Journal of Chromatography B, 949–950 (2014) 109–114

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Application of solidified floating organic drop microextraction method for biomonitoring of chlorpyrifos and its oxon metabolite in urine samples

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ARTICLE INFO

Article history:
Received 18 September 2013
Accepted 4 January 2014
Available online 10 January 2014

Keywords:
Solidified floating organic drop microextraction
Chlorpyrifos
Chlorpyrifos oxon
Pesticide
Gas chromatography
Urine

ABSTRACT

A simple, efficient and green analytical procedure for monitoring sub ppb amounts of chlorpyrifos (CP) and chlorpyrifos-oxon (CPO) in urine samples was reported. The methodology is based on the solidified floating organic drop microextraction of the analytes with a free microdrop of 2-dodecanol. The parameters those can affect the microextraction efficiency, such as solvent type, extraction solvent volume, extraction time and temperature, salt effect, pH and stirring rate on extraction were optimized. The analytes were extracted from the urine samples by using 10 μL of 2-dodecanol for 40 min at 70 °C and then, the extracts were injected to GC-MS column by applying 100 kPa injection pressure. The regression coefficients relating to linearity were at least 0.99. The accuracy of the developed method was tested upon recovery studies for CP and CPO calculated as 100 ± 7% and 110 ± 9% (at 0.1 ng mL^{-1} level), respectively. LOD value for CP was found 4.8 ng L^{-1} and for CPO it was found 3.8 ng L^{-1}. This method can easily be adopted by clinical laboratories for the contribution to policies aiming to reduce exposure of pesticides.

1. Introduction

Public health has gained great interest in recent years for pesticide exposure to children [1]. Biomonitoring studies have confirmed that children are widely exposed to organophosphorus pesticides (OPPs) [2]. These compounds are biotransformed by different reactions within the organisms and the products of OPPs are often more toxic than parent types. Hence, their determination in biological samples is significant.

Being a member of OPP class insecticides, chlorpyrifos (CP) causes thousands of deaths each year in worldwide and it is defined as an endocrine disruptor compound [3,4]. Biomonitoring of the CP exposure is becoming an important issue since it is a pro-poison which requires metabolic activation to become a potent phosphorylating agent, namely chlorpyrifos-oxon (CPO) [5]. CPO occurs by conversion from thion (P=S) to oxon (P=O) species of CP via metabolization by oxidative desulphurization in the body primarily by the action of liver microsomes [6,7]. This compound is about 3000 times more potent than CP in inhibition of acetylcholinesterase, which leads to neurotoxicity [8].

A limited number of analytical methods for the simultaneous determination of residues of CP and its main metabolite CPO in various matrices had been developed. These methods involved extraction of the compounds with appropriate solvents, clean-up of the extract and subsequent determination by chromatographic methods. Simultaneous determinations of those analytes in environmental and food samples were established by using gas chromatography (GC) [9–14]. In clinical samples, liquid chromatographic techniques were the method of choice. These compounds were analyzed in mussels by using diode array detector (DAD) [15], in rat plasma and urine by UV detector [16,17], in urine [18] and rat brain tissue and blood samples [8,19] by MS detector.

Although chromatographic techniques provide powerful separation for multiresidue analysis in complex matrices, the sensitivity and selectivity of the method usually depends on the sample preparation method chosen prior to the analysis. Classical extraction methods (e.g. liquid-liquid extraction (LLE), solid phase extraction (SPE)) used in routine applications display some drawbacks such as large consumption of reagent, low enrichment factor and multistage operation [20]. Due to the demand for faster and greener analysis techniques, microextraction methods such as single drop microextraction (SDME) [21], solid phase microextraction (SPME) [22], dispersive liquid liquid microextraction (DLLME) [23] and ultrasound assisted emulsification microextraction (USAEME) [24] have been developed for biological sample analysis.

A novel liquid phase microextraction method based on solidification of floating organic drop (SFODME) has been introduced by Zanjani et al. as an alternative to liquid microextraction

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http://dx.doi.org/10.1016/j.jchromb.2014.01.004

techniques [25]. In this method, a micro drop of 1-undecanol which has a melting point near to room temperature is used as an extraction solvent. The solvent drop is transferred to the surface of the water sample, while being agitated by a stir bar. After the extraction, the sample vial is cooled down and the solidified micro drop is transferred into a conical vial, where it melts immediately and injected to gas chromatography. A variety of applications for the extraction and preconcentration of different analytes are reported in the literature including 2-pyrazoline derivatives in serum and urine [26], fat-soluble vitamins in urine, tap water and fruit juice [27], trihalomethanes in drinking water [28], dichloro-nitrobenzene and dichloro-nitroaniline [29], organochlorine pesticides [30], benzene, toluene and xylene [31] and polybrominated diphenyl ethers in urine and water samples [32] by using chromatographic systems.

The enhancement in the sensitivity of the method due to the increase in interaction area with the aid of dispersive liquid-liquid microextraction was reported in a number of studies for the analytes including chlorophenols [33], polycyclic aromatic hydrocarbons [34], diethofencarb and pyrimethanil in aqueous samples [35] for halogenated organic compounds [36] and organochlorine pesticides in water samples [37] and for volatile aldehyde biomarkers in human blood [38].

Considering sub ppb levels of oxon metabolite in biological samples, it is clear that more sensitive extraction methods are required for its determination along with the parent molecule to design efficient strategies for the investigation of pesticide metabolite. This study is the first attempt that deals with SFODME for preconcentration and determination of endocrine disruptor pesticide CP and CPO in urine samples in ppt range. The method can easily be adopted by clinical laboratories those are not facilitated with expensive equipment.

2. Experimental

2.1. Chemicals and reagents

Pesticide certified standards of chlorpyrifos and chlorpyrifos-oxon were purchased by Labor Dr Ehrenstrofer-Schafers (Augsburg, Germany). A standard mixture (10 mg/L) was prepared by dissolving the selected pesticides in acetone, and stored in the dark at 4 °C. Diluted working standard solutions were prepared just before use. Organic solvents used in extraction including 1-undecanol, 1-dodecanol and 1-bromohexadecane were obtained from Alfa Aesar (Karlsruhe, Germany). 2-dodecanol and 1-chlorooctadecane were obtained from Sigma-Aldrich (St. Louis, USA). Reagent grade anhydrous sodium chloride was obtained from Merck (Darmstadt, Germany).

The measurements were made at ambient laboratory temperature and all the solutions were allowed to attain this temperature prior to the measurement. Aqueous solutions were prepared with ultra pure water (18.2 M Ω cm^{-1}) from a MilliPore Milli-Q Gradient water purification system (Merck, Darmstadt, Germany). Urine samples were obtained voluntarily.

2.2. Instrumentation

GC–MS analyses were performed using a Thermo Scientific DSQ II (Les Ulis, France) system consisting of a Trace GC Ultra gas chromatograph equipped Triplus autosampler and DSQ II Single Quadropole mass spectrometer (Les Ulis, France). For data processing, Excalibur software from Thermofinnigan was used. A DB-5 MS fused silica capillary column (30 m × 0.32 mm I.D. and film thickness 0.25 μm) was used. The split-splitless injector and MS transfer line were operated at 280 °C. 3.0 μL of sample were injected by splitless injector in the constant flow mode set at 1 mL/min and with an injection purge of 100 kPa. The temperature of the injector was set at 250 °C. The oven temperature program was started as follows: 60 °C for 1 min, rising to 150 °C by 10 °C/min then rising to 250 °C by 30 °C/min finally 70 °C/min ramp was applied until 280 °C, held for 3 min. EI mass spectrum database searches were carried out in a mass spectral library (National Institute for Standard Technology (NIST), search program version.1.5, Gaithersburg, MD, USA).

Quantization of target metabolites was performed using selected ion monitoring (SIM) mode. For SIM analysis the m/z for native and labeled molecular peaks for various target metabolites quantified were: 199, 314 and 316 (CP), 197, 270 and 298 (CPO), respectively.

The extraction procedure was carried out by using a heating magnetic stirrer, purchased from IKA-Werke RT-15 (IKA Labortechnik, HS501 digital, Germany) with a 10 mm stirring bar. 15 samples could be prepared at the same time by using this multi stirrer. For solidification procedure a deep freeze (White Westinghouse) was used. For removing water, mini centrifuge purchased from Combi-Spin FVL 2400 N (Boeco, Germany) was used.

2.3. Sample preparation procedure

Prior to the analysis, urine samples were filtered through 0.45 μm pore-sized cellulose acetate filters. 5.0 mL of standard or sample solutions were transferred to 20 mL vials with a stirring bar. Upon addition of 10.0 μL of 2-dodecanol, the magnetic stirrer was set at 800 rpm and the solution was mixed at 70 °C for 40 min. After the extraction, the sample vials were transferred to a deep freeze for 10 min until the organic solvent was solidified. The solidified solvent drop was then taken into a conical vial, where it melted immediately and any residual water was removed by centrifuging the conical vials at 4000 rpm for 3 min. After the separation of phases, 7 μL of the organic phase was transferred to a new conical vial and injected to the GC–MS system. This procedure can be seen in Supplementary material.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2014.01.004.

3. Results and discussion

3.1. Optimization of injection parameters

Considering the low thermal stability of CPO metabolite it was necessary to apply a pressure during the injection as indicated previously [9]. In fact, the signal had shown an increase with the pressure applied in a range of 50-1000 kPa. On the other hand, it is known that high pressure leads less reproducible signal formation. In order to justify the precision and stability, all the experiments were carried out at 100 kPa GC-MS injector block pressure. In this manner, loss of the signal of the analyte was prevented and large volume injection could be achieved. After injection, the column flow rate was automatically reduced to normal values for chromatographic analysis.

3.2. Optimization of extraction parameters

In the present work, the type of extraction solvent and volume, extraction time and temperature, stirring rate and effect of salt on the extraction efficiency were optimized. The optimization studies were carried out with three parallel measurements and the average values were given. In all optimization studies sample volume was taken as 10 mL and the deep freeze time was set at 10 min. The concentrations of the analytes were 2.0 ng mL^{-1}.