lipophilic structures (World Health Organization 1986; Leikin et al. 2002), and as they are commonly applied as aerosols, inhalation and dermal exposures are the most common in accidental intoxications (Ballesteros and Torrecilla 2000; Leikin et al. 2002). However, the gastric mucosa also presents permeability to these compounds, being the classic choice in case of attempted suicide (World Health Organization 1986; Ballesteros and Torrecilla 2000).

Due to their chemical nature, these compounds have biological half-lives in the order of hours to a few days (Barr et al. 1999), and do not circulate in the bloodstream for longer time periods. These pesticides are distributed throughout the body, especially in adipose tissues (Nshimiyimana et al. 2014), but generally do not accumulate due to their rapid biodegradation, being usually metabolized and excreted from the body within a few days (Barr and Needham 2002).

After the distribution phase, the metabolism phase takes place. Some of these pesticides can be eliminated without metabolism. However, as noted earlier, in most cases rapid metabolism occurs, converting them to specific and non-specific metabolites (World Health Organization 1986; Salm et al. 2009). Most organophosphates are activated by oxidation in the liver, and the enzymes involved in this process are the cytochrome P450 system and flavin-containing monooxigenases. The enzymatic systems involved in detoxification are phosphotriesterases (PTEs), carboxylesterases and glutathione-S transferases (Jokanovic 2001). A major detoxification pathway is the hydrolysis by a type of esterases called PTEs, where the reaction products do not exhibit phosphorylation capability and each enzyme molecule is capable of deactivating many pesticides molecules (Vilanova and Sogorb 1999; Sogorb and Vilanova 2002). Nevertheless, these insecticides are generally metabolized into the more reactive form of oxon, which can bind to cholinesterases or be hydrolyzed into a dialkylphosphate (DAP) and a hydroxylated organic fraction specific of the pesticide. Alternatively, the intact pesticide may undergo hydrolysis prior to any conversion to the oxon form and the polar metabolites are excreted (Barr and Needham 2002).

After metabolism, the metabolites may be excreted in feces and expired air, although in low amounts; the main route of elimination is urine, specifically for the more polar metabolites (World Health Organization 1986; Barr and Needham 2002). As an indication of exposure to these compounds, non-specific DAP metabolites may be measured in urine (Bravo et al. 2002, 2004; Hernández et al. 2002b; Castorina et al. 2003; Wessels et al. 2003; Barr et al. 2004; Eskenazi et al. 2004), and as this conversion does not originate specific metabolites the absorbed pesticide cannot be identified (Salm et al. 2009). Most of these compounds are excreted within 48 hours as the parent pesticide, a mercapturate detoxification product, and as free or conjugated metabolites (glucuronides, sulfates) (Nolan et al. 1984; Van Welie et al.

1991; Vasilić et al. 1992; Drevenkar et al. 1993; Kawabata et al. 1994; Wester et al. 1994; Driskell and Hill 1997; Griffin et al. 1999).

5. Case Reports

Because of their widespread use in agriculture, organophosphate pesticides represent a health problem worldwide, and are involved in suicides, accidental self-poisonings and even homicides (Poklis et al. 1980; Miyazaki et al. 1988; Brunetto et al. 1992; Thompson et al. 1998; Vasilić et al. 1999; Wang et al. 2000; Pavlic et al. 2002; Tarbah et al. 2004, 2007; Kamha et al. 2005; Wu and Deng 2009; Ozdemir et al. 2009; Moffatt et al. 2010; Gul et al. 2012; Dulaurent et al. 2013; Chowdhary et al. 2014; Steinritz et al. 2016; Dhakne et al. 2016; Hamad et al. 2016). In fact, a recent study reports that the annual incidence of poisonings among agricultural workers varies from 3-10% per country (Freire and Koifman 2013). However, and despite these data, there is little published case reports in the literature. The following lines describe all case report cases found in the PubMed database using the following search strings (either alone or in combination): “organophosphorus pesticides” or “organophosphorus insecticides” and the different types of biological specimens.

An example of this is the case of a 79-year-old man found dead at home with a belt tied around his neck. A strong "chemical" odor was also detected. According to statements obtained, the deceased was being treated for prostate disease and depression, having already tried suicide twice. The autopsy findings included features such as edematous and emphysematous lungs, bloodlike fluid from the parenchymal cut, presence of yellowish white mucus in the bronchial tubes and trachea, congested liver, clear liquid with strong solvent odor, as well as walls with signs of erosive gastritis in the stomach and in the esophageal mucosa. Samples of blood and gastric contents were submitted for toxicological analyses. Negative results for drug and alcohol abuse were obtained. The samples were analyzed by gas chromatography-tandem mass spectrometry (GC-MS/MS) and diazinon was detected in blood at a concentration of 6.48 μg/mL (Luzardo et al. 2015).

In another case, a 43-year-old man attempted suicide by ingesting 100 mL of 5% fenitrothion and acephate emulsion. The patient was transferred to an emergency department, and blood samples were collected in dried heparin, treated and stored. The next day, the samples were prepared and analyzed by liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS). The determined serum concentrations of fenitrothion and acephate were 4.5 and 7.2 μg/mL, respectively (Inoue et al. 2007).

An 80-year-old man was found dead in bed, with no evidence of a struggle. A forensic autopsy was performed to elucidate the cause of death, where moderate rigidity was observed in all joints, chemical lesions on the right side of the face and upper portion of the body and congestive organs. The lungs were markedly edematous, the stomach contained a dark gray fluid that smelled strongly of organic solvents and the duodenum and intestine also contained

a white milky liquid with the same odor. No drugs or ethanol were detected in urine and blood samples and the concentrations of methidathion in cardiac and peripheral blood were 66.2 and 8.33 μg/g, respectively. In the 30 g of stomach contents the concentration was 64 mg/g and, in addition, the upper portion of the small intestine had the concentration of 38 mg/g. The samples were analyzed by GC-MS (Takayasu et al. 2012).

Another paragon is that of a 24-year-old woman found dead in bed, with a strong smell of solvents or pesticides and feces. There was a bottle whose label said it contained 200 mg/mL of chlorfenvinphos. Furthermore, it was said by the mother that it would be for the treatment of dog ticks. A suicide note was found, and the deceased had a history of depression, drug abuse and suicide attempts. At the autopsy, congested and edematous lungs were observed. Samples of cardiac blood, liver and stomach contents collected had a greenish-smelling color with strong solvent odor and were analyzed by gas chromatography-flame ionization detector (GC-FID). Chlorfenvinphos was detected at concentrations of 8.6 mg/L in cardiac blood, 60.0 mg/kg in liver and 1.132 mg/L in stomach contents (Martínez and Ballesteros 2012).

An additional case is a 54-year-old man found dead on a football pitch. A bottle with a brownish fluid was found near the victim whose label mentioned “Denkavepon M50” and that it contained 47.5% of dichlorvos. The autopsy revealed diffuse congestion of the internal organs and a hemorrhagic ulcer of the digestive tract. In the stomach were present 150 mL of a volatile liquid. Samples of blood (cardiac and peripheral), urine, gastric contents, heart, lung, kidney and liver were collected and analyzed by GC/MS and liquid chromatography-photodiode array detector (HPLC-PDA). The concentration of dichlorvos in cardiac blood was 4.4 mg/L and that of peripheral blood was 1.3 mg/L. The pesticide was detected in heart tissue at 1400 mg/kg and the kidney and lung concentrations were 1 and 2.1 mg/kg, respectively. In the urine the concentration was 1.3 mg/L and in the stomach it was 253 mg/mL, corresponding to 38 g dichlorvos (Abe et al. 2008).

Finally, the case of a 21-year-old woman, known to be a drug user, that was found dead at home. They found an empty syringe and a bottle of parathion-methyl. The blood sample was tested for the presence of alcohol, and also for cannabinoids, opiates, cocaine, benzodiazepines, barbiturates and amphetamines by fluorescence polarization immunoassay. No evidence was found for the presence of drugs, and the blood alcohol concentration was 1.05 g/L. Organophosphorus pesticides were identified in blood, but in the liver, kidney and stomach contents they were not detected. Parathion-methyl was determined in blood at a concentration of 24 mg/mL using gas chromatography-nitrogen phosphorous detector (GC-NPD). According to the toxicological analysis, due to the absence of stomach contents, it was concluded that the cause of death was acute poisoning by intravenous injection of this organophosphate (Tsoukali et al. 2004).

6. Bioanalytical procedures for organophosphorus pesticides detection in biological specimens

In the field of clinical and forensic toxicology it is essential to perform confirmatory tests for the diagnosis of acute or chronical poisoning situations. The choice of a type of analysis depends on the complexity of the biological specimens used for the detection and/or the determination of the toxic agents involved. The most commonly analyzed specimens are blood, plasma, serum and urine. However, it is also possible to use less conventional matrices such as oral fluid, hair, sweat, tissues, vitreous humor, and bile, among others. These alternative specimens are usually characterized by their complexity, and the toxic substances are usually present at low concentrations; in addition, in some cases, the amount of sample available for analysis is relatively small. Furthermore, *postmortem* specimens can endow some difficulties compared to those obtained in clinical scenarios, namely those resulting from autolytic/putrefactive changes. The use of highly sensitive techniques is therefore necessary. Typically, the detection of toxic compounds in clinical and forensic settings begins with a screening test followed by confirmatory analyses. For this purpose, immunoassays are usually the first approach (Rosado et al. 2018).

In the literature there are also cases of application of immunoassay tests to this class of compounds. An example is the work by Zhang et al. (Zhang et al. 2013), in which a portable immunochromatographic strip-based biosensor was developed for the detection of trichloropyridinol (specific biomarker of exposure to chlorpyrifos) in saliva samples, which was successful in the direct analysis of complicated authentic samples. This immunosensor is based on gold nanoparticles, which are augmented in situations of reduced levels of analytes. These nanoparticles, when captured, can be observed without any equipment and quantified by a colorimetric reader. This biosensor exhibits a linear range and detection limit of 0.625-20 and 0.47 ng/mL, respectively.

Eskandari and Naderi-Darehshori (Eskandari and Naderi-Darehshori 2012) syntetized nanoparticles of a hydrophobic magnetic polymer (poly (styrene-divinylbenzene) and studied their adsorption potential in order to determine trace levels of fenitrothion in both biological and environmental samples. The magnetite nanoparticles are added to the sample solution as a way of preparing a strong local magnetic field, which leads to a faster and more efficient precipitation of the nanoparticles from the extraction mixture. The method has a spectrophotometric determination at 571 nm and has been successfully applied to various samples including plasma and urine. Beer's law was observed in a range of 2-230 ng/mL of compound, with relative standard deviations of 0.9-5.1% and recoveries in the range of 97.2-100.0%.

Lu et al. (Lu et al. 2006) have carried out a study in which the occupational exposure to dizainon bt workers from Nicaraguan plantations, as well as their children, was evaluated, with repeated sample collection over several days and biomonitoring using saliva samples. An enzyme-linked immunosorbant assay (ELISA) was used and a significant correlation was found between the

concentrations of the compound in saliva and plasma samples collected at the same time. Regarding children, this compound was not detected in most of the saliva samples, which was confirmed in urine samples. Consequently, it has been shown that saliva may be used to evaluate human exposure to this compound.

Furthermore, the work of Curwin et al. (Curwin et al. 2010), where a comparison of an ELISA immunoassay and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analytical methods for measuring chlorpyrifos metabolite (TCP) in urine samples from Iowa farmers and non-farmers was presented. For this comparison, different statistical methods were used, and the analytical methods were moderately correlated (0.40-0.49), but the immunoassay method consistently presented significantly higher geometric mean (GM) estimates. This estimate of GM for TCP by immunoassay and LC-MS/MS varied between 14-14 and 2.9-3.0 μg/L, respectively. The limits of detection (LOD) values for this metabolite were 0.50 and 3.32 μg/L by LC-MS/MS and ELISA, respectively.

Another approach to perform a screening in order to detct pesticides is the use of optical sensors. Yan et al. (Yan et al. 2018) carried out a recent review concerning this matter. In this review, new trends in high sensitivity optical sensors and their advantages have been compiled for the detection of pesticides, in particular the class of organophosphorus insecticides, which continue to be a concern because of their residues. Recent developments in optical sensors highlighted fluorescence detection (FL) strategies such as enzyme-mediated methods, antibody-assisted methods, molecularly-imprinted polymers (MIPs)-based methods, aptamer-based and host-guest interaction-based sensors. Another category of sensors is shown by colorimetric (CL) sensing strategy based on enzymes, antibodies and aptamers. Surface enhanced Raman scattering strategies (SERS) focused on sensors based on gold, silver and bimetallic nanomaterials. The last group comprehends surface plasmon resonance sensor (SPR) and chemiluminescence strategy. These sensors bring advantages, compared to traditional methods of analysis, with respect to the sampling approach which becomes easier, faster and less expensive, while maintaining sensitivity of detection. These optical sensors show good performance to quantify pesticide residues in complex environments and food matrices, especially for simplification and visualization design. Since the devices are miniaturized and wireless networking is used, pesticide recognition can be transformed into a digital signal measurable by portable devices, which makes detection feasible outside the laboratory environment with minimal user involvement, representing a new generation of analytical devices for real-time detection. However, the use of this optical sensor has scarce application in human biological specimens.

All positive results require therefore confirmation by a different method, which must be at least as sensitive as the screening test, allowing results with higher levels of confidence. The most common confirmation methods involve LC or GC chromatography coupled to different detectors, but since these compounds are volatile and of non-polar nature, GC is considered the most used instrumentation for analysis.

For the detection of this class of pesticides in biological samples, the existing literature has been compiled in the following table (Table 1). Due to the fact that in most of the articles more than one biological matrices are used, it was not possible to separate this information in different tables as a function of the biological specimen. In this table, and taking into account that there is no published review on the matter, analytical details of published methods for the determination of organophosphorus insecticides are reported in order to aid toxicologists, particularly in the fields of both forensic and clinical toxicology. Literature search was performed using the PubMed database, and the search strings were "organophosphorus pesticides" or "organophosphorus insecticides" in the different types of human biological specimens. The most commonly used samples are blood, urine, plasma and serum, and papers since 2000 have been selected. For the remaining samples, considered as alternative, also papers from previous years were selected. All articles were screened independently by three of the authors to determine their relevance in the framework of the current review and only papers selected by at least by two of them were included.

Among the compilation made, it should be emphasized the articles in which more compounds were analyzed at the same time, such as the work of Kumari et al. (Kumari et al. 2015), Lacassie et al. (Lacassie et al. 2001), Musshoff et al. (Musshoff et al. 2002), Luzardo et al. (Luzardo et al. 2015), Kudo et al. (Kudo et al. 2012), Tarbah et al. (Tarbah et al. 2001), Russo et al. (Russo et al. 2002), Roca et al. (Roca et al. 2014) and Duca et al. (Duca et al. 2014). In these works, analytical methods have been developed for the identification and/or quantification of a large number of organophosphorus compounds, and some of their metabolites as well. According to the consulted literature, the most used sample preparation techniques are liquid-liquid extraction (LLE) and solid phase extraction (SPE). In the former it is usual the use of organic solvent mixtures, such as dichloromethane, ethyl acetate, acetone and hexane. In the case of the SPE, the most commonly cartridges used are reversed phase C8 or C18, hydrophilic-lipophilic balance and in some cases aminopropyl and anion exchange cartridges. However, these two sample preparation procedures use considerable volumes of organic solvents. For this reason, there is a growing trend to use 'greener' extraction procedures, namely fully automatic and/or miniaturized techniques, which provide new operational paradigms. In this review paper, the works in which microextraction techniques were used for sample preparation should also be emphasized. This criterion was used taking into account the advantages of these techniques, particularly low volume of sample and organic solvents, minimization of waste of solvents and consequent environmental advantages and finally the possibility of reusing the extraction device (in some techniques of them). In addition, changes to classical techniques such as SPE, incorporation of automation process, use of MIPs or QuEChERS were also described. Examples of the use of this miniaturized techniques in the determination of organophosphorus pesticides is the published article by Kumari et al. (Kumari et al. 2015). These authors used a technique of fast agitated directly suspended droplet microextraction (FA-DSDME) for the determination of a large number of compounds as mentioned above from a small sample volume of blood. Hernández et al. (Hernández et al. 2002a) and Musshoff et al. (Musshoff et al. 2002)

which, also for blood samples, used the headspace-solid phase microextraction (HS-SPME) technique with difference only in the fiber used. Tsoukali et al. in two different works [134, 142] have used the same technique (HS-SPME), but with the use of different and unusual samples as blood, plasma, kidney, liver, cerebrospinal fluid and stomach contents, using the same fiber and the same instrumentation (GC-NPD). Ebrahimi et al. (Ebrahimi et al. 2012) prepared hair samples by hollow fiber solid phase microextraction (HF-SPME) and analyzed them by HPLC-PDA. Santos et al. (Santos et al. 2018) is the only published paper where the microextraction by packed sorbent (MEPS) approach was used for treating whole blood samples, having the advantage of using a low sample volume. López et al. (López et al. 2001), developed an analytical procedure for serum and urine samples using solid-phase microextraction (SPME) with a polydimethylsiloxane (PDMS) fiber. Yet, Gallardo et al. [146, 147, 148] used the same technique, however using carbowax™/divinylbenzene (CW/DVB) fibers for the preparation of blood and urine samples, using smaller sample volumes. In turn, Yang and Xie (Yang and Xie 2004) used the solid-phase micro-extraction membrane (SPMEM) preparation technique for urine samples for the determination of dichlorvos. Recently, Santos et al (Santos et al. 2018) published an article using a miniaturized version of SPE, MEPS. These authors were able to reach limits of detection in the order of 500 ng/mL for diazinon, chlorpyrifos, chlorfenvinfos, parathion-ethyl and quinalphos using volumes of whole blood as low as 0.1 mL.

Worth noting is the use of a mini QuEChERS method by Srivastava et al. (Srivastava et al. 2017) and regular QuEChERS by Usui et al. (Usui et al. 2012) and Roca et al. (Roca et al. 2014). This technique of sample preparation shows advantages in the versatility of the used sample, in these cases plasma, blood and urine, as well as versatility in the type of equipment used for the analysis, which in the described cases was GC-MS/MS, LC-MS and ultra-high performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS), respectively. It is important to mention that this QuEChERS technique ("Quick, Easy, Cheap, Effective, Rugged, and Safe") has been accepted by many pesticide residue analysts, namely in the agricultural field. However, some modifications to the original QuEChERS method had to be introduced to ensure efficient extraction of pH-dependent compounds, to minimize degradation of some compounds which are susceptible to extraction conditions using acids or bases, and to expand the spectrum of matrices and applications covered (Rosado et al. 2018).

Liao et al. (Liao et al. 2011) used cord blood, which is an uncommon sample, with an online SPE system.

Odetokun et al. (Odetokun et al. 2010), Davis et al. (Davis et al. 2013) and Cequier et al. (Cequier et al. 2016) have dedicated some work to the analysis of metabolites of organophosphorus pesticides, using urine as a biological sample and 96-well plate SPE as preparation technique, differing only in the type of cartridges. The latter used an ultra-performance liquid chromatography-mass spectrometry-quadrupole time of flight (UPLC-MS-QTOF) instrument.

Saito et al. [156, 157] used monolithic extraction for serum and urine preparation in the analysis of four organophosphorus compounds which were afterwards determined by GC-MS.

Jia et al. (Jia et al. 2008) used an innovative technique of urine cloud-point extraction coupled to a microwave-assisted back-extraction for the determination of some of these compounds, however with the disadvantage of the need to use a large sample volume. Recently, Aladaghlo et al. (Aladaghlo et al. 2016) used solvent-assisted dispersive solid-phase extraction (SA-DSPE) technique for the determination of diazinon, however in large volumes of urine. Pelit and Yengin (Pelit and Yengin 2014) developed a method for the determination of chlorpyrifos and one of its metabolites with a solidified floating organic drop microextraction (SFODME) sample preparation technique, but also needing considerable amounts of sample to accomplish the analysis.

Santos et al. (Santos et al. 2012) opted for a different preparation technique, molecularly imprinted solid-phase extraction (MISPE), for the determination of two metabolites of organophosphorus compounds in urine, with the disadvantage of the need for derivatization.

Russo et al. (Russo et al. 2002) have used a gel permeation chromatography for extraction of kidney and liver samples, obtaining good analytical results.

Zhang et al. (Zhang et al. 2014) developed a technique of active magnetic metal-organic framework hybrid material and a magnetic SPE procedure for the preparation of hair samples and urine.

Regarding the used equipment, Pérez et al. (Pérez et al. 2010) and Barr et al. (Barr et al. 2002) are highlighted by the use of a gas chromatography-high-resolution mass spectrometry (GC-HRMS). Hernández et al. (Hernández et al. 2004) and Sancho et al. (Sancho et al. 2000) have used a LC-LC-MS/MS and Oya et al. (Oya et al. 2017) used an UPLC-MS/MS.

It should be emphasized that from a general perspective hair samples require a prior extraction from the hair matrix, as well as meconium, for which a methanolic pre-extraction is often used. Also, in general GC equipment can be pointed out as the most used in the analysis of this class of compounds. A final important note is that for the analysis of the DAPs metabolites, it is always necessary to derivatize the extracts prior to analysis by GC, and the most used derivatizing agent is pentafluorobenzyl bromide (PFBBr).

Table 1. Bioanalytical procedures for determination of organophosphorus pesticides in biological samples.

Compounds	Sample volume (mL or mg); matrix	Sample preparation	Detection mode	LOD (ng/mL); LOQ (ng/mL)	Recovery (%)	Reference
Chlorpyrifos Chlorpyrifos-methyl Dichlorvos Dimethoate Fonofos Ethion Malathion Methidathion Monocrotophos Paraoxon-methyl Phorate Phorate Sulfone Phorate Sulfoxide Phosalone Pirimiphos-ethyl Pirimiphos-methyl Quinalphos Triazophos	0.1; blood	FA-DSDME	LC-MS/MS (ESI)	0.0009; 0.01 (Chlorpyrifos) 0.015; 0.01 (Chlorpyrifos-methyl) 0.002; 0.01 (Dichlorvos, Phorate Sulfoxide) 0.004; 0.01 (Dimethoate) 0.038; 0.01 (Fonofos) 0.023; 0.01 (Ethion) 0.043; 0.01 (Malathion) 0.034; 0.01 (Methidathion) 0.029; 0.01 (Monocrotophos) 0.031; 0.01 (Paraoxon-methyl) 0.041; 0.01 (Phorate, Pirimiphos-ethyl) 0.013; 0.01 (Phorate Sulfone) 0.009; 0.01 (Phosalone) 0.110; 0.01 (Pirimiphos-methyl) 0.007; 0.01 (Quinalphos) 0.122; 0.01 (Triazophos)	102-109 (Chlorpyrifos) 96-104 (Chlorpyrifos-methyl) 98-102 (Dichlorvos) 99-104 (Dimethoate) 88-95 (Fonofos) 96-102 (Ethion) 86-95 (Malathion) 96-103 (Methidathion) 93-96 (Monocrotophos) 88-96 (Paraoxon-methyl) 92-102 (Phorate) 97-106 (Phorate Sulfone) 101-107 (Phorate Sulfoxide) 95-104 (Phosalone) 94-104 (Pirimiphos-ethyl) 86-93 (Pirimiphos-methyl) 96-106 (Quinalphos) 86-96 (Triazophos)	(Kumari et al. 2015)
Dimethoate Fenthion Diazinon Chlorpyrifos	0.1; blood	LLE (zinc sulphate: methanol; 1:5)	LC-MS/MS (ion trap)	0.5; 0.5 for all compounds	97-100 (Dimethoate, Chlorpyrifos) 95-99 (Fenthion) 97-99 (Diazinon)	(Salm et al. 2009)
Chlorpyrifos Diazinon Malathion Parathion	1; blood	SPE (Oasis HLB™)	GC-MS (EI)	80; 150 (Chlorpyrifos) 40; 150 (Diazinon) 90; 170 (Malathion) 90; 130 (Parathion)	83.6-93.8 (Chlorpyrifos) 75.0-87.0 (Diazinon) 75.3-81.3 (Malathion) 71.4-80.4 (Parathion)	(Park et al. 2009)
Fonofos Chlorpyrifos Ethion	0.5; blood	HS-SPME (PA, 85µm)	GC-MS/MS (EI; ion trap)	0.4; 1 (Fonofos) 0.2; 1 (Chlorpyrifos) 0.7; 1 (Ethion)	-	(Hernández et al. 2002a)
Vamidothion Dimethoate Ethoprophos Cadusaphos Mevinphos	2; blood and serum	Sample precipitation with acetonitrile and SPE (Oasis HLB)	GC-MS (EI)	25; 50 (Ethion, Fenithrothion, Parathion-ethyl, Parathion-methyl, Phosalone) 5; 10 for the other compounds	45.0-66.0 (Vamidothion); 72.3-92.9 (Dimethoate); 61.5-92.7 (Ethoprophos); 58.5-80.8 (Cadusaphos); 57.1-95.9 (Mevinphos); 41.2-77.5 (Phorate);	(Lacassie et al. 2001)

Phorate Terbuphos Fonophos Chlorpyriphos-methyl Chlorpyriphos-ethyl Fenithrothion Bromophos-methyl Isophenphos Malathion Parathion-methyl Fenthion Methidathion Parathion-ethyl Pirimiphos-methyl Pirimiphos-ethyl Quinalphos Phenamiphos Phosalone Ethion Phosmet Pyrazophos Azinphos-methyl Azinphos-ethyl Coumaphos		for both samples			42.9-53.6 (Terbuphos); 51.9-73.3 (Fonophos); 56.5-92.0 (Chlorpyriphos-methyl); 45.0-64.3 (Chlorpyriphos-ethyl); 62.3-70.8 (Fenithrothion); 45.3-64.2 (Bromophos-methyl); 56.6-71.3 (Isophenphos); 64.6-90.3 (Malathion); 68.4-77.8 (Parathion-methyl); 49.3-53.9 (Fenthion); 74.5-93.7 (Methidathion); 53.8-62.9 (Parathion-ethyl); 46.6-59.9 (Pirimiphos-methyl); 46.6-50.7 (Pirimiphos-ethyl); 56.5-68.3 (Quinalphos); 56.3-87.4 (Phenamiphos); 52.7-98.1 (Phosalone); 39.5-57.5 (Ethion); 73.3-98.9 (Phosmet); 60.7-92.1 (Pyrazophos); 76.8-96.6 (Azinphos-methyl); 73.1-101.8 (Azinphos-ethyl); 51.1-107.9 (Coumaphos) for blood samples; 81.01-91.4 (Vamidothion); 88.2-90.0 (Dimethoate); 78.6-98.0 (Ethoprophos); 66.9-95.7 (Cadusaphos); 55.5-88.2 (Mevinphos); 61.3-92.2 (Phorate); 74.7-94.5 (Terbuphos); 78.7-97.6 (Fonophos); 84.6-90.6 (Chlorpyriphos-methyl); 95.3-95.8 (Chlorpyriphos-ethyl); 85.5-95.4 (Fenithrothion); 86.2-95.1 (Bromophos-methyl); 92.1-96.6 (Isophenphos); 88.5-98.8 (Malathion); 87.3-95.4 (Parathion-methyl); 88.5-90.7 (Fenthion); 91.9-97.9 (Methidathion); 91.4-98.5 (Parathion-ethyl); 91.8-94.6 (Pirimiphos-methyl); 89.9-95.5	

					(Pirimiphos-ethyl); 95.4-98.6 (Quinalphos); 85.8-95.4 (Phenamiphos); 92.6-97.7 (Phosalone); 88.8-97.9 (Ethion) 94.0-97.8 (Phosmet); 89.7-99.5 (Pyrazophos); 82.7-92.1 (Azinphos-methyl); 93.6-96.5 (Azinphos-ethyl); 87.0-96.8 (Coumaphos) for serum samples	
Omethoate Dimethoate Diazinon Chlorpyrifos Parathion-ethyl Chlorfenvinphos Quinalphos Azinphos-ethyl	0.5; blood	SPE (Oasis® HLB)	GC-MS (EI)	50; 100 (Omethoate) 25; 50 for the other compounds	31.3-67.3 (Omethoate) 87.6-108.4 (Dimethoate) 69.3-71.6 (Diazinon) 45.0-61.4 (Chlorpyrifos) 49.1-60.0 (Parathion-ethyl) 58.1-80.6 (Chlorfenvinphos) 63.4-68.4 (Quinalphos) 86.2-103.6 (Azinphos-ethyl)	(Raposo et al. 2010)
Chlorpyrifos	0.5; blood	LLE (acetonitrile: hexane; 1:8)	GC-MS (EI)	2; 10	89.3-93.8	(Dai et al. 2017)
Bromophos-ethyl, Bromophos-methyl, Chlorfenvinphos, Chlorpyriphos, Demethon-S-methylsulfon, Diazinon, Dichlorvos, Dicrotophos, Dimethoate, Disulfoton, Edifenphos, Fenitrothion, Fenthion, Malathion, Methidathion, Mevinphos, Monocrotophos, Omethoate, Parathion-ethyl, Parathion-methyl, Phosphamidon, Quinalphos	0.5; blood	HS-SPME (PDMS, 100 µm)	GC-MS (EI)	0.04; 0.025 (Bromophos-ethyl) 0.05: 0.025 (Bromophos-methyl, Chlorfenvinphos, Malathion) 0.03; 0.025 (Chlorpyriphos, Parathion-methyl) 0.01; 0.025 (Diazinon, Disulfoton, Fenthion, Quinalphos) 0.10; 0.025 (Dichlorvos, Mevinphos) 0.30; 0.025 (Edifenphos) 0.20; 0.025 (Methidathion) (in µg/g) No data for the remaining compounds	8.2 (Bromophos-ethyl) 9.7 (Bromophos-methyl) 1.7 (Chlorfenvinphos) 8.3 (Chlorpyriphos) 2.9 (Diazinon) 0.1 (Dichlorvos, Mevinphos) 3.8 (Disulfoton) 10.6 (Edifenphos) 2.7 (Fenthion) 6.6 (Malathion) 1.3 (Methidathion, Quinalphos) 4.7 (Parathion-ethyl) 19.6 (Parathion-methyl) No data for the remaining compounds	(Musshoff et al. 2002)
Metamidofos, Phoxim, Acephate, Omethoate, Dimethoate, Profenofos, Dimefox, Dichlorphos, Mevinphos, Chlormephos,	2; blood	LLE (dichloromethane:	GC-MS/MS (EI) and LC-MS/MS (ESI)	-; 10 (Dimefox, Dichlorphos, Mevinphos, Heptenophos, TEPP, Etoprophos, Sulfotep, Dicrotophos, Cadusafos, Phorate, Monocrotophos, Thiometon,	91 (Dimefox, Phosphamidon, Phenthoate, Ethion, Azinphos-methyl, Azinphos-ethyl) 90 (Dichlorphos, Fenitrothion, Quinalphos)	(Luzardo et al. 2015)

Heptenophos, Thionazin, TEPP, Etoprophos, Sulfotep, Dicrotophos, Cadusafos, Phorate, Monocrotophos, Thiometon, Dioxathion, Propetamphos, Terbufos, Chlorfenvinfos, Cyanofos, Fonofos, Diazinon, Disulfoton, Isazophos, Formothion, Phosphamidon, Chlorpyrifos-methyl, Parathion-methyl, Fenitrothion, Pirimifos-methyl, Malathion, Chlorpyrifos, Fenthion, Parathion-ethyl, Trichloronat, Pirimifos-ethyl, Isofenphos, Phenthoate, Quinalphos, Mephospholan, Bromophos-ethyl, Methidathion, Propafos, Tetrachlorvinphos, Fenamiphos, Isoxathion, Fensulfothion, Ethion, Chlorthiophos, Sulprofos, Triazofos, Famphur, Carbophenothion, Ediphenphos, Phosmet, EPN, Leptophos, Phosalone, Azinphos-methyl, Pyrazophos, Azinphos-ethyl		ethyl acetate: acetone; 50:30:20)		Dioxathion, Propetamphos, Terbufos, Chlorfenvinfos, Cyanofos, Fonofos, Diazinon, Disulfoton, Isazophos, Formothion, Phosphamidon, Chlorpyrifos-methyl, Parathion-methyl, Fenitrothion, Pirimifos-methyl, Malathion, Chlorpyrifos, Fenthion, Parathion-ethyl, Pirimifos-ethyl, Isofenphos, Bromophos-ethyl, Methidathion, Propafos, Tetrachlorvinphos, Fenamiphos, Isoxathion, Fensulfothion, Ethion, Chlorthiophos, Sulprofos, Triazofos, Famphur, Carbophenothion, Ediphenphos, Phosmet, Leptophos, Azinphos-methyl, Pyrazophos, Azinphos-ethyl) -; 20 (Chlormephos, Phosalone) -; 50 (Thionazin, Phenthoate) -; 40 (Trichloronat) -; 30 (Quinalphos, Mephospholan) -; 5 (EPN) for GC-MS/MS method, -; 10 (Metamidofos, Phoxim) -; 30 (Acephate, Profenofos) -; 50 (Omethoate, Dimethoate) for LC-MS/MS method	101 (Mevinphos, Dicrotophos, Dioxathion, Terbufos, Isoxathion, Chlorthiophos, Leptophos) 105 (Chlormephos) 93 (Heptenophos, Phorate, Pirimifos-methyl, Trichloronat, Fensulfothion, Sulprofos) 100 (Thionazin) 94 (TEPP, Cadusafos, Propetamphos, Cyanofos, Disulfoton, Formothion, Malathion, Isofenphos, Mephospholan, Tetrachlorvinphos, Fenamiphos, Triazofos, Phosalone, Pyrazophos) 95 (Etoprophos, Pirimifos-ethyl) 92 (Sulfotep, Monocrotophos, Fonofos, Diazinon, Chlorpyrifos, Bromophos-ethyl, Methidathion) 87 (Thiometon, Parathion-ethyl) 103 (Chlorfenvinfos) 84 (Isazophos) 88 (Chlorpyrifos-methyl, Ediphenphos) 89 (Parathion-methyl, Carbophenothion, Phosmet) 81 (Fenthion) 77 (Propafos) 97 (Famphur) 86 (EPN) for GC-MS/MS method; 78 (Metamidofos) 97 (Phoxim) 95 (Acephate) 96 (Omethoate) 92 (Dimethoate) 93 (Profenofos) for LC-MS/MS method	
Butamifos Chlorpyrifos-methyl Cyanophos	1; blood	LLE (1 M acetate buffer: hexane; 1:3)	GC-MS (EI)	-; 10 for all compounds	54.8 (Butamifos); 63.4 (Chlorpyrifos-methyl; 133.5 (Cyanophos); 70.9 (Diazinon);	(Kudo et al. 2012)

Diazinon Dichlorvos Dimethoate Edifenphos EPN Ethylthiomethon Fenitrothion Fenthion Iprobenfos Isoxathion Malathion Methamidophos Methidathion Parathion Parathion-methyl Phenthoate Phosalone Pirimiphos-methyl Propaphos Prothiofos Pyridaphenthion Salithion Thiometon		and SPE (Focus™ column)			57.0 (Dichlorvos); 25.2 (Dimethoate); 64.9 (Edifenphos); 75.2 (EPN); 48.3 (Ethylthiomethon); 75.1 (Fenitrothion); 40.5 (Fenthion); 129.8 (Iprobenfos); 44.1 (Isoxathion); 95.6 (Malathion); - (Methamidophos); 131.8 (Methidathion); 72.0 (Parathion); 45.9 (Parathion-methyl); 75.4 (Phenthoate); 64.7 (Phosalone); 52.7 (Pirimiphos-methyl); 88.2 (Propaphos); 46.3 (Prothiofos); 98.4 (Pyridaphenthion); 84.1 (Salithion); 51.0 (Thiometon) for Hexane method; 22.6 (Butamifos); 1.6 (Chlorpyrifos-methyl); 61.4 (Cyanophos); 47.3 (Diazinon); 11.6 (Dichlorvos); 36.0 (Dimethoate); 42.3 (Edifenphos); 23.1 (EPN); 21.0 (Ethylthiomethon); 42.2 (Fenitrothion); 30.6 (Fenthion); 68.1 (Iprobenfos); 2.8 (Isoxathion); 56.4 (Malathion); 0.1 (Methamidophos); 54.4 (Methidathion); 47.4 (Parathion); 63.2 (Parathion-methyl); 39.3 (Phenthoate); 33.1 (Phosalone); 34.6 (Pirimiphos-methyl); 61.9 (Propaphos); 13.5 (Prothiofos); 48.2 (Pyridaphenthion); 21.5 (Salithion); 14.8 (Thiometon) for Focus method	
Methamidophos Heptenophos Parathion-methyl Fenthion Fenamiphos	1; blood	Sample precipitation with acetone and SPE (Isolute HM-N)	GC-MS (EI)	50; 100 (Methamidophos) 25; 50 (Heptenophos) 40; 50 (Parathion-methyl, Triazophos, Pyrazophos) 30; 50 (Fenthion)	40-51 (Methamidophos) 81-90 (Heptenophos) 88-89 (Parathion-methyl) 90-93 (Fenthion) 93-94 (Fenamiphos)	(Frenzel et al. 2000)

Triazophos Azinphos-methyl Pyrazophos				20; 30 (Fenamiphos) 30; 40 (Azinphos-methyl)	96 (Triazophos) 88-95 (Azinphos-methyl) 95-96 (Pyrazophos)	
Diazinon Malathion Parathion-methyl Chlorpyrifos	6; blood	LLE (hexane)	GC-MS (EI)	-; 2-5 for all compounds	105.2 (Diazinon) 128.1 (Malathion) 103.5 (Chlorpyrifos) Without data for the remaining	(Liu and Pleil 2002)
Azynphos-ethyl Diazinon Chlorpyrifos Chlorfenvinfos Parathion-ethyl Quinalphos	0.1; blood	MEPS (C18)	GC-MS/MS (EI)	2500; 2500 (Azynphos-ethyl) 500; 500 (Diazinon, Chlorpyrifos, Chlorfenvinfos, Parathion-ethyl, Quinalphos)	60.70-68.15 (Azynphos-ethyl) 57.76-77.84 (Diazinon) 59.38-68.28 (Chlorpyrifos) 63.98-73.75 (Chlorfenvinfos) 62.30-76.32 (Parathion-ethyl) 70.48-77.57 (Quinalphos)	(Santos et al. 2018)
Chlorpyrifos	0.3; cord blood	Online SPE (Hypersil GOLD C8)	LC-MS/MS (ESI)	0.01; 0.1	97.2	(Liao et al. 2011)
Diazinon Chlorpyriphos	2; plasma	SPE (Varian ABS ELUT-Nexus 60 mg 3 mL)	GC- HRMS (EI)	0.016; 0.025 (Diazinon) 0.021; 0.025 (Chlorpyriphos)	89-95 (Diazinon) 73-87 (Chlorpyriphos)	(Pérez et al. 2010)
Phorate Phosphomidon Parathion-methyl Malathion Chlorpyrifos-methyl Chlorpyriphos Ethion	1; plasma	mini QuEChERS (3 mL of 2% acidified ethyl acetate and 0.4 g of Magnesium sulfate) and SPE (50 mg of PSA)	GC-MS/MS	0.80; 2.65 (Phorate) 3.64; 12.00 (Phosphomidon) 0.97; 3.21(Parathion-methyl) 0.40; 1.30 (Malathion) 0.92; 3.06 (Chlorpyrifos-methyl) 2.35; 7.75 (Chlorpyriphos) 1.78; 5.86 (Ethion)	91-98 (Phorate) 90-105 (Phosphomidon) 90-101(Parathion-methyl) 86-106 (Malathion) 81-105 (Chlorpyrifos-methyl) 81-102 (Chlorpyriphos) 94-100 (Ethion)	(Srivastava et al. 2017)
Chlorpyrifos Diazinon Dicrotophos Dimethoate Ethion Malathion Parathion-methyl Pirimiphos-ethyl Profenofos Prothiophos Triazophos	2; serum and breast milk	Lipid precipitation with acetone and methylene chloride and SPE (aminopropyl)	GC-FPD and GC-MS/MS (EI)	0.18; 0.8 (Chlorpyrifos) 0.38; 0.4 (Diazinon) 1.36; 2.5 (Dicrotophos) 0.39; 2.0 (Dimethoate) 0.27; 0.3 (Ethion) 0.36; 1.5 (Malathion) 0.26; 0.8 (Parathion-methyl) 0.24; 0.4 (Pirimiphos-ethyl) 0.51; 2.0 (Profenofos) 0.47; 0.5 (Prothiophos) 0.33; 2.5 (Triazophos) for serum samples, 0.22; 0.8 (Chlorpyrifos) 0.66; 0.4 (Diazinon)	53-55 (Chlorpyrifos) 49-57 (Diazinon) 39-50 (Dicrotophos) 37-52 (Dimethoate) 38-51 (Ethion, Prothiophos) 50-51 (Malathion) 49-50 (Parathion-methyl) 54-58 (Pirimiphos-ethyl) 36-46 (Profenofos) 39-43 (Triazophos) for serum samples; 58-74 (Chlorpyrifos) 48-65 (Diazinon) 45-65 (Dicrotophos)	(Naksen et al. 2016)

				2.66; 2.5 (Dicrotophos) 0.84; 2.0 (Dimethoate) 0.57; 0.3 (Ethion) 0.57; 0.5 (Prothiophos) 0.12; 1.5 (Malathion) 0.09; 0.8 (Parathion-methyl) 0.13; 0.4 (Pirimiphos-ethyl) 1.23; 2.0 (Profenofos) 1.22; 2.5 (Triazophos) for milk samples	42-69 (Dimethoate) 45-61 (Ethion) 53-67 (Malathion) 54-71 (Parathion-methyl) 51-68 (Pirimiphos-ethyl) 54-69 (Profenofos) 58-66 (Prothiophos) 51-71 (Triazophos) for milk samples	
Acephate Methidathion Dichlorvos Fenthion EPN Diazinon Phenthoate Malathion Fenitrothion Cyanophos	0.2; serum	Acetonitrile (200 µL)	LC- MS (APCI)	250; 375 (Acephate, Phenthoate, Malathion) 500; 625 (Methidathion, Dichlorvos) 1000; 1250 (Fenthion) 375; 500 (EPN) 125; 250 (Diazinon, Fenitrothion, Cyanophos)	94.2-103 (Acephate) 94.1-106.8 (Methidathion) 99.3-107.1 (Dichlorvos) 92.7-103.7 (Fenthion) 93.7-97.1 (EPN) 94.6-107.2 (Diazinon) 84.3-101.1 (Phenthoate) 82.3-102.3 (Malathion) 87.9-105.8 (Fenitrothion) 82.2-104.1 (Cyanophos)	(Inoue et al. 2007)
Diazinon Parathion-methyl Fenitrothion Malathion Fenthion Chlorpyrifos Methidathion	3; serum and urine (human serum diluted 50-fold and urine diluted 10-fold with ultra-pure water)	SPME (PDMS, 100 µm)	GC-FPD and GC-MS (EI)	1; - (Diazinon) 3; - (Parathion-methyl) 4; - (Fenitrothion, Malathion, Fenthion) 5; - (Chlorpyrifos) 15; - (Methidathion) for serum samples, 0.1; - (Diazinon) 0.3; - (Parathion-methyl, Fenitrothion, Chlorpyrifos) 0.4; - (Malathion) 0.2; - (Fenthion) 20; - (Methidathion) for urine samples	104-115 (Diazinon) 92-111 (Parathion-methyl) 79-83 (Fenitrothion) 92-108 (Malathion) 60-65 (Fenthion) 50-69 (Chlorpyrifos) 145-165 (Methidathion) for serum samples; 82-110 (Diazinon) 116-140 (Parathion-methyl) 108-126 (Fenitrothion) 124-141 (Malathion) 84-95 (Fenthion) 76-92 (Chlorpyrifos) 136-145 (Methidathion) for urine samples	(López et al. 2001)
Azinphos-ethyl, Azinphos-methyl, Bromophos-ethyl, Bromophos-methyl, Chlorfenvinphos, Dialifos,	0.7; urine, plasma, blood, serum and gastric juice	LLE (toluene)	GC-NPD and GC-MS (EI)	-	105 (Azinphos-ethyl); 102 (Azinphos-methyl); 123 (Bromophos-ethyl); 122 (Bromophos-methyl,	(Tarbah et al. 2001)

Dichlorvos, Dimethoate, Etrimfos, Fenthion, Fonofos, Heptenophos, Isofenphos, Metasystox I (demeton-S-methyl), Monocrotophos, Parathion-ethyl, Paraoxon, Phosphamidon, Pirimiphos-methyl, Pyrazophos, Sulfotep, Terbufos, Triazophos					Chlorfenvinphos); 133 (Dialifos); 66 (Dichlorvos); 50 (Dimethoate); 55 (Etrimfos); 65 (Fenthion); 56 (Fonofos, Parathion-ethyl); 60 (Heptenophos); 104 (Isofenphos); 69 (Demeton-S-methyl); 73 (Monocrotophos); 72 (Paraoxon); 93 (Phosphamidon, Pirimiphos-methyl); 82 (Pyrazophos); 83 (Sulfotep); 118 (Terbufos); 88 (Triazophos)	
Chlorpyrifos Diazinon Dichlorvos Fonophos Malathion Parathion-methyl Parathion Phorate Terbufos	4 g; serum and plasma	Sample precipitation with saturated ammonium sulfate and SPE (Oasis)	GC-HRMS (EI)	1; - (Chlorpyrifos, Dichlorvos, Fonophos, Parathion, Phorate, Terbufos) 0.5; - (Diazinon) 12; - (Malathion) 2; - (Parathion-methyl) (in pg/g)	21 (Chlorpyrifos) 27 (Diazinon) 15 (Dichlorvos) 20 (Fonophos) 22 (Malathion) 20 (Parathion-methyl) 20 (Parathion) 21 (Phorate) 17 (Terbufos)	(Barr et al. 2002)
Dimethylthiophosphate Diethylphosphate Diethylthiophosphate Mono-2-chloropropyl phosphate Mono-n-butyl phosphate Diethyldithiophosphate Monophenyl phosphate Monobutoxyethyl phosphate Di-iso-butyl phosphate Di-n-butyl phosphate Diphenyl phosphate Monoethylhexyl phosphate Dibutoxyethyl phosphate Diethylhexyl phosphate	3; urine	Sample precipitation with acetonitrile	LC-MS/MS (ESI)	-; 2 (Dimethylthiophosphate) -; 2(Diethylphosphate) -; 1 (Diethylthiophosphate) -; 6 (Mono-2-chloropropyl phosphate) -; 4 (Mono-n-butyl phosphate) -; 0.6 (Diethyldithiophosphate) -; 9 (Monophenyl phosphate) -; 11 (Monobutoxyethyl phosphate) -; 0.3 (Di-iso-butyl phosphate) -; 0.9 (Diphenyl phosphate) -; 2.5 (Monoethylhexyl phosphate) -; 2.5 (Dibutoxyethyl phosphate) -; 1.5 (Diethylhexyl phosphate) No data for the remaining compounds	99 (Dimethylthiophosphate) 114 (Diethylphosphate) 112 (Diethylthiophosphate) 102 (Mono-2-chloropropyl phosphate, Diphenyl phosphate) 69 (Mono-n-butyl phosphate) 122 (Diethyldithiophosphate) 101 (Monophenyl phosphate) 106 (Monobutoxyethyl phosphate) 100 (Di-iso-butyl phosphate, Di-n-butyl phosphate) 104 (Monoethylhexyl phosphate) 81 (Dibutoxyethyl phosphate) 98 (Diethylhexyl phosphate)	(Reemtsma et al. 2011)

Dimethylphosphate Diethylphosphate Dimethylthiophosphate Diethylthiophosphate Dimethyldithiophosphate Diethyldithiophosphate	5; urine	Lyophilization, acetonitrile and Derivatization with PFBBr	GC-FPD	5; 10 (Dimethylphosphate) 3; 10 (Diethylphosphate, Diethyldithiophosphate) 1; 10 (Dimethylthiophosphate, Diethylthiophosphate) No data for the remaining compounds	60 (Dimethylphosphate) 67.5 (Diethylphosphate) 107.5 (Dimethylthiophosphate) 98.6 (Diethylthiophosphate) 90.3 (Diethyldithiophosphate) No data for the remaining compounds	(Petchuay et al. 2008)
Dimethoate Parathion-methyl Malathion Terbufos Parathion	8 (SPE) and 10 (LLE); plasma	SPE (Octadecyl C18) and LLE for comparison (acetone: water; 30:10)	GC-PFPD	0.01; 25 (Dimethoate) 0.03; 40 (Parathion-methyl, Malathion) 0.04; 75 (Terbufos) 0.02; 40 (Parathion)	95.5-109.1 (Dimethoate) 85.2-89.6 (Parathion-methyl) 91.7-91.8 (Malathion) 84.3-93.0 (Terbufos) 86.4-96.1 (Parathion)	(Wang and Du 2010)
Acephate Methamidophos Omethoate Dimethoate	0.8; urine	Lyophilization followed by LLE (dichloromethane)	LC-MS/MS (ESI)	0.005; 0.005 (Acephate) 0.009; 0.009 (Methamidophos) 0.006; 0.006 (Omethoate) 0.004; 0.004 (Dimethoate)	99.99 (Acephate, Omethoate) 100 (Methamidophos, Dimethoate)	(Jayatilaka et al. 2011)
Dimethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylphosphate Diethylthiophosphate Diethyldithiophosphate	0.6; urine	96-well plate SPE (weak anion exchange)	LC-MS/MS (ESI)	0.468; 0.125 (Dimethylphosphate) 0.066; 0.125 (Dimethylthiophosphate) 0.073; 0.125 (Dimethyldithiophosphate) 0.044; 0.125 (Diethylphosphate) 0.110; 0.125 (Diethylthiophosphate) 1.549; 0.125 (Diethyldithiophosphate)	99.99 (Dimethylphosphate, Dimethyldithiophosphate, Diethyldithiophosphate) 100 (Diethylphosphate, Dimethylthiophosphate, Diethylthiophosphate)	(Odetokun et al. 2010)
Phorate Diazinon Parathion-methyl Fenthion Quinalphos	9; urine	Cloud point extraction coupled with microwave-assisted back-extraction (Triton X-114 and isooctane)	GC-FPD	0.07; 0.21 (Phorate, Fenthion, Quinalphos) 0.04; 0.12 (Diazinon) 0.08; 0.24 (Parathion-methyl)	85-107	(Jia et al. 2008)
Chlorpyrifos 3,5,6-trichloro-2-pyridinol	0.5; urine and serum	Sample precipitation with acetonitrile and urine was	LC-LC-MS/MS (ESI)	0.5; 1 for urine samples, 1.5; 5 for serum samples	98-109 for urine samples; 87-113 for serum samples	(Sancho et al. 2000)

		directly injected				
Dimethylphosphate Dimethylthiophosphate Diethylphosphate Diethylthiophosphate 3,5,6-trichloro-2-pyridinol	10; urine	LLE (hexane: isopropanol; 95:5) and SPE (Oasis WCX) and Derivatization with MTBSTFA	GC-MS/MS (EI)	0.667; 2.000 (Dimethylthiophosphate) 0.067; 0.200 (Diethylphosphate) 0.083; 0.250 (Diethylthiophosphate) 0.200; 0.600 (3,5,6-trichloro-2-pyridinol) No data for the remaining compounds	54.08-66.56 (Dimethylthiophosphate) 55.40-67.86 (Diethylphosphate) 59.95-68.64 (Diethylthiophosphate) 57.15-68.51 (3,5,6-trichloro-2-pyridinol) No data for the remaining compounds	(Guo et al. 2017)
Dimethoate Diazinon Parathion-methyl Fenitrothion Malathion Fenthion Chlorpyrifos Methidathion Phosmet Azinphos-methyl	5; urine 2; serum	SPE (C18) and LLME (dichloromethane)	GC-ECD/NPD	1.4; - (Dimethoate) 0.6; - (Diazinon, Fenitrothion) 1.5; - (Parathion-methyl) 0.5; - (Malathion) 0.8; - (Fenthion) 0.7; - (Chlorpyrifos) 0.9; - (Methidathion) 2.0; - (Phosmet) 1.7; - (Azinphos-methyl) for SPE and urine samples, 2.0; - (Dimethoate, Parathion-methyl, Malathion, Methidathion, Phosmet) 3.0; - (Diazinon) 0.7; - (Fenitrothion, Fenthion) 6.0; - (Azinphos-methyl) for LLME and urine samples, 1; - (Diazinon, Fenitrothion) 5; - (Parathion-methyl) 2; - (Malathion, Fenthion, Chlorpyrifos) 3; - (Methidathion) 10; - (Azinphos-methyl) for SPE and serum samples. No data for the remaining compounds	68-102 (Dimethoate); 96 (Diazinon); 96-103 (Parathion-methyl); 95-103 (Fenitrothion); 90-99 (Malathion); 96-99 (Fenthion); 97 (Chlorpyrifos); 94-108 (Methidathion); 83-109 (Phosmet); 96-107 (Azinphos-methyl) for SPE and urine samples; 72-93 (Dimethoate); 61-77 (Diazinon); 84 (Parathion-methyl); 86-87 (Fenitrothion); 93-94 (Malathion); 85-91 (Fenthion); 50-64 (Chlorpyrifos); 99-107 (Methidathion); 93-114 (Phosmet); 98-109 (Azinphos-methyl) for LLME and urine samples; 86-88 (Diazinon, Fenthion); 91-93 (Parathion-methyl); 92-95 (Fenitrothion); 69-75 (Malathion); 87 (Chlorpyrifos); 100-105 (Methidathion); 57-70 (Phosmet); 119-121 (Azinphos-methyl) for SPE and serum samples; 71-98 (Diazinon); 59-92 (Parathion-methyl); 45-78 (Fenitrothion); 53-75 (Malathion); 98 (Fenthion); 26 (Chlorpyrifos); 93-104 (Methidathion); 46-96	(Pitarch et al. 2001)

					(Phosmet); 53-112 (Azinphos-methyl) for LLME and serum samples. No data for the remaining compounds	
Parathion	0.1; urine and blood	SPME (DI) (CW/DVB, 65-µm)	GC-MS (EI)	3; 10 for urine samples, 25; 50 for blood samples	21.9-45.6 for urine samples; 6.6-6.7 for blood samples	(Gallardo et al. 2006a)
Fenitrothion	0.2; serum and urine	Monolithic extraction (MonoSpin® C18)	GC-MS (EI)	25; 50 for both samples	88.8-106.1 for serum samples; 51.3-90.1 for urine samples	(Saito et al. 2011b)
Diazinon, Parathion-methyl, Fenitrothion, Malathion, Chlorpyrifos, Methidathion, Ethion, Phosmet	1; serum	SPE (Bond Elut C18)	GC-MS/MS (EI)	0.3; 0.9 (Diazinon) 3.0; 9.0 (Parathion-methyl) 1.1; 3.3 (Fenitrothion) 0.2; 0.6 (Chlorpyrifos) 0.1; 0.4 (Ethion) No data for the remaining compounds	71-91 (Diazinon) 78 (Parathion-methyl) 90-92 (Fenitrothion) 83-95 (Chlorpyrifos) 88-102 (Ethion) No data for the remaining compounds	(Pitarch et al. 2003)
Dimethoate	0.1; urine and blood	SPME (DI) (CW/DVB, 65 µm)	GC-MS (EI)	50; 100 for urine samples, 200; 500 for blood samples	1.24 for urine samples; 0.50 for blood samples	(Gallardo et al. 2006b)
3,5,6-trichloro-2-pyridinol	10; urine	SPE (Isolute 101)	GC-MS (EI)	0.05; 0.1	104	(Koch and Angerer 2001)
Dichlorvos	1; urine	SPMEM	GC-MS (EI)	-	-	(Yang and Xie 2004)
Dichlorvos Dimethoate Chlorpyriphos-methyl Chlorpyriphos-ethyl Pirazophos	2; serum	LLE (acetone: dichloromethane: hexane; 50:20:30)	GC-MS (EI)	5; 10 (Dichlorvos, Pirazophos) 2; 5 (Dimethoate, Chlorpyriphos-methyl, Chlorpyriphos-ethyl)	56.59-78.77 (Dichlorvos) 61.26- 92.52 (Dimethoate) 59.97-86.03 (Chlorpyriphos-methyl) 58.75-79.39 (Chlorpyriphos-ethyl) 77.39-81.41 (Pirazophos)	(Araoud et al. 2012)
Dimethylphosphate Diethylphosphate Dimethylthiophosphate Diethylthiophosphate	5; urine	LLE (diethylether: acetonitrile; 1:1) and Derivatization with PFBBr	GC-MS (EI)	0.3; 1.0 (Dimethylphosphate) 0.1; 0.5 (Diethylphosphate) 0.1; 0.3 (Dimethylthiophosphate, Diethylthiophosphate)	56.7-67.7 (Dimethylphosphate) 78.5-85.7 (Diethylphosphate) 88.3-103.9 (Dimethylthiophosphate) 84.2-92.4 (Diethylthiophosphate)	(Ueyama et al. 2006)

Diazinon Parathion-methyl Malathion Parathion	0.3; blood, plasma 1g; kidney, liver and cerebrospinal fluid	HS-SPME (PA, 85 µm)	GC-NPD	3; 20 (Diazinon) 50; 100 (Parathion-methyl) 45; 100 (Malathion) 18; 50 (Parathion) for blood samples, 5; 20 (Diazinon) 40; 100 (Parathion-methyl) 45; 100 (Malathion) 16; 50 (Parathion) for plasma samples, 4; 20 (Diazinon) 35; 100 (Parathion-methyl) 50; 100 (Malathion) 12; 50 (Parathion) for kidney samples (in ng/g), 10; 20 (Diazinon) 55; 100 (Parathion-methyl) 50; 100 (Malathion) 25; 50 (Parathion) for liver samples (in ng/g), 2; 20 (Diazinon) 35; 100 (Parathion-methyl) 40; 100 (Malathion) 8; 50 (Parathion) for cerebrospinal fluid samples	1.7 (Diazinon) 0.24 (Parathion-methyl) 0.14 (Malathion) 0.67 (Parathion) for blood samples; 1.39 (Diazinon) 0.23 (Parathion-methyl) 0.14 (Malathion) 0.78 (Parathion) for plasma samples; 1.34 (Diazinon) 0.36 (Parathion-methyl) 0.10 (Malathion) 0.87 (Parathion) for kidney samples; 0.31 (Diazinon) 0.16 (Parathion-methyl) 0.11 (Malathion) 0.32 (Parathion) for liver samples; 2.5 (Diazinon) 0.35 (Parathion-methyl) 0.28 (Malathion) 1.25 (Parathion) for cerebrospinal fluid samples	(Tsoukali et al. 2005)
Diethylthiophosphate Diethylphosphate 3,5,6-trichloro-2-pyridinol	0.2; urine	LLE (ethylacetate: acetonitrile; 70:30) with and without hydrolysis	LC-MS/MS (ESI)	-; 250 for all compounds	97.6-102.2 (Diethylthiophosphate) 39.4-41.9 (Diethylphosphate) 97.8-104.2 (3,5,6-trichloro-2-pyridinol) without hydrolysis; 47.4-73.6 (Diethylthiophosphate) 47.0-52.0 (Diethylphosphate) 100.6-107.3 (3,5,6-trichloro-2-pyridinol) with hydrolysis	(Bicker et al. 2005)
Dimethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylphosphate Diethylthiophosphate Diethyldithiophosphate	2; urine	Lyophilization and LLE (acetonitrile:ethyl ether; 1:1) and Derivatization	GC-MS/MS (EI)	0.6; - (Dimethylphosphate) 0.2; - (Dimethylthiophosphate, Diethylphosphate) 0.1; - (Dimethyldithiophosphate, Diethylthiophosphate, Diethyldithiophosphate)	94-95 (Dimethylphosphate) 82-100 (Dimethylthiophosphate, Dimethyldithiophosphate) 99 (Diethylphosphate) 82-87 (Diethylthiophosphate) 75-85 (Diethyldithiophosphate)	(Bravo et al. 2004)

		with 1-Chloro-3-Iodopropane				
Acephate Omethoate Phorate-oxon Phorate Dimethoate Propetamphos Terbufos Fonofos Diazinon Paraoxon-methyl Disulfoton Parathion-methyl Malaoxon Paraoxon-ethyl Ronnel Fenitrothion Pirimiphos-ethyl Malathion Fenthion Chlorpyriphos Parathion-ethyl Chlorfenvinphos Isofenphos Methidathion Bromophos-ethyl Profenophos DEF Carbophenothion-methyl Ethion Carbophenothion ENP Oxo-azinphos-methyl Phosalone Azinphos-methyl Azinphos-ethyl Co-ral-O-analog Co-ral (Coumaphos)	2-3 g; kidney and liver	2% ethanol in ethyl acetate, LLE (*n*-hexane) and gel permeation chromatography	GC- MS (NCI)	0.05; 1 (Acephate, Methidathion, Profenophos, Co-ral-O-analog) 0.05; 3 (Propetamphos, Paraoxon-methyl, ENP) 0.05; 2 (Disulfoton, Carbophenothion-methyl) 0.07; 1 (Omethoate) 0.06; 2 (Phorate-oxon) 0.06; 1 (Terbufos) 0.04; 1 (Phorate, Malaoxon, Fenitrothion) 0.04; 3 (Oxo-azinphos-methyl) 0.04; 2 (Isofenphos, Bromophos-ethyl, Paraoxon-ethyl, Azinphos-ethyl, Coumaphos) 0.03; 2 (Dimethoate, Pirimiphos-ethyl, Azinphos-methyl) 0.03; 1 (Malathion) 0.02; 1 (Fonofos, Diazinon, Fenthion, Chlorfenvinphos, Carbophenothion) 0.02; 3 (Parathion-methyl, Chlorpyriphos) 0.02; 2 (Parathion-ethyl) 0.01; 2 (Ronnel) 0.01; 1 (DEF, Ethion, Phosalone)	91-93 (Acephate); 79-80 (Omethoate); 60-61 (Phorate-oxon); 62-64 (Phorate); 80-81 (Dimethoate); 76-78 (Propetamphos); 68-70 (Terbufos); 71-72 (Fonofos); 101-104 (Diazinon); 100-102 (Paraoxon-methyl); 74-76 (Disulfoton); 87-89 (Parathion-methyl); 86-88 (Malaoxon); 91-94 (Paraoxon-ethyl); 95-97 (Ronnel); 89-90 (Fenitrothion); 77-78 (Pirimiphos-ethyl); 96-98 (Malathion); 94-95 (Fenthion, Azinphos-ethyl); 80-84 (Chlorpyriphos); 91-95 (Parathion-ethyl); 89-91 (Chlorfenvinphos); 101-103 (Isofenphos); 101-102 (Methidathion); 88-90 (Bromophos-ethyl); 78-80 (Profenophos); 82-85 (DEF); 76-78 (Carbophenothion-methyl); 80-82 (Ethion); 79 (Carbophenothion); 85-86 (ENP); 98-98 (Oxo-azinphos-methyl); 93-94 (Phosalone, Azinphos-methyl); 90-91 (Co-ral-O-analog); 81-84 (Co-ral (Coumaphos)) for kidney samples; 90-92 (Acephate); 85-86 (Omethoate); 70-72 (Phorate-oxon); 66-68 (Phorate); 104-106 (Dimethoate); 82-86 (Propetamphos); 68-71 (Terbufos); 83-84 (Fonofos); 86-89 (Diazinon); 98-100 (Paraoxon-methyl); 63-67 (Disulfoton); 83-88	(Russo et al. 2002)

					(Parathion-methyl, Parathion-ethyl); 85-90 (Malaoxon); 68-75 (Paraoxon-ethyl); 92-95 (Ronnel, DEF); 90-93 (Fenitrothion); 88-90 (Pirimiphos-ethyl); 96-98 (Malathion, Azinphos-methyl); 81-86 (Fenthion); 80-85 (Chlorpyriphos); 95-97 (Chlorfenvinphos); 98-99 (Isofenphos); 87-91 (Methidathion, Carbophenothion); 86-90 (Bromophos-ethyl); 64-67 (Profenophos); 82-87 (Carbophenothion-methyl); 84-89 (Ethion); 98-101 (ENP); 100-101 (Oxo-azinphos-methyl); 103-104 (Phosalone); 65-75 (Azinphos-ethyl); 91-96 (Co-ral-O-analog); 88-93 (Coumaphos) for liver samples	
4-nitrophenol 3-methyl-4-nitrophenol	1; urine	Without sample preparation (urine hydrolyzed)	LC-LC-MS/MS (ESI)	0.1; 0.5 (4-nitrophenol) 0.2; 0.5 (3-methyl-4-nitrophenol)	83-102 (4-nitrophenol) 89-104 (3-methyl-4-nitrophenol)	(Hernández et al. 2004)
Disulfoton Disulfoton-sulfoxide Disulfoton-sulfone Demeton-S Demeton-S-sulfoxide Demeton-S-sulfone	0.5; blood and urine	QuEChERS (pre-packed Extraction: 6 g of magnesium sulfate and 1.5 g of sodium acetate; SPE sorbent: 25 mg of PSA, 25 mg of end capped octadecylsilane, and 150 mg of magnesium sulfate)	LC-MS/MS (ESI)	0.90; 5.0 (Disulfoton) 0.85; 5.0 (Disulfoton-sulfoxide) 1.15; 5.0 (Disulfoton-sulfone) 0.97; 5.0 (Demeton-S) 0.66; 5.0 (Demeton-S-sulfoxide) 0.92; 5.0 (Demeton-S-sulfone) for blood samples, 0.46; 5.0 (Disulfoton) 0.73; 5.0 (Disulfoton-sulfoxide) 0.79; 5.0 (Disulfoton-sulfone) 0.76; 5.0 (Demeton-S) 0.86; 5.0 (Demeton-S-sulfoxide) 1.05; 5.0 (Demeton-S-sulfone) for urine samples	104-111 (Disulfoton) 99-102 (Disulfoton-sulfoxide) 94-95 (Disulfoton-sulfone) 102-106 (Demeton-S) 96-98 (Demeton-S-sulfoxide) 95-99 (Demeton-S-sulfone) for blood samples; 99-106 (Disulfoton) 92-100 (Disulfoton-sulfoxide) 87-101 (Disulfoton-sulfone) 99-112 (Demeton-S) 102-109 (Demeton-S-sulfoxide) 100-102 (Demeton-S-sulfone) for urine samples	(Usui et al. 2012)

Dimethylphosphate Diethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylthiophosphate Diethyldithiophosphate	2; urine	SPE (Bond Elut PPL) and Derivatization with 1-chloro-3-iodopropane	GC-MS/MS (EI)	0.06; 0.1 (Dimethylphosphate) 0.05; 0.1 (Diethylphosphate, Dimethyldithiophosphate, Diethylthiophosphate) 0.17; 0.1 (Dimethylthiophosphate) 0.07; 0.1 (Diethyldithiophosphate)	105 (Dimethylphosphate, Diethylphosphate) 99 (Dimethylthiophosphate, Diethyldithiophosphate) 98 (Dimethyldithio-phosphate) 102 (Diethylthiophosphate)	(De Alwis et al. 2006)
Metrifonate 2,2-dichlorovinyl Dimethylphosphate	0.2; urine and blood (Metrifonate) 1; urine and 0.25; blood (2,2-dichlorovinyl Dimethylphosphate)	LLE (ethyl acetate:hexane; 70:30) and SPE (SPE-Plus-3ML-C) for Metrifonate in blood; LLE (dichloromethane) for Metrifonate in urine and LLE (cyclohexane) for 2,2-dichlorovinyl Dimethylphosphate in blood and urine	GC-ECD (Metrifonate) and GC-MS (EI) (2,2-dichlorovinyl Dimethylphosphate)	-; 10 (for metrifonate in blood and urine), -; 1.13 (for 2,2-dichlorovinyl Dimethylphosphate in blood), -; 2.44 (for 2,2-dichlorovinyl Dimethylphosphate in urine)	63-83.7 (for metrifonate in blood); 43.8-53.7 (for metrifonate in urine; 41.1-37.4 (for 2,2-dichlorovinyl Dimethylphosphate in blood); 52 (for 2,2-dichlorovinyl Dimethylphosphate in urine)	(Heinig et al. 2000)
2-isopropyl-6-methyl-4-pyrimidiol 2-diethylamino-6-methyl pyrimidin-4-ol 5-chloro-1-isopropyl-[3H]-1,2,4-triazol-3-one, 2-[(dimethoxyphosphorothioyl)sulfanyl]succinic acid 4-nitrophenol 3-chloro-4-methyl-7-hydroxycoumarin 3,5,6-trichloro-2-pyridinol	2; urine	SPE (Oasis HLB)	LC-MS/MS (APCI) and LC-MS/MS (TIS)	0.7; 0.25 (2-isopropyl-6-methyl-4-pyrimidiol) 0.2; 0.25 (2-diethylamino-6-methyl pyrimidin-4-ol, 3-chloro-4-methyl-7-hydroxycoumarin) 1.5; 0.25 (5-chloro-1-isopropyl-[3H]-1,2,4-triazol-3-one) 0.3; 0.25 (2-[(dimethoxyphosphorothioyl)sulfanyl]succinic acid) 0.1; 0.25 (4-nitrophenol) 0.3-0.4; 0.25 (3,5,6-trichloro-2-pyridinol)	81-99 (2-isopropyl-6-methyl-4-pyrimidiol) 95-98 (2-diethylamino-6-methyl pyrimidin-4-ol) 90-98 (5-chloro-1-isopropyl-[3H]-1,2,4-triazol-3-one) 68-75 (2-[(dimethoxyphosphorothioyl)sulfanyl]succinic acid) 93-95 (4-nitrophenol) 95-96 (3-chloro-4-methyl-7-hydroxycoumarin) 88-93 (3,5,6-trichloro-2-pyridinol)	(Olsson et al. 2004)
Chlorfenvinphos	1g; liver	LLE (diethyl ether)	GC-FID GC-MS (EI)	100-300 for all samples	98-99	(Martínez and

	3; stomach content, blood, bile, urine and vitreous humor					Ballesteros 2012)
Dimethylphosphate Diethylphosphate Dimethylthiophosphate Diethylthiophosphate Dimethyldithiophosphate Diethyldithiophosphate	5; urine	LLE (diethylether: acetonitrile; 1:1) and Derivatization with PFBBr	GC-MS (EI)	1; 1 (Dimethylphosphate) 5; 5 for the other compounds	88-108 (Dimethylphosphate) 100-114 (Diethylphosphate) 71-89 (Dimethylthiophosphate) 72-88 (Diethylthiophosphate) 75-85 (Dimethyldithiophosphate) 68-83 (Diethyldithiophosphate)	(Hardt and Angerer 2000)
Dimethylphosphate Dimethylthiophosphate Diethylphosphate Diethylthiophosphate	1; urine	SPE (Strata X-AW)	LC-MS/MS (ESI)	0.4; 1.2 (Dimethylphosphate, Dimethylthiophosphate) 0.2; 0.6 (Diethylphosphate) 0.1; 0.3 (Diethylthiophosphate)	95-100 (Dimethylphosphate) 64-105 (Dimethylthiophosphate) 81-82 (Diethylphosphate) 93-101 (Diethylthiophosphate)	(Ueyama et al. 2014)
Dimethylphosphate Diethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylthiophosphate Diethyldithiophosphate	2; urine	SPE (Bond Elut PPL) and Derivatization with PFBBr	GC-MS/MS (EI)	0.1; 0.1 (Dimethylphosphate, Diethylphosphate, Dimethyldithiophosphate, Diethylthiophosphate, Diethyldithiophosphate) 0.15; 0.1 (Dimethylthiophosphate)	67 (Dimethylphosphate) 109-119 (Diethylphosphate) 77-84 (Dimethylthiophosphate) 58-67 (Dimethyldithiophosphate) 73-75 (Diethylthiophosphate) 55-60 (Diethyldithiophosphate)	(Hemakanthi De Alwis et al. 2008)
Diazinon	10; urine	SA-DSPE (0.5 mL of ethanol (as disperser solvent) containing benzophenone (2% (w/v))	GC-FID	0.3; 2	97.5-99.3	(Aladaghlo et al. 2016)
O, S-dimethyl acetylamidothiophosphate 5-chloro-1, 2-dihydro-1-isopropyl-[3H]-1, 2, 4-triazol-3-one 3-chloro-4-methyl-7-hydroxycoumarin 2-diethylamino-6-methyl pyrimidin-4-ol 1, 2, 3-benzotriazin-4-one	2; urine	Sample hydrolyzed, SPE (Oasis HLB) and SPE (Chem Elute)	LC-MS/MS (ESI)	0.8; - (O, S-dimethyl acetylamidothiophosphate; O,S-dimethyl amidothiophosphate) 1; - (5-chloro-1, 2-dihydro-1-isopropyl-[3H]-1, 2, 4-triazol-3-one) 0.2; - (3-chloro-4-methyl-7-hydroxycoumarin; 2-diethylamino-6-methyl pyrimidin-4-ol; 2-isopropyl-6-methyl-	32-34 (O, S-dimethyl acetylamidothiophosphate) 88-111 (5-chloro-1, 2-dihydro-1-isopropyl-[3H]-1, 2, 4-triazol-3-one) 91-103 (3-chloro-4-methyl-7-hydroxycoumarin) 98-115 (2-diethylamino-6-methyl pyrimidin-4-ol) 90-91 (1, 2, 3-benzotriazin-4-one)	(Olsson et al. 2003)

2-isopropyl-6-methyl-pyrimidin-4-ol 2-[(dimethoxyphosphorothioyl)sulfanyl]succinic acid O,S-dimethyl amidothiophosphate 4-nitrophenol 3, 5, 6-trichloro-2-pyridinol				pyrimidin-4-ol; 2-[(dimethoxyphosphorothioyl)sulfanyl]succinic acid) 6; - (1, 2, 3-benzotriazin-4-one) 0.1; - (4-nitrophenol) 1-8; - (3, 5, 6-trichloro-2-pyridinol)	93-116 (2-isopropyl-6-methyl-pyrimidin-4-ol) 74-86 (2-[(dimethoxyphosphorothioyl)sulfanyl]succinic acid) 18-21 (O,S-dimethyl amidothiophosphate) 94-106 (4-nitrophenol) 95-111 (3, 5, 6-trichloro-2-pyridinol)	
Fenitrothion Malathion Phenthoate	0.2; serum and urine	Monolithic extraction (MonoSpin® C18)	GC-MS (EI)	100; 100 for both samples	12.7-20.0 (Fenitrothion) 39.7-49.5 (Malathion) 23.4-29.2 (Phenthoate) for serum samples; 6.4-9.4 (Fenitrothion) 7.2-9.0 (Malathion) 7.9-9.2 (Phenthoate) for urine samples	(Saito et al. 2011a)
Dimethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylphosphate Diethylthiophosphate Diethyldithiophosphate	-; urine	SPE (Oasis WAX)	UPLC-MS/MS (ESI)	0.47; 1.53 (Dimethylphosphate) 0.03; 0.09 (Dimethylthiophosphate) 0.04; 0.14 (Dimethyldithiophosphate) 1.77; 4.58 (Diethylphosphate) 0.02; 0.08 (Diethylthiophosphate) 0.07; 0.21 (Diethyldithiophosphate)	91.4 (Dimethylphosphate) 72.6 (Dimethylthiophosphate) 92.8 (Dimethyldithiophosphate) 101.4 (Diethylphosphate) 96.7 (Diethylthiophosphate) 54.2 (Diethyldithiophosphate)	(Oya et al. 2017)
Dimethylphosphate Diethylphosphate Dimethylthiophosphate Diethylthiophosphate Dimethyldithiophosphate Diethyldithiophosphate	0.25; urine	LLE (acetonitrile, hexane), SPE (silica) and Derivatization with diazotoluene	GC-MS (EI)	3-6; 18 for all compounds	-	(Kupfermann et al. 2004)
Dimethylphosphate Diethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylthiophosphate Diethyldithiophosphate	2; urine	Lyophilization, acetonitrile and Derivatization with PFBBr	GC-MS/MS (NCI)	0.5; 10 (Dimethylphosphate) 0.1; 10 (Diethylphosphate, Dimethylthiophosphate) 0.04; 10 (Dimethyldithiophosphate, Diethylthiophosphate)	-	(Oglobline et al. 2001a)

				0.02; 10 (Diethyldithiophosphate)		
Acephate Methamidophos Omethoate Dimethoate	2; urine	Lyophilization and LLE (dichloromethane)	HPLC-MS/MS (APCI) with TSQ 7000 or TSQ-Quantum	0.28; 0.250 (Acephate) 0.16; 0.250 (Methamidophos) 0.05; 0.250 (Omethoate) 0.03; 0.250 (Dimethoate) for TSQ 7000, 0.023; 0.250 (Acephate) 0.001; 0.250 (Methamidophos) 0.025; 0.250 (Omethoate) 0.004; 0.250 (Dimethoate) for TSQ-Quantum	52-63	(Montesano et al. 2007)
Dimethylphosphate Diethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylthiophosphate Diethyldithiophosphate	5 for GC-FPD; urine 2.5 for GC-MS; urine	LLE (ethyl acetate: acetone; 1:1) for GC-FPD method; LLE (acetonitrile: diethylether; 1:1) for GC-MS method and Derivatization with PFBBr	GC-FPD and GC-MS (EI)	2.5; 25 (Dimethylphosphate) 0.20; 5.0 (Diethylphosphate) 0.20; 2.0 (Dimethylthiophosphate) 0.20; 1.0 (Dimethyldithiophosphate) 0.20; 2.0 (Diethyldithiophosphate) 0.10; 1.0 (Diethylthiophosphate) for GC-FPD method, 2.5; 0.25 (Dimethylphosphate) 0.25; 0.25 (Diethylphosphate, Dimethylthiophosphate, Dimethyldithiophosphate, Diethylthiophosphate, Diethyldithiophosphate) for GC-MS method	94-103 (Dimethylphosphate) 100-103 (Diethylphosphate) 110-118 (Dimethylthiophosphate) 110-119 (Dimethyldithiophosphate) 103-116 (Diethylthiophosphate) 110 (Diethyldithiophosphate) for GC-FPD method; 94-98 (Dimethylphosphate) 92-96 (Diethylphosphate) 98-103 (Dimethylthiophosphate) 98-100 (Dimethyldithiophosphate, Diethylthiophosphate) 97-99 (Diethyldithiophosphate) for GC-MS method	(Prapamontol et al. 2014)
Dimethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylphosphate Diethylthiophosphate Diethyldithiophosphate	1; urine	Acetonitrile and Derivatization with PFBBr	GC-FPD and GC-MS (EI)	-; 2.0 (Dimethylphosphate) -; 10.0 (Dimethylthiophosphate) -; 8.0 (Dimethyldithiophosphate) -; 4.0 (Diethylphosphate, Diethyldithiophosphate) -; 2.0 (Diethylthiophosphate)	62.5-75.6 (Dimethylphosphate) 63.8-75.6 (Dimethylthiophosphate) 68.1-78.5 (Dimethyldithiophosphate) 84.5-95.3 (Diethylphosphate) 82.7-97.6 (Diethylthiophosphate) 86.1-95.4 (Diethyldithiophosphate)	(Wu et al. 2010)
Dimethylphosphate Dimethylthiophosphate Dimethyldithiophosphate	5; urine	LLE (diethylether, ethyl acetate)	LC-MS/MS (ESI)	1.3; 2 (Dimethylphosphate) 1.1; 2 (Dimethylthiophosphate, Dimethyldithiophosphate)	13.2-19.4 (Dimethylphosphate) 25.2-96.2 (Dimethylthiophosphate)	(Dulaurent et al. 2006)

Diethylphosphate Diethylthiophosphate Diethyldithiophosphate				0.7; 2 (Diethylphosphate) 0.5; 2 (Diethylthiophosphate) 0.9; 2 (Diethyldithiophosphate)	67.5-91.8 (Dimethyldithiophosphate) 50.2-88.5 (Diethylphosphate) 49.5-99.8 (Diethylthiophosphate) 58.5-90.6 (Diethyldithiophosphate)	
Diethylthiophosphate Diethyldithiophosphate	1; urine	MISPE and Derivatization with PFBBr	GC-MS (EI)	3.0; 10.0 for both compounds	28.9-29.7 (Diethylthiophosphate) 31.9-32.7 (Diethyldithiophosphate)	(Santos et al. 2012)
4-nitrophenol 3,5,6-trichloro-2-pyridinol 2-[(dimethylphosphorothioyl)sufanyl]succinic acid 2-isopropyl-6-methyl-4-primidiol 2-diethylamino-6-methyl pyrimidin-4-ol 2,4-dichlorophenoxyacetic acid 2,4,5-trichlorophenoxyacetic acid	1; urine	96-well plate SPE (Oasis HLB)	LC-MS/MS (HESI)	0.3; 0.1 (2-[(dimethylphosphorothioyl)sufanyl]succinic acid) 0.1; 0.1 (2-isopropyl-6-methyl-4-primidiol; 2-diethylamino-6-methyl pyrimidin-4-ol; 4-nitrophenol; 3,5,6-trichloro-2-pyridinol) 0.04; 0.03 (2,4-dichlorophenoxyacetic acid) 0.03; 0.03 (2,4,5-trichlorophenoxyacetic acid)	89.3 (4-nitrophenol) 80.1-81.7 (3,5,6-trichloro-2-pyridinol) 71.4-73.0 (2-[(dimethylphosphorothioyl)sufanyl]succinic acid) 50.5-51.3 (2-isopropyl-6-methyl-4-primidiol) 64.6-67.0 (2-diethylamino-6-methyl pyrimidin-4-ol) 86.7-91.7 (2,4-dichlorophenoxyacetic acid) 96.7-97.5 (2,4,5-trichlorophenoxyacetic acid)	(Davis et al. 2013)
Dimethylphosphate Diethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylthiophosphate Diethyldithiophosphate	4; urine	Acetonitrile and derivatization with 1-chloro-3-iodopropane	GC-MS/MS (EI)	1000; 0.5 (Dimethylphosphate) 720; 0.5 (Diethylphosphate) 1600; 0.5 (Dimethylthiophosphate) 780; 0.5 (Dimethyldithiophosphate) 380; 0.5 (Diethylthiophosphate) 250; 0.5 (Diethyldithiophosphate)	32-45 (Dimethylphosphate) 27-62 (Diethylphosphate) 60-65 (Dimethylthiophosphate) 19-43 (Dimethyldithiophosphate) 17-36 (Diethylthiophosphate) 37-47 (Diethyldithiophosphate)	(Bravo et al. 2002)
Dimethylphosphate Diethylphosphate Dimethylthiophosphate Diethylthiophosphate	2.5; urine	LLE (diethylether: acetonitrile; 1:1) and Derivatization with PFBBr	GC-MS (EI)	0.15; 0.5 (Dimethylphosphate) 0.07; 0.3 (Diethylphosphate) 0.05; 0.2 (Dimethylthiophosphate, Diethylthiophosphate)	62.6-71.3 (Dimethylphosphate) 85.6-90.5 (Diethylphosphate) 83.5-88.4 (Dimethylthiophosphate) 91.6-102.0 (Diethylthiophosphate)	(Ueyama et al. 2010)
Chlorpyrifos Chlorpyrifos-oxon	5; urine	SFODME (2-dodecanol)	GC-MS (EI)	0.0048; 0.0156 (Chlorpyrifos) 0.0038; 0.0129 (Chlorpyrifos-oxon)	100.3 (Chlorpyrifos) 109.6 (Chlorpyrifos-oxon)	(Pelit and Yengin 2014)

Dimethylphosphate Diethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylthiophosphate Diethyldithiophosphate	2; urine	LLE (ethyl ether: acetonitrile; 1:1), SPE (ChemElut) and Lyophilization	GC-MS (EI) and GC-MS/MS (EI)	13.1-644.4; 100 (Dimethylphosphate) 48.9-234.3; 100 (Diethylphosphate) 16.8-153.9; 100 (Dimethylthiophosphate) 22.2-119.4; 100 (Dimethyldithiophosphate) 30.3-129.3; 100 (Diethylthiophosphate) 11.3-108.0; 100 (Diethyldithiophosphate) for GC-MS method, 0.01-4.08; 0.4 (Dimethylphosphate) 1.50-2.97 0.4 (Diethylphosphate) 0.07-1.23; 0.4 (Dimethylthiophosphate) 0.02-1.04; 0.4 (Dimethyldithiophosphate) 0.02-2.19; 0.4 (Diethylthiophosphate) 0.01-2.18; 0.4 (Diethyldithiophosphate) for GC-MS/MS method	76-97 (Dimethylphosphate) 85-115 (Diethylphosphate) 20-24 (Dimethylthiophosphate) 22-28 (Dimethyldithiophosphate) 26-27 (Diethylthiophosphate) 18-25 (Diethyldithiophosphate) for LLE; 89-96 (Dimethylphosphate) 96-134 (Diethylphosphate) 4-6 (Dimethylthiophosphate) 19-22 (Dimethyldithiophosphate) 7-17 (Diethylthiophosphate) 21-29 (Diethyldithiophosphate) for SPE; 88-108 (Dimethylphosphate) 96-106 (Diethylphosphate) 80-122 (Dimethylthiophosphate) 73-78 (Dimethyldithiophosphate) 64-131 (Diethylthiophosphate) 65-119 (Diethyldithiophosphate) for Lyophilization	(Weerasekera et al. 2008)
Dimethylphosphate Dimethylthiophosphate Diethylphosphate Diethylthiophosphate 3,5,6-Trichloro-2-pyridinol 3-Methyl-4-nitrophenol 3-Methyl-4-(methylthio)phenol 2-Isopropyl-6-methyl-4-pyrimidinol 2,4-Dichlorophenol	5; urine	SPE (Isolute ENV+) and Derivatization with MTBSTFA	GC-MS (EI)	-; 1.4 (Dimethylphosphate) -; 0.8 (Dimethylthiophosphate) -; 1.2 (Diethylphosphate) -; 1.6 (Diethylthiophosphate) -; 2.4 (3,5,6-Trichloro-2-pyridinol) -; 3.7 (3-Methyl-4-nitrophenol) -; 2.9 (3-Methyl-4-(methylthio)phenol) -; 1.8 (2-Isopropyl-6-methyl-4-pyrimidinol) 2.7 (2,4-Dichlorophenol), LOD of Dimethylphosphate, Dimethylthiophosphate,	50 (Dimethylphosphate) 76 (Dimethylthiophosphate) 103 (Diethylphosphate) 92 (Diethylthiophosphate) 102 (3,5,6-Trichloro-2-pyridinol) 104 (3-Methyl-4-nitrophenol) 81 (3-Methyl-4-(methylthio)phenol) 84 (2-Isopropyl-6-methyl-4-pyrimidinol) 101 (2,4-Dichlorophenol)	(Yoshida and Yoshida 2012)

				Diethylphosphate and Diethylthiophosphate were 0.2-0.5		
Diazinon Diethylphosphate Diethylthiophosphate 2-isopropyl-4-methyl-6-hydroxypyrimidine	2; serum 5; urine	SPE (Oasis HLB) for serum and LLE (diethylether followed by ethyl acetate) for urine	LC-MS/MS (ESI)	5; 10 (Diazinon, 2-isopropyl-4-methyl-6-hydroxypyrimidine) No data for the remaining compounds	-	(Dulaurent et al. 2013)
Dimethylphosphate Dimethylthiophosphate Diethylphosphate Diethylthiophosphate Diethyldithiophosphate	-; urine	Tetrabutyl ammonium acetate	LC-MS/MS (ESI)	2; 5 (Dimethylthiophosphate) 1; 5 (Diethylphosphate) 1; 5 (Diethylthiophosphate) 1; 5 (Diethyldithiophosphate) No data for the remaining compounds	101-119 (Dimethylthiophosphate) 82-95 (Diethylphosphate) 78-87 (Diethylthiophosphate) 82-90 (Diethyldithiophosphate) No data for the remaining compounds	(Hernández et al. 2002b)
3-methyl-4-nitrophenol *p*-nitrophenol	0.5; urine	Hydrolysis with ß-glucuronidase containing sulfatase, LLE (ethyl acetate) and Derivatization with MTBSTFA	GC-MS (EI)	0.3; 1.0 (3-methyl-4-nitrophenol) 0.5; 2.0 (*p*-nitrophenol)	90.0-117.8 (3-methyl-4-nitrophenol) 68.7-104.3 (*p*-nitrophenol)	(Okamura et al. 2012)
3,5,6-Trichloro-2-pyridinol *p*-nitrophenol 2-Diethylamino-6-methyl-4-pyrimidinol 2-Isopropyl-4-methyl-6-hydroxypyrimidine 3-Chloro-7-hydroxy-4-methylcoumarin (3-Chloro-4-methylumbelliferone) 3-Methyl-4-nitrophenol 1,2,3-Benzotriazin-4-one Dimethoate Omethoate Acephate Methamidophos Malathion dicarboxilic acid	5; urine	Hydrolysis with ß-glucuronidase and QuEChERS (Magnesium sulfate; Sodium chloride; Sodium Citrate; Disodium citrate sesquihydrate)	UHPLC-HRMS (ESI)	-; 0.8 (3,5,6-Trichloro-2-pyridinol; *p*-nitrophenol; Omethoate) -; 1.6 (2-Diethylamino-6-methyl-4- pyrimidinol; 2-Isopropyl-4-methyl-6- hydroxypyrimidine; 3-Methyl-4-nitrophenol; Methamidophos; Dimethylthiophosphate; Dimethyldithiophosphate) -; 2 (3-Chloro-7-hydroxy-4-methylcoumarin) -; 50 (1,2,3-Benzotriazin-4-one) -; 3.2 (Dimethoate; Malathion dicarboxilic acid;	72-106 (3,5,6-Trichloro-2-pyridinol) 90-101 (*p*-nitrophenol) 90-110 (2-Diethylamino-6-methyl-4- pyrimidinol) 40-107 (2-Isopropyl-4-methyl-6-hydroxypyrimidine) 54-80 (3-Chloro-7-hydroxy-4-methylcoumarin) 69-92 (3-Methyl-4-nitrophenol) 98-104 (1,2,3-Benzotriazin-4-one) 101.119 (Dimethoate) 103-125 (Omethoate) 71-86 (Acephate) 61-118 (Methamidophos)	(Roca et al. 2014)

Diethylphosphate Diethylthiophosphate Diethyldithiophosphate Dimethylphosphate Dimethylthiophosphate Dimethyldithiophosphate				Diethylthiophosphate; Diethyldithiophosphate) -; 10 (Acephate; Diethylphosphate; Dimethylphosphate)	61-91 (Malathion dicarboxilic acid) 69-111 (Diethylphosphate) 99-117 (Diethylthiophosphate) 71-85 (Diethyldithiophosphate) 96-122 (Dimethylphosphate) 88-127 (Dimethylthiophosphate) 98-114 (Dimethyldithiophosphate)	
Dimethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylphosphate Diethylthiophosphate Diethyldithiophosphate	2; urine	LLE (hexane) and Derivatization with PFBBr	GC-MS/MS (EI)	-; 0.1 (Dimethylphosphate, Dimethylthiophosphate, Dimethyldithiophosphate, Diethylphosphate, Diethylthiophosphate) -; 0.01 (Diethyldithiophosphate)	91-115	(Spaan et al. 2015)
Dimethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylphosphate Diethylthiophosphate Diethyldithiophosphate	0.3; urine	96-well plate SPE (Strata-X-AW)	UPLC-MS (QTOF) (ESI)	1.2; 4.0 (Dimethylphosphate) 0.20; 0.66 (Dimethylthiophosphate) 0.64; 1.9 (Dimethyldithiophosphate) 0.38; 1.3 (Diethylphosphate) 0.33; 1.0 (Diethylthiophosphate) 0.15; 0.50 (Diethyldithiophosphate)	51-63 (Dimethylphosphate) 87-106 (Dimethylthiophosphate) 49-63 (Dimethyldithiophosphate) 102-112 (Diethylphosphate) 83-100 (Diethylthiophosphate) 39-48 (Diethyldithiophosphate)	(Cequier et al. 2016)
Dimethylphosphate Diethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylthiophosphate Diethyldithiophosphate	2; urine	Derivatization with PFBBr	GC-FPD	50; 500 (Dimethylphosphate) 10; 250 (Diethylphosphate) 10; 100 (Dimethylthiophosphate, Dimethyldithiophosphate) 5; 100 (Diethylthiophosphate, Diethyldithiophosphate)	-	(Oglobline et al. 2001b)
Diethylphosphate Dimethylthiophosphate Dimethyldithiophosphate Diethylthiophosphate Diethyldithiophosphate	1; urine	With or without Silver Hydroxide (AgOH) addition, SPE (SAX disk) and Derivatization with methyl iodide	GC-FPD	50; 50 for all compounds	0.0-25.6 (Diethylphosphate) 92.6-96.4 (Dimethylthiophosphate) 0.0-15.5 (Dimethyldithiophosphate) 84.5-98.4 (Diethylthiophosphate) 11.6-34.5 (Diethyldithiophosphate) with AgOH addition; 0.0-25.9 (Diethylphosphate)	(Lin et al. 2007)

					16.5-38.8 (Dimethylthiophosphate) 80.5-87.7 (Dimethyldithiophosphate) 11.5-13.9 (Diethylthiophosphate) 80.8-94.3Diethyldithiophosphate) without AgOH addition	
Quinalphos	0.1; blood and urine	SPME (DI) (CW/DVB, 65 µm)	GC-MS (EI)	10; 50 for blood samples, 2; 10 for urine samples	12.5-16.9 for blood samples; 24.5-27.7 for urine samples	(Gallardo et al. 2006c)
Dimethylphosphate Diethylphosphate Diethylthiophosphate Diethyldithiophosphate	200-500; hair	Methanolic extraction and Derivatization with PFBBr	GC-MS (EI)	6; 20 (Dimethylphosphate) 5; 10 (Diethylphosphate, Diethylthiophosphate) 3; 5 (Diethyldithiophosphate) (in pg/mg)	84.3 (Dimethylphosphate) 116.1 (Diethylphosphate) 109.0 (Diethylthiophosphate) 91.5 (Diethyldithiophosphate)	(Tsatsakis et al. 2010)
Dichlorvos Methamidophos Dimethoate Parathion-methyl Malathion Parathion	300; hair 3; urine	Active magnetic metal-organic framework hybrid material (Fe_3O_4/MIL-101 composite) and Magnetic SPE procedure	GC-NPD	1.20; 4 (Dichlorvos) 2.28; 8 (Methamidophos) 0.21; 1 (Dimethoate) 0.38; 1 (Parathion-methyl) 0.30; 1 (Malathion) 0.28; 1 (Parathion)	76.8-82.6 (Dichlorvos) 92.1-94.5 (Methamidophos) 88.2-89.8 (Dimethoate) 84.1-86.9 (Parathion-methyl) 78.1-88.8 (Malathion) 87.5-88.6 (Parathion) for hair samples; 74.9-82.3 (Dichlorvos) 79.8-89.5 (Methamidophos) 80.1-83.7 (Dimethoate) 90.6-91.7 (Parathion-methyl) 89.3-90.8 (Malathion) 88.1-92.1 (Parathion) for urine samples	(Zhang et al. 2014)
Diazinon Parathion-methyl Malathion Fenthion	200; hair	Methanolic extraction and LLE (ethyl acetate)	GC-ECD and GC-MS (EI)	2.0; 2.5 (Diazinon) 2.0; 5.0 (Parathion-methyl, Malathion, Fenthion) for GC-ECD method, 2.5; 5.0 (Diazinon) 5.0; 5.0 (Parathion-methyl, Malathion, Fenthion) for GC-MS method, (LOD in ng/mL and LOQ in pg/mg)	121 (Diazinon) 124 (Parathion-methyl) 96 (Malathion) 68 (Fenthion)	(Tsatsakis et al. 2008)

Dimethylphosphate Diethylphosphate Dimethylthiophosphate	50; hair	Water extraction, LLE (diethyl ether: acetonitrile; 1:1 and hexane); clean-up on Florisil/Bondesil -PSA column and Derivatization with PFBBr	GC-MS (EI)	0.10; 0.33 (Dimethylphosphate) 0.02; 0.06 (Diethylphosphate) 0.10; 0.34 (Dimethylthiophosphate) (in ng/mg)	56.1-66.5 (Dimethylphosphate) 61.1-98.9 (Diethylphosphate) 97.7-107.9 (Dimethylthiophosphate)	(Margariti and Tsatsakis 2009)
Diazinon Fenitrothion Malathion	50; hair	HF-SPME	HPLC-PDA	0.6200; 2.000 (Diazinon) 0.0074; 0.024 (Fenitrothion) 1.3000; 4.200 (Malathion) (in ng/g)	83-92	(Ebrahimi et al. 2012)
Dimethylphosphate Diethylphosphate	100; hair	Methanolic extraction and Derivatization with PFBBr	GC-MS (EI)	-	-	(Kanavouras et al. 2011)
Dimethylphosphate Diethylphosphate Diethylthiophosphate Diethyldithiophosphate	100; hair	Methanolic extraction and Derivatization with PFBBr	GC-MS (EI)	6; - (Dimethylphosphate) 5; - (Diethylphosphate, Diethylthiophosphate) 3; - (Diethyldithiophosphate) (in pg/mg)	-	(Knipe et al. 2016)
Diazinon, Chlorpyriphos-methyl, Parathion-methyl, Malathion, Chlorpyrifos-ethyl, Parathion-ethyl, 3-Methyl-4-nitrophenol, Dimethylphosphate, Diethylphosphate, Dimethylthiophosphate, Dimethyldithiophosphate, Diethylthiophosphate, Diethyldithiophosphate, 3,5,6-Trichloro-2-pyridinol, *p*-nitrophenol, Malathion dicarboxilic acid, 2-Isopropyl-4-methyl-6-hydroxypyrimidine	50; hair	Comparison SPE (Florisil/PSA, GCB/PSA and SAX/PSA) and LLE (ethyl acetate, hexane: ethyl acetate; 80:20) or hexane: dichloromethane; 80:20)	GC-MS/MS (EI) and LC-MS/MS (ESI)	-	50-150	(Duca et al. 2014)

Dimethylphosphate Diethylphosphate Diethylthiophosphate Diethyldithiophosphate	100; hair 2; urine	Methanolic extraction for hair, LLE (acetonitrile: diethylether; 1:1) for urine and Derivatization with PFBBr	GC-MS (EI)	4.2; 9.0 (Dimethylphosphate) 2.6; 5.5 (Diethylphosphate) 2.4; 5.2 (Diethylthiophosphate) 1.4; 2.6 (Diethyldithiophosphate) for hair samples (in pg/mg), 0.06; 0.20 (Dimethylphosphate) 0.02; 0.08 (Diethylphosphate) 0.03; 0.10 (Diethylthiophosphate) 0.05; 0.18 (Diethyldithiophosphate) for urine samples (in ng/mL)	106.8 (Dimethylphosphate) 92.0 (Diethylphosphate) 89.1 (Diethylthiophosphate) 74.9 (Diethyldithiophosphate) for hair samples; 83.0 (Dimethylphosphate) 92.0 (Diethylphosphate) 101.7 (Diethylthiophosphate) 90.7 (Diethyldithiophosphate) for urine samples	(Kokkinaki et al. 2014)
Dimethylphosphate Diethylphosphate Dimethylthiophosphate Diethylthiophosphate Diethyldithiophosphate	500; meconium	Methanol, LLE (acetonitrile: diethylether; 1:1) and Derivatization with PFBBr	GC-MS (EI)	5.0; 2.50 (Dimethylphosphate) 5.0; 1.50 (Diethylphosphate) 10.0; 5.00 (Dimethylthiophosphate) 5.0; 1.25 (Diethylthiophosphate) 2.5; 0.50 (Diethyldithiophosphate) (LOD in ng/mL and LOQ in ng/g)	76.9 (Dimethylphosphate) 65.2 (Diethylphosphate) 94.1 (Dimethylthiophosphate) 109.4 (Diethylthiophosphate) 107.2 (Diethyldithiophosphate)	(Tsatsakis et al. 2009)
Diethylphosphate Diethylthiophosphate Diethyldithiophosphate Dimethylphosphate Dimethylthiophosphate Dimethyldithiophosphate	500; meconium	Methanol and Derivatization (without information about the reagent)	GC-MS/MS (ID)	0.2; - (Diethylphosphate) 0.09; - (Diethylthiophosphate) 0.05; - (Diethyldithiophosphate) 0.51; - (Dimethylphosphate) 0.18; - (Dimethylthiophosphate) 0.08; - (Dimethyldithiophosphate) (in µg/g)	26 (Diethylphosphate) 55 (Diethylthiophosphate) 62 (Diethyldithiophosphate) 18 (Dimethylphosphate) 63 (Dimethylthiophosphate) 66 (Dimethyldithiophosphate)	(Whyatt and Barr 2001)
Chlorpyrifos	3; serum and gastric content	LLE (diethyl ether)	GC-FID and GC-MS (EI)	25; 83 for GC-MS method (in ng/mL)	102-104	(Martínez et al. 2004)
Parathion-methyl	2; blood (LLE) 0.3; blood, stomach content, liver and kidney (HS-SPME)	LLE (petroleum ether), purification for TLC and HS-SPME (PA, 85 µm)	GC-NPD	50; 100 (for blood samples and SPME) No data for the remaining compounds	46 for blood samples; 53 for liver samples; 54 for kidney samples. No data for the remaining compounds	(Tsoukali et al. 2004)
Dimethoate Dimethylphosphate Diethylphosphate Dimethylthiophosphate	1; blood, urine and gastric contents	LLE (Toxi® extraction tubes) for dimethoate, LLE	GC-MS (EI)	500; 1500 (for Dimethoate in blood samples) No data for the remaining compounds	-	(Tarbah et al. 2007)

Diethylthiophosphate Dimethyldithiophosphate Diethyldithiophosphate	500; tissues (brain, myocardial muscle, liver, lung, skeletal muscle, kidney and gall bladder) for Dimethoate 0.1; blood, urine and gastric contents, for remaining compounds	(acetonitrile, heptane) and Derivatization with PFBBr for other compounds				
Dimethylphosphate	0.1; blood, urine and gastric fluid	LLE (heptane) and Derivatization with PFBBr	GC-NPD and GC-MS (EI)	60; 500	60	(Tarbah et al. 2004)
Methidathion	500; blood, urine and stomach contents	SPE (Extrelut NT®)	GC-MS (EI)	50 (on-column); 100 (in ng/g)	74.1	(Takayasu et al. 2012)
Dichlorvos	0.25; plasma, urine and stomach content 5000; tissues (heart, lung, liver, kidney)	LLE (dichloromethane: diethyl ether: hexane: isoamylic alcohol; 150:250:100:2.5)	GC-MS (EI) and LC-PDA	275; 1000	100.2-111.9	(Abe et al. 2008)
Malathion	1; blood, liver and gastric contents	LLE (methyl t-butyl ether)	GC-MS (EI)	1000; - (for gastric content samples) (in ng/g) No data for the remaining compounds	-	(Thompson et al. 1998)

Prothiophos	2000; liver 1000; brain, kidney, gastric contents and intestinal contents	SPE (Extrelut®)	GC-FPD and GC-MS (EI)	-; 0.5 (in ng/g)	-	(Miyazaki et al. 1988)

µECD: Microelectron capture detection; **APCI:** Atmospheric pressure chemical ionization; **C8:** Octylsilane; **C18:** Octadecylsilane; **CW/DVB:** Carbowax™/divinylbenzene; **DI:** Desorption ionization; **ECD:** Electron capture detector; **EI:** Electron ionization; **ESI:** Electrospray ionization; **FA-DSDME:** Fast agitated directly suspended droplet microextraction; **FID:** Flame ionization detector; **FPD:** Flame photometric detector; **GC:** Gas chromatography; **HESI:** Heated electrospray ionization; **HF-SPME:** Hollow fiber solid phase microextraction; **HLPC:** High-performance liquid chromatography; **HRMS:** High-resolution mass spectrometry; **HS-SPME:** Headspace-solid phase microextraction; **LC:** Liquid chromatography; **LLE:** Liquid-liquid extraction; **LLME:** Liquid-liquid microextraction; **LOD:** Limit of detection; **LOQ:** Limit of quantitation; **MEPS:** Microextraction by packed sorbent; **MISPE:** Molecularly imprinted solid-phase extraction; **MS:** Mass spectrometry; **MTBSTFA:** *N*-methyl-*N*-(t*ert*-butyldimethylsilyl) trifluoroacetamide; **NCI:** Negative chemical ionization; **NPD:** Nitrogen phosphorous detector; **PA:** Polyacrylate; **PDA:** Photodiode array detector; **PDMS:** Polydimethylsiloxane; **PFBBr:** Pentafluorobenzyl bromide; **PFPD:** Pulse flame photometric detector; **PSA:** Primary-secondary amine; **QTOF:** Quadrupole Time of Flight; **QuEChERS:** Quick, easy, cheap, effective, rugged, and safe; **SA-DSPE:** Solvent-assisted dispersive solid-phase extraction; **SFODME:** Solidified floating organic drop microextraction; **SPE:** Solid-phase extraction; **SPME:** Solid-phase microextraction; **SPMEM:** Solid-phase micro-extraction membrane; **TIS:** Turbo ion spray; **TLC:** Thin layer chromatography; **UHPLC:** Ultra-high performance liquid chromatography; **UPLC:** Ultraperformance liquid chromatography.

In the absence of the LOQ value, the lowest point of the calibration curve was considered.

7. Conclusions and future perspective

The use of pesticides has affected human societies and man worldwide since ancient times to the present day.

This exposure to pesticides, specifically to the organophosphorus class, has been documented through the analysis of biological samples, mainly blood, plasma, serum and urine specimens. These specimens are the most commonly used for analysis in clinical and forensic toxicology. However, it is thought that toxicological studies should not only depend on the analysis of these samples, but may be complemented by the analysis of other non-conventional biological matrices such as oral fluid, hair and nails. One of the advantages of using these samples, compared to the more traditional ones, is that their collection is less invasive, being easier and less uncomfortable for the patient. However, few or no studies and analytical methods exist for the identification of these compounds in these samples.

Over the last few years, most of the procedures for sample preparation involve micro approaches, and there has been developments in GC-MS and LC-MS technologies for the identification of these compounds, being accessible to most laboratories nowadays. These methods contributed to reduce the amount of sample used in the analysis as well as to obtain lower LODs and LOQs.

In the future, instruments are expected to become even more sensitive and accurate, and other analytical technologies can be used. Consequently, as the sensitivity of the analytical equipment increases, there will also be a trend to reduce sample volume, which may be decisive in the case of little matrix availability; this is also important from the analytical point of view, since matrix-borne interferences will affect analysis to a lesser extent.

In addition, more efficient sample cleaning procedures are also being developed, less time consuming and less expensive and harmful to the environment with the use of lower amounts of organic solvents.

Concluding, what is being more and more are rapid, sensitive and specific trails, which are also miniaturized and prone to automation. Analytical methodologies for the identification of these compounds in cases of *ante-* and *postmortem* intoxication should be developed, validated before routine use and the analytical data should be shared through different communication platforms to let the results available quickly enough to contribute to the immediate attention of an intoxicated individual.

Conflict of interest and Ethical standards

The authors declare that they have no conflict of interest. The manuscript does not contain clinical studies or patient data.

Acknowledgments

This work is supported by FEDER funds through the POCI - COMPETE 2020 - Operational Programme Competitiveness and Internationalisation in Axis I - Strengthening research, technological development and innovation (Project POCI-01-0145-FEDER-007491) and National Funds by Fundação para a Ciência e a Tecnologia (FCT) (Project UID/Multi /00709/2013).

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Chapter II: Experimental developments

1. Introduction

Organophosphorus insecticides were created in the 1930s before the World War II and have widespread all over the world due to their high efficiency to control pests affecting crops [1]. Despite several warnings and legislation to control the use of these compounds, organophosphorus pesticides are often implicated in human intoxications [2], being also responsible for many suicide deaths in rural areas [3]. The morbidity and mortality attributable to these substances depend on many variables, such as the level of socioeconomic development, the accessibility of these chemicals and the importance of the agricultural sector; these variables change from country to country. Pesticide fatalities are a consequence of accidents, self-injury or more rarely homicides and range from less than 1% of intoxication deaths in European Union (EU) countries [2, 4] to 71% of all violent deaths in the Western Pacific and Southeast Asia [5]. According to the Laboratory of Forensic Toxicology of the South Branch of the National Institute of Legal Medicine in Portugal, these insecticides were involved in 86 intoxications between 2003 and 2006 (unpublished data). Diazinon, parathion-ethyl, chlorpyrifos, chlorfenvinphos and quinalphos were among the most detected compounds [3], and have been included in this work.

Many publications report different sample cleaning techniques for the determination of these substances in blood, including protein precipitation [6], solid phase microextraction (SPME) [7], liquid-liquid extraction (LLE) [8] and solid phase extraction (SPE) [9]. The use of chromatographic techniques, such as high performance liquid chromatography (HPLC) or gas chromatography (GC), combined with the detection of analytes by tandem mass spectrometry (MS/MS) has been an interesting approach, as well as a very useful tool in forensic toxicology laboratories, allowing high selectivity and very low detection limits. The virtual elimination of isobaric interferences is allowed, as well as a significant decrease of the chemical noise of the matrix [10, 11]. Furthermore, the high speed of acquisition in the multiple reaction monitoring (MRM) mode allows the development of methods for the simultaneous analysis of a huge number of compounds belonging to different chemical classes [12].

Dried blood spots (DBS) sampling is a non-invasive technique that involves a low volume of blood, being obtained directly from a blood sample and dried on a paper substrate before being analysed in a laboratory. This sampling method can be used in neonatal screening for innate diseases [13], and also other applications, such as HIV screening and monitoring [14], in the monitoring of therapeutic drugs as an alternative to the use of venous blood [15]. This approach is now used for research in several areas such as pharmacology [13], epidemiology [16] and anthropology [17]. Some of the advantages of using this sampling technique include increased performance, simplified logistics and reduced costs [18] as well as the need for a smaller sample

volume compared to the use of venous blood. This method also has the advantage of fixing and drying the blood in this support matrix, which simplifies its handling when compared to other samples, namely liquid samples. Also, the ease of sampling is one of the advantages of this procedure. However, in order to replace the use of venous blood in other matrices, under all circumstances, there are still problems to be solved, namely the difficulty in quantitative analysis of blood concentrations, the imprecision of the measured volume [19] and the heterogeneity of the sample [20].

DBS sampling technique is becoming known for bioanalysis, very useful in genetics and screening of metabolic diseases. Recently, emerging applications related to DBS sampling and analysis have been also described, specifically in the field of toxicology as a new paradigm.

This article describes a sensitive method for the determination of the above-mentioned organophosphorus insecticides in whole blood using dried blood spots (DBS) and gas chromatography/tandem mass spectrometry (GC-MS/MS). To our best knowledge, this is the first study to report the determination of these compounds in blood samples by this extraction technique.

2. Experimental

2.1. Reagents and standards

Standard solutions of diazinon (DZN), chlorpyrifos (CLP), parathion-ethyl (PRT), chlorfenvinphos (CLF) and quinalphos (QLP) were acquired from Sigma-Aldrich (Lisbon, Portugal). Ethion (ETH) was acquired from Sigma-Aldrich (Lisbon, Portugal). It is noteworthy that ETH, used as internal standard (IS), is an insecticide not commercially available in Portugal, so it is highly unlikely to appear in an authentic sample, thus impairing quantitative analysis. In addition, the chemical structure of this compound is similar to that of the other compounds studied, allowing to improve linearity, accuracy and precision, while minimizing analyte losses during sample preparation. All standards were acquired at 1 mg/mL. Methanol (Merck Co, Darmstadt, Germany), and acetonitrile (Carlo Erba Reagents, Val de Reuil, France) were both of analytical grade. Dichloromethane, 2-propanol, hexane and ethyl acetate were acquired from Fisher Scientific, Loughborough, UK. The blood was applied to filter paper obtained from Whatman 903 Protein Saver Snap (Cardiff, UK). Working standard solutions were prepared by properly diluting the starting solutions with methanol to the final concentrations of 5, 50 and 100 µg/mL for all analytes. The IS working solution was prepared in methanol at a concentration of 5 µg/mL. All those solutions were stored in the absence of light at 4 °C.

2.2. Sample collection and pre-treatment

Blank blood samples were provided by the exceeding supplies (outdated transfusions) of the local blood banks. Authentic blood samples used for analysis were provided by the emergency services of Centro Hospitalar Cova da Beira, Covilhã, Portugal. These samples were stored frozen at -20 ºC until analysis.

2.3. Gas chromatography and mass spectrometry conditions

Chromatographic analysis was performed using an HP 7890A gas chromatography system equipped with a triple quadrupole mass spectrometer model 7000B, both from Agilent Technologies (Waldbronn, Germany), a MPS2 auto sampler and a PTV-injector from Gerstel (Mülheim an der Ruhr, Germany). For the chromatographic resolution of pesticide substances, a capillary column (30 m x 0.25 mm I.D., 0.25 µm film thickness) with 5% phenylmethylsiloxane (HP-5MS), supplied by J & W Scientific (Folsom, USA), was used.

The initial oven temperature was maintained at 100 °C for 4 minutes, then increased to 200 ºC at 10 °C/min. Finally, the temperature was increased to 270 ºC at 24 ºC/min (maintained for 8 minutes), giving a total run time of 25 minutes. The injection inlet temperature was set at 240 ºC and the detector temperature was set at 280 °C. The sample was introduced into the gas chromatograph by splitless injection mode and the helium flow (carrier gas) was 1.0 mL/min at a constant flow rate. The mass spectrometer was operated with a filament current of 35 µA and electron energy 70 eV in the positive electron ionization mode and nitrogen was used as collision gas at a flow rate of 2.5 mL/min. Data was acquired in the MRM mode using the MassHunter WorkStation Acquisition Software rev. B.02.01 (Agilent Technologies).

Retention times and mass-to-charge ratio (m/z) spectra were initially obtained by individually injecting each of the standard solutions at a high concentration (10 µg/mL) and subsequently used to identify the different compounds. Three transitions were chosen for each of the compounds (one quantitative and two qualitative). The most abundant transition was used for quantitation, while the second and third transitions were for confirmation purposes. This choice was made to obtain better selectivity and sensitivity for the analytes and less interference from the matrix. The choice of the ions for the transitions was based on the higher masses and more abundant mass peaks (including more specific masses for each compound) to maximize signal-to-noise ratio in matrix extracts. Table 1 shows the detection criteria for each analyte, namely retention time, quantifier transitions, qualifier transitions and collision energies selected for each compound.

Table 1. Retention times, selected transitions and collision energy for the identification of the organophosphorus pesticides.

Analyte	Retention time (min)	Quantifier transition (m/z)	Qualifier transitions (m/z)	Collision energy (eV)
DZN	14.84	136.6 – 84.1	303.6 – 179.1 178.3 – 137.1	10 (10 ; 15)
CLP	16.23	196.6 – 168.9	313.1 – 257.8 313.1 – 285.9	15 (15 ; 5)
PRT	16.26	290.8 – 109.0	290.8 – 81.0 290.8 – 137.1	10 (20 ; 5)
CLF	16.72	266.1 – 159.0	322.0 – 266.9 322.0 – 294.9	15 (15 ; 5)
QLP	16.76	156.3 – 129.1	145.8 – 118.1 297.6 – 190.1	10 (10 ; 5)
ETH*	17.75	230.6 – 128.9	-	10

***Internal Standard

2.4. Sample preparation

The final process for the extraction was as follows: 50 µL of blood was applied to Whatman® 903 Proteinsaver snap and dried for 12 hours. After that, 3 mL of methanol:acetonitrile (50:50) was added, followed by slight agitation for 10 minutes at room temperature. The card was removed and the samples were centrifuged for 10 minutes at 3000 rpm. The extract was evaporated to dryness and reconstituted in 65 µL of methanol. The extracts were transferred to autosampler vials and a 2 µL aliquot was injected in the GC-MS/MS system.

3. Results and discussion

3.1. Extraction optimization

The optimization of the extraction process to the DBS began with the appropriate selection of the extraction solvent. The extraction solvent must be able to solubilize the analytes of interest, minimizing the co-extraction of other matrix components that can interfere with chromatographic analysis. When choosing the extraction solvent, it is also important to take into account its compatibility with the analytical technique, as well as its volatility because the concentration of the extracts is often deemed necessary [21].

The polarity of the solvent should be close to those of the target compounds. Mixtures of low- and high-polar solvents can also provide more efficient extractions than single solvents when analytes with a wide range of polarities are extracted [21]. In this study, several solvents were evaluated in order to choose the one allowing obtaining better recoveries of the target analytes. The solvents chosen for this first step of the optimization were methanol,

acetonitrile, dichloromethane, 2-propanol, hexane, ethyl acetate, as well as 2 mixtures, methanol:acetonitrile (1:1) and hexane:ethyl acetate (1:1). The results obtained are shown in Figure 1. It is possible to observe better recoveries of DZN, PRT and CLP when a mixture of methanol:acetonitrile (1:1) was used for their extraction. This mixture seems to be more efficient than using methanol alone, although this is clearly the second best choice for the mentioned analytes. However, methanol seems to provide greater recoveries for CLF and QLP, but no significant differences are observed when compared to the application of methanol:acetonitrile (1:1) [$F(1.4) = 4.15$ $p < 0.05$] for CLF and [$F(1.4) = 6.52$ $p < 0.05$] for QLP. Regarding the solvent mixture of methanol:acetonitrile (1:1), the recoveries obtained for DZN, PRT and CLP were significantly greater when compared to using methanol [$F(1.4) = 110.73$ $p < 0.05$], [$F(1.4) = 58.12$ $p < 0.05$] and [$F(1.4) = 9.42$ $p < 0.05$], respectively.

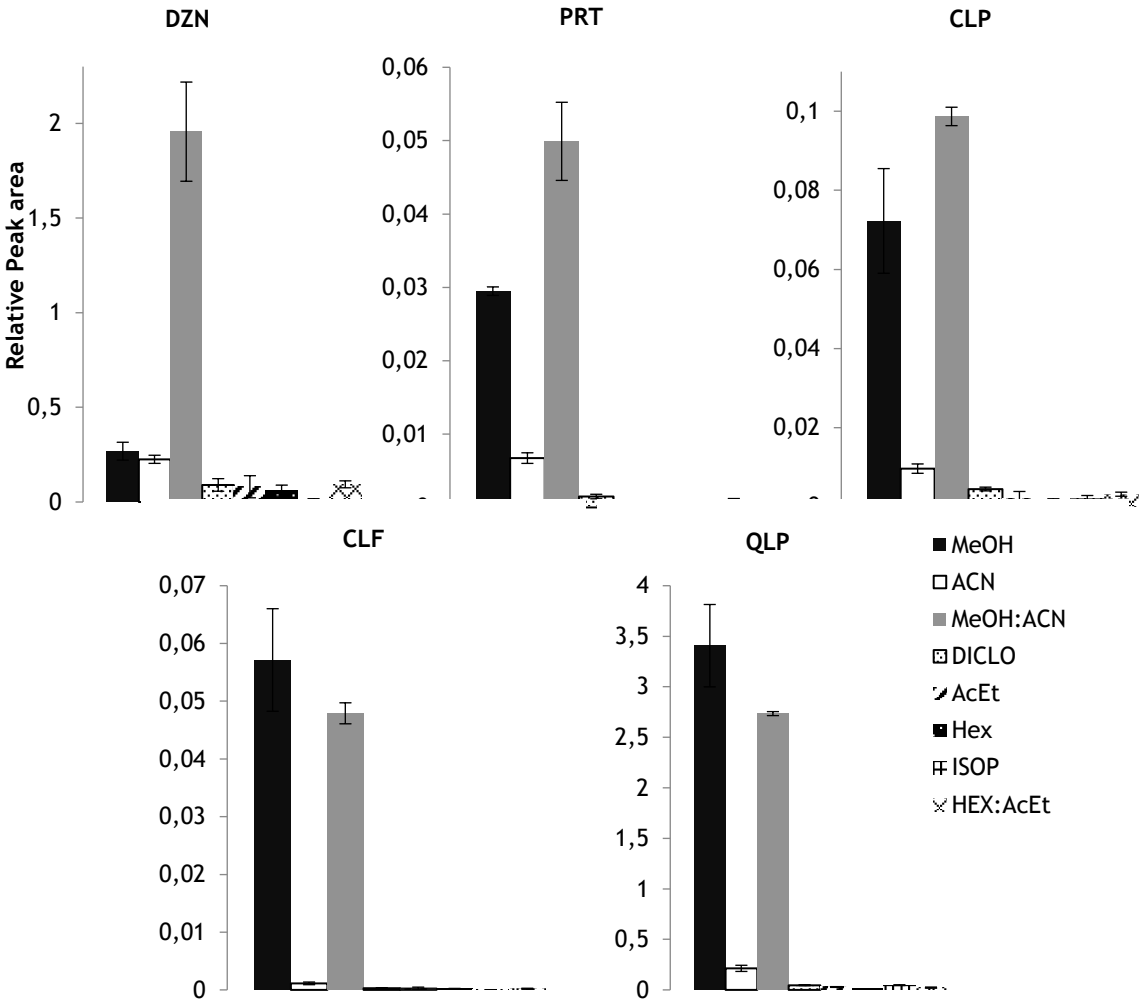


Figure 1. Effects of the different organic solvents and/or mixtures in the extraction process.

Once a solvent mixture seemed to result in greater extraction efficiency for most of the target analytes, it was considered pertinent to evaluate the ideal proportions of the organic solvents

present in the mixture. Subsequently, three different proportions of methanol:acetonitrile mixture were tested (10:90, 50:50 and 90:10). The proportion with better recoveries was 50:50, except for CLF (90:10). Regarding DZN, the extraction efficiency was significantly greater when 50:50 was used instead of 90:10 [$F(1.4) = 55.62$ $p < 0.05$]. For all the other analytes, although greater results were obtained when methanol:acetonitrile (50:50) was used, no significant differences were observed when compared to 90:10. All data is shown in Figure 2.

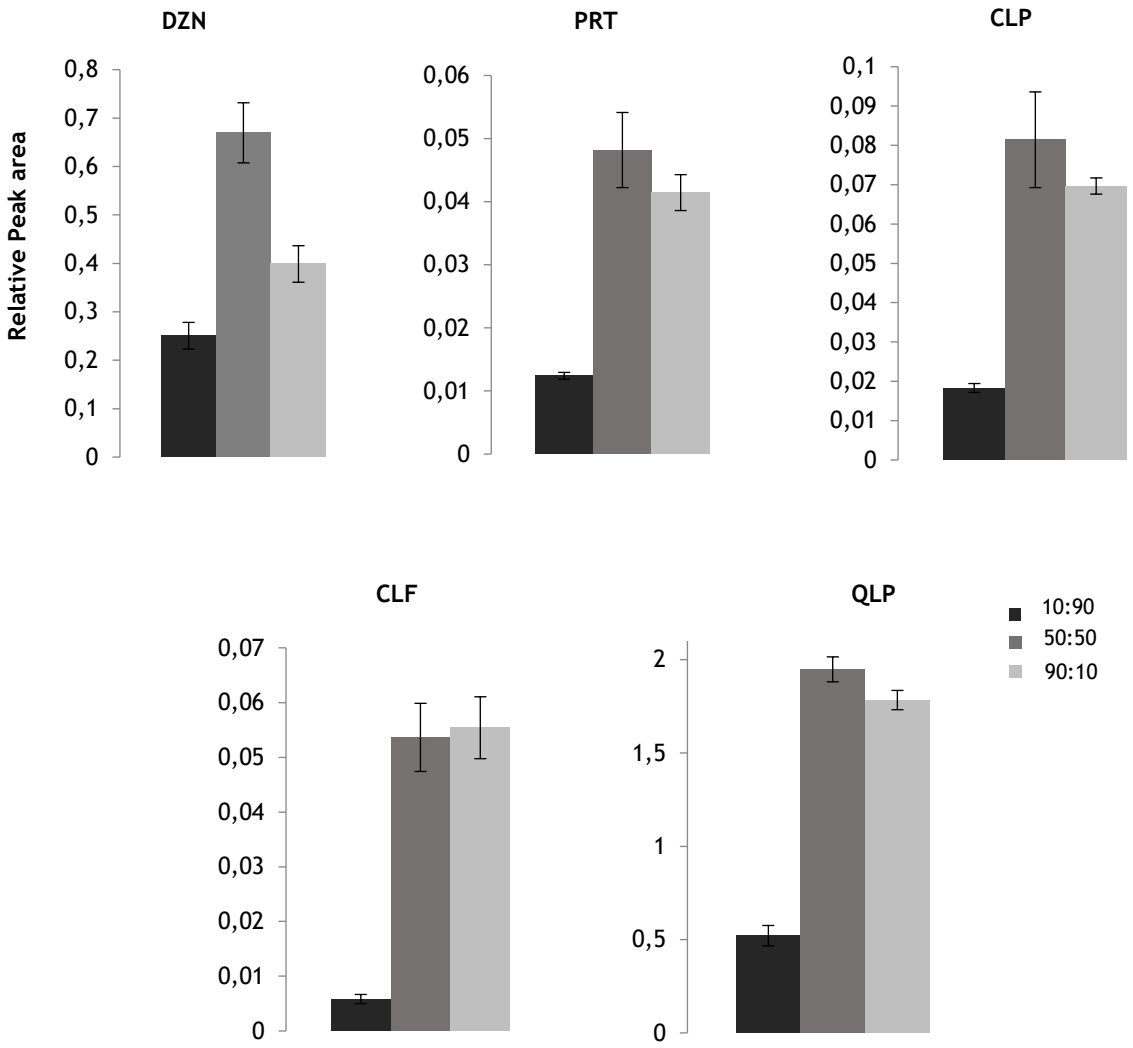


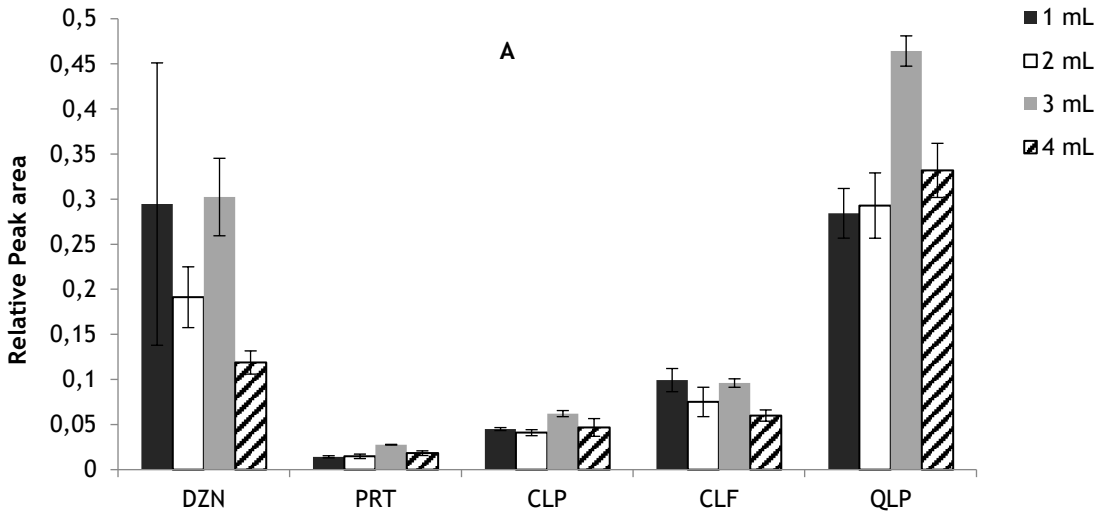
Figure 2. Effects of the different mixtures of methanol:acetonitrile in the extraction process.

The volume of solvent was subsequently evaluated (1 to 4 mL) and the results obtained are shown in Figure 3A. Overall, 3 mL of solvent seem to result in greater recoveries, particularly for QLP, which presents a significant difference when compared to 1 and 4 mL, [$F(1.4) = 117.83$ $p < 0.05$] and [$F(1.4) = 44.49$ $p < 0.05$], respectively. Lower standard deviations and associated errors are also observed when 3 mL of the extraction solvent is used, which also makes it as an excellent option.

Another relevant parameter, also studied, was the extraction time (10 to 40 min) that might result in a greater recovery of the target analytes, as well as influence signal intensity. All studied times of extraction resulted in similar recoveries, with no significant differences between them. The extraction of these compounds appears not to depend on the time of exposure to the solvent mixture. In order to make the process faster, 10 minutes was chosen (Figure 3B).

The temperature's influence on the extraction process (room temperature to 80 ºC) was also studied. The results obtained are shown in Figure 3C. From all the tested temperatures, 50 °C has provided slightly better recoveries when compared to other temperatures. However, for all compounds, there are no significant differences when compared to 25 ºC (room temperature), and therefore this temperature was chosen.

Centrifugation time was optimized in order to obtain cleaner extracts since blood is a very complex biological matrix (10 to 30 min). Regarding this parameter, 30 minutes appear to result in cleaner extracts. However, there are no significant differences when compared to 10 minutes of centrifugation after extraction. This way, the process becomes way simpler and faster if this final procedure takes the least amount of time possible, justifying the choice of 10 minutes. The results obtained are shown in Figure 3D.



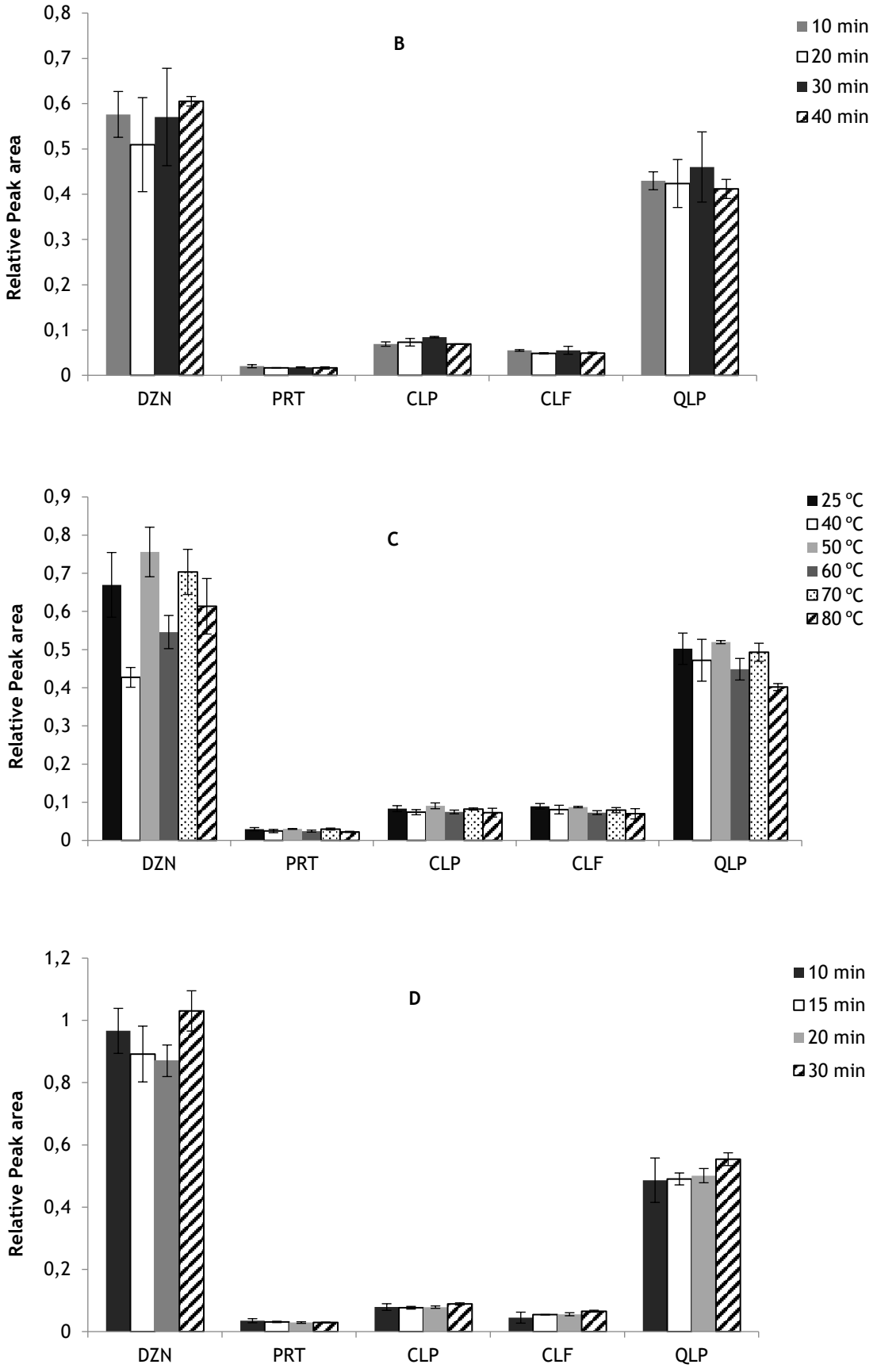


Figure 3. Effects of the different solvent volumes (3A), extraction time (3B), temperature (3C) and centrifugation conditions (3D) in the extraction process.

The period of time during which the blood spot is left to dry in the paper is also of great interest. In fact, with this evaluation, one has to study the influence of the complete drying of the blood spot on the recovery of the target analytes, as well as on the minimization of matrix interferences. This evaluation was performed at five periods of time (from 1h to overnight) (Figure 4A). A drying time of 12 hours or during overnight appear to result in greater extraction efficiencies as well as cleaner extracts. However, between 12 hours and overnight there is no significant difference. Moreover, since the 12 hours have the lowest associated errors and since it facilitates the control of the extraction by the operator, it was the drying time chosen for the extraction process.

The sample volume was also tested to adequate the signal strength and the smallest amount of sample that could be used. For all compounds, the results are significantly greater when 50 µL of sample is used, [$F(1.4) = 596.56$ $p < 0.05$] for DZN, [$F(1.4) = 456.73$ $p < 0.05$] for PRT, [$F(1.4) = 173.79$ $p < 0.05$] for CLP, [$F(1.4) = 464.25$ $p < 0.05$] for CLF and [$F(1.4) = 365.05$ $p < 0.05$] for QLP. The results obtained are shown in Figure 4B.

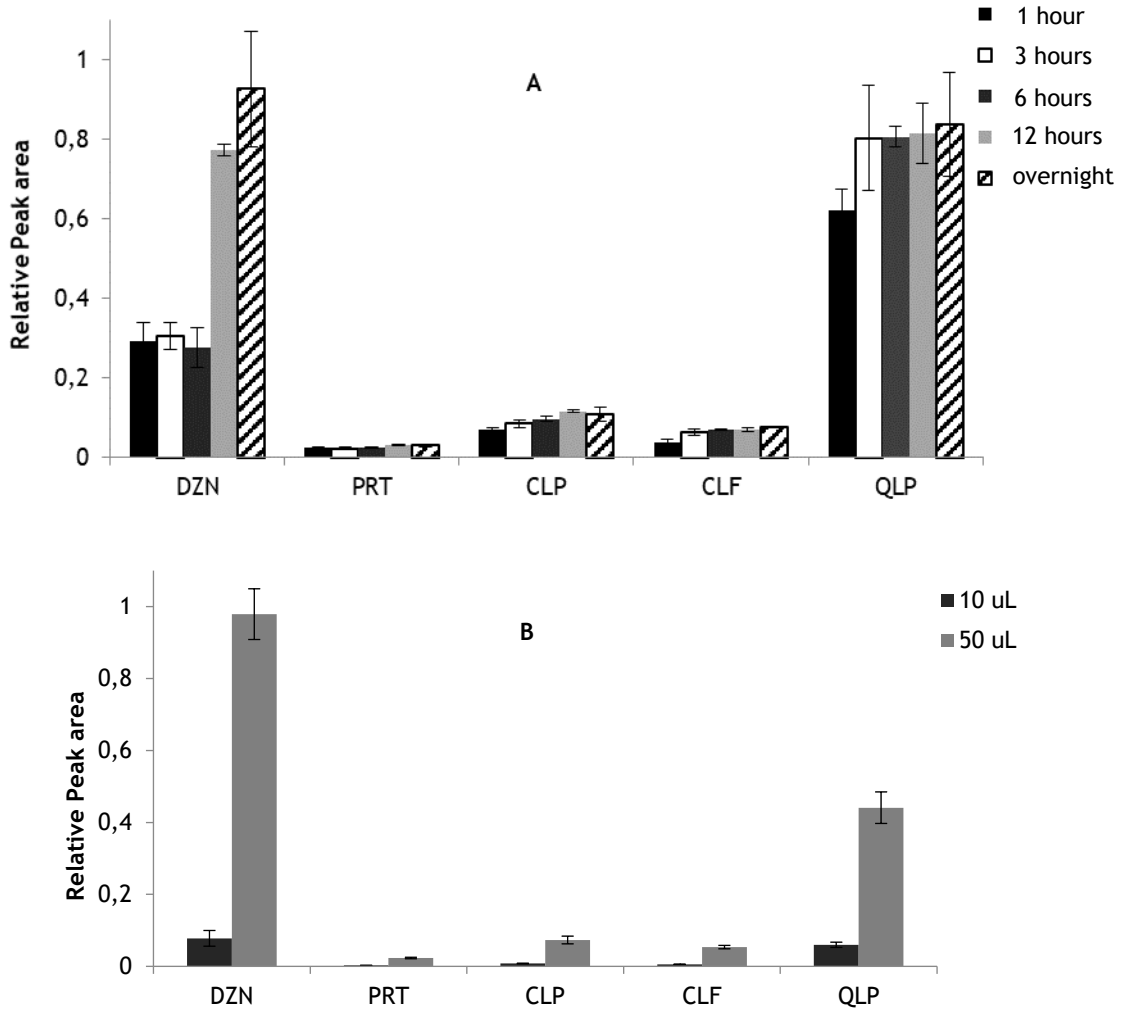


Figure 4. Evaluation of the influence of the drying time of the samples (4A) and the volume of the sample (4B) in the extraction process.

3.2. Validation procedure

The described technique was validated according to the guiding principles of the Food and Drug Administration (FDA) [22], the International Conference on Harmonization (ICH) [23] and the Scientific Working Group for Forensic Toxicology (SWGTOX) [24]. The validation was achieved following a 5-day validation procedure and the studied parameters included selectivity, linearity and limits, intra- and inter-day precision and accuracy, recovery, stability and dilution integrity.

3.3. Selectivity

The selectivity of the method was evaluated by analysing pools of blank blood samples. Each pool contained blood of 10 different origins, in order to investigate possible interferences in the retention times and selected ions of the studied analytes.

The criteria considered for a positive identification with associated confidence included an absolute retention time within 2% or ± 0.1 min of the retention time of the same compound in the control sample and also the presence of three transitions per analyte. The maximum allowable tolerances for the relative ionic intensities between the transitions (as a percentage of the base peak) were: if the relative ion intensity in the control sample was higher than 50%, an absolute tolerance of ± 10% was accepted; if this value was between 25 and 50%, a relative tolerance of ± 20% was permitted; if it was between 5 and 25%, an absolute tolerance of ± 5% was accepted and lastly, for relative ion intensities of 5% or less, a relative tolerance of ± 50% was used [25]. As these previously described criteria, the method would be considered selective if no analyte could be identified in the blank samples.

After the selectivity was evaluated, no interferences from endogenous substances were observed at the retention times and transitions selected for each analyte, and therefore the method was considered selective (Figures 5 and 6 show chromatograms of a blank sample and a sample spiked at the lower limit of quantification (LLOQ)).

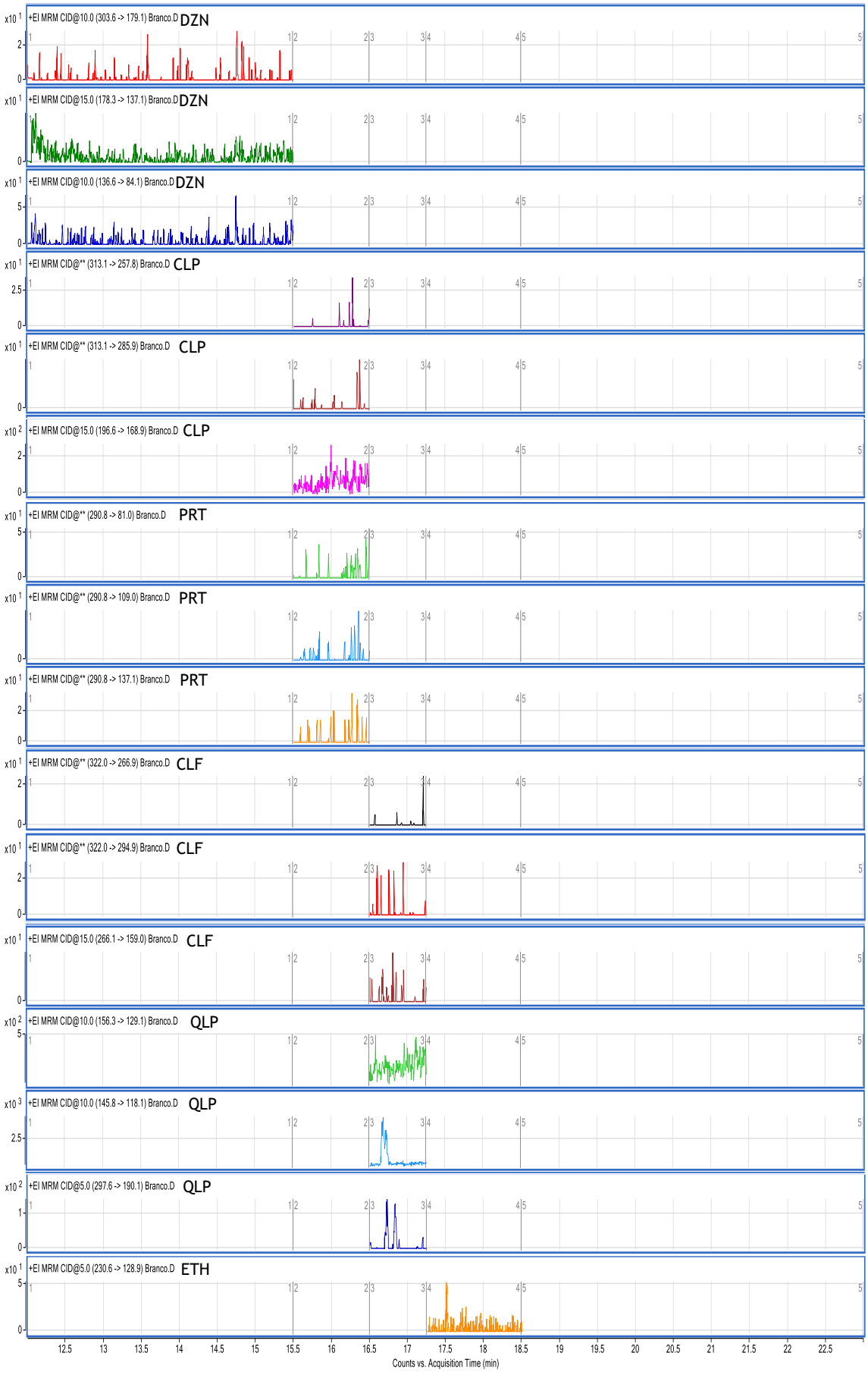


Figure 5. Chromatogram of selected fragments obtained after extraction of a blank sample.

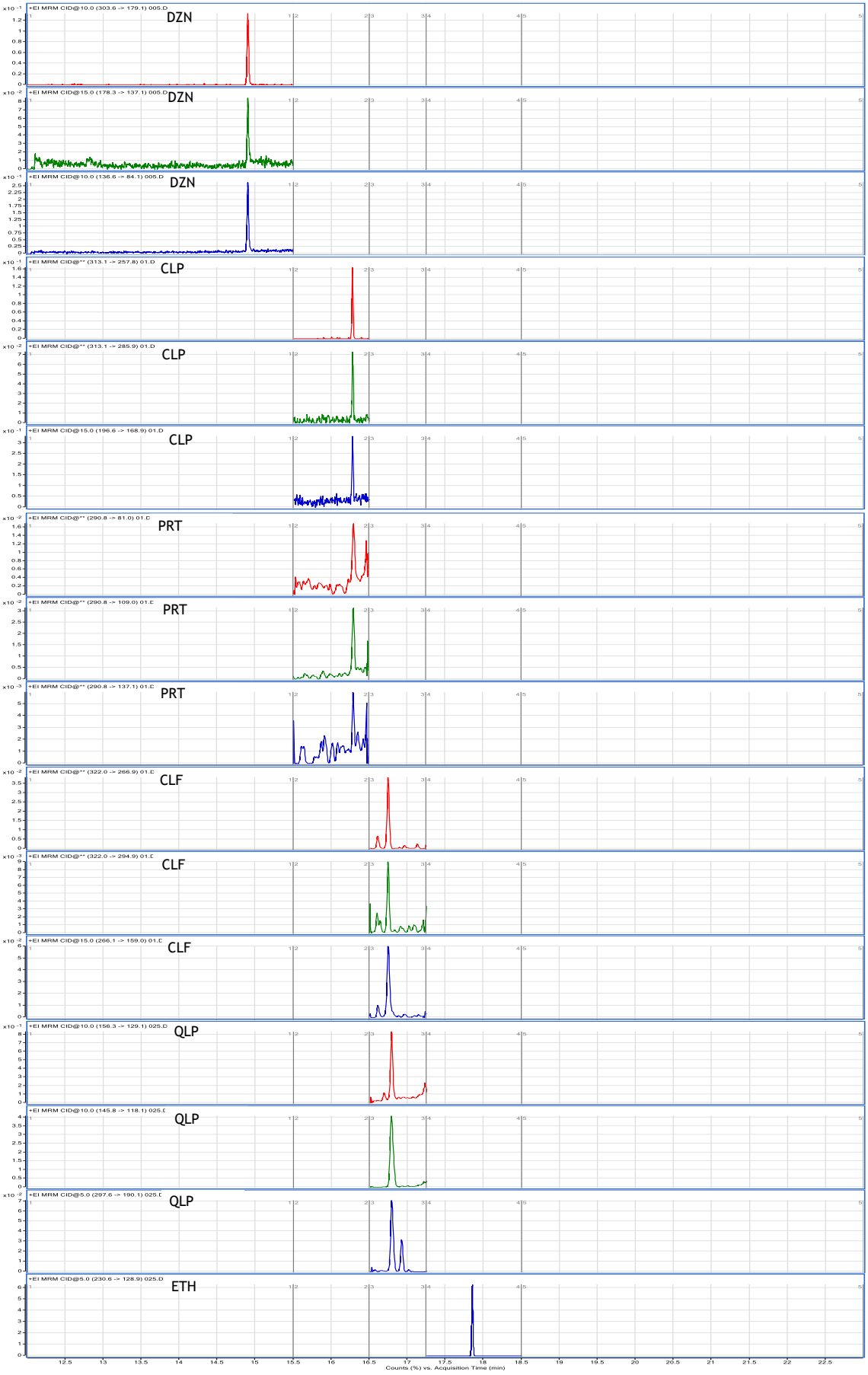


Figure 6. Chromatogram of selected fragments obtained after extraction of samples spiked at the LLOQ.

3.4. Calibration curves and limits

Spiked samples were prepared and analyzed using the above described extraction procedure in the range of 0.1-25 µg/mL for all compounds, except for DZN (0.05-25 µg/mL) and for QLP (0.25-25 µg/mL), establishing the linearity of the method. This was evaluated with five replicates and the calibration curves were obtained by plotting the peak area ratio between each analyte and the IS against analyte concentration. For a calibration curve to be accepted, the applied criteria included (1) a determination coefficient (R^2) value of at least 0.99 and (2) the calibrators' accuracy within ± 15% (except at the LLOQ, where ± 20% was considered acceptable). The adopted calibration ranges were wide and in order to compensate for heteroscedasticity, weighted least squares regressions had to be adopted (six weighting factors were studied: 1/√x, 1/x, 1/x2, 1/√y, 1/y, 1/y2). The weighting factor that resulted in the lower sum of relative errors and simultaneously a mean R^2 value of at least 0.99 was chosen (Table 2).

Regarding LLOQ, this was considered as the lowest concentration that could be measured with acceptable precision and accuracy, i.e. with a coefficient of variation (CV, %) of less than 20% and a relative error (RE, %) within ± 20% of the nominal concentration. The LLOQs were 0.1 µg/mL for most insecticides, except for DZN (0.05 µg/mL) and QLP (0.25 µg/mL). These limits were considered satisfactory, especially when compared to those obtained by other authors. Inoue et al. (2007) [26] applied 200 µL of serum, a greater volume (4 times higher) of a less complex biological sample than blood and the reported limit of quantitation (LOQ) for DZN was greater than that herein presented. Park et. al (2009) [27], used 1 mL (20 times higher) of *postmortem* blood and reported LOQs of 0.15 µg/mL for DZN and CLP. The mentioned authors have used the classic pre-concentration procedure of SPE, that usually requires greater volumes of solvents and specimen; also, it can be considered more laborious when compared to the DBS approach. However, this analytical method was developed on a GC-MS/MS, which can be way more sensitive than the liquid chromatography-mass spectrometry (LC-MS) used by Inoue et al. (2007) [26] and the GC-MS used by Park et. al (2009) [27]. A GC-MS/MS analytical method is reported by Luzardo et. al (2015) [28], and achieved lower LOQs than ours, however using 40 times more blood than our method (2 mL). The latter authors also use a classic extraction procedure, LLE, that can require greater amounts of solvents. This type of pre-concentration is also applied by Zhao et. al (2016) [29], which required 500 µL of sample and resulted in a greater LOQ for CLP (0.2 µg/mL). The remaining publications about the subject report lower LOQs, nevertheless, the authors use a minimum of 100 µL of biological specimen, 2 to 40 times greater than the one required by our method using the DBS approach [3, 6, 9, 30-33].

The limits of detection (LOD) were determined as the lowest concentrations that showed a discrete peak clearly distinguishable from the blank and presented a signal-to-noise ratio of at least 3. These limits were determined by the analysis of six replicates of spiked samples. These data are shown on Table 2.

Table 2. Linearity data (n=5).

Analyte	Weight	Linear range (µg/mL)	Linearity		R^{2*}	LOD
			Slope*	Intercept*		(µg/mL)
DZN	linear	0.05 - 25	0.7985 ± 0.3145	-0.0015 ± 0.0364	0.9988 ± 0.0005	0.05
CLP	1/x	0.1 - 25	0.5473 ± 0.1234	-0,0125 ± 0.0167	0.9981 ± 0.0013	0.1
PRT	1/x	0.1 - 25	0.1957 ± 0,0347	-0.0082 ± 0.0082	0.9972 ± 0.0033	0.1
QLP	1/x	0.25 - 25	1.0019 ± 0.1597	-0.1396 ± 0.1183	0.9958 ± 0.0026	0.1
CLF	1/x	0.1 - 25	0.2435 ± 0.0708	-0.0106 ± 0.0208	0.9967 ± 0.0007	0.1

*Mean values ± standard deviation

3.5. Intermediate, Inter- and intra-day precision and accuracy

Precision of the method was expressed in terms of CV, while accuracy was characterized in terms of the mean RE between the measured and spiked concentrations; the accepted limit was ± 15% for all concentrations, except at the LLOQ, where ± 20% was accepted.

Intermediate precision and accuracy was evaluated by analysis of the quality control samples (5 concentration levels related to the linearity range) which were analyzed in triplicate over the 5-day period (n=15). The obtained CVs were typically lower than 12% with an accuracy within a ± 12.1% interval (Table 3).

Table 3. Intermediate precision and accuracy (n=15) for blood.

Analyte	Spiked (µg/mL)	Measured* (µg/mL)	CV (%)	RE *(%)
DZN	0.4	0.3705 ± 0.0363	9.80	-7.4
	0.8	0.8343 ± 0.0735	8.81	4.3
	1.5	1.4856 ± 0.1602	10.78	-1.0
	8	7.5948 ± 0.8594	11.32	-5.1
	20	20.2971 ± 2.1163	10.43	1.5
CLP	0.4	0.3830 ± 0.0432	11.28	-4.3
	0.8	0.8144 ± 0.0836	10.26	1.8
	1.5	1.4478 ± 0.1543	10.66	-3.5
	8.0	7.7756 ± 0.8300	10.67	-8.6
	20	21.0730 ± 1.3867	6.58	5.4
PRT	0.4	0.3514 ± 0.0105	2.98	-12.1
	0.8	0.7748 ± 0.0758	9.79	-3.1
	1.5	1.3681 ± 0.1317	9.62	-8.8
	8	7.8270 ± 0.9380	11.98	-2.2
	20	20.6918 ± 1.6140	7.80	3.5
QLP	0.4	0.4105 ± 0.0388	9.45	2.6
	0.8	0.7744 ± 0.0724	9.35	-3.2
	1.5	1.3642 ± 0.0884	6.48	-9.1
	8.0	7.6305 ± 0.5235	6.86	-4.6
	20	20.2779 ± 1.3527	6.67	1.4
CLF	0.4	0.3925 ± 0.0335	8.53	-1.9
	0.8	0.7940 ± 0.0767	9.66	-0.8
	1.5	1.4921 ± 0.1765	11.83	-4.0
	8.0	7.2703 ± 0.5679	7.81	-9.0
	20	18.8634 ± 0.8144	4.32	-5.7

All concentrations in µg/mL; CV – Coefficient of variation; RE – Relative error [(measured concentration-spiked concentration/spiked concentration) x 100; *Mean values ± standard deviation.

Inter-day precision was evaluated at a minimum of six concentrations within a 5-day period. The obtained CVs were typically lower than 14% for all analytes at the tested concentration levels, except for CLF at the LLOQ, for which a CV of 17% was obtained. Accuracy was within a ± 14% interval (Table 4).

Table 4. Inter-day precision and accuracy (n=5) for blood.

Analyte	Spiked (µg/mL)	Measured* (µg/mL)	CV (%)	RE *(%)
DZN	0.05	0.048 ± 0.006	13.31	-3.62
	0.1	0.101 ± 0.010	9.90	1.01
	0.25	0.235 ± 0.031	13.18	-5.91
	0.5	0.499 ± 0.064	12.82	-0.23
	0.75	0.737 ± 0.092	12.50	-1.76
	1	1.036 ± 0.127	12.21	3.63
	2.5	2.507 ± 0.192	7.65	0.26
	10	9.907 ± 0.426	4.31	-0.93
	25	24.973 ± 0,373	1.49	-0.11
CLP	0.1	0.098 ± 0.008	8.13	-1.96
	0.25	0.240 ± 0.025	10.36	-4.10
	0.5	0.460 ± 0.027	5.90	-8.06
	0.75	0.712 ± 0.042	5.92	-5.07
	1	0.939 ± 0.093	9.88	-6.11
	2.5	2.363 ± 0.144	6.09	-5.47
	10	9.625 ± 0.644	6.69	-3.75
	25	25.391 ± 0.579	2.28	1.56
PRT	0.1	0.105 ± 0.009	8.18	4.53
	0.25	0.241 ± 0.022	9.00	-5.89
	0.5	0.445 ± 0.044	9.86	-8.27
	0.75	0.716 ± 0.043	5.94	-4.48
	1	0.905 ± 0.028	3.05	-9.47
	2.5	2.385 ± 0.135	5.66	-4.60
	10	9.866 ± 0.334	3.38	-1.34
	25	25.312 ± 0.333	1.31	1.25
QLP	0.25	0.283 ± 0.027	9.70	13.28
	0.5	0.477 ± 0.051	10.74	-4.64
	0.75	0.733 ± 0.074	10.15	-2.32
	1	0.985 ± 0.077	7.81	-1.45
	2.5	2.440 ± 0.212	8.70	-2.41
	10	9.493 ± 0.976	10.28	-5.07
	25	25.621 ± 0.572	2.23	2.48
CLF	0.1	0.109 ± 0.019	17.00	8.95
	0.25	0.278 ± 0.010	3.70	11.07
	0.5	0.468 ± 0.039	8.27	-6.34
	0.75	0.747 ± 0.064	8.54	-0.46
	1	0.951 ± 0.045	4.74	-4.86
	2.5	2.488 ± 0.244	9.80	-0.48
	10	9.253 ± 0.267	2.89	-7.47
	25	26.052 ± 0.304	1.17	4.21

All concentrations in µg/mL; CV – Coefficient of variation; RE – Relative error [(measured concentration-spiked concentration/spiked concentration) x 100]; *Mean values ± standard deviation.

Intra-day precision and accuracy were evaluated by the analysis of 6 replicates at a minimum of 4 concentration levels on the same day. The obtained CVs were lower than 14% at the studied concentration levels, with a mean RE within ± 12% of the nominal concentration (Table 5).

Table 5. Intra-day precision and accuracy (n=6) for blood.

Analyte	Spiked (µg/mL)	Measured* (µg/mL)	CV (%)	RE *(%)
DZN	0.1	0.101 ± 0.008	7.62	0.92
	0.5	0.467 ± 0.036	7.77	-6.59
	2.5	2.535 ± 0.265	10.47	1.40
	15	16.656 ± 1.060	6.36	11.04
	25	22.793 ± 1.491	6.54	-8.83
CLP	0.1	0.101 ± 0.010	10.01	1.02
	0.5	0.452 ± 0.021	4.81	-9.66
	2.5	2.629 ± 0.086	3.28	5.16
	15	15.974 ± 0.886	5.55	6.42
	25	23.534 ± 2.283	9.70	- 5.86
PRT	0.1	0.106 ± 0.005	4.30	5.74
	0.5	0.453± 0.022	4.87	-9.48
	2.5	2.377 ± 0.262	11.04	-4.90
	15	15.758 ± 1.524	9.67	5.06
	25	24.721 ± 1.889	7.64	-1.12
QLP	0.5	0.498 ± 0.047	9.49	-0.31
	2.5	2.397 ± 0.270	11.30	-4.10
	15	15.532 ± 0.749	4.82	3.55
	25	22.949 ± 1.847	8.05	-8.20
CLF	0.1	0.099 ± 0.003	2.85	-3.17
	0.5	0.443 ± 0.022	4.91	-11.30
	2.5	2.689 ± 0.143	5.34	7.55
	15	14.926 ± 1.281	8.58	-0.49
	25	24.935 ± 3.375	13.53	-0.26

All concentrations in µg/mL; CV – Coefficient of variation; RE – Relative error [(measured concentration-spiked concentration/spiked concentration)] x 100; *Mean values ± standard deviation.

3.6. Extraction efficiency

For the study of absolute recovery, two sets of samples (n=3) were prepared by spiking blank blood at three different concentration levels (1, 2.5 and 15 µg/mL). The first set, representing 100% recovery, was prepared spiking the samples after extraction, while in the second the samples were spiked with the analytes before extraction.

The results of the recovery were obtained comparing the relative peak areas of the samples of the second group with those of the analytes from the samples belonging to the first group. The obtained results are shown on Table 6.

Table 6. Recoveries of organophosphorus pesticides (n=3) for blood samples.

Analyte	Recovery (%)		
	1 µg/mL	2.5 µg/mL	15 µg/mL
DZN	5.11 ± 1.46	3.83 ± 1.25	4.56 ± 0.77
PRT	1.19 ± 0.10	0.92 ± 0.25	1.20 ± 0.43
CLP	3.50 ± 0.41	2.53 ± 0.63	2.64 ± 0.30
QLP	11.98 ± 0.20	7.41 ± 0.94	6.35 ± 1.13
CLF	1.58 ± 0.14	1.52 ± 0.51	1.33 ± 0.15

*Mean values ± standard deviation; LSC- Low Spiked Concentration; MSC- Medium Spiked Con centration; HSC- High Spiked Concentration.

Since this study represents a new applicability of DBS to pesticide samples, it is not possible to compare our results to those reported in the scientific literature concerning this sampling approach. However, by approximation, they can be compared to other techniques of microextraction, for instance headspace-solid phase microextraction (HS-SPME); Musshoff et. al (2002) [33], using this latter approach, have obtained a recovery of 2.90% for DZN, despite using a greater sample volume (0.5 mL), whereas in our study a mean recovery of 4.50% was obtained with a sample volume of only 50 µL. The same occurred for QLP, where the previous authors achieved a recovery of 1.30%, whereas in the present study an average recovery of 8.58% was achieved. Similar extraction efficiencies were reported for CLF (1.48%), but for PRT and CLP lower recovery values were reached in this study, 1.10% and 2.89%, respectively [33]. Although extraction efficiencies are low, if they are compared to other microextraction approaches, one can observe that even using a considerably smaller sample volume, values close to or even higher than those already published have been obtained. Furthermore, the method presents good sensitivity, and DBS can be considered a powerful technique, resulting in a fast and efficient extraction of the target analytes with less consumption of sample and solvents.

3.7. Stability

The stability of the compounds was studied at 0.4, 0.8, 1.5, 8 and 20 µg/mL under specific conditions and time intervals, for short-term and freeze/thaw cycles.

Short-term stability was assessed at the above concentration levels (n=3), where the blank samples were spiked and left at room temperature for 24 hours. To study stability after freeze/thaw cycles, the samples were spiked at the above-mentioned concentration levels (n=3) and were stored at -20 °C for 24 hours. After this time, they were thawed at room temperature and refrozen for another 24 hours under the same conditions. This cycle was repeated twice more, and the samples were analyzed at the end.

The samples subjected to the stability study were compared to freshly prepared samples analyzed on the same day and quantified on the same calibration curve. Therefore, the concentrations were compared and the respective RE was calculated relatively to the theoretical concentrations; CVs were calculated as well. The compounds were considered stable if the aforementioned criteria for accuracy and precision (CVs below 15% and REs within ± 15% range) were met.

Long-term stability was also studied, which lasted for 21 days at concentrations of 0.1, 5 and 20 µg/mL. During this period, and every 7 days, samples were dried on DBS, having at the end three sets of samples to be analyzed. At the end of the 21 days, as described for the other forms of stability, the samples were compared to fresh samples, analyzed on the same day and quantified on the same calibration curve. For the analysis of the results, all above-mentioned criteria would have to be met (CVs below 15% and REs within ± 15% interval). It was possible to conclude that PRT is stable only in the first 7 days of study, with significant losses from that time on. It should also be noted that this loss of the compound is more significant at the higher concentrations. The remaining compounds had a stable behavior throughout the study.

3.8. Dilution integrity

This parameter was studied to deal with those situations where the analytes are present in concentrations that exceed the LOQ of the method, if it is possible to dilute the sample. The study was performed for all analytes at the dilution levels of 1:2, 1:5 and 1:10.

For this parameter, blood samples were prepared with concentrations of each analyte at a dilution appropriate to the calibration interval. Therefore, samples were spiked at 40 µg/mL (1:2), 100 µg/mL (1:5) and 200 µg/mL (1:10). Each spiked sample was diluted by the respective factor with blank blood before analysis, in order to fit within the calibration range.

After multiplied by the respective dilution factor, sample dilution would be acceptable if the calculated concentrations were within a ± 15% interval from the spiked value. The obtained REs ranged between -1.75 and 11.02%. Therefore, even overly concentrated samples can be properly analyzed after adequate dilution.

3.9. Method applicability

After validation of this analytical method, in order to demonstrate the applicability of the method, it was successfully applied to routine analysis of hospital samples with suspicion of organophosphorus intoxication. All samples were homogenized for 20 minutes and were analysed in triplicate according to the described method. As an example, Figure 7 shows the chromatogram of an authentic sample, in which DZN was identified at a concentration of 170 µg/mL.

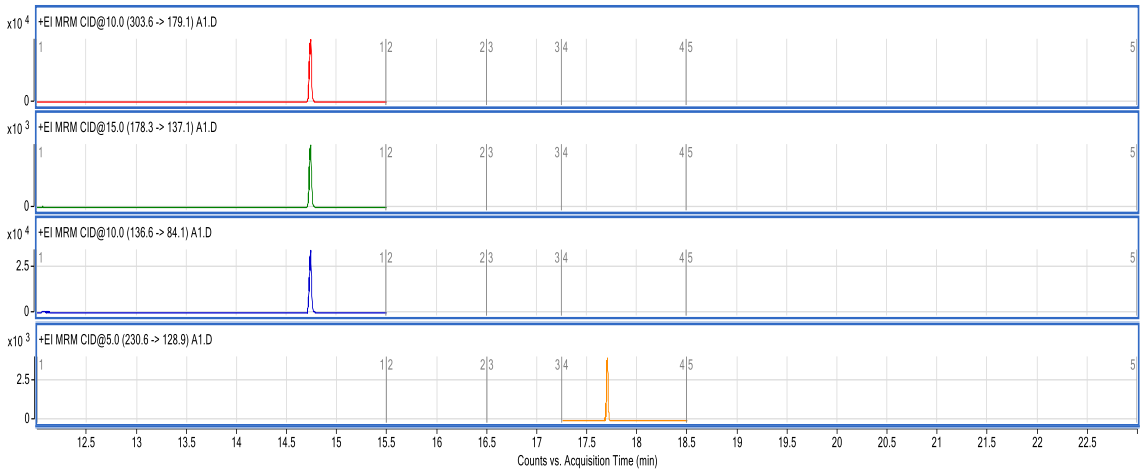


Figure 7. Chromatogram obtained after analysis of an authentic sample positive for DZN (170 µg/mL).

4. Conclusions

An analytical method using DBS extraction and GC-MS/MS was developed and optimized for the simultaneous detection and quantification of five organophosphorus pesticides in blood samples.

The validated method was linear in the range of 0.1-25 µg/mL for all compounds except for DZN (0.05-25 µg/mL) and for QLP (0.25-25 µg/mL), with adequate precision and accuracy. With a LLOQ of 0.1 µg/mL for most of them except DZN (0.05 µg/mL) and for QLP (0.25 µg/mL), using only 50 µL of sample.

This approach was found suitable for the determination of the studied compounds in low volume blood samples due to its sensitivity. This is the first report on the use of this sampling approach for these compounds, considered a better alternative to traditionally used microextraction techniques, resulting in a lower sample and solvent consumption. In addition, the method is faster than the traditional approaches. This feature, together with its ease of operation allows the routine use of this method in the determination of organophosphorus insecticides in both clinical and forensic toxicology situations.

Acknowledgments

The authors acknowledge the POCI - COMPETE 2020 - Operational Programme Competitiveness and Internationalisation in Axis I - Strengthening research, technological development and innovation (Project POCI-01-0145-FEDER-007491), National Funds by FCT - Foundation for Science and Technology (Project UID/Multi /00709/2013).

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Conclusions

A specific analytical method was developed for the determination of five organophosphorus pesticides, diazinon (DZN), chlorpyrifos (CLP), parathion-ethyl (PRT), chlorfenvinphos (CLF) and quinalphos (QLP), using the dried blood spots (DBS) sampling approach to extract the analytes from samples of only 50 µL of blood, which makes this method quick and easy to perform. The analysis for the quantitative and qualitative determination of the compounds was performed by gas chromatography (GC) coupled to tandem mass spectrometry (MS/MS).

The developed and optimized methodology was validated following guidelines internationally accepted for bioanalytical method validation, including the guiding principles of the Food and Drug Administration (FDA), the International Conference on Harmonization (ICH) and the Scientific Working Group for Forensic Toxicology (SWGTOX). Parameters as selectivity, sensitivity, linearity, limits of detection (LOD) and quantification (LLOQ), precision and accuracy as well as stability, dilution integrity and recovery were evaluated.

For the extraction process, all the involving factors were optimized with the purpose of maximizing it, allowing achieving low limits of detection and quantification. The proposed method was linear in the range of 0.1-25 µg/mL for all compounds, except for DZN (0.05-25 µg/mL) and for QLP (0.25-25 µg/mL), with adequate precision and accuracy according to the mentioned guidelines. Even presenting low recoveries (values from 1 to 12%) and despite of using only 50 µL of sample, LLOQ values of 0.1 µg/mL were obtained for all compounds except DZN (0.05 µg/mL) and QLP (0.25 µg/mL).

With the obtained results, we can state that the developed methodology using this type of sampling can be implemented as an alternative to the traditionally used methods, with the advantage of the speed of the extraction process, low volumes of sample and organic solvents, allowing its routine use in clinical and forensic toxicology laboratories.

It should also be noted that this is the first study in which the DBS approach was used for the detection and quantification of organophosphorus compounds in blood samples.

Attachments

The present dissertation was disseminated in different congresses in the area of toxicology, as well as was submitted for publication.

Presentations in congress:

DEVELOPMENT AND OPTIMIZATION OF A NEW METHOD BASED ON DRIED BLOOD SPOTS TO DETERMINE ORGANOPHOSPHOROUS INSECTICIDES BY GC-MS/MS (Oral communication)
Soares S., Castro T., Rosado T., Fernández N., Restolho J., Barroso M., Gallardo E.
54th Annual Meeting of the International Association of Forensic Toxicologists, Brisbane (Australia), Junho 2016

APLICAÇÃO DE DRIED BLOOD SPOTS NA DETERMINAÇÃO DE PESTICIDAS ORGANOFOSFORADOS POR GC-MS/MS (Poster)
Soares S, Castro T, Rosado T, Barroso M, Gallardo E.
III Conferência do Instituto Nacional de Medicina Legal e Ciências Forenses, Coimbra, Novembro 2016

Submitted articles:

TOXICOLOGICAL ASPECTS AND BIOANALYTICAL APPROACHES FOR THE DETERMINATION OF ORGANOPHOSPHORUS INSECTICIDES IN HUMAN BIOLOGICAL SAMPLES
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NEW ANALYTICAL APPROACH TO DETERMINE ORGANOPHOSPHOROUS INSECTICIDES IN BLOOD BY DRIED MATRIX SPOTS SAMPLING AND GC-MS/MS
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