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New Chromogenic Spray Reagent for Thin Layer Chromatographic Detection of Organophosphorus Fungicide.

*Keshav S. Kapgate[1,2], Charansing B Ghoti[2], Priyadarshani N Deshmukh[1], V. J. Thakare[2]

[1]Department of Chemistry, Shivaji Science College Nagpur Maharashtra 440012.

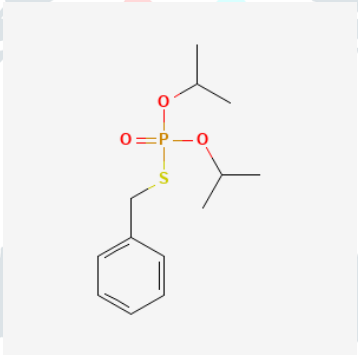
[2]Regional Forensic Science Laboratory Nagpur Maharashtra 440012.

Abstract: Thin layer chromatography(TLC) is a simple, rapid and reliable technique usually used in forensic science laboratory for detection of poison in biological material. It can separate many complex mixtures in a short period of time. In this study an effort has been taken to determine Kitazin organophosphorus fungicide (iprobenfos) from biological samples by using Thin Layer chromatography. A chromogenic reagent cobalt thiocyanate has been used for the detection of Kitazin (iprobenfos) with Hexane :Acetone (8:2) as a solvent system for the separation. a blue coloured co-ordination complex formed.

Keywords: Thin Layer Chromatography, Kitazin, Cobalt Thiocyanate, Spray Reagent

Introduction: Organophosphorus pesticides with insecticide, herbicide and fungicide are applied in very high quantities in world wide and pose a serious threat both to the environment and human health. Pesticide usage has increased to fulfil agricultural demand. Pesticides such as organophosphorus pesticides (OPPs) are ubiquitous in world food production. Their widespread usage has un- avoidable detrimental consequences for humans, wildlife, water, and soil environments. Hence, the development of more convenient and efficient pesticide residue (PR) detection methods is of paramount importance. Organophosphorus fungicide were introduced in the mid to late 1960 to prevent crop losses caused by phytopathogenic fungi[1]. Iprobenfos(KITAZIN) is a broad spectrum , highly effective systemic Fungicide with curative and protectant action for the management of disease caused by a wide range of Fungi on Fruits, Vegetables and Field Crops. Iprobenfos is an organic thiophosphate that is the S-benzyl O,O-diisopropyl ester of phosphorothioic acid with Molecular Weight 288.34 and chemical Molecular Formula C13H21O3PS. It is used as a rice fungicide to control leaf and ear blast, stem rot and sheath blight. It has a role as a phospholipid biosynthesis inhibitor and an antifungal agrochemical. The sensitive detection of iprobenfos (IBF) and edifenphos (EDI) was successfully conducted by using a new aptamer-based colorimetric multi-aptasensor[2]. Visible light-induced catalytic degradation of iprobenfos fungicide was investigated using poly(3-octylthiophene-2,5-diyl) (POTh) film by varying oxygen pressure of bubbling gases[3]. The fluorescence behaviours of poly(3-octylthiophene-2,5-diyl) (POTh) containing photosensitizers such as perylene (Per) and 9-cyanoanthracene (CNA) and the degradation of iprobenfos fungicide by the POTh film

blended with Per were investigated[4]. Differential gene expression profiling was performed in the hepatic tissue of marine medaka fish (Oryzias javanicus) after exposure to an organophosphorus pesticide (OPP), Iprobenfos (IBP), a widely used pesticide in agri- and fish-culture, by heterologous hybridization using a medaka cDNA microarray[5] . Optimum HPTLC separation of ditalimfos (D), edifenfos (E), and tolclofos-methyl (TM), three organophosphorus fungicides (OPF), has been achieved on silica gel 60F_{254} plates with n-hexane-acetone 9:1 (v/v) as mobile phase[6]. Eliasu Issaka et.al. studied[7] Advanced visual sensing techniques for on-site detection of pesticide residue in water environments. Although there are many instrumental techniques for qualitative and quantitative analysis of pesticides and drugs viz. HPLC, IR, NMR, the Thin Layer Chromatography (TLC) is an easy, fast and cost effective technique.It is seen that it is challenging to detect and identify pesticides from post mortem visceral tissues in crime laboratories. There is still a scope for development of new solvent systems and visualising reagents for newly formulated pesticides. people try to use pesticide for suicidal or homicidal purpose under extreme conditions as easily available in farmer house. One of such case came in forensic laboratory along with viscera for poison detection. It was challenging because no Spray Reagent was known in literature review for Iprobenfos . very few studies are available for organophosphorus fungicide. We used cobalt thiocyanate spray reagent for detection of Iprobenfos in biological samples first time and we got good results for this reagent for iprobenfos .



Iprobenfos(Kitazin) structure

Experimental:

Chemicals, reagents and solutions:

All reagents used were of analytical-reagent grade. Standard Iprobenfos(Kitazin) (PI Industries Ltd) solution was prepared in dichloromethane. 1 gram Cobalt chloride and 3 grams Ammonium thiocyanate in 20 ml distilled water are used for spraying .

Dichloromethane is used for extraction of poison.

Extraction of Iprobenfos (Kitazin) from biological Materials:

A portion of about 100 g each of different types of biological tissues (pieces of stomach, intestine, liver, spleen, lungs and kidneys) containing Kitazin was taken. Viscera were cut into fine pieces and minced carefully, 100 ml of Dichloromethane was added to the homogenised visceral sample. The solvent was vigorously mixed with viscera and kept for about 3 hours in separating funnel and then the organic layer liquid was separated and filtered out using whatman filter paper. The extract was transferred to an

evaporating dish and the liquid portion was evaporated. The residue was dissolved in 1 ml DCM and the solution was used for spotting.

Thin Layer Chromatography:

Chromatography was performed on pre-coated Aluminium TLC plate (silica gel 60 F 254, Merck Ltd. Darmstadt. Germany)for detection of Kitazin. The extract of blank viscera and Kitazin containing viscera were spotted on TLC plate along with the spot of Kitazin standard with fine capillary tubes. The plate was dried and developed in a presaturated tank containing Hexane: Acetone (8:2) as a solvent system. After development the plate was removed from the chamber, dried at room temperature. The plate was kept in UV Chamber at 254 nm, bluish coloured spots were observed under UV light as shown in **Figure 2**) and then sprayed with cobalt thiocyanate reagent. After spraying the plate was kept in the air. A Blue colour spot observed at RF = 0.63 (**Figure 1**)

Result and Discussion:

Kitazin is Systemic fungicide which reacts with Cobalt thiocyanate reagent gives blue colour (Figure 1). The posible coloured spot formed may be six centre coordination complex of the Iprobenfos and the central metal cobalt of the reagent as shown in **figure no. 3.**The colour of the spot remains stable. This spray reagent is highly sensitive, stable, easily available and specific for the detection of Kitazin from biological material. Spots were not observed for Endosulfan (Organochlorine insecticide), organophosphorus insecticides dimethoate, phorate, monocrotophos, triazophos, and quinal-phos, pyrethroid Insecticides Fenvalerate, Cypermethrin, Deltamethrin. This spray method is economic, reproducible and does not involve in any critical reaction condition. This reagent can also be used for the quantitative estimation of Kitazin in biological samples. Hence, this reagent can be used routinely for detection of organophosphorus fungicide Iprobenfos (Kitazin). The possible mechanism of reaction of colour spot formed are shown in **Figure No.(3)**.

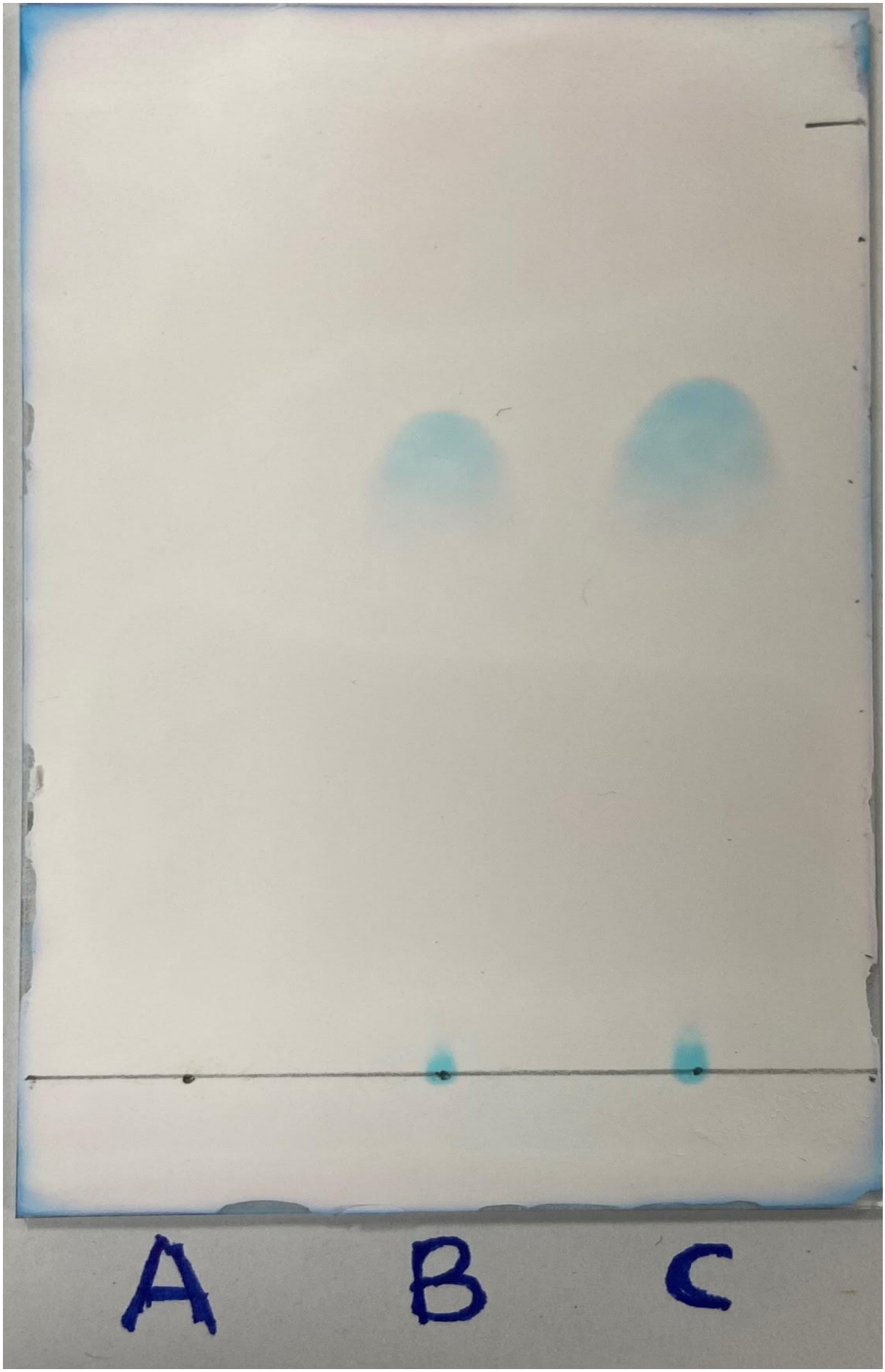


Figure 1. TLC showing spot of Kitazin. A) Blank Viscera B)Kitazin poisoning Viscera Extract C)Kitazin standard

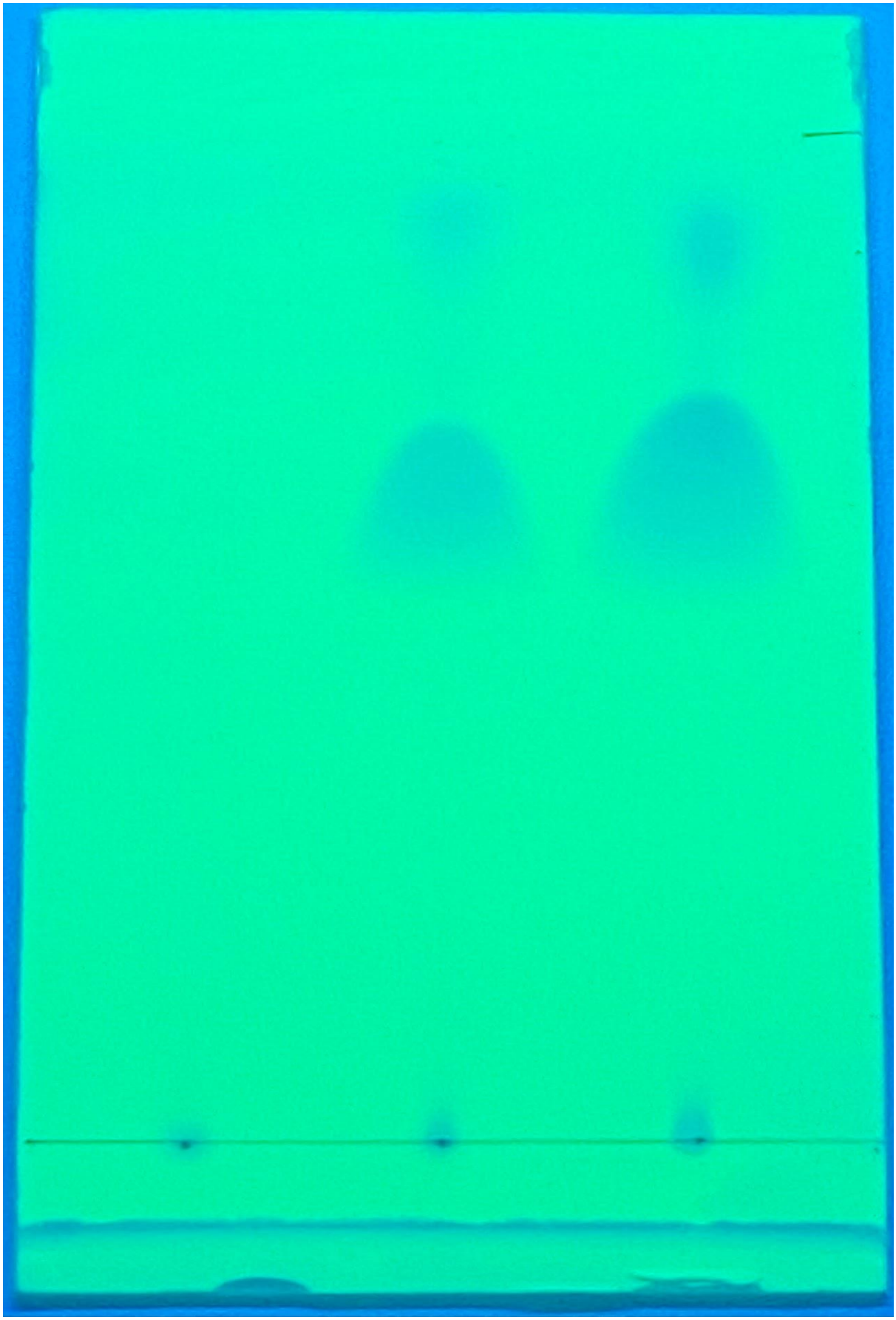
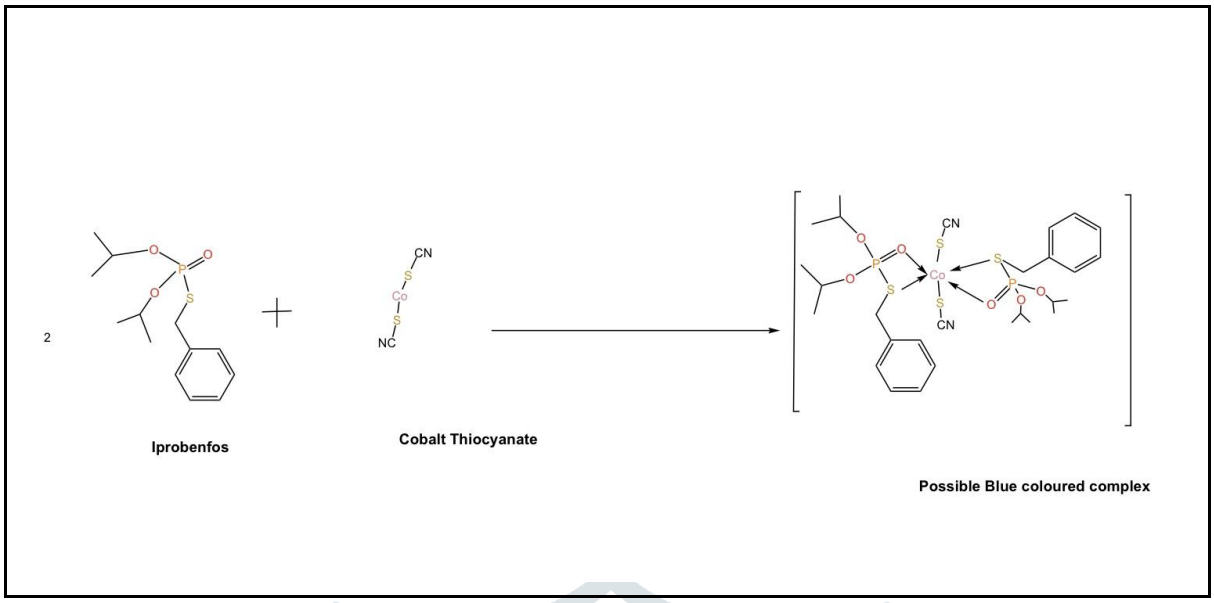


Figure2. TLC showing spot of Kitazin under UV light 254 nm.
A) Blank Viscera B)Kitazin poisoning Viscera Extract C)Kitazin standard

Figure No.(3): Possible mechanism of reaction of coloured spot



Conclusion

To the best of our knowledge, cobalt thiocyanate reagent was used the first time for the detection and identification of Iprobenfos(Kitazin) in post-mortem samples (in fatal poisoning cases of kitazin). The proposed reagent is simple, sensitive, and can be used for routine analysis of oganophoshorous fungicide Iprobenfos. Further this case will become reference for such types of cases received in the laboratory in future.

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