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RESEARCH ARTICLE

QUALITATIVE AND QUANTITATIVE ANALYSIS OF COMMON ORGANOPHOSPHOROUS PESTICIDES IN SPIKED BIOLOGICAL TISSUES USING SPOT TEST AND HPTLC-DENSITOGRAM TECHNIQUE

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ABSTRACT

Poisons like pesticides and insecticides are commonly encountered in suicidal/homicidal cases in India and abroad. Out of the large number of pesticides used for criminal activities Organophosphorous (OP) group of insecticides are largely reported in forensic toxicology cases. Qualitative and the quantitative analysis of the forensic toxicology samples are very important for the medico-legal cases to provide justice to the victims. In the present study we analyzed some of the quinolfos, chlorpyrifos & malathion insecticides in spiked biological tissues. For the qualitative analysis screening by colour/spot test & TLC was performed by using different solvent systems. Further quantitative analysis was done by using HPTLC-densitometer for the confirmatory results.

INTRODUCTION

The number of poisons used by the criminal have not only increased, but also turned complex. There are some newer poisons which are also come in used by the criminals for the homicidal /suicidal purposes (Pal *et al*., 2010). Deaths in suspicious circumstances are normally referred for postmortem and visceral organs/blood etc. is sent to forensic science laboratories for chemical analysis of poison, if any to ascertain the cause of death. There are number of factors which affect the analytical results and no standardized protocols for their identification in the biological samples (Teotia *et al*., 2009). Organophosphorous group of insecticides now become the most widely used insecticides available today. Organophosphorous group of insecticides are commonly used for the control of agricultural pests of the group of coleopteran, diptera, homoptera and lepidoptera in soil or foliage in a wide range of crops, including citrus, nuts crops, vegetables etc. insecticide poisons are also used for the control of house hold pests & mosquitoes. Organophosphates poison insects and mammals primarily by phosphorylation of the Acetylcholinesterase Enzyme (AChE) at nerve endings.

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Organophosphorous compound poisoning from occupational, accidental and intentional exposure is the global problem especially. Toxicity in developing countries (Jaiswal *et al*., 2001, 2006, 2008). may result from an intentional overdose of a substance. This may be due to self administration, with the intention of commit suicide. The victim will select the substances depending on availability and his knowledge about the possible fatal effect of substances. Intentional overdosing occurs in homicidal cases. The route and mode of administration are important aspects in such cases. Accidental and unintentional exposure to a substance is another important aspect. A drug or a substance may be consumed by mistake in place of another. Children are more likely to swallow drug/pesticides accidentally (WHO., 1997). The investigation of poisoning death is a common challenge faced by forensic toxicologists and police investigators. Here colour test, TLC & HPTLC techniques are discussed for the qualitative and quantitative analysis of common organophosphorous pesticides in spiked biological tissues.

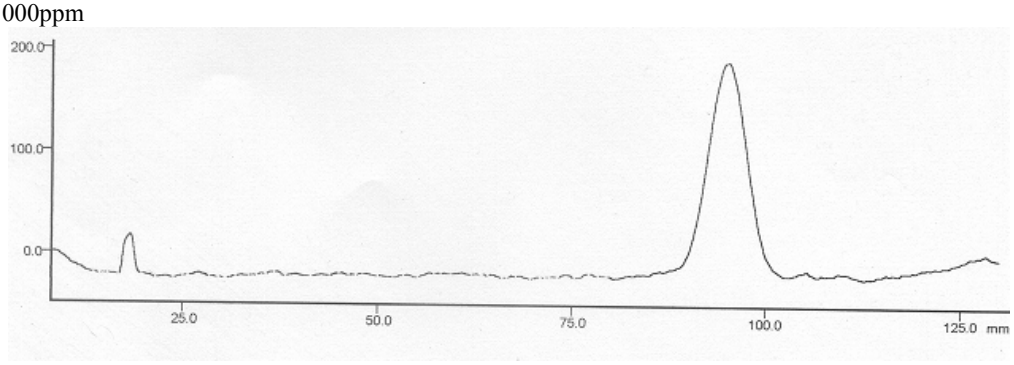
MATERIAL AND METHODS

Chemical

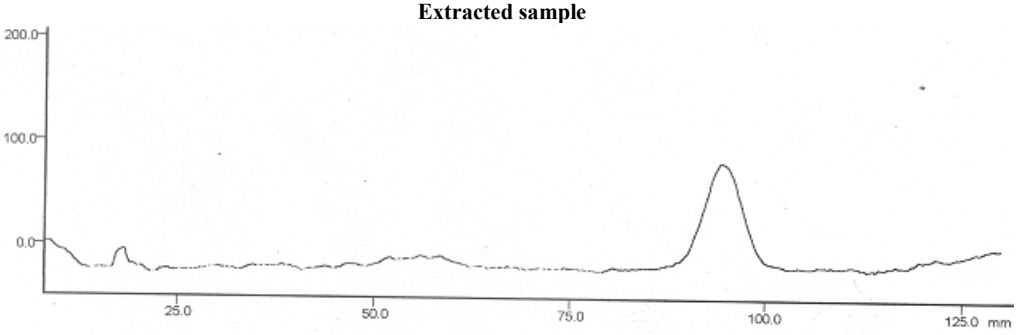
Silica gel G, methanol, ammonia (Glaxo India Ltd. Mumbai), chloroform (Merck Ltd. Mumbai), diethyl ether, ethanol

HPTLC
Densitogram of Chrorpyrifos pesticide

1000ppm

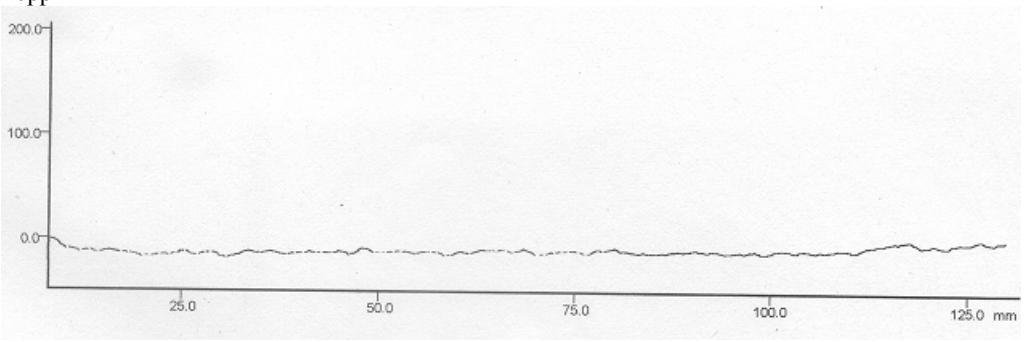


Extracted sample

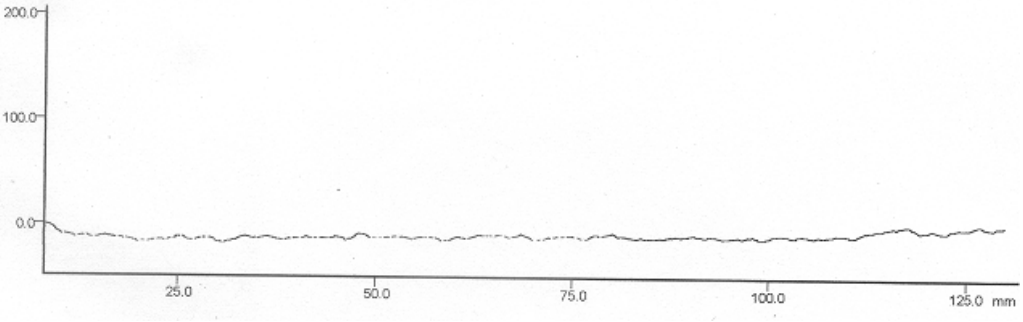


Chlorpyrifos

10ppm

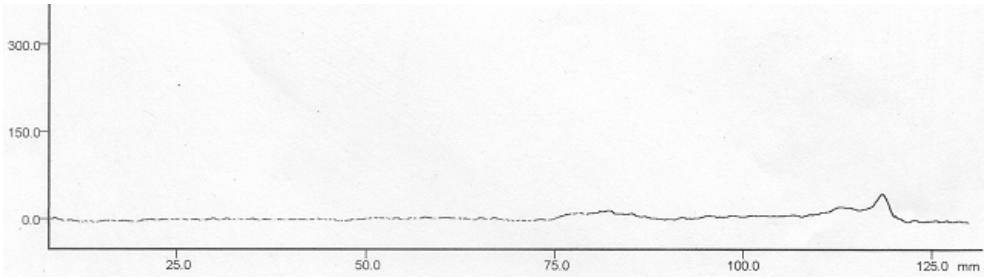


50ppm



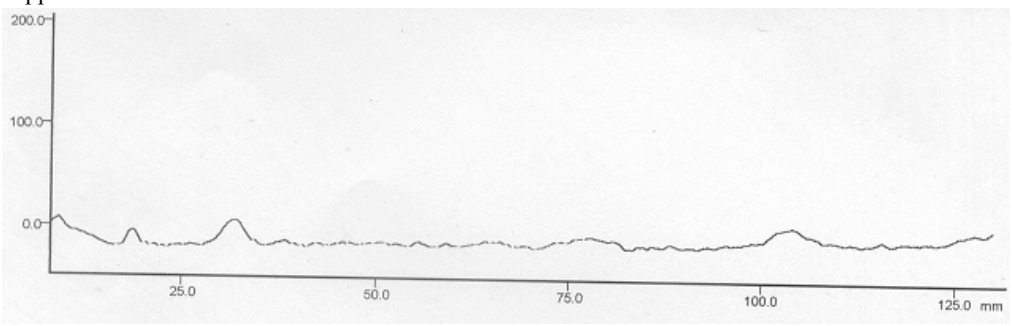
100ppm

Extracted sample from liver tissue of Malathion

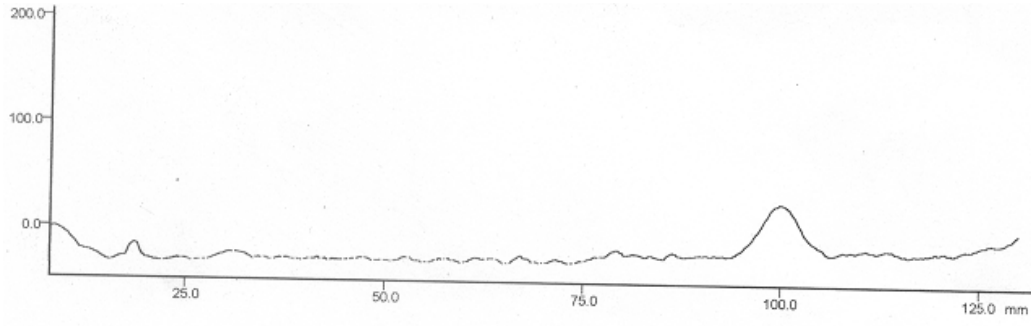


Quinolphos

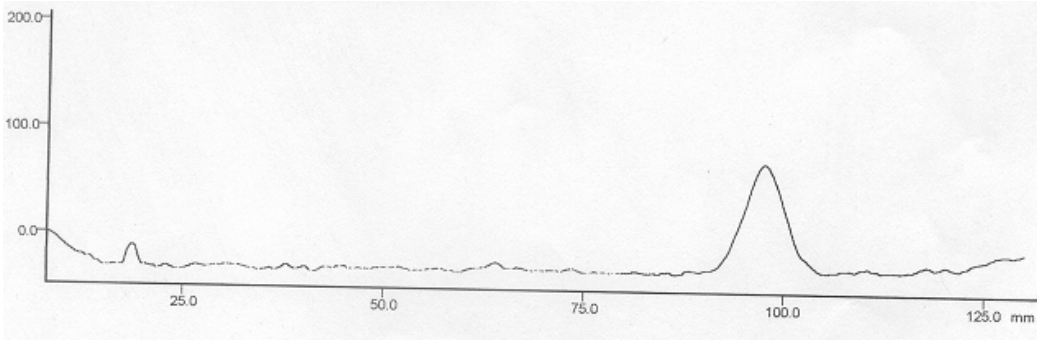
10ppm



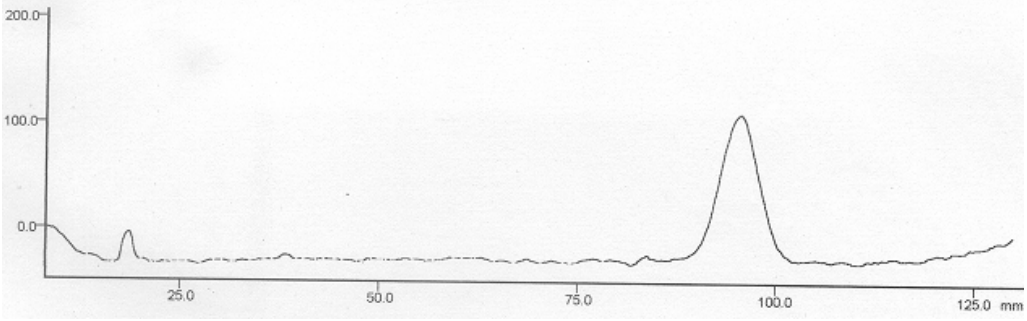
50ppm



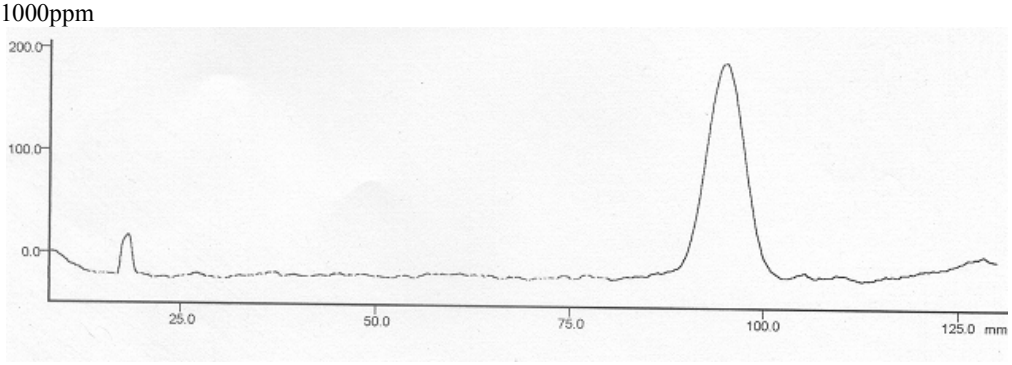
100ppm



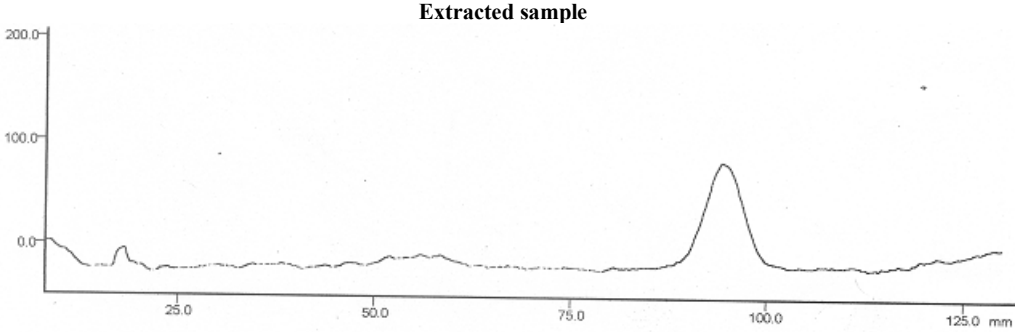
500ppm



1000ppm

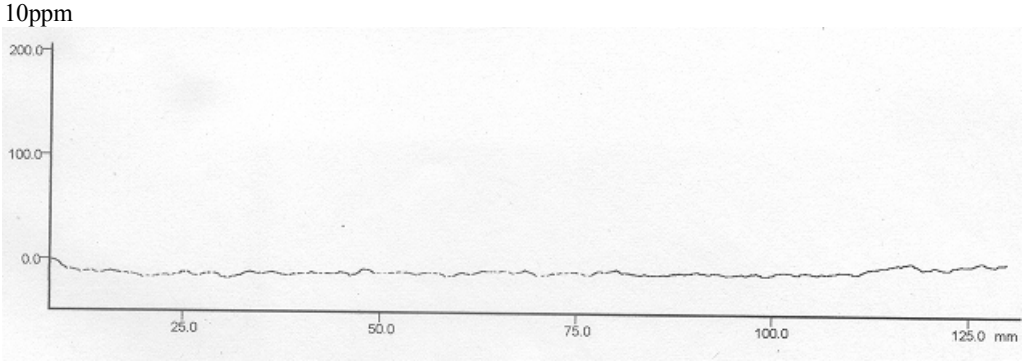


Extracted sample

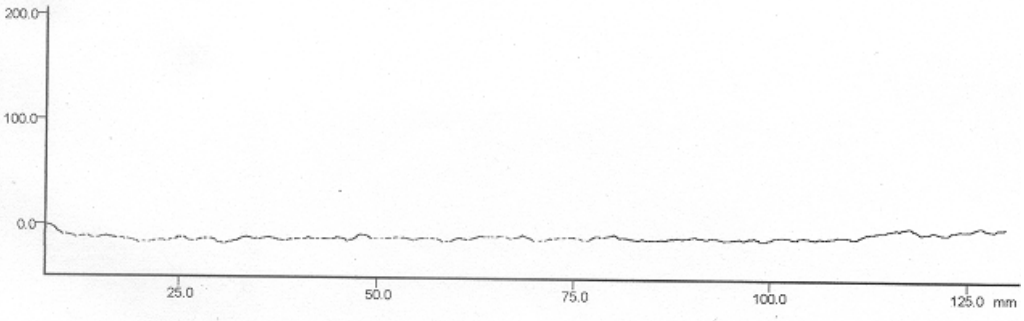


Chlorpyrifos

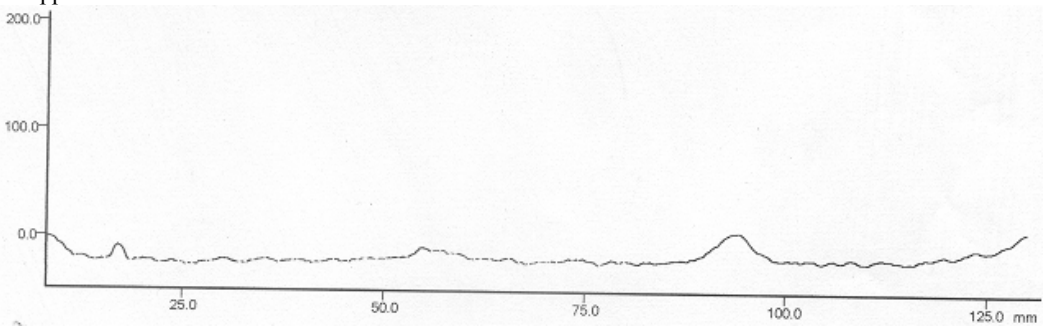
10ppm



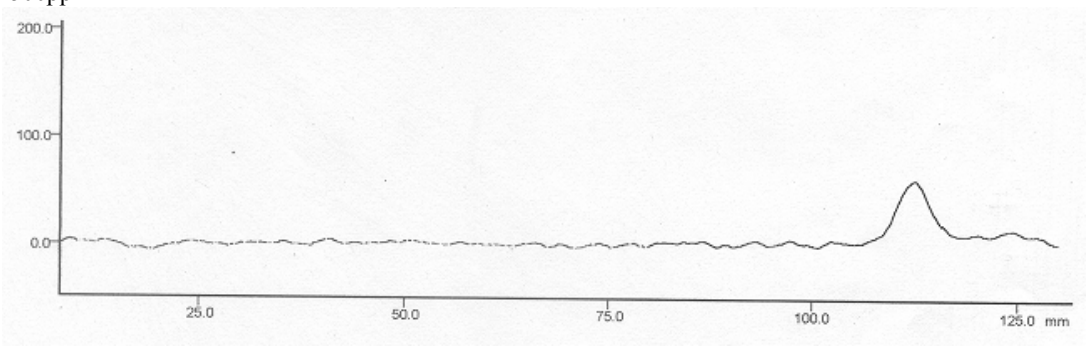
50ppm



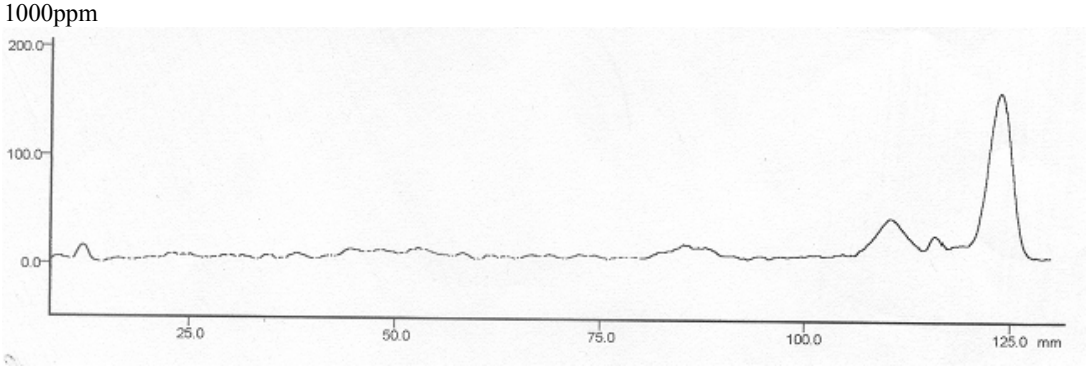
100ppm



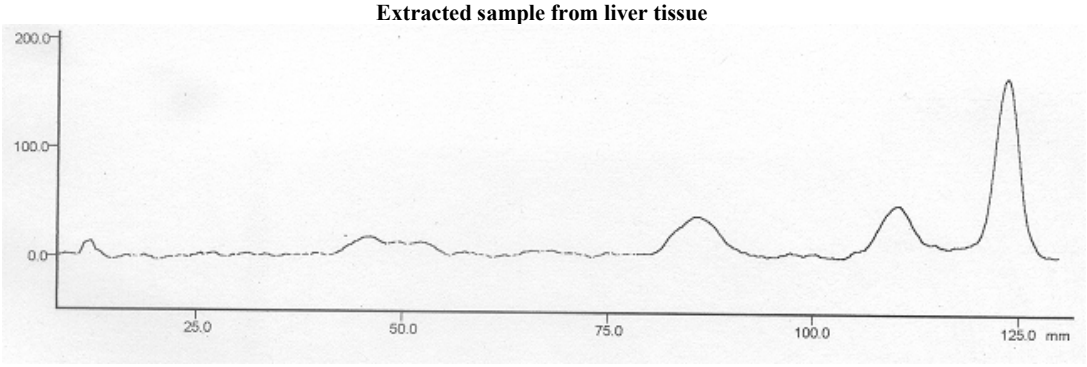
500ppm



1000ppm



Extracted sample from liver tissue



(Merck Germany), ethyl acetate, isobutanol, isopropanol, n-hexane, acetonitrile, diethylamine, alakaline, (galaxosmith cline pharmaceutical ltd Mumbai), cyclohexane, acetone, palladium chloride, dioxane (Merck), n-haxane, concentrated hydrochloric acid, sulphuric acid, anhydrous sodium sulphate, sodium tungustate (E. Merck India Limited). Palladium chloride, Mercurous Nitate.

Preparation of standard solution

The 50, 100, 500, & 1000 ppm solution of Chlorpyrifos, Quinolfos and Malathion was prepared by dissolving 0.1 gm of each insecticides in 100 ml of acetone separately for the preparation of 1000 ppm solution. 1 ml of 1000 ppm solution of each pesticide was used for the purpose of spiking of the 50 gm of biological tissues (goat liver).

Preparation of spraying reagent

0.5 gm of palladium chloride was dissolve in the 100 ml of distilled water and 2-3 drops of concentrated hydrochloric acid was added to it.

Pesticide extraction from viscera

Biological material viscera is macerated into fine slurry by mixing with equal amount of anhydrous sodium sulfate and transferred into the conical flask with an air condenser.50ml of the n-hexane is added to the flask and heated on hot water bath for one hour. The contents are cooled and filtered. The residue slurry is extracted twice with 25 ml portion of n-hexane. The filtered N-hexane portion are combined and taken into the separating funnel. This hexane layer is vigorously shaken with 15ml, 10ml and 10ml again portion of the acetonitrile, which are previously saturated with n-hexane. The acetonitrile layers are mixed and taken into another clean separating funnel and dilute 10 times with distilled water. 25ml of sodium sulfate solution is added to it and extracted thrice with 25ml portion of n-hexane. The n-hexane layers are combined concentrated to 5 ml by evaporating on water bath and 5gm of anhydrous sodium sulfate is added. The extract is evaporated as and when required for analysis.

Preparation of TLC plates

TLC plates were prepared by dissolving 25 gm of silica gel G in 50 ml of distilled water to make slurry. This slurry was

poured on the applicator and the applicator was then moved over the plate in one motion. Plates were allowed to dry at room temperature and then kept in hot air oven at 80 ºC for one hour.

Thin layer chromatography

Pre coated aluminum silica gel plate is used for the TLC. The extracted residue is dissolve in 1ml of n-hexane and an aliquot of it is spotted on silica gel G plate control spots of known organophosphorous pesticide (Std. sample) are also applied on the same TLC plate and spotted plate is developed in using different solvent system. The developed plate is dried and then sprayed with chromogenic reagent (0.5 % palladium chloride) after drying the plate is irradiated to long UV light (254) for ten minutes. The yellowish colour spot was obtained and Rf value was recorded. The results were tabulated and represented as Mean±SD

Spotting of the sample and standard on TLC plates

Poison extracted from the tissues was loaded on the TLC plates along with the standards with the appropriate marking. HPTLC plates were activated at 110 ºC for 30 minutes and then cool at room temperature before spotting. The spotting of the standard sample and extracted sample was done by using the HPTLC sample applicator on the HPTLC plate 20X20 cm. HPTLC plates was then placed in a chromatographic chamber containing different solvent systems (Table-1, 2 & 3). After development plate out was taken out of the chamber, air dried and then analyzed by the attention was paid to the factor which controlled the reproducibility of the results on HPTLC plates. The sample extracted from Viscera along with the standard sample was loaded on the HPTLC plates.

Development of the TLC plates

Spotted plates were developed in different solvent systems taken in different ratios. After developing, TLC plates were taken out from the solvent chamber and air-dried. For visualization the dried developed plates were sprayed with palladium chloride.

HPTLC-Desitometry

All the pesticides extracted from the biological tissues were spotted (10µ) using Hamilton's microsyringe on the HPTLC plates with the help of sample applicator (Desaga Co.) and also the standard samples of 1mg/ml solution of standard pesticide (Sigma Co. USA) The plates were dried and screened from 180 nm to 800 nm using densitogram attached with HPTLC instrument (Desaga Co. Germany). The results were analysed and presented in the form of densitogram.

RESULTS AND DISCUSSION

After the development of the TLC plates are sprayed with spraying reagent such as palladium chloride. There 10 different solvent systems were used (Table 1-3) with different volumetric ratio. The Rf values was also varying with different volumetric ratios. The Rf values of different organophosphorous insecticides extracted from tissue under experimental conditions was found nearly equal to the standard insecticides used. Response of the separation of organophosphorous insecticides in all the 10 solvent systems was analyzed and presented in table 1-3. The HPTLC-densitogram results are represented in the form of graph. It was observed that very small amount of the pesticides extracted from the tissues because of the metabolism in the liver of the victims. So it is very difficult to extract the organophosphorous insecticides for the instrumental analysis. The present studies shown the HPTLC-denstigram technique is very precise and accurate for analysis of the orgnanophosphorous pescticides.

Table 1. Effect of different solvent system on Rf values for Chlorpyrifos analysis by TLC

S. No.	Solvent system used	Rf of standard sample	Rf of extracted sample
1	Hexane : Acetone (9:1)	0.43 ± 0.001	0.42 ± 0.001
2	Hexane : Acetone (8:2)	0.42 ± 0.002	0.43 ± 0.003
3	Hexane : Acetone (7:3)	0.67 ± 0.001	0.67 ± 0.002
4	Acetone : Benzene (8:2)	0.94 ± 0.003	0.95 ± 0.005
5	Benzene : Methanol (4:6)	0.77 ± 0.004	0.77 ± 0.002
	Benzene : Methanol (5:5)	0.83 ± 0.003	0.82 ± 0.003
6	Benzene (100)	0.82 ± 0.004	0.82 ± 0.001
7	Acetone : Benzene (7:3)	0.93 ± 0.004	0.93 ± 0.003
8	Acetone : Benzene (6:4)	0.65 ± 0.003	0.75 ± 0.002
9	Hexane:Benzene:Chroloform (7:2:1)	0.75 ± 0.002	0.75 ± 0.002
10	Acetone : Benzene (5:5)	0.54 ± 0.002	0.54 ± 0.002

Table 2. Effect of different solvent systems on Rf values for Quinolphos analysis by TLC

S. No.	Solvent system used	Rf of standard sample	Rf of extracted sample
1	Hexane : Acetone (9:1)	0.91 ± 0.003	0.90 ± 0.002
2	Hexane : Acetone (8:2)	0.91 ± 0.004	0.92 ± 0.002
3	Hexane : Acetone (7:3)	0.91 ± 0.002	0.91 ± 0.003
4	Acetone : Benzene (8:2)	0.94 ± 0.006	0.95 ± 0.002
5	Hexane:Benzene:Chroloform (7:2:1)	0.39 ± 0.005	0.39 ± 0.001
6	Methanol : Benzene (5:5)	0.81 ± 0.008	0.81 ± 0.003
7	Benzene : Methanol (4:6)	0.82 ± 0.003	0.82 ± 0.003
8	Benzene (100)	0.93 ± 0.001	0.93 ± 0.002
9	Acetone : Benzene (7:3)	0.91 ± 0.004	0.92 ± 0.003
10	Acetone : Benzene (6:4)	0.41 ± 0.003	0.42 ± 0.003

Table 3. Effect of different solvent systems on Rf values for Malathion analysis by TLC

S. No.	Solvent system used	Rf of standard sample	Rf of extracted sample
1	Hexane : Acetone (9:1)	0.82 ± 0.002	0.82 ± 0.003
2	Hexane : Acetone (8:2)	0.81 ± 0.003	0.82 ± 0.002
3	Hexane : Acetone (7:3)	0.80 ± 0.005	0.80 ± 0.004
4	Acetone : Benzene (8:2)	0.91 ± 0.001	0.91 ± 0.005
5	Methanol : Benzene (5:5)	0.89 ± 0.006	0.89 ± 0.006
6	Benzene : Methanol (4:6)	0.42 ± 0.005	0.42 ± 0.002
7	Benzene (100)	0.41 ± 0.002	0.41 ± 0.003
8	Acetone : Benzene (7:3)	0.83 ± 0.003	0.83 ± 0.002
9	Acetone : Benzene (6:4)	0.85 ± 0.005	0.82 ± 0.004
10	Acetone : Benzene (3:7)	0.65 ± 0.004	0.66 ± 0.006

Conclusion

The long time involved in forensic screening of organophosphorous pesticides with analytical techniques like GC, HPLC etc. Thin layer chromatography is a simple method for the qualitative analysis of pesticides in biological tissues.

These results are given in tables (1-3). It takes less time to analyse the sample and simultaneously different samples may be analyzed. For the qualitative and quantitative analysis, high performance thin layer chromatography (HPTLC) attached with densitogram is a simple and useful method for the accurate analysis of organophosphorous pesticides in biological tissues/fluids for the forensic analysis. Micro-nano gram levels can be detected with high precision and accuracy with the HPTLC techniques to provide justice to the victims. Results are self explanatory in the HPTLC-Densitogram.

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