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Original Research Article

Detection of Chlorpyrifos in human blood using thin layer chromatography

Sneha Yadav^{1,2,*}¹National Institute of Criminology and Forensic Science (LNJN), Rohini, New Delhi, India²Dept. of Forensic Science, Galgotias University, Greater Noida, Uttar Pradesh, India

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ABSTRACT

Chlorpyrifos is considered to be a broad-spectrum pesticide. It is a most commonly used kind of pesticides for killing of variety of insects, pest's worms in agricultural sector. It is easily available in market and is highly toxic in nature. The suicidal cases, accidental cases due to poisoning of chlorpyrifos have been reported in large number due to easy to access for this pesticide. Even though many instrumental techniques like UV, HPLC are available for its identification but the analysis cost is high. In this paper we have detected the chlorpyrifos in human blood by using TLC (Thin Layer Chromatography) using different types of solvent system. This technique is fast, reliable, cost effective and less expensive than other sophisticated techniques.

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1. Introduction

Chlorpyrifos is an organophosphate pesticide which is used to killing variety of insects, pests, worms in agricultural sector. The IUPAC name is O, O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate its chemical formula is $C_9H_{11}Cl_3NO_3PS$ with molar mass 350.57g/mol⁹(Eaton et al;2008).¹ It was introduced by dowed chemical company in 1965 specially targeting or controlling mosquitoes, flies many foliage crops pests, household pests and aquatic larvae (Rathod et al;2017).² The high use of chlorpyrifos results into poisoning to non-target species like human beings. It has been observed that in recent years the suicidal cases, accidental cases of poisoning have been increased but still due to being a broad-spectrum pesticide it cannot be eliminated from market (Ambali et al ;2007).³ It has been reported worldwide that chlorpyrifos causes 1000 deaths per year (Pawar et al;2015).⁴

The common names for chlorphyrifos are Brodon, Bolton Insecticide, chlorpyrifos –ethyl, Detmol UA, Dowco 179, Empire, Eradex, Nufos, Tricel, Lorsban, Hatchet etc.

* Corresponding author.

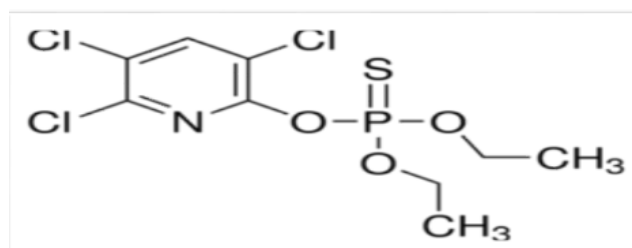
E-mail address: snehay2108@gmail.com (S. Yadav).

Fig. 1: Chemical structure

Chlorpyrifos falls under the category of organophosphate pesticides and is derived through thiophosphoric acid and is a type of neutral non-volatile poison (Racke et al; 1993).⁵ It is available in various formulation like liquids form commonly as sprays, wet table powders, in dry and flow able solutions and many more. The chlorpyrifos powder can be formed in many different colours from colourless crystals, gray crystals, yellow crystals, to various granular form. Mainly it smells like sulphur compounds and is practically insoluble in water and completely soluble in different organic solvents like acetone, ether. Due to the chlorine containing group in its structure the lipid solubility

and half-life in body increases as a result more slow and persistent lowering of enzyme cholinesterase level in body if compared to other thiophosphoric derived pesticides. It has been observed that there are cases of poisoning of chlorpyrifos reported mainly accidental or intentional or suicidal among them homicidal is the rarest form. And there are various factors responsible for effect of this pesticide on body which includes health of individual, various environmental conditions, dose, frequency. This chlorpyrifos works by inhibiting the activity of acetyl cholinesterase as a result it get converted into chlorpyrifos-oxon which is toxic than chlorpyrifos itself and act as respiratory poison and causes more damages to neurons and ganglia (Jung et al; 2009 and Srivastava et al;2011).^{6,7} After poisoning due to ingestion of chlorpyrifos it has been seen that its analysis in of very important for that purpose urine, blood sample is most commonly sent for toxicological analysis. In our study we have extracted the residues of chlorpyrifos from spiked blood sample and identified it the help of thin layer chromatography by using various mobile phases (Jaiswal et al; 2008).⁸

2. Materials and Methods

2.1. Material and reagent

Acetic acid, Acetone, hexane, Amyl alcohol, carbon tetrachloride, chloroform, formaldehyde, methanol, Ethanol absolute, Platinic chloride, Potassium Iodide, Methanol, Hydrochloric acid, Sodium sulphate anhydrous, Sodium Tungstate, sulphuric acid, sulphanilic acid all chemicals use are of Analytical grade /HPLC grade. Stoppered Borosil test tubes(10ml), Borosil test tubes (10ml), Borosil Beakers (250ml, 500ml), Glass rod, China dish, Glassgropper, Conical Flask (250ml), Glass Plate (10cmx10cm), Solvent Chamber, Silica crucible, Borosil Measuring Cylinder (10ml, 100ml), spray bottle(Atomiser). Chlorpyrifos purchased from local market, Blood sample collected from (Jaipur golden Hospital, Delhi)

2.2. Method

The standard stock solution of chlorpyrifos 10ppm was prepared by dissolving 0.01mg of chlorpyrifos in 10 ml of n-hexane. The blood sample solution was prepared by spiking 2mg of Chlorpyrifos in 10ml of human blood and keeping is aside for 24 hrs at 35 degree Celsius.

2.2.1. Preparation of Palladium chloride spraying reagent

Palladium chloride (prepared by 0.5g of Palladium chloride in 100ml of Distilled water containing few drops of (25% HCL).

2.3. Extraction of Pesticide from blood

The 10 ml of blood sample solution was mixed with 10 ml of 10 % sodium tungstate solution and 15 ml of sulphuric acid in a beaker and it is shaken for two minutes and then filtered. The filtrate was kept reserved, the residue was washed with 15 ml portions of 0.1 N sulphuric acid, the washings are collected, mixed with filtrate and then transferred into a separating funnel and then extracted thrice with 20ml portion of n hexane, later on the hexane layers are collected & combined and followed by passing it through anhydrous sodium sulphate and solvent is removed by passing a stream of air.

2.4. Thin layer chromatography

TLC Plates were activated at 110°C for about 25-30 min and then let it be cooled down at room temperature.

2.4.1. Spotting

The extract obtained through extraction is evaporated to half and then this extracted sample was spotted on TLC plates with the help of capillary tube, the spotting was done at 1 cm away from bottom of TLC plate, and the diameter of spot was kept small, one more spot of standard solution of chlorpyrifos was spotted on TLC plate at some distance from sample spot. The spots were allowed to dry for few minutes, at the sample spot, 10-15 times spotting was done by giving time for earlier spot to get dry.

2.4.2. Development

After spotting is completed the TLC plate is kept in Saturated Mobile solvent system in TLC developing chamber, here 9 different types of solvent system were used the plates were run in these solvent systems the plates were removed from chamber when the solvent run almost to the top of plate.

2.4.3. Visualization

The TLC plates were air dried for 5-10 min and then to make the spots visible the spraying reagent palladium chloride was sprayed. Yellow color spot were observed in both sample & standard. This reaction was instantaneous and colour formation was clearly distinct and was not faded out with time.

The R_f value is used in quantifying the movement of the material throughout the plate. R_f is equal to the distance travelled by substance divided by the distance travelled by the solvent, the value lies between 0-1. R_f value of chlorpyrifos extracted from blood was found neatly equal to that of standard used. The 9 different solvent systems were analyzed and found good to run the sample. The R_f values lies between (0.55-0.966). Ethyl Acetate: Cyclohexane (9:1) resulted in highest R_f value i.e. 0.96. Therefore according to highest R_f value it can be considered as good solvent system

Table 1: TLC results obtained with different solvent systems

S. No	Mobile solvent system	Ratio	R _f value Extracted sample	R _f value Standard sample	Colour of spot
1	Hexane: Acetic acid	9:1	0.57	0.599	Yellow
2	Ethyl acetate: Cyclo hexane	9:1	0.966	0.965	Yellow
3	Benzene: Hexane	8:2	0.866	0.855	Yellow
4	Benzene: Carbon tetrachloride	6:4	0.77	0.78	Yellow
5	Formaldehyde: Acetone	6:4	0.85	0.833	Yellow
6	Benzene: hexane	5:5	0.699	0.71	Yellow
7	Amyl alcohol: Cyclo hexane	9:1	0.955	0.977	Yellow
8	Amyl alcohol: chloroform	7:4	0.56	0.55	Yellow
9	Chloroform: Methanol	9:1	0.65	0.67	Yellow

in comparison to other 8 otherwise all 9 solvent system can be used for detection of chlorpyrifos. The calculated R_f value in 9 different solvent systems are summarize in Table 1.

3. Result & Discussion

The TLC of sample and standard solution performed and R_f values calculated which suggest that the R_f values are comparable and thus indicates that the R_f of standard matches with the R_f of sample which confirms that the chlorpyrifos was extracted out successfully and was able to detect by TLC with the help of 9 different solvents systems. Ethyl Acetate: Cyclohexane (9:1) resulted in highest R_f value i.e. 0.96. Therefore, according to highest R_f value it can be considered as good solvent system in comparison to other 8 otherwise all 9 solvent system can be used for detection of chlorpyrifos. The result suggest that the given Mobile phases can be used for detection of chlorpyrifos in blood samples.

4. Conclusion

The present work describes that the detection of chlorpyrifos extracted from blood can be done using TLC which is a less expensive, fast and reliable technique by using 9 different types of solvent system. The main advantage of using TLC is minimum sample preparation is needed and simultaneously many samples can be analyzed.

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6. Conflicts of Interest

All contributing authors declare no conflicts of interest.

7. Source of Funding

None.

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Author biography

Sneha Yadav, Assistant Professor

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