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**NEW DELHI**

# WORKING PROCEDURE MANUAL: TOXICOLOGY

**2021**

**Punya Salila Srivastava, IAS**
**Additional Secretary (WS & IS-I)**
Tel. No.: 011-23092785
e-Mail: aswsis1@mha.gov.in

सत्यमेव जयते

भारत सरकार
GOVERNMENT OF INDIA
गृह मंत्रालय
MINISTRY OF HOME AFFAIRS
NORTH BLOCK
NEW DELHI-110001

## FOREWORD

Forensic Sciences have a critical role to play in criminal justice delivery system. Successful prosecution of offenders hinges on the quality of test reports. Exhibits in crime cases need to be examined timely, precisely and accurately. In order to standardise and benchmark forensic analysis and test reports in respect of crime cases in Forensic Science Laboratories across the country, the Directorate of Forensic Science Services (DFSS), Ministry of Home Affairs has taken the initiative to prepare Working Procedure Manuals (WPMs) for various forensic disciplines.

Due to advancement of technology and its global access, crime is continuously evolving and adapting. New crime trends are emerging with people committing crimes in cyberspace, trafficking occurring in new psychoactive substances and drugs etc. Therefore, incorporation of new technologies in the WPMs to meet the ever increasing challenges for solving crime is the need of the hour, which is systematically and comprehensively reflected in these WPMs.

A long-felt need for such uniform WPMs has been fulfilled and I hope that these will be of immense use to forensic professionals of India. I congratulate the DFSS team for developing these manuals and urge them to keep on updating these at regular intervals.

Punya Salila

**(Punya Salia Srivastava)**

डॉ. एस. के. जैन
निदेशक–सह–मुख्य न्यायालयिक वैज्ञानिक

**Dr. S.K. Jain, M.Sc Ph.D**
Director-cum-Chief Forensic Scientist

सत्यमेव जयते

न्यायालयिक विज्ञान सेवा निदेशालय,
गृह मंत्रालय, भारत सरकार
ब्लॉक–9, तल नं. 8 , केन्द्रीय कार्यालय परिसर
लोधी रोड, नई दिल्ली–110 003

**Directorate of Forensic Science Services,**
**Ministry of Home Affairs, Govt. of India**
**Block No. 9, 8th Floor, C.G.O. Complex**
**Lodhi Road, New Delhi-110 003 (India)**
**Tel. : 011-24362676 Fax : 011-24362819**
**E-mail : cfs-dfss@nic.in**

## PREFACE

The analytical procedures for examination of forensic physical clue materials in forensic science laboratories involve high degree of skill & expertise and play a significant role in a wide range of legal proceedings. The occurrence of error(s) in any of the forensic analytical activities is a serious matter for both laboratories and end users. For a laboratory, it can lead to re-testing of samples, if available, and loss of its credibility. The analytical techniques adopted by the scientist(s) for the forensic analysis may be one of the causes for this serious error.

The risk of committing error can be eliminated if the scientists undertake two or more independent validated techniques while conducting forensic analysis of crime case exhibits in the laboratory. Essentially, the procedures adopted must conform to the quality, sensitivity, repeatability and reproducibility of the examination so that the chances of error are absolutely avoided. It is, therefore, one of the essential requirements of good laboratory practices to introduce a Working Procedure Manual, which contains validated laboratory methods/techniques for forensic analysis of the exhibits. It is also necessary for all the Central/State Forensic Science Laboratories to follow these manuals in the country to maintain uniformity in test reports.

Keeping in view the advancement in science & technology and use of various protocols & procedures in the international arena of forensic science, the Directorate of Forensic Science Services (DFSS), Ministry of Home Affairs(MHA), has taken the initiative for preparing a systematic and comprehensive Working Procedure Manual for the discipline of 'Forensic Toxicology' to bring uniformity and standardization in the examination methods. In this regard, DFSS/MHA formed Scientific Working Groups, comprising eminent forensic scientists from the CFSLs and FSLs for each forensic discipline to compile forensic analytical techniques in the form of Working Procedure Manuals. Several meetings were conducted with detailed deliberations among the scientists at National level and finally the manual has been prepared /updated in the present form.

I am sure that this Working Procedure Manual, which pertains to the discipline of 'Forensic Toxicology' will help the forensic science laboratories to continue to follow standard and latest updated procedures in the examination of clue materials as well as to adopt quality control/ quality assurance in the forensic practices and also for obtaining accreditation from National Accreditation Board for Testing and Calibration of Laboratories (NABL).

I understand that there is always a scope of improvement and perfection can be achieved with collective efforts, therefore, stakeholders are welcome to offer their feedback and suggestion, if any, in this regard.

10/8/2024

**(Dr S K Jain)**
**Director-cum-Chief Forensic Scientist**

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# SECTION- 1: INTRODUCTION

**1.1 Title:** Forensic Toxicology

**1.2 Scope:** Various aspects of toxicological analysis.

**1.3 Purpose:** To provide standard toxicological methods of analysis for uniformity.

**1.4 Definition:**

The word "Toxicology" is derived from the Greek word "Toxicon" which was used as a poisonous substance to arrowheads. Traditionally, the toxicology is defined as the science embodying the knowledge, source, character, fatal effect, lethal dose and analysis of poisons and the remedial measures. A poison is defined as the substance, which is capable of producing injury or death when absorbed. Appropriate dosages can differentiate poison and also the remedial measures. All chemicals can produce injury or death under certain conditions. Hence, a poison can be defined as a substance that is capable of producing detrimental effects on a living organism. As a result, there may be a change in the structure of the substance or functional processes, which may produce injury or even death. The toxicologist is specially a trained expert to examine the role of such substances and their adverse effects. The variety of potential adverse effects and the diversity of chemicals present in our environment contribute to make toxicology a very broad field of science. Therefore, toxicologists are usually specialized to handle various areas of toxicology. The professional activities of toxicologists fall into four main categories i.e. Forensic, Industrial, Clinical and Environmental Toxicology. Forensic toxicology emerged on the hybrid of analytical Chemistry and toxic principle effects. Forensic toxicologists are also primarily concerned with the medico legal aspects of the harmful effects of chemicals on human and animals. The expertise of forensic toxicologists is primarily utilized in establishing the cause of death and elucidating its circumstances in post-mortem investigation. The work of forensic toxicologist is therefore considered as highly complicated as small quantities of poisons and their metabolites are to be isolated, purified and quantified from a highly complex matrices.

**1.5 Objective:**

This manual is aimed to serve as a didactic text to Forensic Experts working as Toxicologists. Due to the preponderance of assorted redundant procedures proclaimed for isolation, analysis and estimation of poisons, the selection of correct procedures become tedious and obfuscating. This entails the need for standardised commensurable and pragmatic procedures with emphasis on accuracy and precision. Having cognisance of these facts, it was decided to orchestrate an adhoc committee of highly experienced and learned experts. This manual assimilates the outcome of the meticulous dialectics of the committee constituting highly experienced and knowledgeable Forensic Scientists from the Forensic Science Laboratories of the country. The committee has developed this abridged yet comprehensive text which encompasses a thorough revision of erstwhile procedures within the ambit of Toxicology and also introduces some new topics. This text is intended to allay disparity and ambiguity concerning the causes of Toxicology in general with special reference to Indian context. This text has been prepared with conscientious appraisal of various procedures described in the sundry reference books

pertaining to Toxicology. We have the propensity to put forward a text that would serve to mitigate the tedious and responsible job of Toxicologist. By providing a handy and consolidated quick reference, we have scrupulously avoided superfluous details and procedures in this manual. Albeit, it cannot substitute for the reference books and textbooks, it should serve as a practical guide to pertinent Forensic Scientists. This manual does not detail the clinical symptoms or pharmacological effects of poisons but rather focuses on the classification, isolation, detection and quantitation of poisons. As stated above, the manual is not directed towards the replacement of accepted procedures/methodologies, but incorporate only those, which are relevant and practicable. However, the options are left open for the Toxicologists to adopt any other testing procedure if required. The committee hopes this manual will be received with fervour and will meet the ends it has envisaged.

## SECTION - 2: ISOLATION AND PURIFICATION OF POISONS

**2.1 Title:** Isolation and purification of poisons.

**2.2 Scope**: General procedures for isolation and purification of poisons.

**2.3 Purpose:** To know the methods adopted for isolation or extraction of poisons from different matrices.

**2.4 Responsibilities:** Gazetted officers and other associated scientific staff.

### 2.5 BASIC STEPS IN ANALYTICAL TOXICOLOGY:

1. Extraction of active constituent i.e, poison in matrices of interest.
2. Stripping or purification of active constituent thus separated.
3. Rapid Screening and Identification
4. Quantitation
5. Interpretation / Conclusion.

### 2.6 GLOSSARY OF TERM RELATED TO EXTRACTION:

**Matrix:** Any material substance wherein the active constituent may be dispersed, accumulated, left, absorbed or chemically bound.

**Active Constituent:** The toxic chemical of interest i.e. poison.

**Stripping:** Purification.

### 2.7 Classification of Matrices:

A. **Biological**: Viscera, blood, urine, saliva, stomach contents, intestinal contents, gastric lavage, vomit, brain matter, stool, faecal matter, bone, nails, hair, skin.

B. **Non Biological matrices**: Water, remnants or traces of poison in small container, food and food products, milk and milk products, fruits, vegetables, tea, coffee, cooked materials, drinks, cereals, pulses, wines, etc.

## 2.8 DIFFERENT CLASSES OF POISON:

For the purpose of chemical analysis, poisons are grouped according to the methods used for isolation of the substance from matrices. These are given below.

- Noxious gases
- Volatile poisons (organic and inorganic).
- Non-volatile poisons (organic and inorganic).
- Plant poisons
- Miscellaneous poisons.

## 2.9 DIFFERENT METHODS OF EXTRACTION:

There are various classical and modern methods of extraction. The selection of proper method of extraction depends on various controlling factors viz. nature of poison, matrix or matrices and also quantity of samples available or forwarded for analysis. The active constituent should be extracted from sample in minimum steps to avoid loss during processing. The extracted material also require proper stripping or purification to avoid interferences of matrices as far as possible. The efficiency of extraction and stripping determines the lower limit of detection, precision and accuracy in the determination. The analyst plays a significant role in the selection of proper methodology on the basis of different parameters viz. case history, amount physical state of matrices, analytical requirements and also infrastructural facilities available. It will be befitting if the methods are presented Schematically depending on the classification of poisons.

| Class of poison | Classical Method | Modern Method |
|---|---|---|
| Gases | Micro-diffusion, Adsorption- desorption | Sensor Based Gas Analyzer,<br><br>Gas Chromatography. |
| Volatile Inorganic | Gutzeit Method, Massh-Berzelius Method, Micro-diffusion, Digestion with specific reagents / under specific conditions of PH, Crystal test, Color tests etc. | Microwave oven technique for digestion followed by<br><br>Ion Chromatography using<br><br>Ion exchange resins,. Spectroscopy etc |
| Volatile Organic | Distillation, Steam Distillation, Diffusion | Gas Chromatography/mass spectrometry. |
| Non-Volatile Inorganic | Dry and wet ashing, Group analysis, Electro-dialysis, Digestion under appropriate analytical conditions, Titrametric, Paper and Thin layer chromatography | Microwave oven technique for digestion followed by Ion Chromatography using<br><br>Ion-exchange resins, Inductively coupled plasma atomic emission spectroscopy, (ICPAES), ICP-MS. |

| | | |
|---|---|---|
| Non-Volatile Organic | Solvent Extraction, Stas- Otto, Digestion with ammonium sulphate, Sodium tungstate or related method. | HPLC, Paired ion extraction /<br><br>Chromatography, HPTLC, Supercritical fluid chromatography, Solid phase extraction, Micellar extraction, Affinity chromatography, , Microwave assisted Reaction system, Accelerated solvent Extractor, Sweep Co-Distillation Universal Trace Residue Extractor. |
| Anion | Dialysis, Chemical Digestion, Paper and Thin Layer Chromatography | Ion-Chromatography by Ion-Exchange resins. |

The above table clearly indicates that there are different methods of extraction both classical and modern. The classical methods are popular in the Indian forensic science laboratories dealing with a large number of toxicological cases. The poisons cover a broad spectrum unlike foreign countries. As Indian laboratories are smarting up with resources, modern methods are gradually replacing traditional methods which require discussions separately.

### 2.9.1 **Unit processes/operation in extraction methods**:

The various unit processes / operation related to extraction are given below for convenience. The details of the above will be available in any standard book on analytical Chemistry.

#### 2.9.1.1 **Solvent Extraction:**

A system of two immiscible liquid is required for the separation of material by solvent extraction. The active constituent should be unevenly soluble in the system thereby facilitating extraction of the constituent from one phase to the other. The efficiency of extraction is determined by distribution co-efficient (D).

$$D = \frac{\text{Total wt(gms.) of solute in the Organic Phase}}{\text{Total wt(gms.) of solute in the aqueous phase}}$$

If one of the two liquids contains a solute and the system is shaken and then allowed to settle, some of the solute will be transferred to other liquid. Each of the liquid in a mixture of two immiscible liquids of this kind is referred to as a phase. Thus, some of the solutes is transferred from one phase to the other in the two phase system. The amount transferred depends on the relative affinity of the solute for each of the two solvents (relative solubility). It is determined by D. Greater the value of D, greater is the efficiency of extraction.

The immiscible system may involve two organic solvents. The extraction for this system may be impaired due to formation of emulsion. Solvent extraction is a common technique in forensic toxicology related to biological matrices. Solvent extraction method has know been upgraded and made automatic viz. accelerated solvent extraction. In case of solid non-biological matrices, continuous extraction by a soxhlet may be employed i.e. continuous extraction.

2.9.1.2 **Distillation**:

The process involves heating a sample of liquid to convert it into vapour which is then allowed to flow in another location, where it is cooled, considering it back into a liquid. Vapour modification of the basic distillation process are used for specific Purpose viz. distillation under reduced pressure, fractional distillation, sweep co-distillation.

**2.9.1.2.1** Steam distillation**:**

Volatile substances can be separated or isolated from blood, urine homogenates or properly minced viscera by the operation of steam distillation. Steam is passed into the solution and the aqueous distillate collected by condensation. Toxicants from acidic distillation process include ethanol, methanol, phenol, halogenated hydrocarbons, cyanides etc. On the other hand toxicants from basic distillation process include basic drugs amphetamine, methadone and also aniline, pyridine, nicotine etc.

**2.9.1.2.2** Fractional Distillation**:**

This is a type of distillation which enable us to separate a mixture of volatile liquid differing in boiling point. A mixture of kerosene oil of mineral turpentine oil in an oil-water emulsion may be separated by steam distillation.

**2.9.1.2.3** Distillation under vacuum**:**

This is another type of distillation which provide separation of a thermally labile volatile compound at a low temperature without decomposition.

**2.9.1.2.4** Sweep co-distillation**:**

It is a special case of distillation that is based on the preferential volatalisation of organic compounds specially pesticides from oil, lipids or plant extracts using a stream of inert gas and subsequent isolation of volatiles on cola traps or solid adsorbent. It is a purge and trap technique involving dispersion of the sample in thin films on deactivated glass beads or florisil or alumina or silica gel or tenax as trapping media at elevated temperature. Universal Trace Residue Extractor (UNITREX) and Accelerated Solvent Extractor (ASE) i.e. the highly automatic extraction systems for rapid extractions of multiple samples work on this principle.

**2.9.1.3** Micro diffusion**:**

Micro – diffusion is a convenient and popular operation that facilitates toxicants (gaseous and volatiles) in blood, urine and gastric, aspirates to be detected or determined after isolation by various techniques. This is done by Conway Micro-diffusion dish (elaborated separately for convenience).

**2.9.1.4** Dialysis**:**

It involves separation of a crystalloid from a colloid by filtering through a semi permeable membrane. This separation method may be employed for the separation toxic cations and anions in a colloidal solution or dispersion or colloidal matrices specially biological materials including blood. The separation process may be accelerated by applying e.m.f. i.e. electro-dialysis.

**2.9.1.5** Sublimation**:**

This is similar to distillation except the sample is a solid to begin with and is converted directly into vapour and then back into solid. Sublimation is applicable to isolate a toxicant in solid matrices viz. naphthalene, an thracene which sublimes.

**2.9.1.6** Digestion or chemical treatment**:**

Sometimes active constituents (Toxicant) are separated on treatment with acid or alkali or digestion on a water bath or muffle furnace viz. biological matrices are digested on a water bath for 1 hour or above or digested in muffle furnace with acid / alkali / or Chemicals to isolate active constituent. Volatile inorganic poisons or phosphine, arsine and hydrogen sulphide are isolated from their salts on treatment with dilute acids.

**2.9.1.7** Microwave Digestion:

Matrices are digested with acids / alkalis in microwave oven to facilitate isolation of inorganic poison in organic matrices under a specific analytical condition of operation viz. operation of over at a specific microwave for sometime. The interaction of microwave with matrices results in production of heat with rise of temperature for which digestion occurs.

**2.9.1.8** Absorption**:**

It is a slow process (compared to adsorption at the surface) involving diffusion of one substance into the interior of absorbent material. Toxic gases and volatiles in oil are entrapped and enriched by the process using a tube containing diverse absorbing materials specific for a particular toxicant and on line detection and determination is facilitated.

**2.9.1.9** Chromatography**:**

Chromatography involves the separation of substances based on their relative affinity for two phases, one stationary and one mobile. Substances which have higher affinity for the mobile phases are moved or carried along with it and are thus separated from those with higher affinities for the stationary phase. Thus, the toxicants in molecular mixtures may be separated convenient under different chromatographic methods and operating condition in a particular Chromatography. There are different controlling parameters viz. nature of toxicant, mobile and stationery phase and temperature. Salient aspects of different Chromatographic methods are given below.

**2.9.1.9.1** Column Chromatography**:**

Separation of active constituent is achieved here by preferential absorption of active constituents on the adsorbent or stationary phase and also adsorption by the mobile phase. In this method a vertical glass tube is filled with a granular adsorbent. This adsorbent acts as the stationary phase. The sample is then added to the top of the column in the form of a solution in a suitable solvent or mixture of solvents. The system of solvent is then added as a mobile phase, which is to selected beforehand. As the solvent system flows through the column under the influence of gravity or pressure, various components of the sample mixture will migrate at different rates and then arrive at the lower end of the column at different rates i.e. time. Fractions collected at various intervals will thus contain the different component separated at different intervals. The fractions thus separated can be subjected to further analysis by different methods. The process is also known as elution.

The efficiency of separation in the column is dependent on the adsorbent material, selection of solvent system, nature of active constituent as well as flow of mobile phase. These controlling factors have been explored to develop different method of chromatography viz. HPLC, HPTLC,

affinity, gel permeation, ion-exchange chromatography and ion chromatography, solid phase extraction and super critical fluid chromatography. These have been discussed separately. The analytical condition required for separation of toxicants are either available in standard texts or references or may be developed conveniently by trial depending on nature of toxicant and matrices.

**2.9.1.9.2** Paper Chromatography**:**

The separation of active constituent occurs on cellulose paper as the stationary phase. This is the primitive method in chromatography methods and used for separation of organic dyes, pigments, inks, cations and anions.

**2.9.1.9.3** Thin Layer Chromatography**:**

The separation takes place kin a thin layer of adsorbent material such as alumina, silica gel G or cellulose coated onto an inert backing material such as glass plate, plastic sheet or alumina foil. The chromatography development is made by applying samples as small spots. The plate is then dipped in a chamber containing the developing solvent. The solvent is allowed to migrate for some distance and separation is achieved. There are different methods that are popular in TLC viz. ascending, decending, two dimensional etc. Quantitative separation is achieved by optimizing analytical conditions.

**2.9.1.9.4** High Performance Thin Layer Chromatography**:**

A type of thin layer chromatography wherein the stationary phase is designed to offer enhanced separation and resolution properties. The enhanced separation is due to special structural feature of adsorbent attained by special processing for which optimum resolution of molecular mixtures (for separation on chromatography) coated with specially prepared adsorbent. The system (HPTLC) is now fully automatic and multiple samples may be handled quickly, precisely and conveniently. Diverse literatures are available on different classes or organic sample for their separation in simple and complex matrices.

**2.9.1.9.5** High Performance Liquid Chromatography:

It is based on the different attraction of non-volatile analytes viz. drugs, pesticide, explosive, organics etc. between a liquid phase ( pumped through a column) and a solid phase ( packed within the column-stationary phase ). The operating parameter include composition of mobile phase, adsorbent, nature of analyte etc. for optimizations of resolution.

**2.9.1.9.6** Ion Chromatography**:**

It works on the same principle as in HPTLC. The composition of stationary phase and also the mobile phase is so selected that cations and anions can be separated conveniently. The active constituents in the matrix material and chemically bonded to molecules which have a fixed charge. In suitable form ion exchange resins can be packed into columns and used for separation of mixture of molecules which have the opposite ( because unlike charges attract ) viz. cations and anions. In some cases, the net charge on the column material or sample molecules or both is dependent on pH giving rise to greater flexibility.

**2.9.1.9.7** Gas Chromatography:

It is a procedure whereby volatile components of a mixture may be separated by partition between a solid or liquid stationary phase and a gaseous mobile phase. The efficiency in the separation is achieved by controlling several factors by the analyst viz. column type (capillary or wide bore), column length, column diameter, nature of liquid phase, carrier gas flow rate and temperature. This technique has now been hyphenated with other technique viz. SFC-GC, HPLC-GC-MS. The method is specially applicable for pesticide, drugs, volatile organic, solvents etc.

**2.9.2** Modern Methods of Extraction:

The methods include various methods in Chromatography as well as special extraction methodologies of the present generation viz. ion-pair formation, solid phase extraction, solid phase micro extraction, micellar extraction. Special types of digestion as a means of isolation of toxicant through chemical processing i.e. micro wave digestion and digestion by plasma source for inorganic samples are also emerging for their consideration in routine toxicological analysis. Thus, there are various methods of extraction under the head " Modern Method" which are either underutilized or unexploited in the Indian perspective due to lack of infrastructural facilities and analytical expertise. As it has become very difficult to cope up with much inflow of exhibits, modern methods are replacing traditional methods of extraction for speedy analysis. A few methods viz. HPLC, HPTLC, GLC, GC-Head Space, Micro Wave Oven Technique have become the methods of choice. The other methods including hyphenated techniques HPLC-GC, SPC-GC, GC-MS are under processing for standardizing in the Indian perspective. The available references in literature are to be followed for standardization or standard analytical conditions are to be arrived at after trials and comparison with other methods. However, the principles of the methods and analyticals concerning a few methods have been covered in the present monograph.

### 2.9.2.1 Head Space Technique:

The headspace method is specially suitable for the very fast separation of volatile components (alcohols, acetone, aldehydes) in complex biological matrices specially blood in mass-liquor and prohibition low related cases. This methods has the advantage that the risk of contamination of non-volatile component may be eliminated for on line analysis of toxicant by gas chromatography. The principle underlying head space analysis is that in a sealed vial at constant temperature, equilibrium is established between the volatile components of a liquid sample in the vial and the gas phase above it ( the head space ). After allowing the time for equilibrium (normally 15 minutes or so for 50 or more samples or more samples in a single run) a portion of the head may be withdrawn one by one from vials using a gas-tight syringe and injected to GC on line for further analysis of the separated components.

2.9.2.2 **Dynamic Headspace, Purge and Trap Technique**:

These techniques are basically modified or higher version of Headspace method to optimize the separation of volatiles for on-line analysis by GC.

In the purge and trap method, volatile compounds (toxicants) are liberated from the sample by bubbling with an inert carrier gas and subsequently either condensed in a receiver cooled usually with solid carbon-dioxide or liquid nitrogen adsorbed on a catridge filled with solid dsorbent material such as Tenax. It is polymer based diphenyl- p-phenylene oxide and is available in various forms. Tenax TA is a highly purified form of the polymer and stable upto375$^{o}$C and gives insignificant bleeds of organics. A material suitable for the recovery of low molecular weight compounds is Tenax GR which contains 23% graphite. This adsorbent is suitable for efficient trapping of compounds of low to medium polarity and recovering them quantitatively by either solvent extraction or thermal desorption. After some time trapped volatile are flash vaporized into a stream of carrier gas for GC analysis. Alternatively, catridges filled with activated charcoal can be used to trap the volatiles which are then extracted into a small volume of carbon disulphide prior to the analysis. This technique is widely used in the analysis of volatile compounds in water samples till date mainly because of the difficulty in interpreting the results at concentration below those which can be measured using ordinary headspace method. Purge and trap technique are similar to dynamic headspace sampling except that the gas is passed through the sample. Clearly any apparatus used for dynamic headspace sampling can also be used for purge sampling by using on appropriate sampling vessel. Both dynamic and purge methods are available in a variety of automatic systems, to enable separation of constituent of concentration in the ppb – ppt range.

2.9.2.3 **Solid Phase Extraction (SPE):**

The old observation in the extraction of drugs by adsorption on solid materials viz. Florisil ( A synthetic magnesium silicate ) or activated charcoal and selective elution by solvent thereafter has given with to solid phase extraction method. In this method, siliceous materials with relatively close size distribution (15 to 100 Mm ) and various silica bonded phases viz. n-octadecyl (C18, ODS), n-octyl (C8), n-hexyl (C6), ethyl (C2), methyl (C1), cyanopropyl (nitrile,

CN) aminopropyl (amino, APS etc.) have been employed as adsorbent with much greater efficiency of separation. The method is based on the use of small tubes or catridges filled with 100-500 mg. Of an appropriate adsorbent as state above. There lies however a recently developed type of SPE catridge in which very small particles of the adsorbent are enriched in a well of PTFE micro fibrils having similar efficiency as in a packed column but require less pressure drop. The sample is applied to the SPE catridge ether from a syringe or sampling manifold by applied pressure or suction at the lower end. Components are subsequently recovered by solvent flashing. The method is attractive because of the small amounts of the sample and materials necessary and also much greater speed of the procedure compared to classical adsorption method.

Analyte concentration may often be achieved more easily with SPE than with liquid-liquid extraction while use of SPE column to concentrate an analyte from solvent extract may provide a quicker and possibly safer alternative to solvent evaporation. A major advantage of SPE is that latch processing can be simplified. Further feature when screening for unknown is that a range of analyte can be extracted simultaneously although this may create problem if analyte of a single component is required. Moreover, SPE columns are expensive and it may not be possible to retain very water soluble analyte. The SPE protocol is now available.

Solid Phase Extraction (SPE) cartridges are used primarily to clean up samples for analysis and/or concentrate samples to improve detection limits. The lack of sufficient sample preparation will result in poor detection limits, identification and quantitation errors, contamination problems and rapid deterioration of GC or HPLC column performance. SPE techniques usually provide better sample cleanup and recoveries than liquid-liquid extraction techniques. SPE uses small volumes of common solvents, requires very simple laboratory skills, does not require the use of highly specialized laboratory equipments and allows rapid sample throughput. A liquid sample or solid sample dissolved in a solvent is poured into the conditioned SPE cartridge. Vacuum or pressure is used to force the sample through the sorbent in the cartridge. In SPE, vacuum manifold is normally used to simultaneously process multiple cartridges. Usually, SPE methods are designed to retain the analytes of interest; other sample components similar to the analytes also will be retained. The analytes of interest are then eluted from the sorbent using another solvent. This solvent is collected for analysis for additional processing.

**SPE Cartridges**

An SPE cartridge is composed of three basic parts:

1) Cartridge or tube body

2) Frits

3) Phase or sorbent

**Cartridge or Tube Body**

The cartridge body usually is a syringe like barrel made of serological grade polypropylene.

**Frits**

The frits are used to hold the sorbent in the barrel and to act as a particulate filter.

**Phase or Sorbent**

The most common SPE phases are bonded silica-based materials. Irregular shaped, 40 µm silica particles with 60Å pores are used as the starting material. Various silanes are used to attach functional groups to the accessible areas of the silica particle. In addition, several non-silica based phases are commonly used. Solvent reservoirs can be used to increase the volume of barrel above the phase. Large amounts of sample or solvent (up to 75 ml) can be added directly to SPE cartridges in one volume instead of in small increments. Coupling fittings are used to attach the reservoirs to the SPE cartridges.

**Phases:**

There are three types of phases: normal, reverse and ion- exchange.

**Normal Phase**

Table 2 lists common normal phase sorbents. All of these phases are polar and are used to retain (extract) polar analytes. For normal phase sorbents, solvent strength increases as the solvent becomes more polar. For example, a retained analyte will completely elute from a normal phase sorbent in a smaller volume of methanol than chloroform. All of the solvents in Table 1 are commonly used with normal phase sorbents. Mixtures of two solvents often are used to refine the solvent strength for optimal sample cleanup and analyte recovery.

**Reverse Phase**

All of these phases are non-polar and will be used to retain (extract) non-polar analytes. For reverse phase sorbents, the solvent strength relationship is the opposite from normal phase sorbents (Table 2). For reverse phase sorbents, solvent strength increases as the solvent becomes more non-polar. For example, a retained analyte completely elutes from the sorbent in a smaller volume of acetonitrile than water. In most cases, the solvents used with reverse phase sorbents are limited to water, methanol, isopropanol and acetonitrile as mentioned in Table 1 (Reverse Phase). On occasion, acetone or dichloromethane may be used as an elution solvent for highly retained analytes.

**Ion Exchange Phase**

Ion exchange phases are more dependent on pH, ionic strength and counter ion strength than solvent strength. Ion exchange phases depend on ionic interactions as the primary retention mechanism. Ionic interactions occur between an analyte molecule carrying a positive or negative charge and a sorbent carrying an opposite charge. There are two groups of ion exchange phases. The cation exchange phases retain positively charged or "cationic" compounds. Amines and carboxylic acid are not charged species. They can be charged by varying pH. The anion exchange phases retain negatively charged or "anionic" compounds. Table 3 lists the classifications and characteristics for several common ion exchange phases charge).

**Table 1: Solvent strengths**

| NORMAL PHASE | Weak | REVERSE PHASE |
|---|---|---|
| Hexane | | Water |
| Isooctane | | Methanol |
| Toluene | | Isopropylalcohol |
| Chloroform | | Acetonitrile |
| Methylene chloride | | Acetone |
| Tetrahydrofuran | | Ethyl acetate |
| Ethylether | | Ethylether |
| Ethyl acetate | | Tetrahydrofuran |
| Acetone | | Methylene chloride |
| Acetonitrile | | Chloroform |
| Isopropylalcohol | | Toluene |
| Methanol | STRONG | Isooctane |
| Water | | Hexane |

Table 2: Sorbents

| |
|---|
| NORMAL PHASE |
| Cyano (CN)* |
| Diol (DIOL) |
| Silica (SI) |
| Amino ($NH_3$) $^{+1}$** |
| REVERSE PHASE |
| Octadecyl (C18 OR ODS) |
| Octyl (C8) |
| Methyl (C 3) |
| Phenyl (Ph) |
| ION EXCHANGE PHASE |
| BENZENE SULFONYLPROPYL (scx) |
| Quaternary amine (SAX) |

*may be used as a reverse phase also

**may be used as an ion exchange also

Relative counter ion exchange.

Table 3: Ion Exchange phases

| CATIONS | | ANIONS | |
|---|---|---|---|
| $Li^{+1}$ $H^{+1}$ | 0.5 | $OH^{-1}$,$F^{-1}$,Propionate | 0.1 |
| $Na^{+1}$ | 1.5 | Acetate,Formate | 0.2 |
| $(NH_4)^{+1}$ | 2.0 | $(HPO_4^{2-})$ ,$(HCO_3^{-1})$ | 0.4 |
| $Mn^{+2}$,$K^{+1}$,$Mg^{+2}$,$Fe^{+2,+3}$ | 2.5 | $CI^{-1}$, $(NO_2)^{-1}$ | 1.0 |
| $Zn^{+2}$,$Co^{+2}$,$Cu^{+1}$,$Cd^{+2}$ | 3.0 | $(HSO_3)^{-1}$ ,$CN^{-1}$ | 1.5 |
| $Ca^{+2}$ | 4.5 | $(NO_2)^{-1}$ | 4.0 |
| $Cu^{+2}$ | 6.0 | $(CIO_3)^{-1}$ | 4.5 |
| $pb^{+1}$,$Ag^{+1}$ | 8.5 | $(HSO_4)^{-1}$ | 5.0 |
| $Ba^{+1}$ | 10.0 | Citrate | 9.5 |
| | | Benzene sulfonate | 10.0 |

For each category, the highest selectivity counter ions were normalized to 10; thus, the values are relative.

### 2.9.2.4 **Solid Phase Micro Extraction (SPME):**

Solid phase micro extraction (SPME) is an extraction technique for organic compounds in aqueous samples in which analytes are adsorbed directly from the sample onto a fused silica fiber that is coated with an appropriate stationary phase. When the fiber is inverted in the sample, the analyte portion from the sample matrix enters the stationary phase until equilibrium is reached. The fiber in then inserted into the injection port of a gas chromatograph (GC) where it is heated and the analytes are rapidly thermally desorbed into a capillary GC column for analysis.

The fiber holder can be used manually with any GC having appropriate straight inlet liner. The holder is designed to be used with a reusable, replaceable fiber assembly. Each fiber can be used for 50 to 100 analysis or more depending on the particular application and the care that is given. The holder consists of a stainless steel barrel, a bisack polymeric plunger on adjustable depth gauge with needle guide a stainless steel relating nut.

Each disposable fiber assembly for manual injection has been outer septum piercing needle with a flanged brass ferrule a gray sealing septum and an inner fiber attachment needle. The fiber attachment needle has a coated fused silica extraction fiber secured at one end and a colour-coded thread at the other. A tensioning spring is located between the nut and the sealing septum. The nut color indicate the type of bonded phase coating on the fiber.

### 2.9.2.5 **Super Critical Fluid Extraction:**

Gases above their critical pressure and temperature are in a supercritical state, intermediate between that of a gas and liquid. Supercritical fluids have strong extraction properties because

the solubility of compounds in fluid is close to that of a true solvent and much lower viscosity allows it to percolate through packed bed of sample. Thus, knot only therein an efficient contact between the extracting fluid and the sample but the fluid is easily removed when it is released from its supercritical state.

Carbon dioxide is nearly always the chosen gas for SPE in view of its innocuous nature and mild critical condition namely critical pressure of 75 bar and a critical temperature of 310C which are relatively easy to achieve at present.

The sample holder is composed of a number of small stainless steel catridges which are filled with the sample in a particular state. Solid sample such as soil or sediment are packed into the cartridges without any pretreatment and aqueous samples can be flashed through the cartridges filled with an appropriate adsorbent to concentrate all of the contaminates. The catridges are subsequently fed into the extraction oven and the carrier gas line which at this stage consists of supercritical carbon dioxide. The extracted compounds are carried to the cold trap and condensed after the heated constriction which restore carbon dioxide to its true gaseous state. After the appropriate extraction period, the circulation of coolant cases and trap is rapidly heated to vaporize the components. At the same time the column temperature is programmed according to the required condition. The adjustable split partitions the sample size to avoid the possibility of overloading effect, SFC is suitable for extraction of pesticide traces in solid and aqueous samples.

2.9.2.6 **Micellar Extraction**:

Micellar extraction is a special type of extraction procedure that appears to be unique in the separation of drugs, plant poisons and pesticides in biological matrices (viscera). In the extraction of active constituents as above, micellar environment of surfactant kef different classes is employed. Surfactant or surface active agents at a particular concentration in solution known as critical micellar concentration (CMC) form micelle or association colloid. At this concentration or above marked changes in the properties viz. viscosity, conductance, electrical conductance are exhibited. Surfactant in solution also acts at the interface of a two phases system of oil and water or organic solvent and water resulting solubilisation of one phase into the other. An emulsion or micro-emulsion is formed by the process. The emulsion may also be stabilized by increasing the ionic concentration of additives including surfactant. Biological matrices (Viscera) in the Indian perspective contains fats, degraded protein and colouring matter etc. resulting extraction of active constituent difficult. In the solvent extraction process if surfactant is added to the extractant (organic solvent) deproteinization and also solubilisation with the formation of emulsion occur due to micellar interaction. The emulsion thus formed is due to solubilisation of fat in biological matrices in the added solvent (containing traces of water) with the simultaneous formation of emulsion occurs due to micellar interactions. The emulsion thus formed due to solubilisation is destabilized on increasing the concentration of surfactant in the system. As a result, fats are separated and separated as semisolid material due to lowering of zeta potential between the electrical double layers of the colloidal system. As protein and fats are separated out, the supernant liquid containing active constituent may be

extracted for poison by organic solvents. The detailed analytical conditions have also been presented at places in the manual.

2.9.2.7 **Microwave Accelerated Reaction System**:

The method of extraction is used for isolating pesticides in biological materials especially in liver and kidneys. In this process, the sample is subjected to rapid heating with organic solvent by microwaves at elevated pressure resulting isolation of active constituent. The biological material (1 – 2 gms.) is placed inside a microwave transparent vessel with a polar solvent o ionic solution (usually an acid) and is subjected to rapid heading by microwave in a Microwave accelerated reaction system (digester). The analytical conditions (temp., time of digestion, pressure) may very depending on active constituent and nature of sample viz. monocrotophos and phosphamidon are successfully extracted within 15-20 minutes from viscera using dichloromethane as a solvent at 80-1000C and 100 Psi. However, optimization of analytical conditions to covers different classes of pesticides are required for a rapid extraction by this method. The method finds application in the digestion of biological materials for isolation of some toxic metals (Cu, As, Pb etc.) and determination by AAS and ICP thereafter.

2.9.2.8 **Universal Trace Residue Extraction**:

It's a system that has been developed for the recovery of pesticides and organic residue from a wide range of samples including biological materials. It is based on the principle of sweep co-distillation that relies on preferential volatalisation of pesticides or other organic chemicals from biological materials, lipids, plant extract using a stream of inert gas and subsequent isolation of volatiles on cold traps of solid adsorbents. It is a purge and trap technique involving dispersion of the sample in thin films on deactivated glass beads at elevated temperatures.

The extractor system is specifically designed to recover volatile, thermally stable organochloro and organophosphorous from lipids, meat, butter, viscera etc. At present the distillation tube does not contain glass beads or glass wool as it renders less recovery. Florisil in conjunction with sodium sulphate has been found satisfactory for trapping many different classes of volatile organic compounds Alumina, Silica gel and Tenax are materials that have potential for used as trapping media with advantages over Florisil in specific applications. The method is expected to fail for thermally labile pesticides. The consumption of solvent is minimum. The method requires optimization of analytical conditions before its application to biological samples (viscera) in forensic cases. However it is a 2 in 1 process i.e. extraction cum clean up in a very short time with a very economical use of organic solvents unlike solvent extraction methods.

2.9.2.9 **Accelerated Solvent Extraction:**

The name of method signifies multiple sample handling in a very short time by a very updated extraction system which also works in the same as in the case of Universal Trace Residue Extractor i.e. Sweep Co-distillation. In this method a commonly used solvent is pumped into an extraction cell containing the sample which is then brought to an elevated temperature and pressure. Minutes later, the extract is transferred from the heated cell to a standard collection

vial for clean up analysis. The entire extraction process is fully automated and performed in minutes for fast and easy extraction of multiple samples with a very minimum solvent consumption. The standard or optimum analytical conditions are to be arrived for its application to biological matrices in forensic toxicological work covering a broad spectrum of pesticides. However, the method has been found to be effective for soil samples.

2.9.2.10 Size Exclusion Chromatography:

The technique can be used to advantage as a preparation technique for the prior fractionation of oils, fats, environmental samples etc. into discrete molecular weight fractions and as a way of removing very high molecular weight material from complex sample.

2.9.2.11 **Ion-Pair Extraction**:

This method is applicable for the extraction of highly water soluble organic compound. These compound can not extracted from samples by direct solvent extraction. The difficulty has been overcome by forming ion pair with a suitable reagent viz. quarternary, ammonium salts as ion-pairing agent. The ion-pair formation is an attraction between a positive and a negative charge. This is not a reaction it is only an attraction. Once this pair is formed it will act exactly like an organic molecule and not like as ionic compound at all. The extraction may be carried out thereafter by direct solvent extraction preferable in presence of a buffer. The method is also applicable for extraction of drugs in the form of quarternary salt in urine or blood. The biological matrices are deproteinized and treated with a dye(selective) in presence of buffer to form drug-dye complex which is extracted by organic solvent. The dye is destroyed by the action of acid or alkali and the drug is isolated for analysis.

2.9.2.12 **Hyphenated Techniques (HPLC-GC AND SFC-GC**):

The hyphenated techniques viz. HPLC-GC and SFE-GC allow complex samples to be pre-fractionated rapidly according to molecular weight or chemical classification on a suitable HPLC pre-column or SFC and appropriate fractions then separated by on line capillary GC. The coupling of two technique has been made by a bypass valve fitted with a sample loop of appropriate volume. Selected fractions are passed to the capillary column via a large retention gap which utilizes the process of concurrent solvent evaporation to retain the fraction in the retention gap thus avoiding any preliminary contact with column must be adjusted suitably.

2.9.3 **Extraction of Volatile Poisons by Distillation**:

The method is applicable for volatile organic or inorganic poison under different conditions of PH acidic and alkaline.

2.9.3.1 **Neutral and Acid Distillation**:

Procedure :

50 gms. of viscera (properly minced), stomach contents, vomit or other materials to be examined are knot to be mixed together but should be examined separately. They should be brought to the consistency of a thin gruel by adding 3-5 times of distilled water and acidified with tartaric or sulphuric acid and submitted to steam distillation. The condenser and the receiving flask should be well cooled especially during the hot season with ice, the outlet of the condenser being dipped in a little water or NaOH solution or any other reagent as necessary. A few pieces of pumice store may be taken in the flask to prevent bumping. It is better to collect the distillate in 4 or 5 fractions, of which the first one should not exceed 20 ml. and the remaining fractions should be 50 ml. each. The flask containing the material should preferably be heated on the water bath. If phosphorous is suspected, the distillation should be carried out in a dark room and a black screen placed between the burner and condenser so that phosphorescence may be seen clearly. The distillate as above contains alcohols, paraldehyde, other aldehydes, acetone, carbolic acid and phenol, carbon disulphide, thymol, camphor, turpentine, nitroglycerine, benzene and other volatile acids etc. For cyanides and carbolic acid, the distillate is collected in 10 ml. of 0.1N sodium hydroxide solution.

2.9.3.2 **Alkaline Distillation**:

After the completion of the acid distillation, the flask is allowed to cool and its contents are rendered alkaline by adding NaOH solution. The alkaline mixture is then distilled again in the same way as before and the distillate collected in two fractions – the first fraction of about 20 ml. and the second fraction of about 50 ml. The distillate from the alkaline mixture may contain aniline, pyridine, nicotine, conine, ammonia and volatile bases.

2.9.4 **Extraction of Toxic Metals in Matrices:**

2.9.4.1 **Non-Biological Matrices**:

The non-biological matrices may be subjected to chemical analysis by preparing solution of samples and their systematic group analysis.

2.9.4.2 **Biological Matrices**:
The extraction of metals in biological matrices may be carried out by the following methods.

Dry Ashing Method;
Wet Digestion or Acid Digestion Method;
Fresenius and Babo Method;
Selective Chemical Treatment.

The organic matter which constitute the bulk portion are destroyed by chemical means to get the active constituents (metal ions) free completely for qualitative and quantitative analysis.

2.9.4.2.1 **Dry Ashing Method**:

About 10 gm. Of tissue or other biological materials is taken in a silica crucible and heated in a Bunsen burner for removing the moisture and partially destroying the organic material. Then the crucible is kept in a muffle furnace. The temperature of the furnace is raised up to 5500C and at this temperature the incineration of the organic matter is performed by keeping the silica crucible for one hour. After incineration in complete, the crucible is taken out. The colour of the residue is noted when hot because in presence of zinc the residue assumes yellow colour while in presence of copper the colour of the residue is somewhat bluish green. The residue in the silica basin is boiled with 10 ml. of 4(N) hydrochloric acid and then filtered. The clear acidic solution is tested for metallic poisons such as copper, bismuth, zinc, barium etc. by performing general group analysis by suing micro methods, chromatographic and instrumental techniques.

2.9.4.2.2 **Wet Digestion Method**:

Procedure : 100 gms. of biological materials or 10 ml. of blood are taken into a large Kjeldahl flask and 20 to 40 ml. of Conc. HNO3 are added to cover the material and flask is gently heated in a small flame when the mass begins to liquefy. The heating is continued until the liquefaction of the material is complete and that must be done in the presence of copious brown fumes of nitrogen dioxide in the flask. At this stage about 20 –30 ml. of Conc. H2SO4 are added and the flask is heated strongly over a wire gauge and Conc. HNO3 is added in drops (by using dropping funnel) to the contents of the flask at the rate of about 10 drops per minute so that the atmosphere in the flask must at no times be free from brown fumes. Heating is continued until all organic matter is destroyed and the liquid becomes clear and colourless or straw coloured.

To find out if the oxidation is complete, the flask is heated without adding any HNO3. If there is any un-burnt organic matter, the liquid begins to darken and if the digestion is complete no darkening takes place and the white fumes of SO3 are given off. In the former case, the addition of HNO3 and heating are continued further till the organic matter is completely oxidized. strong is continued for 15 minutes more to expel the nitric acid completely. Then, after cooling 25 ml. of saturated ammonium, oxalate solution is added. The liquid is boiled until SO3 fumes appear. This ensures complete removal of HNO3. It is then cooled, diluted with an equal volume of water and carefully transferred to a beaker. The beaker is heated on a hot plate or sand bath to expel the excess H2SO4. The solution is cooled and diluted with water in such a way that the strength of acid is in the neighborhood of 10%. At this stage a precipitate may be formed which contains the insoluble salts of lead, bismuth, tin, barium, strontium or silver etc. The precipitate is filtered off and tested for the metals mentioned above. The filtrate will now contain all other metals except mercury. It is subjected to systematic group analysis and quantitative determination thereafter as and required.

2.9.4.2.3 **Fresenium and Babo Method (for Mercury):**

The nitric-sulphuric acid method of destruction of organic matter is not at all suitable for mercury, which is almost completely lost by volatilsation. The method is considered most

suitable for liberation of mercury although there is a possibility of some loss of mercury by vaporisation.

Procedure: A definite amount of biological material viz. 20-25 gms. of viscera or 5-10 ml. of blood is taken in a flask fitted with a reflux condenser. In the case of viscera or other solid material sufficient water is added to make a gruel like consistency. One third of its volume of chemically pure hydrochloric acid and a few gms. of solid KC1O3 are added. The content are mixed by shaking. The mixture is heated over a wire gauge on a burner flame or on a boiling water bath. Small amount of KC1O3 is added time to time and the flask is shaken. Chlorine gas evolves. The heating is continued until the contents of the flask becomes a uniform, straw coloured liquid free from organic matter except some fatty substances in suspension which can not be oxidized. If heating for an hour after the last addition of KC1O3 produces no darkening of the mixture, the oxidation of organic matter may be taken as completed. It takes 4-6 hours to attain the stage. It is filtered and washed with water. The filtrate and washings are collected. Sufficient sodium sulphite or bisulphate is added to reduce the excess of chlorine into hydrochloric acid. The liquid is warmed on water bath and a current of air is passed to expel the excess SO2. The solution is now ready for analysis.

2.9.4.2.4 Selective Chemical Treatment:

A few toxic metals viz. arsenic and antimony in their specific oxidation state (+3) may be subjected to the reduction process by nascent hydrogen (by the reaction between zinc and dil sulphuric acid) for isolation of metals in matrices viz. burnt bones, nail, hair and non-biological matrices like food preparation, drinks, tea, coffee etc. in the form of their volatile hydrides (ASH3 and SbH3). The process may be carried out by Gutzeit or Marsh Berzelius method. This is actually two in one method for isolation and on line detection or determination.

In case of presence of As and Sb in their higher oxidation state (+5), reduction to +3 state is to be carried out prior to chemical treatment. The method and analytical have been presented separately. The analyst should not be biased by positive findings as there are chances of interference due to the presence of Phosphorous, sulphide, As or Sb in the matrix itself. A blank test should invariably be carried out as As or Sb may be present in matrices or the reagent.

2.9.5 **Extraction of Toxic Anions in Forensic Matrices**:

The extraction of toxic anions in non-biological matrices require very minor processing and clean up. In- convenicneces are felt in case of biological matrices viz. viscera, stomach and gastric contents, stomach wash, urine and blood. The extraction procedures include the following method.

Protein Precipitation.
Dialysis.
Selective Chemical Treatment.
Micro Diffusion.
Ion Chromatography

2.9.5.1 **Protein Precipitation**:

A slurry of the sample (minced viscera 10-20 gm. of stomach contents or gastric lavage etc. is prepared. The protein in the sample is coagulated by adding 0.5 – 1.0 gm. of ammonium sulphate. It was filtered through a 2 cm. layer of cotton wool held in the barrel of the syringe. The excess of ammonium sulphate remaining in the filtrate is precipitated by methanol. The supernant liquid is collected and evaporated to a small volume on a water bath. The concentrated liquid is ready for analysis of anions.

2.9.5.2 **Dialysis**:

10 gms, of the tissue is cut into small pieces and placed in a cellophane membrane made into the shape of a bag. The bag is then slowly rotated in a beaker containing 100 ml of distilled water by means of an electrical motor or mechanical device. Dialysis occurs rapidly. After one hour, the water in the beaker is replaced by fresh water and the bag is rotated for further half an hour. The water is then taken out, mixed with the previous fraction and evaporated on water bath to a small volume. This is filtered, if necessary and tested for toxic anions.

2.9.5.3 **Selective Chemical Treatment**:

Phosphides and sulphides present in biological matrices viz. Viscera, stomach content and gastric lavage is subjected to treatment with dilute acid on hot plate or water bath. The toxicants that are liberated in the form PH3 or H2S are subjected to chemical analysis. This has been covered separately in the monograph.

2.9.5.4 **Micro-Diffusion**:

The unit operation may be employed for biological matrices viz. blood, urine and stomach wash and also non-biological matrices viz. water, drinks, tea, coffee containing traces of toxic anions (cyanide, phosphide, sulphide,) by using selective liberating, sealing and detection reagent in Conway Micro-Diffusion assembly. (described as and when required in case of volatile poisons. Viz. ethanol, methanol, acetaldehyde, chloroform, toxic gases viz. carbon monoxide, phosphine, ammonia, hydrogen sulphide), and toxic anions (cyanide, nitrate etc.).

**Description of Conway Micro-Diffusion Assembly:**

The assembly consists of obrink type, polypropylene cells with clear polystyrene covers and the cells have an outer most annular sealing well, an intermediate annular well for the sample and the liberating agent and a center well for the reagent which is used to trap the diffusing gas or vapour.

The sealing agent (usually 2 ml.) is introduced into the outermost sealing well. An approximate amount (1 ml.) of liberating agent (usually same as sealing agent viz. 10% $H_2SO_4$ as sealing and liberating agent for carbon monoxide and saturated sodium carbonate solution for methanol and ethanol etc. is placed as a pool in one half of the intermediate well i.e. in the sample well. Trapping agent (say $PdCl_2$ in care of carbon monoxide turning to black, acidified potassium

dichromate turning to green in case of methanol or ethanol etc.). Sample is introduced (1 ml.) into the other half of sample well taking care that it does not mix with the liberating agent. The cover is placed and cell is rotated to effect airtight seal. The assembly is fitted back & forth to mix the sample and liberating agent. The cell is placed on table or water bath, if needed for reaction. The control sample cell is also prepared using same reagent but without sample i.e. 1 ml. of water in place of sample. The color charge in central compartment is noted.

2.9.5.5 **Ion Chromatography**:

The preferential exchange of ions on ion-exchange resins packed in the column of ion chromatograph renders separation of anions by using mobile phases usually buffers of diverse PH.

2.9.6 **Extraction of Non-volatile Organic Poisons**:

The group includes broad spectrum of pesticides different classes of drugs viz. acidic, basic, neutral and amphoteric, plant poisons (specially alkaloids, glycosides etc.). Out of the above classes of poisons, pesticides account for more than 80% of fatal cases of poisoning in most of the states of India. Thus, the extraction of insecticides requires separate discussion. At the same time the extraction of drugs of diverse classes and plant poisons are to be elaborated for their applications

2.9.6.1 **Extraction Pesticides in Matrices**:

The extraction of pesticides in biological materials viz. viscera, stomach contents, gastric lavage, blood is difficult due to the interferences of fat, degraded protein and colouring matter in the matrices. The extracts require proper clean up except for Micellar method. The extraction in case of non-biological matrices is cumbersome and requires either minor clean up of no clean up. The procedures may be described as follow. The methods described hereunder are based on solvent extraction cum stripping under diverse conditions viz. nature and condition of matrices, use of organic solvent etc.

2.9.6.1.1 **Method – I**

Biological materials (viscera, stomach content or gastric lavage etc.) are macerated into a fine slurry by mixing with equal amount of anhydrous sodium sulphate and transferred into a conical flask with an air condenser. 50 ml of n-hexane are added to the flask and heated on a hot water bath for one hour. The contents are cooled and filtered. The residual slurry is extracted twice with 25 ml portion of n-hexane. The filtered n-hexane fractioned are combined and taken into a separating funnel. This hexane layer is vigorously shaken with 15 ml, 10 ml and 10 ml portion of acetonitrile which are previously saturated with n-hexane. The acetonitrile layers are mixed and taken into another clean separating funnel and diluted 10 times with distilled water. 25 ml of saturated sodium sulphate solution are added to it and extracted thrice with 25 ml portion of n-hexane. The n-hexane layers are combined, concentrated to 5 ml by evaporating on water bath and 5 gms of anhydrous sodium sulphate added. The extract is evaporated as and when required for analysis.

2.9.6.1.2 **Method – II**

50 gms of macerated tissues or biological materials are mixed with equal amount of anhydrous sodium sulphate and 100 ml of acetone in a conical flask and then refluxed on hot water bath for one hour. After cooling the acetone extract is filtered. The residue is extracted twice with further 50 ml portion of acetone. The acetone fractions are combined and concentrated by evaporation up to 50 ml for further processing (clean up). The above acetone extract (50 ml) is taken into a separating funnel and diluted with 150 ml of water. To it 20 ml of saturated solution of sodium sulphate is added. The contents are extracted thrice with 25 ml portions of chloroform with gentle shaking. The chloroform extracts are combined, washed with water – acetone mixture (1 : 1) and finally with 50 ml of water. The washed chloroform layer is passed through anhydrous sodium sulphate and then evaporated to dryness by passing air. The residue is ready for analysis.

2.9.6.1.3 **Method – III**

Extraction of Pesticides in stomach wash, urine and vomit:

The sample (20 ml of stomach wash or urine or 20 gms of vomit) is taken in a conical flask. To it 50 ml of n-hexane is added. It is refluxed on a water bath for half an hour. After cooling the liquid is filtered, mixed with 20 ml of n-hexane and taken in a separating funnel. The n-hexane layer is separated; passed through anhydrous sodium sulphate and evaporated to dryness by passing a current of dry air through it. The residue is ready for analysis.

2.6.1.4 **Method – IV**

Extraction of Pesticides in Blood :

Sample of Blood (20 ml) is mixed with 10 ml of 10% sodium tungstate solution and 15 ml of 1(N) sulphuric acid, shaken for two minutes and then filtered. The filtrate is kept reserved. The residue is washed with two 15 ml portions of 0.1(N) sulphuric acid. The washing are collected, mixed with filtrate (kept reserved), transferred into a separating funnel and extracted thrice with 20 ml portion of n-hexane. The hexane layers are combined, passed through anhydrous sodium sulphate and the solvent is removed by passing a stream of air as stated in the previous methods. The residue is kept reserved for analysis.

2.8.6.1.5 **Method – V**

Direct Solvent Extraction of Pesticides followed by Clean Up for Biological Materials :
The biological materials (50 gms of viscera) are biological materials are mixed with 5 gms of ammonium sulphate and homogenized. After addition of 100 ml of diethyl ether, the mixture is shaken at intervals and kept overnight. It is filtered and concentrated as before. The concentrated extract is cleaned up by passing through a chromatographic column (diameter, 1") containing three successive layers of different lengths viz. 2" layer of alumina (top layer), 1" of

activated charcoal (middle) and 1" layer of anhydrous sodium sulphate (bottom) previously washed with ether. The eluate is evaporated to dryness as before the residue is kept reserved for analysis.

2.8.6.1.6 **Method – VI**

Extraction of Pesticides by Steam Distillation cum Solvent Extraction followed by Clean Up for Biological Materials :
When the biological materials are clean and purified i.e. less degraded and contains very little fat and colouring matter, the following method may be carried out.

Procedure : 50 gms of biological materials are treated with a few drops of phosphoric acid and steam distillation for 15 minutes. The distillate (100 ml) is collected and subjected to solvent extraction with 100 ml of diethyl ether in 20 ml portion. The ethereal layers are collected during extractions, combined and subjected to clean up by passing through Chromatographic column as before.(method V).

2.9.6.1.7 **Method – VII**

Isolation of Pesticides in Non Biological Materials : Matrices : 20 – 25 gms of rice or more, if available, 100 ml of drinking water or tea or coffee or milk, wearing apparel (20 – 25 round pieces cut out from fabric, each of 1" diameter), 20 – 25 gms of soil, sand, grains or cereals.

Procedure : For the above materials direct solvent extraction is carried out with 50 – 100 ml of diethyl ether without adding ammonium sulphate. The ethereal extract is concentrated to 20 ml and cleaned up by column chromatography as stated above. The ethereal extract is collected, evaporated to dryness by passing stream of air. The residue is kept reserved for analysis.

2.9.6.1.8 **Micellar Extraction of Pesticides in Biological Matrices**:

50 gms of biological materials (viscera) are mixed with 5 gms of ammonium sulphate and homogenized. After addition of 100 ml of diethyl ether, the mixture is shaken at intervals and kept overnight. It is filtered. The ethereal extract is taken into a separating funnel. 10 mg of sodium lauryl sulphate (an anionic surfactant found to be most suitable out of different classes of surfactant cationic, anionic and neutral) is added to it and stirred gently. On setting, fat in liquid and semi-solid form is separated and taken off from the system. The addition of surfactant is continued till all the fatty materials and proteins are separated and settled at the bottom. The end is indicated by a charge of dark colour of ethereal layer to colourless. The ethereal extract is shaken with 25 ml portion of water twice. The ethereal layer is collected. In case of emulsion formation, ethereal layer is collected by breaking the emulsion with excess ether and gentle stirring. The collected ethereal layer in dried over anhydrous sodium sulphate to remove traces of water. The ethereal layer is decanted and evaporated to dryness as before. The residue is kept reserved for analysis.

2.9.6.1.9 **Extraction of Pesticides in Fruits, Vegetables, Butter fat by Universal Trace Residue Extractor:**

Procedure : The sample is extracted by direct solvent extraction with dichloromethane. The extract is dried with granular anhydrous sodium sulphate in a column. The dried extract thus obtained is concentrated by a stream of nitrogen. The concentrated extract is then subjected to sweep co-distillation in the Universal Trace Residue Extractor at 230oC for 30 minutes by passing nitrogen (230 ml / min) and using sodium sulphate 10% florisil (1 : 1). Elution is made by 5 ml of 10% acetone in hexane or 5 ml of dichloromethane. The extract is collected for analysis. The applications of this method to different pesticides for their analysis in biological matrices require further standardization to set up optimum analytical conditions to cover different classes of pesticides.

3.9.6.1.10 **Extraction of Pesticides in Sediment, Soil, Drywates and Tissue by Accelerated Solvent Extraction (ASE):**

Procedure : The sample is mixed thoroughly or passed through a 1 mm sieve. Sufficient sample is introduced into the grinding apparatus to yield at least
10 –20 g after grinding. The sample is air dried at room temperature for 48 hours in a glass tray or on hexane cleaned aluminium foil. The drying may also be made by mixing with anhydrous sodium sulphate until a free flowing powder is obtained ( Air drying is not recommended for volatile pesticides Gummy, fibrous or oily materials not amenable to grinding should be cut, shredded or otherwise separated to allow mixing. These may be grinded after mixing with anhydrous sodium sulphate 1 : 1 proportion ). A cellulose disk is placed at the outlet and end of the extraction cell. Approximately 10 g of each sample or 20 g of each sample in 11 ml or 22 ml extraction cell. (Surrogate spikes and matrix spikes may be added to the appropriate sample cell). The extraction cells were placed into the auto-sample tray and the collection trays are loaded in appropriate number (up to 24) kef 40 ml pre-cleaned, chapped vials with septa. The conditions for extraction in ASE are set for extraction of pesticides by using acetone : hexane (1 : 1, v/v) as the solvent. The operating conditions include oven temp. of 100oC, pressure at 1500 psi, oven heat time and static time each of 5 minutes and flush volume in the proportion of 60% of extraction cell volume. The extracts are collected for analysis. 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.(1)

2.9.6.2 **Extraction of Drugs, Glycosides and Plant Poisons:**

This group comprises alkaloids, glucosides, barbiturates, phenothiazines, salicylates, sulphonal groups, sulphonamides drugs of different classes (drugs of abuse), plant poisons and certain animal poisons etc. Te extraction of these poisons depends on their solubility at different pH i.e. at low or high acidic or alkaline condition and also differential solubility in organic solvents. The methods include Stas-Otto or Dragendorff or their different modification, ammonium-sulphate method and modern method especially solid phase extraction of above poisons in biological materials.

The main difficulty in all the methods for extraction of poisons in biological materials is to get rid of fats, degraded protein and pigments which interfere with their isolation pure form and subsequent identification and quantitation. The problem is that quantities of these poison present kin biological matrices are small and multiple steps in the extraction and purification may incur loss of poison giving a minus error. If not sufficiently purified, a positive error arises. This is the reason why the result, of quantitative determination especially the alkaloids are not dependable and the negative findings in many cases do not represent the actual state of affairs. The quantitative result actually represents the recovery not the exact quantity present in the tissues. However, the percentage of recovery is improving excellently by using modifications of the existing methods and also modern methods. The methods are described hereunder with special reference to biological materials.

2.9.6.2.1 **Stas-Otto Process**:

After extraction of insecticides from biological materials (viscera, blood, stomach wash or vomit etc.) the homogenate is made acidic with acetic acid or tartaric acid (pH=2). To this mixture 2-3 times of absolute alcohol are added. The contents are stirred at frequent intervals and kept overnight. This is refluxed thereafter for 30 minutes on hot water bath and filtered through the filter paper pulp to remove precipitated proteins. The filtrate is evaporated on a hot water bath. To this residue is mixed thorough with 100 ml of cold distilled water. The aqueous solution is then processed according to Stas-Otto method in the following manner.

A. The aqueous solution is made acidic with acetic or tartaric acid if not acidic (check up with pH paper). This is then extracted several times with diethyl ether or chloroform. The solvent will dissolve free acid and acidic compounds such as picric acid, salicylic acid, veronal, neutral compounds such as acetanilide and phenacetin and very weak bases such as antipyrene, caffeine, colchicines and narcotine (but in traces).

B. The acid-aqueous part remaining after extraction with ether or chloroform is made strongly alkaline with NaOH solution and again extracted with diethyl ether or chloroform. Free basic substances i.e. most alkaloids except phenolic bases such as morphine and apomorphine are isolated by NaOH and remain in aqueous solution as sodium compounds.

C. The aqueous portion is made neutral by adding ammonium chloride or neutralizing with dilute acids. This is made again alkaline with ammonia solution. The alkaline solution now obtained is extracted as follows:

1. Firstly, the extraction is made with ether for apomorphine and traces of morphine.

2. This is then extracted with hot chloroform or pure amyl alcohol for morphine and narceine.

**2.9.6.2.2 Modified Stas-Otto Method:**

Procedure :

A. 50 gms of biological materials are minced preferably in a mincing machine, mixed with plenty of rectified spirit (about 2-3 times the weight of material) in a flask and acidified with tartaric acid. The mixture is heated on the steam bath for 1-2 hours with thoroughly shaking at frequent intervals. The extraction is then allowed to proceed for about 24 hours with the steam off. It is then filtered through a flutted filter. The filtrate is evaporated and the residue is again extracted with acidulated alcohol in the same way, filtered and washed several times with hot rectified spirit. The combined filtrates are evaporated in a porcelain basin on the steam bath to a syrupy consistency.

B. To the syrupy residue about 100 ml of rectified spirit is now added very slowly with constant stirring so that insoluble matter may be granular and not gummy. It the alcohol is added rapidly or all at a time, the insoluble matter will be gummy causing much loss of alkaloids by enclosing them in the sticking mass. It is warmed with occasioned stirring for about half an hour and filtered. This process is repeated once more and the combined alcoholic extracts are evaporated almost to dryness.

C. The residue is now dissolve in about 50 ml of water acidulated with dilute sulphuric acid and filtered after about an hour. The poisons are thus dissolved out by the aqueous solution which is transferred to a separating funnel and extracted with a suitable solvent such as ether, chloroform etc. in portions of about 25 ml. The solvent would take up the following from the acid solution, colouring matters, toxic oils and resins, salicylic acid and its derivatives (aspirin, salol etc.), barbiturates, sulphonal, acetanilide, narcotine and alkaloids of ergot, certain glucosides such as thevetin and which has escaped initial treatments for purification.

D. The acid aqueous solution is then rendered alkaline with a solution of sodium carbonate or ammonia which would liberate the free base from its salt. The alkaline solution is now extracted with chloroform in the same way as in the previous stage. It will take up all the alkaloids except morphine (only a trace being extracted) and those feebly basic substances which are partially extracted from the acid solution. The extraction is separated 2 or 3 times more.

E. If morphine is suspected, it may be extracted at this stage by amyl alcohol or chloroform – ether (3 : 1) mixture or chloroform – alcohol (9 : 1). Of these, amyl alcohol is the best but as it is prone to form annoying emulsions the chloroform – ether mixture is used by many. If morphine is likely to be the only poison, the chloroform extraction (stage D) described above may be omitted altogether. The combined chloroform or amyl alcohol extracts are evaporated to dryness and the residue is now ready for further purification and analysis.

F. The evaporated chloroform extract is purified by dissolving it in about 20 ml of water acidulated with sulphuric acid and filtering through a small filter. The filtrate is extracted with chloroform, first in acid and then in alkaline medium as in the initial stages of extraction. These extracts are evaporated to dryness for analysis.

2.9.6.2.3 **Further Modification of Stas – Otto Method**:

As there are too many steps in the extraction of non-volatile organic poison by Stas-Otto method and also chances of loss of poison in each of the steps, there may be even a complete failure to detect it in case of considerable loss of poisons. The objectives of modifications are to minimize the steps as far as practicable under special circumstances. The following modifications of the technique are sometimes necessary.

i) The alcoholic extraction of biological materials is to be carried out at room temperature (not exceeding 40°C) i.e. without using steam bath and preferably with absolute alcohol (to prevent hydrolysis) in place of rectified spirit for suspected poisoning by aconite, belladonna, datura or cocaine. The evaporation of alcoholic extract should be done under reduced pressure.

ii) The extraction with rectified spirit is to be done for 48 hours if biological materials are preserved in saturated.

iii) For stomach contents containing too much fluid, the extraction should be done with absolute alcohol 3 or 4 times.

iv) For stomach wash as the sample, extraction with double the quantity of absolute alcohol acidulated by tartaric acid should be done and then allowed to evaporate on a steam bath.

v) For filtration, Buchner funnel is preferred.

vi) To prevent loss due to emulsion formation, agitation with organic solvents, (ether or chloroform or amyl alcohol) should be done gently in the beginning and steadily in an violent manner thereafter (2 – 3 time in the beginning and knot exceeding 12 times at the end). If emulsion persists, it is to be evaporated on a steam bath and the residue taken up in fresh solvent.

2.9.6.2.4 **Ammonium Sulphate Method**:

This method is most useful for preliminary analysis (screening) of barbiturates, alkaloids and tranquilizing drugs etc. and also identification and semi-quantitation.

**Procedure :**

The organic materials (about 100 gms.) are cut into small pieces, macerated, mixed with100 ml of 5 percent acetic acid and taken into a 600 ml beaker. Solid ammonium sulphate is then added to it by frequent shaking to make a saturated solution. The about 20 gms. of solid ammonium chloride are added in excess. The mixture is then heated in a boiling water bath for three hours (for suspected poisoning by aconite the temperature should not exceed 60°C).

The mixture is cooled slightly and filtered through the filter paper pulp. The residue on the funnel is again extracted with two portions of 100 ml of 5 percent acetic acid and filtered as before. The filtrates are combined and taken into a 500 ml separating funnel. The residue slurry

on filter paper is leached with 100 ml of diethyl ether and the same is received in a cold container. The ether fraction is added to the aqueous acidic extract in the separating funnel and shaken for 5 minutes and separated. 100 ml of ether is again added to the acidic layer, shaken for 5 minutes and separated. The ether layers are combined. The acidic ether extract is tested for salicylic acid, aspirin, barbiturates, meprobamates, lysergides, benzo- diazepines etc.

The aqueous solution remaining in the separating funnel after separation of acidic drugs is made alkaline by addition of ammonium hydroxide and extracted three time with 100 ml portions of chloroform ether mixture (1 : 3). The aqueous layer is retained for extraction of opium alkaloids. The organic layers after separation are combined and washed with 50 ml of water. It is extracted three times with 25 ml portions of 10% sulphuric acid. The sulphuric acid fractions are combined and taken into another separating funnel. (Organic layer is discarded) 50 ml mixture of chloroform – ether (1 : 3) are added to it. Ammonium hydroxide solution (dil.) is added to make the solution alkaline shaken for 5 minutes. The organic layer is separated. The extraction is repeated thrice. The organic layer after separation are combined, washed with l50 ml of water and then dried by passing through anhydrous sodium subphate and evaporated to dryness. The extracted is tested for opium, dhatura and aconite alkaloids amphetamines, meprobamate, methaqualone etc.

The acid – ether extract may be further be separated into three fractions.

i) The acid – ether fraction is shaken with 25 ml of 5% sodium bicarbonate solution. The aqueous layer is removed and taken into another separating funnel. It is acidified with dilute sulphuric acid and re-extracted with 25 ml of ether. This ether fraction is passed through on hydrous sodium sulphate and then dried just to dryness. The residue (R1) contains salicylates.

ii) The ether layer (of acid ether fraction) after washing with sodium bicarbonate is extracted twice with 25 ml portions of N.sodium hydroxide solution. The aqueous layer are separated from ether layer, combined and taken into another separating funnel. It is made acidic with dilute sulphuric acid and extracted twice 25 ml portions of ether. This ether fraction is washed with 25 ml of water and then dried by passing through anhydrous sodium sulphate and then evaporated to dryness. The residue (R2) contains barbiturates in relatively purified form.

iii) The ether layer after extracting with NaOH is washed with water and then evaporated to dryness. The residue (R3) contains meprobamate, other carbamates and other neutral drugs.

2.9.6.3 **Extraction of Drugs in Urine**:

Sufficient phosphoric acid or tartaric acid is added to 10 ml of urine to adjust the PH to 3. It is then extracted with two 30 ml portions of ether. The extracts are combined and washed with 5 ml of water. The washings are added to the sample. The aqueous solution is retained for possible presence salicylates (Fraction A).

The etheral solution is extracted with 5 ml of 0.5 M sodium hydroxide and the extract is retained for examination of barbiturates and weakly acid substances (Weak Acid Fraction B – Barbiturates, Glutethimide, Paracetamol, Phenytoin, Phenylbutazone etc.).

The ethereal solution is washed with water. The washing is discarded. The ethereal solution is then dried with anhydrous sodium sulphate and evaporated to dryness. The residue may contain neutral drugs (Neutral Fraction C – Caffeine, Carbromal, Chlordiazepoxide, Flurazopam, Lorazepam, Meprobamate, Methaqualone, Methyprylone, Nitrazepam, Paracetamol).

To the aqueous solution retained after the first extraction sufficient dilute ammonia solution is added to adjust the PH to 8 . It is extracted with two 10 ml portions of chloroform. The combined ether extracts are washed with water, filtered. A little tartaric acid is added to prevent the loss of volatile bases. It is evaporated to dryness. The residue may contain basic drugs (Basic Fraction D – Amphetamine, Amitriptylene, Caffeine, phenothiazines, Ergot Alkaloids, Morphine, Methaqualone, Flurazepam, Lorazepam etc.).

The pH of the aqueous solution obtained after extraction of Fraction D is adjusted to PH 3 by the addition of hydrochloric acid. It is heated at 100°C for 30 minutes, cooled and extracted with two 10 ml portions of ether. The aqueous solution is kept reserved. The combined ether extracts are washed with 5 ml of M. sodium hydroxide and evaporated to dryness. The residue may contain benzodiazepines as benzophenones (Fraction E).

The pH kef reserved aqueous solution is adjusted to PH 9 cooled. It is extracted with a mixture of ethyl acetate and isopropyl alcohol (9 : 1).The solvent layer is separated and evaporated to dryness.The residue may contain opiates (Fraction F).

2.9.6.4 **Extraction of Drugs in Blood**:

As sample-volume in case of blood or serum or plasma is small and only a limited number of drugs may easily be detected and identified in them, the extraction procedure is slightly different from that for urine and stomach contents. Different fractions of extraction viz. A, B, C, D bear the same meaning as stated in 2.9.6.3. i.e. extraction of drugs urine. The initial extraction is carried out at PH 7.4 as many basic drugs are recovered by chloroform extraction at this PH. As a result, the substance looked for is most likely to be found in either fraction B or C and preparation of fraction D is only necessary either to ensure that nothing has been missed or where no drug has been found in fractions B and C.

Procedure : 2 ml of phosphate buffer (pH = 7.4) and 40 ml of chloroform are added to 4 ml of the sample and shaken vigorously. 2 gms of anhydrous sodium sulphate are added and again shaken again to produce a solid cake. The decanted chloroform is passed through a filter and the cake is extracted with a further 20 ml of chloroform. The chloroform extracts are combined.

The chloroform layer is extracted with sodium carbonate to remove salicylate (Strong Acid Fraction A), if detected in the preliminary tests. To the chloroform layer 8 ml of 0.5 N sodium hydroxide solution is added. The mixture is shaken and centrifuged. The sodium hydroxide may contain barbiturates and other weakly acid substances. (Weak Acid Fraction B).

The chloroform layer is washed with a little water. The washing is discarded. The chloroform layer is dried with anhydrous sodium sulphate, filtered and evaporated to dryness. The residue

may contain (caffeine, carbromal, benzodiazepines, meprobamate, phenazone etc.) neutral drugs together with a number of bases (Neutral and Basic Fraction C).

If sufficient of original sample is available, a further portion of it is made alkaline with dilute ammonia solution and extracted with two 10 ml portions of chloroform. The chloroform extract is dried with anhydrous sodium sulphate and evaporated to dryness. The residue may contain basic drugs (Basic Fraction D - as stated in the previous section).

If there is not sufficient of the original sample for the extraction of basic fraction F, the following procedure may be carried out. After fraction C has been chemically examined by UV or Chromatographic methods, the remaining residue, if any is dissolved in chloroform and extracted with 0.5 M sulphuric acid. This extracted portion is added to the sodium sulphate cake retained after the first extraction (for Fraction A). It is made alkaline with dilute ammonia solution and extracted with two 10 ml portions of chloroform. The chloroform layers are collected, dried over anhydrous sodium sulphate and evaporated to dryness. The residue may contain basic drugs (Basic Fraction D - as stated in the previous section).

2.9.6.5 **Solid Phase Extraction of Drugs from Urine**:

5 ml of urine are added to 2 ml of phosphate buffer (0.1 mol./L, PH 6.0) in a glass test tube and the PH is adjusted to PH 5.5 – 6.5 using 0.1 mol./L aqueous sodium hydroxide or 1 mol./L aqueous acetic acid (depending on the nature of the drug). SPE column is inserted into vacuum manifold and washed with 1 ml of methanol and 1 ml of phosphate buffer (0.1 mol./L, PH 6.0). An 8 ml fritted reservoid is attached to the top of the extraction column and urine is added to it. The column is dried under vacuum and washed with 1 ml of phosphate buffer (0.1 mol./L, PH 6.0) followed by 0.5 ml of aqueous acetic acid (1 mol./L, ). The column is dried under vacuum and washed with 1 ml of hexane. The elution for acidic and neutral drugs is made with 4 x 1 ml portions of dichloromethane. The elute is evaporated to dryness under a stream of nitrogen at 30o – 40$^{o}$C. The residue is kept reserved for analysis of acidic and neutral drugs. The column is then washed with methanol (1 ml) and eluation for basic drugs is made with 2 ml of methanolic ammonium hydroxide (.2%, V/V). 3 ml of de-ionised water is added and eluation is made with 0.2 ml of chloroform. The chloroform layer is collected and evaporated to dryness under a stream of nitrogen as above. The residue is kept reserved for analysis of basic drugs.

2.9.6.6 **Tissue Digestion using Proteolytic Enzyme:**

The digestion with proteolytic enzymes after give much improved recovery and has the advantage that once the digest has been prepared, analogous methodology to those used with plasma can be employed. It is obviously important to ensure that use of the enzyme preparation does not introduce interferences. A further potential problems is that conjugates and other metabolites may not survive. The procedure has been described below.

A solution (2 gms/L) of lyophilized subtilisin in sodium dihydrogen orthophosphate / disodium hydrogen orthophosphate buffer (7 mol./L, PH 7.4) 100 mg portions of tissue are dissected and the excess of fluid removed by filter paper. The tissue is added to 10 ml of tapered glass tubes and exact weights are recorded. 1 ml of subtilisin solution is added. The tubes are sealed with

ground glass stroppers and incubated fin a water bath at 50°C for 16 hours. The tubes are cooled. The contents are mixed on a vortex – mixer. Thereafter extraction is done with 0.2 ml portions as for plasma or serum.

2.9.6.7 **Micellar Extraction of Drugs in Biological Materials**:

100 gms of biological materials are finely minced in a mincing machine. An excess of rectified spirit (about 200 ml) is added to it in a flak, mixed thoroughly and made acidic with glacial acetic acid by drop wise addition and stirring. The mixture is then heated on a steam bath for 1 hour with through shaking at intervals and the flask containing the mixture is kept for 24 hours at room temperature. It is then filtered through a Buchner funnel having a bed of sand (0.3 cm) on the filter paper. The filtrate is collected in a separating funnel. After adding of 50 ml of solvent ether, cloudiness is observed. Fine particles of fat separate on standing which is drained off. The contents of separating funnel is shaken with 1 mg portions of sodium lauryl sulphate when semi-solid fat separates. The separation of fat is accelerated by addition of a few drops of water. Reddish brown oily mass of fat settles at the bottom of the separating funnel. This is drained off. The process is separated till the settling of fat ceased (indicated by a clear transparent extract in the separating funnel). The contents of the separating funnel is evaporated to dryness. The residue is extracted with 20 ml portions of water five times. The aqueous extract is made distinctly acidic by drop wise addition of acetic acid and extracted with 80 ml of diethyl ether in 20 ml portions. The aqueous part is kept separately. The combined ether extract as above (Acid-ether part for acidic and neutral drugs) is washed with water till free from acid.

The washings are mixed up with the aqueous part. The combined ethereal extract is dried over anhydrous sodium sulphate. The dried ether extract is decanted off. It is evaporated to dryness. The residue is kept reads for analysis.

The combined aqueous part is made distinctly basic with drop wise addition of ammonia solution. It is extracted with 80 ml of chloroform in 20 ml portion. The combined chloroform extracted is washed with water till free from alkali and dried over anhydrous sodium sulphate. It is decanted and evaporated to dryness. The residue is kept ready for analysis (basic drugs).

2.9.7 **Headspace procedure for Isolation of Volatiles in Biological Materials**:

The principle underlying headspace analysis is that in a sealed vial at constant temperature equilibrium is established between volatile components of a liquid sample in the vial and the gas phase above it (the headspace). After allowing due time for equilibrium (normally 15 minutes or so) a portion of the headspace may be withdrawn using a gas-tight syringe and injected into the GC column.

Procedure: The internal standard solution (25 mg / l ethyl benzene and10 mg / l l, 1, 2 trichloroethane) is added to a 200 µl of a mixture of expired blood and deionised water (1 : 24,v/v ) in a 7 ml glass septum vial using a semi-automatic pipette. The vial is sealed using a crimped on PTFE- lined silicone disc. The vial is incubated at 65$^{o}$C in a heating block and a portion (100-300µl) of headspace is withdrawn using a warmed gas-tight glass syringe for onward analysis in gas chromatograph.(2)

## 2.10 **Clean-Up Procedures**:

In the extraction techniques described it is likely that the extract will contain interfering substances, which will create problems in the analysis in various ways. The occurrence is particularly so in the extracts from samples of effluent, soil, sediment and tissues or organic matrices which contain fats, oils and other naturally occurring substances. The long established procedure for the removal of these substances has been to pass the extract through an alumina column and then to separate the target compounds into different batches by passing the clean up extract through a silica column. The supply of materials by preparative thin layer chromatography is also followed if the active components are identified by preliminary screening. The purification in the same way may also be achieved by gas chromatography or HPLC.

For volatile toxicants, stripping may also be done by GC-Head Space method (Purge or Trap technique). There are different analytical conditions for the Purpose which have been specified in literatures.

In different hyphenated techniques viz. GC-MS, LC-MS, HPLC-IR, the first technique is applied for separation of components in the pure state for their on-line identification and analysis by the other technique.

Recently, the clean up has been streamlined by the use of commercially available solid-phase extraction cartridges or disc.

Different methods are described below in short.

### 2.10.1 **Clean up using Alumina and Silica Column**:

This technique removes interfering compounds by passing the extract through basic and acidic column and then separating active constituents specially pesticides on a silica column.

Preparation of Solid Adsorbents:

The preparation of solid adsorbents – basic alumina, acidic alumina and silica gel is as follows.

i) **Basic Alumina**:

About 100 gms of alumina is placed in a silica dish, heated in muffle furnace for 4 hours at 800oC, cooled to about 200oC and then to room temperature in a desiccator. Water (4%, w/w) is added to the weighed portion in a stoppered flask. It is shaken well, sealed and stored

ii) **Acidic Alumina** :

A porition of the alumina is washed with IM HCI by making a slurry in beaker. It is filtered through a sinter funnel and dried in a silica dish at 150oC for 4 hours and cooled in a desiccator. Water (4% w/w) is added to a weighed portion in a stoppered flask. It is mixed thoroughly and

stored. These alumina preparations will slowly deactivate on exposure to air and should be discarded after 2 weeks.

iii) **Silica Gel** :

About 100 gms of silica gel is heated in a silica dish in a muffle furnace for 2 hours at 500°C, cooled and then placed in a desiccator. A portion is weighed into a stoppered glass container and water equivalent to 3% of the silica is added. The silica gel deactivates more rapidly than the alumina and should preferably be prepared daily.

Procedure:

The column is pre-washed with acetone followed by hexane and allowed to dry. The acid / base alumina column is prepared by first adding 2 gms. of acidic alumina then 1 gm of basic alumina to the column. The column is tapped to settle.

The silica column is prepared by adding 2.5 gms of deactivated silica gel to the column and tapped to settle. 10 ml of hexane is passed through the column (to wet the column) and run off in excess. Untill the hexane meniscus is level with the column material. The organic layer (obtained after extraction of matrices for pesticides or drugs) is then transferred on to the column. The active constituents (pesticide or drugs) are then eluted from the column by hexane or diethyl ether or suitable organic solvent. The eluate is collected. It is then passed through the silica column and elution is made. The eluate is collected and dried over anhydrous sodium sulphate. It is evaporated to dryness. The residue is kept for analysis.

2.10.2 **Modified method for Clean-Up and separation using Alumina / Silica Nitrate and Sliica Gel**:

Preparation of Solid Adsorbent:

(i) **Alumina** – About 100 gms of alumina is heated in a silica dish at 500oC for 4 hours and then cooled. To a weighed portion in a stoppered glass container, deionized water equivalent to 7% (w/w) of the alumina weight. is added and the sample is agitated to mix thoroughly. The alumina is kept in a sealed container. This adsorbent is stable for only about a week once re-exposed to atmosphere.

(ii) **Alumina / Silver Nitrate** - A batch of material for adding to the column is prepared by dissolving 0.75 gm of silver nitrate in 0.75 ml of water 4 ml of acetone is added to it. To this solution in an unstoppered conical flask 10 gms of dried alumina is added and shaken thoroughly. The acetone is allowed to evaporate and the preparation is stored in the dark until ready for use. The adsorbent should be prepared freshly.

Procedure : The chromatographic column is plugged with hexane washed glass wool or cotton wool and 15 ml of hexane is added. 1 gm of alumina / silver nitrate is poured and allowed to settle. Then 2 gms of alumina is added and again allowed to settle. This is then charged with a little anhydrous sodium sulphate. Hexane is run off in excess until the liquid level is at the top of

the column. The concentrated organic layer (containing pesticides or drugs as the active constituents) is added with rinsing to the top of the column. 30 ml of hexane or diethyl ether or any suitable organic solvent (as the case may be is passed through the column and the eluate is collected. The eluate concentrated down to 10 ml. A silica column is then prepared by adding 2 gms of silica to a plugged chromatographic column with a layer of anhydrous sodium sulphate at the top. The concentrated eluate from the alumina / silver nitrate column is added with rinsing to the silica gel column and allowed to be adsorbed. 10 ml of hexane or diethyl ether or any suitable organic solvent or a mixture of solvent is added to the top of the column and the eluate is collected. This is dried over anhydrous sodium sulphate and evaporated to dryness for further analysis.

2.10.3 **Clean – Up by a simple column Chromatographic method**:

The method can be profitably employed to purify the matter after extraction in biological materials for pesticides or drugs.

The concentrated extract (organic layer) is cleaned up by passing through a chromatographic column (dia. 1") containing from top alumina layer of 2", activated charcoal layer of 1" (middle layer) and a anhydrous sodium sulphate layer of 1" (bottom layer) previously arranged after plugging the chromatographic column and washing bag hexane or diethyl ether or any suitable solvent or mixture of solvents (as the case may be). The elution is made with appropriate organic solvent. The eluate is dried over anhydrous sodium sulphate and evaporated to dryness. The residue is kept reserved for analysis.

2.10.4 **Clean-Up using Solid Phase Extraction (SPE) Catridges**:

There are a variety of catridges available viz. Bond Elut from Analytichem International. The catridge is filled with a chemically modified silica adsorbent and the appropriate one selected according to the nature of the material (toxicant) viz. aminopropyl for organo-chloro compounds.

Procedure : A selective catridge is taken with a black adapter fitted on top.A 10 ml glass syringe is fitted onto the adaptor. 5 ml kef methanol is put into the syringe. It is passed through the tube, (tube should be wet always). The tube is then washed with 5 ml of hexane. The syringe is detached. The concentrated organic layer containing active constituent is added to the top of the tube and allowed to pass through it. The clean extract is collected. The tube with the syringe is washed with the same solvent. This is added to the clean extract as above. The combined layer is dried over anhydrous sodium sulphate. It is then evaporated to dryness and the residue is kept reserved for analysis.

2.10.5 **Clean-Up by Preparative TLC method**:

This is applicable if the identity of toxicant is established. Thereafter the chromatogram is developed by applying the same chromatographic condition that was used in the identification i.e. same developing solvent system, adsorbent, temperature etc. The adjoining circular areas

around the components separated at different Rf values are scrapped off the scrapped materials at different zones are eluted with selective organic solvent. This is collected, dried over anhydrous sodium sulphate and evaporated to dryness for analysis.

2.10.6 **Clean-Up by HPLC method**:

This is also applicable as in the case stated above i.e. the identity of toxicant by HPLC method is to be established by noting retention time and other chromatographic conditions viz. solvent system, flow rate, column etc. Again the same HPLC method is employed and the separated components at various time (as per retention time established earlier) are collected for further analysis.

Different methods of extraction / isolation of toxicants in diverse matrices and their stripping methods have been described in the previous paragraphs. The methods are to be employed depending on the nature of toxicant, matrices and availability of infrastructure facilities. In the forthcoming chapter the analysis of different classes of toxicants will be described.

**References:**


1. Applicable Note Nos.320 & 321 on ASE, Diones, USA.
2. Bough P J, Gas Chromatography – A practical approach, p-174, 1st edition, Oxford University Press, NY, 1993.
3. Curry, Alan., Poison Detection in Human Organ. 3rd Edn. Charles C. Thomas, Springfield, 19767.
4. Lundquist, Frank., Methods of Forensic Science, Vol.I-IV, Internclence Publishers, N. York, 1962.
5. Clarke, E.G.C., Isolation and Identification of Drugs, 2nd Edn. The Pharmaceutical Press, London, 1986.
6. Bamford, F. Poisons, Their Isolation and Identifications, J/A/, Churchill Ltd., London, 1940.
7. Stewart, C.P. and Solman, A., Toxicology – Mechanism and Analytical Methods, Vol. 2, Academic Press, N. York, 1961.
8. Stars, J.S., Bull. Acad. Roy Med., 11, 304, 1851.
9. Otto, J., Anal. Chem. Pharm., 100, 39, 1856.
10. Jones, G.R. and Singer, P.P. in Advances in Analytical Toxicology (Bassalt, R.G., ed.), Vol.2, Year Book Medical, Chicugo, 1989.
11. Whelpton, R. in Analytical Methods in Human Toaxicology (Curry, A.S. – ed), Macmillan, London, 1984.
12. Middleditch, B.S., Analytical Artifacts. GC., HPLC, TLC and PC, J. Chromatogr. Libr., Vol.44, Elsevier, Amsterdam, 1989.
13. A.O.A.C., Official and Tentative Methods, 1980.
14. Ghosh, S. and Bagchi, K.N., Organic and Taoxicological Chemistry, Art Press, Calcutta, India, 1948.
15. Maehly, Andreas, Chemical Criminalistics, Springer – Verlag, Berlin, 1981.
16. Stahl, E. Thin Lyer Chromatography, 2nd Edn. Springer – Verlag, Berlin, 1969.
17. Gour, T.H. (Ed.), Guide to Modern Methods of Instrumental Analysis, Wiley. Inter Science, N. York, 1972.
18. Jennings, Walter, Analytical Gas Chromatography, Academic Press, Inco., N. York, 1987.
19. Ewing, Galenwood (Ed), Analytical Instrumentation Hand book, Marcel Dekker, Inc., N. York, 1990.
20. Sethi, P.D., High Performance Thin Layer Chromatography : CBS Publishers and Distributors, India, 1996.
21. Gauglitz, Gunter and VO – Dinn, Tuan (Eds), Handbook of Spectroscopy, Wiley VCH, Germany, 2003.

# SECTION – 3: GASEOUS AND VOLATILE POISONS

**3.1 Title:** Analysis of gaseous and volatile poisons.

**3.2 Scope:** Systematic analysis of gaseous and volatile poisons in various type of formulations, biological materials and non-biological matrices.

**3.3 Purpose:** To identify and estimate gaseous and volatile poisons .

**3.4 Responsibilities:** Gazetted officers and associated scientific staff.

**3.5 VOLATILE POISONS:**

Some of the volatile poisons with their characteristics are given below:-.

**TABLE – 3.1**

| Name | Synonym & Source | Physical Properties | Other Characteristics |
|---|---|---|---|
| Ethyl Alcohol | Ethanol, Methyl Carbinol, Spirit of Wine.<br>Source: As free alcohol in some fruit juices, as ester in some eucalyptus oil, produced by fermentation of starch, molasses, grapes etc. | Transparent, colourless and volatile liquid, having spirituous odour and burning taste. Hygroscopic, boils at $78.4^0$C. | Burns with blue flame. Used as solvent, converted into aldehyde and acetic acid. Absolute alcohol-99.95 % of alcohol. |
| Methyl alcohol | Methanol, Carbinol, wood spirit.<br>Source: The liquid fraction pyro ligneous acid in the destructive distillation of wood contains methanol as a major constituent. Can be synthesized commercially. | A colourless liquid, B.P. $64.7^o$C, mixes with water and organic solvents, peculiar odour and a burning taste. | Extremely poisonous & causes blindness and even death. Metabolised by oxidation to formaldehyde, formic acid & finally formate. |

## 3.6 METHODOLOGY:

The distillation methods for isolation of volatile poison have already been described (section-3). The distillates in two fractions, acid steam distillate and alkaline steam distillate are kept to undertake systematic screening and other detailed analysis as mentioned hereunder.

### 3.6.1 Identification tests for volatiles:

In biological materials (viscera), stomach contents, urine, isolation by acid-distillation as described earlier. The distillate is subjected to the following tests:

#### 3.6.1.1 Test for methanol:

**Isolation:** By acid-distillation. The distillate is subjected to the following tests.

**Chromotropic Acid Test:**

To 0.5 ml. of the distillate in a test tube, 0.2 ml. of 5% potassium permanganate solution is added. After 5 minutes, saturated solution of sodium bisulphite is added drop by drop until the permanganate colour is discharged. If a brown colour still persists, a drop of phosphoric acid is again added to it a drop of sodium bisulphite solution. To this colourless solution, 0.5 ml. of freshly prepared chromotropic acid solution (prepared by dissolving 5 mg. of sodium salt of chromotropic acid in 10 ml. of concentrated sulphuric acid and heating on a water bath at $60^{o}$C for 30 minutes and cooling thereafter. A violet colour is observed.

#### 3.6.1.2 Test for ethyl alcohol:

**Isolation:** Ethyl alcohol is isolated from biological materials by acid distillation. The distillate is subjected to the following tests.

**Dichromate test**

To one ml of the distillate is added 0.2 ml of 2% Potassium dichromate solution followed by one ml of conc sulphuric acid. The yellow colour of the dichromate changes to green or blue. Sensitivity 2 mg.

### 3.6.3 METHODS OF QUANTITATION OF ETHANOL IN BIOLOGICAL MATERIALS:

**Gas Chromatographic determination of Ethanol in Blood/Urine**

**Isolation of Ethanol in blood** : 1 ml. of blood is diluted with 4 ml. of water. It is acidified with a few drops of 5% tartaric acid solution and then distilled. 5 ml of distillate is

collected in ice cold condition and an aliquot (10 µl) of it is injected into the gas chromatograph as per conditions stated below.

**Isolation of Ethanol in Urine**: 1 ml. of urine is taken into a micro centrifuge and centrifuged for 15 minutes. 5 µl of supernatant liquid is injected into the gas chromatograph as per conditions stated below.

Column – Porapak θ – Polymer bead, 80 – 100 mesh,
5' X 4 mm. (id.) glass column.
Column Temperature – 160°C
Carrier Gas - Nitrogen

Gas Flow - Nitrogen - 50 ml./ min.
Hydrogen - 50 ml./ min.
Air - 300 ml./min.

Detector - F.I.D.

**Quantitation :**

The quantitative estimation of volatile substance is made by determining the area of the corresponding peak and calculating the quantity of the sample from the following relation ship.

$$\frac{Ca}{Aa} = \frac{Cb}{Ab}$$

Where,

Ca = Concentration of volatile substance in exhibit.

Aa = Area of peak of volatile substance present in the exhibit.

Cb = Concentration of the standard.

Ab = Peak area of the standard.

Cb is once determined by injecting 10 µl of supernatant prepared by standard blood sample having 150 mg

Ab of volatile substance per 100 ml. of blood (the ratio remains constant).

### 3.6.2.1 ALTERNATIVE METHOD FOR QUANTITATION OF ETHNOL IN BLOOD/URINE BY KOZELKA AND HINE METHOD

This method requires an apparatus, each unit of which consists of four hard glass tubes (2.5 cm dia, 20 cm height) connected with each other by means of quick fit joints.

In first tube 5 ml of 2 percent potassium dichromate in concentrated sulphuric acid are taken, through which air is bubbled to remove the moisture and other organic vapours.

In the second tube, 2 ml of accurately measured blood sample is taken. To it 2 ml of 10 percent sodium tungstate solution and 0.5 ml of 2 N sulphuric acid added to deprotinise the blood. (In case of urine only few drops of 10 percent sodium hydroxide solution are added and no sodium tungstate or sulphuric acid are needed).

In third tube is taken 5 ml of saturated mercuric chloride solution and 5 ml of saturated hydroxide solution. This tube is interposed between the tube of blood samples and the fourth tube.

To the fourth tube are taken 10 ml of 0.1 N Potassium dichromate solution and 10 ml of centrated sulphuric acid.

All the four tubes are arranged so that the first tube remains out of the hot water bath while the rest of the tubes are dipped into the hot water bath. The air is sucked with the help of an aspirator (with a rate of about 25 ml per minute) through the tube containing 2% dichromate-sulphuric acid, to the tube of blood sample, then to alkaline mercuric chloride tube and finally into the 0.1 N dichromate mixture. After one hour, the dichromate solution is taken out of the tube (fourth tube) and made up to 100 ml with distilled water. This solution is titrated iodometrically with 0.1 N hypo solution.

A blank experiment is also performed side by side by taking all the reagents except the blood sample and the similar titration is performed by taking same amount i.e. 10 ml of 0.1 N Potassium dichromate and 10 ml of conc. Sulphuric acid.

Let x ml is the volume of 0.1 N hypo required by blank experiment and y ml is the volume of 0.1 N hypo required by actual alcohol determination experiment.

Then (x-y) ml of 0.1 N hypo solution = (x-y) ml of 0.1 N dichromate used.

But 1.0 ml of 0.1 N $K_2Cr_2O_7$ solution used – 1.15 mg of alcohol. Mg of alcohol per 100 ml blood = $\frac{1.15 \text{ X } (x-y) \text{ x } 100}{V}$

Where V is the volume of blood taken for experiment in the tube.

### 3.6.2.2. ALTERNATIVE METHOD FOR QUANTITATION OF ETHNOL IN BLOOD/URINE BY CAVETT'S MODIFIED METHOD

In Cavett's modified method about 2 ml of blood or urine are taken in a cavett internal dish. It is then placed over 10 ml of potassium dichromate solution (4.9 gs. of dichromate in 1 litre of 50% sulphuric acid). The cavett is then closed and sealed properly. It is left for about 20 hours at room temperature or incubated at about $37^oC$ for 8 hours.
The alcohol in the sample is distilled over and gets oxidized on coming in contact with the solution. An excess of potassium iodide is added to unused dichromate solution. The

solution is titrated with sodium thiosulphate solution using starch as the indicator. The quantity used for alcohol is thus found out.

A blank experiment is also performed side by side by taking all the reagents except the blood sample and the similar titration is performed by taking same amount of potassium dichromate.

Let x ml is the volume of 0.1 N hypo required by blank experiment and y ml is the volume of 0.1 N hypo required by actual alcohol determination experiment.

Then (x-y) ml of 0.1 N hypo solution = (x-y) ml of 0.1 N dichromate used.

But 1.0 ml of 0.1 N $K_2Cr_2O_7$ solution used – 1.15 mg of alcohol. Mg of alcohol per 100 ml blood = $\frac{1.15 \text{ X } (x-y) \text{ x } 100}{V}$

Where V is the volume of blood taken for experiment in the tube.

## 3.7 GASEOUS POISONS:

Various toxic gases are known in forensic toxicology. Although the list includes various types of toxic gases of diverse origin or sources, cases involving a few toxic gases are encountered in day-to-day work.

However, the table – 3.4 indicates their characteristics, origin, properties etc.

**TABLE – 3.2: CHARACTERISTIC OF GASEOUS POISONS**

| NAME | SOURCE | PROPERTIES | OTHER CHARACTERISTICS |
|---|---|---|---|
| Phosphine | Phosphides used as rodenticides, phosphorous-based industries. | Colourless, flammable with odour of decaying fish. | Highly toxic, affects CNS and reacts with haemoglobin. |

### 3.7.1 Identification of some Gaseous Poisons:

**Method – (A): Phosphine in Biological materials:**

25 gms. of biological material is taken in a conical flask filled with a guard tube containing lead acetate soaked cotton. A few ml. of cadmium sulphate solution is added. It is acidified with dilute sulphuric acid. The mixture is heated gently on water bath at 40-60 °C. The gas evolved is allowed to come in contact with $AgNO_3$ paper. It turns grey or yellowish brown or black. (Due to reaction of phosphine with silver nitrate solution). The presence of phosphide is indicated. The paper is dried and cut into pieces and dissolved in dil nitric acid. The extract is evaporated to dryness for 2-3 times. The residue is taken in a few drops of concentrated nitric acid. 1 ml. of ammonium molybdate solution is added and warmed. The formation of canary yellow precipitate confirms the presence of phosphide.

It may also be estimated by trapping the gas in bromine water or sodium hydrochlorite solution, and after elimination of the bromine, determination is done for the presence of phosphate. It may be determined in gases by use of the reaction $PH_3 + 3\ HgCl_2 \rightarrow P(HgCl)_3 + 3\ HCl$, the quantity of acid being proportional to the volume of phosphine present.

**Method – B: By HS-GC-FPD method [19]:**

**Sample preparation**: Transfer 1-5 mL of sample material into 20 mL of head-space vial. Add 10 mL of 0.1 M $H_2SO_4$ and immediately seal the vial for chromatographic analysis. Use aqueous solution of $Zn_3P_2$ as standard and treat in the same way as described.

**HS-GC-FPD analysis**:

Capillary Column: HP PLOT Q (30 m × 0.32 mm × 20 µm) (Bonded polystyrene-divinylbenzene based column)

Carrier gas : Helium

Injection : Split 1:30 at room temperature @150 °C

Injection volume : 0.1 mL

Detector : Flame Photometric Detector (P-Mode) @ 310 °C

Oven Temperature : 70 °C for 0.5 min followed by 15 °C/min up to 100 °C with an 8 min hold;

Retention time : Peak of Phosphine at 1.05 min.

**3.7.2: Identification of carbon monoxide in blood by micro-diffusion method [20]**

- Add 2 mL of $PdCl_2$ solution in the central well of Conway micro-diffusion cell*.
- Add 2 mL of the blood in one side of outer ring
- Add 1 mL of 10% $H_2SO_4$ in another side of outer ring
- Quickly cover the micro-diffusion cell and gently rock/rotate to mix blood with sulfuric acid.
- Allow to diffuse for approximately 1 hour in a oven at ~50°C.
- A silver colored mirror will form in the center well of the dist in positive samples.

***[**0.005 N Palladium Chloride Reagent: Weigh 0.22 g palladium chloride, transfer into a 250 mL volumetric flask and qs to volume with 0.1 N HCl and let stand overnight. Transfer to a 500 mL volumetric flask and qs to volume with 0.1 N HCl. Store at room temperature for up to two years. ]

**ENVIRONMENTAL CONDITIONS:**

Isolation and purification of poisons may be carried out at room temperature.

**METHOD OF QUALITY CHECKS USED:**

Quality checks have been undertaken by one of the following methods.

1. Repeat analysis
2. Comparison with control
3. Use of two different solvent systems in TLC.

**References:**


1. J. Forensic Science 6 :401, 1961.
2. AOAC official methods of analysis 986.20 chapter 10, p- 47-48, (1995),
3. Bureau of Indian Standards Specification, IS 1061 : 1997
4. A S Curry, Poison Detection in Human Organs, Charles C., Thomas, Springfield., England, 1963.
5. E.J.van Kampen and H.Klocwen, Ned. Fidschr. Geneeskd, 195498, 161-164, et al Ree, Trav. Chim.des Pays Bas, 1954, 23, 119 128.
6. Lundquist, Frank, Methods of Forensic Science, Vol.I to IV, Inter Science Publishers, N. York, 19962 (Ref.No.2 in Section 3).
7. Clarke, E.G.C., Isolation and identification of drugs, 2nd Edn., The Pharmaceutical Press, London, 1986. (Ref. No.15 in section 1 and 2)
8. Feigl, F., Spot Test in Organic Analysis, 7th Edn. Elsevier, Amsterdam, 1966.
9. Sunshine, I. (ed), Methodology for Analytical Toxicology, CRC Press, Cleveland, 1975.
10. Braun, R.D., Introduction to Chemical Analysis, McGraw Hill, N. York, 1982.
11. Saferstein, R. (ed), Criminalistics : An Introduction to Forensic Science, 6th Edn., Prentice Hall, New Jersey, 1998.
12. Siegel, J.A., Saukko, P.J. and Knuffer, G.C., Encyclopedia of Forensic Science, Vol.1, Academic Press, N. York, 2000.
13. Maehly, Andreas, Chemical Criminalistics Spinger – Verlag, Berlin, (Ref.No.13 in section 3.)
14. Ghosh, S. and Bagchi, K.N., Organic and Toxicological Chemistry, Art Press, India, 1948. (Ref. No.12 in section 3).
15. Jungreis, Ervim., Spot Test Analysis, John Wiley and Sons, N. York, 1984.
16. Curry, A.S., Advances in Forensic and Clinical Toxicology, CRC Press, Cleveland, 1972 (Ref.No.4 at Page 1)
17. Lundquist, Frank., Methods of Forensic Science, Vol. I-IV, Inter science Publishers, N. York,1962 (Ref. No.2 at Page 2).
18. AOAC, Official and Tentative Methos, 1980 (Ref.No.11 at Page 2). Feigl, Fritz., Spot Test in Organic Analysis, Elsevier, Amsterdam, 1975
19. Mussoff F., Preuss J., Lignitz E. and Madea B., "A gas chromatographic analysis of Phosphine in biological material in a case of suicide" Forensic Science International 177 (2008) e35-e38.
20. B. P. Huddle, J.C. Stephens "Analysis of carbon monoxide in blood", Journal of Chemical Education 80 (2003) 441-443.

# SECTION – 4: ANALYSIS OF INORGANIC POISONS (CATIONS AND ANIONS)

**4.1 Title**: Analysis of inorganic poisons (cations and anions)

**4.2 Scope:** Analysis of inorganic poisons in crime exhibits viz. hair, nail, skin, bone, biological materials, food and food products, meat, milk and milk products, drinks, cereals, grains etc.

**4.3 Purpose:** To detect and determine inorganic poisons (metal cations and different anions or radicals) in exhibits.

**4.4 Responsibilities:** Gazetted Officers and other associated scientific staff.

**4.5 DESCRIPTION OF METALLIC AND NON METALLIC POISONS**

The important poisons in the group of metals include arsenic, antimony, mercury, lead, thallium, zinc, manganese, barium and aluminium. The salts of these metals are toxic. Non-metals or specifically toxic anions include borate, bromide, chlorate, cyanide, fluoride, hypochlorite, iodide, nitrate, nitrite, oxalate, bromate, iodate, sulphide, thiocyanate etc. Generally, the anions of the salts are responsible for the toxic action. Thus, their detection gives a clear idea regarding the source of poison. Sometimes, toxic anions are directly used as active species viz. cyanide, oxalate, borate etc. These cations and anions are to be isolated especially in case of biological materials for onward detection and estimation. There are some classical methods viz. Reinsch test, Gutzeit test, Marsh test that are still followed for the detection of cations. The present day analysis includes micro methods and instrumental techniques. Before elaboration, the description of cations and anions are given below.

**4.5.1 Characteristics of some toxic Cations:**

| NAME | SOURCE | SIGN & SYMPTOMS / TOXICITY | CHARACTERISTICS |
|---|---|---|---|
| Arsenic | Different compounds of arsenic: arsenic trioxide ($As_2O_3$) (white arsenic), chloride, sulphide (Orpiment), red sulphide ($As_2S_3$) (Realgar), copper acetoarsenite (Paris Green, Copper | All arsenicals inhibit sulphydryl enzyme system necessary for cellular metabolism, symptoms of poisoning include faintness, depression, | Arsenic is a grey substance, which is said to be non-poisonous. As it is insoluble in water and therefore incapable of absorption form the alimentary canal. However, it is continuously changing |

| | | | |
|---|---|---|---|
| | arsenite (Schlee's Green), arsenates.<br>Organic Compounds of arsenic: Cacodylic acid, sodium pentaphenyl arsonate (Atoxyl) Dioxy-Diamino Arseno-Benzene Dihydrochloride (Salvarsan) Silver arphenamine (Silver Salvarsan) etc.<br>Proprietory articles containing arsenic.<br>Rough on rats, fly paper, weed killer, fly water, fly powder. | nausea, severe burning pain, contraction of throat, increased salivation and stomatitis. Severe thirst and projectile vomiting. Vomit may contain streaks of blood (distinction from cholera) Urine is suppressed, Skin be – comes cold and clammy. In chronic poisoning red pigmentation (rain drop type) on skin occurs. | into arsenious oxide, which is tasteless and most poisonous. Arsenic causes toxicity by combining with sulphydryl enzymes, thus interfering with cell metabolism. Poisoning is mostly done by arsenious oxide. Such type of poisoning accounted for 90% of cases in the past. This is not common now a days. However, animal poisoning by arsenic occurs frequently. Pentavalent arsenic is to be reduced to the trivalent state for its detection.<br>The organic arsenicals may contain arsenic in trivalent and pentavalent state. As an antidote BAL is used. |
| Mercury | Metallic Mercury: Bright silvery, heavy liquid used in thermometers, barometers, mercury vapour lamps. Inorganic Compounds: Mercuric chloride (corrosive sublimate), cyanide (as fungicide), nitrate, sulphide (Cinnabar, Hingul, Sindoor), sulphate. Organic Compounds: Dimethyl mercury, mercurochlrome, | It affects cellular metabolism and function. The symptoms are due to corrosive sublime. Symptoms start within half hour of intake. The symptoms include acrid, metallic taste, a feeling of constriction or choking. The mouth, tongue and faces are corroded, swollen and coated with a greyish | Constriction of throat is more marked. Irritation of kidney is pronounced. Metallic mercury can hardly be considered to be a poison. It is not absorbed when taken by mouth. Mercury is readily absorbed through skin when rubbed of all compounds. Mercuric chloride and nitrate are responsible for most of the cases of poisoning.<br>Antidote: BAL |

| | | | |
|---|---|---|---|
| | organic mercurical. Preparation: Neptal, thiomerin sodium, mercurophylline Mercurous mercury is not toxic. | white coating. Hot burning pain moth extending to stomach & abdomen followed by nausea, retching and vomiting. Vomit may be accompanied by mucous and blood. This may be followed by diarrhoea with bloodstain. Urine is suppressed and scanty. Pulse becomes quick, small and irregular. Spasms, convulsions may precede the death. | |

### 4.5.1.1 Detection and determination of toxic cations:

The toxic cations are to be detected in biological materials in case of fatal poisoning. This is done by digestion (dry or wet). The extract obtained after digestion is used for chemical test and quantitation.

### 4.5.1.2 ARSENIC:

**Chemical Test for Arsenic:**

**Reinsch's Test:**

About 20 ml. of Conc. HCl (pure for toxicological work) and 100 ml. of water are taken in a porcelain basin in which a bright copper foil (about 3" by ¼") is placed with one of its ends being fixed on the edge kef the basin in the form a loop. It is boiled for about half an hour to see if the copper, basin and the acid are free from the metal to be tested (here it is arsenic). If a stain on copper foil appears, the blank experiment is to be carried out again with fresh materials. If the blank is negative, the suspected material (biological or non-biological) is added and boiled for about an hour or more with occasional addition of water and acid to make up for the loss due to evaporation. A shining steel grain stain appears in a few minutes. Which becomes thick gradually. The stained copper strip obtained by Reinsch test is washed cautiously with water followed by alcohol and finally with ether to remove the adhering fat, if the matrices are biological materials. The strip is

dried by keeping it between filter paper sheets, cut in small pieces of 0.2 mm x 0.2 mm size and taken into Reinsch tube. The tube is heated slowly on the flame of spirit lamp. The blank deposit on the copper strip volatilization and gets deposited on the cooler part of the tube. The tube is cooled and viewed under microscope. Characteristic octahedral crystals of arsenious oxide are seen (sensitivity 10 μg). There are certain limitations of the test viz. negative result may be obtained if oxidizing agent is present on As is in +5 state (it is to be reduced to +3 state) by treatment with sodium sulphite or potassium iodide or stannous chloride or ferrous sulphate as reducing agent. Organic arsenicals do not respond if organic matter is not destroyed. Some organic sulphur compounds produces black stains of copper sulphide, which may be removed by oxidation. The concentration of HCl should not be too low or too high. This test is generally used for rapid screening of As, Sb, Hg.

#### 4.5.1.3 Mercury

Metallic mercury, also known as quicksilver, is a liquid metal having a bright silvery luster. It exist in nature as the metal itself and as the sulphide (cinnabar or ras sindoor). Metallic mercury is not poisonous if taken by mouth because it is not absorbed but causes poisoning if finely divided and inhaled, swallowed or rubbed into the skin. It vaporizes even at room temperature to an extent sufficient to permit the inhalation of toxic amounts. The vapour may be source of danger in industry and even in the research laboratory.

Mercury and its salts are used very extensively in the arts, commerce, dentistry and medicine. It is used in the metallic form as inorganic mercurous (monovalent) and mercuric (divalent) salts, and in combination with organic molecules. Mercuric compounds being soluble are intensely poisonous.

Mercuric chloride obtained in the form of white crystalline powder or tablets are used as a germicide, coloured by cosin, methylene blue or other dye. It has an archid metallic taste, no smell and by far the most common cause of acute poisoning. The other poisonous mercurical salts are mercuric oxide, mercuric ammonium chloride, mercurous chloride or calomel, mercuric potassium iodide, mercuric nitrate and mercuric cyanide.

Fatal dose & fatal period: The fatal dose of corrosive sublimate is about 200 to 300 mg. Death may occur within a few hours but is usually delayed for 3 to 5 days.

**Chemical Tests for Mercury:**

**Reinsch's Test:**

A silvery shining depository on the copper strip indicates the presence of mercury. After necessary cleaning the dried shining copper strip pieces are heated slowly in a Reinsch tube and the deposit on the cooler side is viewed under a microscope. Shining round globules of metallic mercury are observed.

**Micro Test**: A portion of the stained copper strip from Reinsch test is taken into a spotted tile. Few drops of con. Nitric acid at added to dissolve the stain on the copper strip. After evaporation, the residue is taken in dilute hydrochloric acid and spotted on a chromatogram. The presence of mercury is established by spraying the chromatogram with dithizone.

**ENVIRONMENTAL CONDITIONS:**

Isolation and purification of poisons may be carried out at room temperature.

**METHOD OF QUALITY CHECKS USED:**

**Quality checks have been undertaken by one of the following methods.**

1. Repeat analysis
2. Comparison with control
3. Use of two different solvent systems in TLC.

**References:**


1. Modi's textbook of medical jurisprudence and toxicology, 17nth edition, 1969, P-596
2. Clarke's Isolation & Identification of drugs, IInd edition, The Pharmaceutical press, London, 1986, p-58.
3. J.Hoste, Anal.Chim.Acta. 4(1950)23.
4. F.Feigl and V.Anger, Spot Test in Inorganic Analysis, Elsevier Pub.Co.Amsterdam, London VI Ed.1972, p.203.
5. F.Feigl, Ber, 56(1923) 2023; Microchemic, 1(1923)76.
6. L.Kul'berg, Microchem, 20 (1936) 153.
7. Pr. Ray and R.M. Ray, Quart. J.Indian Chem.Soc. 3(1926) 118.
8. Dr. V.B. Patil & D.M. Jogadankar, J.Planar Chromatogr. 7(1994) 166.
9. Clarke's Isolation & Identification of drugs, IInd edition, The Pharmaceutical press, London, 1986, p-59.
10. P.Cazeneuve, Compt.rend, 131 (1900)346.
11. F.Feigl and V.Anger, Spot Tests in Inorganic Analysis …. P.307
12. F.Feigl and V.Anger, Spot Tests in Inorganic Analysis …. P.308.
13. N.A.Tamamaeff.Z.anorg.allgen.Chem.136(1924)373;140(1924)321.
14. Clarke's Isolation & Identification of drugs, IInd edition, The Pharmaceutical press, London, 1986, p-60, 62 and 63.
15. Official Methods of Analysis, AOAC, Washington D.C., 1975.
16. Jungreis, Ervim., Spot Test Analysis, John Wiley & Sons, N. York, 1984.
17. Vogel, A.I., A Text Book of Macro and Semi Micro Qualitative Inorganic Analysis, 4th Edn., Longman, London, 1964.

# SECTION 5: ANALYSIS OF NEUTRAL POISONS (ORGANIC NON-VOLATILE)

**5.1** **Title**: Analysis of Neutral Poisons (organic non-volatile).

**5.2** **Scope:** Analysis of neutral poisons (organic non-volatiles) in biological and non-biological.

**5.3.** **Purpose**: To identify neutral poisons (organic non-volatiles).

**5.4** **Responsibility**: Gazetted Officers and associated scientific staff.

**5.5 ORGANOPHOSPHOROUS INSECTICIDES:**

These are considered as derivatives of the corresponding acids or hydrogen phosphide (phosphine).

**5.5.1 Classification:**

These compounds are the derivatives of oxy acids of phosphorous or thiophosphoric acids. The different acids from which organo-phosphates have been derived are as follows.

1. Phosphoric Acid.
2. Thiophosphoric Acid.
3. Dithiophosphoric Acid.
4. Miscellaneous organo phosphorous compounds

The different derivatives under the above classification are as follows.

**5.5.1.1 Derivatives of Phosphoric Acids**

The insecticidal and acaricidal properties increases when we go from phosphites to phosphates. The important derivatives of phosphoric acid having insecticidal activities are given in the table below:

**TABLE 5.1 : Derivatives of Phosphoric Acids.**

| Sl. No. | Name | Other Names | Chemical Name |
|---|---|---|---|
| 1. | Dichlorovos | Dichlorfos, DDVF DDVP Bromchlophos Dibrom. | O, O-Dimethyl O – 2, 2 dichlorovinyl Phosphate |
| 2. | Naled | Demecron | O, O – Dimethyl – O – 2, 2 Dichloro –1, 2-dibromomethyl Phosphate. |
| 3. | Phosphamidon | | O, O – Dimethyl – O [2-chloro – N, N– dimethyl – carbamoyl] -methylvinyl phosphate. |
| 4. | Phosphinon | | O, O-Diethyl-O (2, 2-dichloro – 1 – chloroethoxylvinyl phosphate. |
| 5. | Phosdrin | | O, O – Dimethyl – O – (1-methyl- 2-carbomethoxy-vinyl) phosphate. |
| 6. | Bidrin | | 3-Dimethoxy phosphinyloxy N, N – dimethyl Crotonamide. |
| 7. | Birlane | | 2-chloro 1-(2, 4- dichldorophenyl) vinyl diethyl phosphate. |

| | | | |
|---|---|---|---|
| 8. | Gardona | | 2-chloro-1 (2, 4, 5- trichlorophenyl) vinyl dimethyl phosphate. |
| 9. | Dimefox | | Bis-dimethyl flurophosphate, |
| 10. | Mipafox | | N,N-di isopropyl phosphorodiamide fluoride. |
| 11. | Avenin | | O, O-Dimethyl-N-(isopropoxy – carbamoyl phosphate). |
| 12. | Cyolane | | Diethyl – N-3-dithioanyl - 2- imino-phosphate. |

**5.5.1.2 Derivatives of Thiophosphoric Acid:**

The replacement of one of the oxygen atoms by sulfur in the derivatives of phosphoric acid decreases the toxicity of the compounds related to mammals without substantial changes in the insecticidal or acaricidal activity. There are now at least 30 derivatives having a thiolo or thiono moiety. Thiolo derivatives are more toxic to mammals compared to thiono derivatives. The thiono compounds are converted to thiolo isomer on being heated or treated by certain reagents. The following compounds are widely used.

**TABLE 5.2 : Derivatives of Thiophosphoric Acid:**

| Sl. No. | Name of Insecticide | Chemical Name |
|---|---|---|
| 1. | Parathion | O, O – Diethyl – O – 4 – nitrophenyl thiophosphate |
| 2. | Methyl Parathion | O, O – Dimethyl-O - 4- nitrophenyl thiophosphate. |
| 3. | Paraoxon | O, O-Dimethyl O-P-nitrophenyl phosphate. |
| 4. | Thiophos ME | O-Methyl – O- ethyl O-4-nitrophenyl thiophosphate |
| 5. | Fenitrothion | O, O-Dimethyl-O-4-nitro-3 methylphenyl thiophos-phate. |
| 6. | Chlorothion | O, O-Dimethyl-O-4-nitro-3chlorophenyl thiophosphate. |
| 7. | Dicapthon | O, O-Dimethyl-2-chloro-4-nitrophenyl thiophosphate. |
| 8. | Ronnel | O, O – dimethyl–O-2, 4, 5-trichlorophenyl thiophosphate. |
| 9. | Bromophos | O, O-Dimethyl-O-2, 5-dichloro-4-bromophenyl thiophosphate. |
| 10. | Fenthion | O, O-Dimethyl-O-(4-methyl mercapto-3-methyl phenyl thiophosphate. |
| 11. | Dasanit | O, O-Diethyl-O-p-(methyl sulphinyl)-phenyl phosphate. |
| 12. | Diazinon | O, O-Diethyl-O(2-isopropyl-4-methyl pyrimidyl) 6-thiophosphate. |
| 13. | Demeton | O, O-Diethyl-2-ethyl mercapto ethyl thiophosphate. |
| 14. | Methyldemeton | O, O-Diethyl-2-ethylmercapto ethyl thiophosphate. |
| 15. | Dursban | O, O-Diethyl O-3, 5, 6-trichloro pyridyl thiophosphate. |
| 16. | Potasan | O, O-Diethyl-O-(4-methyl-coumarinyl 7-thiophos-phate. |
| 17. | Vamidothion | O, O-Dimethyl-S-(5-methoxy-pyronyl-2-methyl) thiophosphate. |
| 18. | Acetophos | O, O-Diethyl –S-Carboethoxy methyl thiophosphate. |
| 19. | Chlorpyrifos | O, O-Diethyl O-3, 5, 6-trichloro 2-pyridyl phospho-rothioate. |

### 5.5.1.3 Derivatives of Dithiophosphoric Acid:

The compounds under the class are more stable but less toxic than the corresponding compound of thiophosphoric acid. More than 25 different derivatives are found effective as insecticide in agricultural applications. The list of some important compounds is furnished below.

**TABLE 5.3 : Derivatives of dithiophosphoric Acid:**

| Sl. No. | Name of Insecticide | Other Name | Chemical Name |
|---|---|---|---|
| 1. | Malathion | Malathon, Carbofos, Mercaptothion, | O, O-Dimethyl S-1, 2-dicarbo – ethoxy ethyl dithiophosphate. |
| 2. | Dimethoate | Maldison Fosfamid | O, O-Dimethyl-S-(N-methyl – carbamoyl methyl) dithiophos – phate. |
| 3. | Morphothion | | O, O-Dimethyl-S-(morpholino – carbamoyl methyl) dithiophos – phate. |
| 4. | Formothion | | O, O-Dimethyl-S-(N-methyl-N-formylcarbamoylmethyl) dithio-phosphate. |
| 5. | Thimet | Phorate, Timet | O, O-Diethyl-S-(ethyl thiomethyl) dithiophosphate. |
| 6. | Ethion | Diethion | O, O, O, O-tetra ethyl-S, S-methylene bisdithiophosphate. |
| 7. | Ekatin | | O, O-Dimethyl-S-(2 ethyl merca-ptoethyl) dithiophosphate. |
| 8. | Disyston | | O, O-Diethyl-S-(2-ethylmercapto-ethyl) dithiophosphate. |
| 9. | Tetrathion | Benzofos | O-Methyl O-ethyl-S(2-ethyl thioethyl) dithiophosphate. |
| 10. | Phosalone | | O, O-Diethyl-S-(6-chlorobenzo-linyl-3-ethyl) dithiophosphate. |
| 11. | Imidan | | O, O-Dimethyl-S(napthyl-imidomethyl) dithiophosphate. |
| 12. | Guthion | | O, O-Dimethyl-S-(3, 4-dichloro- 4-keto-1, 2, 3-benzotriazinyl-3-methyl) dithiophosphate. |
| 13. | Menazon | | O, O-Dimethyl-S-(4, 6-diamino-1, 3, 5-triazinyl-2-methyl) dithio-phosphate. |
| 14. | Edifenphos | | O-Ethyl S, S-diphenyl, phosphorodithioate. |

### 5.5.1.4 Miscellaneous Organo Phosphorous Compounds:

**TABLE 5.4 : Miscellaneous Organo Phosphorous Compounds:**

| Sl. No. | Name of Insecticide | Chemical Name |
|---|---|---|
| 1. | Trichlorofon | O, O-Dimethyl-(1-hydroxy – 2, 2, 2-trichloroethyl) phosphate. |
| 2. | EPN | O-Ethyl-O-(4-nitrophenyl) benzene thiophosphate. |

## 5.6 ANALYSIS OF ORGANO PHOSPHOROUS PESTICIDES:

### 5.6.1 Compounds of Forensic Interest:

Dichlorovos, Phosphamidon, Mevinphos, Methyl Parathion, Fenitrothion, Fenthion, Chlorpyriphos, Quinalphos, Diazinon, Metasystox, Dimethoate, Malathion, Ethion, Thimet, Edifenphos etc.

The analytical steps include extraction of pesticide residue in biological and non-biological materials, their stripping and analysis by different methods.

**Isolation and stripping of Organo-phosphorous insecticides in biological and Non-Biological Matrices:**

For isolation and stripping, refer section 2.

**Procedure:-**

1. Biological materials (having high lipid content) are homogenized and extracted with diethyl ether, hexane, ethyl acetate, petroleum ether or acetone-hexane mixture (diethyl ether is mostly used) as described in Section 2.

2. Samples with low water content or dried samples are homogenized and extracted with binary solvent mixtures viz. hexane-acetone (1 : 1), hexane-isopropanol (1 : 1), hexane-isopropanol (3 : 1) or methanol or acetone or acetonitrile. Presoaking of sample with distilled water for 5 minutes may improve the extraction efficiency.

3. Moist samples such as vegetables, fruits etc. are usually homogenized and extracted with a binary solvent mixture such as hexane-acetone (1 : 1) or (4 : 1), hexane-isopropanol (3 : 1) in the presence of anhydrous sodium sulphate.

4. Soil sample is extracted with acetone-hexane, methanol-acetone or acetonitrile by shaking.

### 5.6.2 Detection and determination of organo-phosphorous insecticides:

The analytical methods for detection and determination of residues of organo-phosphorous insecticides are based on chromatographic methods viz. TLC ( or HPTLC), GLC or HPLC under diverse analytical conditions. The purified extract of samples (biological and non-biological matrices) are subjected to analysis by any of the above methods.

#### 5.6.2.1 Thin layer Chromatography

**TLC conditions and Spray reagents for identification of Organo -phosphorous pesticides:**

Plate: Silica gel G (thickness 0.2 mm).
Solvent System: Hexane : Acetone : : 9 : 1.
Method: Ascending
Identification : Comparing Rf values of unknown and control samples after location of spots by any of the Spray reagents.

**Chromogenic (Spray) Reagents for TLC Identification of Organo-phosphorous Insecticides:**

I. **Cobalt Acetate – O-Toludine Reagent:**

**Preparation of Reagent :**

A. Cobalt Acetate Solution: 5 gms. of cobalt acetate is dissolved in 100 ml. of distilled water.

B. O-Toludine Reagent: 1 gm. of O-toludine is dissolved in 100 ml. of 10% (v/v) acetic acid.

**Method of Spraying and the Colour of Spot** **:** The developed plate is sprayed with 5% sodium hydroxide solution followed by 5% cobalt acetate solution. After 5 minutes the plate is again sprayed with O-toludine in acetic acid. Blue spots are observed.

**Responding Compounds :** Phosphamidon and Endosulfan.

II. **Tollen's Reagent** **:** 10% Ammoniacal silver nitrate solution.

**Preparation of Reagent :** 10 gms. of silver nitrate is dissolved in 100 ml. of water followed by addition of a few drops of nitric acid. After some time ammonia solution is added drop wise when a precipitate appears which dissolves on adding excess of ammonia.

**Method of Spraying and the Colour of Spot.** **:** The plate is sprayed directly with the reagent when black spots are observed.

**Responding Compounds** **:** Phosphamidon, Nuvan, Fenthion (Dalf), Dasnit, Carbofuran, Zineb, Dipteren and other phenolic compounds Propoxur (Baygon), carbaryl (Sevin) .

## Selective detection of Dichlorovos (DDVP):

**Preparation of Reagent:**

1. **1% Phenylhydrazine hydrochloride Solution (w/v):**

   1 gm. of phenyl hydrazine hydrochloride is dissolved in 100 ml. of distilled water and filter.

2. **10% Hydrochloric Acid Solution:**

   10 ml. of 32% hydrochloric acid is diluted to 100 ml.

   **Method of Spraying and Colour of Spot:**

   The developed plate is sprayed with 1% phenyl hydrazine hydrochloride solution. Yellowish-red spots appear after 5 minutes. The spots turn red on spraying with 10% hydrochloric acid solution.

## Selective Detection of Monocrotophos by Diazotised Sulphanilamide or Sulphanilic Acid Reagent:

1. **Diazotised Sulphanilamide / Sulphanilic Acid Reagent:**

   0.5 gms. of sulphanilamide or sulphanilic acid and 1 gm. of sodium nitrite are dissolved in 100 ml. ice cold solution of 10% (v/v) hydrochloric acid.

2. **20% Sodium Hydroxide Solution:**

20 gms. of sodium hydroxide is dissolved in 100 ml. of distilled water.

**Method of Spraying and Colour Spot:**

The developed plate is sprayed with sodium hydroxide solution. After 5 minutes it is sprayed with ice-cold diazotised sulphanilic acid or sulphanilamide.

Orange spots are obtained.

**Responding Compounds:**

In addition to Monocrotophos, phenolic compounds and carbamate insecticides also respond.

**Detection of Monocrotophos by Chloranil reagent:**

**Reagent Solutions**:

(a) Chloranil Reagent (0.5 % w/v) : Dissolve 0.5 g. chloranil in 100 ml acetone.
(b) Anhydrous sodium carbonate solution(20% w/v) :- Dissolve 20 g. of anhydrous sodium
carbonate in 100 ml distilled water.

**Procedure:** Spray the developed plate with 20 % anhydrous sodium carbonate solution, followed by 0.5 % chloranil reagent.

**Color of the spot: RED**
**Solvent system:** Chloroform + Acetone (7 + 3)

**Responding compounds:** Monocrtophos (Nuvacron).

**Other chromogenic reagents for TLC**

a) Palladium (II) chloride.

b) Congo red.

## 5.7 ORGANO CHLORO PESTICIDES:

Organo-chloro insecticides are being extensively used in agriculture and also familiar in domestic applications. New varieties of these insecticides are emerging every year. Owing to easy availability, these insecticides are frequently misused in homicidal and poisoning cases. Accidental poisoning cases are also known. The identification and sometimes quantitation is required which is done by TLC or HPTLC, GLC, HPLC and UV spectroscopy after extraction of pesticide residue in biological and non-biological, matrices. The commonly encountered Organo chloro insecticides are given below.

**TABLE: 5.5 - List of organo- chloro Insecticides**

| Sl. No. | Name of Insecticide | Chemical Name |
|---|---|---|
| 1. | DDT | 1, 1, 1, Trichloro –2, 2 bis (p-chloro phenyl) ethane. |
| 2. | BHC | Gamma isomer of 1, 2, 3, 4, 5, 6 hexachloro cyclohexane (Benzene hexachloride). |
| 3. | Lindane | Gamma isomer of hexachloro cyclohexane. |
| 4. | Endrin. | 1, 2, 3, 4, 10, 10-Hexachloro 6, 7, epoxy 1, 4, 4a, 5, 6, 7, 8, 8a octahydro 1,4,5,8 endo-exo dimethano naphthalene. |
| 5. | Dieldrin | 1, 2, 3, 4, 10, 10-Hexachloro 6, 7, epoxy 1, 4, 4a, 5, 6, 7, 8, 8a octahydro 1,4,5,8 endo-exo dimethano naphthalene. |
| 6. | Endosulfan | 6, 7, 8, 9, 10, 10-Hexachloro 1, 5, 5a, 6, 9, 9a-hexahydro 6, 9 methano 2, 4, 3 – benzo oxathiepin – 3- oxide. |
| 7. | Heptox | 1, 4, 5, 6, 7, 8, 9-Heptachloro 3a, 4, 7, 7a-tetrahydro-4, 7 endo-methanonapthalene. |
| 8. | Chlorodan | 1, 2, 4, 5, 6, 7, 8, 8a-Octachloro 3a, 4, 7, 7a tetrahydro – 4, 7 methano indane. |
| 9. | Toxphene. | Chlorinated comphene. |

| | | |
|---|---|---|
| 10. | Kelthane | 1, 1-Bis (chlorophenyl) 2, 2, 2-trichloro-ethane. |
| 11. | Heptachlor | 1, 4, 5, 6, 7, 8, 8a –Heptachloro 3a, 4, 7, 7a-tetrahydro 4, 7-methano indane. |
| 12. | Methoxychlor | 2, 2 – Bis-(p-chlorophenyl) 1, 1, 1-trichloro-ethane. |

## 5.8 ANALYSIS OF ORGANO CHLORO PESTICIDES:

DDT, Gammaxene, Aldrin, Endrin, Endosulfan are widely used.

### 5.8.1 Isolation and Stripping of Organo chloro insecticide in Biological and Non-Biological Matrices:

The extraction, stripping methods and selection of solvent for extraction have already been covered in sections 2 and also under organo phosphorous compounds. The analytical methods include TLC or HPTLC, GLC, HPLC and UV spectroscopy. A few analytical methods or conditions of chromatography will be covered hereunder.

### 5.8.2 Detection and Identification of Organo Chloro insecticides:

#### 5.8.2.1 TLC conditions and spray reagents for identification of organo-chloro insecticides:

Plate : Silica gel G (thickness 0.2 mm.).

Solvent system : Hexane : Acetone : : 9 : 1.

Method : Ascending.

Identification : Comparing Rf values of unknown and control samples after location of spots by any of the following spray reagents.

**Chromogenic (Spray) Reagents for TLC Identification of Organo-chloro Insecticides:**

I. **Zinc Chloride - Diphenylamine Reagent.**

**Preparation of Reagent:**

0.5 gm of diphenylamine and 0.5gm of zinc chloride are dissolved in 100 ml. of acetone.

**Mode of Spraying:**

The developed plate is sprayed with the reagent solution and then heated for 10 minutes at 110$^{o}$C.

Bluish green spots are obtained.

**Responding Compounds:**

BHC, Endosulfan, DDT, Edrin, Aldrin, Dieldrin, Toxaphen and Heptachlor. A few of organophospho-rous compounds phorate, Sumithion, DDVP, Phosphamidon, Phosalore also respond.

II. **O-Toludine Reagent:** (6)

**Preparation of Reagent:**

0.5 gm. of O-toludine is dissolved in 100 ml. of acetone.

**Mode of Spraying:**

The developed plate is sprayed with O-toludine reagent and then exposed to UV irradiation for 10 minutes.

Bluish green spots are obtained.

**Responding Compounds:**

Endrin, Endosulfan, DDT, BHC, Aldrin, Dieldrin, Heptachlor, Toxaphen.

III. **Nickel-Amine Reagent for Specific Detection of Endosulfan.** (7,8)

**Preparation of Reagent:**

1. **Nickel Amine Reagent:**

Equal volumes of 5% w/v aqueous nickel chloride solution and 30% ammonic (Sp.gr.-0.88) are mixed.

2. **20% Sodium Hydroxide Solution:**

20 gms. of sodium hydroxide is dissolved in 100 ml. of distilled water.

**Mode of Spraying:**

The developed plate is sprayed with 20% solution of sodium hydroxide solution followed by nickel amine reagent.

Greyish-black spots are obtained.

## 5.9 ORGANO CARBAMATE INSECTICIDES:

The widely used carbamates include Propoxur, Carbaryl, Carbafuran and Zineb. The mechanism of toxic manifestation in mammalian system is similar to organo-phosphorous insecticides, but the toxicity of the former is comparatively lesser.

### 5.9.1 Analysis of Carbamates:

The analysis of carbamates is undertaken in the same way as it is done in case of organo chloro and organo-phosphorous insecticides. The steps include extraction of carbamates in traces in biological and non-biological matrices and their stripping for onward analysis by chromatographic and HPLC methods.

### 5.9.2 Extraction of Carbamates in Biological and Non Biological Matrices:

This is done as described in the case of organo-phosphorous and organo-chloro pesticides. The extract is subjected to analysis.

### 5.9.3 Detection of Carbamates :

#### 5.9.3.1 TLC conditions and Spray reagents for Identification of Carbamate insecticides:

Plate : Silica gel G (thickness 0.2 mm.).

Solvent Systems : 1. Hexane : Acetone : : 9 : 1.

2. Hexane : Acetone : : 8 : 2.

Method : Ascending.

Identification : By comparing Rf values of unknown and control samples after location of spots by any of the spray reagents.

**Chromogenic (Spray) Reagents for TLC Identification of Carbamate Insecticides.**

**1. Tollen's Reagent** : 10% ammonical silver nitrate solution.

Mode of Spraying : Direct spraying with the reagent.

Colour of Spot : Black.

Responding Compounds : Propoxum (Baygon), Carbaryl (Serin), Carbofuran Zinel, Dipterex and other Phenolic compounds, Phosphamidon (Demecron), Nuvan, Fenthion (Dalf, Bay tex), Metasystox.

**2. Alkaline Fast Blue B salt :**

**Preparation of Reagent :** 0.1 gm. of fast blue B salt is dissolved in 100 ml. of 10% aqueous sodium hydroxide solution (freshly prepared).

**Mode of Spraying :** Direct Spraying.

**Colour of the Spots :** Red / Violet.

**Responding compounds :** Propoxur, Carbaryl, Carbafuran, other Phenolic compounds.

**3. Phenyl Hydrazine Hydrochloride Reagent for Carbaryl :**

**Preparation of Reagent :** Equal volume of 1% (W/V) aqueous solution of phenyl hydrazine hydrochloride in mixed with 10% (W/V) aqueous solution of sodium hydroxide solution just before use.

**Mode of Spraying :** Direct spraying with the reagent.

**Colour of Spots :** Red.

### 5.9.4 Analysis of methyl parathion in blood and tissue by SPME-GC-NPD method*

**Sample preparation**: Homogenize 1 g of tissue in 2 mL of water. Transfer 300 µL of homogenate / blood into SPME vial. Adjust the total volume up to 3 mL using distilled water.

**SPME parameters:**

| | |
|---|---|
| Fiber | : Polyacrylate 85 µm |
| Extraction mode | : Headspace |
| Incubation time | : 15 min |
| Extraction temp | : 70 °C |
| Extraction time | : 20 min |
| Salt addition | : 0.8 g (NaCl) |
| Desorption temp | : 230 °C |
| Desorption time | : 4 min |

**GC-NPD analysis**:

| | |
|---|---|
| Capillary Column | :EC-5 (30 m × 0.32 mm × 0.25 µm) |
| Carrier gas | : Helium |
| Injection | : Split less @ 230°C |
| Detector | : Nitrogen Phosphorous Detector @ 300 °C |
| Oven Temperature | : 120 °C for 3 min followed by 10 °C/min up to 230 °C with an 4 min hold; |
| Retention time | : Peak of methyl parathion at 10.8 min. |

## ENVIRONMENTAL CONDITIONS:

Isolation and purification of poisons may be carried out at room temperature.

## METHOD OF QUALITY CHECKS USED:

**Quality checks have been undertaken by one of the following methods.**

1. **Repeat analysis**
2. **Comparison with control**
3. **Use of two different solvent systems in TLC.**

***Reference:**

H. Tsoukali, N. Raikos, G. Theodoridis, D. Psaroulis "Headspace solid-phase microextraction for the gas chromatographic analysis of methyl-parathion in post-mortem human samples: Application in a suicide case by intravenous injection" Forensic Science International 143 (2004) 127-132.

**REFERENCES**


1. Kawale, G. B. Joagalekar, V.D. Barve, V.P; and Mahal,H.S. Sci. Cult, (1972), 38, 373
2. Baumler, J.,& Rippstein, U.S., Helv. Chim . Acta, (1961),44, 1162.
3. Walkar, K.G., & Beroxa , M.J., J. Assoc Off. Agric. Chem. 1963, 46, 250.
4. Irudayswamy , A & Natraja, A.R. Analyst( 1965),90 503.
5. Zweig, G., Analytical Methods for pesticides, plant Grouth Regulators and Food Additives, Vol. V , Academic Press New york & London p. 109.
6. J. Kawashira and Y. Hosagai, Shokuhin Eisegatu Zaashi , (1964), 5, 54.
7. D. Kath; J. Chromatogr. (1964) , 15, 269.
8. V.B. Patil, M.t. Sevelkar and S.V. Padalikar.J. Chromatogr. (Netherlands), (1987), 396, 441-443.
9. V. B. Patil and M. S. Shingare., J Chromatogr., (1993), 653, 181-183.
10. V. B. Patil and M. S. Shingare, Analyst (London ) , 1994, 119, 415-416.
11. M.T. Sevelkar, V.B.Patil and M.V.Garad, Pr5oceedings of XI All India Forensic science conference, Bhopal, 1999.
12. Spot test in Organic Analysis by Fritz Feigl, Elsevier Scientific Pub.Comp. Amsterdam, London, Ed.1975, p.304.
13. Spot test in Organic Analysis by Fritz Feigl, Elsevier Sc.Pub.Comp. Amsterdam, London, Ed.1975 p.244.
14. H.Sommer, Ind.Eng.Chem; Anal.Ed; 12(1940) 388.
15. F.Feigl and V.Anger; Spote Tests in Inorganic Analysis Elsevier Pub.Co. Amsterdam, London, 1972 p.433-434.
16. F.Feigl and V.Anger; Spot Test in Inorganic Analysis ….. (1972) p.435.
17. F.Feigl and V.Anger; Spot Test in Inorganic Analysis … p.436.
18. F.Feigl and V.Anger; Spot Test in Inorganic Analysis … p.436.
19. U.S. Shree Ramulu, Chemistry of Insecticides and fungicides. Oxford and IBH pub. Co. New Delhi, 1979 p.244.
20. U.S. Shree Ramulu, Chemistry of Insecticides and fungicides, Oxford and IBH Pub.Co. New Delhi, 1979, p.251.
21. U.S. Shree Ramulu, Chemistry of Insecticides and fungicides, Oxford and IBH Pub.Co. New Delhi, 1979, p.251.
22. U.S. Shree Ramulu, Chemistry of Insecticides and fungicides, Oxford and IBH Pub.Co. New Delhi, 1979, p.244 - 245.

## SECTION – 6: ANALYSIS OF BASIC DRUGS / POISONS (ORGANIC NON-VOLATILES)

**6.1** **Title**: Analysis of basic drugs / poisons (Organic-non-volatiles).

**6.2** **Scope**: Analysis of basic drugs / poisons (Organic non-volatiles) in crime exhibits especially biological samples (viscera, blood, urine etc.) after their extraction.

**6.3** **Purpose:** To identify basic drugs / poisons (organic non-volatile in crime exhibit).

**6.4** **Responsibilities**: Gazetted Officers and the associated Scientific Staffs for the purpose.

**6.5 CLASSIFICATION:**

Drugs which are alkaline in nature are called basic drugs. They contain different moieties having ring structure viz. pyridine, pyrrole, isoquinoline, quinoline i.e. containing hetero atom N, S, O etc. in the ring. These compounds readily react with acids forming salts. Their classification is based on their physiological action they produce as stated hereunder.

1. **Narcotics:**
   Drugs which interact with those receptors in the brain which are responsible for the transmission and response to pain are known as narcotic analgesics or simply narcotics viz. opium alkaloid, both natural and synthetic viz. Morphine, Codeine, mono-acetyl and diacetyl morphine. On the contrary peripheral analgesics (for example aspirin) have no abuse potential. Now a days various designer synthetic analgesics viz. pethidine (meperidine, methadone, fentanyl) have come up.

2. **Deliriant:**

   These drugs affect the vital part of brain viz. atropine, hyoscyamine, cocaine, muscarine (fungi mushrooms).

3. **Spinal poisons/drugs:**

   These affect spinal tract viz. strychnine, brucine (Source Strychnos Nux Vomica).

4. **Cardiac poisons/drugs:**

   They affect functioning of heart viz. Quinine, aconitine, nicotine, digitalis glycosides.

   Drugs of abuse are common with the above drugs. Their identification is of prime importance in clinical toxicology. When these drugs are consumed by the user in more therapeutic dose, the outbreak of sign and symptoms occur and the drugs are likely to be distributed in blood and urine. Thus, clinical toxicological cases blood and urine the main crime exhibit. In acute cases resulting death, stomach contents

and viscera are to be taken into consideration for extraction of drugs along with blood or urine.

## 6.6 EXTRACTION OF DRUGS :

The procedures for extraction have already been covered in Section 2.

## 6.7 DETECTION AND IDENTIFICATION OF BASIC DRUGS:

The identification methods include screening tests, chemical tests, TLC, GLC, HPLC and also UV Spectrophotometry. However, the above cited methods may be employed profitably for characterization of class and the specific member of the class, after a particular or important group has been elaborated to meet the requirement and these methods are applied to sample after their extraction.

### 6.7.1 Colour Tests performed with extracts for Basic drugs:

Colour tests for basic drugs performed with extract.

| ALKALOIDS | COLOUR DEVELOPED WITH REAGENT | | |
|---|---|---|---|
| | MARQUIS | CONC.NITRIC ACID | MANDELIN |
| Morphine | Violet | Bright orange yellow | Dark reddish brown |
| Heroin | Reddish purple | Pale yellow | Reddish brown |
| Codeine | Dark violet | Greenish yellow | Olive green |

### 6.7.2 Colour Tests with Different Reagents:

Various basic drugs respond to different reagents to produce a variety of colors, which sometimes can be useful for a screening test. The following table can be useful to a large extent to identify the group of drugs.

**TABLE: 6.1: Colour Tests with Different Reagents:**

| **Reagent** | **Preparation of Reagent** | **Colour Observed** | **Class of Compound Present** |
|---|---|---|---|
| Formaldehyde – Sulphuric Acid | 4 parts of sulphuric acid + 6 parts of formalin | Red/Pink/Blue/violet/Red-violet/Blue- violet | Benzodiazepines /Phenothiazine |
| FPN Reagent | 5 ml. of ferric chloride sol- ution + 45 ml. of 20% w/v solution of Perchloric acid + 50 ml. of 50% v/v solu- tion of nitric acid. | Orange red / Violet red / Brown red / Orange / Red orange / Pink orange /Blue/ Violet / Red Brown. | Phenothiazines |
| Iodoplatinate Solution. | 2 ml. of 5% solution of platinic chloride in 2N Hcl + 5 gms. of potassium iodide to 98 ml. water with stirring. | Violet / Blue-violet / Brown violet / Grey violet. | Alkaloids. |
| Marquis Reagent | 1 volume of formalin + 9 vols of concentrated sulphuric acid. | Yellow / Orange | Benzodiazepines |
| -do- | -do- | Violet<br>Reddish purple<br>Dark violet | Morphine<br>Heroin<br>Codeine |
| Dragendorff's Reagent | 1 gm of bismuth subnitrate is dissolved in 3 ml. of 10M hydrochloric acid , if necessary by gentle heating. It is diluted to 20 ml. It is dissolved in 1 gm of potassium iodide. If black precipitate of bismuth tri-iodide separates, it is dissolved in 2M hydrochloric acid. | Orange / Red-orange / Brown orange Precipitate. | Alkaloid / primary or Secondary or tertiary amine. |

### 6.7.3 Thin Layer Chromatography

#### 6.7.3.1 TLC conditions and data for screening of some common Basic drugs:

Solvent System : Methanol **:** Ammonia (100 **:** 1.5v/v)

Plate : Silica gel G(0.2 mm thickness)

Development : Ascending technique.

Spray reagent : Iodoplatinate Solution – Violet / Blue violet / Brown violet / (for alkaloids) Grey violet spots.

Dragendorff's Reagent – Orange Spots.

(alkaloids & benzodiazepines).

FPN Reagent - Orange red / Violet red / Brown red / (Phenothiazines)Orange / Red orange / Pink orange / Blue / Violet / Red Brown spot.

Sample : Extract of samples along with control drugs and alkaloids.

#### 6.7.3.2 TLC conditions and data for screening of some Benzodiazepines: (8,9,10,11)

Plate : Silica gel G (0.2 mm. thickness)

Development : By ascending technique.

Solvent systems : No. 1 : Chloroform : Acetone : : 4 : 1

No. 2 : Ethyl acetate : Methanol : Strong Ammonia Solution : : 85 : 10 : 5

No. 3 : Ethyl acetate.

Spray Reagent : Dragendorff's Reagent – Orange, Red – orange / Brown orange spot Other reagents viz. Marquis Reagent or

Iodo platinate & Reagent may be used.

**TABLE:6.2: TLC data of some Benzodiazepines**

| Sl. No. | Compound | hRf Value in Solvent Systems | | |
|---|---|---|---|---|
| | | No. 1 | No. 2 | No. 3 |
| 1. | Bromazepam | 13 | 62 | 20 |
| 2. | Chlordiazepoxide | 10 | 51 | 11 |
| 3. | Clobazam | 53 | 73 | 49 |
| 4. | Clonazepam | 35 | 60 | 45 |
| 5. | Demaxepam | 15 | 44 | 22 |
| 6. | Diazepam | 58 | 77 | 48 |
| 7. | Flunitrazepam | 54 | 77 | 48 |
| 8. | Flurazepam | 03 | 74 | 03 |
| 9. | Ketazolam | 45 | 73 | 45 |
| 10. | Lorazepam | 23 | 45 | 39 |
| 11. | Medazepam | 56 | 79 | 40 |
| 12. | Nitrazepam | 35 | 59 | 45 |
| 13. | Nordazepam | 34 | 67 | 45 |
| 14. | Oxazepam | 22 | 44 | 37 |
| 15. | Prazepam | 64 | 81 | 55 |
| 16. | Temazepam | 51 | 63 | 47 |
| 17. | Triazolam | 05 | 45 | 02 |

### 6.7.3.3 TLC conditions and data for screening of some common drugs of abuse:

Plate : Silica gel G (0.2 mm. thickness)

Development : By ascending technique.

Solvent systems : No. 1 : Cyclohexane : Tolune : Diethylamine
(75 :15 : 10)
No. 2 : Methanol : Conc. Ammonia
(100:1.5)
No. 3 : Chloroform : Methanol : : 90 : 10.

Spray Reagent : 1. Acidified Potassium Iodoplatinate :
Violet / Blue violet / Brown violet / Grey violet

Spot.

2. Dragendorff's Reagent – Orange / Brown

Orange, Reddish Orange spot.

**TABLE:6.3: Rf Values of Some Common Drugs of Abuse:**

| **Serial No.** | **Compound** | **hRf in Solvent Systems** | | |
|---|---|---|---|---|
| | | **No. 1** | **No.2** | **No.3** |
| 1. | Methaqualone | 40 | 74 | 80 |
| 2. | Mecloqualone | 30 | 74 | - |
| 3. | Cocaine | 52 | 67 | 47 |
| 4. | Caffeine | 05 | 63 | 58 |
| 5. | Heroin | 19 | 46 | 38 |
| 6. | Diphenhydramine | 56 | 55 | 33 |
| 7. | Diazepam | 29 | 75 | 73 |

**References:**


1. Gill, R et al, J.chromatogr. 218, 639, 1981
2. Law B et al., J. Chromatogr. 301, 1984
3. Kabraj, P.M et al., Clinica Chim Acta, 111, 1981.
4. Gill R, unpublished data
5. Flanagan et al., J. Chromatogr., 187, 1980.
6. Gill, R. et al., J. Chromatogr., 301, 1984.
7. Pharm.Acta.Helv.61 (1986), pp 167-178
8. J. Chromatography 179 (1979), pp 381-389
9. J. Chromatography 194 (1980), pp 262-269
10. J. Chromatography 179 (1986), pp 169-176

# SECTION – 7: ANALYSIS OF ACIDIC (ORGANIC NON-VOLATILE) POISONS/ DRUGS

**7.1 Title**: Analysis of Acidic (organic non-volatile) poisons/ drugs

**7.2 Scope:** Analysis of acidic organic non volatile poisons / drugs in crime exhibits by different methods.

**7.3 Purpose**: To identify the acidic (organic non volatile) poisons/drugs in crime exhibits.

**7.4 Responsibilities:** Gazetted officers and other scientific staff

**7.5 ACIDIC POISONS/DRUGS:**

**Acidic Drugs:**

Drugs which are acidic in nature are called acidic drugs. These drugs readily react with bases forming salts. The main acidic drugs are barbiturates (substituted malonyl urea) and salicylates etc. and a few compounds other than barbiturate viz. Glutithimide, Meprobamate etc.

**Acidic Poisons**:

Phenolic Compounds viz. phenol, cresols, β-naphthol etc.

### 7.5.1 Barbiturates

Barbiturates (salts of barbituric acid) are the drugs that are associated with criminal poisoning cases (homicidal and suicidal) in the Indian perspective due to their easy availability. Thus, the search for barbiturate in biological materials is of importance in case of suspected poisoning by drugs.

#### 7.5.1.1 Analysis of Barbiturates:

As stated earlier for other toxicants under different classes in the preceding sections, the active constituent i.e. barbiturate is to be extracted in biological and non-biological matrices and stripped thereafter prior to analysis for their identification by diverse methods viz. colour test, screening by UV Spectroscopy, TLC or HPTLC, GLC and HPLC etc.

##### 7.5.1.1.1 Extraction of Barbiturates in Biological and non biological matrices:

This is achieved according to methods described in Section-2. Normally, the acid-ether part is kept reserved for onward analysis. The extract is stripped, dried over anhydrous sodium sulphate and evaporated to dryness as and when required for analysis.

### 7.5.1.1.2 Colour tests for the presence of Barbiturates in extracted materials:

**Colour Test:**

The colour tests are the screening tests in the general method of analysis of toxicants. The positive finding is a presumptive indication for any of the barbiturates necessitating confirmed identification. However, these tests are very important.

1. **Dille – Koppayani Test:**

**Preparation of Reagent:**

A. **Cobalt Acetate Solution:** 1 gm. of cobalt acetate (tetrahydrate) is dissolved in followed by addition of 0.2 ml. of acetic acid.

B. **Isopropylamine Solution:** 5 ml. of isopropylamine is mixed with 100 ml. of methanol.

**Test Procedure No. 1:**

A small amount of extracted material is placed on a spot plate. 3-4 drops of cobalt acetate solution and 3-4 drops of isopropylamine solution are added. The appearance of a purple or blue violet colour indicates the presence of barbiturate.

**Test Procedure No. 2:**

The residue of extract of sample is taken in 1 ml. of chloroform. To a portion of chloroform extract of the sample, 2 drops of freshly prepared 1% cobalt acetate in methanol is added followed by 1% lithium hydroxide in methanol drop by drop. A blue ring at the junction indicates the presence of barbiturates.

### 7.5.1.1.3 Identification of Barbiturates by TLC: (1)

Plate : Silica gel G(0.2 mm thickness).
Development : By ascending technique.

Solvent System : System 1: Ethyl Acetate : Methanol : 25% Ammonia (25 : 50 : 25)

: System 2 : Chloroform : Acetone : : 80 : 20.

: System 3 : Isopropyl Alcohol : Chloroform : ammonia (45 : 45 : 10)

**Spray Reagents and Colour of Spots:**

1. **Mercuric Chloride – Diphenylcarbazone Reagent:**

A. **Diphenyl carbazone Solution:** 0.1 gm. of diphenyl carbazone is dissolved in 50 ml. of ethanol.

B. **Mercuric Chloride Solution:** 0.1 gm. of mercuric chloride is dissolved in 50 ml. of ethanol.

The mixing of A and B is in equal volume is done before spraying (Solution A is to be replaced by a fresh bath after a short interval to get better spots.

**N.B. The spraying reagent contains toxic mercuric salt and is to be done in fume chamber to avoid hazards.**

**Colour of Spots:** Blue violet spots on a pink background are observed for barbiturates.

**TABLE : 7.1**

| **Compound (Barbiturate)** | **hRf in Solvent Systems** | | |
|---|---|---|---|
| | **No. 1** | **No. 2** | **No. 3** |
| Allobarbital. | 31 | 50 | 53 |
| Amobarbital. | 40 | 52 | 74 |
| Barbital. | 33 | 41 | 51 |
| Cyclobarbital. | 35 | 50 | 59 |
| Phenobarbital. | 44 | 55 | 76 |
| Secobarbital. | 29 | 47 | 38 |
| | 42 | 55 | - |

**ENVIRONMENTAL CONDITIONS:**

Isolation and purification of poisons may be carried out at room temperature.

**METHOD OF QUALITY CHECKS USED:**

**Quality checks have been undertaken by one of the following methods.**

1. **Repeat analysis**
2. **Comparison with control**
3. **Use of two different solvent systems in TLC.**

**References:**


1. "Thin Layer Chromatographic Rf Values of Toxicologically relevant substances on standardized systems" – DFG/TIAFT, VCH. Verlagsgeselschaft, (weinheim) 1987.

2. "Clarke, EGC Isolation & Identification of Drugs" 2nd Edition, The Pharmaceutical Press (London), 1986.

3. "Clarke, EGC Isolation & Identification of Drugs" 2nd Edition, The Pharmaceutical Press (London), 1986.

4. J. Chromatography 204 (1901) 275-284

5. J. Chromatography 192 (1980) 363-374

6. J. Chromatography 427 (1988) 172-180 (modified)

7. J. Chromatography 204 (1981) 275-284

8. Clarke, EGC Isolation & Identification of drugs, The Pharmaceutical Press, London, 1986, II edition, p-1156

9. Clarke, EGC Isolation & Identification of drugs, The Pharmaceutical Press, London, 1986, II edition, p-361, 965.

10. British Pharmacopoeia, p-154, 1447

# SECTION 8: LIST OF EQUIPMENTS REQUIRED FOR TOXICOLOGICAL ANALYSIS OF DRUGS AND POISONS

## 8.1 Major Equipments

a) Gas Chromatography – Mass Spectrometry (GC-MS)
b) Head Space Gas Chromatography-Flame Ionization Detection (HS-GC-FID)
c) High Performance Liquid Chromatography (HPLC)
d) Atomic Absorption Spectrophotometer
e) UV-Visible Spectrophotometer
f) FTIR Spectrophotometer

## 8.2 Minor Equipments

a) Thin Layer Chromatography setup
b) Hot air oven
c) Tissue homogenizer
d) Centrifuge
e) Sonicator
f) Vortex shaker
g) UV-Cabinet (254 nm, 265nm and visible lights)
h) Hot plate cum magnetic stirrer
i) Fuming hood
j) Refrigerator
k) Water bath
l) Analytical balance

**Formation of committees at National Level for formulation of SOPs and Manuals:**

**Background:** In view of technological advancements in the scientific arena, the Standard Operating Procedures (SOPs) and Working Procedure Manuals, around which the technical and analytical exercise takes place in the laboratory in the examination of crime exhibits, needs periodical review to keep the laboratory updated.

For uniform SOP/Manuals and reporting pattern in all the CFSLs / State FSLs following committees were formed by JS (PM), MHA by including members from Central and State FSLs in the following areas:

| Discipline | CFSL Member | Member |
|---|---|---|
| Biology/DNA | Dr. A. K. Sharma, Director, CFSL, Kolkata/Guwahati | 1. Sh. Arun Sharma, Director, FSL, HP<br>2. Sh. Srikumar, Director, Chemical Examiner Lab, Thiruvananthapuram. |
| Chemistry/ Narcotics | Sh. K. M. Varshney, Coordinator, CFSL, Pune | 1. Dr. R. K. Gupta, Director, FSL, Chhattisgarh.<br>2. Sh. B Shanmukham, Director, FSL, Puducherry.<br>3. Dr. Harsh Sharma, Director, FSL, Sagar (MP) |
| Explosives | Dr. Sukhminder Kaur, Coordinator CFSL, Pune | 1. One officer from FSL, Delhi<br>2. One officer from FSL, Maharashtra |
| Toxicology | Dr. Vimukti Chauhan, SSO, CFSL, Chandigarh | 1. Dr. K. V. Kulkarni, Director, DFSL, Maharashtra<br>2. One officer from FSL, Karnataka. |
| Ballistics | Sh. S. S. Baisoya, CFSL Chandigarh | 1. Dr. D. K. Kaushal, Director, FSL, Haryana<br>2. Sh. N. P. Waghmare, Director, FSL, Goa<br>3. Dr. S. S. Das, Director, FSL, Odisha |
| Documents | Sh. M. C. Joshi, Dy. Director, CFSL, Chandigarh (Shimla Unit) and Dr. S. Ahmad, DFSS HQs., New Delhi | 1. Ms. Deepa Verma, Director, FSL, Delhi |
| Psychology, Computer, Audio-Video | Dr. S. K. Jain, Director, CFSL, Chandigarh and Sh. M. Krishna, AD, CFSL, Hyderabad | Officers from FSL: HP, Delhi, Gujarat and Maharashtra |
| Crime Scene | Dr. M. Baskar, Dy. Director (Physics), CFSL, Chandigarh | 1. Dr. Harsh Sharma, Director, FSL (MP)<br>2. Sh. R. K. Gupta, Jt. Director, FSL, Chhattisgarh |

The officers of CFSLs will coordinate with the experts of State FSLs for convening of meeting(s) in the state and Central FSLs and finalization of SOPs and manuals.