

Application of HPLC method with diode array for tetramethrin determination in different environmental samples from upper Egypt

Abstract

A specific and precise High-performance liquid chromatography (HPLC) method with diode array detection (DAD) has been sophisticated for tetramethrin determination in environmental water samples. Water samples were collected from two governorates in Upper Egypt: Assiut and Qena. Extraction of tetramethrin from water samples was carried out using liquid-liquid extraction method before the injection of analyte into HPLC-DAD system. The best separation method has been achieved on the Supelcosil TM LC-18-DB column (4.6* 250mm), particle size 5µm, with mobile phase methanol: water (78:22,v/v) at temperature of 300°C and at a flow rate of 0.8mL/min. The detection wavelength of the detector was set at 220nm. Calibration curves obtained over the concentration range of 10–100µg mL⁻¹ were linear. The accuracy ranged from 99.44% to 102.07%. The suggested study showed that most of samples do not contain tetramethrin residues, while few samples contain tetramethrin residues.

Keywords: HPLC-DAD, determination, tetramethrin, environmental samples

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Introduction

Tetramethrin[(1,3-dioxo-4,5,6,7-tetrahydroisoindol-2-yl)methyl-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate] is a broad-spectrum, first-generation synthetic pyrethroid insecticide often used to target insects such as wasps, hornets, roaches, ants, fleas, and mosquitoes. It is oftentimes combined with another active ingredient for more broad range pest control, or for more effective long term treatments.¹ Tetramethrin is commercially available in the form of lotion containing 0.006g of tetramethrin per mL combined with 0.024g of piperonyl butoxide per mL for topical treatment of pediculosis (lice infestations). Tetramethrin has a molecular formula of C₁₉H₂₅NO₄ and molecular weight of 331.41g/mol.² There are numerous different instrumental techniques used for the determination of tetramethrin. The available methods include voltammetric determination,³ capillary electrophoresis,⁴ spectrophotometric,⁵ as well as infrared spectrometric methods.⁶ However, the most widely applied techniques are chromatographic methods, such as gas chromatography (GC) with electron capture detection (ECD)⁷ or with mass spectrometry (MS),⁸ as well as high performance liquid chromatography (HPLC) with diode array (DAD)⁹ or with MS detection.¹⁰

The majority of analytical methods was developed for detection of trace levels of the pesticides in environments like: air,¹¹ soil,¹² water samples or in human fluids.¹³ Liquid-liquid extraction (LLE) and solid phase extraction (SPE) are commonly used for the extraction of tetramethrin.⁴ Literature survey revealed an optimization and validation of HPLC method for tetramethrin determination in human shampoo formulation.¹⁴ Voltammetric determination of the synthetic pyrethroid insecticide tetramethrin in acetonitrile.³ Determination of pesticides in water samples by solid phase extraction and gas chromatography tandem mass spectrometry.¹⁵ Multiresidue Method for Determination of 67 Pesticides in Water Samples Using Solid-Phase Extraction with Centrifugation and Chromatography-Mass Spectrometry¹⁶ and monitoring of pesticides in the Egyptian River

Nile.¹⁷ The end of this proposed study was to develop a HPLC-DAD method for identification of tetramethrin in environmental samples.

Material and methods

Chemicals and Solvents

Tetramethrin 99% was kindly provided by Egyptian international center for import. Structural formula of tetramethrin is shown in (Figure 1). Organic solvents methanol and acetonitrile (HPLC gradient grade) all these chemicals were of analytical grade and all were purchased from Sigma-Aldrich (Steinheim, Germany). Deionized water (WP 4100 reagent grade water purifier-SMEG) was used for standard and sample preparations.

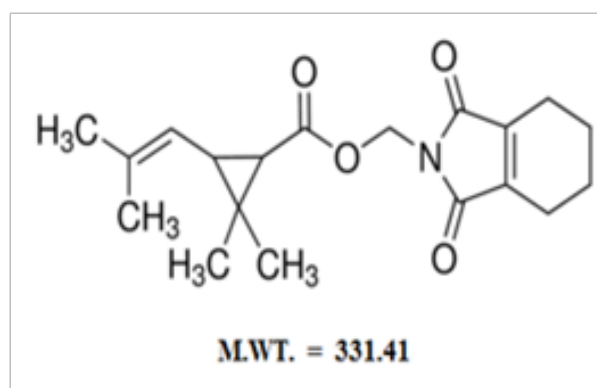


Figure 1 Structural formula of tetramethrin.

Instrumentation

Chromatographic system: HPLC chromatographic apparatus consisted of an HPLC Dionex UltiMate 3000 Series system with a DAD-3000SD/RS detector, auto sampler WPS-3000(T) RS, degasser, binary pump HPG 3200SD/RS and column oven TCC-3000SD (Thermo Scientific, Germany). The system control, data acquisition

and data evaluation were performed by ChromeleonA7 software (Thermo Scientific, Germany).

Chromatography procedure: Mobile phase consisted of acetonitrile and deionized water or methanol and de-ionized water filtered through a 0.45µm filter, degassed in an ultrasonic bath, and pumped at a flow

rate of 0.8mL/min. Injection volume was 20µL. Compared columns were Supelcosil TM LC-18-DB, 4.6*250mm, 5µm particle size (Sigma-Aldrich Co. LLC) and Hypersil GOLD aQ, 3*150mm, 3µm particle size (Thermo Scientific) at 300°C. The detection Wavelength of the detector was set at 220 nm for all used mobile phases. HPLC chromatogram was shown in (Figure 2).

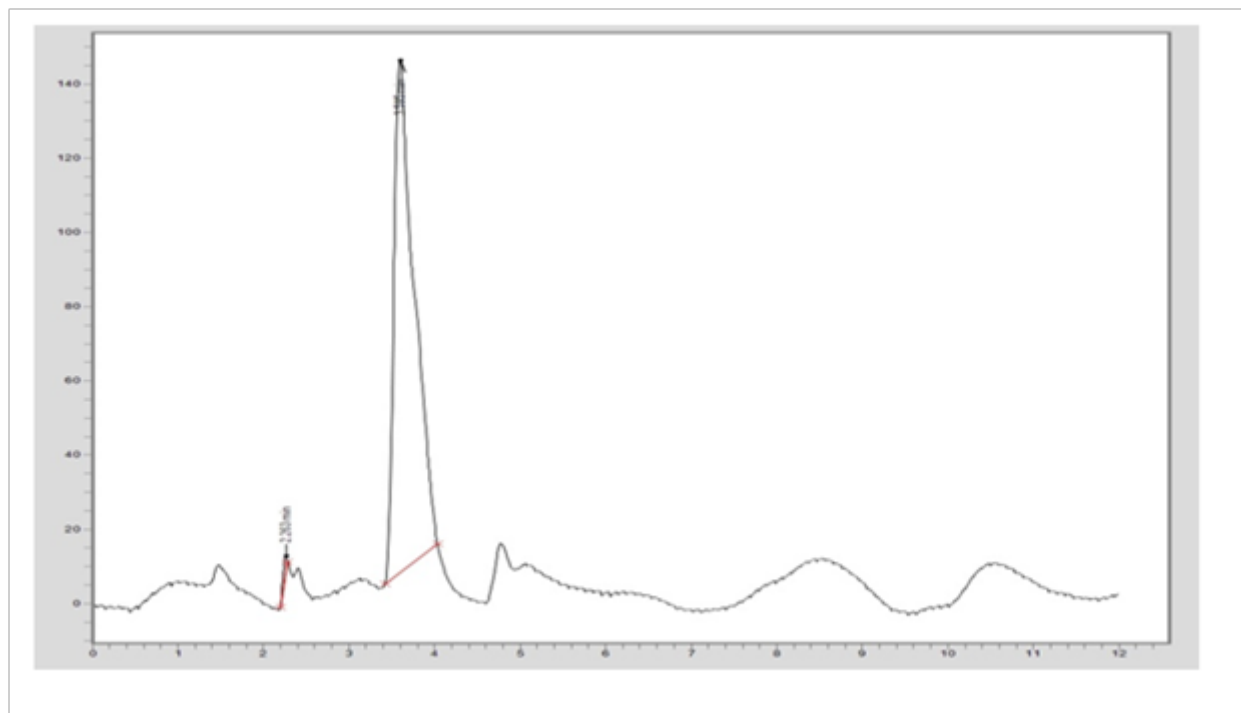


Figure 2 HPLC chromatogram of Tetramethrin standard (50µg mL⁻¹).

Analytical method

Preparation of standard stock solution: Standard stock solution of Tetramethrin was prepared by dissolving 5mg standard tetramethrin in 10mL of methanol. Tetramethrin working solutions in the desired concentration range was prepared by appropriate dilution of standard stock solution with mobile phase. The prepared stock solution was kept at 4°C until use.

Collection of samples: A total of 39 water samples were collected in amber glass bottles in January and February 2018. Samples of collected water were drinking water, agriculture stream water, and River Nile water from two different governorates: (Abo teeg and Al badari) Assiut and (El-Sheikheya and Naga Hammadi) Qena (3 samples each) and waste water sample from skin and leprosy hospital in Qena (three time analysis per each taken sample as drinking, agriculture stream and River Nile water). Coordinates of sampling locations were shown in Table 1.

Sampling: Water samples 2.5L were collected in glass bottles at 50cm below water level. Waters were collected by qualified personnel using standard sampling field protocol. The bottles were covered with screw caps and the samples were then stored at 4°C until extraction and analysis.

Liquid – Liquid Extraction of samples: A measured volume of 1L of the sample was transferred in a 2L separation funnel and was extracted twice with 60mL of 15% methylene chloride in hexane.

The samples were shaken vigorously; the organic layer was taken and dried over anhydrous sodium sulfate and evaporated in a rotating evaporator. The volume of solution was attained to 10mL with mobile phase. 3µl of each extracted solution was injected into the HPLC.¹⁸

Table 1 Coordinates of sampling locations

Sampling locations	E	N
Aboo teeg in Assiut	31.31844	27.04561
Al-badari in Assiut	31.40811	26.99326
El-Sheikheya in Qena	32.78858	25.98312
Naga Hammadi in Qena	32.24374	25.05385
Skin and leprosy hospital in Qena	32.71104	26.1574

Results

Linearity

The linearity was established by injecting the tetramethrin at six different concentrations (10–100µg mL⁻¹) into the HPLC-DAD system. The same procedure was applied three times per concentration. The calibration curve was obtained by plotting the mean peak area against the corresponding concentrations. The analytical parameters of the proposed methods are summarized in Table 2.

Table 2 Analytical parameters and linear regression data of tetramethrin

Parameter	
Linearity range ($\mu\text{g mL}^{-1}$)	10-100
Regression equation*	
Correlation coefficient	0.999
Slope (b)	40853
Intercept (a)	18857

*Y, a + bC; where Y is the peak area and C is the concentration in $\mu\text{g mL}^{-1}$.

Table 3 Intra-day and Inter-day accuracy and precision of tetramethrin

Drug	Concentration ($\mu\text{g/ml}$)	The concentration found ($\mu\text{g/ml}$) \pm SD; RSD			
		Intraday precision	accuracy	Interday precision	accuracy
Tetramethrin	10	10.06 \pm 0.11; 1.09	100.60	9.98 \pm 0.08; 0.80	99.83
	50	50.24 \pm 0.25; 0.49	100.48	51.03 \pm 0.42; 0.82	102.07
	100	100.27 \pm 0.25; 0.25	100.27	99.44 \pm 0.83; 0.83	99.44

Table 4 Mean \pm SD of tetramethrin residue levels ($\mu\text{g/ml}$) in water samples

Sampling locations	Found concentration (mean \pm SD)			
	Drinking water	Agricultural stream sites	River Nile	Waste water
Aboo teeg in Assiut	Not detected	Not detected	Not detected	-----
Al-badari in Assiut	Not detected	Not detected	Not detected	-----
El-Sheikheya in Qena	Not detected	12.05 \pm 0.30	Not detected	-----
Naga Hammadi in Qena	Not detected	Not detected	Not detected	-----
Skin and leprosy hospital in Qena	-----	-----	-----	19.17 \pm 0.42

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The specificity of the proposed method was assured by applying the HPLC method in qualitative and quantitative determination of tetramethrin in water samples according to standard method for water and wastewater analysis.^{18,19} The HPLC chromatogram of the samples shows that the method has sufficient specificity to resolve all related substances and the tetramethrin from each other.

Robustness

Robustness was estimated and expressed in term of % RSDs. The flow rate was changed (0.8 \pm 0.1 mL/min) and temperature of HPLC was studied at 30 \pm 1°C to verify that the proposed method was not affected by these small deliberate variations. The results of Robustness were given in Table 5.

Accuracy and precision

The accuracy and precision were evaluated at three different concentration levels (10, 50, and 100 $\mu\text{g mL}^{-1}$). Accuracy of this study was calculated and expressed as percent recovery, while Precision (repeatability and intermediate) was evaluated and expressed in term of standard deviation. Table 3 summarizes the intra-day (the same day) and inter-day (successive days) accuracy and precision of Tetramethrin. All accuracy and precision values were within acceptable limits. The suggested study has been successfully applied for the determination of tetramethrin in water samples and the results were shown in Table 4.

Table 5 Robustness data

Parameter	Validation	%RSD of the area Tetramethrin
Flow rate	0.7 ml/min (low)	0.82
	0.9 ml/min (high)	0.68
HPLC temperature	29°C (low)	1.27
	31°C (high)	0.54

Discussion

This study aims to detect tetramethrin in environmental samples using the HPLC method. Our new concept is to use a simplified method to detect and determine concentration of tetramethrin in the environment and follow-up development. To achieve rapid and

effective separation of tetramethrin in a limited time, simple mobile phase was used for evaluation. Optimal conditions for the separation of tetramethrin and matrix peaks established were as follows: mobile phase that consisted of methanol:water (78:22, v/v), isocratic elution at a flow rate of 0.8mL/min and temperature of 300°C. According to the requirements of ICH (2005), these conditions were found to be most suitable for separation and quantification of tetramethrin. The results of the analyzes showed that all water samples collected and analyzed were completely free of tetramethrin except samples (El-Sheikheya (agriculture stream site) and Skin and leprosy hospital in Qena (I have sampled random vegetables (potatoes and zucchini) from places where tetramethrin is present and analyzed (El-Sheikheya and Skin and leprosy hospital in Qena (The results of the samples were negative. Tetramethrin was not found in all vegetable samples. These results are consistent with the results of other studies conducted by some colleagues in the field of work. Important step to be followed in the next research is to detect tetramethrin degradation products in environmental samples, especially in water and vegetables. I believe that over time, accumulation of tetramethrin in water and vegetables will lead to major problems.

Limitations of the study

The most important limitations faced by this study are that the samples were withdrawn from a few places. We should have carried out the examination and analysis and extracted samples from more places and large areas covering a large part of the Egyptian countryside in Upper Egypt to obtain more comprehensive and credible results, but that would require a lot of money and Research is not funded by any organization but is a personal effort. Lack of time is also one of the important reasons and obstacles to research.

Conclusion

A validated, sensitive and accurate HPLC analytical method was developed for the analysis of tetramethrin in water samples. The method was fully validated according to the ICH guidelines and presented good linearity, accuracy, precision and specificity. Tetramethrin was observed in agricultural streams samples of El-Sheikheya and waste water sample of Skin and leprosy hospital in Qena due to the use of tetramethrin as a pesticide and lice infestations in these areas. However, it was not observed in drinking water and River Nile water in all sampling locations. The proposed method can be successfully applied for determination of tetramethrin in water samples using LLE- HPLC method.

Data availability

The readers can access the data represented in this research article by contacting with the author on this

Email ahmedabdrabou31@yahoo.com as the data presented is his own personal experiment and work.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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