CHAPTER IV

RESULTS AND DISCUSSION
5.1 Results and Discussion

Analytical toxicology is an important branch of forensic Science. The number of fatal poisoning cases received for toxicological analysis is constantly increasing. There is tremendous rise in the use of insecticide, pesticide and other potentially poisonous substances in last five year for the purpose of crop protection, but their misuse has lead to suicidal, homicidal or accidental poisoning cases. The poison isolated from biological material in poisoning cases generally is in microgram quantities. The conventional methods of chemical analysis are not feasible in such cases; hence micro chemical and instrumental technique has to be developed for detection, isolation and quantitation of isolated poison.

For identification of these substances have been attempt has made to develop new technique by using thin layer chromatography (TLC), HPTLC. These methods are highly sensitive and can be used for unequivocal identification of pesticides/drugs, etc. these method are discuss in this chapter. An attempt is being made to develop a new chromogenic reagent and analytical methods for selective detection of pesticides and drugs of forensic interest.

According to the WHO, about one million serious accidental cases and two million suicidal poisonings cases due to insecticides occur worldwide every year, of which 200,000 patients die with most deaths occurring in developing countries. In India, organocompounds (OPCs), organophosphates and organocarbamates, are the commonest pesticides used and because of their easy availability, there is widespread abuse of these compounds with suicidal and homicidal intent.

Josef, G., et al. have prepared W.H.O. report of cases of acute pesticide poisoning account for significant morbidity and mortality worldwide, especially in developing countries. There are no reliable estimates as to how many people per year suffer from pesticide-related health effects. This is due to several reasons including a lack of standardized case definition. The case definition is inclusive of all circumstances of poisoning including suicide, homicide, and non-intentional (accidental exposure) and occupational.

Studies in developed countries have demonstrated the annual incidence rates of acute poisoning in agricultural workers to be as much as 18.2 per 100 000 full time
In developing countries, where there is insufficient regulation, lack of surveillance systems, less enforcement, lack of training, inadequate access to information systems, poorly maintained or nonexistent personal protective equipment, and larger agriculturally-based populations, the incidences are expected to be higher. The use of pesticides is banned in industrialized countries, in particular, highly toxic pesticides as classified by WHO. Obsolete stockpiles and improper storage techniques may provide unique risks in the developing world.

Since occupational and non-intentional pesticide poisoning require a specific set of prevention and control measures separate from those required for suicidal exposures, it is important to accurately determine the magnitude of the problem through better estimates and identification of cases and deaths resulting from acute poisoning.

From above literature and in day to day life we can see that, In India poising due to pesticides is commonest problem in agriculture society, Students and common man. In a routine life for simple reason people get disturbed, if thing don’t happen as they like, they intend to consume poison. People consume poison in fatal dose and succumb to it. The visceral samples of deceased are forwarded to forensic toxicology division for determination of cause of death.

Research and development work in agricultural pesticides is done in very large scale, because major investment of developing countries is in agricultural production and pest (insect) do not give response for older pesticides. Scientist develops new pesticides to yield more crops. Identification of these pesticides from biological (viscera, blood, stomach wash etc.) material is bigger problem for forensic toxicologist. For this reason we can develop innovative methods for extraction and identification of these pesticides.

The optimum analytical condition for extraction of pesticides in biological material has been developed and discussed in chapter II, section A, by using Accelerated Solvent Extraction (ASE), based on the principal of sweep co-distillation.

There is standard method for extraction of pesticides in biological material by accelerated solvent extraction. These methods as per technical notes of M/S Dionex. The method has been validated for analysis of pesticides in soil, sediment, dry wastes.
and fish tissues. However, further standardization is required for application of ASE to biological matrices in forensic toxicological work. The accuracy of this method is not much more in Indian environment. This author has done several experiments of extraction and then developed very simple, rapid and accurate method for extraction.

**Optimum method of ASE Developed for our study**

The biological materials (50 gm) were mixed up with 5gm of ammonium sulphate and homogenized. After addition of 100 ml diethyl ether, the mixture was shaken at intervals and kept overnight. It was filtered and evaporated to dryness. The residue was mixed up with 10-20 gm of anhydrous sodium sulphate and air dried at room temperature for 24 hours in a glass tray or hexane washed aluminum foil for a free flowing material. A cellulose disc was placed at the outlet and end of the extraction cell. The dried material was taken in 22 ml of extraction cell.

The samples thus prepared were collected in extraction cells. These were placed into the auto sample tray and collection trays were loaded in appropriate number (maximum 24). The tray was loaded with 40 ml pre-cleaned clapped vial with septa. The condition for extraction in ASE was set for extraction by using diethyl ether or hexane or acetone or acetonitrile or their mixture in definite proportion (V/V).

The residues of extract were taken up for detection and determination of poison by chromatographic method. The method developed is much simpler less cumbersome and offers a high recovery (80 ± 5%) for rapid handling of a large number of sample in a single run.

The low recovery on the method of M/S Dinoex has been enhanced by present developed method. The analytical conditions may be randomized for further processing in analysis viz. operating conditions and solvent used specially as mixture. The developed method on ASE showed better performance compared to existing method when applied to actual sample in the work place and also fulfilled the objectives of work i.e. time of analysis for large number of samples in a single run in ASE.

In our study we develop spray reagent for Identification of monocrotophos. Detail about this is discussed in chapter II, section B. Organophosphorous groups
have large application in the field of agriculture and are highly toxic for animal and human beings. A new chromogenic spray reagent for chromatographic detection & identification of monocrotophos, is developed by high performance thin layer chromatographic method. Monocrotophos on alkaline hydrolysis yields one molecule each of o,o-dimethyl phosphoric acid, and N-methyl acetoacetamide. After acidifying, N-methylacetoacetamide gives enol form of monomethylamid which reacts with ferric ions to yeild purple color complex. This spray reagent is very useful for Identification of monocrotophos in a very small amount. The biological impurities such as amino acids, peptides etc. present in visceral material do not interfere in the test.

In our study we developed spray reagent for tridemorph. Detail about this is discussed in chapter II, section C. Tridemorph is a widely used fungicide for crops, and is frequently encountered in forensic toxicology. The fungicide reacts with iodine in chloroform solution giving yellowish addition compound which with starch solution gives an intense blue colored spot with detection limit of 5 µg.

Tridemorph was also detected with methyl orange as yellow spot and with bromophenol blue as intense blue spot at detection limit of 10 µg and 1 µg respectively. The biological impurities such as amino acids, peptides etc. present in visceral material do not interfere in the test.

In our study we also developed a new chromogenic spray reagent for chromatographic detection and identification of carbosulfan. Detail about this is discussed in chapter III, section A. The carbamate insecticide is described by high performance thin layer chromatographic method. Carbosulfan on alkaline hydrolysis yields sodium salt of 2,3-dihydro-2,2-dimethylbenzofuran-7-ol, which form purple color complex with potassium ferricyanide, is thiochrome reaction. Distinguished purple color spot from standard carbosulfan and carbosulfan extracted from visceral sample were observed at hRf 72. There is no specific method for detection and identification of carbosulfan from biological material; we developed a specific and reliable method for identification of this insecticide.

Oxidizines are the newly discovered group of insecticides, highly toxic to animal and insects, widely used in agriculture for the protection of fruits and
vegetables from insects. Indoxacarb is an oxidizine class organic compound indoxacarb (Avant). The ending carb in the name doesn’t mean that it belongs to the carbamate class of insecticide. Owing to its easy availability and extensive use in agriculture by the farmers, are often misused for homicidal or suicidal purposes. There is extensive burden on forensic laboratories due increasing number of biological samples for poison detection. Hence, the forensic toxicology needs to develop new rapid analytical technique of Indoxacarb analysis in biological & non-biological material. High performance thin layer chromatography (HPTLC) is the method of choice because of its speed, and versatility. A new chromogenic spray reagent for chromatographic detection and identification of indoxacarb, an oxidizine insecticide is described by High performance thin layer chromatographic method.\textsuperscript{17} Detail about this is discussed in chapter III, section B. Indoxacarb on acid hydrolysis yields its oxadiazine derivative. The oxadiazine under acidic condition react with diacetylmonoxime and furnish azide derivative of oxadiazine which is being sensitive to heat and light turns black on heating.

Hydrogen cyanamide is used in agriculture as a plant growth regulator and is applied to many deciduous plants to stimulate uniform bud break after dormancy, resulting in uniform flowering and maturity. Dormex\textsuperscript{®} (Degussa AG, Trostberg, Germany), a pesticide producing company has started the production of hydrogen cyanamide in Italy in 2000. Nashik region (Maharashtra state) is famous for grape production and use of hydrogen cyanamide is very common in this region. This laboratory is receiving considerable number of poisoning cases involving hydrogen cyanamide insecticide in routine forensic work. The detection of hydrogen cyanamide is achieved by high performance thin layer chromatography (HPTLC). Detail about this is discussed in chapter III, section C. HPTLC is the most simple, rapid and reliable technique, usually used in forensic laboratory for detection and identification of poison. In our study we demonstrates the development of new selective and specific chromogenic spray reagent for chromatographic detection and identification of Hydrogen cyanamide.\textsuperscript{18,19} Hydrogen cyanamide on alkaline hydrolysis followed by spray of sodium nitroprusside and potassium ferricyanide furnish pink color complex. Such pink color complex is not observed for other insecticide.
Different types of latest complicated cases are received in this laboratory, involving poisoning by newly discovered insecticides and pesticides.

There are so many problems for Identification of these poisoning substances. There are no previous references for identification of such types of cases. By doing research and development work, and using the spray reagent developed by us, these cases are solved. Here are some cases involving our innovative work.

Chapter IV, section A discussed an interesting case solved after our research and development work. In a small village in rural areas of Nanded district there was quarrel between two families, the accused intentionally mixed an unknown poison in the flour of victim family at the flourmill resulting into the poisoning. Samples of flour from flourmill and flour Seized from accused were forwarded to our laboratory. In this case we developed our own method for extraction of insecticide from flour sample. The identification was done with thin layer chromatography. After chemical analysis, of flour from flourmill and flour from accused, it was found to be containing cypermethrin, a pyrethroid insecticide.

Chapter IV, section B discussed second interesting case solved after our research and development work. In widely spread forest of Nashik region in Maharashtra state, leopard is frequently existing wild life species. When leopard catches the pray, he eats it partially. The remnants of prey are left at the site. On next day he revisits the spot to eat the remaining portion. In a present case the modus operandi of accused was similar in three such incidents. The unknown accused persons were poisoning the leopards through the remnants of the prey left over by the animal. In three different cases the dead leopards were found in forests. The visceral samples of three leopards were forwarded to forensic science laboratory for chemical analysis. In all three cases, cypermethrin a pyrethroid insecticide was detected.

Chapter IV, section C discussed third interesting case solved after our research and development work. A female aged 25 year was admitted for severe abdominal pain in a hospital. She was 4 months pregnant. She was willing to terminate her pregnancy due to socioethical reasons. She attempted abortion by inserting a stick covered with monocrotophos in her private parts. Abortion was caused by rupture of the membrane. Excoriation, laceration was produced in the upper part of vagina and
cervix. During treatment the victim died due to severe neurogenic shock. In this case we came across altogether different and peculiar case history. The victim in this case used insecticide monocrotophos an organophosphorous insecticide. The purpose however was not suicide or homicide. She used it externally to facilitate an abortion to terminate the pregnancy for socioethical reason. Death may have occurred instantaneously due to neurogenic shock. Viscera of deceased along with vaginal swab were forwarded to Forensic laboratory for toxicological examination. Analysis of above mentioned samples facilitated the identification of organophosphorous insecticide namely monocrotophos.

Thus the innovative methods applied by the author facilitated the identification of modern day insecticides and pesticides. This is the boost to forensic toxicology division to enhance the analytical methodologies by which the challenge of finding out modern day poisons can be achieved.

Another major thing is that when patient is admitted in hospital for treatment of poisoning, Hospital can’t give correct treatment without knowing the nature of poison. Majority of patient die due to incorrect treatment\(^2\). For correct treatment Identification of these pesticide and insecticide poisons is of great importance. In Rural area of Maharashtra there is unavailability of institute or laboratories which carry the Identification of poison, to facilitate the proper treatment of the victim. For these purpose cheap and innovative techniques developed by the author like TLC and HPTLC for detection and Identification of poison from biological material are of great value.
5.2 References
