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Coupling diazotization method to analysis of chlorpyrifos in environmental and biological samples

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Abstract

A modified and sensitive spectrophotometric coupling diazotization method for the analysis of Organophosphate insecticides i. e., Chlorpyrifos, in sub-microgram levels is described. During alkaline hydrolysis of Chlorpyrifos to produce 1, 2, 4-trichloropyridine (TCP) in alkaline medium, which coupled with diazotized p-aminoacetophenone to form orange-red color dye at measure 505 nm. The color system obeys Beer's law in the following working range of 1.2 to 10.5 µg (0.048 -0.42 ppm) in a final solution of 25 ml. The Molar Absorptivity, Sandell's Sensitivity, Correlation coefficient, Standard deviation and Relative standard deviation have been determined The method is highly reproducible and have been successfully applied for determination of Chlorpyrifos insecticides in environmental and biological samples.

Keywords: spectrophotometry, chlorpyrifos, p-aminoacetophenone, environmental and biological samples

Introduction

Chlorpyrifos is one of the widely used organophosphate insecticides. It is a crystalline and their IUPAC name of chlorpyrifos is O, O-diethyl *O*-3,5,6- trichloro-2-pyridyl phosphorothioate and with molecular formula $C_9H_{11}C_{l3}NO_3PS$. Chlorpyrifos is moderately toxic and chronic exposure has been linked to neurological effects, developmental disorders, and autoimmune disorders. Chlorpyrifos is manufactured by reacting 3,5,6-trichloro-2-pyridinol with diethylthiophosphoryl chloride. Chlorpyrifos is registered only for agricultural use, where it is "one of the most widely used organophosphate insecticides", according to the United States Environmental Protection Agency (EPA). The crops with the most intense chlorpyrifos use are cotton, corn, almonds and fruit trees including oranges and apples. It is produced via a multistep synthesis from 3-methylpyridine [1].

For acute effects, the World Health Organization classifies chlorpyrifos as Class II: moderately hazardous. The oral LD_{50} in experimental animals is 32 to 1000 mg/kg. The dermal LD_{50} in rats is greater than 2000 mg/kg and 1000 to 2000 mg/kg in rabbits. The 4-hour inhalation LC_{50} for chlorpyrifos in rats is greater than 200 mg/m^3. Chlorpyrifos is an organophosphate, with potential for both acute toxicity at larger amounts and neurological effects in fetuses and children even at very small amounts. Recent research indicates that children exposed to chlorpyrifos while in the womb have an increased risk of delays in mental and motor development at age 3 and an increased occurrence of pervasive developmental disorders such as ADHD. An earlier study demonstrated a correlation between prenatal chlorpyrifos exposure and lower weight and smaller head circumference at birth. The use of chlorpyrifos in agriculture can leave chemical residue on food commodities. The FFDCA requires EPA to set limits, known as tolerances, for pesticide residue in human food and animal feed products based on risk quotients for acute and chronic exposure from food in humans. These tolerances limit the amount of chlorpyrifos that can be applied to crops. A study of the effects of chlorpyrifos on humans exposed over time showed that people exposed to high levels have autoimmune antibodies that are common in people with autoimmune disorders. There is a strong correlation to chronic illness associated with autoimmune disorders after exposure to chlorpyrifos [2, 3].

Chlorpyrifos may affect other neurotransmitters, enzymes and cell signaling pathways, potentially at doses below those that substantially inhibit acetyl cholinesterase. The extent of and mechanisms for these effects remain to be fully characterized. Chlorpyrifos is used for termite control in construction, forestry and field crops [4]. The immediate health hazard from air born chlorpyrifos in the examined houses was negligible, but the findings suggest that it is necessary to monitor chemicals which may contaminate indoor air and to assess the risk of prolonged exposure to such chemicals. The measuring of urinary metabolite TCP of chlorpyrifos via biological monitoring would be useful, allowing comprehensive evaluation of the exposure to chlorpyrifos in indoor air [5-7].

Numerous instrumental methods have been described for the determination of chlorpyrifos generally analyzed by spectrophotometry [8, 9], thin layer chromatography (TLC) [10] and GC-MS [11-13] and liquid chromatography-mass spectrometry [14-16]. In this paper the author developed a spectrophotometric method based on diazotization with diazotized p-aminoacetophenone and determined after extraction of chlorpyrifos Insecticides in Environmental and Biological Samples.

Methods and Materials

Apparatus: 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 g with fixed swing out rotors was used for centrifugation. All reagents used were of Anala R grade or of the best available quality. Double distilled demineralized water was used throughout.

Reagents: Chlorpyrifos (Rallts, Tata Enterprise). A stock solution of 1 mg mL^{-1} was prepared in ethanol. Working standard solutions were prepared by appropriate dilution of the stock standard solution with water.

Sodium Hydroxide: A 20% aqueous solution was used. Anhydrous sodium sulphate (AR) from E Merck used for residue extraction.

Sodium Nitrite: A 1% m/v solution was prepared in 10 v/v hydrochloric acid.
P-aminoacetophenone, [PAAP] (E. Merck, Germany): 0.1% (m/v) solution of the reagent was prepared by dissolving 500 mg of p-aminoacetophenone in 50 ml ethanol.
Diazotized p- amino benzoic acid [DPAAP]: To 10 mL of p-aminoacetophenone, 1 ml of 1% sodium nitrite in hydrochloric acid was added and the solution was kept in a brown bottle. This was stable for 4 h when kept in cold.

Preparation of calibration graph: An aliquot of test solution containing 1.2 to 10.5 µg of Chlorpyrifos was taken in a 25 ml calibrated flask, 4.0 mL of 20% sodium hydroxide was added. The solution was then heated for 5 minutes for complete hydrolysis. Then, 1 ml of diazotized p-aminoacetophenone was added and shaken thoroughly and kept at 0-5ºC for 10 minutes for full color development and orange-red color was obtained. The solution was then diluted to the mark with water and absorbance was measured 505 nm against a reagent blank.

Spectral Characteristics: The color system shows maximum absorption at 505 nm for Chlorpyrifos. All spectral measurements were carried out against demineralized water as the reagent blank showed negligible absorption at this wavelength. The molar absorptivity, Sandell's sensitivity and Correlation coefficient was found to be 6.1×10^5 (±100) mol^{-1}cm^{-1}, 0.035 µg cm^{-2} and 0.9985 respectively. The Standard deviation and Relative standard deviation are 0.065 and 0.45 %.

Conditions for the color development: Maximum hydrolysis was observed with 20% sodium hydroxide at temperature range of 50 - 70ºC as it gave maximum absorbance values, good stability and quantitative results. It was observed that 1 ml of diazotized p-aminoacetophenone was sufficient for complete color reaction.

Effect of pH: The effect of pH on the color reaction was studied and it was found that constant absorbance values were obtained at pH range of ~ 9.5 – 11.0 and no buffer solution was required to stabilize the color. The colored species remain stable for more than 7 hr. under optimum conditions.

Effect of foreign species: The effect of common foreign species and pesticides was studied to assess the validity of the method. Known amount of metal ions, and pesticides were added to the standard 5.0 µg/25 ml of chlorpyrifos before 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 pesticide (Table-01).

Application

The proposed method was applied satisfactorily, for the analysis of chlorpyrifos in various samples i.e. polluted water, vegetables, fruits and biological fluids. The amount of chlorpyrifos found in various samples i.e. water, rice, soil, spinach, brinjal, coriander, orange were purchased from market respectively. Results is analyzed in Table-02 and Table-03.

Analysis of chlorpyrifos in polluted water

Water samples from rivers receiving run off from various agricultural fields, where chlorpyrifos were sprayed are collected. Then these samples are filtered through Whatman No. 40 filter paper. Now the water is evaporated to dryness and the residue was dissolved in 10ml of double distilled water. Aliquot of water samples were taken in 100 ml graduated tube, followed by the addition of 4.0 mL of 20% sodium hydroxide and analyzed as described above.

Analysis of Chlorpyrifos in Different Fruit, Vegetables and soil

Various samples of vegetables, fruits and soil each 10 gm were collected from agricultural field, where chlorpyrifos had been sprayed as an insecticide. The sample were macerated with 50 ml portions of ethanol: double distilled water (1:1) filtered through a Whatman filter paper No. 40 and the filtrate was centrifuged at 1850 rpm for 10 minutes. In case of vegetables and fruits, the filtrate was quantitavely transferred in to 50 ml calibrated flask and made up to the mark with distilled water. 10 ml aliquot were taken in a beaker, added 4.0 ml of 20% sodium hydroxide and heated for 10 minutes at 50-70ºC under optimum condition for complete hydrolysis. Then 1.0 ml diazotized p-aminoacetophenone was added. Shaken thoroughly and kept at 0-5ºC for 10 minutes for full color development.

Analysis 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. Five replicate analysis were done and given in Table-03.

Conclusions

The proposed method is rapid, simple, sensitive and reagent described here is sensitive and selective for analysis of chlorpyrifos and method is more selective than other reported method i.e. Anthranilic acid [4], Congored [5] and p-amino benzoic acid [6]. The proposed method has been applied satisfactorily to the analysis of chlorpyrifos in various samples of polluted water, vegetables, fruits and biological fluids samples.

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Table 1: Effect of foreign species Chlorpyrifos, Conc., 5.0 µg in 25 mL solution

Foreign species	Tolerance limit* µg mL^{-1}	Foreign species	Tolerance limit* µg mL^{-1}
Ca^{2+}, glucose, sucrose, citric acid Cypermethrin,	2800	Nicotinamide, Nicotine	4000
SO$_4^{2-}$, PO$_4^{3-}$, Paraquat	2300	Ferrous fumarate as per ion form	3200
Tartaric acid, malic acid, Dichlorvos, Glyphospate	1000	Calcium pantothenate	800
Al^{3+}, Fe^{3+}, Zn^{2+}	500	Vitamins	500
		B$_1$, B$_2$	
		B$_6$	400
		B$_{12}$	200
		Folic acid	100

*Amount of foreign species causing ± 2% in absorbance values

Table 2: Recovery and analysis of Chlorpyrifos in various environmental and agricultural samples

Samples*	Chlorpyrifos originally found*		Ascorbic acid added, µg (b)	Total Chlorpyrifos by proposed method (c)	Difference (c-a)	Recovery% $\frac{(c-a) \times 100}{b}$
	Proposed method, µg (a)	Reported method µg				
Polluted water**	5.39	5.23	3.0	8.23	2.84	94.6
Rice***	5.92	5.85	3.0	8.90	2.98	99.3
Soil***	3.97	3.92	2.0	5.86	1.89	94.5
Spinach***	2.93	2.78	2.0	4.83	1.90	95.0
Brinjal***	2.93	2.78	2.0	4.91	1.98	99.0
Coriander***	5.92	5.85	3.0	8.90	2.98	99.3
Apple***	3.97	3.92	2.0	5.86	1.89	94.5
Orange***	2.93	2.78	2.0	4.83	1.90	95.0

* Mean of three replicate analysis.

** Water sample 100 mL; after treatment 100 mL aliquot was analyzed.

*** Sample 10 gm (Rice, Soil, Spinach, Coriander taken from agriculture field and Brinjal, Apple, Orange purchased from market, 100 mL aliquot of sample was analyzed after treatment as described in procedure section.)

Table 3: Results of analysis of Chlorpyrifos in blood and urine samples

Samples*	Chlorpyrifos originally found*		Chlorpyrifos added µg (b)	Total Chlorpyrifos by proposed method** (c)	Difference (c-a)	Recovery% $\frac{(c-a) \times 100}{b}$
	Proposed method, µg (a)	Reported method µg				
Blood Sample	3.66	3.48	1.5	5.02	1.36	90.6
	1.75	1.39	2.0	3.68	1.93	96.5
Urine	2.36	2.48	2.0	4.24	1.88	94.0

*Mean of five replicate analyses. In µg 20 mL^{-1}

** Amount of biological Samples= 20 mL, after treatment as described in procedure section.

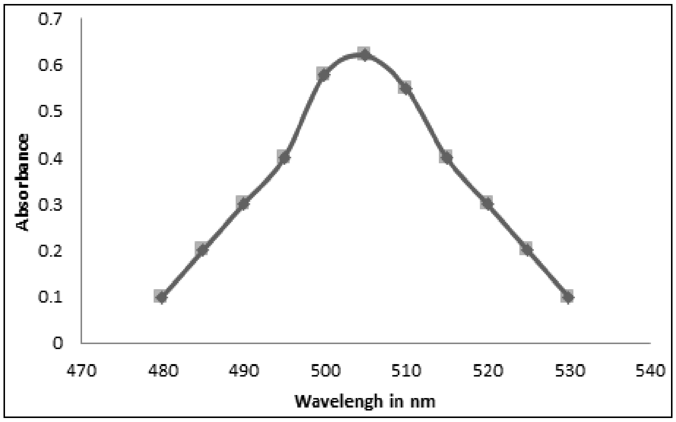

Fig 1: Absorption curve of Chlorpyrifos

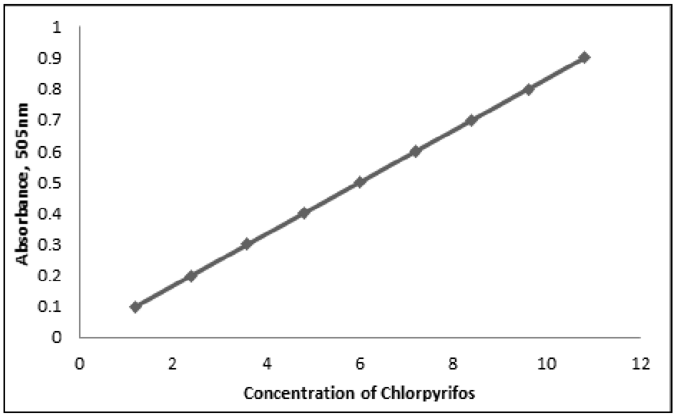

Fig 2: Beer's Law verification of Chlorpyrifos

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