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SPECTROPHOTOMETRIC DETERMINATION OF ORGANOPHOSPHATE INSECTICIDE (CHLORPYRIFOS) BASED ON DIAZOTISATION WITH ANTHRANILIC ACID

(Penentuan Spektrofotometri Racun Serangga Organofosfat (Chlorpyrifos) Berdasarkan Pendiazoan dengan Asid Antranilik)

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Abstract

New reaction system was found for spectrophotometric determination of chlorpyrifos pesticide. This is based on reaction of chlorpyrifos with diazotized anthranilic acid in alkaline medium to form an orange-red color. The solution containing 200ppm chlorpyrifos pesticide and 2 ml of decinormal sodium hydroxide is colorless and with the addition of diazotized anthranilic acid, an immediate orange-red dye is formed. The method is rapid, simple and easy to carry out. The absorbance maximum was observed at 450nm.The Beers law is obeyed up to 8.18 ppm for chlorpyrifos standard solution. Water and vegetable samples were collected in different areas of Visakhapatnam district, Andhra Pradesh, India to determine the chlorpyrifos and found low levels in the range up to 0.04ppm. Interference study was carried for other pesticides and ions.

Keywords: chlorpyrifos, anthranilic acid, diazotization, water and vegetables

Abstrak

Suatu sistem tindak balas baru telah ditemui bagi penentuan spektrofotometri racun perosak chlorpyrifos. Ia berasaskan tindakbalas pendiazoan dengan asid antranilik dengan pembentukan larutan berwarna oren-merah. Larutan yang mengandungi 200 ppm racun perosak chlorpyrifos dan 2 ml natrium hidroksida desinormal adalah tidak berwarna dan dengan penambahan asid antranilik akan membentuk larutan berwarna oren-merah. Kaedah yang digunapakai adalah pantas, ringkas dan mudah untuk dijalankan. Nilai serapan maksimum diperhatikan pada 440 nm. Hukum Beers dipatuhi sehingga 8 ppm terhadap larutan piawai chlorpyrifos. Sampel air dan sayuran telah dikumpul dari kawasan yang berbeza di daerah Visakhapatnam, Andhra Pradesh, India dalam penentuan kandungan chloropyrifos dan mendapati kandungannya rendah dan berjulat sehingga 0.04 ppm. Kajian gangguan oleh racun perosak dan ion lain telah dijalankan.

Kata kunci: chlorpyrifos, asid antranilik, pendiazoan, air dan sayuran

Introduction

Chlorpyrifos is a crystalline organophosphate insecticide. The IUPAC name of chlorpyrifos is O, O-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate and with molecular formula $C_9H_{11}Cl_3NO_3PS$. 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[1]. 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. An example of the consumption pattern of the pesticide is given in Figure 1 [2].

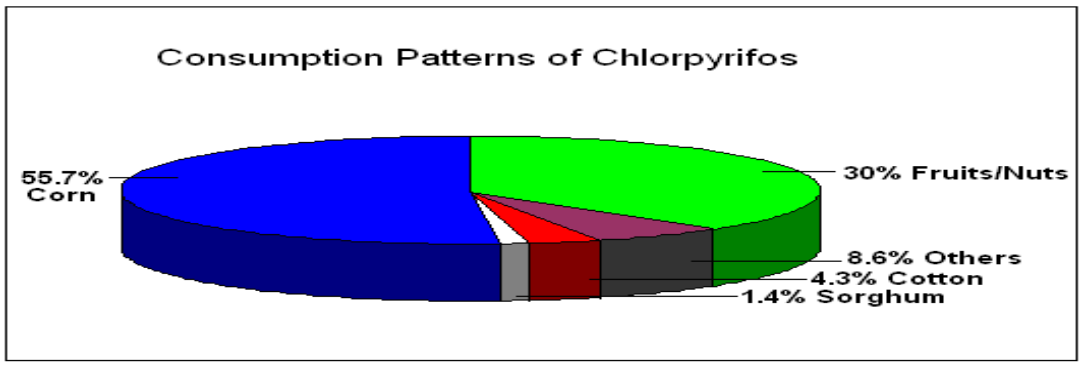


Figure 1. Consumption pattern of pesticides

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 [3]. An earlier study demonstrated a correlation between prenatal chlorpyrifos exposure and lower weight and smaller head circumference at birth [4]. 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 [5].

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 [6].Chlorpyrifos is used for termite control in construction,forestry and field crops.

Numerous instrumental methods have been described for the detection of chloropyrifos generally analyzed by spectrophotometry [7-8], thin layer chromatography (TLC) [9] and GC-MS [10], liquid chromatography-mass spectrometry[11], and gas chromatography[12]. In this paper the author developed a spectrophotometric method based on diazotization with anthranilic acid and determined after extraction of chloropyrifos pesticide in water and vegetables.

Materials and Methods

Chemical and reagents

A JASCO (Model UVIDEC-610) UV-VIS Spectrophotometer with 1cm matched quartz cuvettes was used for all absorbance measurements. Systronics pH Meter (Model 331) is used. All chemicals and reagents used are AnalR grade. The organic solvents like dichloromethane, ethyl acetate and hexane used were HPLC grade and purchased from E Merck. Technical grade pesticide standards were used for standardizations. Anhydrous sodium sulphate (AR) from E Merck used for residue extraction.

Extraction and clean up

Each vegetable was chopped into small pieces. All vegetables and fruits were collected from agricultural fields near Sabbavaram area, Visakhapatnam district, India. A representative sample (50 gm) was taken with 5-10 gm anhydrous sodium sulphate in blending machine to make fine paste. The sample was extracted with 100 ml of ethyl acetate hexane or dichloromethane on mechanical shaker for one hour, extract was filtered, concentrated up to 5 ml on rotary evaporator. The clean-up of chlorpyrifos was performed out by using column chromatography, packed

with Florisil. The extract was eluted with ethyl acetate, hexane or dichloromethane. Elute was concentrated to 5-10 ml on rotary evaporator.

Sampling

Several samples of water and vegetables were collected from agricultural fields in Sabbavaram, Visakhapatnam district. Samples of one kilogram of carrot, cabbage (vegetables) were procured and kept in refrigerator till analysis.

Spectrophotomeric Method

2 mL of decimolar sodium hydroxide was added to an aliquot of working standard of chloropyrifos (0.5-8.0 µg/mL). To this colorless solution, 1ml of diazotized anthranilic acid is added to give orange-red color. The solution was kept aside for 5 min before taking absorbance and absorbance was measured at 450 nm against reagent blank. The absorbance corresponding to the bleached color which in turn corresponds to the analyte chloropyrifos concentration was obtained by subtracting the absorbance of the colorless blank solution from that of test solution. The method was applied to various samples collected in different areas in Visakhapatnam district.

Results and Discussion

The method is based on the reaction of chlorpyrifos with diazotized anthranilic acid in presence of sodium hydroxide forms an orange red color and measured at 450 nm.The absorption maximum is shown in Figure 2. The decrease tendency in absorbance is proportional to chlorpyrifos. The Beer's law is obeyed in the range of 0.5 -8.18 µg/mL.

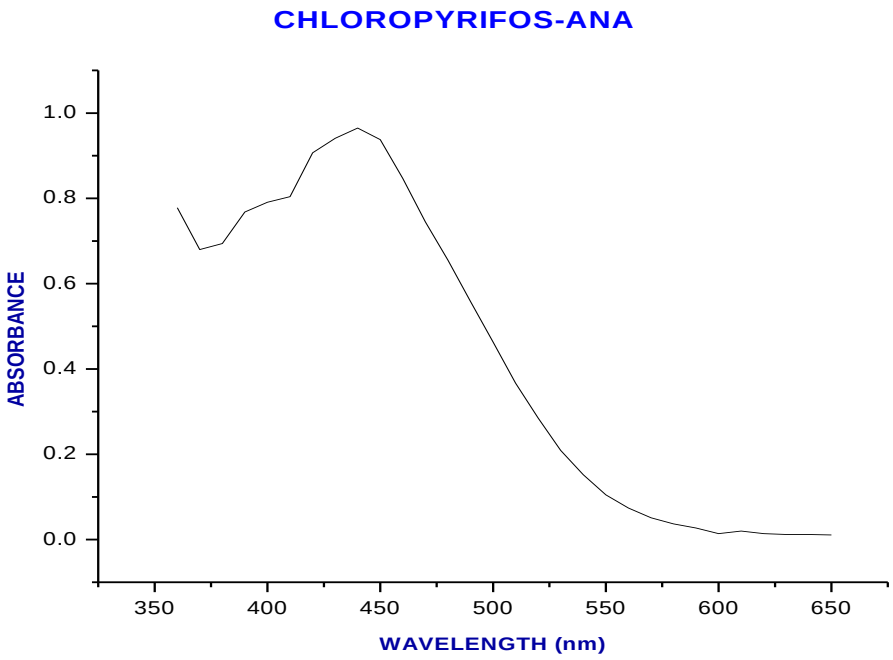
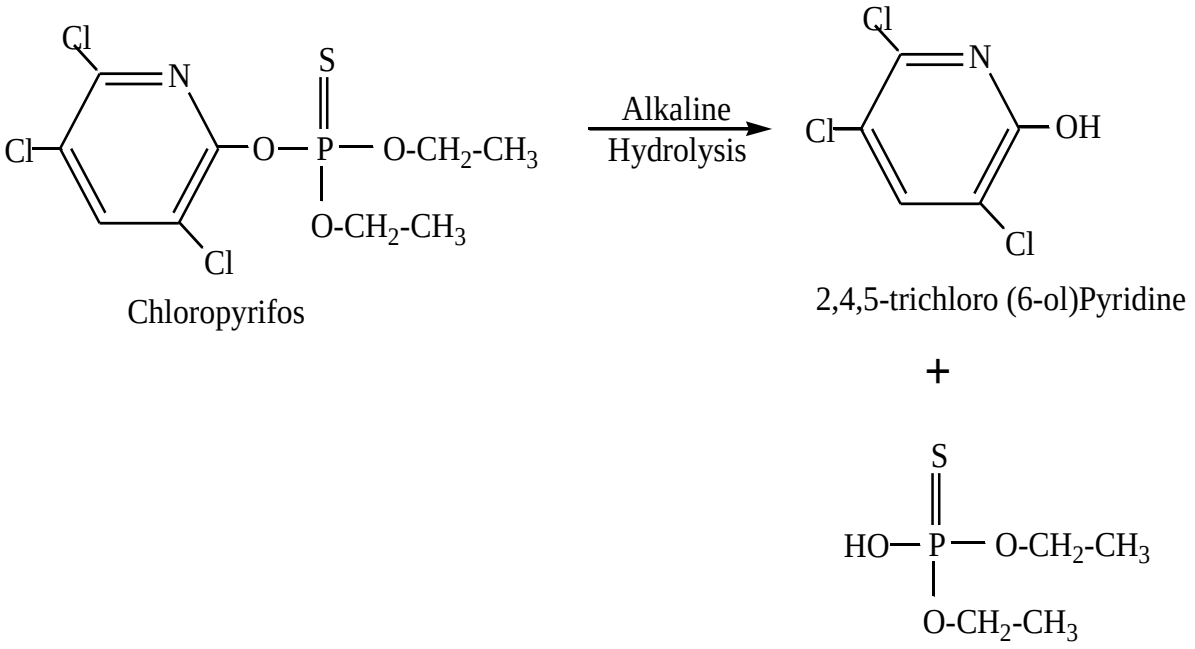


Figure 2. Absorption maxima of chlorpyrifos

The reaction scheme involved is as given below:

Step 1:

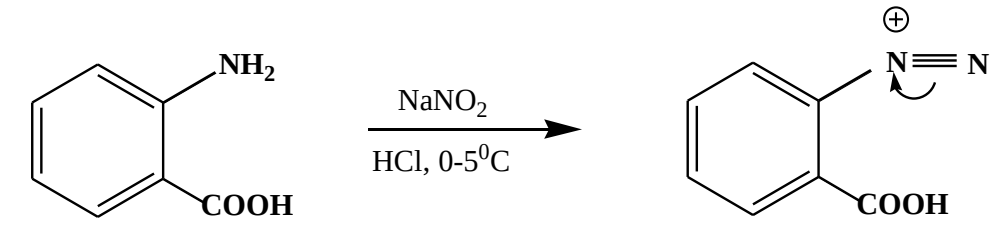


Chloropyrifos

2,4,5-trichloro (6-ol)Pyridine

+

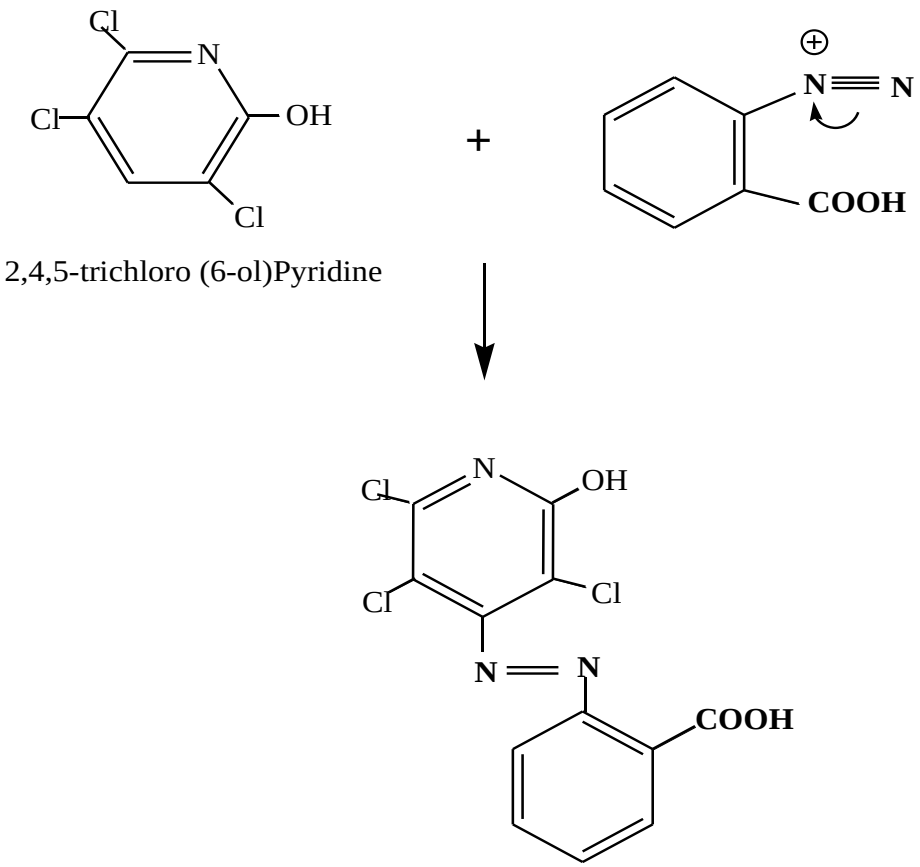
Step 2:



Anthranilic acid

Diazotization of Anthranilic acid

Step 3:



Presence of pesticide residues in water and vegetables has become global phenomenon. For the development of solid phase extraction conditions, a volatile solvent system must be used, as rapid evaporation of a large volume would be required in sample preparation without causing loss of volatile pesticides. The solvent system must be sufficiently polar to extract most polar pesticides. A flow rate of 0.5rnL/min was sufficient to recover all the pesticides except hexaconazole. It was noted that the solid phase extraction should not be left dry after conditioning. This could result in a significant loss of pesticides. It was observed that increasing the polarity of solvent gives lower recoveries. Twelve samples of water and vegetables were collected from agricultural fields in Sabbavaram, Visakhapatnam district for the determination of chlorpyrifos are tabulated and the optical characteristics are given in Table 1 and Table 2 respectively. Very low concentrations were observed in few samples.

Table 1. Optical characters, precision and accuracy

Parameter	**Values**
Maximum absorption	450nm
Color	Orange-red
Relative standard deviation (RSD)	0.74%
% range of error (confideny limit) at 95% confidence level	10±0.025

Table 2. Chlorpyrifos in water and vegetables collected in Sabbavaram, Visakhapatnam

S. No.		Name of the item	Pesticide residue, mg/Kg
1	Water	Rice field	0.30, 0.29, 0.27
		Banana field	5.12, 5.06, 4.08
2	Vegetables	Carrot	8.18, 6.11, 8.17
		Cabbage	5.60, 7.76, 8.14

Conclusion

The present method was tailor-made in view of the previous information about the most prevalent pesticides in the area. The method is rapid, simple and easy to carry out. Application of the method for analysis of chlorpyrifos in water and vegetables from Sabbavaram show the presence of chlorpyrifos in the range of 0.27 – 8.18 mg/ kg.

Acknowledgement

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