exposure have impaired fetal growth.[14] They inhibit an enzyme, acteylcholinesterase (AChE), that breaks down acetylcholine, chemical involves in transmitting nerve impulses across the junctions between nerves. Without functioning AChE, acetylcholine accumulates; producing rapid twitching of involuntary muscles convulsions, paralysis, and ultimately death.[3] Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, incoordination, headache, dizziness, tremor,

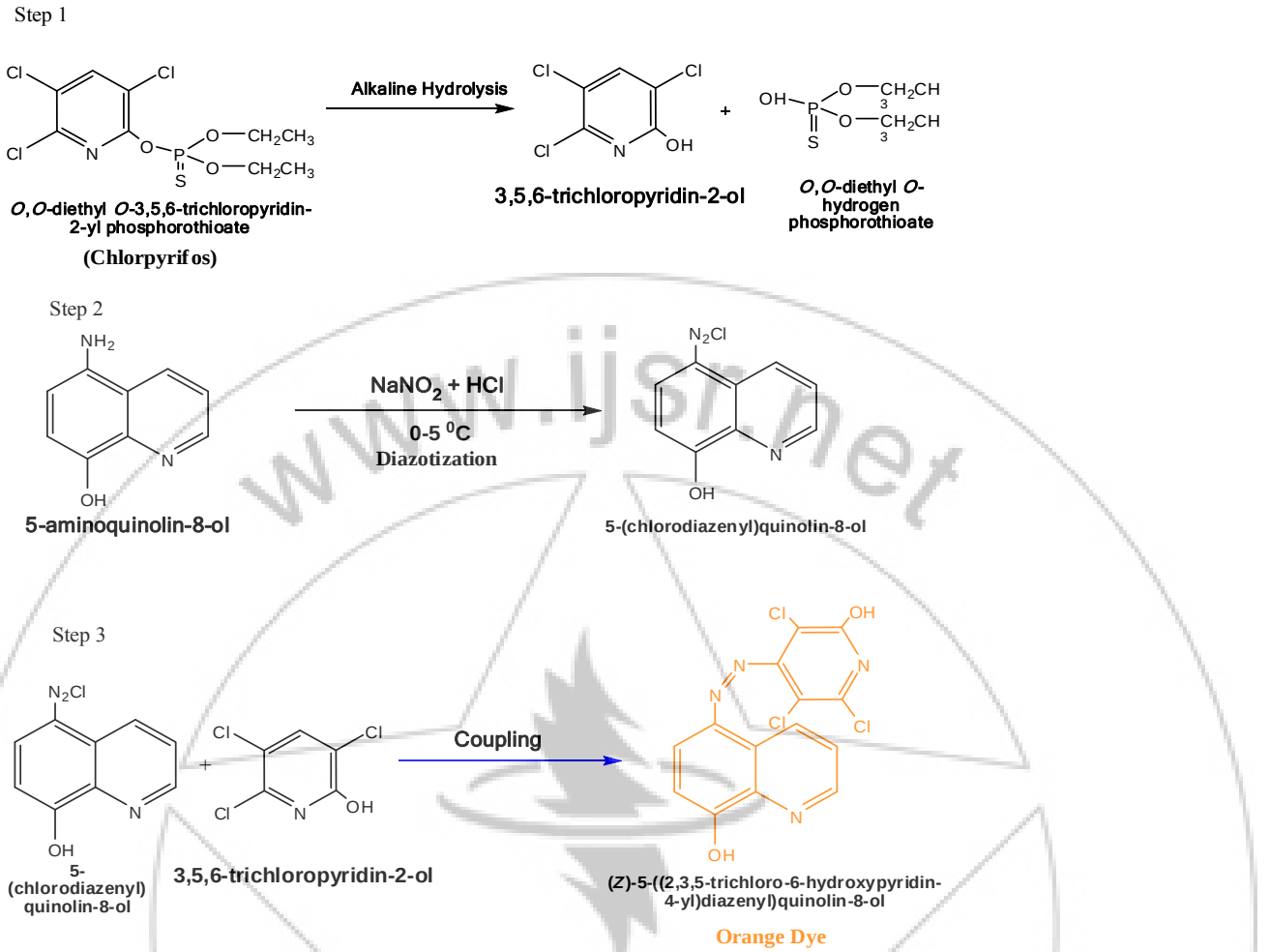
of 2% sodium nitrite was added and the solution was kept in a brown bottle. This remained stable for 4 hr when kept at 0-5 ^{o}C.

2.3 Preparation of Analytical Curve

An aliquot of test solution containing 1 to 8 µg in 10 mL of chlorpyrifos was taken in a 25 mL graduated tube and 10 mL of 8 mole L^{-1} sodium hydroxide was added to it. The solution was then heated for 3 minutes for complete hydrolysis. Then, 1 mL of diazotized 2-amino- 8-hydroxy

deproteination with trichloroacetic acid was done and analyzed after applying the described process. Three replicate analysis were done and given in Table-2.

Reactionmechanism



Scheme 1: Colour reaction. The colour reaction involves the following steps: Coupling of diazotized 2-amino-8-hydroxy quinoline with 1, 2, 4-trichloropyrdine (TCP) to form orange dye showing a λ_{max} at 420 nm.

3. Results and Discussion

3.1 Spectral Characteristics

The orange color dye formed in the proposed reaction shows maximum absorption at 420 nm. All spectral analysis was carried with double distilled water as the reagent blank showed negligible absorption at this wavelength. The color system obeys beer's law in the range of 1µg to 8 µg in a final solution of 10mL at 420 nm. Fig 2 shows absorbance and concentration of chlorpyrifos ranging from 1 to 8 µg per 10 mL. The molar absorptivity, Sandell's sensitivity and correlation coefficient, were found to be 9.04 $\times 10^9$ mol^{-1} cm^{-1}, 0.010 µ g cm^{-2} and 0.979 respectively.

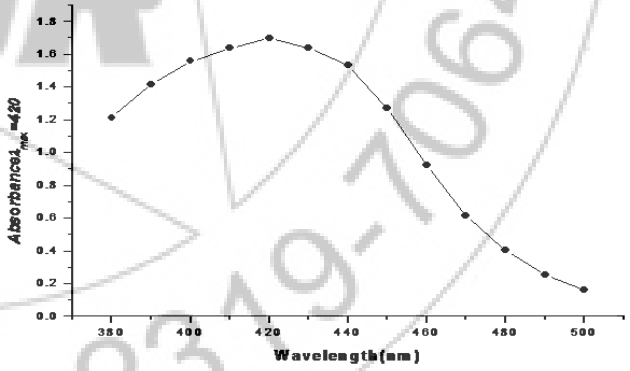


Figure 1: Absorbance curve of chlorpyrifos drawn between wavelength and Absorbance. λ_{max} obtained is 420 nm of concentration 10 µg.

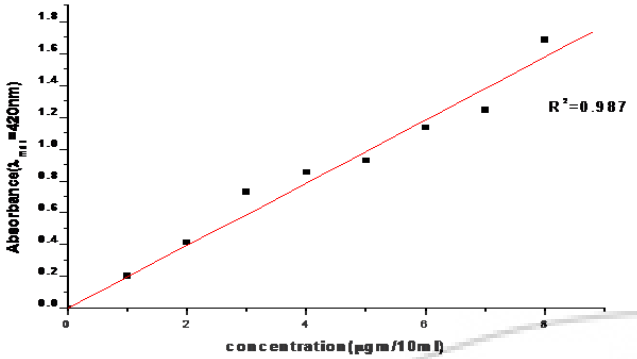


Figure 2: Calibration curve is plotted between absorbance and concentration of chlorpyrifos ranging from 1 to 8 µg per 10 mL.

3.2 Effect of pH

The effect of pH on the color reaction was studied and was found constant absorbance value obtained at a pH range of 10.5- 12 and no buffer solution was needed to stabilize the color. At pH lower and higher than this, the absorbance values decreased. It was observed that about to 10min we required for complete color development and the color remained stable for several days.

3.3 Effect of Temperature

Maximum color intensity was observed when the solution containing orange dye was observed at different temperature. The dye shows maximum absorbance at 45 ^{o}C and then gradually decreases is shown in Fig 3. The color remains at room temperature for 4days.

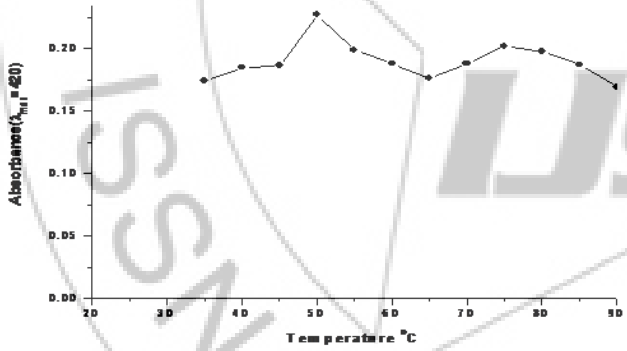


Figure 3: Effect of temperature upon the dye formed for determination of chlorpyrifos is shown with the graph plotted between absorbance and temperature in ^{o}C.

3.4 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 2 µg 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-1).

Table 1: Effect of Foreign Species i.e metal ion, and pesticide (Concentration of chlorpyrifos 2 µg in 10 mL)

Foreign	*Tolerance*
Cypermethrin	500
Paraquat	250
Dichlorvos	500
Glyphosphate	300
Zn^{2+}	400
Cu^{2+}	250
Pb^{2+}	500
Fe^{2+}	300

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

3.5 Precision

The precision of the method was checked by determining 2 µg of chlorpyrifos in 10 mL of final solution over a period of 7days. The standard deviation and relative standard deviation of absorbance values were 0.0811 and 2.45% respectively.

3.6 Application

The proposed method was applied satisfactorily, for the determination of chlorpyrifos in various samples of polluted water, vegetables, fruits and biological fluids. The amount of chlorpyrifos found in various matrix i.e. water, rice, soil, spinach, brinjal, coriander, orange, were 1.25-1.54 µg in 5g, 3.64-3.79 µg in 5 g, 2.23-2.48 µg in 5g, 3.81-4.02 in 5g collected from field using chlorpyrifos, 4.62-4.87 µg in 5 g, 3.15-3.52 µg in 5g, 2.10-2.38 µg in 5g, 1.01-1.27 µg in 5 g purchased from market respectively.(Table-2) and (Table-3).

Table 2: Chlorpyrifos,in the biological samples (urine and blood)

Samples		*Amount of Chlorpyrifos added(µg)*		*Chlorpyrifos Found** (µg)*		*Recovery %*	
		X	*Y*	*X*	*Y*	*X*	*Y*
Urine	A	1	1	0.94	0.96	94	96
	B	2	2	1.68	1.83	84	91
Blood	A	1	1	0.91	0.93	91	93
	B	2	2	1.83	1.88	91.5	94

X,Y= Samples added .
*Mean of three replicate analyses.
**In µg 10 mL^{-1}
*** Amount of biological Samples=5 mL, after treatment as described in procedure section.

Table 3: Recovery of Chlorpyrifos in various environmental and agricultural samples

Samples	*Chlorpyrifos originally found*(µg) A*	*Chlorpyrifos added (µg) solution prepared in 250ml. B*	*Total Chlorpyrifos found by proposed method**** C*	*Difference (d =c-a)*	*Recovery % (d/b x 100)*
Polluted water**	1.25	1	2.17	0.92	92
	1.54	2	3.43	1.89	94
Rice***	3.64	1	4.49	0.85	85
	3.79	2	5.71	1.92	96
Soil***	2.23	1	3.18	0.95	95
	2.48	2	4.34	1.86	93
Spinach***	3.81	1	4.72	0.91	91
	4.02	2	5.96	1.94	96
Brinjal***	4.62	1	5.57	0.95	95
	4.87	2	6.51	1.64	82
Coriander***	3.15	1	4.07	0.92	92
	3.52	2	5.25	1.73	86
Apple***	2.10	1	3.01	0.91	91
	2.38	2	4.25	1.87	93
Orange***	1.01	1	1.97	0.96	96
	1.27	2	3.23	1.96	98

*Mean of three replicate analysis.** Water sample 5 mL; after treatment 5 mL aliquot was analyzed. *** Sample 5 g (Rice, Soil, Palak, Coriander taken from agriculture field and Brinjal, Apple, Orange purchased from market, 5 mL aliquot of sample was analyzed after treatment as described in procedure section.)
**** In µg per 10 mL

Table 4: Comparison with various extractive spectrophotometric methods

Method	*Reference*	*λmax(nm)*	*Beer's Law range detection limit (ppm)*	*Remark*
Anthranilic acid	(19)	450	0.5-8.18	Less sensitive
Congored	(20)	605	0.5-5.7	Toxic reagent
p-amino benzoic acid	(21)	520	0.048-0.72	Less sensitive
2-amino8-hydroxy quinoline (Proposed method)		420	0.01-0.08	Highly sensitive, selective.

4. Conclusion

The present method is sensitive, selective and cheaper spectrophotometric method for determination of chlorpyrifos in various environmental and biological samples requiring no extraction steps and thereby avoiding the toxic organic solvents.

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