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Cloud point Extraction and Preconcentration of trace amount of α -cypermethrin in Environmental samples and determination using high – pressure liquid chromatography.

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ABSTRACT

A new, effective cloud – point extraction (CPE) method for determination of the pesticide α – cypermethrin from the pyrethroid group, in environmental water samples, by high – pressure liquid chromatography with UV spectrophotometric detection, was developed and validated. The key extraction conditions were as follows: surfactant 0.4% (v/v) Triton X – 114, temperature and incubation time of 65°C and 20min, respectively, and 100 μ l organic solvent (acetonitrile) for dissolving the micellar phase. The acetonitrile micellar phase with the isolated α – cypermethrin was analyzed by reverse phase high – pressure liquid chromatography using the isocratic flow of mobile phase consisting of water and acetonitrile .The Preconcentration and enrichment factors of the method were found to be of 28.57 and 29.35 fold, respectively, achieving the detection limit of (0.018) μ g.ml⁻¹ with linear range of(0.06 – 3) μ g.ml⁻¹and correlation coefficient (0.99945) . The proposed method was successfully applied to the determination of traces amount of cypermethrin in water samples.

Keywords: α – cypermethrin, cloud point extraction, HPLC, surfactant, water sample.

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INTRODUCTION

A pesticide is substance or mixtures of substance intended for preventing, repelling destroying or mitigating pests. pesticides may be a chemical substance having antimicrobial or a biological agent (bacteria or such as a virus) or disinfectant properties, or a device used against pests. Pests include insects, plant pathogens, weeds, mollusks, birds, mammals, fish, nematodes and microbes that destroy property spread disease, act as vectors for disease or cause a nuisance. The word “pesticide” is an umbrella term for all insecticides fungicides, herbicides, rodenticides; wood preservative and house hold disinfectants that may be used to kill some pests [1,2]. There are four main groups of chemical classification of pesticides and they are organo chlorine, organo phosphorous, Carbamate and pyrethroid [3-5] . Pyrethroid is one of most important class of pesticide which introduced in the early 1970s; Permethrin the first pyrethroid is synthesized from insecticidal plant called pyrethrum [6]. The pyrethroid pesticides, as neurotoxic pesticides which are high lipophilicity, poor water solubility, slow metabolic rate in the environment with long residues [7].

Cypermethrin is a type 2 synthetic pyrethroid as shown in figure 1 , it is widely used as pest control in forestry, agriculture, horticulture, health programs and private homes. Cypermethrin is neurotoxic insecticide either inhibiting neurotransmitters or affecting voltage – gated sodium channels. As pyrethroid, Alpha cypermethrin interfere with sodium (Na) channels in the tail (axon) of neurons, by blocking these gates open they elongate the time of nerve impulse. This lead to muscular paralysis and death [8-10].

The α – cypermethrin molecule contains in contrast to type 1 pyrethroid, an α – cyano group in the phenoxy benzyl alcohol moiety, which seems to be responsible for production of long – lasting prolongation of sodium permeability, clinically characterized by choreoathetosis and salivation [11,12] US Environmental protection agency (EPA) has also classified cypermethrin as a possible human carcinogen [13]. Cypermethrin is practically non – toxic to birds but is highly toxic to fish and aquatic invertebrates and this is mainly because it is metabolized and eliminated significantly more slowly by fish than mammals or birds, it is also highly toxic to bees [14,15] .

Various methods used for determination of cypermethrin have been reported, such as , gas chromatography – mass spectrometry after QuEChERS method [16], gas chromatography with electron capture detection (GC – ECD) [17,18], Dispersive liquid – liquid micro extraction (DLLME) coupled to high performance liquid chromatography (HPLC) with UV detection [19], Spectrophotometric method [20-22]. Recently cloud point extraction (CPE) has been accepted as an effective method for sample preparation. CPE offers good (features) as follow: simple, use less solvent and nonvolatile surfactant that are friendly to the environment. Moreover, CPE can be used for extraction of the analytes and remove the sample matrices in one step [23].

In CPE, phase separation is achieved by formation of micellar phase using surfactant after changing temperature or adding salt to an aqueous solution. The aggregation of micelles depends on the minimum concentration of surfactant which is called as the critical micelle concentration (CMC) [24-26].

EXPERIMENTAL

Materials and measurements

The materials and reagents used in this work with high purity, doubly distilled water was used in the preparation of all solutions and for final rinsing of glass wares, standard Alpha-cypermethrin was purchased from Dr. Ehrenstorfer (Augsburg, Germany). The stock standard solutions of (100 mg ml⁻¹) of Alpha cypermethrin were prepared in methanol. The stock solution and diluted standard solutions were stored in glass volumetric flasks in the dark at (4°C), Triton X – 114 was purchased from Amresco LLC (Solone, USA) and used without further purification. Various concentrations (v/v) of aqueous surfactant solutions were prepared by dissolving appropriate amounts of surfactants in distilled water. Methanol and acetonitrile (gradient grade) were purchased from Scharlau- Spain

The chromatographic analysis was performed on Shimadzu LC –20 AD liquid chromatography with the shaking water bath SW23 microprocessor with PID temperature control (JULABO GmbH, Germany) was employed during the course of CPE experiments. For the solution PH measurement, a portable pH – meter microprocessor (HANNA, Germany) was used, centrifuge was used to accelerate the phase separation process.

CPE procedure: Atypical cloud point experiment has been carried out according to following procedure: to an aliquot of 10ml of a solution containing known amount of alpha cypermethrin (CYM) standard ($1\mu\text{g.ml}^{-1}$) or sample solution were taken into 10ml graduated centrifugal tubes. Then adding 1ml of buffer solution (pH=7). After that, 0.4ml of 10% (v/v) Triton X – 114 were added and diluted to mark with water. The content of each tube was transferred to water bath at 65c° for 20min to form cloudy solution. The separation of the phases was accelerated by centrifuging at 3500 rpm for 20 min. The whole system was cooled in an ice bath so that the surfactant rich phase would regain its viscosity and the bulk aqueous phase was easily decanted. The surfactant rich phase was diluted with $100\mu\text{l}$ acetonitrile to reduce its viscosity before HPLC analysis and then it was transferred to the HPLC sample vials.

HPLC conditions: The HPLC separation was performed on (Reposil 100 C18, $5\mu\text{m}$) column the mobile phase at isocratic elution was pumped at flow rate of 1ml.ml^{-1} . Acetonitrile: water (95:5 v/v) was used as mobile phase. The column temperature was set at 40c° . Each sample was injected three times. The injection volume of sample was $20\mu\text{l}$.

Analysis of water sample: Tap water samples were collected from the laboratory. River water samples were collected from the river in Baghdad, Iraq. All water samples were filtered using a $0.45\mu\text{m}$ nylon membrane filter to remove suspended particulate matter and then stored at 4c° in the dark. An appropriate amount of standard solutions of different concentrations were added to the water samples for recovery tests.

RESULTS AND DISCUSSION

In order to obtain the best extraction efficiency, several experimental parameters including surfactant concentration, equilibration temperature, equilibration time, solution pH and time of centrifugation were investigated.

Effect of surfactant concentration

In the CPE system, the concentration of surfactant is essential for sufficient extraction of target analytcs and the Preconcentration factor depends on the amount of surfactant. Therefore, the effect of the surfactant concentration is investigated in the range of 0.2 – 1.2 % (v/v), while the other conditions are as follows equilibration at 50C° for 20min followed by 3500 rpm centrifugation for 30min. As can be seen from (fig.1) 0.4% (v/v) Triton X – 114 yields the highest response signal. Below this concentration, the volume of surfactant – rich phase is too small to separate from the bulk solution accurately, which makes it more difficult for subsequent analysis, above this concentration the response decreases gradually due to the fact that the volume of the surfactant – rich phase increase as the concentration of surfactant increases, which results in the decrease Preconcentration factor. On the other hand, excessive amount of surfactant can interfere with the back – extraction process. Based on these experimental results, 0.4% (v/v) Triton X – 114 was selected for the following experiments.

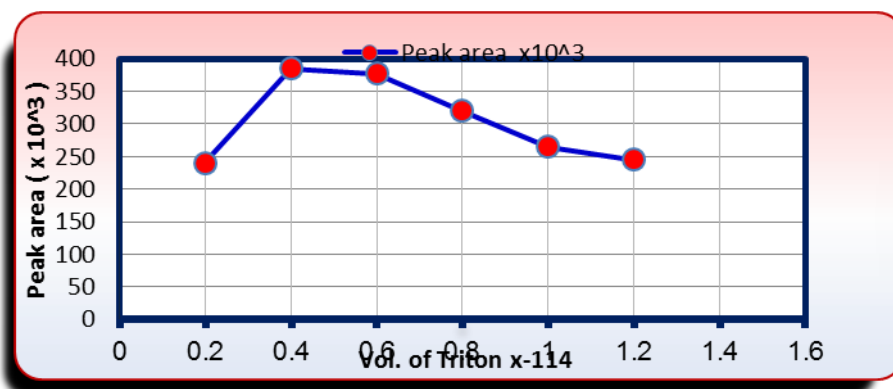


Figure 1. Effect of surfactant concentration on the extraction of cypermethrin by CPE method

Effect of equilibration temperature and Time

Fig. 2 shows the impact of equilibration temperature on the extraction of cypermethrin by varying the temperature from 25 to 75°C at incubation time of 25min and keeping other variables constant. It was observed that peak area for cypermethrin increased as the temperature was increased up 65°C. Beyond this point, the signals gradually decreased. Therefore, 65°C was selected. The effect of incubation time (5 – 30 min) at 65°C was also investigated, the highest (signal response) value reached when extracted for 20min. When the extraction time was longer than 20min, the peak area of the studied insecticide decreased shown in fig.3

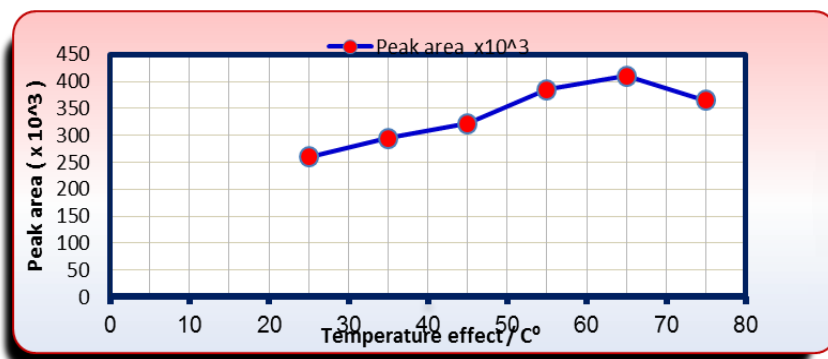


Figure 2. Effect of equilibration temperature on the extraction of cypermethrin by CPE method.

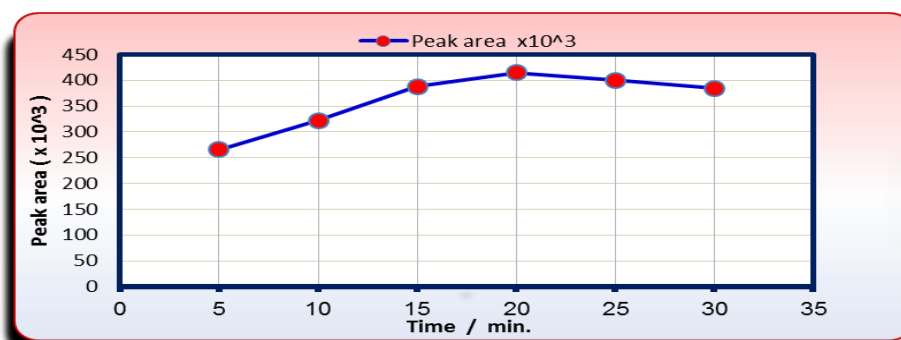


Figure 3. Effect of incubation time on the extraction of cypermethrin by CPE method.

Effect of sample pH

When the aforementioned conditions were fixed, the effect of sample pH was studied in the pH range of 2 – 10 by using the phosphate buffer solutions. In the case of cypermethrin insecticide, the optimum sample pH value of 7 was selected for the analysis; the results are shown in fig.4.

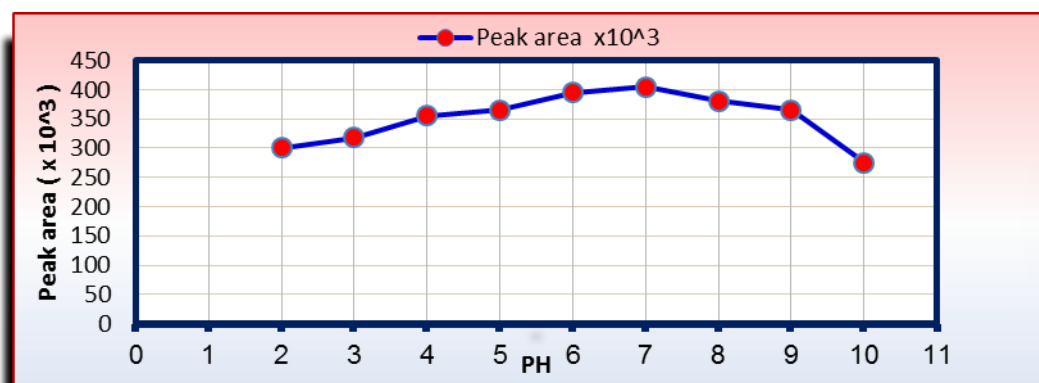


Figure 4. Effect of pH on the extraction of cypermethrin by CPE method.

Effect of centrifuging time

Centrifuging time has an important effect on extraction efficiency. In this study, the effect of centrifuging time was tested at the constant centrifuge speed of 3500 rpm. The dependence of extraction of sample species on centrifuge time (5 – 30 min) was presented in fig.5. This figure shows that the best separation was obtained with centrifugation time of 20min, at shorter times the separation was not complete and it is probably the longer centrifugation time that caused the partial back extraction to the aqueous phase (28).

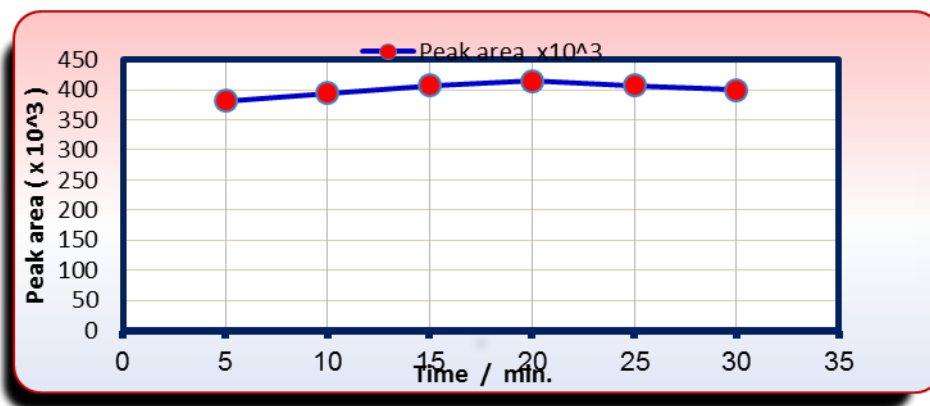


Figure 5. Effect of centrifuging time on the extraction of cypermethrin by CPE method.

Analytical characteristics and method validation

The analytical performances and validation of the proposed method such as linearity, limit of detection (LOD) limit of (LOQ) were determined. The results are shown in table 1. In order to determine the linearity of the proposed method calibration curves were constructed by triplicate injections of standard (CYM) insecticide obtained after CPE at different concentrations of CYM. Peak area of CYM was plotted against its concentration ($\mu\text{g}.\text{ml}^{-1}$). Determination of limits of detection (LOD) was carried out by identifying the concentration giving the signal – to – noise ratio (s/n) of (s/n=3). To compare the sensitivity of the CPE and HPLC method, enrichment factor (EF) is defined as the ratio of slope obtained from CPE – HPLC and HPLC (direct injection).

Table 1. Analytical parameter and statistical data of two methods

	Without CPE	With CPE
Linearity range ($\mu\text{g}/\text{ml}$)	0.5-10	0.06-3
Linear equation	$y=13.807x + 1.3731$	$y=405.33x - 9.2673$
R²	99.93%	99.89%
r	0.999649939	0.999449849
LOD ($\mu\text{g}/\text{ml}$)	0.15	0.018
LOQ($\mu\text{g}/\text{ml}$)	0.5	0.06
Preconcentration Factor(PF)	28.57	
Enrichment Factor (Enr.F)	29.35	

Application to water samples

In order to test the reliability of the proposed methodology for assay of CYM insecticide, it was applied to the determination of concentration this insecticide in water samples including tap, river water under optimum conditions. The developed methodology was validated by recovery studies. Good recoveries were achieved for all analyzed sample. All the recoveries obtained in this work were calculated as average values, the standard deviation from three replicate analyses, each with three injections.

Table 2. Recovery of cypermethrin in spiked water samples (river water, tap water)

sample	Added concentration Mg.L ⁻¹	Found Mg/L	Recovery %	RSD %
River water	0.8	0.769	96.1	1.85
	1.0	0.982	98.2	2.23
	2.0	1.958	97.9	2.74
Tap water	0.8	0.775	96.9	2.171.03
	1.0	0.973	97.3	0.85
	2.0	1.963	98.2	

The result obtained is shown in (table 2). It is apparent that recoveries of the method are satisfactory and precision is good, with relative standard deviation 2.74%. (fig.6): show the chromatograms obtained from (a) blank real sample ,(b)real sample spiked with 0.1 µg.ml⁻¹, (c) cypermethrin standard solution. The analytic was completely separated from the surfactant and the peak shapes were acceptable and enabled precise quantitative. River, tap water sample were collected in 1L amber glass bottle (without any further treatment) and cooled in refrigerator. Prior to performing CPE, each sample was filtered through a 0.45µm membrane filter and then was used for extraction. The spiked samples were extracted using the optimized CPE procedure and then analyzed using HPLC. The real aqueous samples were spiking with standard at three concentration level at 0.8µg.ml⁻¹,1µg.ml⁻¹ and 2µg.ml⁻¹. The spiked samples were extracted using the optimized CPE procedure and the analyzed using HPLC.

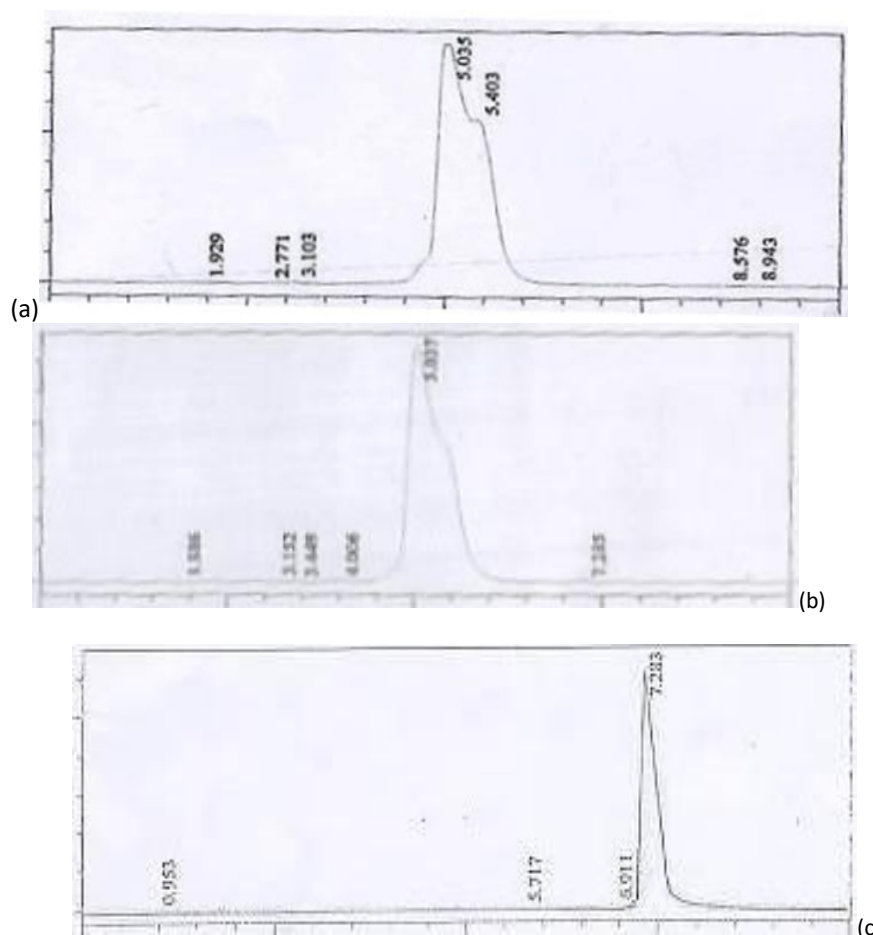


Figure 6. HPLC chromatogram of (a) river water blank (b) river water spiked with 0.1µg.ml⁻¹ (c) Cypermethrin standard solution.

CONCLUSION

In this paper we introduced a novel and sensitive cloud point extraction procedure as a rapid, safe and inexpensive method for the extraction, pre concentration and determination of cypermethrin. This method provides excellent linear range, limits of detection (LOD). The use of CPE method as alternatives to other techniques of separation and pre concentration offers several advantages including low cost, safety and high capacity to pre concentration variety organic compounds with high recoveries and good enrichment factors.

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