



# SPECTROPHOTOMETRIC DETERMINATION OF TRICHLORFON INSECTICIDE AND ITS APPLICATION IN AGRICULTURAL AND ENVIRONMENTAL SAMPLES

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**Abstract:** A spectrophotometric method has been developed based on the modification of the Fujiwara reaction for the determination of trichlorfon at ppm level. Trichlorfon on its alkaline hydrolysis gives chloroform, which react with pyridine to produce pink color. In the present method the pink color acquired in the preceding reaction is discharged with a few drops of acetic acid, followed by the addition of p-nitro aniline reagent, which produces a yellow color dye. The absorption maxima of the yellow color dye formed is measured at 430 nm. Beer's law is obeyed in the range of 2.0-5.0 µg/10 mL of Trichlorfon. The molar absorptivity of the colored system is  $4 \times 10^{-5} \text{ L mol}^{-1} \text{ cm}^{-1}$  and Sandell's sensitivity is  $0.20 \times 10^7 \text{ µg cm}^{-2}$ . This method is free from interference by other pollutants and can be successfully applied for the determination of trichlorfon in various environmental samples.

**Index Terms - Pesticide, Trichlorfon, Detection Techniques, Environmental Samples.**

## I. INTRODUCTION

Pesticides are organic toxic compounds used against a wide range of pests that can enter the food chain through a variety of routes, creating health issues in both human and animals. Varieties of ailments are caused due to pesticide exposure [1]. These have been widely utilized to prevent or reduce damage caused by pests, weeds, and illnesses, so benefiting agricultural manufacturing. These are used by farmers in the agriculture industry for crop protection, pre- and post-harvesting. Pesticide use is expected to protect roughly 30% of global agricultural production. [2]. Organophosphate insecticides have significantly increased agricultural output. These are commonly employed in pre and post harvest treatments to combat fruit and vegetable diseases [3]. Because pesticides are among the most dangerous chemical industry products, there is a high demand for disposable, low-cost, and simple monitoring instruments [4].

Organophosphate pesticides have been widely used in recent decades. Apart from agricultural benefits, OPs have negative toxicological consequences on both animal and human populations [5]. Neurotoxins derived from organophosphates are amongst the most dangerous chemicals known. These chemicals have been extensively utilized in modern agriculture as pesticides and insecticides, as well as chemical warfare agents in terrorist attacks or military operations [6]. Trichlorfon [O,O-dimethyl-(2,2,2-trichloro-1-hydroxyethyl)-phosphonate] is an organophosphate insecticide that is used to combat cockroaches, crickets, silverfish, bedbugs, cattle grubs, flies, ticks, leaf-miners, and leaf-hoppers [7]. It is crucial for the non-destructive and quick detection of trichlorfon insecticide in fruits, because of the intricacy of the matrix found in vegetables, particularly spicy foods, determining tiny levels of trichlorfon is difficult. [7,8]. However, it is toxic to humans via ingestion and dermal absorption and has the potential to cause tumors, genetic mutations and to affect the reproductive system. Trichlorfon is an organophosphorus ester insecticide that is mildly toxic. Overexposure from manufacturing or use, as well as accidental or purposeful intake, can result in serious poisoning. Trichlorfon is unlikely to constitute a hazard to persons who are occupationally exposed if acceptable work practices, hygienic measures, and safety precautions are followed. Despite its severe toxicity to non-target arthropods, trichlorfon has been utilized with few or no detrimental impacts on environmental creature populations. Trichlorfon has very strong dermal and inhalation effects. It is poisonous to humans and other warm-blooded animals. The oral LD50 for rats is  $630 \text{ mg kg}^{-1}$ . Organophosphate pesticide poisoning has been a major cause of concern around the world due to its severe effects on the nervous and reproductive systems of living beings [9]. For agricultural and domestic application, several organophosphorus pesticide formulations are available. Every year, our laboratory receives reports of these insecticides being misused in a number of poisoning instances, including suicide and homicide. Because of the extensive usage of these insecticides, a simple and specific method of detection is required [10]. The detection of human beneficial substances, contaminants as well as pollutants from environmental samples is very necessary and many researchers have reported their work in these regards [11-13]. Previously, a method that

couples capillary electrophoresis (CE) with UV-absorption for GLYP analysis using online copper (II)-GLYP derivatization and successfully detects GLYP in tea beverage samples has been reported [14]. Recently, our research group reported the spectrophotometric detection of glyphosate insecticide in environmental and agricultural samples [15]. In addition, techniques such as electro chromatography [16], Gas Chromatography with  $\mu$ -Electron Capture Detection and GC-MS method [17], solid-phase extraction coupled with high-performance liquid chromatography [18] etc., have been reported for the detection of pesticides in food and environmental samples.

In the present work, a new and sensitive spectrophotometric method has been developed for the determination of trichlorfon insecticide in agricultural and environmental samples. The reaction is based on the modification of the Fujiwara reaction for the determination of trichlorfon at ppm level. Different parameters like effect of pH, temperature, reagent concentration and effect of foreign species etc., were also studied.

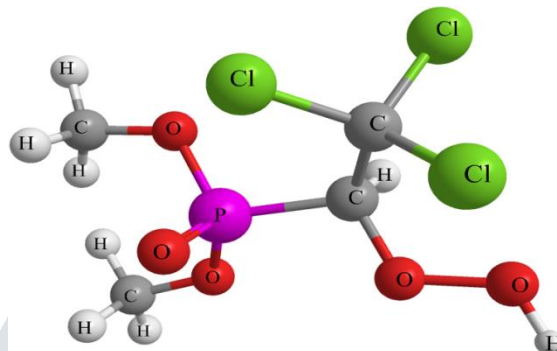


Fig. 1. Structure of trichlorfon

Table 1. Specific information of trichlorfon

Trade name	Trichlorfon
IUPAC Name	(2,2,2-Trichloro-1-hydroxyethyl) phosphonic acid dimethyl ester, Metrifonate
Color	Colorless crystalline powder; stable at room temperature slowly hydrolysed in acid media pH 1-5 (20°C)
Molecular formula	$C_4H_8Cl_3O_4P$
Molecular weight	$257.44 \text{ g mol}^{-1}$
Water solubility	15.4 g in 100 ml at 25°C
Melting point	83-84°C
LD50	LD50 for rats is $630 \text{ mg kg}^{-1}$ , orally
Half-life	526 days

## II. EXPERIMENTAL SECTION

### 2.1 Instruments

UV-Visible absorption spectrum was performed on double beam spectrophotometer make Cary 60 UV-Visible Spectrophotometer (Agilent technologies). The pH measurements were performed with a basic pH meter pH 700 EUTECH instruments and REMI R-4C centrifuge machine was used to centrifuge the samples.

### 2.2 Chemicals and solution preparation

The insecticide trichlorfon was purchased from Makhteshim-agan India PVT. LTD. A stock solution of 1 mg/ml solution of trichlorfon is prepared in water. Working standard solution is prepared by appropriate dilution of the stock solution. Sodium Hydroxide was purchased from Oster Chemical and Pharmaceutical works PVT LTD., Calcutta; a  $5.0 \text{ mol L}^{-1}$  aqueous solution is used. The solution of Reagent p-nitroaniline was prepared with 0.001 M solution of ethanol. Similarly, 10 M aqueous solution Hydrochloric Acid was prepared using ultrapure water. Pyridine and acetic acid used were of A.R grade.

### 2.3 Procedure

An aliquot containing 0.5 to 5.0  $\mu\text{g}$  of trichlorfon was taken in a 25 mL calibrated test tube. The solution of the test tube was evaporated off up to 0.5 mL in a water bath. To this, 1 mL of pyridine followed by 2 mL of 5 M sodium hydroxide were added, and the test tube was kept in a boiling water bath for 2-5 minutes. The pink colored dye thus obtained was cooled in ice cold water and then decolorized with a few drops of glacial acetic acid. Then 1 mL of p-nitroaniline reagent and 10 M HCl were added and the solution was kept for 10 minutes for full color development. The volume of the test tube was made up to 10 mL with distilled water and the absorbance of the yellow colored dye was measured at 430 nm against a reagent blank.

### III. RESULT AND DISCUSSION

#### 3.1 Reaction Mechanism

Three steps are involved in the reaction. In the first step, trichlorfon is hydrolyzed by NaOH to produce chloroform (I), which is used in the subsequent reaction. Next, chloroform reacts with pyridine in an alkaline medium to form Schiff's base of glutamic aldehyde. In the third stage, the pink color of Schiff's base of glutamic aldehyde is transformed into glutamic aldehyde (II), which generates a yellow colored dye with p-nitro aniline reagent (Scheme 1).

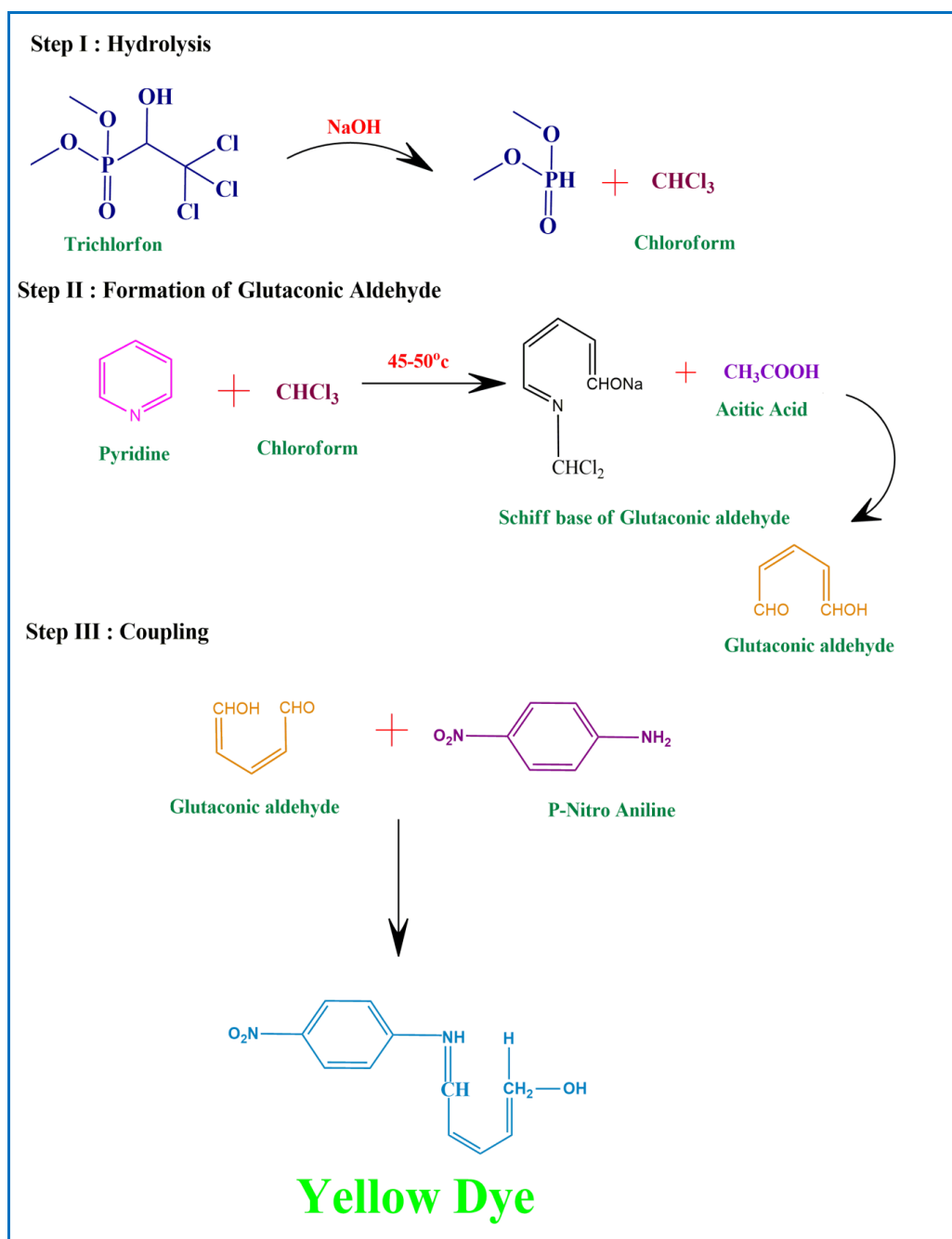


Fig. 2. Reaction mechanism for the determination of trichlorfon.

#### 3.2 Optimization parameters

##### 3.2.1 Effect of pH

The effect of pH on the proposed reaction was studied and it was noticed that the maximum value of absorbance was obtained at a pH 4.5, as shown in Fig. 3(a). A buffer solution was not required for color stability. It was also discovered that 15 minutes were required for full color development, and the color remained stable for several days.

##### 3.2.2 Effect of Temperature

It was found that 2-5 minutes in a water bath at a temperature range of 30°C was sufficient for the complete color development. Further increasing the temperature of the solution decreases the absorbance value. The results are shown in Fig. 3(b).

### 3.2.3 Effect of Pyridine

The hydrolysis of trichlorfon to chloroform and the reaction of chloroform with pyridine to form a pink-colored dye were optimized using standard reaction conditions. In addition, the effect of the concentration of pyridine and the effect of NaOH were also checked. 1 mL of pyridine was sufficient for complete color development. The absorbance value decreases as the amount of pyridine increases or decreases [Fig. 3(c)].

### 3.2.4 Effect of *p*-nitroaniline

It was found that 1.0 mL of *p*-nitroaniline was sufficient for complete color development. On increasing the amount of *p*-nitroaniline, the absorbance value decreases. The results are depicted in Fig. 3 (d). Moreover, 5 M (2 mL) of NaOH was sufficient for full color development. A higher concentration of these reagents decreases the absorbance value.

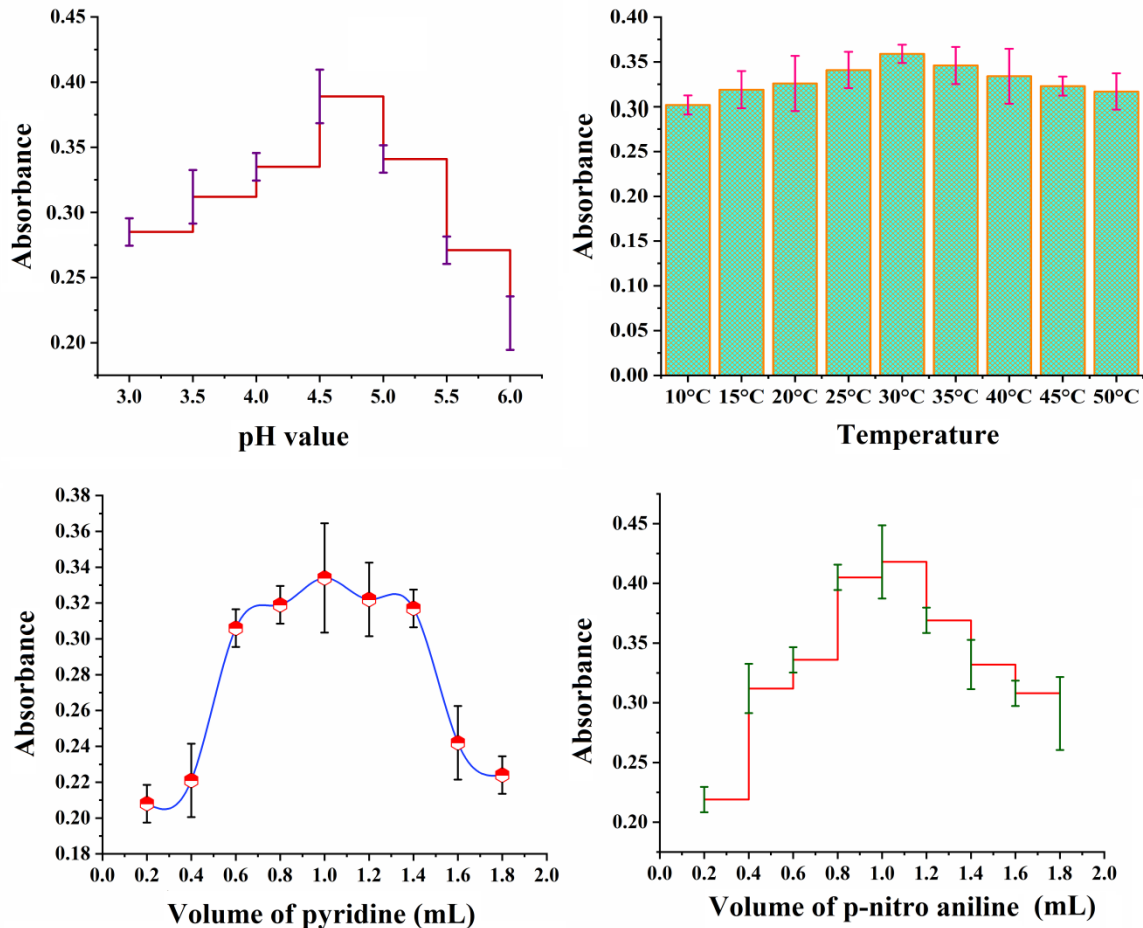


Fig. 3. Effect of parameters on trichlorfon determination

### 3.2.5 Effect of Foreign Species

The effect of various pollutants and pesticides on the determination of trichlorfon was studied (Fig. 4). A known amount of trichlorfon pesticide was added, to a sample containing foreign species. The trichlorfon was analyzed as described above. The method was found to be free from interference by other pesticides and pollutants normally given in Table 2.

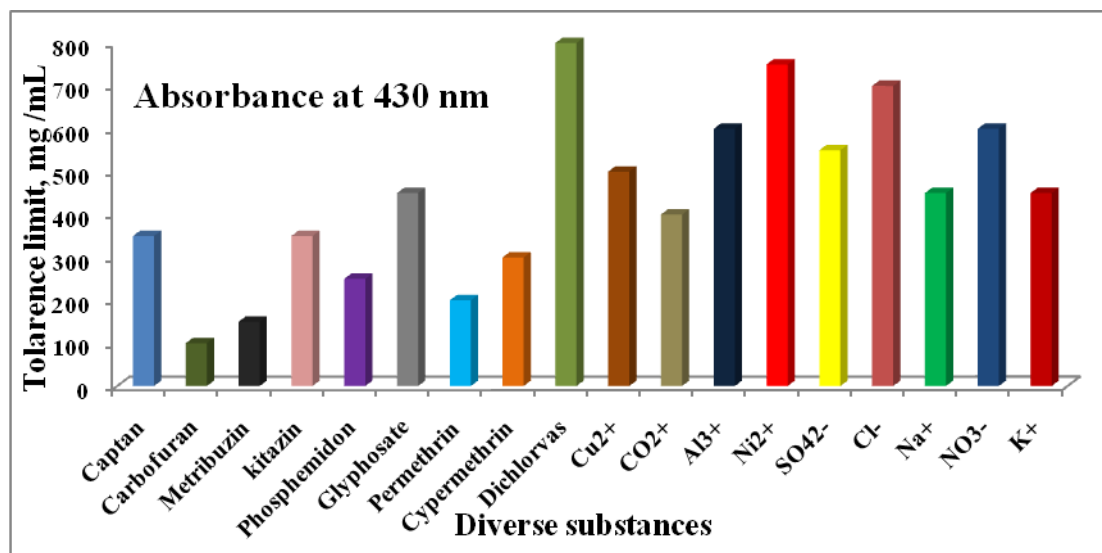


Fig. 4. Effect of various pollutants and pesticides

Table 2. Effect of foreign species

Foreign species	Tolerance limit in mg/L	Foreign species	Tolerance limit in mg/L
Kitazin	600	Cu <sup>2+</sup>	800
Phosphemidon	350	CO <sup>2+</sup>	500
Glyphosate	250	Al <sup>3+</sup>	400
Permethrin	450	Ni <sup>2+</sup>	600
Cypermethrin	200	SO <sub>4</sub> <sup>2-</sup>	750
Dichlorvas	300	Cl <sup>-</sup>	550
Flonicamid	300	Na <sup>+</sup>	450
Carbofuran	100	NO <sub>3</sub> <sup>-</sup>	600
Metribuzin	150	K <sup>+</sup>	450

### 3.3 Analytical validation of the method

The UV–Visible absorbance spectra of yellow colored dye complex showed maximum absorption at 430 nm (Fig. 5). The reagent blank had negligible absorbance at this wavelength. At 430 nm, Beer's law is obeyed over the concentration range of 2.0–5.0  $\mu\text{g}$  of Trichlorfon per 10 mL of the final solution. The molar absorptivity of yellow color dye was found to be  $4 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ . Sandell's sensitivity of yellow color dye was found to be  $0.20 \times 10^7 \mu\text{g cm}^{-2}$ . The reproducibility of the method was evaluated by the replicate analysis of 2.0  $\mu\text{g}$  of trichlorfon in 10 mL of final solution over a period of 7 days. The standard deviation and relative standard deviation are indicated in table 3.

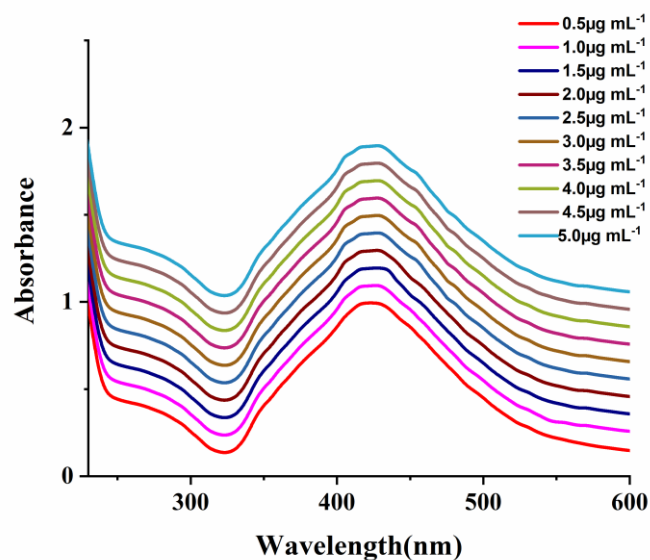


Fig. 5. UV–Visible absorption spectrum of yellow colored dye complex

Table 3. Reproducibility of the method

No. of days	Absorbance (at 430nm)
1	0.312
2	0.314
3	0.316
4	0.315
5	0.317
6	0.318
7	0.321
Mean	0.316
Standard deviation	0.002
Relative standard deviation	0.63%

Table 4. Optical characteristics and statistical data of the regression equations for the method

S.No.	Parameters	Values for the reaction
1.	$\lambda_{\text{max}}$	430 nm
2.	Beer's law limit	2-5 $\mu\text{g}$ in 10 mL
3.	Molar absorptivity	$4 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$
4.	Sandell's sensitivity	$0.20 \times 10^7 \mu\text{g cm}^{-2}$
5.	Relative standard deviation	0.63%
6.	Correlation coefficient( $R^2$ )	0.986
7.	Intercept	0.495
8.	Slope	0.569
9.	Limit of Quantification (LOQ)	$0.540 \mu\text{g mL}^{-1}$
10.	Limit of Detection (LOD)	$0.178 \mu\text{g mL}^{-1}$

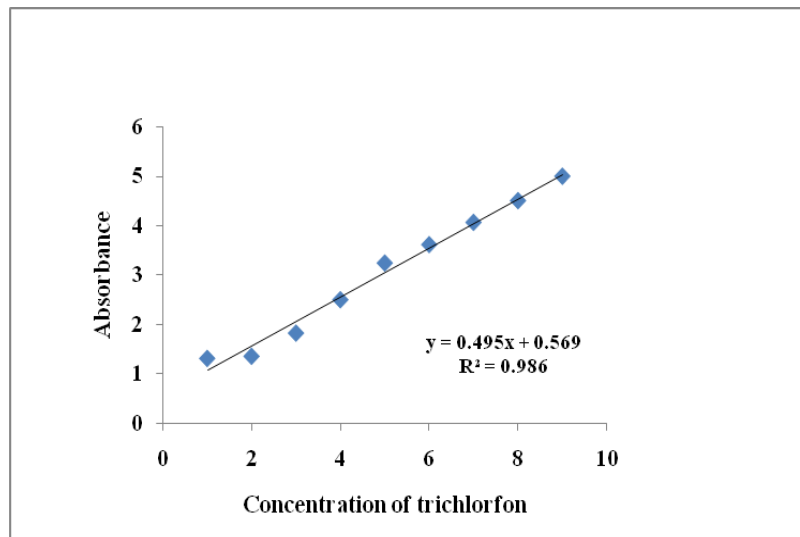


Fig. 6. Calibration curve for the determination of trichlorfon

### 3.4 Application of the method

#### 3.4.1 Determination of trichlorfon in Water Sample

A 100 mL water sample was taken, a known amount of trichlorfon was added and stored for 24 hours. Next, trichlorfon was extracted into hexane. Hexane was evaporated and trichlorfon was measured using both the proposed and reported methods. The recoveries are shown in Table 5.

#### 3.4.2 Determination of trichlorfon in vegetables, fruits, grains and soil

Water, Soil, Soyabean, cauliflower, sweet corn, Tomato, Cabbage and Pumpkin were weighed (5gm), crushed and spiked with known amount of Trichlorfon. After 24 hours trichlorfon was extracted in hexane. Hexane was evaporated, and trichlorfon was determined by using both, proposed as well as reported method. The recoveries are shown in Table 5. According to the method of the present investigation, the recovery rate is in the range of 90.85 to 109.4%.

Table 5. Recovery of trichlorfon in environmental samples

Sample	Trichlorfon originally found* ( $\mu\text{g}$ ) $X_1$	Trichlorfon added ( $\mu\text{g}$ ) $Y$	Total Trichlorfon found* $X_2$	Difference( $\mu\text{g}$ ) $X_2 - X_1$	Recovery (%) $(X_2 - X_1) \times 100 / Y$
Water	5.513	2	7.583	2.07	103.5
Soil	3.59	2	5.513	1.923	96.15
Soyabean	3.663	2	5.85	2.18	109.4
Cauliflower	3.51	2	5.513	2.00	100.4
Sweetcorn	3.148	2	5.103	1.955	97.75
Tomato	4.773	2	6.623	1.89	94.50
Cabbage	3.623	2	5.44	1.817	90.85
Pumpkin	3.363	2	5.518	2.155	107.75

Means of three replicate analysis 50 mL; Amount of sample 5 mL; Amount of sample 5 gm

### 3.5 Comparison of the proposed method with other reported methods

This method has been compared with other reported methods for the determination of trichlorfon and it has been found that this method is more sensitive, inexpensive, and simple than the other methods, by simple modification of discharging the color obtained by Fujiwara reaction with acetic acid and later regeneration of the color with p-nitroaniline reagent. The comparison of the present method with the GC-MS (Reference) method is shown in table 6.

Table 6. Comparison of the present method with the GC-MS method (reference method)

Statistical parameters	Statistical data analysis	Statistical data analysis
	UV (Present method)	GC-MS (Reference method)
Linear range ( $\mu\text{g mL}^{-1}$ )	2-5	25-250
RSD (%)	0.63	3.6-6.7
Correlation coefficient ( $R^2$ )	0.986	0.9988

Intercept	0.495	3.022
Slope	0.569	3.9634
Mean Recovery (%)	90.85-109.4	92.4-103.6
LOD ( $\mu\text{g mL}^{-1}$ )	0.178	3.7
LOQ ( $\mu\text{g mL}^{-1}$ )	0.540	11.1

**Table 7.** Comparison of proposed trichlorfon method with other reported methods

Methods	Correlation coefficient	LOD ( $\mu\text{g L}^{-1}$ )	LOQ ( $\mu\text{g L}^{-1}$ )	Average recovery (%)	RSD range	Reference
dispersive-solid phase extraction with liquid chromatography–tandem mass spectrometry	-	0.20-2.0	0.50-5.0	74.8-110	-	19
cloud point method	0.9905 - 0.9964	0.045– 0.03	3.54	87.2–111.2	6.6–7.4	20
solid-phase extraction coupled with high-performance liquid chromatography	-	1.2 -4.2 ng/g	-	88.5-94.2	-	21
Gas Chromatography with $\mu$ -Electron Capture Detection and GC-MS	0.9988	3.7	11.1 ng/L	92.4-103.6	-	17
Liquid Chromatography Mass Spectrometry	0.999	0.5 g/kg	1.7 g/kg	98.8-105	1.1-2.3	22
Spectrophotometric	0.986	0.178	0.540	90.85-109.4	0.63	Present method

#### IV. CONCLUSION

In this paper, we describe a novel and sensitive spectrophotometric approach for determining trichlorfon insecticide in agricultural and environmental materials, which is based on the Fujiwara reaction for determining trichlorfon at ppm level. The reaction was carried out at many optimal settings such as pH, temperature, reagent concentration, effect of foreign species, and so on. The current method is less expensive, simpler, and more sensitive than previous spectrophotometric methods for determining trichlorfon. It is capable of detecting trichlorfon in a variety of agricultural and environmental materials (i.e., soybean, cauliflower, sweetcorn, tomato, cabbage, pumpkin, water, and soil).

#### V. ACKNOWLEDGMENT

The authors are grateful to the Head, School of Studies in chemistry, Pt. Ravishankar Shukla University Raipur for providing lab facilities.

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