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Research Paper

Application of Thin layer Chromatography for Pesticide Detection in Fish Tissues

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Abstract: Chromatography is one of the most useful group of techniques available for the separation of closely related compound is preset in a mixture. Here the separation is effected by differences in the equilibrium and distribution of the components between two immiscible phase, namely the stationary and the mobile phases. Thin layer chromatography (TCL) is a technique, easy to perform and requires a simple apparatus. It readily provides qualitative information and it is possible to obtain quantitative data also with careful attention to details. The present papers deals with the application of the TCL technique for qualitative detection of pesticide residues in fish. This technique has been proved to be specific, rapid and sensitive in qualitative determination of pesticide in fish tissues. In the present study TLC was applied to fish treated with carbamate (carbaryl) organochloro (Endosulfan) and organophosphorous (malathion) pesticides. In all studies it has been observed that this technique is a simple easy one, specific and it is possible to detect quantities of pesticides hence, its use in strongly recommended for the detection of these poisonous chemical in fish.
Keywords: Thin layer Chromatography, Fish, Pesticides

INTRODUCTION

Thin layer chromatography (TCL) is a technique, easy to perform and requires a simple apparatus. It readily Provides qualitative information and it is possible to obtain quantitative data also with careful attention to details. (Singh, 1985) TLC is one of the most useful group of techniques available for the separation of closely related compound is present in a mixture. Here the separation is effected by differences in the equilibrium and distribution of the components between two immiscible phases, namely the stationary and the mobile phases (Abbout *et. Al.,* 1964) In this technique one can easily alter the various parameters to achieve a particular separation. Technique has therefore become immensely valuable to biomedical research and forensic studies.

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The present papers deals with application of the TCL technique for qualitative detection of pesticide residues in fish tissues. This technique has been proved to be specific, rapid and sensitive in qualitative determination of pesticides in these tissues. Its application in forensic investigations for detection and confirmation of organophosphorous and organochloro pesticide is well known in cases of poisoning with these chemicals. In the present study TLC was applied to fish *Rashora daniconius* (Ham) treated with carbamate (carbaryl) organochloro (Endosulfan) and organophosphorous (malathion) pesticides.

METHODOLOGY

The TLC method given by Walker and Beroza (1963) for insecticide analysis was employed with several modifications depending on the pesticides and fish tissues.

Sampling of Fish Tissues :-

The tissues after removal from fishes exposed to sublethal concentration of pesticides were thoroughly washed and dried in the oven. These tissues mainly gills, liver, kidney, gonads and Brain were kept in Hexane: Ether (90:10) mixture for organo chloropesticides. Hexane: Acetone (80:20) mixture for organophosphorous pesticides and in Acetone for carbamate pesticides for fixation (fixed for 24 hrs).

The tissue sample (0.5 gm) was homogenized in ethanol. The solid parts were dissolved in 5 ml of fixative solution and this solution was transferred to a 12 ml centrifuge tube. The tube was then shaken for 2 minutes and centrifuged. The tube along with the material was chilled in ethanol and then it was decanted ad fixative solution was added. After 1 hr this extract was used for TLC. In this method separation of different components of pesticide was done on silica gel G.

Apparatus and Requirements :-

The apparatus consist of a rectangular glass jar (30X15X12cm) with a ground rim on which a glass lid can be placed. Glass plates of size 22X10 cm. glass capillaries, sprayer suitable solvents of analytical grades and authentic samples of pesticides.

Preparation of TLC Plates :-

Rectangular glass plates of size 22X10 cm were thoroughly washed with chromic acid and then with detergent solution. The plates were finally rinsed with distilled water and dried. Thirty gms Silica gel G and 60 ml of water are stirred and the plates coated to a thickness of 250 u with this adsorbent. The plates were then allowed to dry at room temperature.

Spotting of Plates :-

Solutions of samples to be analysed were applied on the glass plates by capillary tubes. The area of application should be kept as small as possible because the smaller the area of application the sharper will be the re-1 solution. In order to keep the size of the spot small, a series of applications are made by allowing the solvent to evaporate after each application. Finally the spots were made approximately 1.5 cm above from the bottom of plates.

Development of TLC Plates :-

After the mixture to be analysed is spotted on the plates, it is dipped into the desired solvent in a closed chamber such that the spot is above the solvent level. The solvent rises by capillary action as in TCL and the mixture is resolved into discrete spots, when the solvent front approaches the top which normally taken 30-60 mts, the plate is dried and the position of separated components are located by spraying with a suitable indicator or spray reagent. Spraying was done with the help of a glass automizer. RF values of spots were calculated as follows:-

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RF = Distance of Spot centre From Start Point/Distance of Solvent Front From Start Point

RF values very with layer thickness hence, in this study layer thickness was always kept constant at 250 u.

Solvent systems and spray reagent -

The following solvent systems were used for the development of chromatograms -

a-For organochloo pesticides

1. n - hexane : ether (90:10)
2. n - hexane : Benzene (50:50)

b-For organophosphorous pesticide -

1. n - hexane : acetone (80:20)
2. Benzene : Ethyl acetate (90:10)

c-For CarbamaTe pesticide -

1. Cyclohexane : acetone (80:20)
2. Benzene : acetone (95:05)

Spray Reagent -

a-For organo chloropesticide: 500 mg silver nitrate dissolved in 100 ml of 90% ethyl alcohol. It is sprayed over the developed chromatogram, dried for 5 mts at 100^0C and then sprayed with a 0.2% Solution of bromophenol blue and 0.5% of silver nitrate in a mixture of ethanol : ethyl acetate (1:1), After drying for 10 mts at 100^0C the pesticide appears as bright blue or yellow spots on a blue background.

b-For organophosphorous pesticide: 0.5 gm palladium chloride was dissolved in 100 ml water containing a few drops of 25/Hcl. This reagent was sprayed over developed chromatograms. Yellow coloured spots are observed.

c-For carbomate pesticide: 1 gm silver nitrate was dissolved in 100 ml of distilled water. Gradually liquid ammonia solution was added till the solution become colourless. This resultant solution was sprayed over the developed chromatogram. Golden yellow spots were observed.

RESULTS

The separation of Endosulfan an organochlorine pesticide in the different tissues of *Rashora daniconius* using two different solvent systems is given in Table 1 and separation of malathion, an organophosphorous pesticide in the brain of *Rashora daniconius* using different solvent systems is given in table 2.

Table: 1 Shows Separation of Endosulfan in the different tissues of *Rashora daniconius*

Hexane : Ether Solvent System (90:10)

Days of Exposure	Colour of Spot	RF values					
		Gill	Liver	Kidney	Testis	Ovary	Brain
96 hr	Yellow	ND	0.75	ND	ND	ND	0.75
10 days	Yellow	0.75	0.75	0.74	0.74	0.73	0.75
15 days	Yellow	0.75	0.75	0.74	0.74	0.73	0.75
	Yellow	0.98	0.97	0.98	0.98	0.97	0.98
30 days	Faint Yellow	ND	0.32	0.32	ND	ND	0.30
	Yellow	0.75	0.75	0.74	0.74	0.73	0.75
	Yellow	0.98	0.97	0.98	0.98	0.97	0.98

Hexane : Benzene Solvent System (50:50)

96 hr	Yellow	ND	0.34	0.35	ND	ND	0.35
10 days	Yellow	0.35	0.34	0.35	0.34	0.34	0.35
15 days	Yellow	0.35	0.34	0.35	0.34	0.34	0.35
	Faint Yellow	0.90	0.91	0.91	0.90	0.90	0.90
30 days	Yellow	0.35	0.34	0.35	0.34	0.34	0.35
	Faint Yellow	ND	0.72	ND	ND	ND	0.72
	Faint Yellow	0.90	0.91	0.91	0.90	0.91	0.90

ND - Not detected

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Table: 2 - Shows separation of malathion in the brain of *Rashora danicanius* with the different solvent systems used

Days of exposure	Colour of Spot	Rf Values of metabolites in the brain	
		Hexane : Acetone (80:20)	Benzene : Ethyl acetate
96 hr	Yellow	0.34	0.26
10 days	Yellow	0.34	0.26
15 days	Yellow Bright Yellow Faint Yellow	0.34 0.68 0.85	0.26 - 0.52
30 days	Yellow Bright Yellow Faint Yellow Faint Yellow	0.34 0.68 0.85 -	0.26 - 0.52 0.85

Pesticides were identified on thin layer chromatograms by comparing the Rf values and colour of this resolved spots of unknown and authentic samples.

DISCUSSION

The separation and identification of pesticides by TLC have been observed and reported by many workers as part of chemical analysis of compounds (Walker and Beroza 1963). Significant studies on TLC determination and distribution of pesticides in autopsy tissues and their toxicological analysis have been reported by Singh (1985) in forensic studies.

Pesticides used in agriculture have been known to accumulate in various tissues of both aquatic and terrestrial animals. (Sahai, 1990) Identification of malathion residues in *Rashora daniconius* and accumulation of malathion in the gills, liver and kidney of fishes were assessed by TLC. (Singh 2014) Metabolism of BHC, Endosulfan and molathion in various tissues of *P. ticto* (Singh and Sahai 1987) were studied applying TLC. Pesticide metabolites in animal tissues have been investigated by Joseph Sharma (2003), Shahid Mehboob (2011) and Joseph Sharma (2017) have been studies by TLC. In the present investigation accumulation of Endosulfan in some important organs of *Rashora daniconius* and malathion in the Brain of *Rashora daniconius* have been studied in (Table 1 & 2) In all these studies it has been observed that this technique is a simple, easy one, specific and it is possible to detect minute quantities of pesticides, Hence, its used is strongly recommended for the detection of these poisonous chemical in fish. More investigation on other animal tissues applying TLC with modifications accordingly, would be helpful validating this technique for biological researches.

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