



RESIDUE ANALYSIS METHOD FOR LAMBDA-CYHALOTHRIN IN VARIOUS ENVIRONMENTAL SAMPLES

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ARTICLE INFO

Article History:

Received 15th July, 2017

Received in revised form 19th

August, 2017 Accepted 25th September, 2017

Published online 28th October, 2017

Key words:

Lambda-cyhalothrin, spectrophotometry, bromination, environmental sample, residue analysis.

ABSTRACT

A simple, sensitive and inexpensive spectrophotometric method was developed for the determination of Lambda-cyhalothrin is based on bromination followed by violet complex formation with leucocrystal violet. The method obeys Beers Law within the range of 0.6-9.0 $\mu\text{g mL}^{-1}$ at λ_{max} 590 nm. The molar absorptivity and Sandal's sensitivity were found to be $0.491 \times 10^6 \text{ L mol}^{-1}\text{cm}^{-1}$ and $5.40 \times 10^{-4} \mu\text{g cm}^{-2}$ respectively. The standard deviation, relative standard deviation, detection and quantification limits were also calculated. The interfering effect of various foreign species was also investigated. The results of the analysis were validated statistically and the proposed method was successfully applied to determination of Lambda-cyhalothrin in various environmental samples.

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INTRODUCTION

Although in terms of prosperity, green revolution has brought a windfall to the farmers in the field of agriculture, it is now showing its side-effects in the form of large-scale environmental degradation with chemical pesticides leading to the contamination of water, food and air. There has been indiscriminate use of pesticides in agriculture as well as in the household and this is one of the major health issues in both developing and developed countries (Ghosh *et al.*, 2016). Pesticides have been used for the welfare of human beings, but their adverse impacts on non-target organisms are significant because they are considered among the most potentially harmful chemicals introduced into the environment (Mohamed *et al.*, 2016).

Pyrethroids, as an important type of synthetic pesticide, are widely used in agricultural production, because of their advantages of efficient and broad-spectrum de-insectization (Xudong *et al.*, 2016). Unfortunately, pyrethroids are highly toxic to a number of non-target organisms such as bees, freshwater fish and other aquatic organisms even at very low concentrations (Oudou *et al.*, 2004). Lambda-cyhalothrin is an insecticide registered by the USEPA in 1998. It belongs to a group of chemicals called pyrethroids (Manigandan *et al.*, 2013). Lambda-cyhalothrin

(LTC) [α -cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclo-propanecarboxylate] is commonly used in residential and agricultural areas and may cause serious environmental pollution and health problems (Bakhta *et al.*, 2017) (Ghosh *et al.*, 2016). Lambda-cyhalothrin (LCT) is generally used to control a wide range of pests that affect cotton, cereals and vegetables as well as control insects, ticks, and flies, which may act as disease vectors and have harmful effects on public health (Zeinab, 2012) (Mohamed *et al.*, 2016). LCT is moderately persistent in the soil environment with field half-lives ranging from 4 to 12 weeks. In water sediment mixtures the half-life decreases to less than 20 days (Oudou *et al.*, 2004).

Exposure to lambda-cyhalothrin poses both acute and chronic risks. Acute effects include skin and eye irritation, non-cardiogenic pulmonary edema, cardiovascular toxicity, coma, convulsions, hepatopancreatic intoxication of lambda-cyhalothrin and severe muscle fasciculation (Manal *et al.*, 2015). Thus, a rapid, sensitive, and selective analytical technique for the determination of pesticide residues in the environmental samples has been long-cherished for practical purposes. The literature revealed many efforts made to determine lambda-cyhalothrin (LCT) in various environmental samples. The methods include gas chromatography tandem mass spectroscopy (Robert, 2002), gas-chromatography (Hayam *et al.*, 2013), solid-phase extraction with centrifugation (Kouzayha *et al.*, 2012), gas-chromatography with electron capture detection (Muhamad *et*

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al., 2012), GC- μ ECD or HPLC-UV (Hem *et al.*, 2010), NMR (Li *et al.*, 2014), voltametric (Oudou *et al.*, 2004), nuclear and nucleolar biomarkers (Cava and Ergene, 2003). In spite of their versatile uses, these techniques have a lot of drawbacks such as tedious procedure, non-linearity of calibration curve and matrix interference, also these techniques required large number of solvents for the extraction and also some limitations in term of high cost instruments used in routine analysis and matrix effects. In the proposed work first time a spectrophotometric method is developed for the determination of lambda-cyhalothrin (LCT) in various environmental samples as the literature review suggested no single spectrophotometric method is reported for estimation of LCT. Due to common availability of the instrumentation, simplicity of the procedure, speed, precision and accuracy, spectrophotometric methods enjoy wide popularity. In addition, they are more economical and simple compared to other analytical methods. The present communication involves the method based on the hydrolysis of the resultant aldehyde group with leucocrystal violet. The developed method has been successfully employed for the determination of lambda-cyhalothrin in environmental samples.

MATERIALS AND METHOD

Apparatus

In the present study a systronics UV-Vis spectrophotometer model-104 (Gujarat, 10 India) with 10 mm matched silica glass cell was used to quantify the absorbance. pH meter model Thermo fisher Orion star A211 (India) was utilized for pH resoluteness. A Remi C-854/4 clinical centrifuge force of 1850 g (India) with permanent swing out rotors was utilized for centrifugation. Calibrated glassware were utilized after getting cleaned and marinating in acidified solution of potassium dichromate and rinsing two times with distilled water.

Reagents and Materials

All reagents used were of Anala. R. grade and double distilled water was used throughout the experiment. Special care was taken during the handling of all glassware's to avoid any possible contamination and to maintain the sensitivity of the method. Lambda-cyhalothrin [Isagro (Asia) Agrochemicals Pvt. Ltd.] A stock solution of 1 ppm lambda-cyhalothrin is prepared in Acetate Buffer (pH=4.5). It is prepared by dissolving 34 g sodium acetate trihydrate in 200 mL of water with acetic acid and mixture was diluted to 100 mL with water. Working standard solution was prepared by appropriate dilution of stock. Potassium hydroxide (Loba Chemie, Mumbai, CAS No. 1310-58-3) 2% alcoholic solution were prepared, saturated solution of bromine in water was prepared. The solution was prepared daily. 90% (v/v) solution of acetic acid (Merck, CAS No. 64-19-7) was prepared. Potassium iodide (BDH, CAS No. 7681-11-0) 0.1 mol l⁻¹ aqueous solution and Potassium iodate (Merck, CAS No. 7758-05-6) 0.2 mol l⁻¹ aqueous solution were prepared. Leucocrystal violet (LCV) (Sigma- Aldrich S. Germany, CAS No. 603-48-5) was prepared adding to a 1 litre volumetric flask 200 mL of water, 3 mL of 85% phosphoric acid and 250 mg of Leuco crystal (4,4',4''-methylidynetris, N,N',-dimethyl aniline) (CH[C₆H₄N(CH₃)₂]₃). It was shaken gently until the dye gets

dissolved. The content of the flask was then diluted to 1 Litre with water (Janghel *et al.*, 2007).

Procedure

An aliquot of the test solution containing 0.6 to 9.0 μ g of lambda-cyhalothrin was taken in a 10 mL graduated tube and to it 2 mL 2% alcoholic potassium hydroxide solution was added and allowed to stand for 10 min for complete hydrolysis and then 0.5 mL bromine water was added and shakes well for 10 minutes. Then add 4 drops of acetic acid to remove excess of bromine. Then 0.5 mL potassium iodide-potassium iodate mixture (5:1) and 1.0 mL of leucocrystal violet was added and heat for 2 min and then leave for 10 min for complete color development. A violet colored dye obtained. The solution was then diluted with distilled water and absorbance was measured at 590 nm against a reagent blank (scheme).

Application to environmental samples analysis

Determination of lambda-cyhalothrin in water Sample

The analytical method was applied in the actual water sample. The water sample was taken from a local river receiving runoff water from the agricultural land. 50 mL of water sample was taken in PTFE bottle and fortified with known amount of lambda-cyhalothrin and kept for 3-4 hours, lambda-cyhalothrin was determined by the proposed method. Firstly filter the sample and add 1 mL volume of 5% EDTA to remove the metal ion present. Later pass the sample through silica gel column. Lambda-cyhalothrin was absorbed by the silica gel and then extracted by allowing through saturated solution of ammonium chloride. The extract was collected in 50 mL calibrated flask and made up to mark by adding water. 5 mL from this aliquot was taken for analysis of lambda-cyhalothrin through the above proposed method.

Determination of lambda-cyhalothrin in soil, fruits and vegetables samples

Weighted (10 g) soil, tomato, carrot, apple, potato etc. collected from various agricultural fields where lambda-cyhalothrin had been sprayed as an insecticide, were crushed first (5 g) then after washing with 50% ethanol filtered and centrifuged and was used to check the originally found concentration by proposed method. While another 5 g spiked with known amount of lambda-cyhalothrin and kept 2-3 hours. The samples were then washed with 50% ethanol and the washing collected in a volumetric flask. Aliquots of these washings were used for the determination of lambda-cyhalothrin by the proposed method (Tamrakara *et al.*, 2012). With 5 mg silica gel column was filled and foliage filtrate was passed through it, for exclusion of lambda-cyhalothrin and other intrusive materials in the extracted sample. With 10 mL of 50% ethanol the column was washed; washings were collected and analysed by above proposed method.

RESULTS AND DISCUSSION

Analytical features of the method

The absorption spectrum of the reaction product of lambda-cyhalothrin with leucocrystal violet is shown in Fig. 1 with maximum absorption at 590 nm. The reagent blank had negligible absorbance at this wavelength. All spectral measurements carried out against double distilled water. Beer's law was obeyed and the linearity graph is shown in

Fig. 2 in the concentration range of 0.6- 9.0 $\mu\text{g mL}^{-1}$ of lambda-cyhalothrin.

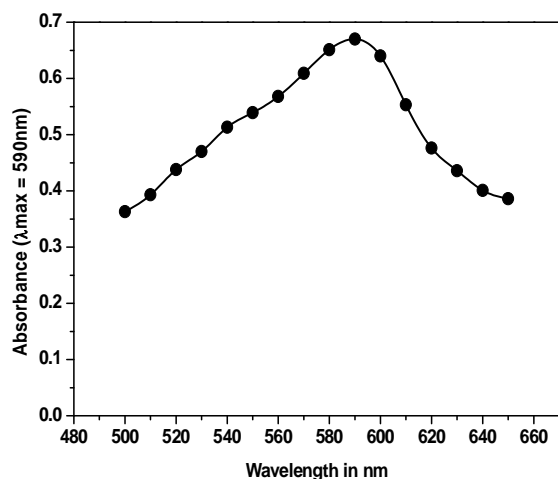


Figure 1 Absorbance spectra of the reaction of lambda-cyhalothrin.

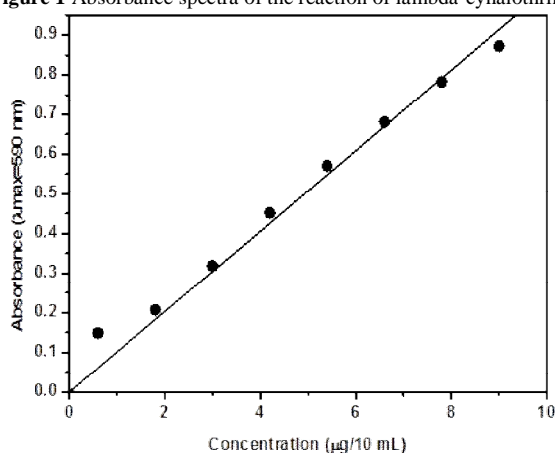


Figure 2 Calibration Curve of lambda- Cyhalothrin.

The curve was found to be linear with a good correlation coefficient. The reproducibility of the method was checked by seven replicate analyses. The optical characteristics and the precision data summarized in Table 1.

Table 1 Optical characteristics and validation data of the proposed spectrophotometric method

Optical characteristics	Parameters
Volume of the dye (mL)	10
Color	Violet
Detection limits, $\mu\text{g mL}^{-1}$	0.121
Quantification limits, $\mu\text{g mL}^{-1}$	0.367
Molar absorptivity $\times 10^6$, $\text{L mol}^{-1} \text{cm}^{-1}$	0.491×10^6
Sandell's sensitivity $\times 10^4$, $\mu\text{g cm}^{-2}$	5.40×10^4
Regression equation, $y = mx + c$	$Y=0.159x+0.068$
Intercept (c)	0.068
Slope (m)	0.159
Correlation coefficient (r^2)	0.997
Standard deviation (S.D.)	5.86×10^{-3}
Relative standard deviation (%)	0.994

$y = \text{Absorbance}; x = \text{Concentration in } \mu\text{g mL}^{-1}$

Optimum reaction conditions for dye formation

The optimization of the method was carefully studied to achieve the complete reaction formation, highest sensitivity and maximum absorbance. Optimum reaction conditions were found by studying with preliminary experiments such as pH effect, the effect of reagents, reaction time and temperature for the completion of the reaction using a lambda-cyhalothrin solution with a concentration of $5 \mu\text{g mL}^{-1}$.

Effect of pH

The effect of pH on the absorbance of the product was studied. It was noticed that the maximum color intensity and highest absorbance value were observed at pH 6 (Fig. 3). And on further increasing the pH value from 6 the absorbance value decreases.

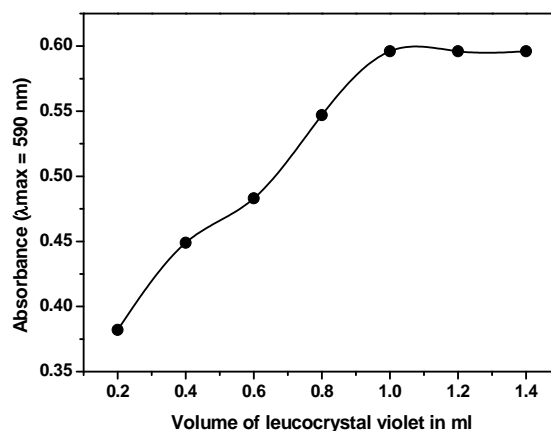


Figure 4 Effect of volume of leucocrystal violet.

Effect of reagents

The effect of reagents was studied by measuring the absorbance of a solution containing a fixed concentration of the compound and varied amount of the reagents. The maximum color intensity was achieved with 1.0 mL of leucocrystal violet. After this volume the absorbance remains constant by increasing the volume of the leucocrystal violet (Fig. 4). So an excess of leucocrystal violet has no effect on the reaction. It was also observed that 0.5 mL each of bromine water (Fig. 5) and the mixture of potassium iodide and potassium iodate solution were sufficient for the complete reaction.

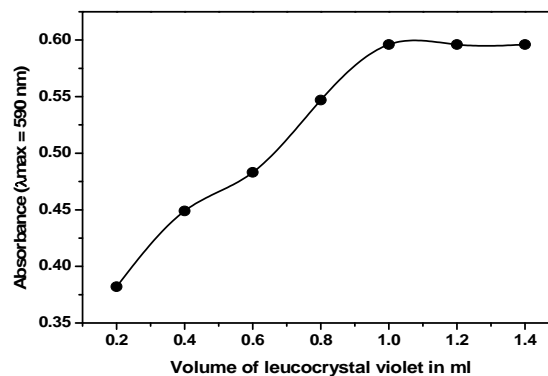


Figure 4 Effect of volume of leucocrystal violet

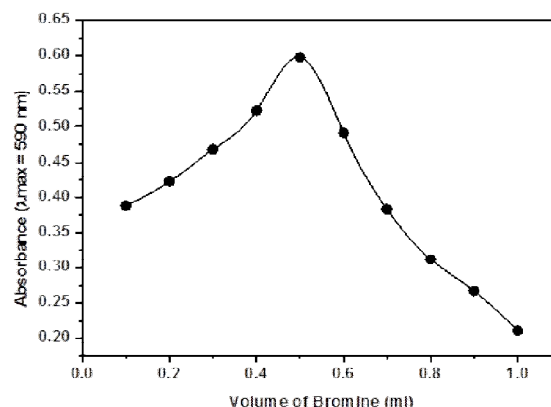


Figure 5 Effect of volume of bromine on the reaction

Effect of time and temperature

Maximum color intensity was observed when the solution containing violet color dye was observed at different temperature. The dye shows maximum absorbance at 34° C and then gradually decreases as shown in Fig. 6. It was found that 30 minute was required for the full color development and the color was stable for 48 hours at room temperature.

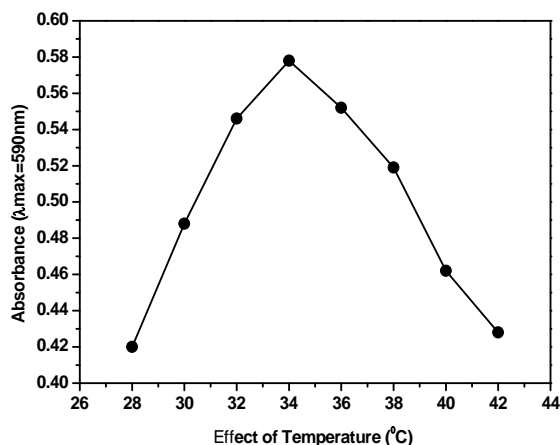


Figure 6 Effect of temperature (°C) on the reaction.

Effect of interference

For interference study the influence of frequently encountered foreign ions and species were studied by analysing sample solution containing 5 µg mL⁻¹ of lambda-cyhalothrin with different amounts of possible interfering. The tolerance limit was taken as the concentration causing an error of ±2% in the determination of the pesticide. The tolerance limit for the foreign ions and species studied are shown in Table 2. The results showed that the most of the foreign ions and species tested did not interfere with their concentrations.

Table 2 Tolerance limit of interfering ions and species on determination of Lambda-cyhalothrin using spectrophotometric method

Foreign Species	Tolerance Limit* µg mL ⁻¹	Foreign Ions	Tolerance Limit* µg mL ⁻¹
Thiocloprid,	850	SO ₄ ²⁻	700
Bifenthrin			
Acetamiprid	800	Fe ²⁺	650
Isoprothiolane,	700	Zn ²⁺	600
Benzene			
Dicofol	650	Cu ²⁺	500
Pyridine	500	Mg ²⁺	400
Baygon	400	Pb ²⁺ , CH ³ COO ⁻	350

* Tolerance limit is the amount of foreign species that causes an error of ±2% in absorbance value.

Validation of the method

Calibration curve, limit of detection (LOD) and limit of quantification (LOQ)

Under the selected conditions, calibration curve for the determination of lambda-cyhalothrin by its reaction with leucocrystal violet was constructed by plotting the absorbance as a function of the corresponding concentrations. A linear relationship between the absorbance, A, of the product and the concentration, C, of lambda-cyhalothrin is obtained in the range of 0.6-9.0 µg mL⁻¹. The linear regression equation obtained from the calibration curve is: Y=0.159 x + 0.068 with a correlation coefficient, r², of 0.997.

The limit of detection (LOD) calculated based on 3.3 σ/s was 0.121µg mL⁻¹ and limit of quantification (LOQ) calculated based on 10σ/s was 0.367 µg mL⁻¹ (Wani *et al.*, 2017).

Table 3 Determination of Lambda-cyhalothrin in various environmental samples by proposed method

Sample	Originally found*	Added (µg/mL) b	Total found (µg/mL) c	Recovery (% ± R.S.D.) (d/b)×100
	A			
Water**	0.40	3	3.29	2.89
	1.02	6	6.91	5.89
Soil***	0.32	3	3.19	2.87
	0.89	6	6.76	5.87
Rice***	0.50	3	3.42	2.92
	0.82	6	6.72	5.90
Beans***	0.71	3	3.66	2.95
	1.20	6	7.17	5.93
Potato***	0.83	3	3.77	2.94
	1.32	6	7.27	5.95
Brinjal***	0.96	3	3.90	2.94
	1.25	6	7.20	5.95
Cabbage***	0.81	3	3.75	2.94
	1.37	6	7.18	5.81
Spinach***	0.92	3	3.83	2.91
	1.52	6	7.34	5.82
Tomato***	0.89	3	3.65	2.76
	1.47	6	7.29	5.82
Apple***	0.76	3	3.61	2.85
	1.21	6	7.12	5.91
Grapes***	0.83	3	3.78	2.95
	1.48	6	7.23	5.75

*Mean of three replicate analyses.

**Water sample 50 mL.

***Sample taken 10 g (taken from agricultural field), Values are mean ± R.S.D. for three determinations.

Precision

The precision and accuracy of the proposed method was determined by replicate analysis of 3 separate solutions of working standard at different concentration levels over a 7 trials. The method gave satisfactory results; RSD did not exceed 2% indicating the good precision of the proposed method.

Robustness

For this proposed method the robustness was studied by evaluating the impact of a small deviation in the method variables on its analytical performance. In this experiment, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that a small deviation in the method variables did not significantly affect the procedures, recovery values were recorded in Table 4 (Shazalia *et al.*, 2015).

Table 4 Robustness of the proposed spectrophotometric method

Recommended condition	Lambda-cyhalothrin	Recovery% ± RSD*
Standard pH	6	96.83±0.800
	5	95.66±0.465
Temperature (°C)	34	97.00±0.527
	35	97.83±0.730
Reagent (mL)	1.0	98.00±0.639
	1.2	98.16±0.165

*Values are mean of three determinations

Comparison with other analytical methods

For determination of lambda-cyhalothrin using leucocrystal violet is detailed here as an efficient method for the

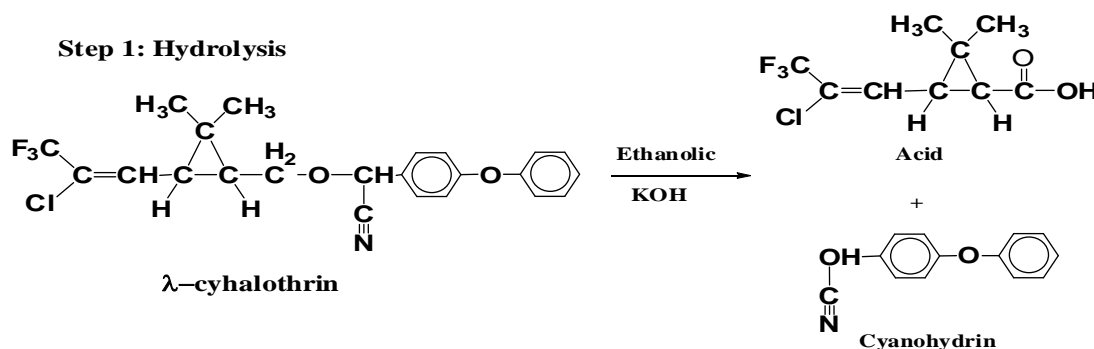
determination of lambda-cyhalothrin. It could be used as an alternative against expensive instrumental techniques. The present method is more convenient and cost beneficial than earlier reported analytical methods, this method is also beneficial to the account of easy availability of reagent used and free from drastic experimental conditions. Comparisons with other methods published and observed are shown in Table 5.

Analyzing environmental samples by proposed method

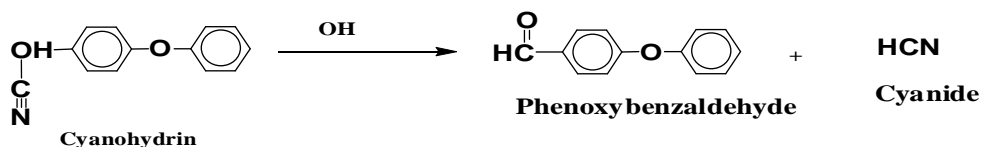
For the determination of lambda-cyhalothrin in various samples of vegetables, fruits, soil and polluted water gathered from different agricultural fields (Raipur, India) where lambda-cyhalothrin was utilized as an insecticide the proposed method was applied most efficiently. Known amount of lambda-cyhalothrin was mixed with different samples of soil,

Table 5 Comparison of proposed method with other analytical methods

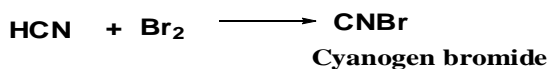
Methods	Reagents	Limit of detection (LOD)	Remarks
Voltammetric(Oudou <i>et al.</i> , 2004)	3-phenoxybenzaldehyde	2.5 µg L ⁻¹	Reagent used is very toxic
Gas Chromatography (Lofty <i>et al.</i> , 2013)	Acetonitrile	0.001 ppm	Reagent used is toxic
Colorimetric Sensors (Yuling <i>et al.</i> , 2011)	Ag@SiO ₂ -NH ₂	1.0×10 ⁻⁶ M	Reagent used is very expensive
Spectrophotometric (Proposed Method)	Leucocrystal violet	0.121 µg mL ⁻¹	Developed colour is highly stable, cost effective, no need of extraction into organic phase and applicable to wide range of samples



Step 2: Formation of Phenoxybenzaldehyde.



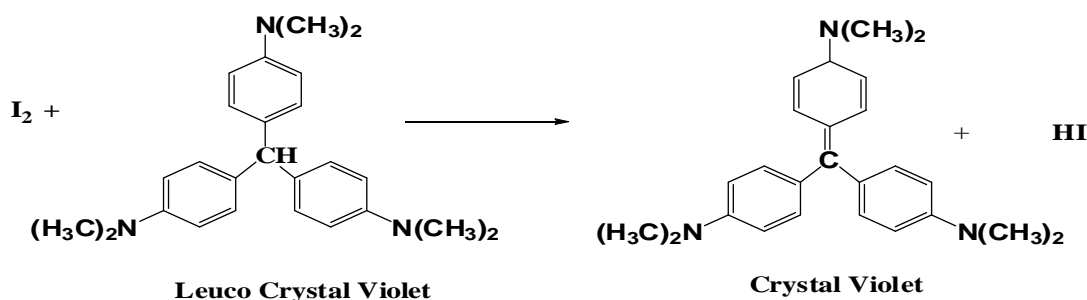
Step 3: Formation of Cynogen bromide.



Step 4: Reaction with Potassium Iodide and Potassium Iodate with Cynogen bromide to release Iodine.



Step 5: Reaction showing formation of blue dye due to reaction between Leuco crystal violet and liberated Iodine



Scheme Chemical reaction for determination of Lambda-cyhalothrin.

water and agricultural products, etc. and then analysed by the proposed method for the determination of lambda-cyhalothrin. And the recovery percentage of the analysed samples are summarised in Table 3.

CONCLUSION

The proposed spectrophotometric method is rapid, simple, sensitive and cost beneficial. The results presented in this work demonstrate that spectrophotometric is a very efficient method for the determination of lambda-cyhalothrin at traces levels in various environmental samples. The most attractive feature of this method is its relative freedom from the interference from different foreign ions and species. Although various analytical methods have been reported for the determination of lambda-cyhalothrin in different environmental samples, to the best of our knowledge this is the first spectrophotometric method for lambda-cyhalothrin. Spectrophotometric detection has the merits of simplicity, cheapness, and portability. Therefore, the method is practical and valuable for routine application in laboratories for the analysis of lambda-cyhalothrin residues in various environmental samples.

ACKNOWLEDGMENT

Authors are grateful to the Head School of Studies in Chemistry Pt. Ravishankar Shukla University, Raipur, India and Director General, Chhattisgarh Council of Science and Technology, Raipur, India for providing laboratory facilities and financial support for this work. The first author is thankful to Rajiv Gandhi National Fellowship for SC, University Grant Commission for Senior Research Fellowship.

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How to cite this article:

Kalpana Wani *et al* (2017) 'Residue Analysis Method For lambda-Cyhalothrin in Various Environmental Samples', *International Journal of Current Advanced Research*, 06(10), pp. 6556-6562.
DOI: <http://dx.doi.org/10.24327/ijcar.2017.6562.0966>
