

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development of new methodology for preconcentration of Ultra trace amount of pyrethroid pesticide in real samples by Cloud point Extraction and determination by high – pressure liquid chromatography with UV detector.

Hassan M Basheer Hassan^{1*}, Abdul Karim JawadAli², and Seham Alwan Shukur¹.

¹Department of Chemistry, College of Science for Women, University of Baghdad, Jadiyahira, Baghdad, Iraq

²Ministry of Agriculture, State Board for Plant Protection, Abu-Ghraib, Baghdad, Iraq

ABSTRACT

Effect of different salts with varying concentrations on cloud point extraction (CPE) and preconcentration of the insecticide alphacypermethrin from the pyrethroid pesticide was studied. Types of salts (Na_2CO_3 , CaCl_2 , MgSO_4 , Na_2SO_4 , NaHCO_3 and NaCl) and concentrations were studied. The extracted target compound was analyzed using high – pressure liquid chromatography with UV detector. Among the salts studied Na_2SO_4 was found to be the most effective salt for salting out of alphacypermethrin, the optimum CPE conditions were 0.4ml (10%) Triton X-114, 3.0% (w/v) Na_2SO_4 , and 25 min incubation time at 55 °C. Under the selected conditions, the linear range of (0.03-3.0) $\mu\text{g}\cdot\text{ml}^{-1}$ was found with correlation coefficient (0.9997) and correlation rate was ($R^2= 99.98\%$) and enrichment factor (Enr.F), Preconcentration factor (PF) and Enhancement Factor (Enh. F) Of the method were found to be of (53.10), (30.30) and (47.79) fold respectively compared to extraction without preconcentration, achieving the limit of detection (LOD) was (0.009) $\mu\text{g}\cdot\text{ml}^{-1}$, limit of quantitation (LOQ) was (0.03) $\mu\text{g}\cdot\text{ml}^{-1}$. The proposed method to be simple green, rapid, sensitive, and the accuracy was assessed through recovery experiments. And was successfully applied for the determination alphacypermethrin pesticide in environmental water sample which provided high recoveries, the recovery rate was given between (95- 95.98%) and a standard deviation of (0.4% -0.76%).

Keywords: salts effect, non-ionic surfactant, Triton X- 114, cloud point extraction, alphacypermethrin insecticide, High – pressure liquid chromatography with UV detector.

**Corresponding author*

INTRODUCTION

Pesticides are used in agriculture to control weeds (herbicides), pests including insects (insecticides) and plant diseases (fungicides)[1, 2].

Pyrethroid insecticides are derived from natural compounds (the pyrethrins), isolated from *Chrysanthemum* genus of plants. Pyrethroid pesticides, widely used on crops like cotton, fruits and lettuce, appear to be a significant source of sediment toxicity in urban and agriculturally dominated streams. Pyrethroids act as neurotoxins and target the central nervous system of insects[3, 4]. Pyrethroids as shown in figure1 [15] are powerful insecticides that rapidly immobilize and paralyze target insects.

Pyrethroids are neurotoxins, which interfere with the messages sent along nerves (by maintaining sodium channels in an open position, they allow repetitive nervous influx, or depolarization, which leads to different symptoms such as tremors, involuntary movements and enhanced salivation in animals [5, 6,7].

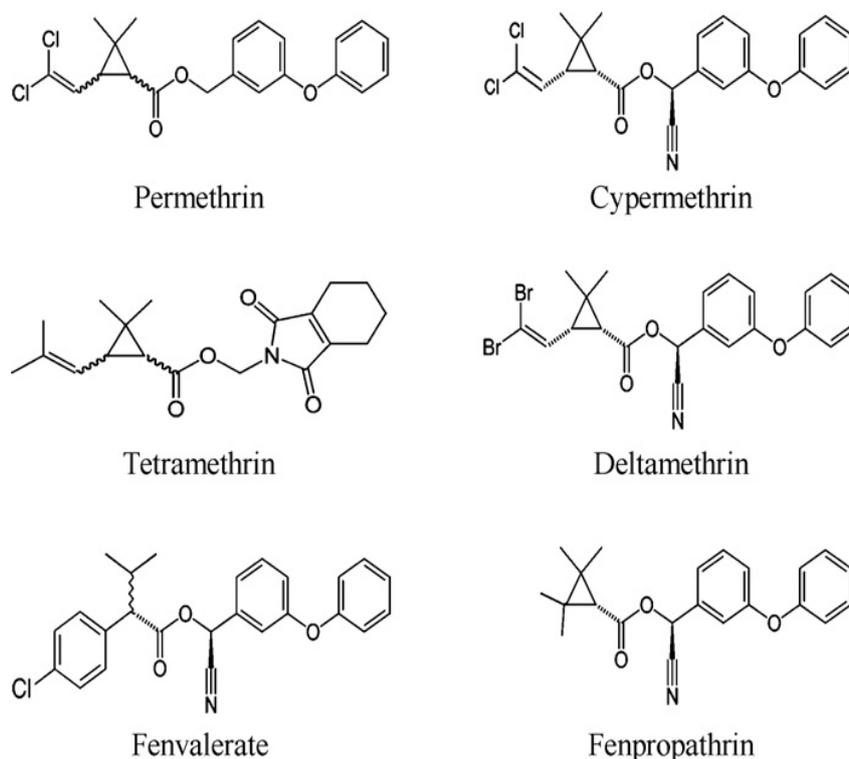


Figure 1: Structure of some pyrethroids insecticides

Cypermethrin a (Cyano-(3-phenoxyphenyl)methyl)[3-(2,2-dichloroethyl)-2,2-dimethylcyclopropane-1-carboxylate] is a synthetic pyrethroid insecticide extensively used for pest control in domestic, industrial and agricultural situations. (8,9,10).

Various methods used for determination of cypermethrin have been reported, such as Spectrophotometric method [11, 12, 13], gas chromatography – mass spectrometry after QUEChERS method [14], Dispersive liquid – liquid micro extraction (DLLME) coupled to high performance liquid chromatography (HPLC) with UV detection [15], gas chromatography with electron capture detection (GC–ECD) [16,17].

Compared with the traditional liquid –liquid extraction, cloud point extraction requires very small amount of relatively nonflammable and nonvolatile surfactants friendly to the environment. Another important merit is that no solvent concentration procedures, which may cause analyte loss, are needed under appropriate conditions such as temperature, concentration of surfactant and Na_2SO_4 , equilibrium time. The solution containing the surfactant becomes turbid and separates into two phases: a surfactant –rich phase (very small volume) and larger volume aqueous solution phase (bulk amount) with diluted surfactant concentration which approximates to its critical micelle concentration (CMC). The hydrophobic analytes of the solution are

extracted into the small volume of surfactant – rich phase with a high enrichment factor. As a promising alternative to traditional solvent extraction [18, 19, 20].

Generally, CPE can be carried out using proper concentration and temperature conditions without additives, but in many cases, the addition of salts (e.g., NaCl and Na₂SO₄) can facilitate phase separation and improve extraction efficiency [21,23]. The phenomenon is based on salting out and salting in of cations and anions that are present in the aqueous micellar solution. Thus, the appropriate selection of salts to induce CPE has been investigated to improve the capability of the extracting target analytes [24, 25, 26].

EXPERIMENTAL

Materials and measurements

All reagents were of analytical grade; doubly distilled water was used in the preparation of all solutions and for final rinsing of glass wares, standard Alphacypermethrin 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 (4C°), 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 µg.ml⁻¹) or sample solution were taken into 10ml graduated centrifugal tubes. Then adding 3ml of sodium sulphate 10% (w/v) 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 55C° for 25min to form cloudy solution. The separation of the phases was accelerated by centrifuging at 3500 rpm for 20min. 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 100µl 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, 5µm) column, the mobile phase at isocratic elution was pumped at flow rate of 1ml.mmin⁻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 20µl.

Analysis of water Sample

Tap water samples were collected from the laboratory. River water samples were collected from the Tigris River in Baghdad, Iraq. All water samples were filtered using a 0.45 µm 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

Effect of salt additives

The addition of salt has been reported to facilitate phase separation in CPE. The presence of salt can increase the incompatibility between the water structures in hydration shells of the analytes and surfactant macromolecules which can reduce the concentration of "free water" in surfactant – rich phase and consequently reduce the volume of the phase [27].

Effect of salt type

To study the effect of salts on the extraction of cypermethrin, different salts of mono- and divalent ions, including Na_2CO_3 , CaCl_2 , MgSO_4 , Na_2SO_4 , NaHCO_3 and NaCl (2.0%, w/v), for each salt studied in 10 mL of diluted sample solution containing ($1\mu\text{g}\cdot\text{mL}^{-1}$) cypermethrin were tested and compared to solutions without salt addition. The results (Figure 2) demonstrate that Na_2SO_4 and NaCl provided higher responses (i.e., peak area) for the analytes when compared to those obtained from the other salts or those without salt addition.

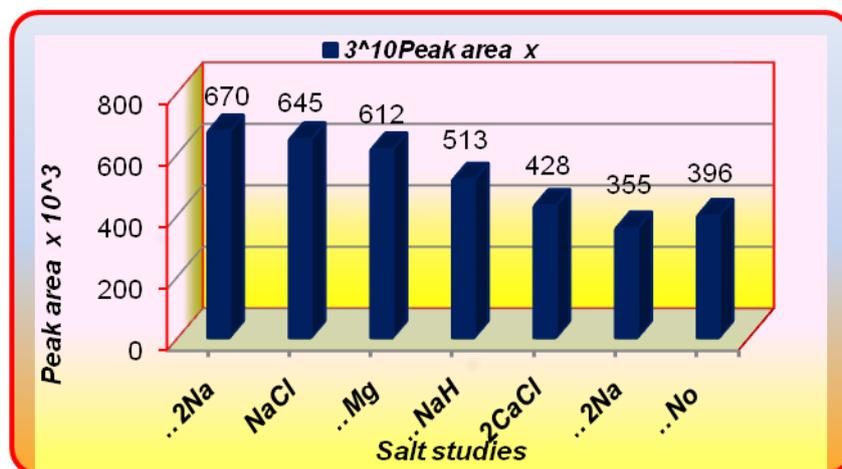


Figure 2: Effect of the addition of various salts on the peak area of CYM compared to that of without salt addition. Conditions: $1.0\mu\text{g}\cdot\text{mL}^{-1}$ Insecticide, 0.4% (v/v) Triton X-114, 2.0% (w/v) salt, 20-min equilibrated at 65°C 20 min centrifuged at 3500 rpm

The effect of the studied salts on analytical signal of insecticide alphacypermethrin can be ordered as follows $\text{Na}_2\text{SO}_4 > \text{NaCl} > \text{MgSO}_4 > \text{NaHCO}_3 > \text{CaCl}_2 \approx \text{No salt addition} > \text{Na}_2\text{CO}_3$. It has been reported that the addition of salts to the surfactant micellar solution can increase or decrease its cloud point temperature (CPT); Na_2SO_4 was found to be more effective than NaCl in decreasing CPT. It has also been reported that the ionic strength of the solution can alter the CPT and facilitate the separation of two phases by altering the density of the bulk aqueous phase (24,28). As results show, salts derived from mono- and divalent ion pairs like ($2\text{Na}^+/\text{SO}_4^{2-}$) gave higher peak responses than monovalent ions (e.g., Na^+/Cl^- and $\text{Na}^+/\text{HCO}_3^-$) or divalent ions (e.g., $\text{Mg}^{2+}/\text{SO}_4^{2-}$). An exception were the salts $2\text{Na}^+/\text{CO}_3^{2-}$ and $\text{Ca}^{2+}/2\text{Cl}^-$ which gave the lowest response (peak area) and this may be due to hydrolysis the insecticide cypermethrin in basic conditions ($\text{pH} > 7$) though cause the addition of the salts can alter the pH. To extent the investigation, the effect of pH of the solution after the addition of salts was also considered because the cypermethrin insecticide can be hydrolyzed to the corresponding 3-phenoxy benzaldehyde and cyanide ion under basic conditions (12, 13). In this study, salts studied can be classified into two groups, as follows

- 1- The salts that gave neutral solutions ($\text{pH} = 7$), i.e., Na_2SO_4 , NaCl and MgSO_4
- 2- The salts that gave alkaline solutions ($\text{pH} > 7$), i.e. CaCl_2 ($\text{pH} 10.8$) and Na_2CO_3 ($\text{pH} 11$)

Effect of Na_2SO_4 concentration

Because Na_2SO_4 gave a higher response (peak area) for the analytes than the other studied salts, the effect of Na_2SO_4 concentration in the range 1–7.0 % (w/v) was investigated. It was clear as show from fig.3 that the peak area of the analytes increased with an increase in salt concentration up to 3.0% (w/v) compared to those without salt (0%). Above 7.0% (w/v), a strong salting-out effect produced an SRP that became milky and moved to the surface of the solution, making it difficult to measure and to handle the volume. Therefore, 3.0% (w/v) Na_2SO_4 was selected as the optimum content.

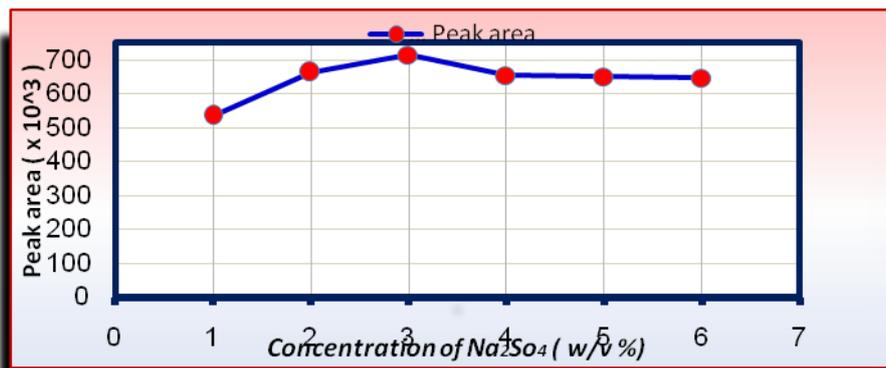


Figure 3: Effect of the addition of various concentration of Na₂SO₄ on the peak area of CYM Conditions: 1.0 µg.ml⁻¹ Insecticide, 0.4% (v/v) Triton X-114, 20-min equilibrated at 65 °C

Effect of equilibration temperature, time

When the cloud point extraction procedure was processed at equilibration temperature of the surfactant, the best extraction efficiency was achieved if the temperature is lower than the cloud point; the phase separation is difficult to be formed. In order to obtain the maximum phase separation, the lowest equilibration temperature needs to be examined. Theoretically, the optimal equilibration temperature of the extraction occurs when the equilibration temperature is 15–20°C higher than the cloud point temperature of surfactant (Raymond et al) The effect of equilibration temperature on phase separation was studied in the range of 30-65 °C. The results (fig. 4 a) showed 55 °C is available for a complete phase separation.

The effect of equilibration time on phase separation was studied in the range of 5–30 min at 55 °C. The results (fig. 4 b) showed 25 min is available for a complete phase separation

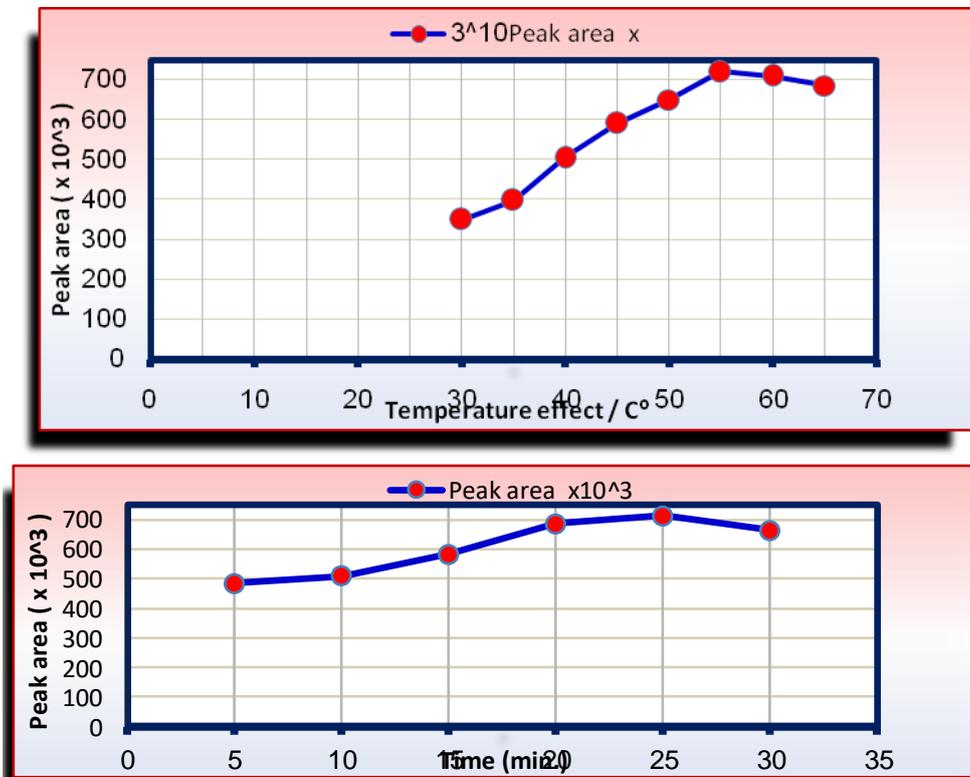


Fig 4: (a) shows the effects of equilibration temperature on the extraction efficiency. The maximum signals were presented between 55–60°C. Therefore, 55°C was selected as the working equilibration temperature (b) shows the effects of equilibration time on the extraction efficiency. The maximum signals were presented at 25min. Therefore, 25 min was selected as the working equilibration time

The analytical performances and validation of the proposed method, analytical parameters such as linearity, LOD and limit of quantitation (LOQ) of the standards obtained from CPE induced by Na₂SO₄ (CPE - Na₂SO₄), were compared with those from experiments without addition salts. These parameters are summarized in Table I. The lowest concentration of the linear range of calibration graph obtained from the method using Na₂SO₄ was 0.03 mg/L, which is double lower than the analysis without addition salts. The enrichment factor (EF) was calculated as the ratio of the slope of the calibration graph obtained using CPE and without preconcentration (Table 1).

Table 1: Calibration and Analytical Parameters of the Studied Insecticides Obtained from the Proposed CPE-salt addition method and without salt addition

	CPE - HPLC	
	Without salt addition	With salt addition
Linearity range (µg/ml)	0.06 -3	0.03- 3
Linear equation	y= 405.33 x - 9.2673	y=733167 x-7578.6
R ²	99.89%	99.98%
R	0.999449849	0.9997
LOD (µg/ml)	0.018	0.009
LOQ(µg/ml)	0.06	0.03
Preconcentration Factor(PF)	28.57	30.30
Enrichment Factor (Enr.F)	29.35	53.10

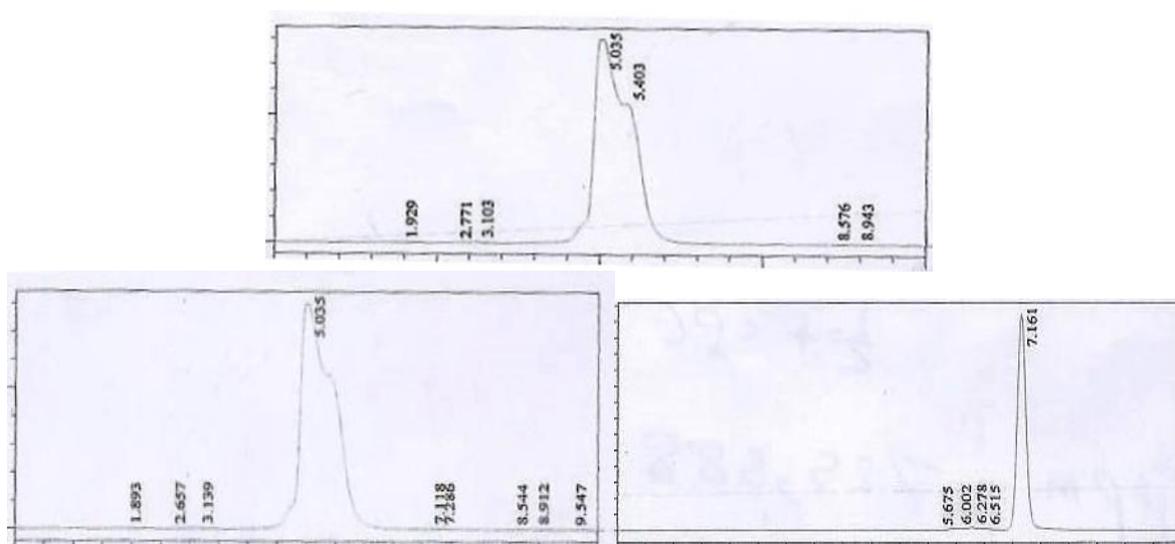


Figure 5: Typical chromatograms of (a) real water sample. (1) Blank sample, and (2) spiked 1µg.ml⁻¹ (b) cypermethrin standard solution

Analysis of real samples

No CYM were detected out in the selected real water sample .In order to validate the accuracy and precision of the proposed method under the selected conditions spiked real samples had been tested by HPLC-UV. All the recoveries obtained in this work were calculated as average values, the standard deviation from three replicate analyses, each with three injections. The results are shown in Table 2.

Table 2: Determination and recoveries of cypermethrin in spiked water samples

Real Sample	Injected ($\mu\text{g}\cdot\text{mL}^{-1}$)	Mean \bar{x} found	RSD%	Recovery%
River Water	0.1	0.095	4.05	95.0
	0.5	0.485	2.65	97.0
	2.0	1.976	2.12	98.8
Tap Water	0.1	0.096	3.55	96.0
	0.5	0.479	1.57	95.8
	2.0	1.978	0.76	98.9

*Average of three measurements

CONCLUSIONS

In this study, the addition of Na_2SO_4 in cloud point extraction was found to improve the extraction efficiency of cypermethrin by promoting the salting-out effect. High extraction efficiency was obtained CPE using Na_2SO_4 salt additive and/or without preconcentration Enrichment factors of 29 were achieved. The proposed method is simple, rapid and effective and involves nontoxic organic solvents and offers good analytical features for the samples studied.

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