Journal of Chromatography B 1092 (2018) 459–465

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

An effective and high-throughput analytical methodology for pesticide screening in human urine by disposable pipette extraction and gas chromatography – mass spectrometry

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ARTICLE INFO

Keywords:
Pesticides
Forensic toxicology
Urine
Disposable pipette extraction
DPX
GC–MS

ABSTRACT

In this paper, the use of disposable pipette extraction (DPX) for the determination of pesticides in human urine in possible cases of poisoning is proposed for the first time. The pesticides studied were oxamyl, propoxur, carbofuran, 3-hydroxycarbofuran, carbaryl, methiocarb, terbufos, parathion methyl, malathion, chlorpyrifos and endosulfan. The pipette tip used for the extraction of these compounds was commercially acquired. It has a capacity of 5 mL and contains 20 mg of sorbent material (styrene-divinylbenzene). The optimization of the main parameters that can influence the extraction efficiency of this sample preparation technique was performed with univariate and multivariate approaches. The analytes were separated and identified by gas chromatography coupled to mass spectrometry (GC–MS). The optimal extraction conditions were 5 extraction cycles of 30 s and 5 desorption cycles of 15 s with 250 µL of ethyl acetate. Elution of the extract was performed in a vial containing 100 mg of anhydrous sodium sulfate. The method developed was validated, providing correlation coefficients higher than 0.9955 for all analytes, limits of detection (LOD) of 0.76 to 1.52 µg L^{-1}, limits of quantification (LOQ) of 2.5 to 5.0 µg L^{-1}, relative recoveries of 63 to 118%, intra-day precision of 0.7 to 15.3% and inter-day precision of 4.9 to 13.1%. An effective and rapid method for the analysis of human urine for the identification of possible cases of poisoning by pesticides was successful developed.

1. Introduction

Pesticides are chemical compounds which have been extensively used to control or destroy agricultural pests that affect crops, which results in the improvement of food production throughout the world [1]. Since these compounds have become widely available commercially they have been used irregularly in many countries around the world, which has increased (un)intentional cases of poisoning [2]. Reports published in several countries show that there are around 250,000 to 370,000 deaths associated with this type of poisoning each year [3]. The World Health Organization (WHO) reports that death by poisoning through contact with pesticides is one of the most common methods of suicide worldwide and, therefore, it is considered a public health problem [4].

Fatalities involving pesticides are a consequence of different factors, such as accidents, self-injury and homicides. In all of these cases, chemical analysis is required to investigate the substance which caused the poisoning. In this regard, the forensic laboratories face a difficult challenge because in most cases there is no information on the substance involved in the intoxication [5]. Many different methods for the determination of pesticides have been reported in the literature to analyze biological samples including blood [5–7], plasma [8, 9], urine [10–14], serum [9, 12, 15] and hair [16, 17]. The chemical groups of pesticides most frequently found in the toxicological analysis of these biological samples are organophosphates, organochlorides and carbamates.

Biological matrices are very complex samples and therefore a sample preparation step, performed prior to the sample injection into the analytical instrument, is often required [18]. Variety sample preparation techniques used for the determination of pesticides in biological samples are liquid-liquid extraction (LLE) [5], solid phase extraction (SPE) [6, 7, 11, 16] and solid phase microextraction (SPME) [12]. Recently, a technique called disposable pipette extraction (DPX) has been widely used for the determination of pesticides in food residues [1, 19–23] and environmental samples [24, 25]. However, no study has been reported in the literature using DPX in the analysis of pesticides in biological samples.

The DPX technique is based on solid-phase extraction and was

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https://doi.org/10.1016/j.jchromb.2018.06.047
Received 25 May 2018; Received in revised form 19 June 2018; Accepted 21 June 2018
Available online 23 June 2018

developed by Dr. William E. Brewer at the University of South Carolina in 2003 [26]. This technique involves a modification of the conventional SPE technique with a reduced amount of sorbent material used as the extraction phase. In general, the extraction time applied in DPX is shorter and the volume of solvents used for the elution step is lower compared to classical SPE approaches [27, 28]. A very recent modification adopted in DPX is the use of a conventional pipette tip with a capacity of 1 or 5 mL, containing a known amount of sorbent material placed between two filters: one inserted at the lower end and the other at the upper end of the pipette tip [29–31]. The type of sorbent material used in the DPX procedure is dependent on the analyte characteristics and a wide range of different materials is commercially available [27, 29]. For the extraction of pesticides, the use of DPX-RP (reverse phase), which contains styrene-divinylbenzene as the sorbent material, is appropriate, since this extraction phase is recommended for the extraction of non-polar and slightly polar compounds [1].

In general, the DPX technique exhibits a number of advantages, such as the possibility of using multi-channel devices to increase the analysis throughput, the liquid flow can be bidirectional and the contamination is less frequent because the pipette tips are disposable. Since smaller masses of sorbent material are used, small amounts of sample and organic solvent are required in the extraction step. In addition, the extraction system can be automated to meet larger analytical demands, which also allows for more precise and accurate analysis [27, 28].

The aim of this study was to carry out the development, optimization and in-house validation of a rapid and straightforward method for the determination of 11 pesticides (oxamyl, propoxur, carbofuran, 3-hydroxycarbofuran, carbaryl, methiocarb, terbufos, parathion methyl, malathion, chlorpyrifos and endosulfan) in human urine by DPX with separation/detection performed by GC–MS. These analytes are of important biological interest since they can be associated with cases of poisoning that require the aid of forensic analysis. Therefore, simple, rapid and accurate analytical methodologies are needed to enable the determination of these compounds in complex samples such as urine.

2. Experimental

2.1. Material and reagents

An analytical standard containing a mixture of carbamate pesticides (oxamyl, propoxur, carbofuran, 3-hydroxycarbofuran, carbaryl and methiocarb) was purchased from Sigma-Aldrich (Milwaukee, WI, USA) at a concentration of 100 mg L^{-1} in methanol. Analytical standards of terbufos, parathion methyl, malathion, chlorpyrifos and endosulfan were also purchased from Sigma-Aldrich. From these standards, individual stock solutions of each analyte were prepared at a concentration of 100 mg L^{-1} in methanol. From these solutions, a working solution containing a mixture of all analytes, with 5 mg L^{-1} of the carbamate analytes and 10 mg L^{-1} of the other analytes, was prepared in methanol. Methanol (HPLC grade) was purchased from J.T. Baker (Mallinckrodt, NJ, USA), ethyl acetate (HPLC grade) from Sigma-Aldrich and acetonitrile (HPLC grade) from Merck (Darmstadt, Germany). The anhydrous sodium sulfate used in the sample elution vial was purchased from Vetec (Rio de Janeiro, Brazil). The pipette tips (1 mL of 20 mg DPX-RP and 5 mL without sorbent) were purchased from DPX Labs (Columbia, SC, USA). A 10 µL microsyringe was purchased from Hamilton (Nevada, USA) and 5 mL disposable syringes were obtained from Bd Solomed (São Paulo, Brazil). The ultrapure water used in the experiments was purified by a Mega purity system (Billerica, USA).

2.2. Urine samples

Initially, the optimization assays were performed with ultrapure water samples spiked with the analytes at concentrations of 150 and 300 µg L^{-1}. Later, after all of the parameters had been optimized, the method was validated using blank urine samples donated by healthy volunteers from our research group aged 22–23 years. No discomfort or risks were associated with the sample collection. These samples were collected in 40 mL glass bottles and stored for one day under refrigeration at 4 °C until the analysis. The extractions were performed using 100 µL of the urine sample, which was diluted with the addition of ultrapure water to the vial up to a final volume of 5 mL. The analysis was performed at room temperature.

2.3. Instrumental and chromatographic conditions

The chromatographic analysis was performed on a GC–MS-QP 2010 Plus gas chromatograph coupled to mass spectrometry (Shimadzu, Kyoto, Japan). Chromatographic separation was carried out in a Restek capillary column (Torrance, CA, USA) model Rtx®-5MS (30 m × 0.25 mm × 0.25 µm film thickness). Ultrapure helium was used as the carrier gas at a constant flow of 1 mL min^{-1}. The injector temperature was set at 250 °C and the initial oven temperature was initially set at 50 °C, held for 1 min, increased to 200 °C at a rate of 10 °C min^{-1} and then increased to 255 °C at a rate of 7 °C min^{-1}, totalizing 24 min of chromatographic run. A manual injection using a volume of 1 µL in splitless mode was performed. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV. The temperature of the ion source was set at 230 °C and the interface at 280 °C, with a solvent cut-off time of 2.5 min. The quantification of the analytes was performed in selected ion monitoring (SIM) mode using the highest intensity ion. Table 1 shows some physicochemical properties of the analytes, as well as the ions monitored for the identification and quantification.

2.4. Preparation of the DPX pipette tips

For the development of the methodology, a 5 mL pipette tip containing no sorbent material was used. Firstly, the upper filter was

Table 1
Class, toxicity, pKa value, log *P* value and MS information for the target analytes.

Number	Analytes	Class[a]	Toxicity LD_{50} (mg kg^{-1}) [5]	pKa [34]	log P [34]	Identification ions (*m/z*)	Quantification ion (*m/z*)
1	Oxamyl	CA	30	−2.11	−0.44	42, 69, 72	72
2	Propoxur	CA	51.2	–	0.14	27, 110, 152	110
3	Carbofuran	CA	10.2	–	1.8	122, 149, 164	164
4	3-hydroxycarbofuran	CA	7	3.6	1.7	137, 147, 180	147
5	Carbaryl	CA	150	10.4	2.36	115, 116, 144	144
6	Methiocarb	CA	16	–	3.18	109, 153, 168	168
7	Terbufos	OP	3.5	–	4.51	57, 97, 231	97
8	Parathion methyl	OP	57	–	1.80	109, 125, 263	109
9	Malathion	OP	53	–	2.75	125, 127, 173	173
10	Chlorpyrifos	OP	60	–	4.70	97, 197, 199	97
11	Endosulfan	OC	26	–	4.75	195, 197, 241	241

[a] CA: carbamate pesticide, OP: organophosphate pesticide, OC: organochlorine pesticide.