

A New Spectrophotometric Method for the Determination of Isoprothiolane in Environmental Samples

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Abstract: A new spectrophotometric method for the determination of fungicide isoprothiolane. The method is based on the bromination of isoprothiolane to form dibromoisoprothiolane which react with Potassium iodide-Potassium iodate mixture in the presence of leucocrystal violet (LCV) to form a violet colored complex which is soluble in 30% alcohol. The complex shows maximum absorbance at 600 nm. Beer's law obeyed over the concentration range of 5.0-25 µg in a final solution volume of 10 ml. The molar absorptivity of the colored system is $4.6 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and Sendell's sensitivity is $2.0 \times 10^{-3} \text{ µg cm}^{-2}$. The reproducibility assessed by carrying out seven days replicate analysis of a solution containing 20 µg of isoprothiolane in a final solution volume of 10 ml. The standard deviation and relative standard deviation for the absorbance value were found to be $\pm 1.33 \times 10^{-4}$ and $4.1 \times 10^{-2}\%$ respectively. The proposed method is free from the interference of other toxicants. The analytical parameters were optimized and the method was applied to the determination of isoprothiolane in water, food and human sample.

Keywords:- Spectrophotometry, Isoprothiolane, Bromination, Leucocrystal violet, Violet Dye.

1. Introduction

Isoprothiolane (diisopropyl 1, 3, - dithiolane-2 - ylidene malonate, trade name - fujion) a related dithiolane fungicide, belong to organosulphur group of compound is also used as an insecticide (1). These are mainly used to control rise blast disease and to reduce the population of brown plant hopper and leaf hopper in rice plants (2). The LD₅₀ was estimated to be between 300 and 2000 mg/kg. On rice plants it is used to control the following fungi: *Pyricularia oryzae*, *Helminthosporium sigmoideum*, *Fusarium nivale*. It is also used on rice to control *Delphacidae* as well as to accelerate the rooting (3). This fungicide has adverse effects on humans resulting in eye irritation, serious eye damage and severe acute toxicity. Isoprothiolane residues in water and soil are of concern as their uptake can lead to the accumulation of primary products (4), and results in the toxic effect to the non target species such as humans through food chain (2).

Because of the wide applicability and toxicity of isoprothiolane, numerous instrumental method have been described for the detection/determination of isoprothiolane such as gas chromatography (5) and high performance liquid chromatography (6) have also been reported.

2. Experimental

2.1 Apparatus

A Systronics UV-Vis spectrophotometer model – 104 with matched silica cell was used for all spectral measurements. A Systronic pH meter model – 335 was used for pH measurements.

2.2 Reagents

All reagents used were of Anala. R. grade and Double Distilled water was used throughout. Isoprothiolane (Rallis

India Limited) A stock solution of 1 ppm isoprothiolane is prepared in 30% alcohol. Working standard solution was prepared by appropriate dilution of stock standard solution with 30 % alcohol.

- **Bromine Water** – A Saturated solution of bromine in water was prepared. This solution was prepared daily.
- **Formic Acid**- 90% solution was prepared.
- **Potassium iodide**-Potassium iodate mixture: prepared by mixing 0.1 mol L⁻¹ Potassium iodide and 0.2 mol L⁻¹ Potassium iodate in 5:1 ratio (7).
- **Leuco crystal violet (Sigma- Aldrich S. Germany)** – Leuco crystal violet (LCV) was prepared adding to a 1 Liter volumetric flask 200 ml of water, 3 mL of 85% phosphoric acid and 250 mg of Leuco crystal (4,4',4''-methylidynetris, N,N', - dimethyl aniline) (CH[C₆H₄N(CH₃)₂]₃). It was shaken gently until the dye gets dissolved. The content of the flask was then diluted to 1 Liter with water (8).

2.3 Procedures

An aliquot of the test solution containing 5.0 to 25 µg of isoprothiolane was taken in a 10 mL graduated tube and to it 0.5 ml bromine water was added and shakes well for 10 minutes. Then add 2 drops of formic acid to remove excess of bromine. Then 0.5 mL Potassium iodide-Potassium iodate mixture and 1mL Leuco crystal violet was added and leave for 15 min for complete color development. A violet dye obtained. The solution was then diluted with 30 % alcohol and absorbance was measured at 600 nm against a reagent blank.

3. Result and Discussion

3.1 Spectral characteristics

The absorption spectrum of violet color dye shows maximum absorbance at 600 nm. The reagent blank had

negligible absorbance at this wavelength. fig (1). All spectral measurements carried out against double distilled water as the reagent blank shows negligible absorption at this wavelength. The color system obeys the Beer's law in the range of 0.5 to 25 μg of isoprothiolane in 10 mL of final solution at 600 nm. The molar absorptivity and Sandell's sensitivity were found to be $4.6 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $2.0 \times 10^{-3} \mu\text{g cm}^{-2}$ respectively.

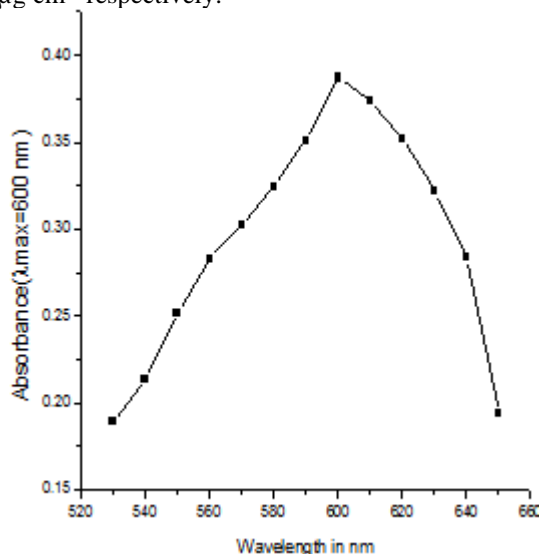


Figure 1: Absorbance Curve of the Isoprothiolane

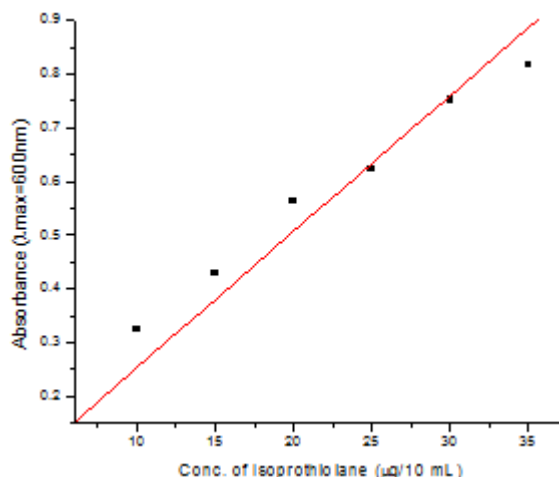


Figure 2: Calibration curve for the determination of Isoprothiolane

3.2 Optimization of Condition

Bromination of isoprothiolane to dibromoisoprothiolane was studied at different temperature and acidity. It was observed that acidic condition was required for the bromination. Maximum bromination was observed with saturated solution of bromine in water at a temperature of 25-30°C as it gave maximum absorbance values, good stability and quantitative results. It was observed that 1 mL of Leuco crystal violet was sufficient for complete color reaction.

3.3 Effect of Temperature and pH

It was found that 45 minute was required for full color development; the color was stable for several days. The maximum absorbance of the dye was obtained at 30°C. At

higher temperatures the absorbance value increased (fig 3). The effect of pH on the color reaction was studied, and it was found that constant absorbance values were obtained at the pH range of ~ 5 – 6 and no buffer solution was required to stabilize the color. At lower pH absorbance values decreased and higher pH absorbance value increased (fig. 3). Precision of the method was checked by the replicate analysis of working standard solution containing 20 $\mu\text{g mL}^{-1}$ of isoprothiolane in 10 mL final solution over a period of 7 days. The standard deviation and relative standard deviation for the absorbance value were found to be $\pm 1.33 \times 10^{-4}$ and $4.1 \times 10^{-2}\%$ respectively.

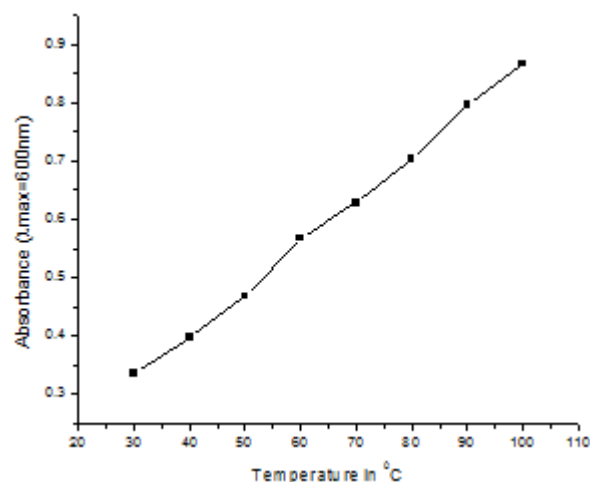


Figure 3: Effect of Temperature

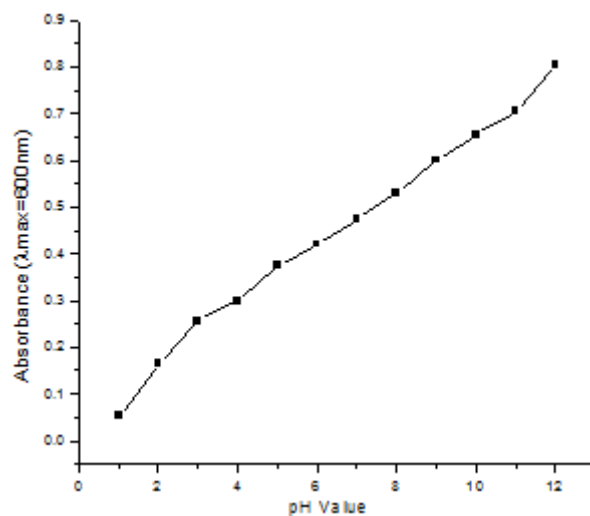


Figure 4: Effect of pH

3.4 Effect of foreign species

The effect of common foreign species and pesticides were studied to assess the validity of the method. Known amount of foreign species and pesticides were added to the standard solution containing 10 μg of isoprothiolane prior to bromination, and the solution was analysed by the proposed method. The method was found to be free from interferences of most of the foreign species and pesticides (Table-1).

Table 1: Effect of foreign species and pesticides

Foreign Species	Tolerance Limit $\mu\text{g mL}^{-1}$	Foreign Species	Tolerance Limit $\mu\text{g mL}^{-1}$
Glyphosat	1500	SO_4^{2-}	2000
Phoaphamidon	1200	Fe^{++}	1300
Bifenenthrin, Acetamiprid, Benzene	1000	Zn^{++}	900
Butachlor	900	Cu^{++}	500
Pryridine	500	Mg^{++}	300
Profenofos	400		

3.5 Application

3.6 Determination of Isoprothiolane in Water Sample

River water samples, receiving run-off water from agricultural fields sprayed with isoprothiolane, were

collected. These samples were extracted with 2×25 mL portion of diethyl ether. The ether solution was evaporated to dryness, and the residue was dissolved in 50 mL of ethanol. Aliquots were then analyzed as described above.

3.7 Determination of Isoprothiolane in soil and vegetables

Various samples such as soil, Potato, apple and rice were collected from the field where isoprothiolane was used as fungicide. The sample was weighted (50 g), crushed and extracted with 2×25 mL portion of diethyl ether. The ether solution was evaporated to dryness, and the residue was dissolved in 50 mL of ethanol. Aliquots were then analyzed as described above (Table-2).

Table 2: Determination of Isoprothiolane in various environmental samples

Sample	Isoprothiolane originally found	Isoprothiolane added (μg) B	Total isoprothiolane found by proposed method (c)	Difference (c - a)	Recovery $\frac{c-a}{b} \times 100$
	Proposed method (μg) A				
Water	2.93	2.5	4.82	2.46	98.40
Soil	2.12	2.25	4.54	2.42	96.80
Potato	2.57	4.0	6.68	3.91	97.70
Rice	2.72	4.0	6.67	3.96	98.72
Apple	3.8	2.5	6.27	2.47	98.80

4. Conclusion

The present method is Cheapest and more sensitive for the determination of isoprothiolane. The advantage to the proposed method is mainly its sensitivity, simplicity and higher stability of the colored solution. The proposed method is successfully applied for the determination of isoprothiolane in water, soil, fruit, vegetables and biological sample.

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