Original Paper

A New Thin Layer Chromatographic System for the Analysis of Some Commercially Available Mosquito Repellents

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ABSTRACT
Use of mosquito repellents is a popular way of avoiding mosquito bites. These repellents are quite effective, but can be toxic when misused. These substances are periodically involved in accidental, homicidal and suicidal poisoning cases.

The study being presented deals with thin layer chromatographic (TLC) analysis of six samples of different brands of liquid mosquito repellents available in the local markets of India. The results reveal a discernible separation of the components of the selected samples. This could help forensic toxicologists in the quick and economical analysis of samples of mosquito repellents.

Key Words: Mosquito repellent; Thin layer chromatography

Introduction

Mosquitoes are responsible for the spread of diseases such as malaria, filariasis, Japanese encephalitis, dengue haemorrhagic fever, yellow fever, etc. Carbon dioxide and lactic acid produced by human beings are the two best-studied mosquito attractants. Mosquitoes have chemoreceptors on their antennae that are stimulated by lactic acid. These same receptors may be inhibited by N,N-diethyl-3-methyl-benzamide (DEET)-based insect repellents.[1] A variety of mosquito repellents in various forms like mats, coils, lotions and vapourizers are available in the market. Pyrethroids, herbs, oils or diethyl toluamide (DEET) are the usual active ingredients in these repellents. The use of mosquito repellents is now the method of choice amongst consumers to protect themselves from insect bites. Allethrin and prallethrin are the chief constituents of various mosquito repellent- insecticides in India.[2]

While mosquito repellents are quite efficacious, several studies have revealed that they could be harmful to human beings.[3-6] Owing to their toxic properties, these substances can be a cause of human poisoning in the form of accident, suicide or homicide. One study revealed that one of the causes of poisoning in children was the ingestion of mosquito repellents, especially certain brands that are attractively packaged.[7,8]

In forensic cases of poisoning, it is important not only to identify and estimate the amount of poison consumed, but the method of identification must preferably be simple, economical and accurate. Analysis of these mosquito repellents can be accomplished by thin layer chromatography,[9,10] gas liquid chromatography,[11,12] high performance liquid chromatography,[13] mass and ultraviolet (UV) spectrometry,[12] and NMR spectroscopy.[14]

In this study, analysis of six liquid mosquito repellents was carried out with the help of thin layer chromatography (TLC), which is economical and easily employed. The main aim of this study was to refine the already established analytical procedure of TLC. Fifteen TLC solvent systems were used for the separation of six commercially available mosquito repellents.

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Materials and Methods

Sample Collection: Six different brands of mosquito repellent, namely Casper, Needs, Good Knight, Mortein, Odomos and Allout were procured from local markets, and were marked as A, B, C, D, E and F respectively. Details of the information regarding their names and manufacturers have been given in **Table 1**.

TLC Analysis of Samples: Extracts of the samples were prepared by dissolving them in appropriate solvent systems. These were then spotted on a 20x20 pre-coated (*Silica gel-G*) TLC plates with the help of fine capillary tubes. The spots were allowed to dry for a few minutes. The TLC developing chambers were properly saturated with solvent systems and the spotted TLC plates were placed in it. The chambers were properly covered with lids. The solvent system was allowed to run for a distance of 10 cm from the point of spotting. The plates were then removed from the chamber and dried at room temperature. The separated components were observed in sunlight, under UV light, and lastly with iodine fuming method.

Results and Discussion

Fifteen solvent systems were evaluated for the separation of selected samples. The list of solvent systems employed have been mentioned in **Table 2**. Solvent system 12, comprising xylene:toluene:petroleum ether (70:20:10) was found to be the most useful and suited for the separation of all the samples (**Plate 1**), while solvent system 10 comprising hexane: methanol (85:15) also produced good results (**Plate 2**). Solvent system 1 comprising cyclohexane:acetone (85:15) was also found satisfactory (**Plate 3**). Visualization of the separated components was clear only with iodine fuming, and no coloured or fluorescent spots could be seen under visible or UV light. The hRf (100 x retention factor) values of the spots of the samples with respective solvent systems are listed in **Tables 3, 4** and **5**.

In earlier studies, separation of pyrethrins was made by TLC, and the spots were visualized under UV light and fluorescein bromine reagent.[9] Their results were also satisfactory. Thin layer chromatography of pyrethrin and allethrin on silica gel plates with ether:hexane (2:1), and staining with phosphomolybdic acid have been reported.[15] In this study, a different solvent system comprising hexane:methanol (85:15) was done, which provided good results. TLC detection of pyrethroids using palladium chloride as a chromogenic reagent has also been reported,[16] as also the use of phosphomolybdic acid for cypermethrin and deltamethrin.[17] Silver nitrate impregnated alumina-G plates followed by irradiation with UV light has been done for the detection of halogenated synthetic pyrethroids,[18] while silica gel has been made use of for the separation of pyrethrins.[19] In this study, no attempt was made to use any spraying reagent for the development of spots, but iodine fuming proved to be better than UV interpretation.

Conclusion

The present study reveals that solvent system 12, comprising xylene:toluene:petroleum ether (70:20:10) is an appropriate and superior solvent system for the TLC analysis of ceratin liquid mosquito repellents. Solvent systems 1 and 10 have also been found acceptable, particularly for a few individual samples, but solvent system 12 appears suitable as a single solvent system for the col-

Table 1 Details of Samples Used in the Study

Sample No.	Brand Name	Manufacturer
A	Casper (Liquidator)	Tainwala Personal Care Products Pvt Ltd., Andheri (E), Mumbai-400093
B	Needs (Liquidator)	Vijay International, Bhiwadi Industrial Area, Bhiwadi, Distt. Alwar, Rajasthan
C	Good Knight (Liquidator)	Godrej Sara Lee Ltd., Eastern Express Highway, Vikhroli (East), Mumbai-400079
D	Mortein (Liquidator)	Reckitt Benckiser (India) Ltd., IGC Samba, Phase II, Jammu-184121.
E	Odomos (Cream)	Dabur India Ltd., Unit II, Phase II, SIDCO Industrial Complex, Bari Brahmna, Jammu & Kashmir-181133
F	Allout (Mosquito Coil)	Bharat Box Factory Ltd., Unit II, Industrial Growth Centre, Samba, Phase II, Jammu-184121.

Table 2 List of Solvent Systems Used in Thin Layer Chromatographic Separation of Mosquito Repellents

Solvent System Codes	Solvent System	Ratio
1	*Cyclohexane:Acetone*	85:15
2	*Cyclohexane:Chloroform*	70:30
3	*Chloroform:Methanol*	90:10
4	*Chloroform:Acetone:Methanol*	60:10:30
5	*Toluene:Ethyl acetate*	60:10
6	*Carbon tetrachloride:Petroleum ether*	60:10
7	*Hexane*	—
8	*Hexane:Diethyl ether:Acetic acid*	80:10:10
9	*Hexane:Acetone:Water*	80:10:10
10	*Hexane:Methanol*	85:15
11	*Chloroform:Acetone*	80:20
12	*Xylene:Toluene:Petroleum ether*	70:20:10
13	*Hexane:Acetone:Butanol*	80:10:10
14	*Hexane:Petroleum ether*	50:50
15	*Cyclohexane:Toluene*	70:30

Table 3 Results of hRf Obtained with Solvent System 12

Sample Code	Solvent System 12 Visualization with Iodine Fuming		
	No. of Spots	Colour of Spots	hRf
A	3	Brown	19,27,80
B	4	Brown	14,34,65,80
C	3	Brown	7,34,58
D	2	Brown	7,80
E	3	Brown	12,17,90
F	2	Brown	16,90

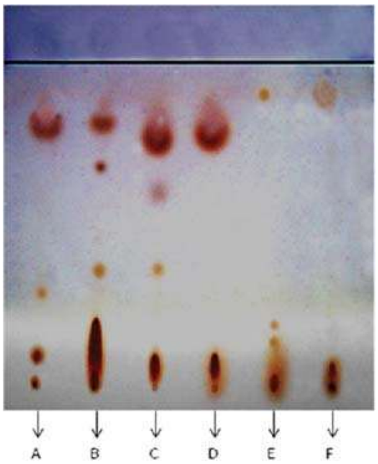
Table 4 Results of hRf Obtained with Solvent System 10

Sample Code	Solvent System 10 Visualization with Iodine Fuming		
	No. of Spots	Colour of Spots	hRf
A	2	Yellow	44,92
B	3	Yellow	5,75,92
C	2	Yellow	65,92
D	2	Yellow	64,92
E	3	Yellow	23,39,88
F	1	Yellow	6

Table 5 Results of hRf Obtained with Solvent System 1

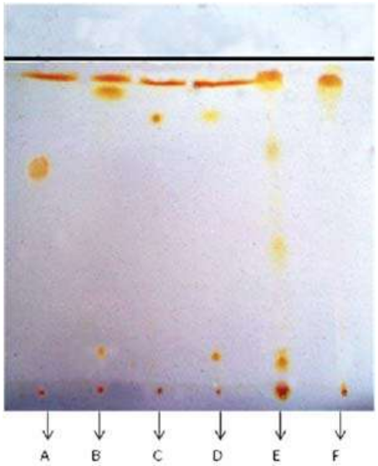
Sample Code	Solvent System 1 Visualization with Iodine fuming		
	No. of Spots	**Colour of Spots**	**hRf**
A	2	Brown	57, 86
B	3	Brown	12,75,86
C	2	Brown	65, 86
D	2	Brown	65, 86
E	3	Brown	9,38,62
F	1	Brown	95

Plate 1
Chromatographic Plate Developed with Solvent System 12
Xylene:Toluene:Petroleum ether (70:20:10)
Visualized by Iodine Fuming



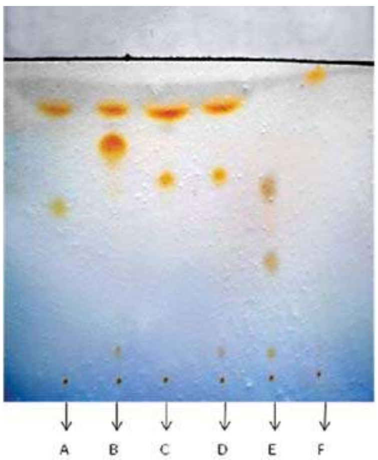
SAMPLE DETAILS: A - Casper (Liquidator); B - Needs (Liquidator); C - Good Knight (Liquidator); D - Mortein (Liquidator); E - Odomos (Mosquito Repellent Cream); F - Allout (Mosquito Coil)

Plate 2
Chromatographic Plate Developed with Solvent System 10
Hexane:Methanol (85:15)
Visualized by Iodine Fuming



SAMPLE DETAILS: Same as for **Plate 1**

Plate 3
Chromatographic Plate Developed with Solvent System 1
Cyclohexane:Acetone (85:15) ***Visualized by Iodine Fuming***



SAMPLE DETAILS: Same as for Plate 1

lective screening of all the samples. Therefore, this particular solvent system can be preferred by forensic scientists for the forensic analysis of mosquito repellents.

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