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Forensic Medicine

DETECTION OF PESTICIDES IN VISCERA SAMPLES BY THIN LAYER CHROMATOGRAPHY

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ABSTRACT Pesticides of various categories viz. organophosphates, organochlorines and carbamates are used as suicidal and homicidal poisons across the country. They are isolated from postmortem viscera samples by direct extraction and steam distillation. The detection and identification of 11 pesticide residues from 70 postmortem viscera samples was done by thin layer chromatography using different solvent systems and spraying by appropriate chromogenic reagents. Malathion, Methyl Parathion and Phorate were detected in maximum samples indicating their extensive use as suicidal and homicidal poison by people of eastern Uttar Pradesh.

KEYWORDS : Pesticide, viscera samples, Thin layer chromatography, solvent system, chromogenic reagent.

1. Introduction

Pesticides are used all over the world for protection of plants against pests, microbes and pathogenic agents. The common categories comprise of organophosphates (OPPs), organochlorines and carbamates. Pesticides are commonly used as suicidal or homicidal poisons across the country and the poisoning cases referred for postmortem examination comprise majority of deaths due to pesticide poisoning. Among which oragnophosphorus pesticides are most commonly used agricultural pesticides in India [1-3]. These pesticides have different adverse effects on human physiology, which include neuropathic, mutagenic and teratogenic effects. OPPs inhibit acetylcholinesterase enzyme activity by preventing its reuptake at synapses and by modifying the cholinergic signaling which leads to paralysis and death [4-12]. Pesticides are generally preferred due to their ease of availability, fast actions and small lethal doses. Therefore in death due to suspected poisoning cases, the estimation of poisons from visceral samples such as stomach, intestine, heart, liver, kidney and spleen is done by Thin Layer Chromatography for the qualitative estimation of pesticides. Thin layer chromatography is most common, economic, sensitive and a fast method for preliminary estimation of pesticides from visceral samples. The detection is based on the active constituents of the particular pesticide and appropriate use of spraying reagents and solvent systems [1,13-17]. The organophosphates are derivatives of phosphoric, dithiophosphoric or thiophosphoric acids, while the carbamates are carbamic acid derivatives. In this article, the procedures for extraction of pesticides from viscera samples, purification of extracted sample and their identification by thin layer chromatography using chromogenic reagents is mentioned.

2. Material and Methods

2.1. Extraction Procedures:

The pesticides were extracted from visceral samples by steam distillation and solvent extraction. The steam distillation procedure is carried out by preparation of slurry of 50 gram visceral tissue. 100 ml of acetone was added as extraction solvent to visceral slurry and transferred to distillation unit. Sample was boiled for one hour and upper layer was filtered. This extraction procedure was repeated two times and the resulting filtrates were combined. The clean-up procedure was done by passing it through chromatographic column containing successive layers of alumina, activated charcoal and anhydrous sodium sulfate each of 2.5 cm thickness. The resulting solution was evaporated to dryness on hot water bath and 1 ml of acetone is added to the resulting residue [18-21].

The solvent extraction procedure is based on the separation of analytes in a system of two immiscible liquids and the analyte is unevenly soluble in the solvent system to facilitate extraction. The visceral samples were extracted using hexane-acetone solvent system. The tissues were homogenized and 10 g of anhydrous sodium sulfate was added. The solvent system was added and shaken for some time and allowed to settle which facilitates the transfer of solutes from one phase to another [22-24]. It is filtered, concentrated, and cleaned up by passing through column containing successive layers of alumina, activated charcoal and anhydrous sodium sulfate each of 2.5 cm thickness. The resulting filtrate was evaporated to dryness and 1 ml of acetone was added for spotting on TLC plate.

2.2. Preparation of TLC plates:

50 gram of silica gel G was taken and 100 ml of distilled water was added to it and the resulting slurry is stirred to obtain a homogeneous mixture. The slurry is loaded in the preparator and coated on the glass plates producing a uniform thickness of 0.25 mm. The plates were air dried and then incubated at 110^0 C for 45 minutes for activation. The plates were cooled at room temperature before using [25].

2.3. Solvent systems used:

i) n-hexane :acetone (6:1)
ii) Cyclohexane : chloroform (7:3)
iii) n-hexane :benzene (3:1)
iv) Benzene : chloroform (1:9)
v) Acetone: carbon tetrachloride (1:9)

2.4. Chromogenic reagents used:

i) Palladium chloride: 0.5 % palladium chloride in 20 % HCl [26]
ii) Rhodamine B:0.1 % ethanolic solution of Rhodamine blue [27]
iii) Mercurous nitrate reagent: 1 gram of Mercurous nitrate in 100 ml distilled water followed by addition of 0.5 ml conc. nitric acid [28]
iv) Griess reagent: 5% sodium nitrite solution (in 10% acetic acid), 5% stannous chloride solution (in 50 % HCl) and 0.1 % napthyl amine solution (in 10 ml glacial acetic acid) [29]
v) Phenylhydrazine hydrochloride and HCl: 10 ml 10 % HCl diluted to 100 ml and 1 gm. of Phenylhydrazine hydrochloride in 100 ml of water. [30]
vi) Tollen's reagent: 10 % ammonical silver nitrate in 100 ml of distilled water, nitric acid was added drop wise followed by addition of ammonia. [31]

2.5. Thin Layer Chromatography:

1 ml of acetone is added to extracted sample and a solution is made. 10 µl of aliquot samples were taken and spotted on TLC plate about 1 cm above the base of plate along with the control pesticide samples of varying concentrations. The plates were placed in the developing chamber containing the appropriate solvent system based on the affinity of pesticides and allowed to develop by ascending chromatographic technique. After a run of 5 cm, the plates were removed from the chamber and air-dried. [232,33]

2.6. Visualization of spots:

The developed plates were irradiated with UV light at 254 nm after

which the organophosphates with p-nitrophenyl group showed dark spots. After UV irradiation, the TLC plates were sprayed with appropriate reagents for development of colored spots.

2.7. Calculation of Rf value

Rf (retention factor) is the ratio of distance travelled by solute front to distance travelled by solvent front. The Rf value is different for different residues and also depends on the solvent system used.

$$R_f = \frac{\text{distance travelled by solute front}}{\text{distance travelled by solvent front}}$$

3. Results

By using thin layer chromatography a variety of pesticide residues were isolated and identified from visceral samples. Total 70 samples suspected of poisoning were analyzed in which 40 samples gave positive results for pesticide residues of oragnophosphorus, organochlorines and carbamate pesticides as listed in Table 2. Best results were obtained using silica gel G plates and hexane: acetone as solvent system. However, for identification of some pesticide residues other solvent systems were used for better separation and identification. The residues were identified by application of various chromogenic reagents on the developed plates and measuring their R_f values followed by comparison with the standard known samples. Total 11 different pesticide residues were detected using various solvent systems and spraying with chromogenic reagents, which are listed in Table 1.

For the development of colored spots for Malathion, Diazinon and Chlorpyrifos, palladium chloride reagent was used. Similarly, Mercurous reagent was used for Chlorothion, Dimethoate and Phorate. Griess reagent was used for visualization of Paraoxon and Methyl Parathion. Baygon was resolved using Rhodamine B, Dichlorvos was resolved using Phenylhydrazine and HCl. Tollen's reagent was used for visualization of Fenthion. Dimethoate, Chlorothion and Phorate gave black spots with Mercurous nitrate reagent. Diazinon and Chlorpyrifos gave brown spots with palladium chloride while Malathion gave yellow spot with palladium chloride. Paraoxon and Methyl Parathion gave pink-orange spots with Griess reagent. Baygon gave red color spot with Rhodamine B whereas Dichlorvos gave yellowish red spot with Phenylhydrazine hydrochloride solution with hydrochloric acid.

Hexane-Acetone solvent system was suitable for a large group of pesticides which included Malathion, Baygon, Dichlorvos, Methyl Parathion, Dimethoate, Paraoxon and Chlorothion. Diazinon residue was developed in Acetone-Carbon tetrachloride and Chlorpyrifos residue was developed in Benzene-Chloroform.

Table 1:

Pesticide	Constituent	Solvent systems used	Chromogenic reagent	Color of spots	R_f value
Malathion	O,O-Dimethyl S1,2-Dicarbo-ethoxy ethyl dithiophosphate	n-hexane :acetone (6:1)	Palladium chloride	Yellow spot	0.50
Baygon	O-Isopro poxyphenyl methyl ((2-methyl phenyl) thio) carbamate	n-hexane :acetone (6:1)	Rhodamine B	Red color spot	0.16
Dichlorvos	O,O-Dimethyl O -2,2 dichlorovinyl phosphate	n-hexane :acetone (6:1)	Phenylhydrazine hydrochloride solution and HCl	Yellowish red spot	0.13
Parathion methyl	O,O-Dimethyl O-(4-nitrophenyl) phosphorothioate	n-hexane :acetone (6:1)	Griess reagent	Pink – orange spot	0.65
Fenthion	O,O-Dimethyl O-[3-methyl-4-(methylsulfanyl)phenyl] phosphorothioate	Cyclohexane : chloroform (7:3)	Tollen's Reagent	Black spot	0.80
Diazinon	O,O- Diethyl-O(2-isopropyl-4-methylpyrimidyl)6-thiophosphate	Acetone: carbon tetrachloride (1:9)	Palladium chloride	Brown spot	0.30
Dimethoate	O,O-Dimethyl-S-(N-methyl-carbamyol methyl) dithiophosphate	n-hexane :acetone (6:1)	Mercurous nitrate reagent	Black spot	0.50
Paraoxon	O,O- Dimethyl-O-P-nitrophenyl phosphate	n-hexane :acetone (6:1)	Griess reagent	Pink – orange spot	0.10
Chlorothion	O,O- Dimethyl-O-4-nitro-3chlorophenyl thiophosphate	n-hexane :acetone (6:1)	Mercurous nitrate reagent	Black spot	0.35
Phorate	O,O-Diethyl-S-(ethyl thiomethyl) dithiophosphate	n-hexane :benzene (3:1)	Mercurous nitrate reagent	Black spot	0.65
Chlorpyrifos	O,O-Diethyl O-3,5,6-trichloro 2-pyridyl phosphorothioate	Benzene : chloroform (1:9)	Palladium chloride	Brown spot	0.90

Table 2

Pesticide residue	Number of samples	Pesticide residue	Pesticide residue
Malathion	10	Dichlorvos	3
Phorate	6	Chlorpyrifos	2
Methyl Parathion	6	Diazinon	1
Dimethoate	4	Baygon	1
Paraoxon	3	Fenthion	1
Chlorothion	3		

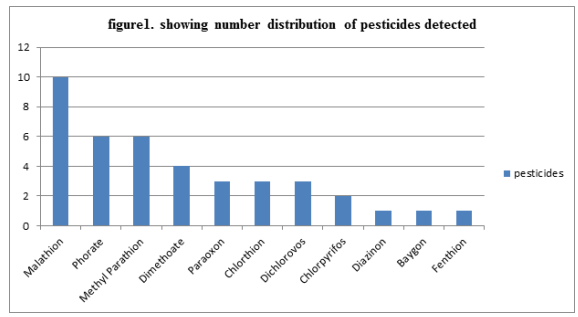
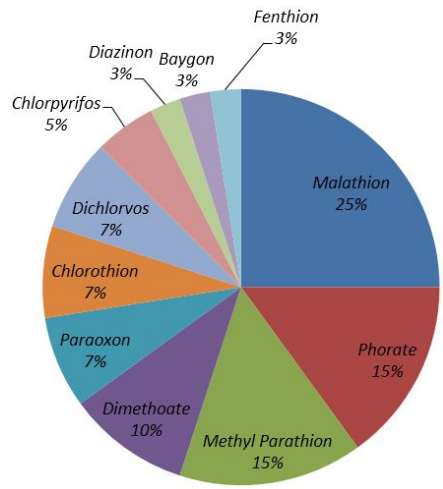


Figure2. showing percentage distribution of pesticides detected



4. Discussion

Thin layer chromatography was used for the identification of pesticides in cases of poisoning from visceral samples viz. stomach, intestine, spleen, liver, kidney and heart. The process involved use of various solvent systems as no single solvent was perfect for isolation of all pesticide categories. Some pesticides residues remained on the base

line or barely ascended from base line with a particular solvent system. The R_f values below 0.10 and above 0.90 were not considered appropriate for comparison and detection of pesticides. Thus, the choice of solvent varied with different categories. Also due to presence of impurities like proteins and fats, the R_f value and development of colored spots were affected. Similarly, no single chromogenic reagent was suitable for visualization of spots. Some pesticides did not responded to palladium chloride, which gave colored spots with most of residues. The organophosphates containing sulfur responded to mercuric nitrate reagent whereas thio group containing pesticides such as Dimethoate, Chlorothion and Phorate responded to palladium chloride reagent. Pesticides containing Nitro and aromatic amino group like Methyl parathion and Paraoxon, responded to Griess reagent. The carbamate pesticide Baygon responded to Rhodamine B. [1-3]

Malathion, Phorate and Methyl parathion were detected in maximum samples which suggested that they are most commonly used and easily available poisons in Eastern Uttar Pradesh which were detected in suicidal and homicidal cases.

5. Conclusion

Being an agricultural belt and low socio-economic area, pesticides are preferably used by people for suicidal and homicidal purposes, due to their low cost, fast action and high lethality. Thin Layer Chromatography can detect pesticide poisoning qualitatively and quantitatively which is cost effective and time saving. For highly accurate and reliable estimations Gas Chromatography, GC-MS and techniques that are more advanced, are used.

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7. References

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