A New Spectrophotometric Determination of Chlorpyriphos in Environmental and Biological Samples

[1]S. KHAN, [1]M. K. RAI*, [1]V. K. GUPTA, [2]E. K. JANGHEL AND [2]J. K. RAI
[1]*School of Studies in Chemistry*
Pt. Ravishankar Shukla University, Raipur (Chhattisgarh), 492010 India.
[2]*Chhattisgarh Council of Science and Technology*
Pt. Ravishankar Shukla University Campus, Raipur (Chhattisgarh), 492010 India.

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Summary: A new and highly sensitive spectrophotometric method was developed for the determination of widely used organophosphorous insecticide chlorpyriphos. The method is based on alkaline hydrolysis of chlorpyriphos to 1,2,4-trichlropyridine, followed by coupling with diazotized p-aminobenzoic acid in alkaline medium. The absorption maxima of the wine red dye compound formed was measured at 520nm. Beer's law was obeyed over the concentration range of 1.2 to 18 μg in a final solution volume of 25 ml (0.048-0.72 ppm). The molar absorptivity, Sandell's sensitivity and correlation coefficient were found to be 1.8×10^4 (+ 100) $mole^{-1}$ cm^{-1}, 0.011 μg cm^{-2} and 0.9989, respectively. The standard deviation and relative standard deviation were found to be ± 0.005 and 2.02 %, respectively. The method was simple sensitive and free from interferences of other pesticides and diverse ions. Other organophosphorous pesticides did not interfere with the proposed method. The method was satisfactorily applied to the determination of chlorpyriphos in environmental and biological samples.

Introduction

Chlorpyriphos ($C_{10}H_{11}O_3C_{13}PSN$), an organophosphorous insecticide is widely used in agriculture in India. It is used for termite control in construction, forestry and field crops. It is effective against various sucking, boring and leaf-feeding insects on sugar-cane, cotton, pulse oil, seeds, vegetables and fruit-trees [1]. The main symptoms manifested after the treatment with chlorpyriphos are headache, nausea, vomiting, blurred vision and salivation [2]. Chlorpyriphos is a systemic and contact insecticide. It is particularly effective against lepidoptera, homoptera and certain coleoptera. The acute oral LD_{50} for rats is 24 mg/ kg [3, 4].

Due to the wide applicability and high toxicity of chlorpyriphos, numerous instrumental methods have been described for the detection / determination of chlorpyriphos, such as, liquid chromatography mass spectrometry [5], a method which uses semipermeable membrane devices [6] and Gas chromatography [7] etc.

Results and Discussion

Spectral Characteristics

The wine-red color formed in the proposed reaction showed maximum absorption at 520 nm. All spectral measurements were carried out against demineralized water as the reagent blank showed negligible followed the absorption at this wavelength. The color system obeys Beer's law in the range of 1.2 to 18 μg of chlorpyriphos per 25 ml of final solution at 520 nm. The molar absorptivity and Sandell's sensitivity were found to be 1.8×10^4 (± 100) l mol^{-1} cm^{-1} and 0.011 μg cm^{-2} respectively.

Optimization of Conditions

Hydrolysis of chlorpyriphos to 1,2,4-trichloropyridine was studied at different temperatures and alkalinity. It was observed that alkaline conditions were required for the hydrolysis. Maximum hydrolysis was observed with 5.0 mol l^{-1} sodium hydroxide at a temperature range of 30-40 °C, as it gave maximum absorbance values, good stability and quantitative results. It was observed that 1 ml of diazotized p-aminobenzoic acid (DPABA) was sufficient for complete color reaction.

The effect of pH on the color reaction was studied, and it was found that constant absorbance values were obtained at a pH range of 10.5-12 and no buffer solution was required to stabilize the color. At pH lower and higher than this, the absorbance values

*To whom all correspondence should be addressed.

decreased. It was found that 15 min were required for full color development; the color remained stable for several days.

Precision of the method was checked by the replicate analysis of working standard solution containing 10 μg of chlorpyriphos in 25 ml final solution over a period of 7 days. The standard deviation and relative standard deviation were found to be ± 0.005 and 2.05 %, respectively.

Effect of Foreign Species

The effect of common foreign species and pesticides was studied to assess the validity of the method. Known amounts of metal ions, organic pollutant and pesticides were added to the standard solution containing 10μg of chlorpyriphos prior to hydrolysis and the solution was analyzed by the proposed method. The method was found to be free from interferences of most of the foreign species and pesticides (Table-1).

Table-1: Effect of foreign species i. e., metal ions, organic pollutants and pesticides (concentration of chlorpyriphos 10 μg in 25 ml)

Foreign species	Tolerance limit* μg in 25ml	Foreign species	Tolerance limit* μg in 25ml
Methanol, Acetone	2000	Fe^{3+} Fe^{2+}, Ce^{3+}	400
D.D.T, BHC	1200	Ni^{2+}, Pb^{2+}, Se^{2+}	200
Formaldehyde, Cypermethin,	800	SO_4^{2-}, PO_4^{3-}	150
Malathion, Parathion	600	Zn^{2+} Co^{2+} Cu^{2+}	100
Phorate, Quinolphos	400		
Benzene, Toluene, Xylene	300		

* The amount causing an error of ±2% in absorbance value.

Application

The proposed method was applied, satisfactorily, for the determination of chlorpyriphos in various samples of polluted water, vegetables, fruits, foliages and biological fluids. The amount of chlorpyriphos found in various matrix, i.e, water, banana, canesugar, apple and soil were 2.23-2.72 μg in 25 ml, 4.10-2.73 μg in 25 gm, 3.8-2.57 μg in 25 gm, 4.23-3.37μg in 25 gm and 2.12-2.57 μg in 25 gm, respectively (Table-2).

To check the recoveries, known amounts of chlorpyriphos were added to various samples of vegetables, fruits, soil and biological fluids and then analyzed by the proposed method (Tables-2 and -3). The recoveries were found to be 96 to 99 %.

Experimental

Apparatus

A systronics UV-Vis spectrophotometric model 104 with matched silica cells was used for all spectral measurements. A systronics pH meter model 335 was used for pH measurements. A Remi C-854/ 4 clinical centrifuge force of 1850 rpm with fixed swingout rotors was used for centrifugation.

Reagents

All the reagents used were of Anala R grade or of the best available quality. Double distilled deionized water was used throughout the procedure.

Chlorpyriphos (Hindustan Ciba-Geigy Bombay, India): A stock solution of 1mg ml^{-1} was prepared in ethanol. Working standard solutions were prepared by appropriate dilution of the stock standard solution with ethanol.

Sodium hydroxide: A 5.0 mol l^{-1} aqueous solution was used.

Sodium nitrite: A 1 % m/v solution was prepared in 10 v/v hydrochloric acid.

p-Aminobenzoic acid (E. Merck, Germany) [PABA]: 5 % solution prepared in 100 % ethanol. [8].

Diazotized p-aminobenzoic acid (DPABA): To 10 ml of p-aminobenzoic acid, 1 ml of 1 % sodium nitrite was added and the solution was kept in a brown bottle. This remained stable for 4 hr. when kept at 0–5 °C.

Procedure

Preparation of Calibration Graph

An aliquot of test solution containing 1.2 to 18 μg of chlorpyriphos [9, 10] was taken in a 25 ml graduated tube and 1.0 ml of 5.0 mol l^{-1} sodium hydroxide was added to it. The solution was kept for 20 min at room temperature for complete hydrolysis. Then, 1 ml of diazotized p-amino benzoic acid was added and shaken thoroughly and kept at 0–5 °C for 15 min for full color development and wine-red color was obtained. The solution was then diluted to the mark with water and absorbance was measured at 520 nm against a reagent blank.

Table-2: Determination of chlorpyriphos in various environmental and agricultural samples.

Samples	Chlorpyriphos originally found* Proposed method (μg) (a)	Chlorpyriphos added (μg) (b)	Total chlorpyriphos found by proposed method (c)	Difference (c-a)	Recovery % $\frac{(c-a)}{b} \times 100$
Polluted water**	2.23	2.5	4.69	2.46	98.40
	2.72	4.0	6.67	3.96	98.75
Banana***	4.10	2.5	6.50	2.40	96.00
	2.73	4.0	6.64	3.91	97.75
Apple***	3.8	2.5	6.27	2.47	98.80
	2.57	4.0	6.48	3.91	97.70
Sugarcane***	4.23	2.5	6.63	1.42	96.00
	3.37	4.0	7.23	3.86	96.50
Soil***	2.12	2.5	4.54	2.42	96.80
	2.57	4.0	6.48	3.91	97.70

* Mean of three replicate analyses.
** Water sample 25 ml ; after treatment 1 ml aliquot was analyzed.
*** Sample 25 gm (taken from agriculture field, 1 ml aliquot of sample was analyzed after treatment as described in procedure section.)

Table-3: Recovery from biological sample.

Samples		Amount of chlorpyriphos added (μg)		Chlorpyriphos found**(μg)		Recovery %	
		X	Y	X	Y	X	Y
Urine**	A	2.5	2.5	2.40	2.37	96.00	94.80
	B	5.0	5.0	4.75	4.78	95.00	95.60
Blood**	A	2.5	2.5	2.41	2.26	96.40	90.40
	B	5.0	5.0	4.80	4.89	97.8	97.80

X, Y = samples added
* Mean of three replicate analyses.
** Amount of biological samples =1ml, after treatment as described in procedure section.

Determination of Chlorpyriphos in Vegetables, Fruits and Soil

Various samples of vegetables, fruits and soil each of 25 gm were taken, collected from agricultural field, where chlorpyriphos had been sprayed as an insecticide. The samples were macerated with two 20 ml portions of ethanol-demineralized water (1+1), filtered through a Whatman filter paper No. 40 and the filtrate was centrifuged at 1850 rpm for 10 min. In case of vegetables and fruits, the filtrate was quantitatively transferred into a 50 ml calibrated flask and made up to the mark with 50 % ethanol. (a) One milliliter aliquots were taken in a 25 ml graduated tube and 1.0 ml of 5.0 mol l^{-1} sodium hydroxide were added to it and kept for 20 min in 30-40 $^{\circ}$C under optimum condition for complete hydrolysis. Then 1 ml of diazotized p-amino benzoic acid was added,

shaken thoroughly and kept at 0–5 C for 15 min for full color development. A wine-red color was obtained. The solution was then diluted to the mark with water and absorbance was measured at 520 nm against a reagent blank [11].

Determination of Chlorpyriphos in Polluted Water

River water samples, which received run off water from agricultural fields, sprayed with chlorpyriphos were collected. These samples were filtered through a Whatman No. 40 filter paper and were extracted with 2 × 10 ml portions of diethyl ether. The ether solution was evaporated to dryness and the residue was dissolved in 25 ml of ethanol. Aliquots of water samples were taken in a 25 ml graduated tube, followed by the addition of sodium hydroxide and were analyzed as described above.

Determination of Chlorpyriphos in Biological Samples

The method was applied for the determination of chlorpyriphos in biological samples. Synthetic samples were prepared by adding known amounts of chlorpyriphos to these samples and then analyzed after deproteination with trichloroacetic acid [12, 13] as described above.

Conclusion

The present method provides a new and selective spectrophotometric procedure for quantitative determination of chlorpyriphos in environmental and biological samples, requiring no extraction step, and thereby avoiding the use of organic solvent.

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