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Analysis of organophosphorus pesticides in whole blood by GC-MS-μECD with forensic purposes

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ABSTRACT

In the present work, two multi-residue methods for the determination of ten organophosphorus pesticides (OPs), namely chlorfenvinphos, chlorpyrifos, diazinon, dimethoate, fenthion, malathion, parathion, phosalone, pirimiphos-methyl and quinalphos, in post-mortem whole blood samples are presented.

The adopted procedure uses GC–MS for screening and quantitation, and GC-μECD (electron capture detector) for compound confirmation. Three different Solid Phase Extraction (SPE) procedures for OPs with Oasis® hydrophilic lipophilic balanced (HLB) and Sep-Pak® C18 cartridges were tested, and followed by GC-μECD and GC–MS analysis. The Sep-Pak® C18 cartridges extraction procedure was selected since it generated analytical signals 5 times higher than those obtained with the two different Oasis® HLB cartridges extraction procedures. The method has shown to be selective for the isolation of selected OPs as well as to the chosen internal standard (ethion) in postmortem blood samples. Calibration curves between 50 and 5000 ng/mL were prepared using weighted linear regression models ($1/x^2$). It was not possible to establish a working range for fenthion by GC-μECD due to the lower sensitivity of the detector to this compound, whereas for pirimiphos-methyl it was set between 500 and 5000 ng/mL. The limit of quantitation was established at 50 ng/mL for all analytes, except for pirimiphos-methyl by GC-μECD analysis (500 ng/mL). The average extraction efficiency ranged from 72 to 102%.

The developed methods were considered robust and fit for the purpose, and had already been adopted in the laboratory routine analysis.

1. Introduction

Organophosphorus pesticides (OPs) were developed to protect crops against damage by insects. However, their usage has been reported in suicide attempts in rural areas, despite European legislation prohibiting the use of certain pesticides, namely the most dangerous ones. This happens because, despite restrictive legislation, small farmers and Portuguese domestic users tend to keep the stock they already have when new prohibiting laws are approved.

When ingested, OPs are rapidly absorbed and distributed throughout the body, binding and therefore inhibiting acetylcholinesterase enzymes, causing accumulation of acetylcholine, which is essential for nerve impulse transmission in both vertebrate and invertebrate species. This accumulation leads to the disruption of the normal functioning of the nervous system, producing typical

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cholinergic symptoms (i.e. hyperactivity tremors, convulsions, paralysis and ultimately death).[1]

Since in suicide attempts people tend to ingest large amounts of pesticides, they eventually die. In order to confirm the cause of death, whole blood samples are collected and sent to toxicological analysis, requiring a sample clean-up step. Known clean-up methodologies involve Liquid–Liquid Extraction (LLE),[2,3] Solid Phase Extraction (SPE),[4–7] and Solid Phase Micro-Extraction (SPME).[8–10]

The analytical determination of OPs in blood was first carried out by gas chromatography (GC) with flame ionization detector (FID),[5,11] nitrogen–phosphorus detector (NPD),[9,10,12,13] and flame photometric detector (FPD).[14,15] However, in the last few decades, mass spectrometry has become the main detection technique, due to its confidence in the confirmation of peak identity and quantitation, especially if isotopically labelled internal standards are available.[2,4,6–9] High performance liquid chromatography coupled with mass spectrometry has also been used to detect this group of compounds.[3,16] The use of electron capture detector (ECD) to detect OPs has been too scarce; indeed, Heinig et al.[17] analysed only metrifonate and one metabolite in whole blood and urine, while Pitarch et al.[18] developed two procedures for organochlorine and organophosphorus pesticides determination but in serum and urine samples.

The aim of the present study was the development, optimization, and validation of a method for the determination of 10 organophosphorus pesticides (chlorfenvinphos, chlorpyrifos, diazinon, dimethoate, fenthion, malathion, parathion, phosalone, pirimiphos-methyl and quinalphos) in whole blood by SPE procedure. These pesticides were selected due to their toxicity, their presence in previously reported intoxication cases in the Chemistry and Forensic Sciences Service, and their commercialization history. Whole blood was used due to the fact that plausible binding of pesticides to erythrocytes, haemolysis and microbial degradation avoids the isolation of serum or plasma, allowing, contemporaneously, the possibility to perform screening, confirmation and quantitation procedures in the same sample.[19–21] The used apparatus consisted on single equipment, containing two independent separation systems, GC–MS and GC-μECD, offering high sensitivity and detection based on different compound properties. Moreover, and according to good laboratory practises and internal guidelines, two different analytical procedures (granted by these two systems) are needed to issue/emit a positive result.

2. Material and methods

2.1. Reagents and materials

Pesticides standards (chlorfenvinphos 97.5%, chlorpyrifos 99.9%, diazinon 99.0%, fenthion 98.3%, malathion 97.2%, parathion 99.5%, phosalone 99.5%, pirimiphos-methyl 99.5% and quinalphos 96.2%) were purchased from Fluka. Dimethoate 96.2% and ethion 99.3% (the later was chosen as internal standard as its commercialization in Portugal is prohibited since 2003), were purchased from Supelco (Saint Louis, USA). Ammonium acetate, acetic acid, potassium chloride, sodium chloride, chloroform, diethyl ether, n-hexane, isooctane, potassium dihydrogen phosphate, di-sodium hydrogen phosphate, isopropanol were purchased from Merck Co. (Darmstadt, Germany). Acetonitrile and methanol were purchased from Fisher Chemical and Sigma–Aldrich, respectively. Water was purified by a Milli-Q system obtained from Millipore (Molsheim, France). All solvents were of analytical or gradient grade. Extraction procedures were performed in a manual SPE equipment, using Oasis® HLB (3 cc, 60 mg) and Sep-Pak® C18 (3 cc, 500 mg) SPE cartridges, obtained from Waters™ (WATERS Corporation, USA).

2.2. Standard solutions preparation

Stock solutions of each analyte were prepared in the lab and then diluted to get appropriate pesticide standard solutions (100 and 1 μg/mL). An internal standard stock solution was also prepared and properly diluted to get a solution with a final concentration of 100 μg/mL. These solutions were stored at −20 °C. A 1 L ammonium acetate buffer solution (0.1 M) was prepared by mixing 3.3 mL acetic acid and 7700 mg ammonium acetate. The volume was adjusted with deionised water (pH 4.9). A 1 L Phosphate Buffer Saline (PBS) solution was prepared by mixing 200 mg of KCl, 8000 mg NaCl, 200 mg of KH_2PO_4 and 1150 mg of Na_2HPO_4. The volume was adjusted with deionised water.

2.3. Extraction procedures

In this work, five different SPE procedures were tested. Two of them used Oasis® HLB cartridges, were adapted from Raposo et al.[7] and Park et al.,[6] and were named by “HLB_A” and “HLB_B” procedures, respectively. Other three procedures, involving the use of Sep-Pak® C18 cartridges and based on the work of Liu et al.,[5] were tested. They differed on the volume (10 mL, 5 mL and 2 mL) of solvent or mixture used in the conditioning step, and were named “Sep-Pak_10”, “Sep-Pak_5” and “Sep-Pak_2”, respectively. As an example, it is presented the procedure Sep-Pak_2, which was validated during this work.

Blood samples (0.5 mL) were mixed with 5 mL deionised water and centrifuged at 4000 rpm during 30 min. Meanwhile, for cartridge conditioning, 2 mL of chloroform:isopropanol mixture (V:V 9:1), 2 mL of acetonitrile, 2 mL of acetonitrile:water mixture (V:V 1:1) and 2 mL of deionised water were passed through the cartridges. The supernatant was loaded onto the conditioned cartridge and allowed to drain at a 1–2 mL/min flow. The cartridge was then washed with 10 mL of water and vacuum dried during 15 min. After that, 3 mL of chloroform:isopropanol mixture (V:V 9:1) was passed through the cartridge to recover the analytes for a glass tube. After solvent evaporation the residues were redissolved with 100 μL isooctane, transferred to a vial with insert and injected into the GC system.

2.4. Instrumental conditions

The GC used in this work was an Agilent 6890N (Agilent Technologies, Santa Clara, CA, USA), equipped with a Agilent 7683 series liquid autosampler, two split/splitless injectors, and two independent separation and detection systems (MS and μECD).

The front system was equipped with an HP-5MS Agilent column (30 m × 0.25 mm × 0.25 μm) and an Agilent 5973N series mass selective detector (MS), containing a single quadrupole. Injector and MS transfer line temperatures were both set at 280 °C. The oven temperature was initially held at 80 °C for 1.0 min, and then increased to 227 °C (35 °C/min), held for 6.0 min, and then increased to 275 °C (10 °C/min) and held for 2.0 min (total run time: 18.0 min). Gas chromatograph was operated in split mode (ratio 38.5:1) and a Helium BIP gas flow of 1.3 mL/min (constant flow mode) was used. The MS was used in SIM mode both for qualitative and for quantitative analysis, with a solvent delay of 5.00 min, and at least three ions were monitored for each analyte, except for the internal standard (Table 1). Chlorfenvinphos standard showed two chromatographic peaks due to its *E* and *Z* isomers.

The back system was equipped with a Factor Four VF-5MS column from Varian (60 m × 0.32 mm × 0.25 μm) and a G2397A micro-cell Electron Capture Detector (μECD) from Agilent. Injector and μECD temperatures were set at 280 and 320 °C, respectively. The column temperature was initially held at 80 °C for 1.0 min,