Case Report

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Qualitative estimation of pesticides in biological matrices using thin layer chromatography method of analysis

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Abstract

Pesticides are very frequently used to keep away any kind of pests, but as we all know everything has its pros and cons, so are the pesticides. Though the main purpose of developing chemical pesticides was to strengthen the agriculture field to full-fill the need of ever increasing population but their uncurbed use leads to serious issues like health issues and their easy availability made it very popular in suicide and homicide poisoning.

The qualitative estimation of pesticides in different biological matrices (viscera, blood, vomit) was done by thin layer chromatography technique. TLC is one of the oldest and cheapest method of quantitative estimation of any kind of drugs or poisons. TLC is also found to be very sensitive and reliable method for pesticides detection. This study aims to detect the presence of pesticides in biological matrices and to find out the most suitable one. The sample was taken from the case samples send to laboratory for the examination of suspected poisoning. Pesticides used as control were Malathion, Dicholorovos, Baygon, and Lindane. Solvent extraction method was used for the extraction of pesticides form different biological matrices.

Keywords: TLC, Malathion, DDVP, Baygon, Lindane, Biological Matrices, Rf value.

Introduction

"Pesticides are any substances or mixture of substances intended for preventing, destroying, repelling or mitigating any pest." According to Environmental Protection Agency (EPA).[1] Although there are many benefits of using pesticides but these have limitations as well, such as potential toxicity to humans and other animals. Because of their toxic nature, these substances are commonly used as poison in homicides suicides and in cases of crime against animals.

According to insecticide Act 1968, there are 186 chemical pesticides registered for use in India under section 9(3), [2] it is very difficult to detect the presence of each pesticides when there is such a huge range of chemicals. Another factor that interferes with the detection of presence of pesticides is the change in their chemical structure and composition after they are been exposed to environment or human body.

Pesticides can be classified on the basis of various categories, one of the very common classification used here is – classification on the basis of active ingredients.

1. Organophosphorous (e.g. Malathion, DDVP)
2. Organochloro (Lindane, DDT)
3. Carbamates (Baygon, Carbaryl)
4. Pyrethroid (Fenevalrate, Permethrin)

These are the some commonly used pesticides and because of their easy accessibility they are very frequently used by people for suicidal or homicidal purposes. In this study we only included certain pesticides namely- Malathion [2-(Dimethoxyphosphinothioylthio) butanedioic acid diethyl ester], DDVP [2,2- Dichlorovinyl dimethyl phosphate], Lindane [Hexachlorocyclohexane] and Baygon . The reason for selecting these pesticides was – they were very easily available, very frequently used in agriculture and were also very cheap and thought to be very common for poisoning purposes.

Pesticides get metabolized in human body and affects different organ and organ system. Such as organophosphorous pesticides affects the nervous system by inhibiting the acetylcholinesterase activity.[3] Pesticides can be present in different organs with variable concentration thus in this study we had taken different biological matrices through which pesticide's detection was possible. These biological matrices include viscera (liver, stomach, kidney) blood and vomit. These are the frequently examined biological matrices in suspected cases of poisoning send after post-mortem examination. All these biological matrices have proteins and fats which may interfere with the TLC results,

possible efforts were made /adopted such as using suitable extraction method to overcome with these shortcomings. In this study, Thin Layer Chromatography had been preferred method of analysis because of its merits like affordable, easily available and being quick and very efficient for the determination of different types of compounds [4]. Besides TLC there many other techniques being very sensitive to the identification of pesticides such as spectrophotometric method, High Performance Liquid Chromatography (HPLC) method, HPLC-UV method, HPLC –MS and many more in different matrices.[5-9]

MATERIALS AND METHODS

The deductive method of study was used for the detection of pesticides. The purpose of this study was to detect the possible pesticide causing poisoning and the most suitable biological material for their proper extraction and detection. The samples send to laboratory for suspected poisoning were analyzed. The chain of custody was maintained throughout the analysis process, right from the opening of sample boxes till the results were finalized.

In this study the technique used for the analysis was Thin Layer Chromatography because of its being available to all the laboratory and being cost effective as well. Along with all this, this technique is most preferable for the detection of pesticides is because of its being very sensitive to pesticide detection and also using proper extraction solvent, interferences caused by different biological materials can be avoided. For the analysis and comparison the Rf value and the color appeared after spraying with their respective reagent were observed and compared with the control sample.

RETENTION FACTOR (Rf) – The retention factor of a particular material is the ratio of the distance the spot moved above the origin to the distance the solvent front moved above the origin.

Here is an overview of how Rf value is calculated ;

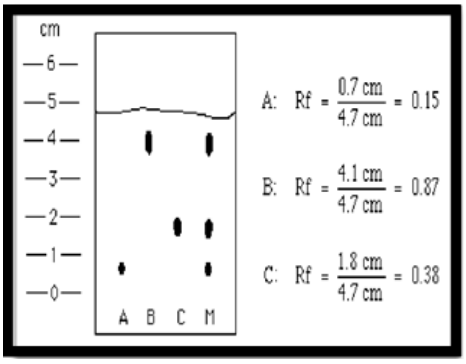

Figure 1: showing Rf value for different spots

Though different compounds can have the same Rf value for a particular solvent, but unlikely to have similar Rf value for a number of different solvents.

MATERIAL REQUIRED

Sample (viscera and biological fluid) ,Standard solution, Thin layer chromatography (TLC) plate made up of silica gel G, fine glass Capillary for the spotting in TLC , Chemicals for solvent system , TLC developing chamber, automizer for spraying the TLC.

SAMPLE PREPARATION- Samples (viscera, blood and vomit) obtained for examination were collected and labelled were finally set for the extraction process.

EXTRACTION PROCESS

Samples like viscera, stomach content (vomit) and blood requires proper cleaning before examination. All these needs to be passed through a proper extraction process so that the undesirable substances like fat, degraded proteins and coloring matter in the matrices do not interfere with findings. The different methods of pesticide extraction and purification are –

I] Solvent extraction

Ii] Stas-otto method of extraction

Iii] Digestion with ammonium sulphate and Sodium tungstate etc...

SOLVENT EXTRACTION METHOD

A. Extraction from viscera :

50gm of biological material (viscera), was mascerated into fine slurry by mixing with equal amount of anhydrous sodium sulphate and was transfered into a conical flask with an air condenser.

Then we added 50 ml of n-hexane to the flask and heated on hot water bath for 1 hour. The content were cooled and filtered. And the residual slurry was extracted twice with 25ml portion of n-hexane. The filtered n-hexane layers were combined and taken in to a separating funnel. This n-hexane layer was vigorously shaken with 15ml, 10ml and again 10ml portion of acetonitrile, which were previously saturated with n-hexane. The acetonitrile layers were mixed and taken into another clean separating funnel and diluted 10 times with distilled water. 25ml of saturated sodium sulfate solution was added to it and extracted thrice with 25ml portion of n-hexane. The n-hexane layer were combined, concentrated to 5 ml by evaporating on water bath/ sunlight and 5gm of anhydrous sodium sulfate was added. The extract was evaporated as and when required for analysis.

B. Extraction from VOMIT

The sample (20 ml vomit/stomach wash) was collected in a conical flask. 50ml oh n-hexane was added. Then sample was heated on a water bath for half-an-hour. After cooling, the liquid was filtered mixed with 20 ml of n-hexane and was collected in a separating funnel. The n-hexane layer was collected left for getting concentrated for some time. Then used for examination.

C. Extraction in BLOOD

10ml of 10% sodium tungstate solution and 15ml of 1N sulfuric acid was added to 20ml blood of, shaken for 2 minutes and then filtered and then transferred into a separating funnel and extracted thrice with 20ml portions of n-hexane.

The n-hexane layers was combined, passed through anhydrous sodium sulfate and the solvent was removed by passing a stream of air. After being passed through the process of extraction, extracted sample were kept to get concentrated in sunlight on the basis of requirement and then spotted on the TLC plate along with the control/standard.

STANDARD SOLUTION - 1000pm solution of standard pesticides in acetone was prepared by dissolving 0.1gm of standard in 100ml acetone. Only 2-3 spots of control/standard were enough to give results.

TLC PLATE – The study was performed on the ready-made silica gel G plates.

SOLVENT SYSTEM- Hexane : Acetone (8:2)

SPRAY REAGENT-

1. Malathion - PdCl2 (appearance of yellow spot surrounded by orange color ring)

2.Dichlorovos – 4,4 Nitrobenzylidine pyridine followed by Heating TLC plate 100c for 10 min. and then spraying with Tetra ethylene pentaamine (appearance of blue color spot)

3.Baygon – Tollen's Reagent (Bluish green spot are obtained)

4.Lindane – 0.5 gm Zinc Chloride + 0.5 Diphenyl amine in 100 ml of Acetone (Black color spot appears)

RESULT

Table 1: Rf Values of Malathion

TRIALS	Rf of Standard	Rf of Visceral Extract	Rf of Blood Extract	Rf of Vomit extract
1.	0.48	0.47	0.45	0.48
2.	0.48	0.46	0.45	-
3.	0.48	0.47	0.45	-
4.	0.48	0.47	0.45	0.48
5.	0.48	0.78	0.80	-
6.	0.48	0.69	0.67	-
7.	0.48	0.42	0.45	-
8.	0.48	0.46	0.46	-
9.	0.48	0.47	0.40	0.47
10.	0.48	0.32	0.51	-

In the above table the Rf value of standard Malathion pesticide is found to be 0.48 and Rf value of other extracted samples are compared.

On observing above table and comparing the Rf values of the Sample extract and the standard, it can be said that TRIALS 1, 4, 9 of visceral and vomit extract shows their Rf value approximately similar to the Rf value of standard malathion pesticide of organophosphorous class.

In other samples where vomit was not available for examination, visceral extract also shows somewhat similar Rf value with the standard. The malathion is very low in toxicity when ingested. The acurate lethal dose is 5400mg/kg in males and 5700mg/kg in females. Very little dose sometimes be unidentified due to interference by other biological materials, needs to be send for more sensitive instrumental analysis as per the need.

Table: 2 Rf Value of DDVP

TRIALS	Rf of Standard	Rf of Visceral Extract	Rf of Blood Extract	Rf of Vomit extract
1.	0.46	0.47	0.45	0.48
2.	0.46	0.46	0.45	-
3.	0.46	0.47	0.45	-
4.	0.46	0.47	0.45	0.49
5.	0.46	0.78	0.80	-
6.	0.46	0.69	0.67	-
7.	0.46	0.42	0.45	-
8.	0.46	0.46	0.46	-
9.	0.46	0.47	0.40	0.47
10.	0.46	0.32	0.51	-

The above table shows the Rf value of DDVP and sample extracts. DDVP is also an organophosphorous pesticide, very commonly used as fumigant and because of its easy availability used for suicidal purpose too.

The table shows that the Rf value of standard Ddvp and Rf of trial 2 and 8 of visceral extract shows exactly same values, on the basis of which we can say that the these two sample have DDVP pesticide poisoning.

DDVP an important pest control pesticide, is a colorless , aromatic liquid. Oral toxicity dose or lethal dose include 56-108mg/kg (rat)

Table 3: Rf Value of Lindane

TRIALS	Rf of Standard	Rf of Visceral Extract	Rf of Blood Extract	Rf of Vomit extract
1.	0.42	0.46	0.45	0.48
2.	0.42	0.46	0.45	-
3.	0.42	0.48	0.45	-
4.	0.42	0.47	0.45	0.49
5.	0.42	0.78	0.80	-
6.	0.42	0.68	0.67	-
7.	0.42	0.44	0.45	-
8.	0.42	0.46	0.46	-
9.	0.42	0.47	0.40	0.47
10.	0.42	0.32	0.51	-

On observing above table we found that there is no Rf value of sample extract which coincide with the Rf value of the standard pesticide Lindane. Also on developing TLC on developing the TLC plate of Lindane and spraying with their respective spraying reagent Zinc Chloride-Diphenylamine reagent (0.5gm of diphenylamine and 0.5gm of zinc chloride are dissolved in 100ml of acetone) only standard Lindane spot showed the bluishgreen color appearance.

It is an organochloro pesticide, it is a persistent organic pollutant. The EPA nad WHO both classify lindane as "moderately" acutely toxic. It has an oral lethal dose of 88mg/kg in rats.

Its production and use is banned since 2009.

Table 4: Rf Value of Baygon

TRIALS	Rf of Standard	Rf of Visceral Extract	Rf of Blood Extract	Rf of Vomit extract
1.	0.23	0.62	0.42	0.45
2.	0.23	0.62	0.47	0.45
3.	0.23	0.62	0.71	0.45
4.	0.23	0.62	0.48	0.45
5.	0.23	0.62	0.51	0.49
6.	0.23	0.62	0.62	0.63
7.	0.23	0.62	0.45	0.45
8.	0.23	0.62	0.46	0.46
9.	0.23	0.62	0.48	0.40
10.	0.23	0.62	0.39	0.51

In the TLC plate of Baygon (propoxure) two spots were observe from the origin point of standard showing different Rf values was noticed.

Out of all the trials, trial 6 showed similarity with the Rf value of the standard.

Baygon products contain the pyrethroids cyfluthrin, transfluthrin, prallethrin and the carbamates propxure and organophosphorous chlorpyrifos, as active ingredients. Since it is a mixture of many pesticides, to find the main consttituent, it was further analysed by other instrumental techniques for more specific result. Since other instrument were not part of this study, result of that is not included.

DISCUSSION

The report and analytical findings of forensic toxicologist is having significant role in crime detection specially in homicidal cases. The number of fatal poisoning cases received for toxicological analysis is constantly increasing. Since, last 5 years insecticides, pesticides and some other poisonous substances were prodigiously been used and their easy accessibility leads to suicidal, homicidal or accidental poisoning cases.

For identification of these substances thin layer chromatography (TLC) is the most widely used separation technique. This study is under taken for detection of pesticides using thin layer chromatography in different biological samples (viscera, gastric lavage, blood and vomit) in order to deduce which one of them gives better results. Thin layer chromatography is the preferable technique in this study because, it is cost effective, less time consuming, requires only easily available chemicals and precise too.

Since the analysis of pesticide residue possess an entirely different types of problems for the toxicologists because these residues are present in extremely small quantity in heterogeneous materials including the biological materials.

The determination of the pesticide in various biological materials often faced with the large problem of determining the minute quantity of insecticides mixed with large amount of extraneous material or intermixing. Quantitative and qualitative methods are required to be applied keeping in mind both the sensitivity and specificity of the methods on one hand and nature of pesticides on the other hand. The technique of thin layer chromatography has been proved to be practicable.

This study proved to be an additional literature which support thin layer chromatography as a consistently good analytical technique for pesticide detection. We also concluded that visceral part and vomit best suited sample for pesticide detection even if the pesticide is taken in very little amount and this study also support to the study of WHO according to which “suicidal poisoning with OP compounds has increased incidence and carries 4-30% mortality in Indian studies”, since the most of the pesticides detected in biological matrices of samples belongs to this organophosphorous class only.

CONCLUSION

In forensic toxicology detection of pesticides in biological matrices by Thin Layer Chromatography is very preferable method of examination because of its being very simple and reliable. Out of all the above biological matrices choosen for examination, visceral part and vomit has been found to give very required results and is also found to be least affected by interfering materials.

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