International Seminar On Non-Conventional Energy Sources for Sustainable Development of Rural Areas,
IJAERD- International Journal of Advance Engineering & Research Development
e-ISSN: 2348-4470, p-ISSN:2348-6406
Impact Factor: 4.72, DOI:10.21090/ijaerd

Investigation Of Chlorpyrifos By Sensitive And Selective Spectrophotometric Method In Different Samples

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Abstract :-*A new and highly sensitive spectrophotometric method for the determination of widely used organophosphorus insecticide chlorpyrifosis done. The method is based on alkaline hydrolysis of chlorpyrifos to 1,2,4 trichloropyridine, followed by coupling with diazotized p-aminoacetophenone in alkaline medium. The absorption maxima of the yellowish-orange dye formed is measured at 450 nm. Beer's law is obeyed over the concentration range of 4 to 35µg in a final solution volume of 25 ml (0.16 - 1.4ppm). The molar absorptivity, sandell's sensitivity, correlation coefficient have been determined. The method is simple sensitive and free from interferences of other pesticides and diverse ions. The method has been satisfactorily applied to the determination of chlorpyrfos in different environmental and biological samples.*

Keywords: *Spectrophotometer, chlorpyrifos, p-aminoacetophenone, environmental and biological samples.*

INTRODUCTION

Chloropyrifos is a crystalline organophosphate insecticide. The IUPAC name of chloropyrifos is O, O-diethyl O-3, 5, 6-trichloro-2- pyridyl phosphorothioate and with molecular formula C9H11Cl3NO3PS. Chlorpyrifos is moderately toxic and chronic exposure has been linked to neurological effects, developmental disorders, and autoimmune disorders. (1)

Most of the chlorpyrifos products are used in the kitchen, bathroom and agricultural field still though it is banned for residential use. (2) Chorpyrifos is a insecticide also used for soil treatment as pre-planting and planting course and seed treatment as a foliar spray and dormant spray. Chorpyrifos has been registered in India in 1968 under insecticides Act of India for regular use in the country.(3) Investigations shows that chlorpyrifos affects the human nervous system inhibits the cholinesterase enzyme , chemist studied their use as nerve gases. (4) It persists in soil for 60–120 days and degrades there primarily through microbial action. (5)

These analytical techniques include HPLC with UV detection (6), liquid chromatography-tandem mass spectrometry (7), HPTLC (8), gas chromatography-mass spectrometry (9), gas chromatography with electron capture detection (10,11),spectrophotometry (12), reflectance near-infrared spectroscopy (13), chemiluminescence assay (14), immunoassay (15) and capillary electrochromatography (16). But this sophisticated equipment need much care and maintenance which is not possible in every laboratory and is too costly to be in reaches of common man. So some alternative methods using easily available reagents and equipment such as spectrophotometer are on process. And in this queue one more finding is discussed hereby so that everyone can protect oneself from such harmful chemical spread around in the environment.

Here a simple and sensitive spectrophotometric method is described for the determination of widely used organophosphorus insecticide chlorpyrifos. The method is based on alkaline hydrolysis of chlorpyrifos to 1, 2, 4 trichloropyridine, followed by coupling with diazotized p-aminoacetophenone in alkaline medium. The absorption maxima of the yellowish-orange dye formed is measured at 450 nm. Beer's law is obeyed over the concentration range of 4 to 35µg in a final solution volume of 25 ml (0.16 - 1.4ppm). The molar absorptivity, sandell's sensitivity, correlation coefficient have been determined. The method is simple sensitive and free from interferences of other

pesticides and diverse ions. The method has been satisfactorily applied to the determination of chlorpyrfos in different environmental and biological samples.

MATERIALS AND METHOD

Apparatus

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

Reagents

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

Chlorpyrifos (Excel crop care limited, Mumbai (Maharashtra)): 1 mg mL-1 was prepared in double distilled water. Working solutions were prepared by dilution of the stock solution with distilled water.

Sodium hydroxide: 8 mole L^{-1} aqueous solutions were used.

Sodium nitrite: 2% m / v solution was prepared.

P-aminoacetophenone (E. Merck, Germany): 0.05% (m/v) solution of the reagent was prepared by dissolving 0.05 gm of p-aminoacetophenone in 100 ml ethanol.

It was observed that 1 ml of diazotized p- aminoacetophenone was sufficient for complete color reaction.

Procedure

An aliquot of the test solution containing 4 to 35 µg of chlorpyrifos was taken in a 25 ml graduated tube and to it; 1.0 ml of 1.0 mol l^{-1} sodium hydroxide was added. The solution was kept for 30 min at room temperature for complete hydrolysis. Then 1 ml of diazotized p-aminoacetophenone was added. The solution was kept for 45 min for full colour development. A yellowish-orange dye is obtained. The solution was then diluted to the mark with distill water and absorbance was measured at 450 nm against a reagent blank.

Determination of chlorpyrifos in water

River water samples, receiving runoff water from agricultural fields, sprayed with chlorpyrifos were collected. These samples were extracted with 2 × 25 ml portions of diethyl ether. Ether solution was evaporated to dryness and residue was dissolved in 50 ml of ethanol. Aliquot were than analysed as described above and in parallel by reported method.

Determination of chlorpyrifos in soil and vegetables

Various samples such as soil, cauliflower and tomato were collected from the fields were dichlorvos was used as an insecticide. The samples were weighed (25g) crushed and extracted with 2 × 25 ml portions of diethyl ether. Diethyl ether was then evaporated to dryness and residue was dissolved in 50 ml of ethanol. Aliquot were than analysed as described above.

Determination of chlorpyrifos in Biological Samples

For the determination of chlorpyrifos in various biological samples i.e. urine and blood, this method was applied. Synthetic samples were prepared by adding known amounts of chlorpyrifos to these samples and then deproteination with trichloroacetic acid was done and analyzed after applying the described process. Three replicate analyses were done and given in Table.

RESULTS AND DISCUSSION

Spectral characteristics

The orange-red dye formed in the given reaction shows maximum absorption at 450 nm. All spectral measurements were carried out against deionised water as the reagent blank showed negligible absorption at this wavelength. The colour system obeys Beer's law in the range of 4 to 35 µg of chlorpyrifos in 25 ml of final solution at 450 nm .The molar absorptivity, Sandell's sensitivity and correlation coefficient were found to be $2.2x10^4$ l $mol^{-1}cm^{-1}$, 0.012 µg and 0.9984 cm^{-2}, respectively.

Optimization of conditions

Hydrolysis of chlorpyrifos to dichloroacetaldehyde was studied at different temperatures and alkalinity. It was observed that alkaline conditions were required for the hydrolysis. Maximum hydrolysis was observed with 1.0 mol l^{-1} sodium hydroxide at temperature of up to 30^oC as it gave maximum absorbance values, good stability and quantitative results .It was observed that 1ml diazotized p-aminoacetophenone was sufficient for complete color reaction.
Precision of the method was checked by the replicate analysis of working standard solution containing 10µg ml^{-1} of chlorpyrifos in 25 ml final solution over a period of 7 days. The standard deviation and relative standard deviation were found to be ± 0.002 and 2.60% respectively.

Effect of foreign species

The effect of common foreign species and pesticides were studied to assess the validity of the method. Known amounts of foreign species and pesticides were added to the standard solution containing 10 µg of chlorpyrifos 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**)

Application

The proposed method has been applied satisfactorily to the determination of chlorpyrifos in water, vegetables, soil and various biological samples. The results are in a good agreement with the reported method.
To check the recoveries, known amount of chlorpyrifos were added to these samples and then analysed by the proposed as well as the reported method (**Table 3 & Table 4**). The recoveries were found to be 88 to 99%.

CONCLUSION

The method is compared with other spectrophotometric methods and found to be more sensitive and selective (**Table 2**). This method is a good alternative to some of the reported costly instrument method. The advantage of the proposed method is mainly its sensitivity, simplicity and selectivity and higher stability of the colored solution. The proposed method has been successfully applied for the determination of chlorpyrifos in water, soil and vegetable samples.

ACKNOWLEDGEMENTS

Authors are thankful to the Head School of Studies in Chemistry Pt. Ravishankar Shukla University, Raipur and Director Shri Shankracharya Institute of Professional Managemant and Technology, Raipur for providing research facilities and funds.

Table -1: **Effect of various pesticides and pollutants. (Concentration of Chlorpyrifos 10µg in 25ml).**

Foreign species	Tolerance limit* ppm	Foreign species	Tolerance limit* ppm
BHC, DDT	400	SO_4^{--}	450
Benzene, ether	500	CO_3^{--}	360
Malathion	350	NO_2^{-}	140
2; 4-D, 2:4:5-T	300	NO_3^{-}	130
Cyanide	250	Cu^{2+},Pb^{2+}	50
Kelthane, Fluoride,chloroform	100	Sb^{3+}	80
Parathion	50	Ca^{2+},Mg^{2+},Cd^{2+}	50
K^{+}, Cl^{-}	20		

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

Table- 2:Comparison with other reported reagents.

Reagent	λmax	Beer's law range (ppm)	Interference
Anthranilic acid	450	0.5-8.18	Less Sensitive
Congo-red	605	0.5-5.7	Toxic Reagent
P-aminobenzoic acid	520	0.048-0.72	Less sensitive
2-amino 8-hydroxy quinoline	420	0.01-0.08	Less sensitive
P-aminoacetophenone	450	0.16 - 1.4	Highly sensitive, selective

Table- 3: Determination of Chlorpyrifos in various environmental and agricultural samples. (10µg of dichlorvos in 25 ml)

Samples	Chlorpyrifos originally found*	Chlorpyrifos added (µg)	Total chlorpyrifos found by proposed* method	Difference	Recovery % ((c-a)/b) X100
	Proposed Method (µg) (a)	(b)	(c)	(c-a)	
Agricultural waste water [a]	5.96	10	15.81	9.93	98.50
	4.55	20	23.92	19.37	96.85
Soil [b]	4.68	10	13.69	9.01	90.10
	4.89	20	24.54	19.65	98.25
Cauliflower [c]	5.22	10	14.27	9.05	90.50
	5.57	20	25.23	19.66	98.30
Tomato [d]	6.83	10	16.71	9.95	98.80
	4.34	20	23.37	19.03	95.15

* Mean of three replicate analysis.
a = Water sample 25ml, after treatment 1 ml aliquot was analysed.
b, c and d = Sample 25gm (taken from agriculture field, 1 ml aliquot of sample was analysed after treatment as described in procedure section

Table-4: Determination of chlorpyrifos in biological samples.

Samples		Amount of Chlorpyrifos added(µg) X	Y	Chlorpyrifos Found * (µg) X	Y	Recovery % X	Y
Urine**	A	5	5	4.92	4.83	98.4	96.6
	B	10	10	9.87	9.85	98.7	98.5
Blood**	A	5	5	4.89	4.81	97.8	96.2
	B	10	10	9.86	9.87	98.6	98.7

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

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