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Practical comparison of three principles for the extraction of residual pyrethroids from aqueous matrices: Relative merits and demerits

Saeed S.Albaseer

Department of Chemistry, Faculty of Education, Thamar University, Thamar, (YEMEN)

E-mail : sshalbaseer@yahoo.co.uk

ABSTRACT

The choice of a sample preparation method is not an easy task, as many aspects should be accounted for. These aspects are either related to the analyte(s) of interest or the method and its compatibility with the sample matrix. Several methods, however, can be applied for the extraction of pyrethroids from aqueous samples but none is free of drawbacks. In this paper, three recently developed principles for the extraction of synthetic pyrethroids from aqueous samples have been practically and statistically evaluated. Merits and demerits have been discussed. It has been found that the method extraction capability is not the solely characteristic that should be looked at when choosing an extraction method. Other characteristics such as method detection limits, sample preparation time, and stability of the analytes in the extracting phase are also important. Pyrethroids are very hydrophobic and adsorb strongly onto walls of devices used for extraction and, hence, the kind of extraction vessel material is important.

KEYWORDS

Pyrethroids;
Environmental analysis;
SPE;
MLLE;
DLLME.

INTRODUCTION

The use of pesticides to protect agricultural products has been critical. In fact, it has become difficult to dispense with such chemicals in agricultural activities as their use brings several benefits such as the increase of agricultural production by protecting commodity yield and quality. Pyrethroid insecticides are a class of pesticides whose use in agricultural activities has seen a considerable increase in the recent decades due to several advantages they offer. These compounds, however, are not free of adverse effects. The accumulation of these chemicals in the environment, although at low concentrations, is sufficient to cause lethal effects, especially, on honeybees and fish (LC_{50} values <1.0 μg/L)[1].

The Groundwater Threshold Values proposed by (The Draft European Communities Environmental Objectives (Groundwater) Regulations) are as low as 0.075 μg/L for cypermethrin[1], and 20 μg/L for permethrin[2]. However, most synthetic pyrethroids are photodegraded by sunlight. In general, the degradative processes, which occur in the environment, lead to less toxic products. Permethrin, for instance, disappears rapidly from the environment, in 6-24 hours from ponds and streams, seven days from pond sediment, and 58 days from foliage and soil in forest[3].

Determination of synthetic pyrethroids in natural water is, however, a difficult analytical task, because of the very low detection limits required, the complexity of the matrix and the very hydrophobic nature of these

chemicals. Thus, in general, complicated, time-consuming extraction and purification processes, based on various extraction principles, are necessary before final determination step. This paper presents a practical comparison of three sample preparation techniques (micro liquid-liquid extraction (MLLE), dispersive liquid-liquid microextraction (DLLME) and solid phase extraction (SPE)) applied for the determination of synthetic pyrethroids in surface water. These three methods have been developed in our laboratory and are based on different principles. MLLE is essentially a simultaneous extraction and concentration procedure suitable for determination of a wide range of organic compounds in water. The principle of MLLE is the use of a very small volume of solvent for the extraction of target analytes from a large volume of sample (e.g., water) to give an extract that can be directly injected into a chromatographic column without any further treatment. DLLME is operated by the rapid addition of a small amount of a mixture of two selected solvents (extracting solvent and dispersing solvent) to an aqueous sample (in a conical test tube). The rapid addition of the solvent mixture results in forming a cloudy solution of small droplets of extracting solvent which are dispersed throughout the aqueous phase. In consequence of the very large surface area formed between the two phases, hydrophobic solutes (such as synthetic pyrethroids) are rapidly and efficiently enriched in the extracting solvent very quickly. SPE utilizes the affinity of analytes to adsorb onto a certain solid material (adsorbent) that are then eluted later with a small amount of an organic solvent.

In this paper, merits and demerits of these principles regarding their use for determination of synthetic pyrethroids in surface water are discussed.

MATERIALS AND METHODS

Chemicals and materials

Permethrin (PER) and Resmethrin (RES) (PESTANAL®, analytical reagent grade, 98.2% and 98.5% purity, respectively) were obtained from Sigma-Aldrich Co., USA. Cypermethrin (CYP) (technical grade, 95% purity) was donated by Hyderabad Chemicals Pvt. Ltd, Hyderabad, India. HPLC grade acetonitrile, methanol, n-hexane and acetone were obtained from MERCK (Merck Specialties Pvt. Ltd, Mumbai, India). Analytical reagent grade sodium chloride (NaCl) and ethanol were obtained from MERCK (Merck Specialties Pvt. Ltd, Mumbai, India). Analytical reagent grade carbon tetrachloride and tetrachloroethane were obtained from RFCL Pvt. Ltd, New Delhi, India. HPLC grade tetrachloromethane and chlorobenzene were obtained from MERCK (Merck Specialties Pvt. Ltd, Mumbai, India). All reagents were used without further purification.

Instrumentation

The chromatographic analysis was performed on an ultra performance liquid chromatography (UPLC) system (LC-20AT Prominence, Shimadzu, Japan), equipped with a binary solvent delivery system, an in-line degasser, an injection valve with a 20 µL sample loop, and a UV-diode array detector model SPD-M 20A Prominence. The chromatographic separation was performed on a Phenomenex Luna C18, (250 x 4.6 mm I.D., 5-µm particle size, Torrance-CA, USA). Mobile phase was acetonitrile- methanol-water, 20:60:20 (v/v). Ultrapure water, purified by a Milli-Q water purification system, Millipore (Bedford, MA, USA) was used throughout the experiments unless stated, and was collected on daily basis and degassed with a vacuum pump and further filtered through 0.45 µm membrane (Hydrophilic Nylon, Millipore). Samples of 20 µl volume were injected at a mobile phase flow rate of 1.0 mL min^{-1}. Diode array detector was set at 220 nm. Centrifugation was performed on a refrigerated centrifuge model Sigma 4-16K (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany).

Sample preparation

The stock solutions of the individual standard solutions and mixtures of three SPs, that is, cypermethrin, resmethrin and permethrin were prepared in HPLC grade acetonitrile and kept in amber reagent bottles refrigerated at +4° C. Working standard solutions of concentrations ranged from 50–2000 μgL^{-1} were prepared by dilution of the above stock solutions in pure HPLC grade acetonitrile and used for constructing the standard calibration curve. Spiked samples in distilled water were prepared on daily basis and used for method optimization and validation. Tap water samples were collected from our lab and surface water samples were

collected from an agricultural runoff-fed-pond located near our laboratory.

Extraction procedure

Three extraction procedures of the three methods can be briefly described as follows:

For DLLME, 5.0 mL water sample was placed in a 15 mL screw cap polypropylene tube with a conical bottom; the sample was spiked with the analytes and 0.25 g NaCl (5%, w/v) was added and dissolved. A mixture of 65.0 µL carbon tetrachloride (CCl_4) and 1.0 mL acetonitrile was rapidly added into the aqueous solution using a 1.0 mL micropipette. A cloudy solution (water, acetonitrile and carbon tetrachloride) was immediately formed in the tube. Then, the mixture was gently shaken for 5 min by hand. In this step, SPs were extracted into the fine droplets of carbon tetrachloride. The mixture was then centrifuged for 3 min at 5000 rpm (refrigerated centrifuge, 27° C); the carbon tetrachloride phase was sedimented at the bottom of the centrifuge tube (~30 µL). The upper aqueous solution was removed and 20 µL of the sedimented phase was injected into the HPLC system for analysis using a 25 µL microsyrange (Hamilton, Switzerland).

MLLE was operated using a specially designed 100 ml volumetric flask. The procedure was performed as follows: A spiked100 mL water sample was added to an especially designed extraction flask. Accurately weighed 0.5 g NaCl (10%, w/v) was added and dissolved by shaking the flask. 100 µl of HPLC-grade n-hexane was added to the flask. The flask was sealed with a glass stopper and manually shaken for 2 minutes and then allowed to stand until complete phase separation was observed. Saturated salt solutions was added slowly and carefully from the tube side (6 mm I.D.) connected to the bottom of the flask to raise the organic layer to the narrow neck where it is being withdrawn using a 25 µL Hamilton microsyringe, a volume of 20 µl was injected into RP-HPLC system. SPE was operated as follows: Commercial Teflon (6.0 g) was cut into small pellets and was packed in a glass column with a stopcock. A spiked100 mL water sample was transferred to the column and the stopcock was opened. The sample was poured out the column at a flow rate of 5 mL min^{-1}. 2.5 mL of methanol (desorption solvent) was added to the column which was then immersed in a sonication bath for 5 min to completely recover the adsorbed phase. The organic phase was evaporated until near dryness and 0.5 mL methanol was added to

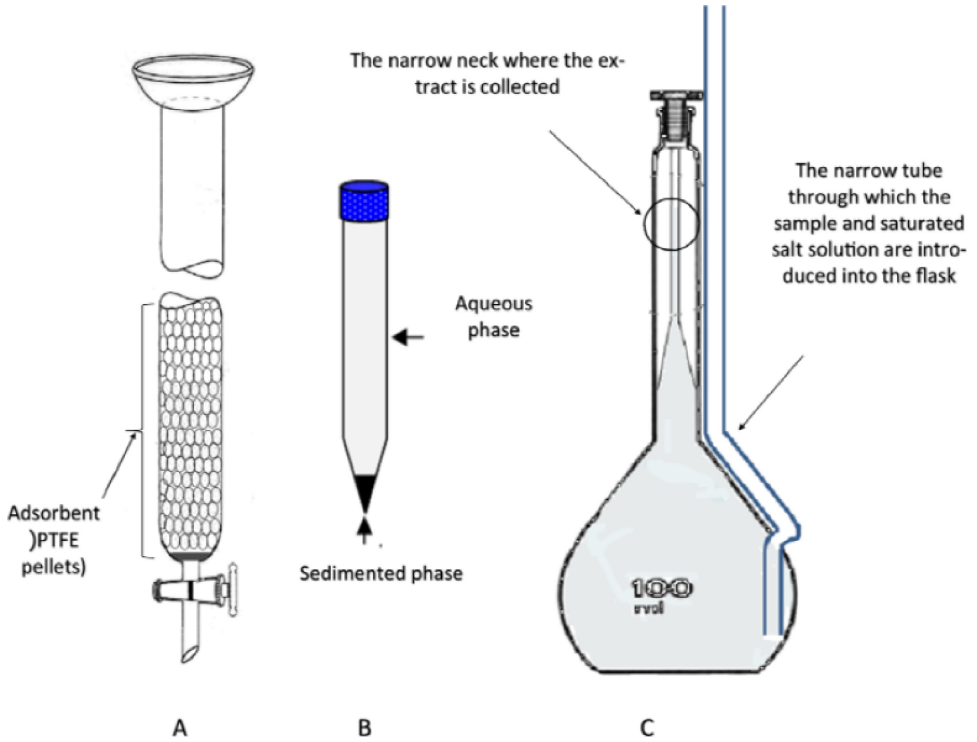

Figure 1 : Apparatuses used to conduct (A) solid phase extraction, (B) dispersive liquid-liquid microextraction, and (C) micro liquid-liquid extraction

dissolve the analyte extract, of which a volume of 20 µl was injected into RP-HPLC system. For DLLME operations, 5.0 mL water sample was placed in a 15 mL screw cap polypropylene tube with conical bottom; and 0.25 mg NaCl (5%, w/v) was added and dissolved. A mixture of 65.0 µL carbon tetrachloride (CCl_4) and 1.0 mL acetonitrile was rapidly added into the sample using a 1.0 mL micropipette. A cloudy solution (water, acetonitrile and carbon tetrachloride) was immediately formed in the tube.

Then, the mixture was gently shaken for 5 min by hand. In this step, synthetic pyrethroids were extracted into the fine droplets of carbon tetrachloride. The mixture was then centrifuged for 3 min at 5000 rpm (refrigerated centrifuge, 27 C); the carbon tetrachloride phase was sedimented at the bottom of the centrifuge tube (≈30 µL). The upper aqueous solution was removed, using a 1.0 ml micropipette and 20-µL of the sedimented phase was injected into the HPLC system for analysis using a 25-µL microsyrange (Hamilton, Switzerland). Figure 1. illustrates the apparatuses used or conducting the described methods.

RESULTS AND DISCUSSION

Recovery studies and matrix effect

Recovery studies were conducted using distilled water as well as surface water to investigate possible matrix effect.

Results illustrated in TABLE 1 show that the extraction efficiency of micro liquid-liquid extraction method (MLLE) was negatively affected by sample matrix, especially for CYP and PER. Interestingly, in case of DLLME; the matrix effect was positive where recoveries of all synthetic pyrethroids improved when real samples were analyzed. However, SPE efficiency was not significantly affected by the matrix effect. These observations could be explained as follows: surface water contains solid particulates and organic matter onto which synthetic pyrethroids adsorb strongly and instantly[4], and thus the dissolved phase is reduced in such matrices in comparison to distilled water. Hence, the lower recoveries obtained by MLLE in case of surface waters came as a result of its inability to extract the adsorbed phase of synthetic pyrethroids, i.e., only freely dissolved phase is extracted using MLLE method. Therefore, it is expected that the recoveries of the synthetic pyrethroids investigated will be further reduced if the natural water sample contains a higher percentage of solid particulates and/or organic matter. Although this can be considered as a drawback of MLLE, it could, on the other side, be an advantage in case only freely dissolved phase is targeted. Several studies[e.g., 5,6] have shown that only freely dissolved phase of synthetic pyrethroids are bioavailable to water organisms and thus, toxic effects of synthetic pyrethroids are directly associated with the freely dissolved phase but not the total concentration of synthetic pyrethroids. On contrary to MLLE, dispersive liquid-liquid microextraction (DLLME) showed higher extraction efficiency when applied to natural surface water samples. In any case, the efficiency of DLLME may improve if glass tubes were used instead of plastic tubes because synthetic pyrethroids associate to glass walls at a very slow rate[7-11].

In case of SPE, the recovery results indicate that the method is efficient and not affected by matrix composition. The almost identical efficiency of SPE for both distilled and surface water samples may indicate that synthetic pyrethroids adsorb preferentially onto PTFE surface (adsorbent material) rather than onto surfaces of solid particulates, organic matter or container walls. So, synthetic pyrethroids adsorption onto PTFE is not significantly affected by the composition of water sample.

TABLE 1: Efficiency of MLLE, DLLME and SPE for the extraction of pyrethroids from water

Extraction technique	Distilled Water			Surface water		
	R%			R%		
	CYP	RES	PER	CYP	RES	PER
MLLE	87.5%	94.5%	84.7 %	77.0	94.0	78.0
DLLME	94.6	75.3	74.5	98.1	82.5	89.6
ic-SPE	94.1	72.5	86.8	101.7	72.8	89.2

Detection and quantification limits

The results illustrated in TABLE 2 show clearly that DLLME is inferior to MLLE and SPE in respect to detection and quantification limits. This could be attributed to the fact that both MLLE and SPE allow the use of relatively larger sample volume (100 mL), while DLLME utilizes only 5 mL sample volume. Although,

the use of a small-volume sample may be advantageous, especially because it will reduce the troubles associated with collection, handling and preservation of large sample volumes, the method, on the other side, should be capable of providing detection limits that are low enough to allow monitoring of synthetic pyrethroids at levels that are toxic to aquatic organisms. The European Directive on Drinking Water Quality (98/83/CE) has established 0.10 μg/L as a maximum contaminant level (MCL) for individual SP, and 0.50 μg/L for total SP pesticides[12]. Therefore, it is clear that DLLME with detection limits in the range of 0.4-0.53 μg/L should not be the preferred choice. However, MLLE and SPE with detection limits in the range of 0.05"0.08 and 0.03"0.06 μg/L, respectively, will suit for this purpose with more reliable results are achieved with the latter.

Online coupling

Online coupling of extraction methods with analytical instruments such as HPLC and GC has become a trend in analytical chemistry due to several advantages the coupled systems offer. These advantages include reduction of analysis time, prevention of sample contamination, minimization of analyte losses, and possible improvement of precision and accuracy. It is obvious that LLME is not suitable for online coupling due to some technical difficulties, although it is characterized by being a one-step procedure. As for DLLME, the difficulties facing coupling lie in the fact that it involves centrifugation. On the other hand, SPE coupling is possible with slight modifications, especially because SPE has the advantage that the desorbing solvent can be used as an elution solvent when coupled to RP-HPLC.

Method automation

Automated methods have the advantage of being fully monitored through automated machines leading to minimized errors. Our attempts to design an automated instrumental setup for MLLE were unsuccessful. For DLLME and SPE, on the other hand, several research papers have reported the design of automatable setup that can ease the application of such methods for monitoring environmental contaminants[e.g.,13-15].

TABLE 2 : Comparison of features of MLLE, DLLME and ic-SPE for the extraction of pyrethroids from water

Parameter	Extraction technique		
	MLLE	DLLME	SPE
Matrix	Water	Water	Water
Sample volume	100 mL	5 mL	100 mL
Sample preparation time	~ 10 min	~ 20 min	~ 40 min
Recovery (R %)	84.7–94.5	74.5–94.6	72.5–94.1
Enrichment factor (EF)	1058–1181	109–135	1882–3318
RSD%	3–10	5.6–10.8	3.2–9.4
MDL (μg L-1)	0.05-0.08	0.4-0.53	0.03-0.06

On -site implementation

Analysis of environmental samples involves collecting samples with subsequent sample handling, transport and preservation until the time of analysis. During this time, however, the sample integrity may be affected because several chemical and physical processes such as photodecomposition, adsorption, vaporization, thermal decomposition, microbial action and chemical reaction may occur in the sample between the time of sample collection and analysis. The on-site analysis approach has several advantages such as: i) minimization of errors associated with sample transport and storage, and hence, more accurate and precise data[16,17], ii) shorter time analysis, and iii) possibility of immediate monitoring of environmental contamination. A close look at the three methods, i.e., DLLME, MLLE and SPE reveals that only SPE can be used for on-site extraction. This is because it has been demonstrated that the adsorbed phase of synthetic pyrethroids shows higher stability than dissolved phase[18]. Hence, sample can be extracted on-site by adsorbing them on to PTFE column, which is then transferred to the lab where analytes are desorbed and quantified using an appropriate analytical method.

CONCLUSIONS

The choice of an extraction or sample preparation method is not an easy task, as many aspects should be considered. These aspects are either related to the analyte(s) of interest or the method and its compatibility with the sample matrix. Several methods, however, can be applied for the extraction of synthetic pyrethroids from aqueous samples but none is free of drawbacks, and hence, it is the experience of the analyst that determines which method is more appropriate for a particular extraction system. In general, the following guidelines may be useful when choosing an extraction method

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for the determination of synthetic pyrethroids:

i. the extraction time should be short (10 – 30 min),
ii. all vessels used for extraction and other sample preparation should be made of glass,
iii. although sample acidification is useful for preserving synthetic pyrethroids in environmental samples, the sample acidity during extraction depends on the extraction technique applied; in general, efficiency of sorbent-based extraction techniques will improve at high sample acidity and vice-versa for partition-based extraction techniques, and
iv. if very low LOD is required then extraction methods which utilize large sample volumes would be preferred.

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