Original Article

Extraction and Identification of 'Finit' In Biological Samples Using Different Solvent Systems of TLC

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ABSTRACT

'FINIT' comes under a broad spectrum of common household insecticide which chemically comprises of Malathion and Pyrethrins. Commonly it is used in prevention of insects such as flies, mosquitos, moths, cockroaches and ants etc. Its unsupervised use leads to accidental poisoning along with the intentional suicidal poisoning and hence its analysis is very important for medico legal purposes. Routinely, High Performance Liquid Chromatography, Gas Chromatography, Gas Chromatography-Mass-Spectroscopy and Liquid Chromatography-Mass Spectroscopy are used for analysis of Malathion & Pyrethrins. These techniques are not only costly but also require more sophisticated instruments. An attempt has been made to develop a new method for analysis of 'FINIT' in biological samples namely blood and urine using the different solvent system as mobile phase of Thin Layer Chromatography (TLC). 'FINIT' was extracted from blood and urine sample using liquid-liquid extraction method and analyzed by TLC. Developed plates were viewed under UV light followed by spray of chromogenic reagents which successfully increased the sensitivity without dispensing with the simplicity of the method. The method developed is a simple, rapid, inexpensive, non-destructive and reproducible which can be performed in any laboratory easily.

Keywords: finit; malathion; pyrethrins; extraction; thin layer chromatography

INTRODUCTION

"FINIT" comes under a broad spectrum of common household insecticide. It is commonly used in prevention of insects such as flies, mosquitoes, moth, cockroaches and ants etc. Chemically "FINIT" comprises of two pesticides viz. Malathion and Pyrethrins with kerosene base.[1,2] One litre container of "FINIT" contains Pyrethrins-0.05% wt/wt, Malathion-1.0% wt/wt, Kerosene and perfume®. Now-a-days there are various household insecticides available in market under the name of various brands like "Mortein,Baygon, HIT," etc. Basically, these household insecticides contain Pyrethroids viz Cyfluthrin, Transfluthrin, Prallethrin etc., Carbamates viz-Propoxur and Organophosphorus pesticide viz-Chlorpyrifos etc.[3] In market these are available in different combination and concentration. Some household insecticides chemically contain Allethrin (2.09 g/kg), Resmethrin (0.39 g/kg),[4] others contain Deltamethrin (0.07%), Allethrin (0.05%), Imiprothrin (0.07%), Cypermethrin (0.02%), Benzyl salicylate, Isopropyl alcohol etc. and d-Trans Allethrin (0.25% w/w), Synergist(0.50% w/w) etc.

MATERIAL & METHODS

1. Reagent/Chemicals/Glassware

   a. Malathion standard was obtained from Hindustan Insecticides Limited, R&D centre Gurgaon, Haryana India.

   b. One litre container of "FINIT" Space spray manufactured by Hindustan Petroleum

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Corporation Limited was procured from the market which contained Pyrethrins-0.05% wt/wt, Malathion-1.0% wt/wt, Kerosene and perfume- Rose

c. Palladium(II)Chloride, Sodium tungstate, Conc Sulphuric acid, n-Hexane, Acetone, Toluene, Conc Ammonia, Carbon tetra chloride, Ethyl methyl ketone, Ethyl acetate, Chloroform, Amyl alcohol & Cyclohexane of analytical grade from Merck India

d. Pre-coated Thin Layer Chromatographic plates (silica gel G60 F254 DC Kiesel gel 60 F254 CCM Gel silica gel 60 F254) from Merck Germany.

e. Glass chromatographic chamber, Beaker, conical flask, separating funnel, evaporating bowl and pipettes from borosil India.

2. Preparation of standard solution: 1000ppm solution of Malathion solution was prepared in acetone.

3. Preparation of spray reagent15: 0.5 gm of palladium chloride was dissolved in 100 ml of distilled water and 2N HCl was added to maintain pH.

4. Spiking of Sample:

   a. Spiking of blood sample: 5ml of blood was spiked with 1ml of "FINIT" then it was kept overnight in incubator.

   b. Spiking of urine sample: 5 ml of urine was spiked with 1ml of "FINIT" then it was kept overnight in incubator.

5. Preparation of samples :

   a. Extraction of active constituent from blood[14]: 100 mg of sodium tungstate and 3ml of sulphuric acid was added to the blood and mixed thoroughly. The solution was heated at 60°C and filtered. The filtrate was subjected to liquid-liquid extraction with 20 ml of n-hexane. After that organic layer was collected and passed through anhydrous sodium sulphate. The procedure was repeated thrice and then the filtrate was air-dried and concentrated upto 1ml and used for TLC.

   b. Extraction of active constituent from urine2: Mixture of spiked urine and 25 ml of n-hexane was subjected to reflux for half an hour and then filtered. The filtrate was subjected to liquid-liquid extraction method with 20ml of n-hexane. Then the organic layer was collected and passed through the anhydrous sodium sulphate. The procedure was repeated thrice and then the filtrate was air dried and concentrated up to 1 ml and used for TLC.

6. Activation of TLC Plates/Saturation of TLC developing chamber: TLC plates were placed at 105°C for 30 min for activation. The TLC developing chamber was saturated for 30 min with different reagent as per **Table-1.**

7. Spotting of samples and standards on TLC plate: Extracted samples were loaded on the TLC plates along with the standard, using fine capillaries with appropriate marking. Loaded plates were developed in 14 solvent systems as per **Table-1.**

RESULTS

The developed plates were first air-dried and then viewed under ultraviolet light at 254 nm. After that plates were sprayed with palladium chloride as chromogenic reagents. After exposure with palladium chloride yellow coloured spots appeared on the TLC plates on respective position of samples and standard of Malathion. As the quantity of Pyrethrin in the standard preparation was low (0.05% in 1L), it could not be extracted by the liquid-liquid extraction method attempted in the laboratory conditions; hence only Malathion was identified and used as a standard. Positions of samples were compared with standard of Malathion. Retention factor of samples and the standard were calculated and matched with each other as per **Table-2.**

The Rf values of samples and standard as obtained in various solvent systems are depicted in **Figure 1-14.**

Table-1: Showing various solvent systems used in experiment

S.No.	Solvent System	Ratio(v/v)
1.	Toluene: Conc Ammonia	100:0.5
2.	Toluene: Carbon tetra chloride: Ethyl methyl ketone	50:30:20
3.	Toluene: Carbon tetra chloride : Ethyl methyl ketone	30:50:20
4.	Ethyl acetate: Chloroform	50:50
5.	Ethyl acetate: Chloroform	40:60
6.	Ethyl acetate: Chloroform	60:40
7.	Ethyl acetate: Chloroform	20:40
8.	Ethyl acetate: Chloroform	80:20
9.	Amyl alcohol: Cyclohexane	50:50
10.	Ethyl acetate: Cyclohexane	50:50
11.	Ethyl acetate: Cyclohexane	60:40
12.	Ethyl acetate: Cyclohexane	40:60
13.	Ethyl acetate: Cyclohexane	20:80
14.	Ethyl acetate: Cyclohexane	80:20

Table 2: Showing Rf value of samples and standard in various solvent systems.

S.No.	Solvent System	Blood	Urine	Finit	Std Malathion
1.	Solvent System 1	0.15	0.16	0.16	0.20
2.	Solvent System 2	0.83	0.83	0.82	0.84
3.	Solvent System 3	0.80	0.80	0.78	0.82
4.	Solvent System 4	0.88	0.89	0.88	0.91
5.	Solvent System 5	0.84	0.85	0.84	0.87
6.	Solvent System 6	0.85	0.85	0.85	0.86
7.	Solvent System 7	0.88	0.89	0.89	0.90
8.	Solvent System 8	0.85	0.85	0.84	0.86
9.	Solvent System 9	0.87	0.89	0.91	0.93
10.	Solvent System 10	0.78	0.77	0.77	0.85
11.	Solvent System 11	0.84	0.83	0.84	0.85
12.	Solvent System 12	0.84	0.81	0.89	0.92
13.	Solvent System 13	0.35	0.32	0.32	0.34
14.	Solvent System 14	0.68	0.68	0.68	0.72

Fig 1-14 : The *Rf*-values of Samples and Standard as obtained in various Solvent Systems

Solvent System 1

Solvent System 2

Solvent System 3

Solvent System 4

Solvent System 5

Solvent System 6

Solvent System 7

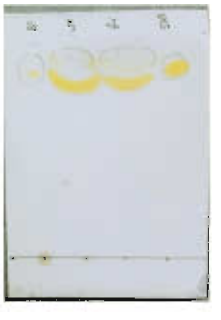
Solvent System 8

Solvent System 9

Solvent System 10

Solvent System 11

Solvent System 12



Solvent System 13

Solvent System 14

DISCUSSION

Malathion belongs to organophosphate insecticide group.[5] Generally it is used in prevention of insects such as mosquitoes, flies, aphids, spider mites etc.[6] Malaxon, a by-product of Malathion is chiefly responsible for toxicity by Malathion. Nausea, head-ache, dizziness, lacrimation, salivation, diarrhoea, urination, convulsions, incoordination, blurred vision, pupil constriction, abdominal cramps, slowed heartbeat, depressed respiratory system, skeletal muscle damage, episodes of rapid twitching, incoordination, paralysis followed by death are the acute effect of malathion exposure.[7] For rat acute oral dose LD_{50} of malathion is 1375-2800mg/kg.[2] For human the lethal dose is about 60 g.[8]

Pyrethrin is another active chemical constituent present in "FINIT". 'Pyrethrin' is the term used for six insecticidal components which are present in the extracts of different species of pyrethrum flower.[2] It consists of three closely related insecticidal esters of chrysanthemic acid i.e. cinerin I, jasmolin I & pyrethrin I and three closely related insecticidal esters of pyrethric acid i.e. cinerin II, jasmolin II & pyrethrin II. First three insecticidal esters collectively known as pyrethrin I and later ones are known as pyrethrin II.[9] Skin burning itching, dizziness are the initial symptoms of occupational poisoning. Other symptoms of pyrethrin poisoning are headache, nausea, anorexia, fatigue, fasciculation in large muscles of the extremities.[10] In pyrethroids poisoning, the target organ for its toxic effect is the nervous system but respiratory tract can also be affected causing oedema of lungs.[11] For male rat acute oral LD_{50} is 2370 mg/kg and for female rat it is 1030mg/kg.[2]

Unsupervised use of "FINIT" leads to accidental poisoning along with suicidal poisoning; so its analysis is very important from the medicolegal point of view. Routinely, High Performance Liquid Chromato-graphy, Gas Chromatography, Gas Chromatogarphy-Mass Spectroscopy and Liquid Chromatography-Mass Spectroscopy are used for analysis of Malathion & Pyrethrins. These techniques are not only costly but also require sophisticated instruments. Generally Hexane: Acetone (9:1) is used for the identification of insecticides in the biological samples by Thin Layer Chromatography.[12] In our study an attempt has been made to develop a new method for analysis of 'FINIT' in biological samples namely blood and urine using different solvent system as mobile phase of TLC.

CONCLUSION

With this study, new solvent systems were developed for the identification of active constituent of FINIT from the biological samples viz- blood and urine. All the solvent systems used in the study showed clear spots of active constitute of samples which are matched with standard Malathion. These TLC solvent systems can be used as alternative solvent systems for separation of active constituent of "FINIT" in a mixture of constituents. The method is very cost-effective, easy to demonstrate in any laboratory with the help of easily available chemical and glassware.

CONFLICTS OF INTEREST

Declared none

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