Extraction and determination of alpha-Cypermethrin in environmental samples from Kerbala city / Iraq and in its formulation using high performance liquid chromatography (HPLC).

Ihsan M Shaheed¹ and Saadiyah A Dhahir²

¹ Department of Chemistry, Faculty of Science, University of Baghdad, Baghdad, Iraq.
² Department of Chemistry, Faculty of Science for women, University of Baghdad, Baghdad, Iraq.

E-mail: ihsan.aldahan@uokerbala.edu.iq

Abstract: Synthetic perothroid, Alpha-cypermethrin (α-CY), was determined in both river water samples collected from different agriculture areas in Kerbala city / Iraq and in some of its formulation which include α-cypermethrin as an active gradient. The method is based on the development of the analysis using high performance liquid chromatography (HPLC) with ultraviolet detection. Dispersive liquid-liquid microextraction (DLLME) was also developed to extract α-cypermethrin from river water samples by using chloroform as extraction solvent and acetonitrile (ACN) as dispersant. A mixture of acetonitrile/methanol (50:50v/v) was optimized as a mobile phase and C18 column (250mmx4.6mm,5µm) was chosen as a stationary phase, at pH 7.0 and flow rate 0.5 mL.min⁻¹. Linearity of calibration curve ranged from (0.1-70)µg mL⁻¹ was applied. Limit of detection and limit of quantification are (0.047) and (0.157)µg.mL⁻¹, respectively. Validation of this method was maintained for three concentration (0.5, 10 and 50) µg mL⁻¹. Relative standard deviation were (0.823, 0.113 and 0.150) µg mL⁻¹, respectively for the three level of concentrations . Three spiked level were applied for recovery study (1.0, 5.0, and 10) µg.mL⁻¹. Recovery ranged from (83.704 - 96.972%) for the three spiked level. The developed method could be provide an acceptable results for determination of α-cypermethrin in its formulation and river water samples.

Key words: Alpha-cypermethrin, dispersive, formulation, HPLC and spiked.

1. Introduction

Perothroids have taken great interest by researcher as it have become an alternative to classical pesticides[1]. Pyrethrin was the precursor of perothroids which extracted from Chrysanthemum genus of plants. Perothroids classified into two types due to the absence of cyano group(CN). These two types have different action. Type I, which consist of two chiral centers and no cyano group, causes T-syndrome effect and type II, which contain two chiral centers in addition to other chiral center on the α-carbon bonded to the CN, causes CS-syndrome effects[2, 3].

α-Cypermethrin, (RS)-α-Cyano-3-phenoxybenzyl(1RS,3RS; 1RS,3SR)- 3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate(figure 1), was one of the synthetic perothroids (Type II) used to control different types of insects in crops as well as in houses and other places. Cis and trans isomers of cypermethrin have similar chemical properties and physiological effect[4].
α-Cypermethrin should be under controlled when used against pests due to its probability to accumulate in human body causing different side effects on reproductive, cardiovascular, nervous and immune systems[5-8].

α-Cypermethrin can easily absorbed in soil and sediment due to its high hydrophobicity and high octanol/water partition coefficient($K_{ow}$). α-Cypermethrin was stable in light, neutral and weakly acidic media also it is stable when expose to heat up to 220 $^\circ$C [9, 10].

Different techniques were used for analysis of α-cypermethrin residue in different environmental samples, some of these techniques are high performance liquid chromatography[4, 11, 12], gas chromatography[12-14], gas chromatography-mass spectroscopy[15, 16], flow injection analysis[17], spectrophotometric methods[18, 19] and liquid chromatography-mass spectroscopy[20].

Many extraction methods were developed in order to extract the perothroids in very low amount with less time and less consuming of solvent. Some of these extraction methods are solid phase microextraction[21], microwave assisted extraction[22], ultrasonic solvent extraction[23], stir bar sorptive extraction[24, 25] and QuEchERS method[26]. The aim of this study is to determine α-cypermethrin in its formulation and river water samples.

2. Materials and methods

2.1. Reagents and chemicals

α-Cypermethrin standard (purity> 99%) was purchased from Dr. Ehrenstorfer GmbH company. Acetonitrile, water and methanol for HPLC analysis with purity (> 99.9% ) were purchased from sigma-Aldrich company. Formulation of α-Cypermethrin (Alpahsin 10% ) was supplied from Kerbala Agriculture Department. Sodium hydroxide (99%) from B.H.D company, phosphoric acid (85%) was supplied from Merck (Darmstadt, Germany) and hydrochloric acid (36w/v%) from B.H.D company.

2.2. Preparation of Formulation Solution

Formulation solution of cypermethrin prepared by taking 1mL from stock solution (10%) into 100mL volumetric flask and made up to the mark with methanol, then taking 1 mL from this solution and diluted with 100mL methanol in volumetric flask. From final solution an accurate volume 3mL was diluted to 5 mL with methanol and injected to HPLC instrument for analysis.

2.3. Preparation of Standards Solutions

Stock solution of α-cypermethrin (1000µg mL$^{-1}$) was prepared by dissolving (0.1g) in (100mL) methanol, from this stock solution series of solutions were prepared to study the calibration curve (0.1 -100) µg mL$^{-1}$. 

Figure 1. Chemical structure of cypermethrin.
2.4. Modified Extraction Method for Samples

A portion of river water samples, 5mL, was transferred to 10 mL screw glass test tube and spiked with three concentration levels (1.0, 5.0 and 10 ) µg mL\(^{-1}\). Salting out agent sodium chloride (5% v/v) was added to the spiked samples. A mixture of chloroform (100µL) , as extraction solvent and acetonitrile (1 mL), as dispersant solvent, were added to the spiked samples. The mixture was vortexed for 1 min. and then shook by hand for 10 min. to extract \(\alpha\)-Cypermethrin in to fine droplet of chloroform, Then the mixture was centrifuged at 3000 rpm for 10 min. The upper aqueous layer removed gently by micropipette . The chloroform phase contained \(\alpha\)-Cypermethrin was diluted with 0.5 mL acetonitrile and injected to the HPLC system[27]. The same procedure was applied for extraction of \(\alpha\)-cypermethrin from water samples collected from different agriculture areas in Kerbala City/ Iraq.

2.5. Instrumentation

Ultra High-Performance Liquid Chromatography(UFLC) Shimadzu 20AD was used for analysis. Double beam Ultraviolet – spectrophotometer -1800, Shimadzu equipped with quartz cell (1cm) for UV scanning . Digital Balance, Sartorius for weighing . pH-meter, Hanna-pH211 for adjusting the pH. Ultrasonic cleaner, KQ200E for dissolving and cleaning and centrifuge, Pro-sepE, U.K.

2.6. Chromatographic conditions

Auto sampler high performance liquid chromatography system (LC-20AD, SHIMADZU Corporation) supplied with ODS C18 analytical column (250mmx4.6mm,5μm) was used for analysis. Mobile phase composition was acetonitril:methanol (50:50 v/v) at pH 7.0 adjusted using H\(_3\)PO\(_4\)/NaOH (0. 1M). The flow rate was 0.5mL/min., the injected volume was 15µL with UV detection at 220 nm.

2.7. Wavelength selection

From prepared stock solution different concentrations of \(\alpha\)-Cypermethrin (10,50,400 µg mL\(^{-1}\)) were prepared in methanol and scanned from 190-800 nm to choose \(\lambda_{\text{max}}\). It was found that there are two peaks for \(\alpha\)-cypermethrin , very strong absorption at 220 nm and weak absorption at 268 nm (figure 2). On the basis of high response peak at 220nm was chosen for optimization.

![Figure 2. Absorption maximum of \(\alpha\)-Cypermethrin standard.](image-url)
2.8. **Limit of Detection (LOD) and Limit of Quantification (LOQ)**

LOD refer to the lowest concentration of analyte can be detected, but not necessary can be quantified. LOQ was the lowest concentration of analyte that can be detected with good accuracy and precision. LOD and LOQ were studied on the bases of ratio of baseline noise to the signal obtained, LOD calculated when baseline noise was three times the signal of analyte while LOQ as ten times of the signal[28].

2.9. **Precision and Accuracy**

Precision is represented by repeatability for same sample injected (n=5) and expressed in term of relative standard deviation (RSD%), while accuracy is represented by recovery[29]. For validation study three different level of concentration of standard solutions within the calibration curve of α-Cypermethrin (0.5,10 and 50)µg mL⁻¹ were injected (n=5).

3. **Results and discussion**

3.1. **Method development**

Preliminary studies were made for method development includes investigation two type s of reverse phase column C18 and C8 to obtain high response with acceptable capacity and limited tailing effect, also various compositions of mobile phase were tested with different ratio to chose appropriate composition of mobile phase, this includes testing of different ratio of acetonitrile:water, methanol:water and acetonitrile:methanol. It was found that the best result obtained with composition acetonitrile:methanol(50:50 v/v). Testing done at 1 mL/min, the injected volume 20µL and the absorption at λ_max 220nm.

3.2. **Method validation**

3.2.1. **Effect of pH.**

Mobile phases with different pH (3.0-8.0) were tested in HPLC system. α-Cypermethrin(50µg mL⁻¹) was injected, using phosphoric acid (0.1M)/NaOH solution (0.1M) for adjustment the pH.

| pH  | Retention time(t_R) (min.) | Height  | Capacity (k) | Tailing factor (TF) |
|-----|---------------------------|---------|-------------|---------------------|
| 3.0 | 3.391                     | 136768  | 0.247       | 1.149               |
| 4.0 | 3.023                     | 163400  | 0.130       | 1.151               |
| 5.0 | 2.904                     | 172014  | 0.090       | 1.171               |
| 6.0 | 2.844                     | 167850  | 0.224       | 1.195               |
| 7.0 | **2.721**                 | **242683** | **1.103** | **1.010**         |
| 8.0 | 2.946                     | 167880  | 0.274       | 1.160               |
Results illustrated in Table 1 explained that there is a good response at pH 7.0 with more symmetric shape and acceptable capacity obtained, for that pH 7.0 was chosen for optimization. In addition to that, at pH 7.0 possibility of ionization of silanol group in stationary phase was limited[30].

3.2.2. Effect of flow rate.

At pH 7.0, twenty µL of α-Cypermethrin (50µg mL⁻¹) was injected in HPLC system at different flow rates ranged from (0.3-1.5 mL/min.).

| Flow rate mL/min. | Retention time(t<sub>R</sub>) (min.) | Height | Capacity (k) | Tailing factor (TF) |
|------------------|-------------------------------|--------|--------------|---------------------|
| 0.5              | 9.758                         | 217124 | 0.094        | 1.129               |
| 0.5              | 5.415                         | 272253 | 1.101        | 0.906               |
| 0.8              | 3.390                         | 242347 | 0.704        | 0.903               |
| 1.0              | 2.719                         | 217949 | 0.733        | 0.908               |
| 1.3              | 2.096                         | 204289 | 0.739        | 0.932               |
| 1.5              | 1.818                         | 190935 | 0.744        | 0.951               |

Results illustrated in table 2 explained that the flow rate at 0.5 mL/min. gives high response with symmetric shape and acceptable value of capacity, for that 0.5mL/min. was adopted for optimization.

3.2.3. Effect of volumes injection.

Various volumes (5.0-25.0µL) of α-Cypermethrin (50µg mL⁻¹) were injected at optimum conditions. Results sorted in table 3.

| Volume injection (µL) | Retention time(t<sub>R</sub>) (min.) | Height | Capacity (k) | Tailing factor (TF) |
|----------------------|-------------------------------|--------|--------------|---------------------|
| 5                    | 5.303                         | 43762  | 0.791        | 1.900               |
| 10                   | 5.391                         | 101191 | 0.805        | 0.951               |
| 15                   | 5.400                         | 197465 | 1.168        | 0.998               |
| 20                   | 5.454                         | 263891 | 1.241        | 0.810               |
| 25                   | 5.484                         | 324703 | 1.130        | 0.720               |

It was found from that the maximum response for injected α-Cypermethrin (50µg mL⁻¹) with good symmetric shape was achieved at 15 µL, after that volume overloaded the column which affected shape of peak and baseline stability.

3.2.4. Linearity.

Series solutions with different concentration ranging from (0.1-70 µg mL⁻¹) were prepared from stock solution to study the linearity. Volume of 15µL from each concentration filtered through 0.45
μm Millipore filter and injected (n=3) into HPLC system. Peak area for each concentration was plotted against the concentration to obtain the calibration curve (figure 3) and (table 4).

![Calibration curve for α-Cypermethrin pesticides.](image)

**Figure 3.** Calibration curve for α-Cypermethrin standards.

**Table 4.** Analytical parameter for α-Cypermethrin calibration curve.

| \( \lambda_{\text{max}} \) (nm) | Linearity range (μg mL\(^{-1}\)) | Limit of detection (μg mL\(^{-1}\)) | Limit of quantification (μg mL\(^{-1}\)) | Regression Equation | Slope | Correlation coefficient (R\(^2\)) |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|-------------------|-------|-------------------------------|
| 220                             | 0.1-70                          | 0.028                           | 0.0949                          | 107836x + 196530  | 107836| 0.9999                        |

3.2.5. Precision and Accuracy.

Three concentration levels of α-Cypermethrin (0.5, 10 and 50μg mL\(^{-1}\)) were injected to the HPLC system at optimum condition. Results illustrated in table 5.

**Table 5.** Precision and Accuracy for modified method.

| Concentration (μg mL\(^{-1}\)) | Retention time\( (t_R) \) (min) | Peak area (n=5) | Recovery% | RSD% |
|---------------------------------|---------------------------------|-----------------|-----------|------|
| 0.5                             | 5.258                           | 234763          | 100,005   | 0.823|
| 10                              | 5.389                           | 1284271         | 99.998    | 0.113|
| 50                              | 5.399                           | 5631449         | 99.999    | 0.150|

Results in table 5 explained that the method was accurate and precise due to excellent recovery and good value of RSD%.

4. Applicability of Method
Optimized method was successfully applied for determination of α-cypermethrin in spiked river water samples collected from different agriculture area in Kerbala City / Iraq and also in its formulation. Water samples spiked with three different concentration levels (1.0, 5.0 and 10) µg mL⁻¹. Recoveries for three spiked samples and for two different prepared concentrations of formulation illustrated in table 6 and 7.

Table 6. Recovery of α-cypermethrin in water samples spiked with three different levels.

| Spiked level (µg mL⁻¹) | Amount found (µg mL⁻¹) | Recovery % (n=5) |
|------------------------|------------------------|------------------|
| 0                      | Not detect             | -                |
| 1.0                    | 0.838                  | 83.704           |
| 5.0                    | 4.717                  | 94.340           |
| 10.0                   | 9.698                  | 96.972           |

Table 7. Determination of α-cypermethrin in formulation.

| Conc. (µg mL⁻¹) | Amount found (µg mL⁻¹) | Recovery % (n=5) |
|-----------------|------------------------|------------------|
| 10              | 9.803                  | 98.030           |
| 30              | 29.911                 | 99.703           |

5. Conclusion

Extraction of α-cypermethrin by modified dispersion liquid-liquid microextraction and determination by optimised HPLC method can be applied successfully for the determination of α-cypermethrin in water samples with high sensitivity, selectivity and specificity. α-cypermethrin was not detected in 6 water samples collected from different agriculture area in Kerbala City / Iraq.

6. Acknowledgments

The authors thank the support from the staff of College of Science for Women/ Chemistry Department for providing requirements.

References

[1] Harm D and Ansari B 2011 Res. J. Chem. Sci. 1 125-134.
[2] Ray D E, Ray D and Forshaw P J 2000 J. Tox: Clin. Tox 3 895-101.
[3] Pérez F V, García M Á, and Marina M L 2010 1217 J Chro. A 968-989.
[4] Li J, Lin D, Ji R, Yao K, Deng W q, Yuan H 2016 J chrom Sci 54 1584-1592.
[5] Wolansky M J and Harrill J 2008 Neuro tera. 30 55-78.
[6] Kolaczinski J and Curtis C 2004 Foo and Chem. Tox. 42 697-706.
[7] Zhang J, Zhu W, Zheng Y, Yang J, and Zhu X 2008 Rep Toxi 25 491-496.
[8] Ortiz-Pérez M D, Torres A, Batres L E, López-Guzmán O D, Grimaldo M and Carranza C 2005 Enviro. health per. 113 782-786.
[9] Fernandez A M, Llompart M, Lamas J P, Lores M, Garcia C. Cela R 2008 J. Chro. A 1188 154-163.
[10] Oudou H, Alonso R and Hansen H B 2004 *Anal. Chim. Acta* **523** 69-74.

[11] Muhamad H, Zainudin B H, Zulhilmi Z and Abu Bakar N K 2015 *J. Oil Palm Research* **27** 377-386.

[12] Abdulra'uf L B, Lawal A R, Adeyemo F A and Atanda F B 2019 *Nig. J. Basic and App. Sci.* **27** 34-40.

[13] Sharif Z, Man Y B C, Hamid N S A and Keat C C 2006 *J. Chrom. A* **1127** 254-261.

[14] Tankiewicz M 2019 *Molecules* **24** 417.

[15] Feo M L, Eljarrat E, and Barceló D 2010 *J. Chrom. A* **1217** 2248-2253.

[16] Hernandes T, Dores E F, Ribeiro M L, Rossignoli P A and Malm O 2014 *J. Bra. Chem. Soc.* **25** 1656-1661.

[17] Pervez Y, Janghel E K and Sar S K 2016 *Asian J. Chem.* **28** 484-488.

[18] Janghel E, Rai J Rai M and Gupta V 2007 *J. Bra. Chem. Soc.* **18** 590-594.

[19] Tamrakar U, Gupta V and Pillai A K 2012 *J. Anal. Chem* **67** 437-442.

[20] Liao H T, Hsieh C J, Chiang S Y, Lin M H, Chen P C and Wu K Y 2011 *J. Chrom. B* **879** 961-1966.

[21] Abdulra'uf L B, Chai M K and Tan G H 2012 *J.AOAC International* **95** 1272-1290.

[22] El-Saeid M, Al-Turki , Al-Wable M, and Abdel-Nasser G 2011 *Res. J. Env. Scie.* **5** 171-178.

[23] Castro J, Sánchez-Brunete C and Tadeo J 2001 *J. Chrom. A* **918** 371-380.

[24] Van Hoeck E, David F and Sandra P 2007 *J. Chrom. A* **1157** 1-9.

[25] Ochiai N K, Sasamoto Kanda H and Pfannkoch E 2008 *J. Chrom. A* **1200** 72-79.

[26] Anastassiades M, Lehotay S J, Štajnbaher D and Schenck F J 2003 *J. AOAC Inter.* **86** 412-431.

[27] Albaseera S S, Raob R N, Swamyc Y and Mukkantia K 2011 *Glo. J. of Anal. Chem.* **2** 224-231.

[28] Ribani M, Collins C H, and Bottoli C B 2007 *J. Chrom. A* **1156** 201-205.

[29] Zanella R, Primel E, Gonçalves F and Martins A 2000 *J. Chrom. A* **904** 257-262.

[30] Sun S, Liu G and Wang Y 2006 *Chromatographia* **64** 719-724.