Original Article

Chemical Analysis of Toxicological Sample: The Need of the Day

Manish Nigam*#, Rashmi Kulkarni*

ABSTRACT

Acute, deliberate self-poisoning with agricultural pesticides is a global public health problem. There is much need for early diagnosis and proper management in poisoning cases using supportive care and antidotes. In this view the analytical toxicology lab is very useful. The present study highlights the role of analysis of biological samples in toxicology labs of a tertiary care centre by summarizing a few interesting cases out of total 101 cases analyzed during a period between March 2011 to Feb 2014. This study also gives a review of certain important tests carried out in the toxicology lab and their outcome.

In the poisoning cases, organophosphates accounted for the maximum number, while most of the remaining comprised Aluminium phosphide, zinc phosphide, alcohol and pyrethroids. The role of establishing poison control center/ Poison information centre in the region is also emphasized in preventing and controlling such poisoning events.

Keywords: chemical analysis; poisoning; poison control unit

INTRODUCTION

Based on various published studies and our records it has been observed that there is an increasing trend towards suicidal poisoning cases. Acute pesticide poisoning is now an important cause of morbidity and mortality worldwide.[1] WHO report suggests that pesticides are the most common method of suicide worldwide.[2] As per the National Crime Records Bureau of India average suicide reported among farmers is 15,750 per year during 1996-2001, which substantially increased to 17,366 per year during 2002-2007. This is almost equivalent to one suicide every 30 minutes. The figures are very depressing and horrifying, which may be correlated with increasing stress to fulfill present day needs.[3]

Poisoning patients are one of the most undervalued patients, reasons being cost on their treatment, medico legal component & very unpredictable outcome. It has been seen that most of the poisons remain undetected even after death or discharge of patient, due to non availability of proper analytical lab to assist the physician. The increasing load of poisoning cases, in emergency department demands the identification of factors causing high mortality and application of comprehensive strategies to improve the outcomes related to poisoning events.

MATERIAL & METHODS

We have analyzed the biological samples (Blood, gastric lavage, urine) of suspected cases of poisoning, which were sent to analytical toxicology laboratory of the department of forensic medicine and toxicology, Sri Aurobindo Medical College Hospital & P.G Institute, Indore from emergency ward, general ICU, medical ICU, and Pediatric ICU etc. of not only our own hospitals, but also other hospitals of the city.

In the present study we have included the sample from March 2011 to February 2014. Total 101 Samples of poisoned patients were analyzed out of 286 recorded cases of poisoning.

Following test were utilized for qualitative analysis of poisons in the toxicology laboratory.

- Silver nitrate test for phosphine detection.[4] In this test the phosphine gas reacts with silver nitrate to form black colored silver phosphide.

#(Author for correspondence): Email: jurimanish@gmail.com
*Department of Forensic Medicine & Toxicology, Sri Aurobindo Medical College and PG Institute, Indore.

- Ammonium molybdate test for organo-phosphorus.[5] In this test the phosphate ion reacts with ammonium molybdate in acidic solution to form phosphomolybdic acid, which upon reduction with ascorbic acid produces an intensely blue complex.

- Distillation & dichromate oxidation test for ethyl alcohol.[6] In this test ethanol reacts with potassium dichromate in acidic condition to form acetic acid and chromic acid.

- Chemical test for pyrethroids.[7] The cyanide group of pyrethroids reacts in alkaline medium to give pink color with resorcinol.

- Marquis test for different alkaloids.[8] Many alkaloids react with marquis reagent to produce different color.

- Renich's test for heavy metals.[9] The test based on staining of the copper foil. It shows different color for different metal present like, Purple black for antimony, Dull black for arsenic ,Shiny black for bismuth, Silvery for mercury

- Some other Color chemical test were done for identifications of many other compounds.[10]

- Thin layer chromatography is used to confirm many toxic compounds like organophosphates, carbamates, alkaloids, pharmaceutical drugs, pyrethroids etc. The separation of a mixture is based on a difference in the degree of attraction between the components and the stationary and mobile phases using specific solvents and spray reagents like dragandroff for alkaloids, resorcinol for pyrethroids, palladium chloride for organophosphates and toilets' reagent for carbamate etc.

Table 1 shows the recorded data of poisoning patient (286), from March 2011 to Feb 2014, the highest number of poisoning cases (121) recorded in duration between 1st March 2012 to 28th Feb 2013. The highest number poisoning cases recorded are of Organophosphorus compounds and second in the list is phosphide compound. (celphos-53. Zinc phosphide-30) The brought dead patients of poisoning and patients of poisoning discharge from emergency ward were excluded

Table 2 shows total of 101 samples were analyzed using qualitative chemical test or thin layer chromatography in the sample like gastric aspirate/ lavage, blood and urine forwarded to toxicology lab. It is important to note here that all three samples were not necessarily received in every case. The positive result indicated that at least one of the three samples showed the presences of particular toxicant. Majority of bulk (84 samples) tested positive for either the organophosphorus, celphos and ethyl alcohol.

DISCUSSION

Pesticide poisoning is a major concern in developing countries which could be due to occupational, accidental and intentional exposure. India being a developing country has similar picture because the majority of the population is employed in agriculture and hence exposure to pesticides and agrochemical products is more common.[11] Our study shows an important pattern in poisoning cases brought to emergency department. A total of 286 cases of poisoning were recorded from March 2011 to Feb 2014 (Table- I), out of which majority of cases showed poisoning with Organophosphorus compounds (108), celphos (53) and rat killer (30). Several studies like Ramesha KN et al, Jaiprakash H et al, Sinha US et al, Jesslin J et al; done in India have shown organophos-phates as the commonest agents of poisoning which coincides with our findings.[12-15]

It has been said by various researchers that the pattern of poisoning in a region depends on various factors such as availability, cost and access to toxic agents, socioeconomic status, cultural and religious characteristics of people, and regional considerations.[16-18] In same line some recent studies have found change in the trend of poisoning in northern and southern parts of India with increasing incidence of poisoning with aluminum phosphide.[19,20] This is suggestive of easy accessibility to Aluminium phosphide and thus stands next to Organophosphate compounds in incidence of poisoning.

The ethyl alcohol was the next major group of intoxicant which was found positive in 16 cases **(Table 2)** out of 101cases. This must have been ingested with some other poison in many occasions. This observation has explained the higher incidence of alcohol consumption in the population.[21] Seventeen

Table 1 : Recorded cases of poisoning in each year and type of poison as per history

Year	Different poisons					Sub-total
	OP/OC/ Carbamates	Celphos	Zinc phosphide	Miscellaneous	Unknown	
1st March 2011-29th Feb 2012	27	13	16	8	34	98
1st March 2012-28th Feb 2013	64	20	14	15	8	121
1st March 2013-28th Feb 2014	17	20	0	11	19	67
Total	108	53	30	34	61	286

Table 2: Number of cases analyzed in Toxicology Lab

Body Fluid Samples Tested Positive for different Poison					
OPP	Unknown	Celphos	Rat killer	Ethyl alcohol	Miscellaneous
33	10	19	6	16	17

Table 3: Types of miscellaneous poisons detected out of 101 poisoning cases

Types of Miscellaneous poisons detected			
Name of poison	**Cases**	**Name of poison**	**Cases**
Turpentine oil	1	Opium	1
Mercury	2	Pyrethroids	3
Naphthalene	2	alkaloids	4
EDB	2	Benzodiazepines	2

miscellaneous poisons were analyzed **(Table -3)** which included Turpentine oil, Naphthalene, Mercury compounds and pyrethroids Our study showed increasing pattern of household poisoning, which is similar to other study.[22] Ignorance about proper storage in households and easy availability could be mainly responsible for both suicidal and accidental poisoning.

Among numerous cases analyzed in our toxicological laboratory few interesting cases are summarized below

1. Received the sample of one and half year old male child from a hospital situated 15 km away from our centre, having no history of poisoning as per relatives, but with alleged suspicion of poisoning by physician. His sample was analyzed by color test for common pesticides like organophosphate, pyrethroids, carbamate and organochlorines etc, based on clinical history taken. All common screening tests were negative. His clinical history revealed presence of pin point pupil, later blood was extracted for thin layer chromatography analysis and it indicated poisoning with organophosphates compound (malathione). The boy survived after that due to early treatment by specific antitode.

2. A lady 34 year old having history of ingestion of unknown liquid was admitted to our hospital. We analysed her sample for common pesticides, the results were negative. As it was a case of household poisoning the sample was analyzed further for hair dye, bleach and kerosene and turpentine oil. Later the test showed positive for turpentine oil.

3. One Year-old child was admitted to the emergency ward with vomiting and seizures to emergency ward. After telephonic discussion with the treating physician the sample was analyzed for common pesticides, the results were negative. The gastric lavage had camphor like smell. Naphthalene test was carried out which showed crystals of naphthalene picrate microscopically.

4. A 22 year girl whose clinical examination revealed facial swelling with edematous lips, swollen neck and tongue was admitted to emergency ward. Her sample was send to our analytical lab which was anlysed for corrosive agents, bleach and dye. The vanilli- isopropanol chemical test for paraphenyl diamine, which is the major content of many dyes was carried out and was found positive and later on was confirmed by thin layer chromatography.

5. Many cases of poisoning with phosphide were easily detected bed side with the help of silver nitrate test as it is very simple, quick and yet reliable method.

6. Many natural insecticides which contains alkaloids, auxins, cytokines etc. gives similar clinical picture upon poisoning as synthetic pesticide, but the antitode varies, hence the extraction and detection using thin layer chromatography is useful in treatment planning.

Important national efforts and support from WHO to South East Asia Region countries have been undertaken in the last fifteen years, as awareness campaigns and capacity building in the use of a harmonized data collection system, common treatment protocols and guidelines, as well as some laboratory support. Yet, the response capacity to manage poisonings is still far from adequate. Many researchers[23-30] had focused to resolve this issue and so emphasize the need for establishing a poison control centers or at least separate toxicological unit exclusively dealing with clinical poisoning cases in the hospitals, which is helpful in early and accurate diagnosis of poisoning cases, for provision of prompt and adequate treatment. One such well equipped poison information centre is situated at AIMS Cochin working effectively in this field.

CONCLUSION

The Medical management of poisoning patients is difficult because there are lesser options with which to determine, the best strategies for treatment. Analysis of poison in toxicology laboratory will act as a useful planning tool for providing healthcare facilities to reduce the poisoning associated mortality rate. The health and hospital authorities should take initiatives in creating awareness about the establishment of the toxicology analysis unit. Establishment of a poison control center / poison information centre in the region will also help in preventing and controlling such poisoning events.

CONFLICTS OF INTEREST

Declared none

REFERENCES

1. Jeyaratnam J. Health problems of pesticide usage in the Third World. Br J Ind Med 1985;42:505-506.

2. Bertolote J, Fleischhmann A, Eddelston M, Gunnell D. Death from pesticides poisoning: a global response. Br J Psychiatry 2006;189:201-03.

3. World Health Organization, Regional Office for South-East Asia, 2009 Health implications from monocrotophos use: a review of the evidence in India.

4. Singh BG , Kewal K , Kanchan T , Sharma M , Singh SG. Phosphide poisoning. A review of literature Forensic Science International 2012;214:1–6.

5. Khan MH, Azad AK, Chowdhury AZ, Rahman MS, Malek MA. Evaluation of TLC (Thin Layer Chromatography) Methods for Pesticide. Journal of Applied Science and Technology 2010;7(2):91-94.

6. Sidney K and Harvery BH. Determination of ethyl alchol in blood. Journal of forensic medicine 1954;1(6):373-381.

7. Vaishya S, Chandel RS, Singh RA. New Thin Layer Chromatographic System for the Analysis of Some commercially Available Mosquito Repellents. Journal of the Indian Society of Toxicology 2010;6(2):10-15.

8. Rezai BM, Ghazi KM, Faghih A, Sadeghi M, Lotfalizadeh N, Eghbal M, et al . Screening of Morphine & Codeine in Urine of Opioid Abusers by Rapid and TLC Analysis. Eur J Gen Med 2010;7(2):192-96.

9. Pillay VV. Textbook of Forensic medicine and Toxicology. 15th edition, Paras Medical Publisher 2009:395-421.

10. Moffat AC, Osselton MD, and Widdop B. Clark's Analysis of Drugs and Poisons. 3rd edition vol. 2. London, UK: The Pharmaceutical Press; 2004.

11. Aaron R, Joseph A, Abraham S, Muliyil J, George K, Prasad J, et al. Suicides in young people in rural southern India. Lancet 2004;363(9415):1117-8.

12. Ramesha KN, Rao KB, Kumar GS. Pattern and outcome of acute poisoning cases in a tertiary care hospital in Karnataka, India. Indian J Crit Care Med 2009; 13(3): 152-5.

13. Jaiprakash H, Sarala N, Venkatarathnamma PN, Kumar TN. Analysis of different types of poisoning in a tertiary care hospital in rural south India. Food Chem Toxicol 2011; 49(1):248-50.

14. Sinha US, Kapoor AK, Agnihotri AK, Shrivastava PC. A profile of poisoning cases admitted in SRN Hospital, Allahabad with special reference to Aluminium Phosphide poisoning. Journal of forensic medicine and toxicology 1999;16(1) :40-43 .

15. Jesslin J, Adepu R, Churi S. Assessment of prevalence and mortality incidence due to poisoning in a south Indian tertiary care teaching hospital. Indian J Pharm Sci 2010;72(5):587-91.

16. Guntheti BK, Singh UP. The Pattern of Poisoning in Khammam. J Indian Acad Forensic Med 2011;33(4): 296-300.

17. Maharani B, Vijayakumari N. Profile of poisoning cases in atertiary care Hospital, Tamil Nadu, India. J App Pharm Sci 2013;3(1):91-4.

18. Sawalha AF, Sweileh WM, Tufaha MT, Al-Jabi DY. Analysis of the pattern of acute poisoning in patients admitted to a governmental hospital in Palestine. Basic Clin Pharmacol Toxicol 2010;107(5):914-8.

19. Murari A, Sharma GK. A Comparative study of poisoning cases autopsied in LHMC New Delhi and JIPMER. Journal of forensic medicine and toxicology 2008;19:19-21.

20. Singh D, Jit I, Tyagi S. Changing trends in acute poisoning in Chandigarh zone: a 25-year autopsy experience from a tertiary care hospital in northern India. Am J Forensic Med Pathol 1999;20(2):203-10.

21. Non- Integrated Disease Surveillance Project (IDSP), ICMR 2007-08 Madhya Pradesh.

22. Rosaura DF, Presgrave L, Antônio B, Camacho M, Helena S, Villas B. A profile of unintentional poisoning caused by household cleaning products, disinfectants and pesticides. Cad. Saúde Pública, Rio de Janeiro 2008;24(12):2901-08.

23. Dash SK, Raju AS, Mohanty MK, Patnaik KK, Mohanty S. Socio-demographic profile of poisoning cases. J Indian Acad Forensic Med 2005;27(3):133-8.

24. Patil A, Peddawad R, Verma V, Gandhi H. Profile of acute poisoning cases treated in a tertiary care hospital: a study in navi mumbai. Asia pac j med toxicol 2014;3:36-40.

25. Sheetu MK , Naik JD , Thakur MS, Langare SD, Pandey VO. Retrospective analysis of poisoning cases admitted in a tertiary care hospital international journal of recent trends in science and technology 2014;10(2):365-68.

26. Prajapati K , Merchant SP, Patel PR. Trends of suicidal poisoning in Ahmadabad (retrospective study) NHL journal of medical sciences 2012;1(1):18-22.

27. Ramanath KV, naveen kumar HD. study the assessment of poisoning cases in a rural tertiary care teaching hospital by a clinical pharmacist. Asian j pharm clin res 2012;5(2): 138-41.

28. Zaheer MS, Aslam M, Gupta V, Sharma V and Khan SA. Profile of poisoning cases at a north Indian tertiary care hospital Health and Population. Perspectives and Issues 2009;32 (4):176-83.

29. Pillay VV, Arathy SL, Vijesh KP, Vipin KG. Five Year Survey of Toxicological Testing of Clinical Body Fluid Samples at the Poison Control Centre in the Indian State of Kerala. J Indian Acad Forensic Med 2010;32(1):51-55.

30. Prajapati T, Prajapati K, Tandon RN, Merchant S. A study of Acute Poisoning Cases Excluding Animal Bites at Civil Hospital, Ahmedabad. J Indian Acad Forensic Med 2013;35(2):120-22.

Original Article

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

Pallavi Choudhary*, A.K. Jaiswal*, Adarsh Kumar*#, Sudhir Kumar Gupta*

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

*#(Author for correspondence): Email: dradarshk@yahoo.com
*Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029

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

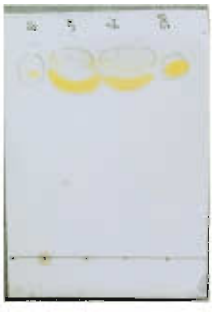
Solvent System 8

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

REFERENCES

1. Hindustan petroleum [Internet]. India: HP finit, Inc.; c2013[cited 2014 April 2]. Available at: http://www.hindustanpetroleum.com/hpclfinit.
2. Jaiswal AK, Nand Lal. Analysis of finit in urine sample using Thin Layer Chromatography.J Environ Science & Engg 2012;54(1):159-161.
3. Wikipedia.org [Internet]. India: Baygon, Inc.; c2014[cited 2014 May 22]. Available at: https://en.wikipedia.org/wiki/Baygon?oldid=654745804.
4. Wikipedia.org [Internet]. India: Mortein, Inc.; c2015[cited 2014 May 22]. Available at: https://en.wikipedia.org/wiki/Mortein.
5. Venugopal NVS, Sumalatha B, Bano S. Spectro photometric determination of malathion in environmental samples. eJournal of Chemistry 2012;(2):857-862.
6. Brenner L. Malathion Fact sheet. Journal of Pesticide Reform 12;1992;4.
7. Bennett J. Sierra Club [internet]. Canada: Pest Control Products Act 'act'- John Bennett. 2015 Feb 4 [cited 2014 April 2]. Available at: www.sierraclub.ca/national/programs/health-environment/pesticides/malathion fact sheet. shtml.

8. Thompson TS, Treble RG, Magliocco A, Roettger JR, Eichhorst JC. Case study:Fatal poisoning by malathion. Forensic Science International 1998;95:89-98.

9. Essig K, Zhao Z. Method development & validation of a High-Performance Liquid Chromatographic method for pyrethrum extract. Journal of Chromatographic Science 2001;39: 473-480.

10. World Health Organisation. Safety of pyrethroids for public health use. Italy: WHO Pesticide Evaluation Scheme (WHOPES); 2005. Available at: http://whqlib doc.who.int/hq/ 2005/ WHO_ CDS_ WHOPES_ GCDPP _2005.10.pdf.

11. Laboratory procedure manual, Directorate of Forensic Science, New Delhi. 2005.

12. Jaiswal AK, Millo T, Murty OP. Thin Layer Chromatography (TLC) an important tool for forensic scientists toxicology-A review. Journal of Forensic Medicine & Toxicology 2009;26(1): 21-33.