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Evaluation of Liquid-Liquid Extraction and Different Solid Phase Extraction Cartridges for Determination of Selected Synthetic Pyrethroid Insecticides in Whole Blood

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In this study, the comparison of classical liquid-liquid extraction (LLE) and different solid phase extraction (SPE) cartridges for determination of some synthetic pyrethroid insecticides in whole blood was aimed. Six synthetic pyrethroids (α-cypermethrin, permethrin, S-bioallethrin, cyphenothrin, cyfluthrin and tetramethrin) were spiked at three different concentration levels (250, 500 and 1000 ng/mL) in 1 mL rabbit blood and each dilution of the insecticides was applied classical LLE and three different SPE procedures (florisil, C_{18} and silica). Final solutions were analyzed with gas chromatography equipped with electron capture detector. Detection limits of the insecticides were found between 3.55 and 16.55 ng/mL. Recovery percentages were determined generally higher than 100 % and matrix effect was observed in LLE procedures of all pyrethroids. Solid phase extraction cartridges decreased this failure and SPE applications may evaluate beneficial for synthetic pyrethroid analysis in whole blood. However, the reduction of the matrix effect was occurred different grades for used SPE cartridges and it was insufficient in some procedures. Florisil cartridges were determined better than the other sorbents for providing good recoveries. This study may be analytical data for pyrethroid analysis using combinations of classical LLE and different SPE sorbents. It was also concluded that further studies should be performed with different LLE procedures and solutions of elution and extraction for determination of the optimal technique.

Key Words: Synthetic pyrethroids, Liquid-liquid extraction, Solid phase extraction, Blood.

INTRODUCTION

Synthetic pyrethroid insecticides show favourable selective toxicity towards insect pests and low toxicity to mammals and birds. Thus, they are widely used for control of many insects in agriculture, forestry, veterinary medicine, public health and in similar contexts. The presence of residual concentrations of the pyrethroids in environment due to the use of different formulations may possibly contribute to humans and domestic animals exposure either by inhalation or skin resorption[1,2]. The evaluation of residues in blood and body fluids gives an indication about the extent of exposure. Therefore, typical human and animal exposure assessment approach is to measure metabolic biomarkers in blood, urine or other biological fluids[3-6].

Liquid-liquid extraction (LLE) is one of the fundamental techniques for the separation of chemical species from a medium or other coexisting component. The most widely used procedure is extraction from an aqueous medium into a properly chosen organic solvent, particularly for partial purification of a biological fluid, such as blood containing toxic chemicals and has been used routinely in the pharmaceutical industry for drug analysis in body fluids[5]. However, analysis of blood for pesticide residues has some difficulties. Firstly, the toxicant concentrations are very low[4]. Also, blood is a complex matrix and especially, elimination of lipids and proteins is a big problem[5-8]. Therefore, usually, the key step in analytical procedures has not been the instrumental analysis but sample pre-treatment for isolation of the pesticides from the matrix. For this reason, most of the analytical procedures reported serum and blood require the application of clean-up steps to remove interferences and to improve detection limits[6,8,9].

Different clean-up techniques, such as treatment with sulphuric acid has been performed, but in the mid 1970's, an alternative approach, solid phase extraction (SPE) was introduced. This concept is basis for the design of a practical sample preparation technique consisting of small, disposable extraction columns filled with a variety of sorbents. Today, SPE is a powerful method for sample preparation and is used by most laboratories. Compared to classical LLE, SPE is faster, uses less solvent, eliminates emulsions and saves money[6,10,11].

For humans, synthetic pyrethroids are usually monitored in urine and less frequently in blood. Because, there is some difficulties are in the blood analysis. The major disadvantages of blood measurements are the venipuncture required to obtain the sample and low toxicant concentrations. Also, blood is a more complex matrix than urine[3,9]. However, generally the parent compound, instead of a metabolite can be detected directly in blood samples and the development of a blood analysis method may not require detailed information on the metabolism[3]. The classical LLE or SPE usage in pesticide analysis has been reported previously, but there have been limited reports of the combined use of SPE and LLE for the determination of pyrethroids in whole blood. Also, many methods have been published for measuring pesticides in human and animal matrices, there will be a continual need for methods that assess exposure to emerging pesticides. The aim of this study is to compare of classical LLE and different SPE cartridges for determination of some synthetic pyrethroid insecticides in whole blood.

EXPERIMENTAL

Fresh rabbit blood collected from healthy animals and analyzed for pyrethroid contamination before the study. Six synthetic pyrethroids (α-cypermethrin, permethrin, S-bioallethrin, cyphenothrin, cyfluthrin and tetramethrin) were spiked at three different concentration levels (250, 500 and 1000 ng/mL) in 1 mL blood, did not contain pyrethroids. Each dilution of the insecticides was applied classical

LLE and three different SPE procedures. Final solutions were analyzed with gas chromatography method and limit of detections (LODs), limit of quantifications (LOQs) and recovery percentages were calculated.

Reference analytical standards were used in the study. Cyphenothrin (95 %), α-cypermethrin (95 %), tetramethrin (98 %) and permethrin (94.5 %) were obtained from Ehrenstorfer GmbH (Augsburg, Germany). S-bioallethrin (95 %) was purchased from Shenzen Production Materials CO. Ltd. (Shenzen, China) and cyfluthrin (92 %) was obtained from Changzhou Ltd. (Changzhou, China). Acetone and *n*-hexane (GC analysis gradient) were obtained from Merck (Darmstadt, Germany).

A Shimadzu GC-17A equipped with electron capture detector (GC-ECD) was used for gas chromatographic analysis. Analysis was carried out on 30 mm × 0.32 mm i.d. fused silica capillary column with 0.25 μm film of 95 % dimethyl polysiloxane and 5 % diphenyl (Teknokroma, Spain). As a carrier gas nitrogen was used at constant flow 1.49 mL/min. Standards and samples were splitless injected 1 μL with the auto injector system. The same instrument conditions were applied for analysis of all samples. The detector temperature was set at 300 ºC, the injector temperature was set at 240 ºC and the column oven temperature program was as follows; the initial temperature was 100 ºC, hold for 4 min; increased at 10 ºC/min to 160 ºC; increased at 2 ºC/min to 250 ºC, hold for 20 min. Total analysis time was 75 min.

The method reported by Ramesh and Ravi[12] was used for LLE procedure, with some modifications. Pyrethroid spiked blood (1 mL) mixed well and extracted with 5 mL of *n*-hexane-acetone mixture (8:2, v/v) by vigorous shaking for 10 min. The sample was centrifuged for 5 min at 5000 rpm. Collected the supernatant and the sample was again re-extracted. Combined supernatants were centrifuged again at the same conditions. The supernatant was collected in a graduated test tube and concentrated to 0.5 mL at 40 ºC under gentle stream of nitrogen. Final volume was made up to 1 mL using *n*-hexane.

Florisil (Strata FL-PR, 1000 mg/6 mL), C_{18} (Strata C_{18}-E, 1000 mg/6 mL) and Silica (Strata SI-1, 1000 mg/6 mL) SPE cartridges were obtained from phenomenex (USA). Same extraction procedure was performed for all SPE applications. Firstly, LLE procedure indicated above performed and after the last centrifugation, the collected supernatant was concentrated to 2 mL at 40 ºC under gentle stream of nitrogen. Solid phase extraction cartridges were conditioned with 10 mL *n*-hexane. The sample was loaded on the conditioned cartridge and eluted with 10 mL *n*-hexane-acetone mixture (8:2, v/v). The eluate was concentrated to 0.5 mL at 40 ºC under gentle stream of nitrogen and final volume was made up to 1 mL using *n*-hexane.

RESULTS AND DISCUSSION

Retention time, LODs, LOQs of the insecticides and calibration results are shown in Table-1 and recovery percentages of classical LLE and different SPE sorbents are shown in Table-2.

TABLE-1
RETENTION TIME, LODS, LOQS AND CALIBRATION RESULTS

Insecticide	Retention time (min)	Detection limit (ng/mL)	Quantification limit (ng/mL)	Equation*	R^2
S-Bioallethrin	32.028	5.71	19.04	y = 50.108x + 3766.3	0.9934
Tetramethrin	47.810	15.90	52.99	y = 17.693x + 764.74	0.9926
Cyphenothrin	55.447	13.01	43.37	y = 50.667x + 365.92	0.9980
Permethrin	59.665	4.39	14.63	y = 35.523x + 106.23	1.0000
α-Cypermethrin	63.089	3.55	11.84	y = 93.175x - 504.10	0.9963
Cyfluthrin	63.339	16.55	55.18	y = 53.452x - 1395.5	0.9969

*: Average of five standard dilutions and five replications, x: concentration (ng/mL), y: response area.

TABLE-2
RECOVERY PERCENTAGES OF CLASSICAL LLE AND DIFFERENT SPE CARTRIDGES

Insecticide	Recovery (%)			
	LLE	Florisil	C_{18}	Silica
S-Bioallethrin	157.67 ± 22.68	121.00 ± 11.79	145.00 ± 18.68	148.00 ± 14.42
Tetramethrin	300.33 ± 105.08	272.00 ± 16.00	290.00 ± 35.04	283.00 ± 80.89
Cyphenothrin	141.33 ± 12.86	136.33 ± 8.50	139.33 ± 12.06	136.67 ± 18.15
Permethrin	108.67 ± 15.53	94.67 ± 16.65	114.33 ± 15.37	99.00 ± 8.66
Cyfluthrin	309.67 ± 40.08	185.67 ± 12.90	244.00 ± 55.03	241.00 ± 25.24
α-Cypermethrin	173.67 ± 28.15	164.67 ± 18.48	153.67 ± 23.03	154.67 ± 15.01

Average of three replications (mean ± SD).

Limit of detections (LODs) of the insecticides were determined between 3.55 and 16.55 ng/mL. Although sophisticated gas chromatography-mass spectrometry (GC-MS) based techniques enable very low LODs, such as 0.001 ng/mL[4] or 0.1-1.0 ng/mL[12]. These values may be acceptable for a GC-ECD performance. Leng *et al.*[1] and Lacassie *et al.*[13] determined LOD values 5 ng/mL for different pyrethroids in plasma and serum by using GC-ECD and GC-MS.

Exposure to hazardous pesticides is of great concern to the general population, because of the widespread use of the compounds in agriculture, human public health, home gardening, veterinary medicine and industry. The best way to determine human and domestic animal exposure to pesticides is to measure them (or their metabolites) directly in biological fluids (biological monitoring). This requires the development of accurate analytical procedures for the determination of pesticides and/or metabolites at the low levels found in these samples[14,15].

However, contamination of the analyte with impurities is a big problem in the analysis of biological samples. The matrixes are heterogeneous in nature and contain many different types of compounds which are difficult to separate and are sometimes very similar to the compounds of interest (have same retention time of target compounds). Due to this effect, called matrix effect, heights and areas of peaks of the target compounds increase on the chromatogram and concentrations are calculated

higher than real values. The impact of matrix effects on the accuracy, precision and robustness of bioanalytical methods is of growing concern in the pharmaceutical industry. Furthermore, the impurities include proteins, carbohydrates, lipids, enzymes, other drugs and exogenous compounds such as plasticizers, colouring, food additives can cause a serious damage to the column and detectors. Same problems might be seen at the synthetic pyrethroid insecticide analysis in body fluids. Thus, sample clean-up is very important in ensuring smooth running of the analysis and a good reliable result. The most conventional extraction and clean-up techniques are LLE and SPE[8,12,16,17].

The matrix effect problem was generally occurred in LLE of pesticides from biological fluids[8,16,18]. However, Ramesh and Ravi[12], simultaneously detected 13 different pyrethroids (include cyfluthrin, cyphenothrin and permethrin) from whole blood, using a LLE procedure and GC-MS in selective ion monitoring (SIM) mode and recovery experiments conducted in whole blood samples at the fortification level 1-1000 ng/mL showed 91-103 % recovery. The matrix effect was not seen and the recovery percentages for cyfluthrin, cyphenothrin and permethrin were found 94-96, 95-97 and 93-97, respectively. In present study, recovery percentage of permethrin was close to that study (96-126, mean 108.67 ± 15.53), but recovery percentages of cyfluthrin and tetramethrin were higher (276-354 (309.67 ± 40.08) and 180-374 (300.33 ± 105.05), respectively). Although the LLE procedure was very similar, generally an important matrix effect was observed for LLE of all synthetic pyrethroid insecticides in the present study. Probably, the instrumental analysis method (GC-MS) and mode (SIM), used by Ramesh and Ravi[12], were affected recovery performance and less matrix effect occurrence.

Corrion *et al.*[19] developed a sensitive method to detect several classes of pesticides (including pyrethroids, bioallethrin, cyfluthrin, cypermethrin and transfluthrin) and their metabolites in maternal and cord whole blood using LLE and electron impact GC-MS. In the quality control samples, recovery percentages of bioallethrin, cyfluthrin, cypermethrin and transfluthrin were detected upper than 100 (116.9, 122.1, 107.7 and 124.1, respectively). Similar to present study, the matrix effect was observed in various grades. In contrast, Liu and Pleil[5] performed a screening method using LLE and GC-MS for persistent organic pollutants (organochlorine, organophosphate and synthetic pyretroid pesticides and polychlorinated biphenyls) for human blood and low recovery percentages obtained. Recovery percentages of permethrin, cyhalothrin and cypermethrin were 18.8, 30.3 and 35.0, respectively.

Due to matrix effect problem, other clean-up methods, such as SPE, were used in many pesticide analytical procedures. Solid phase extraction provides the elimination of the co-eluted impurities and cleaner analytes[8,16,18]. Ogata-Kawata *et al.*[11] determined a direct method for permethrins in human blood using SPE (Oasis®-HLB cartridge) and GC-MS. A good SPE recovery efficiency have been obtained for *cis*-permethrin (97.7 ± 7.7 %) and *trans*-permethrin (99.7 ± 7.7 %). Lacassie *et al.*[13] also used same Oasis® SPE cartridges for detection of pesticides of various classes

(including bifenthrin) from human biological samples and the authors indicated that extraction recovery in serum studied at the lower and upper limits of linearity range varied owing to the pesticide studied, but was satisfactory for most of them (between 40 and 99 %).

However, sometimes insufficient recovery results were observed in SPE applications for synthetic pyrethroids[4,5,20]. Barr *et al*.[4] developed an analytical method for quantifying 29 contemporary pesticides (including *cis*- and *trans*-permethrin) using two different SPE sorbents and high-resolution mass spectrometry. In the end of that study, low recovery percentages were found for *cis*-permethrin (11 ± 7 and 13 ± 5 for C_{18} and Oasis®) and *trans*-permethrin (12 ± 4 and 14 ± 5 for C_{18} and Oasis®). Liu and Suzuki[19] analyzed three organophosphates and four synthetic pyrethroids (methothrin, cyhalothrin, fenopropathrin and fenvalerate) in human urine and plasma by C_{18} and gas chromatography with flame ionization detector. Different methanol concentration of the diluent to be mixed human plasma at the initial step was determined very effective factor on elution recoveries from SPE cartridges. Good recovery could be obtained with diluent at less than 10 % of methanol for methothrin, however other three pyrethroids equally showed low recovery at 0 % of methanol, the maximal recovery around 70 % and again low recovery at 100 %.

Due to, different (sometimes low) recoveries might have been obtained in SPE applications for detection of synthetic pyrethroids, LLE and SPE performed together in some concepts[18]. In the present study, classical LLE and combined three different LLE/SPE procedures for synthetic pyrethroid extraction from whole blood were compared and recoveries were determined generally higher than 100 %. The most proper recovery values were seen in permethrin [99.00 ± 8.66 (silica) and 108.67 ± 15.53 (LLE)]. However, recovery percentages were determined 241.00 ± 25.24 (silica) -309.67 ± 40.08 (LLE) and 283.00 ± 80.89 (silica) -300.33 ± 105.08 (LLE) for cyfluthrin and tetramethrin, respectively. Indeed, matrix effect was observed LLE procedures of all pyrethroids. Solid phase extraction applications may evaluate beneficial for synthetic pyrethroid analysis in whole blood. However, the reduction of the matrix effect was occurred different grades for used SPE sorbents and it is insufficient in some procedures. Although extreme values were observed in silica cartridges, generally florisil cartridges were determined better than other sorbents for providing good recoveries.

A lot of factors affect the performance of an analytical method for detection of pesticides in biological fluids. The recoveries are inversely related to the polarity of analytes. Also, extraction or elution solutions are very important factors[4,16,18]. Liu and Suzuki[20] reported that only by changing methanol concentration of sample diluent affected the recoveries of various organophosphate and pyrethroids. Also, there is need for developing new methods that pesticide analysis in body fluids, continuously. This study may be analytical data for pyrethroid analysis using combinations of classical LLE and different SPE cartridges. It was also concluded that further studies should be performed with different LLE procedures and extraction and elution solutions for determination of the optimal technique.

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