Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Effect of Storage on the Stability and Toxicity of Phoxim and Deltamethrin Insecticides with Repeated Use

Seif, E.Salem* and Ahmed, M.Elmahdy

Department of Biochemistry, Toxicology and Feed Deficiency, Animal Health Research Institute (AHRI), AgriculturalResearch Center (ARC),7 Nadi ElSaid St.Dokki,p.o 12618, Giza, Egypt

Abstract

Objective: To study the effect of storage temperature on the concentration of the active principles of pesticides phoxim and deltamethrin collected from the Egyptian market. Two packages of phoxim and deltamethrin were analyzed for the active ingredients by using validated HPLC methods with a limit of detection = 0.82 microgram per milliliter and 0.27 microgram per milliliter and limit of quantification = 2.47 micrograms per milliliter and 0.83 microgram per milliliter for phoxim and deltamethrin respectively which indicates its sutability for stability investigation of insecticides, after 21 days of storage at 54 °C and 3 months at room temperature and tested for acute toxicity in albino rats at the beginning and the end of the experiment (3 months). Storage of pesticides decreases their concentrations. The initial concentration of phoxim(500 mg/ml) decreased to 403,364 and 311 mg /ml while that of deltamethrin (50 mg/ml) decreased to 38.8, 35.5, and 29.5 mg/ml after 1 week,2 weeks, and 3 weeks respectively after storage at 54 ^{o}c while phoxim concentrations decreased to 460,410, and 395 mg/ml and deltamethrin to 40.3, 36.4, and 31.15 mg/ml after 1, 2, and 3 months at room temperature respectively.. Concerning LD_{50}, phoxim LD_{50} at the beginning of the experiment was 1660 mg/kg.b.wt and decreased to 831.7 mg/kg.b.wt while deltamethrin LD_{50} was 17.37 mg/kg.b.wt at the beginning of the research and increased to 20.89.mg/ kg.b.wt.In conclusion: Phoxim and deltamethrin pesticides are degraded by temperature. We should apply the optimum storage conditions and analysis of the marketable pesticides.

Keywords: storage, phoxim, deltamethrin, HPLC, toxicity

1-Introduction

In agricultural and public health, pesticides play a critical role. They have an important role in raising food and fiber production as well as improving human health by reducing the spread of vector-borne diseases. **[1].**

Due to wide pesticide applications, problems like more production without good marketing practices and illegal trading of pesticides lead to the accumulation of stocks. Worldwide, it is estimated that there are over half a million tons of banned, obsolete, unusable formulas and containers, broken and deteriorated items, unclear products, and active ingredients or technical formulations past their expiry date **[2].**

Manufacturers of pesticides usually have to give a certain shelf life for their products, which in many products is for two years. At the end of this storage period, the product can still be used without any trouble and still able to produce its full biological activity against the pests which it is applied to control. Although this requirement is so simple, it causes large problems especially for those firms which export their pesticides to many countries where the products are then stored under greatly varying conditions. To evaluate the storage stability of a product, attention should be given to those factors capable of changing the properties of the product over time. The factors responsible for degradation are light, temperature, acidity, alkalinity, oxidation, and humidity. [3]**.**

Phoxim is a pesticide based on organophosphorus (OPs) are a type of pesticide that acts as a cholinesterase inhibitor and has been widely employed in agriculture. [4]. The extensive use of OPs makes them common pollutants in aqueous environments including ground, surface, and drinking water. There is now an increasing concern for these compounds because of their potential side effects on aquatic organisms and humans [5]**.** Deltamethrin a synthetic type II pyrethroid pesticide is commonly employed to control a wide range of pest

*Corresponding author: seif_salem2007@yahoo.com (Seif,E.Salem)
Receive Date: 14 December 2021, **Revise Date:** 24 February 2022, **Accept Date:** 01 March 2022
First Publish Date: 14 November 2022
DOI: 10.21608/EJCHEM.2022.108060.5064

insects in agriculture. **[6]** Due to their short biodegradation period and low tendency to accumulate in organisms **[7]**. In addition, it is used as an alternative pesticide for Ectoparasite control in cattle, sheep, and poultry on a topical basis **[8].** Due to over usage, these pesticides are transferred to the aquatic system either from leakage of Chemicals that can be extracted from the soil or sprayed directly on the organisms in concern. Pyrethroid insecticides, such as deltamethrin, have a short half-life, low water solubility, and high soil and sediment adsorption. **[9].**

860

The aimed target of this work is to investigate and study the stability of two commercial emulsifiable concentrate formulations of deltamethrin and phoxim collected from the Egyptian market exposed to different storage periods at 54 ºC, and at room temperature for 3 months and evaluated their LD_{50} in albino rats, in addition, to quantify the active ingredient concentration by HPLC.

2-Experimental

2-1 Chemicals

Phoxim,(MAXIMA®,500mg/mL)was purchased from Pioneer Company(Cairo, Egypt) is a commercially available veterinary product. Deltamethrin (Fact®50mg/ml) was acquired from Magico Group Co. (Cairo, Egypt) The standard phoxim (97.4 %) was acquired from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The standard deltamethrin (100%) was purchased from Tagros chemicals India.

Methanol, acetonitrile, and acetone were all supplied in HPLC grade by Fisher Scientific, Throughout the study, high-quality water collected from a Millipore device was used. The reagents and high-quality water were filtered through a 0.22-μm membrane filter to eliminate particulate impurity.

2-2 Experimental animals and experimental design

Apparently, healthy adult Wister Albino rats, males, weighing 150 g on average were used in this study. The Egyptian Organization for Biological Products and Vaccines provided them to us. The animals were then housed in metal cages at a temperature of 25± 2 °C, on a 12:12 h light/dark cycle, with free access to water and a standard pellet diet. The Phoxim group rats were separated into five groups, each with four rats, and given a three-day treatment. The first group was used as a control group, and only the second was given water., third, fourth, and fifth groups of rats received 500 milligrams per kilogram of body weight, 1000 milligrams per kilogram of body weight, 2000 milligrams per kilogram of body weight, and 4000 milligrams per kilogram of body weight, respectively using metallic stomach tube at the beginning of the experiment and 250, 500, 1000, 2000 milligrams per kilogram of body weight at the experiment's end. (After 3 months).

For the determination of LD_{50} of the deltamethrin, group rats were separated into five groups, each with four rats, and given a three-day treatment. The first group functioned as a control group, receiving merely water. the second, third, fourth, and fifth groups of rats received 6.25 milligrams per kilogram of body weight, 12.5 milligrams per kilogram of body weight, 25 milligrams per kilogram of body weight, and 50 milligrams per kilogram of body weight, respectively at the beginning and the end of the experiment (after 3 months).

The Research Ethical Committee at the Faculty of Veterinary Medicine, Suez Canal University, and Ismailia, Egypt, approved a guide for the care and use of laboratory animals, which was followed in the treatment of all the animals. Before beginning the experiment, all rats were given a week of acclimatization under the same settings.

2-3Analytical procedures

2-3-1 Insecticide Storage stability tests:

The previously pesticide formulations were stored in an oven at 54 ºC for 7, 14 days according to the **[10]** and completed to 21 days storage. During the storage period, the samples were collected at zero, seven, fourteen, and twenty-one days of storage to determine the active ingredient concentrations. Other samples from the pesticides formulations were analyzed for their active ingredient concentrations after 3 months of storage at room temperature (30 ^{O}C)

2-3-2 Short-term toxicity study:
Determination of acute oral 72-hr LD_{50}:

The determination of 72-hr LD_{50} of phoxim and deltamethrin was performed mathematically according to **[11]**

The LD_{50} was calculated according to the following formula:

$$m = X1 + \tfrac{1}{2}\, d - \frac{dr1}{N}$$

Where:

m = Log LD_{50}.

X1 = Log dose causing 100% mortality.

d = logarithmic intervals of doses.

r1 = sum of the number of rats dead at each of the individual dose levels.

N = number of rats on each of the dose levels.

LD_{50} = Antilog of m.

Confidence limits:

$$Variance = \frac{d^2}{N^2\,(N-1)}\, X\, r\,(N-r)$$

Where:

r = No. of dead rats in each group.
95% confidence limits = $m \pm t.S.E.$
S.E. = Square root of variance.

2-3-3 Standard solution:

The standard phoxim and deltamethrin (50 milligrams) was precisely weighed and dissolved in 50 ml acetone and 50 ml acetonitrile respectively at a volumetric flask to provide the stock standard solution in a concentration of 1mg/ml (which can be kept stable for at least 6 months if stored at -18°C in a dark place), the working standards of phoxim and deltamethrin were freshly prepared from stock solution by dissolving in acetone and acetonitrile respectively to produce concentrations of one, five, ten, twenty, fifty, and one hundred micrograms per milliliter

2-3-4 Calibration curve:

The calibration curves for phoxim and deltamethrin were created using six different concentrations ranging from one to one hundred micrograms per milliliter in acetone and acetonitrile respectively. The equation was computed using the least-squares approach and linear regression after graphing the phoxim and deltamethrin peak regions versus known concentrations to produce a calibration curve. The standard curves of phoxim in acetone and deltamethrin in acetonitrile were linear between one and one hundred micrograms per milliliter, the value of the correlation coefficient was less than 0.999.

2-3-5 Sample preparation for HPLC:

Sample of phoxim and deltamethrin were prepared in acetone and acetonitrile respectively with a concentration of 20 micrograms per milliliter, each time the sample was prepared and assayed 3 times (triplicate) with an HPLC device.

2-3-6 HPLC apparatus and chromatographic conditions:

The high-performance liquid chromatography device used was Agilent 1200 series equipped with 4 mobile phase channels, automatic degasser, quaternary gradient pump, auto-sampler, reversed-phase column C18,4.6 mm, 250 mm,5 µm, with adjustable controlled heater and ultraviolet – visual detector, the aforementioned device is controlled through computerized chem-station soft wear, The analyses of phoxim sample were performed with chromatographic condition according to **[12]**, Under isocratic conditions, the mobile phase was a seventy: thirty volume ratio mixture of methanol and water, with a flow rate of 0.8 mL/min. Phoxim was identified using an ultraviolet– visual detector tuned to 280 nm. The samples were transferred into disposable auto-sampler vials before being loaded into the HPLC auto-sampler. A 20 microliters sample volume was successively injected. The analyses of the deltamethrin sample were performed according to **[13]**, under isocratic conditions at a flow rate of 1.0 mL/min, the mobile phase acetonitrile-distilled-deionized water (eighty: twenty), The column temperature was 25 degrees celsius, and deltamethrin was detected using an ultraviolet – visual detector set at 266 nanometers. The samples were placed in disposable auto-sampler vials before being loaded into the HPLC auto-sampler. A total of 20 microliters of sample volume was injected progressively.

2-3-7 Validation of the HPLC Method:

It is a technique used in laboratory research to ensure that the method's performance characteristics meet the requirements for the planned analytical application. **[14] and [15].**

2-3-7-1 Selectivity and specificity:

The standard addition on drug samples is used to assess selectivity and specificity. Acceptance criteria include the absence of interference between the pure standard peaks and any impurity peaks.

2-3-7-2 Linearity and range:

Linearity is established by producing six different drug standard concentrations. Linearity is measured by the squared correlation coefficient, which should be greater than or equal to 0.999.

2-3-7-3The detection limit (DL) and the quantification limit (QL)

They were calculated based on the standard deviation of intercept (S) and slope (b)
DL = 3.3×S/b
QL = 10×S/b

2-3-7-4 Method Precision

It is commonly reported as the relative standard deviation after 6 replicates of standard solutions are used (RSD percent) of a sequence of measurements. It may be a measure of the degree of frequency (interday precision) or the average accuracy that expresses variances within the laboratory, as is the case on different days (interday precision). Acceptance criteria: Relative standard deviation percentage (RSD) was less than or equal to 2%.

2-3-7-5 Accuracy and recovery

Standard additions at various concentrations are made by adding known amounts of standard solution to drug samples. These samples were compared to standard solutions of similar concentrations. The accuracy is then estimated as a percentage recovery based on the test

findings. Acceptance criteria: recovery percentage is ranged from 98 -102% for each compound**. [14&16]**

2-3-7-5 Robustness:

862 The robustness of an analytical method is the degree to which it can withstand slight but deliberate changes in the system's parameters and maintain its reliability throughout routine use. For this study, the detection wavelength (nm), temperature (°C), and mobile phase composition were chosen as parameters. Acceptance criteria: Every change item has a relative standard deviation percentage which was less than or equal to 2%.

2-3-7-6 System Suitability Test:

To assess system suitability, relative standard deviations of retention time, tailing factor, the number of theoretical plates, and peak area were calculated as specified in **[15]**. Criteria for acceptance: theoretical plate ≥ 2000, RSD % of peak area ≤ 2%, tailing factor ≤ 2, and RSD % of retention time ≤ 1 %.**[16]**

3-Statistical Analysis:

The data of Storage stability tests were recorded as mean ± Standard error to express the differences between groups, the obtained data were statistically evaluated using the student-test **[17].**

4- Results and Discussion

In this work, two commercial deltamethrin and phoxim concentrate (EC) formulations were collected from the Egyptian market to determine the degradation of deltamethrin and phoxim after storage at fifty-four Celsius after 1, 7, 14, 21 days, and at room temperature for 3 months, also identification of their active principles by HPLC.

4-1-Determination of LD_{50} of phoxim:

The estimation of acute oral LD_{50} of phoxim in male albino rats was found to be 1660 milligrams per kilogram of body weight at the beginning of the experiment (Table 1)

This result agreed with **[18]** who reported that LD_{50} of phoxim as 2000 milligrams per kilogram of body weight in male albino rats and 1400 milligrams per kilogram of body weight in female albino rats.

At the end of the experiment, the LD 50 of phoxim decreased to 831.77 mg/kg.bwt (Table 2)

This increase in toxicity agreed with **[19] and [5]** who reported that the toxicity of the breakdown products is greater than that of the parent chemical. Furthermore, the toxicity of OPs is exacerbated by their breakdown products, which can be bio-activated within an organism or exposed to sunlight. The degradation route is expected to be sulfur substitution by oxygen in the P=S bond, pyrimidine ester bond breaking, and isopropyl group oxidation. **[20] and [21].** As a result, identifying pesticide breakdown products after their release into the environment is required for assessing the toxicity of Ops. Also, these results agreed with **[22]** who investigated the effects of pH, temperature, and photoirradiation on the degradation of phoxim in river water. The results indicated that the degradation was characterized by a first-order process; UV irradiation and the increase of pH and temperature substantially accelerated the degradation.

Table (1) Determination of the acute oral LD_{50} Phoxim in male albino rats at the beginning of the experiment:

Groups	Dose (mg/kg B.wt.)	Log. Dose	Log dose interval	No. of rats in each group	No of dead rats	Mortality %
1	0	Control	0	4	0	0
2	500	2.698970	0.30103	4	0	0
3	1000	3.00	0.30103	4	1	25
4	2000	3.30102	0.30103	4	2	50
5	4000	3.602059	0.30103	4	4	100
Total				20	7	

LD_{50} of phoxim= 1660 mg/kg.b.wt - Variance =0.01312 S.E=0.11
Confidence limit= 3.22±1.96x 0.11 - LD_{50} with confidence limit =1660(1009.3-2691.5)

Table (2) Determination of the acute oral LD_{50} Phoxim in male albino rats at the end of the experiment:

Groups	Dose (mg/kg B.wt.)	Log. Dose	Log dose interval	No. of rats in each group	No of dead rats	Mortality %
1	0	Control	0	4	0	0
2	250	2.39794	0.30103	4	0	0
3	500	2.69897	0.30103	4	1	25
4	1000	3.00	0.30103	4	2	50
5	2000	3.301029	0.30103	4	4	100
Total				20	7	

LD_{50} of phoxim = 831.77 mg/kg.b.wt Variance =0.01312 S.E=0.11 Confidence limit= 2.92±1.96x 0.11
LD_{50} with confidence limit =831.77(501.2-1349)

4-2 Determination of LD_{50} of deltamethrin

The estimation of acute oral LD_{50} of deltamethrin in male albino rats was found to be 17.37 mg/kg.bwt at the beginning of the experiment (Table 3)

This result agreed with **[23]** who reported the LD_{50} of rats as low as 9.36 milligrams per kilogram of body weight at the end of the work, the LD_{50} increased to 20.89 milligrams per kilogram of body weight (Table 4)

This rise in LD_{50} value was consistent with **[24]**, who indicated that environmental temperature can alter pesticide toxicity. The temperature coefficient (TC) of a pesticide is used to quantify this impact. A positive TC indicates that a pesticide becomes more toxic as the temperature rises, whereas a negative TC shows that an insecticide kills more insects at lower temperatures. Pyrethroids, like deltamethrin, the primary pesticide class now used to combat malaria, have a negative TC. Carbamates and organophosphates, on the other hand (two and three of the 12 recommended compounds for indoor residual sprays (IRS) generally have a positive TC, This theory is confirmed by **[25]** who reported that Because both pyrethroid and organophosphate pesticides are often found in sediments and their temperature sensitivities differ, the temperature Toxicity Identification Evaluation can easily distinguish between the two types of chemicals (TIE). The variation in phoxim and deltamethrin acute toxicities after exposure to storage at room temperature as phoxim toxicity increased while the toxicity of deltamethrin decreased is also confirmed by **[26]**who determined the temperature-toxicity relationship in house flies, the effect of post-bioassay temperature (range, 20–34°C), and the efficacy of seven insecticides from organophosphate (chlorpyrifos, profenofos), pyrethroid (cypermethrin, deltamethrin), and new chemical (emamectin benzoate, fipronil, spinosad), where the toxicities of chlorpyrifos, profenofos, emamectin, and fipronil increased 2.10, 2.93, 2.40 and 3.82 fold (i.e. positive temperature coefficient), respectively. However, the toxicity of cypermethrin, deltamethrin, and spinosad reduced 2.21, 2.42, and 3.16 fold (i.e. negative temperature coefficient), respectively.

4-3 HPLC methods validation:

Many researchers have used the HPLC technique to quantitate phoxim and deltamethrin such as**[12]** who described an improved method for detecting the phoxim residual in whole wheat grain (not milled) using high-performance liquid chromatography (HPLC) with a diode array detector (DAD) at 280 nm**., [27]** measured phoxim in water samples using a novel liquid-phase microextraction technology, continuous-flow microextraction (CFME), in conjunction with high-performance liquid chromatography and variable-wavelength detection, **[28]** who described the first method for determining deltamethrin and its main metabolite 3-phenoxybenzoic acid at the same time (3-PBAcid). Protein precipitation and high-performance liquid chromatography are used in this method to support a toxicokinetic investigation in rats and **[29]** who described a faster and more sensitive high-performance liquid chromatography (HPLC) approach for detecting DLM in plasma and tissues. In our research, the methods for phoxim and deltamethrin were evaluated and validated to study their suitability for current work.

Table (3) Determination of LD_{50} of Deltamethrin at the beginning of the experiment:

Groups	Dose (mg/kg B.wt.)	Log. Dose	Log dose interval	No. of rats in each group	No of dead rats	Mortality %
1	0	Control	0	4	0	0
2	6.25	0.79588	0.30103	4	0	0
3	12.5	1.09691	0.30103	4	1	25
4	25	1.39794	0.30103	4	3	75
5	50	1.69891	0.30103	4	4	100
Total				20	8	

LD_{50} of deltametrin= 17.37 mg/kg.b.wt LD_{50} of phoxim= 17.37 mg/kg.b.wt Variance =0.0113 S.E=0.10 Confidence limit= 1.24±1.96x 0.10 LD_{50} with confidence limit =17.37(11.07-27.29)

Table (4) Determination of LD_{50} of Deltamethrin at the end of the experiment:

Groups	Dose (mg/kg B.wt.)	Log. Dose	Log dose interval	No. of rats in each group	No of dead rats	Mortality %
1	0	Control	0	4	0	0
2	6.25	0.79588	0.30103	4	0	0
3	12.5	1.09691	0.30103	4	1	25
4	25	1.39794	0.30103	4	2	50
5	50	1.69891	0.30103	4	4	100
Total				20	7	

LD_{50} of deltametrin= 20.89 mg/kg.b.wt Variance =0.0113 S.E=0.11
Confidence limit= 1.24±1.96x 0.11 LD_{50} with confidence limit =20.89 (9.54-28.18)

4-3-1 specificity and Selectivity:

To demonstrate selectivity and specificity, the technique must be unaffected by the presence of contaminants or excipients. There are no 864interferences on the chromatograms since no interfering peaks were detected with the same retention period of the pesticides examined. The retention time for phoxim and deltamethrin were 14.92 minutes and 8.28 minutes respectively, the results were illustrated in Table 5 and figure 1, 2.

Table 5: Time of retention data

Pesticide name	Time of retention
Phoxim	14.92
Deltamethrin	8.28

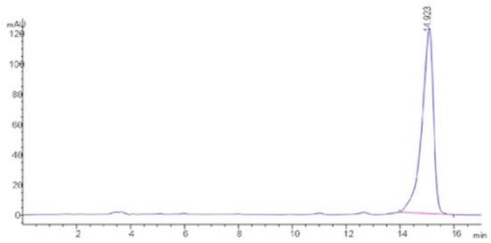

Figure 1: the standard solution chromatogram of phoxim in a concentration of 100 µg /ml

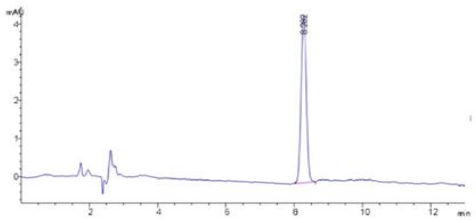

Figure 2: the standard solution chromatogram of deltamethrin in a concentration of 10 µg /ml

4-3-2 Linearity and Range:

In the concentration range of 1 microgram per milliliter to 100 micrograms per milliliter, a linear correlation was found between peak area and phoxim and deltamethrin standard solution concentration. The linearity of the calibration curves was validated by the value of correlation coefficients of the regression (r2), the result was found to be 1 for phoxim and 0.999 for deltamethrin. The results were illustrated in table 6 and figure 3, 4.

Table 6: Regression statistics

Pesticide name	Regression equation
Phoxim	y = 31.715*x-5.6568
Deltamethrin	y = 5.0885*x -0.5992

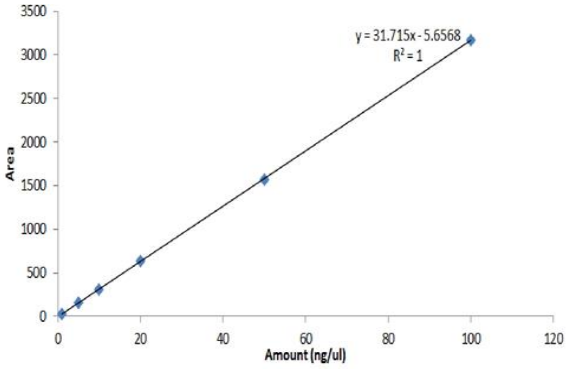

Figure (3): standard calibration curve of phoxim

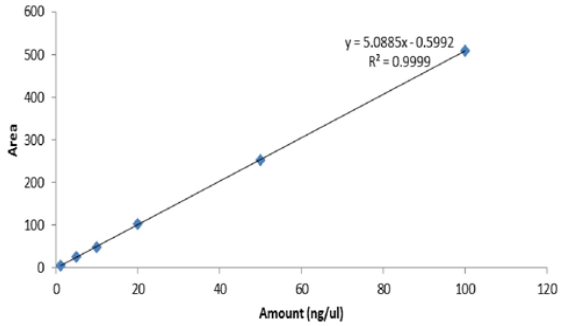

Figure (4): Standard calibration curve of deltamethrin

4-3-3 detection limit and quantification limit:

Based on standard deviation (S) of response and slope (b) of phoxim and deltamethrin using the linear range for the lowest concentration levels under the specified experimental conditions (DL= σ /S × 3.3 and QL= σ /S ×10), The DL for phoxim and deltamethrin were 0.82 microgram per milliliter and 0.27 microgram per milliliter respectively and the QL for phoxim and deltamethrin were 2.47 micrograms per milliliter and 0.83 microgram per milliliter respectively The results were illustrated in table 7.

Table 7: DL and QL results:

Pesticide name	*DL (µg/ml)*	*QL(µg/ml)*
Phoxim	0.82	2.47
Deltamethrin	0.27	0.83

5-3-4 Precision

The method's intra-day and inter-day precision were determined by utilizing six replicate injections of 100 percent test concentration (20 micrograms per milliliter) for each insecticide and analyzing them on the same day (repeatability) and six different days (reproducibility). The Intra-day precision RSD% for phoxim and deltamethrin were 0.133 and 0.73 respectively, while the Inter-day precision RSD% for phoxim and deltamethrin were 0.16 and 0.99 respectively.Acceptance criteria: Relative standard deviation (RSD) ≤ 2.0%.The RSD values for intra-day

and inter-day precision study were < 2.0 % for the studied group of drugs. Which confirms that the method was precise. The results were illustrated in table 8.

4-3-5 Accuracy and recovery

To assess the accuracy, samples at three different concentration levels (50 percent, 100 percent, and 150 percent), corresponding to concentrations (10 micrograms per milliliter, 20 micrograms per milliliter, and 30 micrograms per milliliter, respectively, were prepared by standard addition and then analyzed against a standard solution of the same concentration, in triplicate for each level of concentration. The mean recoveries for three distinct concentrations of phoxim ranged from 98.80 to 100.83 percent. The mean deltamethrin recoveries for three distinct concentrations ranged from 98.20 percent to 100.50 percent. Acceptance criteria include a recovery percentage of 98-102 percent for each chemical**. [14&16]**

The results show that the method was correct. The results were illustrated in table 9.

4-3-6 Robustness:

The robustness of the method was investigated by varying procedure parameters and observing how sensitive the responses are to slight changes in the set conditions. The number of replicates (6) for the concentration level 100 percent was analyzed based on the evaluation of system suitability parameters on recovered amounts, compared to data obtained using the original method. The following modifications were made separately, for phoxim including the composition of mobile phase (72 methanol:28 water) and (68 methanol: 32 water), the wavelength of the detector (283 nm – 277nm) and temperature (30°C –20°C), and for deltamethrin including the composition of mobile phase (88 acetonitrile:22 water) and (82 acetonitrile: 18 water), detector wavelength (269 nm – 263 nm) and temperature (30°C –20°C). The results demonstrated that the values of the test preparation solutions were unaffected by any variance conditions. The change in all tested parameters RSD% were ≤ 2%, As a result, the analytical procedure is assumed to be robust. The results (expressed as mean±SD) were illustrated in table 10.

4-3-7 System Suitability Test:

The system suitability test, which defines the compatibility and efficacy of the system utilized, is a critical part of an analytical procedure. System-suitability tests were carried out following **[15]**. Retention time, tailing factor, column efficiency, and peak area were the distinguishing characteristics. relative standard deviation percentage of peak area for phoxim and deltamethrin were ٦٣١,٥٦ ± 0.84 and 103.04 ± 0.75 respectively, there result were ≤ 2%, Theoretical plate for phoxim and deltamethrin were 6115±4.60 and 3136±3.58 respectively, these results were ≥ 2000. The tailing factor for phoxim and deltamethrin were 0.78 ± 0.006 and 0.98 ± 0.006 respectively, these results were ≤ 2. The values obtained (expressed as mean ±SD) are shown in Table 11.

Table 8: Results of precision study

Compound	Intra-day precision			Inter-day precision		
	Mean(peak areas n=6)	SD	RSD%	Mean(peak areas n=6)	SD	RSD%
Phoxim	631.65	0.84	0.133	631.22	1.00	0.16
Deltamethrin	103.04	0.75	0.73	102.78	1.02	0.99

Table (9): Accuracy and recovery

Compound	Level Conc. (µg/ml)	Mean (N=3)	SD	RSD	Average Recovery%
Phoxim	10	9.88	0.095	0.966	98.80%
	20	19.92	0.055	0.28	99.60%
	30	30.25	0.21	0.69	100.83%
Deltamethrin	10	9.82	0.075	0.768	98.20%
	20	19.90	0.065	0.327	99.50%
	30	30.15	0.17	0.564	100.50%

Table 10: results of robustness study:

Compound	Parameter	Standard	Composition of mobile phase		Wavelength		Temperature	
	Variation	-------	+2	-2	+3	-3	+5	-5
Phoxim	Mean (n=6)	19.88±0.028	19.87±0.076	19.86±0.072	19.86±0.065	19.85±0.074	19.88±0.041	19.87±0.077
	RSD%	0.14	0.38	0.36	0.33	0.37	0.21	0.39
Deltamethrin	Mean (n=6)	19.92±0.046	19.88±0.066	19.90±0.054	19.94±0.058	19.94±0.089	19.90±0.039	19.91±0.046
	RSD%	0.23	0.36	0.27	0.29	0.45	0.20	0.23

Table 11: Summary of System Suitability Test

866

Compound	Parameter	Retention time	Tailing factor	Theoretical plate	Peak area
Phoxim	Mean (n=6)	14.92±0.065	0.78±0.006	6115±4.60	٦٣١,٥٦±0.84
	RSD %	0.43	0.81	0.08	0.13
Deltamethrin	Mean (n=6)	8.28±0.018	0.98±0.006	3136±3.58	103.04±0.75
	RSD %	0.22	0.62	0.11	0.73

5- Estimation of phoxim and deltamethrin concentrations by HPLC after storage at 54 oc or room temperature:

The initial concentration of phoxim(500 mg/ml) is significantly decreased to 403,364 and 311 mg /ml (P<0.001) after 1 week,2 weeks, and 3 weeks respectively with storage at 54 ºc .While at room temperature it significantly decreased to 460(P<0.05), and 410, and 395 mg/ml(P<0.001) after 1 month,2 months, and 3 months respectively (Table 12,13 and figure 5,6). The initial concentration of deltamethrin (50 mg/ml) significantly decreased to 38.8, 35.5, and 29.5 mg/ml (P<0.001) after 1 week, 2 weeks, and 3 weeks respectively with storage at 54oc while at room temperature it is also significantly decreased to 40.3, 36.4 and 31.15 mg/ml (p<0.001) after 1 month, 2 months and 3 months respectively. (Table 14, 15 and figure 7, 8)

Table 12 Phoxim concentrations after heat degradation at 54ºc(mean±SE)

Period	Concentration (mg/ml)
Initial concentration	500.00 ±17.500
After 1 week	403.00 ± 3.58***
After 2 weeks	364.00 ± 2.10 ***
After 3 weeks	311.50 ± 1.19 ***

**Results are expressed as means ± SE (n =3), student t-test*
** P <0.05 **P<0.01 ***P<0.001*

Table 13: Phoxim concentrations after natural degradation at room temperature(mean±SE)

Period	Concentration (mg/ml)
Initial concentration	500.00 ±17.500
After 1 month	460.00 ± 5.79*
After 2 months	410.00 ± 1.19***
After 3 months	395.00 ± 3.47 ***

**Results are expressed as means ± SE (n =3), student t-test*
** P <0.05 **P<0.01 ***P<0.001*

Table14:Deltamethrin concentrations after heat degradation at 54ºc:(mean±SE)

Period	Concentration (mg/ml)
Initial conc.	50.00 ± 0.331
After 1 week	38.80 ± 0.137 ***
After 2 weeks	35.50 ± 0.064 ***
After 3 weeks	29.50 ±0.059***

**Results are expressed as means ± SE (n =3), student t-test*
** P <0.05 **P<0.01 ***P<0.001*

Table 15: Deltamethrin concentrations after natural degradation at room temperature (mean±SE)

Period	Concentration (mg/ml)
Initial conc.	50.00 ± 0.331 mg/ml
After 1 month	40.30 ± 0.332***
After 2 months	36.40 ± 0.210 ***
After 3 months	31.15 ± 0.119***

**Results are expressed as means ± SE (n =3), student t-test*
** P <0.05 **P<0.01 ***P<0.001*

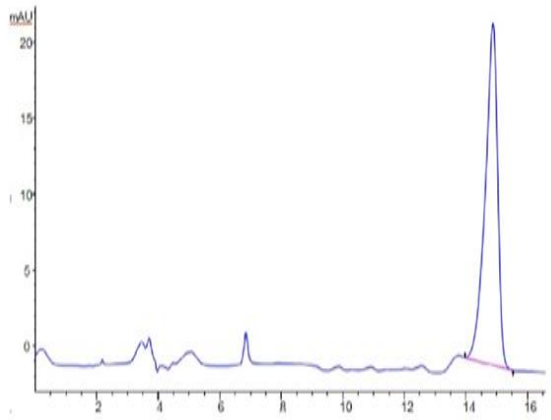

Figure 5: Chromatogram shows a sample of phoxim conc. 50 %

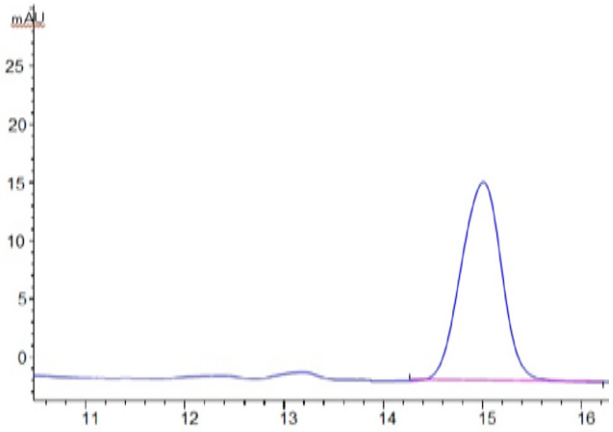

Figure6: Chromatogram shows a sample of phoxim conc. 40.3 %

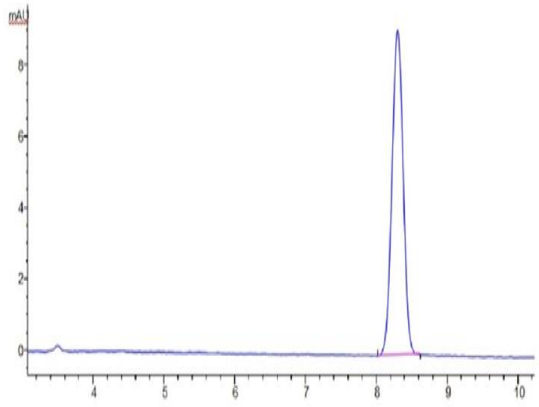

Figure 7: Chromatogram shows a sample of deltamethrin conc. 5%

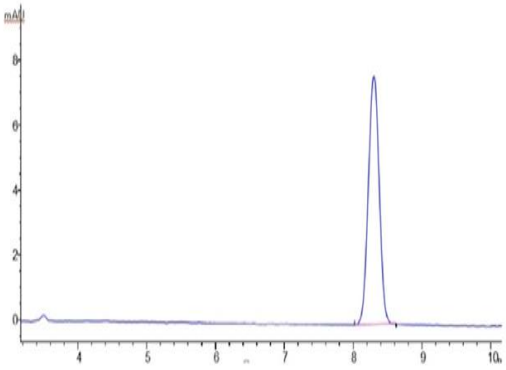

Figure 8: Chromatogram shows a sample of deltamethrin conc. 3.88 %

These results are confirmed by **[30]** who examined the impact of storage on stability and effect of the formulations and the active ingredients of the insecticides dimethoate , diazinon, profenofos , carbosulfan , thiamethoxam, acetamiprid , abamectin - benzoate and lufenuron after storage at 54°C for 0,7,14 and 21 days, and the biological effectiveness against mango scale Aulacaspes tubercularis Newstand (Hemiptera: Diaspidae) to follow the toxicity changes when it is being stored. The results illustrated that the LC50 values of all tested insecticides continued to gradually increase from 0 day to 21 days which indicated that the toxicity was affected by the heat with the long duration of storage. The active ingredient amount of dimethoate, diazinon, and profenofos were 399.2, 499.3, and 716.3 g/l before storage respectively, and reached 361.2, 481.7, and 688.3 g/l respectively after 21 days of storage.

While our results are partially agreed with **[31]** who investigated the degradation of diazinon 60% (collected from the Egyptian market) After seventy days of storage at fifty-four ºC, twelve months of storage at ambient temperature, and six months of exposure to sunlight, several degradation products were identified by GC-MS. The results showed that diazinon was more stable after storage at 54 ºC and room temperature, but degraded faster after exposure to sunlight. GC-MS analysis of materials exposed to sunlight revealed three degradation products: diazoxon, hydroxydiazinon, and 2-isopropyl-6-methyl-4-pyrimidinol (IMP).

6- Conclusions and recommendations

The availability of these easy, highly sensitive, and selective phoxim and deltamethrin testing protocols will be immensely helpful. These approaches, which have been approved following the International Conference on Harmonization (ICH, 2005) and the United States Pharmacopeia (USP Pharmacopeia, 2021), can also be used for stability investigation. The pesticide shelf-life is the amount of time a pesticide can be stored before deterioration occurs. Most pesticides have a stated shelf-life of at least two years from the date of manufacturing; however, shelf-life will be lowered if pesticides are not stored properly, particularly at high temperatures), As a result, do not order more than one year's worth of supplies. The manufacture date and shelf life should be mentioned on the container. as well as the optimum storage conditions should be applied to avoid disposal problems and financial loss.

7 -Conflicts of Interest:

There are no conflicts to declare

8-Formatting of funding sources:

Biochemistry department in Animal Health Research Institute. Dokki.Giza.

9- Acknowledgments:
Many thanks to Prof .Dr.Hany Helmy Halim and Prof.Dr.Elham Mobarez for reviewing this research**.**

10-References

[1] K.M. Blindauer, R.J. Jackson, M. McGeehin, C. Pertowski, C. Rubin, Environmental pesticide illness and injury: The need for a national surveillance system, Journal of Enviromental Health 61(10) (1999) 9.

[2] N. Jayashree, Are pesticides turning into poison spray?, The journal of agriculture and environment 12(10) (2010).

[3] H.J. Niessen, Importance of storage stability studies in the development of pesticide formulations, Pesticide Science 6(2) (1975) 181-188.

[4] M.D. LaGrega, P.L. Buckingham, J.C. Evans, Hazardous waste management, Waveland Press2010

[5] W.G. Aker, X. Hu, P. Wang, H.M. Hwang, Comparing the relative toxicity of malathion and malaoxon in blue catfish Ictalurus furcatus, Enviromental Toxocology 23(4) (2008) 548-554.

868**[6]** A. Marques, M. Custódio, S. Guilherme, I. Gaivão, M.A. Santos, M.J.P.b. Pacheco, physiology, Assessment of chromosomal damage induced by a deltamethrin-based insecticide in fish (Anguilla anguilla L.)–A follow-up study upon exposure and post-exposure periods, Pesticide Biochemistry Physiology 113 (2014) 40-46.

[7] D.A. Laskowski, toxicology, Physical and chemical properties of pyrethroids, Reviews of Enviromental Contamination Toxicology (2002) 49-170.

[8] S.K. Whyte, J.D. Westcott, D. Jimenez, C.W. Revie, K.L. Hammell, Assessment of sea lice (Lepeophtheirus salmonis) management in New Brunswick, Canada using deltamethrin (AlphaMax®) through clinical field treatment and laboratory bioassay responses, Aquaculture 422 (2014) 54-62.

[9] WHO, Environmental health criteria 97: deltamethrin, International Programme on Chemical Safety (IPCS), World Health Organization, 1990.

[10] FAO, Specifications and Evaluations for Agricultural Pesticides, Stability at elevated temperature, 2000.

[11] D.J. Finney, Statistical method in biological assay, Charles Griffin & Company.1978.

[12] Lv Z., Gao L., Gao H., Hou Z., and Zhang B., Improved Determination of Phoxim Residue in Stored Wheat by HPLC with DAD" Journal Of Food Science,74(5),37-41(2009).

[13] D.Z. Bissacot, I. Vassilieff, HPLC determination of flumethrin, deltamethrin, cypermethrin, and cyhalothrin residues in the milk and blood of lactating dairy cows, Journal of Analytical Toxicology 21(5) (1997) 397-402.

[14] International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. ICH harmonised tripartite guideline. Guideline for good clinical practice, 2005.

[15] U.S. Pharmacopeia, Validation of compendial procedures, United States Pharmacopeial Convention Rockville, 2021.

[16] M. Abd Elhafeez, E. Mobarez, Evaluation of analytical method for determination of tetracyclines in their dosage forms by HPLC using different types of chromatographic stationary phases (RP-C8 and RP-C18), Egyptian Journal of Animal Health 1(2) (2021) 1-10.

[17] S.A. Cochran, Snedecor and Cochran's Statistical Methods, Blackwell Pub, 1980.

[18] Flucke, W. and Thyssen, J.: Active ingredient presolution. Acute toxicity studies. Unpublished report (no. 8081) from Bayer AG, Institut für Toxikologie, Wuppertal-Elberfeld, Germany. Submitted to WHO by Bayer AG, Leverkusen, Germany(1979).

[19] Y. Hirahara, H. Ueno and K Nakamuro, Physiological degradation of organophosphorus insecticides in the environment, . Nippon Shokuhin Kagaku Gakkaishi 11(3) (2004)127-136.

[20] V.N. Kouloumbos, D.F. Tsipi, A.E. Hiskia, D. Nikolic, R.B. van Breemen, Identification of photocatalytic degradation products of diazinon in TiO2 aqueous suspensions using GC/MS/MS and LC/MS with quadrupole time-of-flight mass spectrometry, Journal of the American Society for Mass Spectrometry 14(8) (2003) 803-817.

[21] H. Shemer, K.G. Linden, Degradation and by-product formation of diazinon in water during UV and UV/H2O2 treatment, Journal of Hazardous Material 136(3) (2006) 553-559.

[22] B. Lin, Y. Yu, X. Hu, D. Deng, L. Zhu, W. Wang, Degradation mechanisms of phoxim in river water, Journal of Agricultural Food Chemistry 59(1) (2011) 312-321.

[23] British Pharmacopeia, Safety data sheet according to 1907/2006/EC, 2013.

[24] K.D. Glunt, J.I. Blanford, K.P. Paaijmans, Chemicals, climate, and control: increasing the effectiveness of malaria vector control tools by considering relevant temperatures, PLOS pathogens 9(10) (2013).

[25] A.D. Harwood, J. You, M.J. Lydy, Temperature as a toxicity identification evaluation tool for pyrethroid insecticides: toxicokinetic confirmation, Environmental Toxicology and Chemistry, 28(5) (2009) 1051-1058.

[26] H.A.A. Khan, W. Akram, The effect of temperature on the toxicity of insecticides against Musca domestica L.: implications for the effective management of diarrhea, PLoS One 9(4) (2014) 1-6.

[27] L. Guo, P. Liang, T. Zhang, Y. Liu, S.J.C. Liu, Use of continuous-flow microextraction and liquid chromatography for determination of phoxim in water samples, 61(9) (2005) 523-526.

[28] Y. Ding, C.A. White, S. Muralidhara, J.V. Bruckner, M.G. Bartlett, Determination of deltamethrin and its metabolite 3-phenoxybenzoic acid in male rat plasma by high-performance liquid chromatography, Journal of Chromatography B 810(2) (2004) 221-227.

[29] K.B. Kim, M.G. Bartlett, S.S. Anand, J.V. Bruckner, H.J. Kim, Rapid determination of the synthetic pyrethroid insecticide, deltamethrin, in rat plasma and tissues by HPLC, Journal of Chromatography B 834(1-2) (2006) 141-148.

[30] M. Hala, S. Hanan, K. Naglaa, Effects of storage on the stability an d biological effectiveness of some insecticides, Biol.Chem.Environ.Sci 11 (2016) 265-282.

[31] M. Saleh, M. Arief, I. Ismail, Study the relation between storage and stability of diazinonemulsifiable concentrate formulations in the formation of its impurities and major degradation products, International Journal of Advanced Research 3(2) (2015) 247-261.