**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 in arrowheads. Traditionally, the toxicology is defined as the science embodying the knowledge, source, character, fatal effect, lethal dose, 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 specialists 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 as 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 toxicologistsis 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 introduced 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 meet the ends it has envisaged.

**Section –2: CLASSIFICATION OF POISONS AND POISONING**

**2.1 Title:** Classification of poisons and poisoning.

**2.2 Scope:** Poisons encountered in the toxicological analysis

**2.3 Purpose:** To classify the poisons and isolate it from the exhibits.

**2.4 CLASSIFICATION:**

Poisons can be classified in a variety of ways, depending upon the interests and needs of classifier e.g. classification may be done on the basis of mode of action of poison, toxicity rating, in terms of their physical states, their labeling requirements, their chemistry etc. For the purpose of toxicological analysis, poison are classified on the basis of their chemical properties and method of isolation from tissues and other biological fluids which are given below:

**2.4.1 Noxious Gases:**

(1) Carbon Monoxide (CO), (2) Carbon dioxide (CO2), (3) Hydrogensulphide (H2S), (4) Sulphurdioxide (SO2), (5) Chlorine (Cl2), (6) Nitrous oxide (N2O), (7) Methane (CH4), (8) Methylisocyanide (CH3NCO), (9) War gases, (10) Ammonia, (11) Tear gas (chloracetophenone).

* + 1. **Volatile Inorganic:**

(1) Cyanide (2) Phosphine (3) Arsine (4) Phosgene, (5) Stilbine (6) Carbonyl chloride (8) Flurocarbon (9) Isocyanide.

**2.4.3 Volatile Organic:**

(1)Methanol (2) Ethanol (3) Formaldehyde (4)Acetaldehyde (5) Chloral hydrate (6) Pyridine (7)Ketones (8) Hydrocarbons (9) Chloroform

**2.4.4 Non –volatile Inorganic:**

**2.4.4.1 Anions:**

(1) Halides (2) Selenide (3) Dichromate (4) Chlorate (5) Azide (6) Nitrite (7) Sulphide (8) Sulphate (9) Nitrtate (10) Phosphide (11) Cyanide etc.

**2.4.4.2 Cations:**

(1) Mercury (2) Arsenic (3) Barium (4) Thallium (5) Lead (6) Antimony

(7) Bismuth (8) Copper (9) Aluminium (10) Zinc etc.

**2.4.5 Non-volatile Organic Neutral Compounds:**

(1) Pesticides (Organophosphorous, Organochloro, Carbamates and Pyrethroids) (2) Neutral Compounds.

**2.4.6 Non-volatile Organic Acidic Compounds:**

Acidic Drugs like barbiturates, sulpha and phenolic compounds, salicylates etc.

* + 1. **Non-Volatile Organic Alkaline Compounds:**

All basic drugs like benzodiazepines, alkaloids etc.

**2.4.8 Plant Poisons**:

Dhatura, Aconite, Oleander, Nuxvomica, Abrus Precatorius etc.

**2.4.9 Miscellaneous Poisons:**

(A) Mechanical: Glass powder, Diamond dust, chopped hair etc.

(B) Food poisons / mycotoxins.

(C) Animal/insect poisons.

(D) Water soluble compounds.

**2.5 POISONING:**

Poisons are commonly involved in homicidal, accidental or suicidal cases. These are also used to destroy the animals and plants.

* + 1. **Accidental poisoning:**

The accidental poisoning commonly takes place as a result of the carelessness/ negligence. The common accidental poisoning cases are:

a) Coal is allowed to burn in a room unknowingly giving rise to the production of poisonous carbon monoxide gas.

b) Workers many times unknowingly go into abandoned wells or gutters and die due to the presence of poisonous gases.

c) Poisonous materials like insecticides are carelessly sprayed in the farms and due to accidental inhalation, many times may cause the death of farmers/workers.

d) Children handle some poisonous articles and develop poisonous symptoms.

e) Overdoses of medicines like barbiturates or fake medicines wrongly prescribed**.**

f) Allergic conditions.

g) Bites by poisonous insects and snakes**.**

h) Accidental cattle or animal poisoning cases.

**2.5.2 Homicidal Poisoning:**

Owing to small fatal doses, tasteless and odourless properties, miscibility with drink and common availability, some poisons are mainly used for homicidal purposes e.g. arsenic salts, mercury salts, cyanides, sodium nitrite, methyl alcohol, dhatura seeds, phosphides etc. Rarely, cultures of disease germs are also used for homicidal purposes. In certain parts of the country, opium is used as infanticidal poison.

**2.5.3 Suicidal Poisoning:**

Some poisons are mainly popular for suicidal purposes because of their availability in house or the working place like cyanide in electroplating units, insecticidal compounds mostly available with farming communities, sodium nitrite in dyeing industries. Barbiturates are normally used by the educated people. The cases of committing suicides by a few doctors by injecting anaesthetic agents like thiopental have been reported.

**2.5.4 Miscellaneous Poisoning:**

Cases of poisoning other than the above are prevalent all over India which are caused by stupefying agents. The intention behind this is to stupefy the person and commit robbery or other crimes. However, many a times person dies because of overdoses. These poisons are cigarettes containing dhatura, cannabis, drugs, arsenic etc; sweets containing phenobarbitone or other psychotropic substances like lorazepam etc. Some times, chloral hydrate mixed with drinks is also similarly used.

**2.6 ACTIONS AND ADMINISTRATION OF POISONS:**

The action of almost all the poisons is the same. They either stop the supply of oxygen to the body tissues or inhibit the enzymes associated with the respiration mechanism and the person dies due to stoppage of oxygen availability. However, the mode of stoppage varies e.g. insecticides and pesticides are powerful inhibitors of cholinesterase. The site of action is said to be at the myoneural junctions and synapses of the ganglions. At these sites, normally, acetylcholine is liberated from nerve stimulation. The liberated acetylcholine is hydrolysed into choline and acetic acid by cholinesterase. In poisoning by insecticides and pesticides, the activity of cholinesterase is inhibited, acetylcholine therefore accumulates and results in hyper excitation of the voluntary and involuntary muscles. A drop in the activity of cholinesterase to 30 per cent of normal or lower is associated with toxic symptoms and leads to death resulting from respiratory failure or circulatory arrest.

In carbon monoxide poisoning, the gas having greater affinity combines easily with haemoglobin and make them unable to carry oxygen to various tissues of the body. The glycoside poisons affect the heart muscles and the pumping of blood is stopped. Opiates, alcohols, barbiturates, dhatura etc. paralyse the respiratory centers of the brain resulting in respiratory failure.

The action of poisons may be local, remote, local & remote combined or general. The local action means its direct action on the tissues and cause corrosion e.g. strong mineral acids and alkalis, irritation and inflammation in cantharides or some nervous effects as in the case of dilation of the pupil by atropine. Remote action results from the absorption of the poison into the system e.g. alcohol, alkaloids or other drugs affect the organs after being absorbed into the system. Some poisons produce both local and remote actions. In such cases, there is destructive action on the tissue with which they come in contact and there is also a toxic affect after absorption e.g. oxalic acid. General action results when the absorbed poison evokes responses from a wide variety of tissues beyond one or two systems e.g. arsenic, mercury, insecticidal compounds etc.

Most of the poison affects the person immediately, if not, the poison is eliminated from the body by excretions or gets metabolised or detoxified into non-toxic metabolite which is eliminated slowly. However, some poisons have tendency to get accumulated in the body tissues and when a fatal level is reached, the person dies. Arsenic and DDT are among such poisons, which have been reportedly used as slow poisonssince ages for homicidal purposes. The absorption of DDT through skin andaccumulation behaviour may result into accidental death.

* 1. **FACTORS AFFECTING THE INTENSITY OF POISONING:**
     1. **Dose:**

As a general rule, the deleterious effects of a poison depend on its dose. The larger the dose, more severe will be the symptoms.

* + 1. **Hypersensitivity:**

Some persons show abnormal response (idiosyncrasy) to a drug like morphine, quinine, aspirin etc. due to inherent personal hypersensitivity**.**

* + 1. **Allergy:**

Some persons are allergic (acquired hypersensitivity) towards certain drugs like penicillin, sulpha etc**.**

**2.7.4 Incompatible combinations:**

Ingestion of certain medications like anti-ulcerous gels with aspirin may lead to fatal effects.

**2.7.5 Tolerance**

People develop a marked tolerance in the case of opium, alcohol, strychnine, tobacco, arsenic and some other narcotic drugs by repeated and continued use. The snake charmers develop complete immunity from snakebite by getting snakebites regularly.

**2.7.6 Synergism:**

Two poisons e.g. alcohol and barbiturates or cannabis drugs and dhatura in non-toxic doses, when taken together, may cause severe toxic symptoms due to synergism**.**

**2.7.7 Cumulative action:**

The repeated small doses of cumulative poisons like arsenic, lead, mercury, strychnine, digitalis etc. may cause death or chronic poisoning by cumulative action.

**2.7.8 Shock:**

Some times, a large dose of a poison acts differently from a small dose e.g. a large dose of arsenic may cause death by shock while a small dose results in diarrhoea**.**

**2.8 FORMS OF POISON:**

Gases or vapours act more quickly than solid and liquid poisons because they are absorbed immediately. Liquid poisons act more quickly than solids, of which fine powders act more quickly than coarse ones. Some substances in certain combinations become inert e.g. acids and alkalies, strychnine with tannic acid and silver nitrate with hydrochloric acid. Some substances in certain combinations become poisonous, such as lead carbonate and copper arsenite which are insoluble in water but soluble in hydrochloric acid in the stomach and becomes poisons. Similarly, the action of a poison is altered when combined mechanically with inert substances e.g. alkaloids when taken with animal charcoal fail to act. Poison act slowly when the stomach is full with fatty food. A poisonous powder does not sink or float when given with a fluid having nearly same specific gravity. For this reason, arsenic when administered for homicidal purposes, is usually mixed with milk.

**2.9 METHODS OF ADMINISTRATION:**

A poison acts more rapidly when inhaled in gaseous form or when injected intravenously, next when injected intramuscularly or subcutaneously and least rapidly when swallowed or applied to skin. Some poisons act differently when given through different routes. Snake venom is highly poisonous when injected but harmless when ingested orally, if there is no any internal injuries.

**2.9.1 Condition of the Body:**

The age, health and individual allergy towards certain drugs and poisons affect the action of poisons. As a general rule, children, old and weaker persons are affected more severely with low doses than young and healthy adults**.**

**2.9.2 Fate of poisons in the body:**

A poison after being absorbed into the system, a part of it is excreted in the urine unchanged. More frequently, it will be partly or completely metabolized. Normally the poison is not completely destroyed by the metabolic processes and it may be detected in the original form or in the form of intermediate products in the tissue or the excreta. The liver is the guardian of the body against poisons. Therefore, the chances of getting maximum amount of poisons or its metabolites are in liver. From liver, the poison passes in general circulation and exerts its action either on cells of a particular organ or tissues for which it has an affinity, or on the cellular tissues in general.

The main routes of excretion of poison or its end products is the urinary tract, but other channels are the intestine, the bile ducts, the sweat glands, saliva, mucous or serous outflows, breast tissue during lactation and the lungs. The exo and endo skeleton such as epidermis, nails, hair and bony skeleton retain inorganic metals for long periods even after the same have been eliminated from rest of the body tissues.

**2.10 DIAGNOSIS OF POISONING:**

Diagnosis of poisoning is often difficult and has to be made on the available evidences. In many cases, little or no toxicity occurs and the patient, parents or relatives are reassured. Sometimes, the history may not be available or unreliable. Many cases of poisoning present vague symptoms and in some fatal acute poisoning, symptoms may be delayed for many hours or days even e.g. the symptoms in ethylene glycol may be delayed by 6 hours; metal vapours- 8 hours; methanol- 48 hours; paracetamol-36 hours; paraquate-48 hours; salicylates-12 hours; thallium-4 days; arsine and stibine-24 hrs. Some poisons develop deceptive symptoms e.g. gastro-intestinal type of arsenical poisoning may be mistaken for symptoms of cholera or food poisoning. Hence, a working diagnosis has to be made based on clinical features and laboratory investigations. The articles or the containers recovered from the scene of crime or the possession of the victim may provide help in case of suspected poisoning when the diagnosis is not immediately apparent. There are several symptom patterns which are typical for different types of poisoning and can be useful guide to the nature of poison, the laboratory tests needed and the treatment required. The list given below is illustrative and further advice may be required in difficult cases. The examinations should also include a search for signs of trauma and systemic disease because many organic illnesses enter into the differential diagnosis of poisoning. The symptoms, clinical examinations and the patterns of poisoning by some commonly used drugs and poisons are given below which are essential in assessing the patient and may help to identify the agent and severity of the problem.

|  |  |
| --- | --- |
| ***(A) Symptoms*** | ***Poisons commonly involved*** |
| Vomiting | Irritant poisons like arsenic acids, alkalies  excess of liquor and some metallic salts. |
| Diarrhoea | Usually poisons causing vomiting also cause  diarrhoea. |
| Cramps | Metallic poisons like arsenic, lead, antimony,  mercury etc. |
| Delirium | Dhatura, cannabis drugs, alcohol, atropine,  hyoscine, LSD etc. |
| Convulsions | Strychnine, nicotine, cyanides, tricyclic antidepressants, phenothiazines, carbonmonoxide, ethyleneglycol, opioids, organophosphate insecticides, salicylates. |
| Paralysis | Lithium, amphetamines, lead, arsenic, aconite,  snake venom etc. |
| Coma | Barbiturates, carbon monoxide, chloroform, trichloro-ethanol, opioids and excess of liquors. |
| ***(B) Clinical Findings*** | ***Poisons commonly involved*** |
| Skin color  Cherry Pink  Flushed pink skin  Jaundice  Central cyanosis | Carbon monoxide  Alcohol, cocaine, cyanide and anti-cholinergic  agents  Hepatotoxic agents like paracetamol  A sign of hypoxia but methaemoglobinaemia  also causes similar colour. |
| Skin changes  Cutaneous bullae  Sweating | Barbiturates, glutethimide, sedative  overdoses, tricyclic antidepressants and carbon  monoxide.  Organic conditions like hypoglycaemia,  myocardial infarction and pyrexia due to infarctions and poisoning by salicylates, organo phosphates, or monoamine oxidase inhibitors. |
| Pupils  Dilation  Constriction | Pupils dilate in severe hypoxia and in hypothermia. Drugs such as tricyclic antidepressants also cause dilation. Glutethimide and monoamine oxidase inhibitors produce wide dilation.  Opioids typically cause pin point pupils. Organophosphate insecticides and trichloroethanol poisoning cause very small pupils. In barbiturate poisoning the pupils may vary in size at times being small, at others dilated. |
| Body temperature  Hypothermia  Hyperthermia | Comatose condition for sometime may cause hypothermia. Sedative and hypnotic drugs, trichloroethanol, ethanol and opioids cause hypothermia.  Hyperthermia may be caused by heat stroke and meningitis and poisoning by anti-cholinergic agents, tricyclic anti-depressants, monoamineoxidase, inhibitors, carbonmonoxide, dhatura, phenols and salicylates. |
| Breath odour | Alcohol, acetone (diabetic ketoacidosis and starvation) solvents such as toluene, trichloroethane, ether, turpentine, petrol, kerosene, cyanide and methyl salicylate. |
| Appearance of  blood, urine and vomit | Red venous blood may suggest cyanide or carbonmonoxide poisoning.  Brown arterial or venous blood may suggest methaemoglobinaemia.  Vomit or gastric lavage containing blood may suggest repeated vomiting, corrosives, paraquat, coumarin, anticoagulant, irritants, iron and non-steroidal anti-inflammatory drugs.  Many drugs turn urine black e.g. metronidazole.  Urine may be cloudy or red or brown due to haematuria, haemoglobinuria or myoglobinuria. |
| Blood pressure  Hypotension  Hypertension | Almost all sedatives, hypnotics, dehydration, lengthy coma, vomiting and sweating may cause hypotension  Amphetamines, cocaine, phencyclidine, sympathometics and anti-cholinergic agents. |
| Cardiac arrhythmias | Changes in heart rate or rhythm may be caused by beta blocking drugs, organophosphates, theophylline, tricyclic antidepressants, sympathometics, barbiturates  etc. |
| Pulmonary oedema | Petroleum products, organophosphates, ethyleneglycol, irritant gases (metal vapour), salicylates, opioids etc. |
| Rhabdomyolitis | Patient lying in coma for a long time on hard surface may develop it due to pressure necrosis of muscle, which may lead to renal failure. The agents mostly responsible for the same are barbiturates, opioids, ethanol and carbon monoxide. Rhabdomyolysis can also occur after prolonged and severe muscle spasm, due to poisoning by strychnine, phencyclidine and monoamine oxidase inhibitors. |
| ***(C) Pattern*** | ***Poisons commonly involved*** |
| Coma, Hypotension,  Flaccidity. | Barbiturates, benzodiazepines, glutethimide, trichloro- ethanol, ethanol, opiods etc. |
| Coma, hypertension, techycardia, dilated pupils. | Tricyclic antidepressants, anti-cholinergic agents, phenothiazines. |
| Malaise, restlessness, nausea, weakness. | Carbon monoxide, solvents, insecticides, lead, mercury, arsenic. |
| Restlessness, hypertonia, hyperreflexia, pyrexia. | Monoamine oxidase inhibitors, anti-cholinergic agents, strychnine, phencyclidine, amphetamines. |
| Behavioural  Disturbances | Psychotropic drugs, anticholinergic drugs, corticosteroides, solvent abuse, psilocybin-mushrooms. |
| Burns in mouth,  disphagia, abdominal pain,  distension. | Corrosives, caustics, paraquat. |
| Renal failure | Paracetamol, mercurial compound, acids  (phosphoric, oxalic, formic), phenols, arsine,  stibine, lead. |
| Jaundice, hepatic  failure. | Paracetamol, carbon tetrachloride,  phosphorous, organic lead. |

**2.11 Post mortem findings in poisoning:**

* A peculiar smell on opening the body: The substance detectable by their smell are alcohol, cyanide, carbolic acid, petroleum products, camphor, nicotine, opium, paraldehyde, phosphorus insecticides, pesticides etc.
* Presence of any foreign material in the form of powder, capsules, tablets, leaves or seeds in the stomach.
* Irritation, ulceration and perforation or discoloration and change in color or softening of the mucous membrane of the stomach.
* Laryngeal oedema commonly in the death is due to alcohol and barbiturates**.**
* Acute lung congestion and oedema**.**
* Acute swelling of brain with or without a pressure cone**.**
* Distended urinary bladder**.**
* Intravascular sickling.
* Negative evidence such as no trauma or no sign of disease in any organ to account for the cause of death.
* Body is highly decomposed.
* Dilation or contraction of pupils.

**2.12 Management and Medicolegal aspects of poisoning cases:**

The principle of management of poisoned patients is careful attention and treatment to prevent cerebral anoxia, respiratory, cardiovascular, neurological and other complications. The main aim is to help the patient to stay alive. Most patients need only supportive care. The initial management should always be active until the contributions of drug induced damage and pre-existingorganic disease are established.

The main objects of treatment of poisoning are

**\*** Removal of unabsorbed poison from the system

**\*** Administration of antidotes

**\*** Elimination of absorbed poison

\* Symptomatic treatment

\* Maintenance of the patient’s general condition.

It should always be remembered that over-treatment of the patient with large doses of antidotes, sedatives or stimulants does far more damage than the poison itself. The judicious use of drugs and necessary therapeutic measures are required.

**2.13 Removal of unabsorbed Poison:**

**2.13.1 Inhaled Poisons:**

In the cases of poisoning by inhalation, the patient should immediately be removed to fresh air. A clear airway should be ensured and artificial respiration commenced at once. In some cases the respiratory centre may be stimulated by a mixture of 95% of oxygen and 5% of carbon dioxide.

**2.13.2 Injected Poisons:**

The use of tourniquet above the site of injection may slow the absorption. The tourniquet should be loosened every 10 min. for 2 min. suck the poison by making incisions at the site and use chemical antidotes, cold packing and vasoconstrictors e.g. injection of epinephrine to prevent absorption. The common examples of injected poisons are hypnotics, insulin, snake or other insects bites etc.

**2.13.3 Contact Poisons:**

If the poison is applied to eyes, skin, wound or inserted, the poison is removed with specific antidotes.

**2.13.4 Ingested Poisons:**

Gastric lavage or emesis may be induced before gastric emptying time usually 4 to 6 hours after ingestion.

**2.13.5 Emetic:**

When the poison has been ingested, early vomiting (emesis) is of greater value in avoiding absorption than the most energetic gastric lavage carried out after some delay. Emetics are the substances, which produce vomiting. Most poisons are themselves emetics and may cause vomiting. However, it is advisable to give an emetic to ensure a more thorough emptying of the stomach. Emesis is easier than gastric lavage and less traumatic for the patient, if the patient is conscious and co-operative and vomiting is not contra-indicated (like corrosives, strychnine, petroleum distillates and coma). Emesis should be avoided in corrosive poisoning forfear of damage ofoesophagus and stomach. It must also not be used when there is danger of aspiration into lungs, either from volatile poisons like petroleum distillates or from inhalation of gastric contents due to impaired consciousness. Emesis should be induced either by tickling the fauces or by emetics. The household emetics are:

* Copious lukewarm water.
* 15 gm of mustard powder in 200 ml of water.
* About 30 gms of common salt in 200 ml of water. Normally this emetic agent should be avoided because it is a poor emetic and excessive doses are easily given, resulting in hypernatraemia. There are many documented deaths from this cause even in recent years.
* Zinc sulphate: 1-2 gms in 200 ml of water.
* Apomorphine has a remarkable selective action on vomiting centre. It is used in a dose of 6 mg by sub-cutaneous injection for quick emesis followed by levallorphan 1-2 mg, if necessary or preferably naloxone hydrochloride 5-10 mg intramuscular or intravenous to counteract its narcotic effects. Now- a- days, it is widely accepted.

**2.13.6 Gastric Aspiration and Lavage:**

Gastric aspiration and lavage is the only suitable method of emptying the stomach of an unconscious patient. In unconsciousness, protect airway with cuffed endotracheal tube. Place the head of the patient over the end or side of the bed so that mouth and throat are below the larynx and trachea. Use a wide bore tube lubricated with vaseline or glycerin (Jacques gauge 50cm in adults, 50 cms will reach the stomach). Ensure the tube is not in the trachea. Aspirate and save the first sample for analysis. Use 300-600 ml. of water at body temperature for washing. If the tube becomes blocked, gentle suction can be applied. Continue stomach wash with water or saturated lime or starch water or 1:5000 potassium permanganate or 4% tannic acid etc. till a clear odourless fluid comes out. Leave some amount of antidote in the stomach for neutralizing left over poison. It is best method, if undertaken early i.e. within about 4-6 hours after ingestion of poison. After then, it may not be useful.

Certain extra precautions are necessary for gastric lavage in cases of coma, petroleum distillates and strychnine poisoning. Following the ingestion of corrosive, passage of stomach tube may lead to perforation. Lavage is dangerous in cases of petroleum distillates which may be inhaled rapidly, may cause fatal results unless the glottis is sealed. In coma, there is a serious risk of aspiration pneumonia, due to depression of cough reflex, unless the airway is sealed by cuffed intubations by an anesthetist. In the cases of strychnine ingestion, a convulsion may be induced by this method unless the patient was first anaesthetized.

**2.13.7 Use of Antidotes:**

Antidotes are remedies to counteract the effects of poison. They are used because the poison may not have been completely removed by emesis or lavage or the poison is already absorbed or it has been administered by other route than ingestion. According to their mode of action, they are divided into the following classes:

**2.13.7.1 Mechanical or Physical:**

These are the substances, which tend to impede the absorption of poisons by their presence; they are:

**Activated Charcoal:**

It is recommended as a general-purpose oral adsorbent. Activated means the brand of charcoal which meets certain adsorbance standards. To be fully effective, a ratio of about 10 parts of charcoal to 1 part of poison is needed. It is more beneficial for adsorbance of substances which are toxic in small amounts such as tricyclic antidepressants and alkaloids like strychnine; but less effective when large amount of poison has been ingested e.g. in aspirin or paracetamol poisoning.

The treatment with charcoal is most effective during the first four to six hours after ingestion. However, some poisons are also adsorbed by oral use of activated charcoal many hours after ingestion and even after intravenous administration, presumably by back diffusion or interruption of enterohepatic circulation. Aspiration of charcoal into the lungs is a risk that should be guarded against. Normally charcoal is given in a dose of 4-8 gms.

**Demulcents and Bulky Foods:**

These are the substances whichprevent the absorption of the poison by forming a coating on the mucous membrane of the stomach. In doing so, they act both in corrosive and irritant poisoning except in phosphorus poisoning as phosphorus is soluble in them thereby increasing the absorption. The common demulcents are fats, oils, milk and egg albumin. Bulky food like banana acts as a mechanical antidote to glass by imprisoning its particles and thus preventing its action.

**2.13.7.2 Chemical:**

These are the substances which act chemically to form a non toxic compound by forming insoluble compound or by oxidizing the poison to non toxic constituents e.g. dilute acetic acid neutralizes alkalis. Magnesium oxide or calcium oxide will neutralize acids. Similarly, lime can be used for oxalic acid, magnesium sulphate for carbolic acid, copper sulphate for phosphorus, sulphates of alkalis for lead and freshly precipitated iron oxide for arsenic. Tannin produces insoluble compounds with most alkaloids glucosides and metals.

Potassium permanganate being oxidizing agent reacts with organic substances, a 1:1000 strength aqueous solution of potassium permanganate is effective against all oxidizable poisons like alkaloids, amidopyrin, antipyrin, barbiturates, phosphorus, cyanides etc.

**2.13.7.3 Physiological or Pharmacological:**

These chemicals donot enter into any chemical combination, but produce opposite effects to that of the poison. Hence, they are known as physiological antagonists e.g. atropine for pilocarpine, chloroform for strychnine, caffeine for morphine, atropine and oximes for organophosphorous compounds. However, the antagonism is usually not complete and the remedy may itself produce most undesirable results. Nalorphine has provided itself as a very valuable specific antidote against morphine and its group.

Certain chelating agents are widely used as specific antidotes against some heavy metals. These substances produce a firm non-ionized cyclic complex (chelate) with cations. Such compounds can form stable, solutble non-toxic complexes with calcium and certain heavy metals. They are BAL (Dimercaprol), EDTA (Ethylene diamine tetra acetate), pencilamine and desferrioxamine which are used in heavy metal poisoning.

**2.13.7.4 Universal Antidotes:**

It is used when the identity of poison is not known or when a combination of poisons is suspected. It consists of a mixture of the following substances:

|  |  |  |
| --- | --- | --- |
| I Powdered animal charcoal  (or burnt toast ) | 2 parts | Adsorbs alkaloids |
| II Magnesium oxide | 1 part | Neutralizes acids |
| III Tannic acid (or strong tea ) | 1 part | Precipitates alkaloids,  certain glucosides &  many metals. |

The mixture is given in doses of a tablespoonful stirred up in 2 ltrs. of water. This dose may be repeated once or twice, if necessary. Besides, some household products may be used as safe antidotes which are as follows.

* Strong liquid tea precipitates many alkaloids and metal poisons.
* Milk and raw egg white, being protein rich, precipitates mercury, arsenic and other heavy metals. In addition, they have excellent demulcent properties.
* Mashed potatoes in water are also good adsorbents and can be used in place of charcoal when it is not available.
* Milk of magnesia or soap solution may be used in poisoning due to acids.
* Tinned juice or vinegar can be used in poisoning due to alkalis.

**2.14 Elimination of absorbed poisons:**

After a considerable absorption of poison into the blood stream has occurred; procedures must then be employed to accelerate the excretion of the toxic agent mainly through urine. Elimination by catharsis when not contra-indicated and by sweating by means of hot packs may be encouraged. Fluid administration to maintain adequate renal function with periods of dialysis will be beneficial. In cases of aspirin and barbiturate poisoning, forced diuresis using intravenous chlorothiazide and/ormannitol infusion have been proved valuable. Peritoneal dialysis has been recommended for salicylate poisoning in children. Haemodialysis has been used for eliminating barbiturates, bromides, glutethimide, methanol, salicylates and thiocyanates for the blood. Exchange transfusion is only feasible with small children and has been applied to poisoning by salicylates, barbiturates, iron salts, carbon monoxide etc. All toxic substances are removed by this technique.

**2.15 Treatment of general symptoms:**

The treatment should be applied as indicated by the symptoms. In unknown poisoning cases, the symptoms are the clues to the treatment of the case. Strong analgesic should be given for pain and oxygen for artificial respiration in respiratory failure. Antibiotics should not be given routinely, but only if infection is suggested by purulent sputum, pyrexia and leucocytosis treatment is required. Cardiac stimulants in circulatory failure and anaesthetic for convulsions should be given. The symptomatic effect of the poison should be treated by general means. Saline infusion is useful in counteracting dehydration and encouraging diuresis. However, careful attention must be paid to water metabolism as over hydration may lead to pulmonary oedema and circulatory impairment. The addition of sodium bicarbonate to the infusion may be of value when the alkali reserve is diminished. Administration of glucose will combat depletion of liver glycogen, and the restoration of potassium - sodium imbalance may be necessary.

**2.16 Maintenance of the patient’s general condition:**

The patient should be kept warm and comfortable. After coming out of the effects of poison, one of the main dangers is the subsequent development of upper respiratory tract infection., this is a special hazard in elderly people, who had a respiration infection before, and who inhaled vomitus. Hence, if infection is suggested, antibiotic should be given. Good environment and nursing care should be provided to the patient (especially, if unconscious). All patients who have attempted suicide should not be allowed to leave the hospital without being interviewed by psychiatrist who can give further necessary supportive psychotherapy.

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SECTION - 3: ISOLATION AND PURIFICATION OF POISONS

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

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

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

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

**3.5** **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.

**3.6 Glossary of term related to extraction:**

**Matrix:** Any material substance in the universe 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.

**3.7 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. |
| C. | **Viscera**: Internal organs viz. liver, kidney, stomach, intestine, gall bladder, uterus, heart, lungs, brain etc. |

**3.8 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.

**3.9 DIFFERENT METHODS OF EXTRACTION** (1, 2, 3, 4, 5, 6, 7)**:**

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,  Gas Chromatography. |
| Volatile Inorganic | Gutzeit Method, Marsh-Berzelius Method, Micro-diffusion, Digestion with specific reagents / under specific conditions of PH, | Microwave oven technique for digestion followed by  Ion Chromatography, Spectroscopy(Mass) etc |
| Volatile Organic | Distillation, Steam Distillation, Diffusion | Chromatographic methods |
| Non-Volatile Inorganic | Dry and wet ashing, Group analysis, Electro-dialysis, Digestion under appropriate analytical conditions, Paper and Thin layer chromatography | Microwave oven technique for digestion followed by Ion Chromatography using  Ion-exchange resins |
| Non-Volatile Organic | Solvent Extraction, Stas- Otto, Digestion with ammonium sulphate, sodium tungstate or other modified methods of the above | HPLC, Paired ion extraction Chromatography, HPTLC, Supercritical fluid chromatography, Solid phase extraction, Micellar extraction, Affinity chromatography, Microwave Assisted Reaction System, Accelerated solvent Extraction, Sweep Co-Distillation Universal Trace Residue Extraction. |
| Anion | Dialysis, Chemical Digestion, Paper and Thin Layer Chromatography | Ion-Chromatography by Ion-Exchange resins. |

**3.9.1 Unit processes/operation in extraction methods:**

The various unit processes / operations related to extraction are as given below:

**3.9.1.1 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).

Total wt (gms.) of solute in the organic phase

D = ------------------------------------------------------

Total wt (gms.) of solute in the aqueous phase

If one of the two liquids contains a solute, this method is found to be more suitable. The system, in this case is first shaken and then allowed to settle. Some of the solute is transferred to the 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 another phase. 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 now 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.

3.9.1.2 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, condensing it back into a liquid. Various modifications of the basic distillation process are used for specific purpose viz. steam distillation, fractional distillation, distillation under reduced pressure, sweep co-distillation etc.

3.9.1.2.1 Steam Distillation:

Steam volatile substances can be separated or isolated from blood, urine and properly minced viscera by steam distillation. Steam is passed into the sample and the aqueous distillate is 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 such as amphetamine, methadone and also aniline, pyridine, nicotine etc.

3.9.1.2.2 Fractional Distillation:

This is a type of distillation, which enables separation of a mixture of volatile liquid differing marginally in boiling point. A mixture of kerosene oil or mineral turpentine oil in an oil-water emulsion may be separated by this method.

3.9.1.2.3 Distillation Under Vacuum:

This is another type of distillation method which provides separation of thermally labile volatile compounds at a low temperature without decomposition.

3.9.1.2.4 Sweep Co-distillation:

This is a special type of distillation based on the preferential volatilization of organic compounds specially pesticides from oil, lipids or plant extracts, using a stream of inert gas and subsequent isolation of volatiles on cold 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 temperatures. Universal Trace Residue Extractor (UNITREX) and Accelerated Solvent Extractor (ASE) i.e. the highly automatic extraction system for rapid extractions of multiple samples work on this principle.

3.9.1.3 Micro-diffusion:

Micro-diffusion is a convenient and popular method that facilitates toxicants (gaseous and volatiles) in blood, urine and gastric aspirates to be isolated, detected and determined. This is done by Conway Micro-diffusion dish (elaborated separately for convenience in section 4.7.3).

3.9.1.4 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 of toxic cations and anions in a colloidal solution or dispersion or colloidal matrices, especially biological materials. The separation process may be accelerated by applying e.m.f. i.e. electro-dialysis.

3.9.1.5 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, anthracene which sublimes.

3.9.1.6 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 inorganic metals. Volatile inorganic poisons viz. phosphine, arsine and hydrogen sulphide are isolated from their salts on treatment with dilute acids.

3.9.1.7 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 oven 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.

3.9.1.8 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.

3.9.1.9 Chromatography:

Chromatography involves the separation of substances based on their relative affinity for two phases viz. stationary and 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 conveniently under different chromatographic methods and operating conditions in a particular chromatography. There are different controlling parameters viz. nature of toxicant, mobile and stationary phase and temperature. Salient aspects of different chromatographic methods are given below.

3.9.1.9.1 Column Chromatography:

Separation of active constituent is achieved here by preferential absorption of active constituents on the adsorbent or stationary phase. 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 be 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 components separated at different intervals.

The efficiency of separation in the column is dependent on the adsorbent material, selection of solvent system, nature of active constituent as well as mobile phase. These controlling factors have been explored to develop different method of chromatography viz. HPLC, HPTLC, affinity, gel permeation, ion-exchange and ion chromatography, solid phase extraction and super critical fluid chromatography. These have been discussed separately. The analytical conditions required for separation of toxicants are either available in standard texts or references or may be developed conveniently by standardization depending on nature of toxicant and matrices.

3.9.1.9.2 Paper Chromatography:

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

3.9.1.9.3 Thin Layer Chromatography:

The separation takes place on a thin layer of adsorbent material such as alumina, silica gel G or cellulose coated on to an inert support material such as glass plate, plastic or aluminium sheet. There are different methods that are popular in TLC viz. ascending, descending, two dimensional etc. Quantitative separation is achieved by optimizing analytical conditions.

3.9.1.9.4 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 by chromatography) occur on plate coated with specially prepared adsorbent. The system (HPTLC) is now fully automatic and multiple samples may be handled quickly, precisely and conveniently.

3.9.1.9.5 High Performance Liquid Chromatography:

It is based on the different attractions 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 parameters includes composition of mobile phase, adsorbent, nature of analyte etc. for optimizations of resolution.

3.9.1.9.6 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 are 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 molecular mixtures which have the opposite charges (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 analytical flexibility.

3.9.1.9.7 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 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 techniques viz. SFC-GC, GC-MS.

3.9.2 Modern Methods of Extraction (8, 9):

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. microwave 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.

3.9.2.1 Head Space Technique (16):

The headspace method is especially suitable for the very fast separation of volatile components (alcohols, acetone, aldehydes) in complex biological matrices specially blood in mass-liquor and prohibition law related cases. This method has the advantage of avoiding the risk of contamination of non-volatile components, which may be eliminated due to on-line analysis by gas chromatography. The principle underlying the headspace analysis is that in a sealed vial at constant temperature, equilibrium is established between the volatile components of a liquid sample 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 in a single run) a portion of the headspace may be withdrawn one by one from vials using a gas-tight syringe and injected to GC for on-line analysis.

**3.9.2.2 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 cartridge filled with solid adsorbent material such as Tenax. It is polymer based diphenyl-p-phenylene oxide and available in various forms. Tenax TA is a highly purified form of the polymer and stable upto375oC 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. Alternatively, cartridges 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.

**3.9.2.3 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 rise to solid phase extraction method. In this method, siliceous materials with relatively close size distribution (15 to 100μ ) 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 cartridges filled with 100-500 mg. of an appropriate adsorbent as stated above. There lies, however, a recently developed type of SPE cartridge in which very small particles of the adsorbent are enriched in a well of PTFE microfibrils having similar efficiency as in a packed column but require less pressure drop. The sample is applied to the SPE cartridge either from a syringe or sampling manifold by applied pressure or suction at the lower end. Components are subsequently recovered by solvent flushing. The method is attractive because of the small amounts of the sample and materials are 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 as 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 batch 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 (NH3) +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 |
| (NH4) +1 | 2.0 | (HPO42-),(HCO3 –1) | 0.4 |
| Mn+2,K+1,Mg+2,Fe+2,+3 | 2.5 | CI –1,(NO2) -1 | 1.0 |
| Zn+2,Co+2,Cu+1,Cd+2 | 3.0 | (HSO3) -1 ,CN -1 | 1.5 |
| Ca+2 | 4.5 | (NO2) -1 | 4.0 |
| Cu+2 | 6.0 | (CIO3) –1 | 4.5 |
| pb+1,Ag+1 | 8.5 | (HSO4) -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.

**3.9.2.4 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 inserted 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 desorbed thermally into a capillary GC column for analysis.

**3.9.2.5 Super Critical Fluid Extraction (SFC):**

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, not only there is 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 SFC 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 cartridges, 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 cartridges 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 ceases 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.

**3.9.2.6 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 of 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 two phase 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 be destabilized by increasing the ionic concentration of additives including surfactant. Biological matrices (Viscera) contains fats, degraded protein and colouring matter etc. makes the 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 will 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). 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 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 supernatant liquid containing active constituent may be extracted for poison by organic solvents. The detailed analytical conditions have also been presented at appropriate places in this manual.

**3.9.2.7 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 (5– 10 gms.) is placed inside a microwave transparent vessel with a polar solvent or ionic solution (usually an acid) and is subjected to rapid heating by microwave in a microwave accelerated reaction system (digester). The analytical conditions (temp., time of digestion, pressure) may vary 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.) .

**3.9.2.8 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 volatilization of pesticides or other organic chemicals from biological materials, lipids, plant extracts 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 pesticides 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 use 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.

**3.9.2.9 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 on the same principle as 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.

**3.9.2.10 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.

**3.9.2.11 Ion-Pair Extraction:**

This method is applicable for the extraction of highly water-soluble organic compound. These compounds cannot be extracted from samples by direct solvent extraction. The difficulty has been overcome by forming ion pair with a suitable reagent viz. quaternary ammonium salts as ion-pairing agent. The ion-pair formation is an attraction between a positive and a negative charge. 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 preferably in presence of a buffer. The method is also applicable for extraction of drugs in the form of quaternary salts 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.

**3.9.2.12 Hyphenated Techniques (HPLC-GC AND SFC-GC)** (10)**:**

The hyphenated techniques viz. HPLC-GC and SFC-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 techniques 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.

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

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

**3.9.3.1 Neutral and Acid Distillation:**

**Procedure:**

50-100 gms. of viscera (properly minced), stomach contents, vomit or other materials are to 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 with ice especially during the hot season, 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 stone 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 is placed between the burner and condenser so that phosphorescence may be seen clearly. The distillate as stated above contains alcohols, paraldehyde, other aldehydes, acetone, carbolic acid and phenolic derivatives, carbon disulphide, thymol, camphor, turpentine, nitroglycerine, benzene, other volatile acids etc. For cyanides and carbolic acid, the distillate is collected in 10 ml. of 0.1N sodium hydroxide solution.

**3.9.3.2 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, coniine, ammonia and volatile bases.

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

**3.9.4.1 Non-Biological Matrices:**

The non-biological matrices such as tablets, powders may be subjected to chemical analysis by preparing solution of samples and their systematic group separation.

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

**3.9.4.2.1 Dry Ashing Method:**

About 10-50 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 550°C 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 to be noted as 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 4N 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 using semi-micro methods, chromatographic and instrumental techniques.

**3.9.4.2.2 Wet Digestion Method:**

**Procedure :**

About 50gms. of biological material or 10 ml. of blood is taken into a large Kjeldahl flask and 20 to 40 ml. of Conc. HNO3 is 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 liquefication 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 is 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. Heating 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 about10%. 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.

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

The nitric-sulphuric acid (wet digestion) method of destruction of organic matter is not at all suitable for mercury, which is almost completely lost by volatilization. This 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 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 bisulphite 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.

**3.9.4.2.4 Selective Chemical Treatment:**

A few toxic metals viz. arsenic and antimony in their specific oxidation state (+5) 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.

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 analyticals 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.

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

The extractions of toxic anions in non-biological matrices require very minor processing and clean up. Inconveniences is 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

**3.9.5.1 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 is 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 supernatant liquid is collected and evaporated to a small volume on a water bath. The concentrated liquid is ready for analysis of anions.

**3.9.5.2 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 to a small volume on water bath. This is filtered, if necessary and tested for toxic anions.

**3.9.5.3 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.

**3.9.5.4 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 sealing, liberating and detection reagent in Conway Micro-Diffusion assembly (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.).

**Conway Micro-Diffusion Assembly:**

The assembly consists of brink type, polypropylene cells with clear polystyrene covers. The cells have an outermost annular sealing well (No.1), an intermediate annular well(No.2) for the sample and the liberating agent and a center well ( No.3) 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(No.1). An approximate amount (1 ml.) of liberating agent (usually same as sealing agent) viz. 10% H2SO4 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 (No.2). Trapping agent 2ml say PdCl2 in case of carbon monoxide turns to black, acidified potassium dichromate turns to green in case of methanol or ethanol etc is placed in the centre well(No.3). Sample(1 ml.) is introduced into the other half of sample well(No.2) 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 change in central compartment containing trapping agent (No.1) is noted.

**3.9.5.5 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.

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

The group includes pesticides, drugs (acidic, basic, neutral and amphoteric) and 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 India thereby requiring special attention to it..

**3.9.6.1 Extraction of Pesticides in Matrices:**

The extraction of pesticides in biological materials viz. viscera, stomach contents, gastric lavage and blood is difficult due to the interferences of fat, degraded protein and colouring matter in the matrices. The extracts require proper cleanup except for Micellar method. The extraction in case of non-biological matrices is less cumbersome and requires either minor cleanup or no cleanup. 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.

**3.9.6.1.1 Method-I:**

Biological materials (viscera, stomach content or gastric lavage etc.) are macerated into 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 is 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 fractions are combined and taken into a separating funnel. This hexane layer is vigorously shaken with 15 ml, 10 ml and again 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 is 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 is added. The extract is evaporated as and when required for analysis.

**3.9.6.1.2 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 (cleanup). The above acetone extract (50 ml) is taken into a separating funnel and diluted with 150 ml of water. Add 20 ml of saturated solution of sodium sulphate to the same. 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.

**3.9.6.1.3 Method – III:**

**Extraction of Pesticides in stomach-wash, urine and vomit:**

The sample (20 ml of stomach wash or urine or10- 20 gms of vomit) is taken in a conical flask. 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.

**3.9.6.1.4 Method-IV:**

**Extraction of Pesticides in Blood:**

20 ml of blood is mixed with 10 ml of 10% sodium tungstate solution and 15 ml of 1N sulphuric acid, shaken for two minutes and then filtered. The filtrate is kept reserved. The residue is washed with two 15 ml portions of 0.1N sulphuric acid. The washings 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.

**3.9.6.1.5 Method-V:**

**Direct Solvent Extraction:**

The biological materials (50 gms of 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 and concentrated as before. The concentrated extract is cleaned up by passing through a chromatographic column (diameter 2.5cm) containing three successive layers of different lengths viz. 5cm layer of alumina (top layer), 2.5cm of activated charcoal (middle) and 2.5cm layer of anhydrous sodium sulphate (bottom) previously washed with ether. The elute is evaporated to dryness as before.

**3.9.6.1.6 Method-VI:**

**Extraction by Steam Distillation cum Solvent Extraction:**

When the biological materials are clean and purified i.e. less degraded and contain very little fat and colouring matter, the following method may be adopted.

**Procedure:**

50 gms of biological material is treated with a few drops of phosphoric acid and steam distilled 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).

**3.9.6.1.7 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 2.5cm 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.

**3.9.6.1.8 Micellar Extraction in Biological Matrices:**

50 gms of biological material (viscera) is mixed with 5 gms of ammonium sulphate and homogenized. After addition of 100 ml of diethyl ether, the mixture is shaken at intervals, kept overnight and filtered. The ethereal extract is taken into a separating funnel. 10 mg of sodium lauryl sulphate is added and stirred gently. On settling, 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-point is indicated by a change of dark colour of ethereal layer to colourless. The ethereal extract is shaken with 25 ml portion of water twice gently. 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.

**3.9.6.1.9 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 over 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, Dry Waste 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 gm 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 gm of each sample in 11 ml or 20 gm of each sample in 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). The tray is loaded with 40 ml pre-cleaned, clapped 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)

**3.9.6.2 Extraction of Drugs and Poisons of Plant Origin in Biological Matrices:**

This group comprises alkaloids, glycosides, barbiturates, phenothiazines, salicylates, sulphonal groups of different classes and plant poisons. The 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 modifications, ammonium sulphate method and modern method especially solid phase extraction micellar and enzyme digestion for the 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 in pure form and subsequent identification and quantitation. The problem is that quantities of these poisons present in 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.

**3.9.6.2.1 Modified Stas-Otto Method:**

**Procedure:**

A. 50 gms of biological material is minced, 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 thorough shakings at frequent intervals. The extraction is then allowed to proceed for about 24 hours with steam off. It is then filtered through a flutted filter paper. 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 added very slowly with constant stirring so that insoluble matter may be granular and not gummy. If 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 sticky mass. It is warmed with occasional 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 dissolved 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 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 glycosides such as thevetin 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 25ml portions of 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 repeated 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.

**3.9.6.2.2 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 40oC) 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 saline solution.

iii) For stomach contents comprise off too much of fluid, the extraction should be done with absolute alcohol for 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 not 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.

**3.9.6.2.3 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 visceral materials (about 50- 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. 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. 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, benzodiazepines 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) is added to it. Dilute ammonium hydroxide solution 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 sulphate and evaporated to dryness. The extract is tested for opium, datura and aconite alkaloids, amphetamines, meprobamate and 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 fraction 1 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 fraction 2 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 fraction 3 contains meprobamate, other carbamates and other neutral drugs.

**3.9.6.3 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 ethereal solution is extracted with 5 ml of 0.5 M sodium hydroxide and the extract is retained for examination of barbiturates and weakly acidic 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 over anhydrous sodium sulphate and evaporated to dryness. The residue may contain neutral drugs (Neutral Fraction C – Caffeine, Carbromal, Chlordiazepoxide, Flurazepam, 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 1 M. sodium hydroxide and evaporated to dryness. The residue may contain benzodiazepines as benzophenones (Fraction E).

The pH of the aqueous solution is adjusted to pH 9 and 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).

**3.9.6.4 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 3.8.6.3. i.e. extraction of drugs in 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 is added and shaken again to produce a solid cake. The decanted chloroform is passed through a filter paper 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 solutions 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 over 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 over 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).

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

5 ml of urine is 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 fitted reservoir 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 eluate is evaporated to dryness under a stream of nitrogen at 30 – 40oC. The residue is kept reserved for analysis of acidic and neutral drugs. The column is then washed with methanol (1 ml) and elution for basic drugs is made with 2 ml of methanolic ammonium hydroxide (2%, V/V). 3 ml of de-ionised water is added and elution 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.

**3.9.6.6 Tissue Digestion using Proteolytic Enzyme:**

The digestion with proteolytic enzymes gives 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 problem 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 in 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.

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

50-100 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 flask, 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 repeated 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 gently 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 portion . 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 ether extract is dried over anhydrous sodium sulphate. The dried ether extract is decanted off. It is evaporated to dryness. The residue is kept ready 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 extract 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).

**3.9.7 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 and 10 mg / ι l, 1, 2 trichloroethane is added to a 200μl of a mixture of expired blood and deionised water (1:24, v/v) in a 7ml 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 65oC 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)

**3.10 CLEAN-UP PROCEDURES:**

In the extraction techniques described above, 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 is to pass the extract through an alumina column and then to separate the target compounds into different batches by passing the cleanup 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 GC 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 literature.

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

Recently, the cleanup has been streamlined by the use of commercially available solid-phase extraction cartridges or discs (described earlier).

Different methods of stripping are described below in short.

**3.10.1 Cleanup 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 a muffle furnace for 4 hrs at 800oC, cooled to about 200oC and then to room temperature in a dessicator. Water (4%, w/w) is added to the weighed portion in a stoppered flask which is shaken well, sealed and then stored.

ii) Acidic Alumina:

A portion of the alumina is washed with 1N HCl by making slurry in a beaker. It is filtered through a sintered 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 get slowly deactivated on exposure to air and should be discarded after two 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 filled by adding 2.5 gms of silica gel prepared as stated above and tapped to settle. 10 ml of hexane is passed through the column (to wet the column) and run off in excess till the hexane meniscus is at 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 elute is collected. It is then passed through the silica column. The elute is collected and dried over anhydrous sodium sulphate. It is evaporated to dryness.

**3.10.2 Modified method for Clean-Up and separation using Alumina / Silica Gel:**

**Preparation of Solid Adsorbent:**

1. 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) weight of alumina. 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 followed by the addition of 4 ml of acetone . To this solution in an unstoppered conical flask, 10 gms of dried alumina is added and shaken thoroughly. The acetone is allowed to evaporate. The preparation is stored in the dark until ready for use. The adsorbent should be prepared freshly.

**Procedure:**

The chromatographic column is plugged at the base with hexane washed glass wool or cotton wool . 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 elute is collected. The elute is concentrated further 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 elute 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 solvents is added to the top of the column and the elute is collected. This is dried over anhydrous sodium sulphate and evaporated to dryness for further analysis.

**3.10.3 Cleanup by a Simple Column Chromatographic method:**

The method can be profitably employed to purify the material after extraction from biological materials for pesticides or drugs. The concentrated extract (organic layer) is cleaned up by passing through a chromatographic column (dia.2.5cm) containing from top alumina layer of 5cm, activated charcoal layer of 2.5cm (middle layer) and a anhydrous sodium sulphate layer of 2.5cm (bottom layer) previously arranged after plugging the chromatographic column and washing by 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 elute is dried over anhydrous sodium sulphate and evaporated to dryness.

**3.10.4 Cleanup using Solid Phase Extraction (SPE) Cartridges:**

There are a variety of cartridges available viz. Bond Elute from *Analytic Chem International*. The cartridge is filled with a chemically modified silica adsorbent and the appropriate one is selected according to the nature of the material (toxicant) viz. aminopropyl for organo-chloro compounds.

**Procedure:**

A selective cartridge is taken with an adapter fitted on top. A 10 ml glass syringe is fitted onto the adaptor. 5 ml of methanol is put into the syringe. It is passed through the tube . 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. 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.

**3.10.5 Cleanup by Preparative TLC Method** (14)**:**

This is applicable if the identity of toxicant is to be known. Thereafter, by screening of the TLC plate developed by applying similar conditions i.e. developing solvent system, adsorbent, temperature etc. The spots containing the active components separated at different Rf values on the developed plates 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.

**3.10.6 Cleanup by HPLC Method:**

In this method also, the identity of toxicant is to be known by noting retention time under specific chromatographic conditions viz. solvent system, flow rate, column etc. Again the same HPLC method is employed and the separated components at various retention times (as 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 chapters the analysis of different classes of toxicants will be described.

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SECTION – 4: GASEOUS AND VOLATILE POISONS

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

**4.2 Scope:** Systematic analysis of gaseous and volatile poisons in various

type of formulations, biological materials and non-biological

matrices.

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

* 1. **Responsibilities:** Gazetted officers and associated scientific staff.
  2. **VOLATILE POISONS:**

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

TABLE – 4.1

|  |  |  |  |
| --- | --- | --- | --- |
| Name | Synonym & Source | Physical Properties | Other Characteristics |
| Acetaldehyde | Acetic aldehyde ethanal, Ethyl aldehyde.  Source: By-product in alcoholic fermentation. | Colourless, inflammable liquid, B.P. 20.2oC, miscible with water, ethanol and ether. | Metabolized through oxidation to acid and eventually to  carbondioxide and water. More toxic than ethanol or acetic acid. |
| Acetone | Cetona, Dimethyl Ketone,  2-Propanone.  Source: A product of destructive distillation of wood, a by-product in the fermentation of starch. | A clear, colourless, volatile, inflammable liquid, B.P.56oc  Miscible with water, ethanol, chloroform and ether. | Large amounts are excreted unchanged, but small doses may be oxidized to carbon dioxide. Acetone is the main metabolite of isopropyl alcohol, occurs naturally in blood and urine of diabetics, abused in glue sniffing. |
| Aniline | Aminobenzene, Phenylamine, | Liquid, colorless but on keeping turns to brown due to formation of nitroso compound . B.P.1840C. Miscible with alcohol, benzene, chloroform and other organic solvents. Insoluble in water. Forms salts with mineral acids. | Intoxication occurs by inhalation, ingestion or cutaneous absorption. Toxic symptoms include cyanosis, methemoglobinamea, vertigo headache and confusion. |
| Benzene | Phenyl hydride  Source: In light oil fraction obtained by distillation of coal tar. | A clear, colourless, inflammable liquid, characteristic  aromatic smell, B.P.-80oC, practically immiscible with water, miscible with dehydrated alcohol, acetone, ether and glacial acetic acid. | Benzene is absorbed from the gastro-intestinal tract, thro- ugh skin and lungs. 50% of inhaled benzene may be eliminated via the lungs; small amount is excreted unchanged in the urine mainly as phenol, catechol and quinol in the conjugated form. |
| Benzyl alcohol | Phenylcarbinol,  Phenylmethanol  Source: As ester of cinnamic acid & benzoic acid in Balsam of Tolu and Peru. | A colourless liquid with a faint aromatic odour, B.P.-  203oC. Soluble in water, methanol chloroform and ether. | Use:  Local anaesthetic Disinfectant. |
| Carbon disulphide | Carbon disulphide, Carbonic anhydride  Source: Coal tar. | A colourless liquid with a faint aromatic odour, B.P.- 203-208oC soluble in water, miscible with ethanol, chloroform and ether. | Used in the synthesis of different dithiocarbamates. |
| Carbon tetrachloride | Carboneum tetrachloratum medicinale  Source: Synthesis by chlorination of hydrocarbon etc. | A heavy, clear, colourless liquid with chloroform like odour, volatile. Almost insoluble in water, miscible with dehydrated alcohol, chloroform and ether. | Non-inflammable, In contact with a flame it decomposes and gives toxic product of acrid odour, absorbed through skin and by inhalation. Extracted mostly from lungs as such, in urine as urea. Nephrotoxic. |
| Chloral hydrate | Aquachloral, Chloradom, Chlo- ralex, Dormel, Elix-Nocte Rec-tules(- to be checked), Micky, Finn, Knock-out drops.  Source: Synthetic | Colourless or white crystals, it liquefies between 50oC and boils at 98oC, soluble in ether, chloroform and partially soluble in water.  Ethyl alcohol- solubility? | Readily absorbed by oral administration, readily metabolised by reduction to trichloroethanol and finally to trichloroacetic acid by steps.  Used as hypnotic as well as sedative. |
| Chloroform | Chloroformium, Anaesthesicum, Chloromormum, Pro narcosi | A colourless volatile liquid B.P.- about 61oC, insoluble in water, miscible with dehydrated alcohol and ether. | Absorbed by oral administration and inhalation. It is exhaled unchanged between 20% to 70%. General anaesthetic. |
| Cresols  (A mixture of o-, m- & p-cresols) | Synonym :  Cresylic Acid, Tricresol, Proprietary Name- Lyseptol.  Source: Occurs in coal tar, in pine and bleached wood tar, in Lysol (cresol and soap solution containing 50% v/v cresol). | An almost colourless to pale brownish yellow liquid becoming darker with age or on exposure to light. Almost completely soluble in water, miscible with ethanol, chloroform and ether. | Absorbed after ingestion and through skin and mucous membrane. It is metabolised by conjugation and oxidation. |
| Ethyl Alcohol | Ethanol, Methyl Carbinol, Spirit of Wine.  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 78.40C. | Burns with blue flame. Used as solvent, converted into aldehyde and acetic acid. Absolute alcohol-99.95 % of alcohol. |
| Formaldehyde | Formic aldehyde, Methylene oxide, Oxamethane | A colourless gas, soluble in water, slightly soluble in ethanol and ether. | Inflammable, irritant to mucous membrane, inhalation through respiratory tract may cause bronchitis and pneumonia, rapidly metabolised in the body tissue to formic acid and methanol. |
| Hydrocyanic acid | Hydrogen cyanide prussic acid  Source:  Natural: Cherry, apricot, peach bears, bitter almond, Cyanogenetic glycosides. | Smell like bitter almonds. Salts are highly soluble in water and alkaline in nature. | Haemoglobin is converted to cyano haemoglobin. Constriction of throat, dizziness, vertigo. |
| Isopropyl alcohol | Dimethylcarbinol, 2-Propanol. Proprietary names - Alcojel, Avantine Sterets.  Source: Fermentation. | A clear, colourless volatile liquid B.P.-81 – 83oC, miscible with water, ethanol, chloroform and ether. | Inflammable, readily absorbed by oral administration, slowly metabolized than ethanol, largely converted to acetone, then to acetate and formate. |
| Kerosene | Coal oil, Varsol.  Source: By fractionation of petroleum. | Oily liquid of characteristic odour, boiling range:  150-300oC. | Inflammable, consumption or exposure causes irritation to eyes, headache and blurred vision. |
| Methyl alcohol | Methanol, Carbinol, wood spirit.  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. 64.7oC, 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. |
| Naphthalene | Middle oil fraction of coal tar distillation. | Colourless transparent scales, M.P.- 80oC, B.P.- 208oC, practically insoluble in water, soluble in ethanol and Chloroform, highly soluble in ether. | Burns with a smoky flame, used as insecticide and in the preparation of dyes such as indigo azo-dyes. |
| Nitrobenzene | Nitrobenzol, oil of Mirbane  Source: produced by nitration of benzene. | A pale yellow oily liquid, practically insoluble in water, miscible with ether and methanol, B.P.- 206oC, characteristic odour of bitter almonds (shoe-polish). | Used for preparation of aniline. Poisonous. |
| Paraldehyde | Paracetaldehyde  Source: Polymerization of acetaldehyde. | A clear colourless or pale yellow liquid, B.P. 123-126oC, soluble in water, miscible with ethanol, chloroform and ether. | Used as hypnotic, readily absorbed on administration, metabolised to accetaldehyde by depolymerisation & then oxidized to acetic acid and finally carbon dioxide. |
| Phenol | Carbolic acid, Fenol, Phenyl hydrate.  Source: Light oil fraction of coal tar distillation. | Colourless or faintly pink, deliquescent crystals or crystalline mass, which becomes pink on storage, B.P.181oC, partially soluble in water, soluble in ethanol, chloroform and ether. | Phenol denatures and precipitates cellular protein, which may rapidly cause poisoning. Rapidly absorbed through G.I. tract or penetrate skin, metabolised to phenyl glucuronide and phenyl sulphate and small amounts are oxidised to catechol & quinol (conjugated form). |
| Turpentine | Oil of Turpentine, Oil of Pine  Source: Pine tree. | Colourless oil with peculiar odour, immiscible with water but miscible with alcohol, ether and chloroform. | Absorbed through skin or by inhalation. |

**4.6 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.

**4.6.1 Preliminary screening of Volatile Poisons:**

The screening is done in three steps viz,

1. Smell
2. Colour tests
3. UV-spectra

A small portion of the distillate may also be subjected to GC/GC Headspace examination and the retention time of the peaks can be compared with the standard references available in literature and confirmed by the injection of a control. For this purpose, normal Flame Ionization Detector is used and the columns used normally are Carbowax or Hellcomid.

λ max (nm) values of some commonly known volatile in water are given below:-

**TABLE – 4.2**

Name of the poison λmax (nm)

Aniline 280

Benzene 228, 223, 238, 242, 248, 254, 266.

Camphor 282

Cresol 273

Kerosene 270 (may vary depending on inter batch

variations)

Methylsalicylate 238, 303

Naphthalene 265, 275, 283, 206

Nitrobenzene 274

P-Dichlorobenzene 270

Phenol 264, 271, 279

Thymol 270

Colour tests of some commonly known volatile poisons are given below:-

**TABLE – 4.3**

|  |  |  |  |
| --- | --- | --- | --- |
| **S.No** | Reagent | **Observation** | **Probable poisons** |
| 1. | Addition of bromine water | Decolouration | Aniline, Phenol, Cresol |
| 2. | Addition of Millon’s Reagent & heating | Red or Orange red colouration | Aniline, Phenol, Cresol |
| 3. | Addition of AgNO3 | Grey / Black ppt. | Cyanides, Phosphorous, Phosphine. |
| 4. | Aniline + Alcoholic NaOH + Heat | Obnoxious smell | Chloroform, Chloral hydrate, Carbon tetrachloride. |
| 5. | Pyridine + Alcoholic NaOH + Heat | Red colouration | Chloroform, Chloral hydrate, Carbon tetrachloride. |
| 6. | Direct Schiff’s Reagent | Restoration of pink colour | Acetaldehyde, Formaldehyde |
| 7. | Schiff’s Reagent added after Oxidation | Restoration of pink colour | Ethanol, Methanol, Acetaldehyde, Formaldehyde. |
| 8. | Direct addition of Chromotropic acid | Pink-violet colour | Formaldehyde. |
| 9. | Chromotropic acid added after oxidation of sample | Pink-violet colour | Methylalcohol and Formaldehyde. |
| 10. | Chloroform + Alcoholic NaOH + Heat | Red colouration with obnoxious smell | Pyridine. |
| 11. | Direct addition of Deniges Reagent +Heat | Yellow ppt. | Acetone. |
| 12. | Deniges Reagent added after oxidation | Yellow ppt. | Isopropyl alcohol, Acetone. |
| 13. | Lead Acetate + KOH soln.+ Formaldehyde + Heat | Blackening | Carbondisulphide |
| 14. | Prussian Blue Test | Blue ppt or colouration | Cyanide. |

* + 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:

**4.6.2.1** **Tests for acetone:**

1. **Iodoform Test:**

A. **With I2 + NaOH**:

To 1 ml. of the distillate, a few drops of 10% NaOH is added. This is followed by dropwise addition till the solution becomes brown. The mixture is warmed on low flame. A few drops of NaOH solution are added to change the colour of the solution from brown to yellow (addition of a few drops of iodine solution is required, if on warming the solution becomes colourless). It is cooled. The precipitate is observed under microscope. Crystals are formed often on standing. Characteristic hexagonal crystals of iodoform are seen (also positive in presence of ethanol, acetaldehyde, iso-propanol).

B. **With I2 + NH4OH**:

Positive only in case of acetone by using NH4OH, in place of NaOH, as above, i.e hexagonal crystals of iodoform are located by microscopical examination.

2. **Legal’s Test :**

To 1 ml. of the distillate, 2 drops of saturated solution of sodium nitroprusside (freshly prepared) and 1 drop of 10% sodium hydroxide is added. A red or yellowish red colour is produced. A few drops of glacial acetic acid is then added to acidify the solution which changes the colour to carmine or purplish red. Subsequently the solution is heated which produces violet colour.

**4.6.2.2** **Test for acetaldehyde:**

It can be isolated from tissue and other biological material by acid-distillation. The distillate may be subjected to the following tests.

1. **Iodoform Test:**

With I2 + NaOH – Positive (Also given by ethanol isopropanol, acetone).

1. **Schiff’s reagent Test:**

To 1 ml. of the distillate, 2 ml. of Schiff’s reagent (decolourised basic fuchsine) is added and kept for 30 minutes. A purple colour is obtained (formaldehyde also gives purple colour). To the developed purple colour 0.5 ml. of conc. sulphuric acid is added. The purple colour disappears, if acetaldehyde is present.

3. To 1 ml. of the distillate, a few drops of freshly prepared sodium nitroprusside solution and piperidine are added. The formation of blue colour indicates the presence of acetaldehyde.

* + - 1. **Tests for formaldehyde:**

Formaldehyde is isolated from biological materials by acid-distillation. The distillate is subjected to the following tests.

1. **Schiff’s reagent Test:**

To 1 ml. of the distillate, 2 ml. of Schiff’s reagent is added and kept aside for 30 minutes. Purple colour is formed. If the colour persists even after addition of 0.5 ml. of conc. sulphuric acid, the presence of formaldehyde is confirmed.

2. **Chromotropic acid Test:**

To 0.5 ml. of the distillate, 5 ml. of Chromotropic acid solution (prepared by dissolving 0.5 mg. of sodium salt of Chromotropic acid in 100 ml. of Conc. sulphuric acid) is added. The contents are heated on a hot water bath at 60oC for 30 minutes and then cooled. Violet colour is produced (λmax of the coloured complex =570 mµ).

**4.6.2.4 Test for ethyl alcohol:**

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

1. **Iodoform Test:**

With I2 + NaOH – Positive (also given by acetone acetaldehyde and iso-propanol).

1. **Sulphomolybdic Acid** – (1 gm of molybdic acid in 25 ml. of Conc. sulphuric acid):

To 2 ml. of the distillate, 2 ml. of the hot reagent is added. A deep blue ring appears at once at the junction of two liquids. On shaking, the whole mixture becomes deep blue. It indicates the presence of ethyl alcohol (This test is very sensitive and is negative with acetone, acetaldehyde and dilute solution of methyl alcohol. Strong solution of methyl alcohol gives only a light blue colour after several minutes).

3. **Ethyl Benzoate Test:**

2 drops of Benzoyl Chloride are added to 2 ml. of the distillate. The mixture is made alkaline by adding 10% solution of sodium hydroxide drop by drop. On warming, irritating smell of Benzoyl Chloride disappears and the sweet fruity odour of ethyl benzoate appears (methyl alcohol also gives this test but it does not give the iodoform test).

**4.6.2.5** **Tests for isopropyl alcohol:**

The acid-distillation is used to isolate isopropyl alcohol from tissue and other biological material. The distillate is subjected to the following tests.

1. **Iodoform Test:**

With iodine + NaOH Soln.: Positive (presence of isopropyl alcohol along with acetaldehyde, acetone and ethanol).

1. 2 ml. of distillate is taken in each of two test tubes. 3 drops of KMnO4 in phosphoric acid (by dissolving 3 gms. of KMnO4 and 15 ml. of syrupy phosphoric acid in 85 ml. of water) in one of the test tubes. This is allowed to stand for 5 minutes. The colour, if any left after oxidation is decolourised with a pinch of sodium bisulphite. 1 ml of 10% NaOH and 1 ml. of 5% furfural are added to both the test tubes. The contents of each of the test tubes are filtered into test tubes containing 2 ml. of conc. hydrochloric acid. A pink ring is formed at the junction, (containing oxidised product by KMnO4) indicates the presence of isopropanol. A pink colour in the other test tube indicates presence of acetone.

**4.6.2.6** **Test for methanol:**

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

1. **Schiff’s reagent Test:**

To 4 - 5 ml. of the distillate, 0.5 ml. of ethyl alcohol, 2 ml. of 3% KMnO4 solution and 0.2 ml. Of phosphoric acid are added and kept aside for 10 minutes. The 1 ml. of 10% oxalic acid is added followed by 1 ml. of conc. sulphuric acid and the contents are cooled to room temperature. 5 ml. of Schiff’s reagent is added to it and the colour is observed after 30 minutes. Purple colour appears if methanol is present (confirms the presence of methanol in presence of ethanol). The blank run is also made side by side. The quantitation may also be made by matching the intensity of colour developed with different known standards of methanol.

2. **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 60oC for 30 minutes and cooling thereafter. A violet colour is observed.

**4.6.2.7** **Test for chloroform:**

Chloroform is isolated from biological materials by acid-distillation. The distillate is subjected to the following tests:

1. **Fujiwara Test:**

To 1 ml. of the distillate, 1 ml. of pyridine and 2 ml. of 20% sodium hydroxide solution are added in a test tube and this is heated on a water bath for 1 minute. A pink to red colour indicates the presence of chloroform (chloroform and polyhalogenated compounds respond to this test).

2. To 1 ml. of the distillate, 1 ml. of alcoholic solution of potassium hydroxide (10%) is added, followed by 1 drop of aniline. The mixture is heated. A disagreeable odour of phenyl isocyanide evolves.

3. To 1 ml. of the distillate, 1 ml. of Nessler’s reagent is added. No brown precipitate is formed (differentiation from chloral hydrate).

4. To 1 ml. of the distillate, 1 ml. of strongly alkaline solution of β-naphthol is added and then heated. A blue colour turning to green and finally brown is observed.

**4.6.2.8** **Test for turpentine:**

Turpentine is isolated by acid distillation. The distillate is collected in an ice cooled receiver and extracted four times with 5 ml. portion of diethyl ether. This extract is dried by passing through anhydrous sodium sulphate and kept reserved for the following tests.

1. 2 ml. of ether extract is evaporated to dryness on a spotting tile. 1 drop of conc. sulphuric acid is added. A deep reddish brown colour is produced.

2. 2 ml. of ether extract is evaporated to dryness. To this residue, a few drops of Marquis reagent is added and warmed. A pink-red colour is observed.

3. 2 ml. of ether extract is evaporated to dryness. To this residue, 2 ml. of ethanol followed by 1 ml. of 1% Vanillin in conc. HCl is added. The mixture is heated. Green colouration turning to blue is observed.

**4.6.2.9** **Test for paraldehyde:**

Paraldehyde is isolated from biological materials by acid-distillation. The distillate is subjected to the following tests.

1. 0.5 ml. of distillate is added to 5 ml. of chilled 2% sodium bisulphite solution in an ice-bath. To 1 ml. of the chilled mixture, 1 drop of freshly prepared p-hydroxydiphenyl solution is added (1 gm in 25 ml. of hot 2N. NaOH is added and diluted to 100 ml.). Violet colouration (λ-max at 560 nm) is observed.

**4.6.2.10** **Test for carbon disulphide:**

Carbon disulphide is isolated by acid-distillation. The distillation occurs slowly (due to presence of carbon disulphide). It is therefore better to collect 250 ml. in two fractions (latter portion contains much higher proportion of carbon disulphide, if present). The distillate is subjected to the following tests.

1. **Lead acetate Test:**

2 drops of lead acetate is added to the solution to be tested. An excess of potassium hydroxide solution is added and boiled. A black precipitate is obtained if carbon disulphide is present.

2. **Thiocyanate Test:**

To a portion of the distillate, 1 ml. of ammonium hydroxide and 1 ml. of ethyl alcohol are added. It is then boiled for 5 minutes, concentrated to 1 ml. and acidified with dilute hydrochloric acid. 1 drop of ferric chloride solution is added. The formation of red colour indicates the presence of carbon disulphide.

3. **Castiglionis Test:**

To 1 ml. of distillate, 1 ml. of alcoholic solution is added. The formation of yellow precipitate indicates the presence of carbon disulphide.

**4.6.2.11** **Test for carbon tetra chloride:**

Carbon tetra chloride is isolated by acid distillation. The distillate is subjected to the following tests:

1. **Pyridine Test:**

5 ml. of 30% NaOH and 5 ml. of purified pyridine are added to 10 ml. of distillate. It is gently heated for 5 to 10 minutes on a steam bath. A pink red colour indicates presence of carbon tetra chloride (also given by chloroform, chloral hydrate and other chlorinated compounds such as DDT, trichloro ethanol). If the distillate is very dilute, extraction for carbon tetra chloride may be made with n-heptane and test carried out with the residue of extract).

2. **Phenyl Isocyanide Test:**

To 10 ml. of the distillate, 1 ml. of distilled or purified aniline and 2 ml. of 20% sodium hydroxide are added. The mixture is heated for a few minutes. The evolution of foul smell of phenyl isocyanide indicates presence of carbon tetra chloride (chloral hydrate and chloroform also respond).

**4.6.2.12 Tests for chloral hydrate:**

Chloral hydrate is isolated by acid-distillation. The distillate is subjected to the following tests.

1. **Fujiwara Test:**

As stated in case of test for chloroform.

2. **Nessler’s reagent Test:**

A few drops of the reagent are added to a few drops of the distillate. A yellow or reddish brown precipitate changing to grey or black is formed (negative in case of chloroform).

3. **Resorcinol – Potassium bromide Test:**

To 2 drops of reagent (0.2 gm of resorcinol and 1 gm of potassium bromide in 10 ml of water), 2 ml of conc. sulphuric acid is added carefully followed by 2 ml of distillate. This is then heated on a water bath. Change of colour is observed as yellow to pink and finally to violet at 900C. At 1000C blue colour appears which turns to orange-red by adding 2 ml of water. On adding strong KOH solution, the colour again changes to violet and finally to a reddish tint (not given by chloroform).

**4.6.2.13** **Tests for benzene:**

The acid distillation is used to isolate benzene from tissue and other biological materials. The distillate is extracted with diethyl ether and the ethereal extract is dried over anhydrous sodium sulphate.

1. 2 ml. of nitrating mixture (1 ml. of Conc. nitric acid + 1 ml. of conc. sulphuric acid) is cooled in an ice bath and then added to residue obtained after evaporation of 2 ml. of ethereal extract from the distillate. The contents are transferred to a hard glass test tube and then heated by keeping it in a hot water bath. After that the contents are cooled and diluted with water. Due to the formation of nitrobenzene, bitter almond odour is obtained.

2. The procedure as above is to form nitrobenzene by nitration. The following test may be carried out to confirm the presence of nitrobenzene i.e. the presence of benzene in distillate.

The mixture obtained after nitration is reduced on a boiling water bath by using tin and hydrochloric acid. After the reaction is ceased (usually after 5 minutes), 1 ml. of aniline and 1 ml. of alcoholic potassium hydroxide solution are added. The mixture is heated. Obnoxious smell of phenyl isocyanide indicates the presence of benzene.

Gas chromatography: The retention index and retention time of some common volatile poisons are given below:

**TABLE – 4.4**

Compound Retention Index Retention Time

in System 1 in System 2 *(in Minute)*

Acetaldehyde 372 0.70

Benzene 660 14.8

Carbon tetrachloride 659 8.6

Chloral hydrate 695 12.5

Chloroform 605 6.2

Ethanol 421 1.9

Isopropyl alcohol 530 4.0

Methanol 491 0.7

Paraldehyde 786 23.2

**System – 1:**

Column – 2.5% SE 30 on 80 – 100 mesh Chromosorb G (acid washed and dimethyl dichlorosilane treated), 2m x 4 mm I.D. glass column. The support should be fully deactivated.

Column Temperature : 100 – 300oC

Carrier Gas : Nitrogen at 45 ml / min.

Reference : n-Alkanes with an even number of Carbon

atoms.

**System – 2:**

Column – 0.3% Carbowax 20 M on 80 –100 mesh Carbopak C, 2m x 2 mm I.D. glass column.

Column Temperature: 35°C per 2 minute and then programmed at 5°C per

minute to 175°C and hold for at least 8 minutes.

Gas Carrier : Nitrogen at 30 ml / min.

**4.6.2.14** **Tests for Hydrocyanic acid:**

Hydrocyanic acid, may be detected by screening tests with test papers by placing in air tight conditions inside the jar containing biological materials.

1. Guaiacum – Copper sulphate test paper turns blue after 30 minutes (test paper is prepared by dipping strips of No.1 filter paper first in 0.1% aqueous solution of copper sulphate and tincture of guaiacum (10%).
2. Picric acid test paper turns from yellow to tan or brown within 5 minutes (picric acid test paper is prepared by dipping strips of No.1 filter paper into a solution containing 1 gm of picric acid in 10% sodium carbonate solution).

After preliminary screening, hydrocyanic acid is isolated by acid distillation and the distillate is collected in ice-cold condition in 5 ml. of 1N sodium hydroxide solution. The distillate thus collected is subjected to the following tests.

1. **Prussian blue Test:**

To 2 ml. of concentrated alkaline distillate (concentrated by heating on water bath), a few drops of freshly prepared 5% solution of ferrous sulphate and 1 drop of ferric chloride solution are added. The mixture is heated just to boiling followed by the addition of dilute sulphuric acid dropwise to make the solution just acidic and warmed. A Prussian blue colour formation indicates the presence of hydrocyanic acid. On keeping the content over night, a precipitate may be seen.

1. **Nitroprusside Test (Vortman’s):**

To 5 ml. of the alkaline distillate, a few drops of a freshly prepared aqueous potassium nitrite solution is added followed by the addition of four drops of ferric chloride solution. Then dilute sulphuric acid is added while shaking till the mixture assumes a bright yellow colour. The mixture is then heated to boiling. Subsequently it is cooled and dilute ammonium hydroxide solution is added to precipitate out excess of iron and then filtered. To the clear filtrate , a few drops of dilute solution of ammonium sulphide are added. A violet colour is obtained which changes gradually to blue green and finally to yellow (in presence of very small amount of hydrocyanic acid, a bluish green colour is observed instead of violet colour).

3. **Sulphocyanate Test:**

To 2 ml. of concentrated alkaline distillate, 1ml. of yellow ammonium sulphide is added in a porcelain basin and evaporated to dryness on a boiling water bath. Then the residue is dissolved in 1 ml. of acidulated water (containing 2 drops of hydrochloric acid). It is warmed and filtered. To the clear filtrate , a few drops of very dilute ferric chloride solution is added. A blood red colour is obtained in presence of hydrocyanic acid. On adding a few drops of mercuric chloride solution, the red colour disappears.

**4.6.2.15** **Test for Phenol:**

Phenol is isolated in biological materials by acid distillation (Preferably with 1: 4 sulphuric acid). The distillate is collected in 5 ml. of 1N NaOH solution in ice-cooled condition and is subjected to the following tests.

1. **Ferric chloride Test:**

To about 2 ml. of distillate, 2 drops of a very dilute solution of ferric chloride solution are added. A violet or bluish violet colouration is produced. (cresol, other phenols and salicylic acid gives this test with the shades of colour – violet to bluish violet, being different in each case). Antipyrine, pyramidon and similar non-phenolic compounds also give this test. In case of phenol, the colour produced disappears on adding alcohol.

2. **Libermann’s Test:**

5 ml. of alkaline distillate is evaporated to dryness over a small flame in a porcelain dish. It is cooled. A pinch of sodium nitrite is added. Thereafter conc. sulphuric acid is added. On mixing carefully, blue or green colouration is observed. It turns to red on adding water to it, which turns green if made alkaline by sodium hydroxide or ammonia solution. (Cresol and other phenols also respond to this test).

3. **Ware’s nitrite – nitrate Test:**

To 5 ml of the alkaline distillate in a mortar, 10 ml. of conc. hydrochloric acid and 0.5 gms. of nitrite-nitrate mixture (sodium nitrite-1 part, sodium nitrate or potassium nitrate-1 part and anhydrous sodium sulphate 2 parts) are added . Then it is mixed thoroughly to dissolve the contents and thereafter allowed to stand for 2-5 minutes.

In the presence of carbolic acid, a rich crimson or blood red colour appears. 1 ml. of this blood – red or crimson coloured mixture is added drop by drop to 10% ammonia solution. A deep emerald green colour is produced.

Alternatively, 2 drops of formalin are added to 2 ml. of the blood red mixture as above. The colour changes to purple. This mixture is added drop by drop in to 10% ammonia solution. Greenish blue or deep blue colour is produced. (In presence of cresols, no blood red colour is produced. Only brown or reddish brown colour appears. Neither addition of formalin nor presence of ammonia solution changes the colour in case of cresols).

**4.6.2.16 Tests for Cresol:**

Cresols are isolated by acid distillation. The distillate is collected in 5 ml. of 1 N. sodium hydroxide solution in ice-cold condition. The distillate thus collected is subjected to the following tests:

1. **Ferric chloride Test:**

As described in the test for phenol.

2. **Libermann’s Test:**

As described in the test for phenol

1. **Ware’s nitrite – nitrate Test**

As described in the detection of phenol, brown or reddish brown colour appears (phenol gives blood red colour) which does not change on adding a few drops of formalin or 10% ammonia solution (distinction from phenol).

* + - 1. **Tests for Aniline:**

As aniline is basic, it is isolated in biological materials by alkaline steam distillation. The distillate is collected in ice cold condition in 5 ml. of 1 N sodium hydroxide solution. The alkaline distillate is extracted with three, 10 ml. portions of diethyl ether. The ether is dried by passing through anhydrous sodium sulphate and is used for following tests.

1. **Dichromate Test:**

A portion of ether extract is evaporated to dryness in a porcelain basin. 5 drops of conc. sulphuric acid and 1 drop of potassium dichromate solution are added. In a few minutes, the edge of the mixture begins to show a blue colour, which becomes uniformly blue on adding a few drops of water.

2. **Phenyl isocyanide Test:**

As stated in a case of chloroform, obnoxious smell of phenyl isocyanide evolves.

3. **Hypochlorite Test:**

A portion of ether extract is evaporated to dryness. The residue is taken up in water. To it a few drops of sodium hypochlorite or freshly prepared solution of bleaching powder is added. A purple or violet-blue colour appears which changes to reddish brown or dirty red. On adding a few drops of a dilute aqueous solution of phenol containing some ammonia, the violet or dirty red solution changes to blue (indophenol).

**4.6.2.18**  **Test for Nitrobenzene:**

Nitrobenzene in biological materials is isolated by alkaline distillation. The distillate is collected in ice-cold condition. It is extracted four times by 10 ml. portions of diethyl ether and the extract is passed through anhydrous sodium sulphate which is preserved for the following tests.

1. A portion of ether extract is evaporated to dryness. To the dried residue, 3 ml. of concentrated hydrochloric acid and metallic tin are added. This is warmed on a hot water bath for 5 minutes and filtered. To the filtrate , 2 ml of chloroform and 2 ml of alcoholic caustic potash solution are added. The mixture is heated strongly. The evolution of obnoxious smell of phenyl isocyanate indicates the presence of nitrobenzene.

2. A portion of ether extract is evaporated to dryness. To the dried residue in a test tube, 5 ml. of ammonium chloride solution and zinc dust are added. The mixture is heated on a water bath for 5 minutes. To the mixture, 1 ml. of ammoniacal silver nitrate solution is added and heated on water bath for 5 minutes. The formation of mirror on walls of test tube or formation of black precipitate indicates the presence of nitrobenzene.

**4.6.2.19** **Test for Naphthalene:**

Naphthalene in biological materials is isolated by steam distillation. The distillate is collected in ice cold condition (Smell of naphthalene may be felt). The distillate is extracted 4 times with 10 ml. portion of ether. The combined ethereal layers are dried by passing through anhydrous sodium sulphate and kept reserved for tests.

1. **Test with picric Acid:**

A portion of ether extract is evaporated to dryness. To the dried mass, a few drops of picric acid solution is added. Yellow crystals of naphthalene picrate are formed ,which show characteristic shape under microscope.

2. **UV Spectrum:**

A portion of ether extract is evaporated to dryness. The residue is dissolved in *spectra pure* ethanol. It shows maximas at 266 nm and 288 nm in UV Spectra.

**4.6.2.20** **Test for Kerosene oil:**

Kerosene oil in biological materials is isolated by steam distillation. The distillate is collected in ice cold condition in 20 ml saturated solution of sodium chloride. The top layer (1 cm.) of the distillate is taken out after some time and extracted four times with 10 ml. portions of diethyl ether. The combined ethereal extracts are dried by passing through anhydrous sodium sulphate, evaporated at room temperature and subjected to the following tests.

1. **UV Spectra:**

A portion of ethereal extract is evaporated. To the residue, water is added. On scanning in UV Spectrophotometer, maximas are observed at 223 – 228 μm and also at 366 nm (weak band).

**4.6.2.21** **Tests for Nicotine:**

Nicotine (only steam volatile alkaloid) is isolated by alkaline distillation. The distillate is collected in ice cold condition in 10 ml of 10% hydrochloric acid. It is made alkaline and extracted 4 times with 10 ml. portions of diethyl ether. The ether extracts are combined and dried over anhydrous sodium sulphate. The extract is kept reserved for the following tests.

1. **Roussin’s Test:**

To the ethereal extract, 1 ml. of ether solution of iodine is added. A brownish red amorphous precipitate is formed which turns to crystalline ruby red needle shape crystals after sometime.

2. **Schindelmeister Test:**

A portion of ethereal extract is evaporated to dryness. To the , 1 drop of 30% formaldehyde solution (free from formic acid) and a drop of concentrated sulphuric acid are added. The mixture becomes rose red.

**4.6.2.22 Ethylene Dibromide:**

**Synonyms:** 1,2-Dibromoethane; Ethylene bromide, EDB

Ethylene dibromide is a pesticide used as fumigant for storage of food grains. It is a heavy liquid having the odour like chloroform, soluble in about 250 parts of water and miscible in alcohol, ether. Ethylenedibromide is potentially a cause of a variety of acute health effects including damage to liver, stomach and adrenal cortex along with significant reproductive system toxicity, particularly in testes.

**Gas Chromatographic method:**

Column : Glass column packed with 10%SP-1000 (1.8mX 4mm Glass, 80-100 mesh)

Detector : ECD Carrier gas – CH4:Ar (5:95) , F/R 40ml/min.

Injector temp: 2000C Injection quantity - 5μl

Oven temp. : 1150C

Detector temp. 3500C Limit of quantitation – 2ppb (2)

**4.6.2.23 Phenyl: (Household disinfectant)**

These are homogenous solutions of coal tar acids or similar acids derived from petroleum, with or without hydrocarbons, or other phenolic compounds including substituted phenolic compounds, or a mixture of these and a suitable emulsifier. It is black in colour and on dilution with water gives translucent off-white turbidity (3). Phenyl is used as disinfectant in houses, hospitals, and laboratories for sanitary purposes. The tests prescribed for phenols and cresols should be applied.

**4.6.3 mETHODS OF QUANTITATION OF SOME VOLATILES IN BIOLOGICAL MATERIALS:**

**4.6.3.1 Determination of Ethyl Alcohol in blood and urine.**

1. **Modified** **Kozelka – Hine Method:**

The apparatus used in this method consists of four hard glass tubes (length 20 cm, dia 2.5 cm) connected with each other by means of quick fit joints. In the first tube, 5 ml of 2% potassium dichromate in concentrated sulphuric acid is taken through which air is bubbled to remove the moisture and other organic volatiles. In the second tube, 2 ml. of blood sample or 10 ml. of urine sample is taken. To this sample, 2 ml. of 10% sodium tungstate and 0.5 ml. of 2 N sulphuric acids are added to deproteinize the blood (deproteinization is not required for urine sample).

In the third tube, 5 ml. of saturated mercuric chloride and 5 ml. of saturated sodium hydroxide solution are added and mixed uniformly when a yellow precipitate of hydrated mercuric oxide is obtained (used to trap acetone, acetaldehyde etc.). This tube is interposed between the tube of blood or urine samples and the fourth tube. To the fourth tube, 10 ml. of 0.1N potassium dichromate solution and 10 ml. of concentrated sulphuric acid are added.

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

A blank experiment is also performed side by side by taking all the reagents except the blood sample

Calculation:- Considering redox reaction,

1.0 ml. of 0.1 K2Cr2O7 solution = 1.15 mg. of ethanol.

Therefore, blood alcohol % = 1.15 x (X – Y) x 100 mg per 100 ml. of blood. V

Where,

V = Volume ( ml.) of blood taken for the experiment.

X = Volume (ml.) of 0.1N sodium thiosulphate required in blank experiment.

Y = Volume ( ml.) of 0.1N sodium thiosulphate solution required in

the experimentation with the blood sample.

2. **Determination of alcohol in blood by head space gas chromatographic method:**

1. 0.5 ml. of blood and 0.5 ml. of internal standard (0.2% w/v solution of n-propanol in distilled water) is taken into the glass vial and placed into the turntable, head space sampler thermostat at 70oC for 10 minutes. The head space sample is withdrawn and subjected to gas chromatography.

1. Similar sets of experiment are made with different standards of alcohol for calibration purpose as per the following chromatographic conditions.

**Chromatographic conditions**

1. Column : 2m x 1/8” O.D. Stainless steel column. P/W 3.8% Halcomid M-8 on Chromosorb

W(mesh:100-120).

2. Detector : F.I.D.

3. Carried Gas : Nitrogen (32 lbs / sq. inch.).

4. Temperature : Injection temperature : 100°C

Transfer temperature : 125°C

Column temperature : 65°C

F.I.D. temperature : 150°C

5. Programme : Constant thermostating time: 10 minutes

Injection time : 0.1 minute

Pressurisation time : 0.1 minute

Cycle time : 4.5 minutes

**Quantitation:**

Done by peak area method using standards of ethanol up to 400 mg% to ensure linearity.

3. **Alternate head space GLC method for determination of ethanol in blood**

A. Reagents : Calibration Reference Materials (Calibrators).

Ethanol : 0.10, 0.20, 0.40% w/v (1.00, 2.00, 4.00 g / L) aqueous

solutions.

Mixed Calibrator : Low (Acetone 1.00 g / ι, Ethanol 2.00 g / ι,

Isopropanol 1.00 g / ι, Methanol -1.00 g/ι,

aqueous solutions).

Mixed Calibrator : High (Acetone 1.00 g / ι, Ethanol 4.00 g / ι,

Isopropanol 1.00 g / ι, Methanol 1.00 g / ι,

aqueous solutions).

B. Internal Standard solution:

Acetonitrile: 0.15%, v/v (1.5 ml. / ι aqueous solution).

C. Sodium Chloride.

All the reagents should be of A.R. Grade.

**Operation:**

The liquid sample (20 μl to 1ml.) is placed into a glass septum vial together with sufficient sodium chloride to assure saturation of the liquid (and after dilution in fixed proportion with the internal standard solution when that procedural modification is used) Reference samples are treated identically and all sealed vials are inserted into the thermo stated water bath. After a 45 minute equilibration period, a fixed quantity of the head space vapour of each septum vial is sequentially sampled with a gas tight syringe and injected into the preset gas chromatograph. The analysis proceeds for a fixed time interval and the response of detector is recorded as a function of time.

**Gas Chromatographic Conditions:**

Column : Carbowax 1500 (0.4%) on 60 / 80 mesh.

Carbopack C 1.8 M x 3.2 mm O.D. (6 ft. x 1/8 in.) stainless steel column.

Carrier Gas : Helium : Inlet pressure : 55 psig.

Flow rate : 45 ml./ min.

Temperature : Column Oven : 80°C

Injection port : 175°C

Detector : 225°C

F.I.D. : Hydrogen : Inlet pressure : 20 psig

Flow rate : 45 ml./ min.

Air : Inlet pressure : 15 psig.

Flow rate : 330 ml./min.

Temperature Programme : Isothermal.

Chart Speed : 5 mm./ min.

**Procedure :**

1. A well mixed aliquot of the blood specimen is mixed an identical volume of acetonitrile internal standard solution using fully quantitation technique. Normally the respective volumes are both 500 μl. The sample measurement and internal standard solution measurement and mixing are carried out preferably with an automatic dilute and the sample internal standard mixture is delivered directly into one clear, dry numbered septum vial containing 1 gm of crystalline sodium chloride.

2. Ethanol or mixed volatiles reference solutions (usually low and high calibrators) are treated identically.

3. After the minimum equilibration period, a fixed identical volume of the headspace vapour is withdrawn using the gas-tight syringe adjusted to fixed volume (usually 250 μl) with the chaney adapter or otherwise, the syringe having been preheated to 38oC in the incubator or similar device. Repeated rapid pumping of the syringe is employed to obtain a fully representative aliquot of the headspace vapour, which is then immediately injected into the gas chromatograph immediately following the headspace sample withdrawal, each septum vial is vented with a 25 gauge needle to reestablish atmospheric pressure. The syringe and needle are thoroughly purged by rapid, repetitive intake and expulsion of room air and the syringe is returned to the incubator until its meet use.

4. The analyte is allowed to proceed for approximately l5 minutes (or 3 minutes) if ethanol is the only compound.

5. The same gas tight syringe with volume calibration intact is used for the entire unknown and reference sample. Each specimen is analysed by this procedure in at least independent duplicate, as in each calibrator after allowing each septum vial to re-equilibrate for at least 20 minutes after the prior headspace sample withdrawal.

6. The analysis record is appropriately identified for computation of result as follows.

A. **Results Computation by Peak Height Measurement.**

R1

Blood Alcohol concentration (BAC) = ------- X Conc. Ethanol, Calibrator R2

Where, PH = peak height of the indicated detector response recording (e.g. PH i.s. = Peak height of the acetonitrile internal standard response).

PH Ethanol = R1 For unknown sample -internal standard mixture

PH I.S

PH Ethanol = R2 For the Calibrator sample -internal standard mixture

PH I.S

B. **Result Computation by Peak Area Measurement.**

R1

BAC unknown = ------ X Conc. Ethanol, Calibrator. R2

Where, PA = Peak area of the indicated detector response recording

(e.g. PA i.s. – Peak area of the acetonitrile internal standard response).

PA Ethanol

R1 = for the unknown - sample internal

standard mixture.

PA i.s.

PA Ethanol

R2 = P.A. i.s. for the Calibrator – internal standard

mixture.

**C. Result, computation may also be made for other volatiles accordingly.**

# 4. Method For The Determination Of Alcohol In Blood By GC-HS

A volume of 0.5 ml of blood and 0.5 ml of internal standard (0.2 w/ v solution of n-propanol in distilled water) was taken into the glass vial and placed in to the turntable of head space sampler, thermo stated at 70 c for 20 minutes, vapors were injected into the column and analysed. The conditions for analysis are as below.

Gas – Chromatographic Condition:

Instrument: - Perkin - Elmer Clarius Gas Chromatograph coupled

with 110 Head Space sampler and analytical data

Station. Perkin-Elmer 8410,8600 GC coupled with

HS- 101 is also used for this purpose.

Column: - 1/8”o.d. s.s. column 2 meter, p/w 3.8 %halcomid

m-18 on chromosorb w (hp) mess range 100-120

Detector: - Flame ionization detector.

Carrier Gas**: -** Nitrogen 30 lbf/inch.

Temperature: - Injection temp. = 080 c

Transfer temp. = 087 c

Column temp. = 067 c

FID temp. = 150 c

Sample temp. = 065 c

Programme: - Thermostat time = 20 min.

Injection time = 0.05 min.

Pressurization time = 0.1 min.

Cycle time = 4.0 min.

Calculation: - cubic + origin fit type multilevel using peak

area at 5 different levels. This ensures the

linearity fit curve to 400 mg % of ethanol

check standard are used to verify the initial

calibration

**4.6.3.2** **Gas Chromatographic determination of Ethanol and other Volatiles in Blood/Urine/visceral distillate.**

**Isolation of volatiles 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 volatiles 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.

**TABLE : 4.5 :Retention Time of Different Volatile Substances Relative to n-Propanol.**

Sl. No. Substance Relative Relation time (RRT)

1. Ethanol 2.30

2. Methanol 2.80

3. Acetone 2.80

4. Isopropanol 2.90

5. Ether 5.10

6. Chloroform 6.00

7. Trichloromethane 0.44

8. Acetaldehyde 0.21

9. Paraldehyde 0.70

10. Benzene 0.76

11. Amyl Alcohol 0.98

12. Toluene 1.45

The presence of a volatile / volatiles may be made from the above study.

**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.

Ca Cb

----- = ------

Aa 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).

**4.6.3.3** **Determination of cyanide:**

I. **By Argentometric Titration**

All silver salts except the sulphide are readily soluble in excess of a solution of alkali cyanide. Hence chloride, bromide and iodide do not interfere. The only difficulty in obtaining a sharp end point lies in the fact that silver cyanide is often precipitated in curdy forms, which do not readily re-dissolve, and moreover the end point is not easy to detect with accuracy. There are two methods for overcoming these disadvantages. In the first, the precipitation of silver argentocyanide at the end point can be avoided by the addition of ammonia solution in which it is readily soluble. If a little potassium iodide solution is added before the titration is commenced, the sparingly soluble silver iodide, which is insoluble in ammonia solution, will be precipitated at the end point.

Ag [Ag (CN)2] + 4NH3 = 2 [Ag(NH3)2] + +2CN −

[Ag (NH3)2]+ + I−  = AgI + 2NH3

At the end point, silver iodide in precipitated. Viewing against a black background best sees the precipitation. In the second method diphenyl carbazide is employed as an adsorption indicator. The end point is marked by the pink colour becoming pale violet (almost colourless) on the colloidal precipitate in dilute solution before the opalescence is visible. The colour change is observed on the precipitated particles of silver argentocyanide.

**Preparation of solutions:**

**0.1N Silver Nitrate solution**: 8.494 gms. of A.R. grade silver nitrate is weighed accurately, dissolved in distilled water and volume is made up to 500 ml. volumetric flask. This gives 0.1N silver nitrate solution.

Alternatively, about 8.5 gms. of pure dry silver nitrate is weighed out accurately and dissolved in 500 ml. of water in a graduated flask. The molar concentration is calculated from the weight of silver nitrate employed.

Diphenyl Carbazide Solution: It is prepared by dissolving 0.1 gm of the DPC(solid) in 100 ml. of ethanol.

On the above basis, cyanide is determined as follows in salts as well as in biological materials.

1) **Determination of Cyanide in suspected salt:**

**Procedure:**

The sample is weighed accurately and dissolved in 250 ml. of water in a volumetric flask. It is shaken well. From the stock solution thus prepared cyanide may be determined by any of the two methods.

**Method I:**

25 ml. of the stock solution is transferred to a 250 ml. conical flask by means of a burette (not by a pipette due to toxicity of cyanide). 75 ml. of water, 5-6 ml. of 6 M ammonia solution and 2 ml. of 10% potassium iodide solution are added. The flask is placed on sheet of black paper and the content of the flask is titrated against 0.1N silver nitrate solutions. The silver nitrate solution is added drop wise till sign of persistence of yellow colour of silver iodide is observed. When a further drop of silver nitrate produces a permanent turbidity, the end point is noted.

**Method II:**

25 ml. of the stock solution of sample is transferred to a 250 ml. conical flask as above. 75 ml. of water, 5-6 ml. of 6 M ammonia solution and 2 ml. of 10% potassium iodide solution are added. The content of the flask is titrated against 0.1 N silver nitrate solution using 2-3 drops of diphenyl carbazide as an indicator. The end point is recorded when a permanent violet colour is produced by drop wise addition of silver nitrate solution.

**Calculations:**

The amount of cyanide or potassium cyanide may be calculated as follows:

1 ml. of 1 M AgNO3 ≡ 0.05204 g of CN−

≡ 0.1304 g of KCN.

**2) Determination of Cyanide in Biological Materials:**

**Procedure:**

100 gms. of visceral material is placed in a distilling flask of the standard fit distillation unit and acidified with tartaric acid and distilled. The distillate is collected in ice cold condition in a graduated conical flask containing 10 ml. of 6M ammonia solution. 100 ml. of distillate is collected. While collecting the distillate in the receiver, tube should be dipped into the ammonia solution. 2 ml. of 10% potassium iodide solution is added . It is then placed on a sheet of black paper and titrated against 0.1 M silver nitrate solution by drop wise addition and shaking till the persistence of yellow colour of silver iodide is observed. The addition of drops of silver nitrate solution is done carefully. When a drop produces a permanent turbidity, the end point is reached and noted. The relation calculates the amount of cyanide as follows.

1 ml. of 1 M AgNO3 ≡ 0.05204 g of CN−

≡ 0.1304 g of KCN.

The above titration can also be carried out by using diphenyl carbazide solution as indicator.

II. **By Spectrophotometric Method:**

**Scoggin’s Method :**

Cyanide in post-mortem materials is isolated by acid distillation and hydrocyanic acid thus liberated is collected either in 1% Na2CO3 or 0.2 N NaOH (recovery 70%) or 2 x 10 –2 M nickel ammine (recovery 90%) directly. Pre-washing of the collected material by chloroform gives minimal background. By this method cyanide is reacted with nickel amine to form nickel cyanide complex having λmax at 268.8 nm. Computing from Beer’s curve for the known amount of cyanides does the quantitation.

**Procedure:**

10 gms. of homogenized visceral matter is mixed up with 50 ml. of water and acidified with 1 gm of tartaric acid. It is distilled over a low flame. The distillate (1st 30 ml.) is collected at room temperature in a mixture of 40 ml. nickel amine solution (prepared by mixing 20 ml. of 1 M nickel chloride and 20 ml. of 30% ammonia solution) or alternatively in 25 ml. of 1% sodium carbonate solution. In either case, the volume is made up to 100 ml. with 15% ammonia solution or 1% sodium carbonate solution as the case may be. The distillate is washed with chloroform. 1 ml. of the distillate is mixed up with 1 ml. of 2 x 10 –2 M nickel amine solution and made up to 10 ml. In case of distillate collected directly in nickel amine, further dilution (10 times) with water is made. The blank in the case of absorption in sodium carbonate solution has practically zero background in the spectral region in question. Whereas the blank in the case of absorption into nickel ammine, a slight background is shown even when CN– is absent.

The absorbance of nickel cyanide is measured by taking appropriate volume of stock solution of nickel cyanide (prepared from the distillate) at 268.8 nm. From the Beer’s curve, thus prepared the amount of cyanide in biological material (unknown sample) is computed.

**4.7** **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 – 4.6 indicates their characteristics, origin, properties etc.

**TABLE – 4.6: CHARACTERISTIC OF GASEOUS POISONS:**

|  |  |  |  |
| --- | --- | --- | --- |
| **NAME** | **SOURCE** | **PROPERTIES** | **OTHER CHARACTERISTICS** |
| Carbon Dioxide | Atmosphere (0.4%), lime kiln industrial soda water, dry ice. | Colourless, odourless, slightly acid taste, heavier than air. very soluble in water. | Symptoms are giddiness, muscular weakness, drowsiness, stertoyous breathing coma etc. |
| Carbon Monoxide | Decomposition of oxalic and formic acid by sulphuric acid, incomplete combustion in confined space, coalgas, automobile exhausts, refineries etc. | Colourless, tasteless, odourless gas, lighter than air, insoluble in water, combines with chlorine to form phosgene. | Oxygen carrying capacity of blood is lost due to formation of carboxy haemoglobin. 240 times more affinity for haemoglobin than that of oxygen, symptoms include sudden weakness, dizziness, coma and death. Blood becomes deep cherry red, pink discolouration of skin. |
| Hydrogen sulphide | By decomposition of organic substances containing sulphur, by- product in some sulphur based industries & in sewers. | Colourless having odour of rotten eggs. | Converts methaemoglobin to sulphamethemoglobin. Symptoms include conjunctivitis, headache, cough, anemia, offensive smell is felt on opening the body. |
| Ammonia | In industries (fertilizers, ice cream, refrigeration). | Colourless, pungent smell, soluble in water. | Symptoms include choking, coughing, pulmonary odema followed by bronchopneumonia. |
| Phosphine | Phosphides used as rodenticides, phosphorous-based industries. | Colourless, flammable with odour of decaying fish. | Highly toxic, affects CNS and reacts with haemoglobin. |
| Phosgene | Industries (manufacture of insecticide, war gases etc.). | Colourless gas, suffocating . | Suffocation, coughing, edema of lungs. |
| Sulphur dioxide | Burning of sulphur, sulphide ores of zinc, iron and lead, volcanoes. | Heavy, colourless, pungent smelling gas soluble in water. | Irritable to the air passage, suffocating, symptoms include coughing, sneezing, bronchopneumonia oedema. |
| Chlorine | Bleaching powder in disinfecting formulation. | Greenish yellow gas, unpleasant odour, soluble in water. | Irritates the mucous membrane & respiratory tract. Causes cyanosis and suffocation. |

**4.7.1 Identification of some toxic gases by Conway Micro Diffusion technique (blood, stomach wash or urine):**

1. **Conway micro diffusion assembly:**

It is of a brink type, polypropylene cells with clear polystyrene covers having an outermost annular sealing well, an intermediate annular well for the sample and the liberating agent and a center well for the reagent (detection reagent) which is used to trap the diffusing gas or vapour.

**General procedure:**

1. In the center well (sample well) 1ml. of sample (urine or blood) is introduced into the one half and 1ml. of liberating agent in the other half (sample and liberating agent should not be mixed at this moment).

2. 2ml. of sealing agent (sample as the liberating agent as stated above) is added into the outermost sealing well.

3. 2ml. of trapping agent (absorbs vapour or gas liberated by diffusion and indicates the presence of volatile component or gas present in sample by specific colour change or formation of coloured precipitate) is taken in the center well.

4. The cover is now placed and the assembly is rotated to effect an air tight liquid seal.

5. The sealed or closed Conway is tilted back and forth to mix the sample and liberating agent uniformly throughout the sample well area.

6. The assembly is then placed on a table for the reaction.

7. Another Conway assembly is taken to carryout the above step for the control i.e. by taking 1ml. of water instead of sample for comparison.

8. The sample and the control containing assemblies may be placed on water bath to facilitate reaction.

9. At the end of the reaction, the Conway assemblies (sample as well as control) are removed with great care so as to prevent any sealing agent splashing or dipping from the edge of the cover into the center well.

10. The contents of the center well containing the reaction products formed may now be removed for colorimetric or other appropriate measurement.

Using the following table indicating sealing and trapping agents and detection thereof may conveniently carry out the detection of toxic gases or vapours

**Table – 4.7: MICRO-DIFFUSION-ANALYTICAL CONDITIONS FOR TOXIC GASES/VOLATILES.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Poison** | **Sealing/Liberating Agent** | **Absorbent/ reactant** | **Detection** |
| Carbon Monoxide | 10% Sulphuric acid | Palladium chloride solution (0.5%) in 2N HCl-acts as detection reagent. | Palladium Chloride solution turns grey to black. |
| Ethanol | Saturated sodium carbonate solution | Potassium dichromate solution in sulphuric acid. | orange to green. |
| Cyanide | 10% Sulphuric acid | 10% NaOH solution (absorb HCN gas). | Alkaline extract is subjected to Prussian blue test using FeSO4 and HCl – Prussian blue ppt. or colouration. |
| Sulphur Dioxide | 10% H2SO4 | 10% NaOH | Formation of black ppt with lead acetate.. |
| Phenol | 10% H2SO4 | 10% NaOH | By adding Fehling’s reagent to alkaline extract – blue colouration. |
| Paraldehyde | 10% H2SO4 | 10% NaHSO3 | By adding p-hydroxydiphenyl in H2SO4.- blue colour |

**4.7.2 Identification of some Gaseous Poisons:**

1. **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 AgNO3 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.

1. It may 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,

PH3 + 3 HgCl2🡪 P(HgCl)3 + 3 HCl, the quantity of acid being proportional to the volume of phosphine present.

2. **Phosgene (COCl2) in Biological Materials:** (4)

10 gms. of viscera is taken in a conical flask fitted with a guard tube whose bulb is filled with lead acetate soaked cotton. The other end of the tube is put into a receiving flask containing a solution as absorbent for the evolved gas. 20-25 ml. of 1(N) sulphuric acid is added. The flask is now heated slowly and the evolved gas is collected in any of the following solution for carrying out tests.

1. The liberated gas is trapped in 5 ml. of 5% silver nitrate solution. The solution turns black or yellowish-brown or brown indicating presence of arsine or phosphine or stilbine (The blackening of lead acetate cotton in guard tube shows interference due to sulphide). To the solution is added a few drops of concentrated nitric acid followed by 0.5ml of 5% ammonium molybdate solution in nitric acid (Reagent: Appendix 393 Vogel’s). Canary yellow colouration is observed which turns blue on adding an alkaline solution of benzidine.

**Sensitivity:** 0.1 mg phosphorous

2. The gas evolved is trapped in a solution consisting of a mixture of p-nitrobenzylpyridine and n-benzylaniline. The formation of red colour indicates the presence of phosgene.

* + 1. **Detection of toxic gases in air:**

The detection of toxic gases in air is an area related to air pollution. However, for the toxicological purposes, multi gas detectors may be used.

* + 1. **Detection of Carbon monoxide in blood:**

Carbon monoxide has more affinity for blood to form toxic carboxy-haemoglobin. Carbon monoxide has an affinity for combining with hemoglobin more than 240 times greater than oxygen. This causes sinister toxicity by preventing normal transportation and supply of oxygen to cells. Further, a person surviving several hours after an exposure has already eliminated a major percentage of carbon monoxide from his body.

**Identification:**

1. Take two small porcelain-evaporating dishes. Place 1 ml of normal blood into one dish and 1 ml of suspected blood in another dish. Heat both the dishes gently. The normal blood will change to a brown black whereas the blood having carbon monoxide will become brick red. This test is sensitive to 40% carbon monoxide.

2. Take a drop of blood and dilute with 10-15 ml of water. Compare with blood diluted in the same manner. Blood containing carbon monoxide is pink. This test is sensitive to 50% carbon monoxide.

3. Micro diffusion technique (Feld Stein):

Outer compartment : 1 ml on one side plus 1 ml of 10% sulphuric

acid on other side.

Inner compartment : 2 ml of palladium chloride (11mg palladium

chloride in 25 ml of 0.01 N hydrochloric acid; gently heat to dissolve) prepare fresh on need. Seal with 10% sulphuric acid.

At room temperature (25-300C) diffusion time is two hours. Reduction of palladium chloride to grey or black is positive for carbon monoxide. Putrefaction of blood interferes

This test is sensitive between 5 and 10% saturation.

**4.7.4.1 Quantitative estimation of Carbon Monoxide in Blood:-**

**Method - 1**

The method depends on the fact that normal blood contains several forms of hemoglobin, e.g., the reduced form, the oxygenated form, and a small amount of methohaemoglobin. If a reducing agent such as sodium dithionite is added to the blood, both the oxygenated form and the methohaemoglobin are quantitatively converted to the reduced form, which has a visible spectrum. Carbon monoxide has a much greater affinity for hemoglobin than oxygen and carboxyhaemoglobin is not reduced by sodium dithionite. Thus, even when treated with sodium dithionite, carboxyhaemoglobin retains its normal twin-peaked spectrum. The wavelength of maximum absorbance difference for spectra A and B is at 540 nm, whilst at 579 nm, the spectra have the same absorbance (isobestic point). The percentage carbon monoxide-saturation of a blood sample can be calculated from measurements of the absorbance at these wavelengths of the carbon monoxide-saturated sample (A), the carbon monoxide-free sample (B), and the untreated sample(C), after reduction of each with sodium dithionite.(5)

**Procedure:**

Dilute 0.2 ml of the blood sample (mixed well) with 25 ml of a 0.1% solution of ammonia and divide the resulting solution into three approximately equal parts labeled A, B and C. Saturate solution A with carbon monoxide by bubbling the gas through it in a wide container and at a rate, which minimizes frothing. A few minutes bubbling should suffice. Saturate solution B with pure oxygen for 10 minutes to displace all the strongly bound carbon monoxide.

Add a small amount of sodium dithionite to each of the solutions A, B and C and also to 10 ml of 0.1% ammonia solution, and mix well. Place matched 1 cm cells containing the ammonia solution into the sample and reference beams of a spectrophotometer which has been set to record between the wavelengths 500 and 650 nm, in the superimposed, repeat-scan mode on a range of 0 to 2 absorbance units. Record the baseline for the determination. Then record the absorbance spectra for solutions A, B and C. Wash out the sample cell thoroughly between the recordings and wash the cell with a little of the solution whose absorbance is about to be recorded.

If the blood sample was from a person who died from inhalation of carbon monoxide, a set of spectra will be obtained.

Measure the absorbance of each solution at 540 nm and 579 nm and calculate the ratio of absorbance at 540 to that at 579 nm for each of the solutions A, B and C.

The percentage carboxy-hemoglobin-saturation is calculated as follows:

ratio for C – ratio for B

% saturation = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ X 100

ratio for A – ratio for B

Approximate normal values for the ratios of absorbance are: saturated carboxy-hemoglobin 1.5, and reduced hemoglobin 1.1.

Note that the hemoglobin content of blood can vary and therefore, the volume of diluent may also need to be varied. A dilution giving a maximum absorbance of about 1 is ideal.

Carbon monoxide in blood may also be quantified using an automated visible spectrophotometer (IL 282 Co-oximeter), which utilizes four wavelengths to measure hemoglobin, oxyhemoglobin, carboxy-hemoglobin and methaemoglobin in small blood samples. Reference standards are commercially available.

**Method – 2**

**Principle:**

Carbon monoxide is liberated from carboxy-hemoglobin by 10% sulphuric acid. The gas is trapped by palladium chloride solution and reduces yellow tan palladium chloride solution to metallic palladium, which floats as a black silver film on the solution. Carbon monoxide is oxidized to carbon dioxide in this process. The unreacted palladium chloride is measured.

**Materials :**1) 30% CO Hb blood standard b) Unknown Sealing agent – 2 ml. of 10% sulphuric acid sample. Liberating agent – 1 ml. of 10% sulphuric acid (v/v).

**Trapping agent:** 2 ml. of palladium chloride solution (0.22 gms of palladium chloride in 250 ml. of 0.01N hydrochloric acid).

**Procedure:**

1. The reagents are mixed for micro-diffusion as stated earlier in the general procedure for micro-diffusion and allowed to stand for one hour.

2. The extent of reaction is noted at 10, 20, 30 and 60 minutes interval by taking the contents of the center well by a dropper. It is filtered. To 0.1 ml. of the filtrate, 1 ml. of 15% potassium iodide, 8 ml. of water and 1 ml. of 1% starch solution are added. It is mixed thoroughly and absorbance is measured at 500 nm.

3. A blank run is made for all the observations.

4. The percentage of carbon monoxide is calculated as follows.

1.0 ml. of PdCl2 solution ≡ 0.528 mg of palladium

1 mg of palladium ≡ 0.21 mg of carbon monoxide

Assuming a total haemoglobin concentration of 15 g / 100 ml. of blood,

O.D. of reagent blank – O.D. of sample

% CO haemoglobin = X 9

O.D. of reagent blank

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**SECTION – 5: ANALYSIS OF INORGANIC POISONS**

**(CATIONS AND ANIONS)**

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

**5.2 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, water etc.

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

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

**5.5 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 in 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.

**5.5.1 Characteristics of some toxic Cations:**

|  |  |  |  |
| --- | --- | --- | --- |
| **NAME** | **SOURCE** | **SIGN & SYMPTOMS / TOXICITY** | **CHARACTERISTICS** |
| Arsenic | Different compounds of arsenic: Arsenic trioxide (As2O3) (White arsenic), chloride, sulphide (Orpiment), red sulphide (As2S3) (Realgar), copper acetoarsenite (Paris Green), copper arsenite (Schlee’s Green), arsenates.  Organic compounds of arsenic: Cacodylic acid, sodium pentaphenyl arsonate (Atoxyl), dioxy-diamino arseno-benzene dihydrochloride (Salvarsan) Silver arphenamine (Silver Salvarsan) etc.  Proprietory articles containing arsenic.  Rough on rats, fly paper, weed killer, fly water, fly powder. | All arsenicals inhibit  sulphydryl enzyme system necessary for cellular metabolism, symptoms of poisoning include faintness, depression, nausea, severe burning pain, constriction of throat, increased salivation and stomatitis. Severe thirst and projectile vomiting. Vomit may contain streaks of blood (distinction from cholera), urine is suppressed, skin becomes cold and clammy.  In chronic poisoning red  pigmentation (rain drop type) on skin occurs. | 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 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.  The organic arsenicals may contain arsenic in trivalent and pentavalent state. As an antidote BAL is used. |
| Antimony | Antimony occurs in the form of an oxide and sulphide and is present as an impurity in many mineral ores. Antimony is used in alloys, foil, type metal, plating batteries, Ceramics and pigments, safety matches and ant paste.  Inorganic compounds: Antimony potassium tartarate (tartar emetic), antimony trioxide, antimony trichloride (butter of antimony), antimony trisulphide (black antimony), antimony hydride (stibine).  Organic Compounds:  Preparations: Stibenyl, Stibamine, Urea stibamine, Heyden etc. | Symptoms are similar to  arsenic poisoning but appears sometime later than arsenic. The symptoms include a strong metallic taste followed by a burning sensation in the mouth and Oesophagus, Constriction of throat followed by nausea, incessant vomiting with pain in stomach and abdomen. Vomit may contain blood in latter stage. Respiration is laboured. Death occurs by cardiac failure | Antimony as a metal is not considered as poison but when inhaled in the form of vapour it is said to have produced dangerous symptoms. Poisoning by tartar emetic due to overdose may occur.  Accidental poisoning by antimony trichloride is known (used in arts as a bronzing liquid). As an antidote BAL is used. The mechanism of poisoning is similar with that of arsenic poison by combining with sulphydryl enzymes and thus interfering with cell metabolism. |
| 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, mercurochrome, organic mercurial.  Preparation: Neptal, thiomerin sodium, mercurophylline Mercurous mercury is not toxic. | 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 white coating. Hot burning pain mouth 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. | 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.  Antidote: BAL. |
| Copper | Inorganic Salts: Copper sulphate, carbonate, sub-acetate. | After swallowing an astringent taste is felt. The symptoms include metallic taste in the mouth, salivation, burning pain in stomach, nausea, repeated vomiting. Vomited matter is blue or green turns to deep blue on adding ammonia solution (distinction from bile). Oliguria, haematuria, al-buminuria, uremia may occur. Jaundice is common in severe cases. Cramps and convulsion also occur. | Skin may be yellow due to jaundice Greenish-blue forth may be coming out of mouth and nostril.  The contents of stomach is green or blue in colour.  Antidote: Calcium EDTA or BAL. |
| Lead | Inorganic Salts: Lead acetate, sub-acetate, nitrate, sulphate, chromate, chloride, monoxide. The find use in oil painting, calico printing, dye and pigment etc. | Sensation of burning, dryness of throat, salivation, intense thirst after swallowing the poison. Vomit may contain blood. Colicky pain. The other symptoms include nausea, headache, vertigo, cramps, convulsion, numbness followed by hemolysis and oliguria. | Chronic poisoning are marked by Colic, lead line on gum, anemia etc.  Antidote: Calcium EDTA. |
| Thallium | As salt :  Thallium acetate, sulphate | Joints pain in leg and feet, stomatitis, drowsiness, dryness of mouth, vomiting, diarrhoea, pain, pulmonary oedema, cyanosis and respiratory failure. | Poisoning is rare.  The falling of hair is the typical symptoms. Fusiform swelling of the root of hair occurs.  Antidote: Prussian blue, potassium iodide or chloride. |
| Zinc | As Salts:  Zinc chloride (soldering fluid), sulphate (wood preservative), oxide (pigment), phosphide (rodenticide) etc. | Metallic taste, salivation, vomiting, abdominal pain, purging convulsion, collapse and death. | Poisoning is rare.  With zinc chloride corrosive symptoms occur.  Phosphine is the active constituent, which is liberated by action of acid in body system when phosphide is consumed. |
| Bismuth | As salts:  Carbonate (used for treatment of diarrhoea), sub-nitrate (cosmetic). Organic salts of bismuth. Bismuth salicylate (dermatol), orphenamine sulphonate (bismarsen). | Metallic taste, salivation, pain in throat and abdomen, vomiting, purging, scanty urine collapse and death. | Poisoning is rare.  Bismuth subnitrate (used for X-ray examination)  In bismuth poisoning face is black or dark brown in colour.  Antidote : BAL. |
| Manganese | As salts:  Potassium permanganate (as bleaching agent in textile, abortificient, oxidant), dioxide (in dry cell). | Burning pain in the mouth and throat, abdomen, difficulty in swallowing, continuous vomiting, difficulty in breathing death occurs due to circulatory failure. | Poisoning is rare.  Tongue and pharynx are stained (black or dark brown)  Antidote: Calcium gluconate or edenate. |
| Chromium | As salts:  Potassium chromate (dye) dichromate (dye, furniture stainer etc.). | Bitter metallic taste, intense pain in stomach, giddiness, vomiting, diarrhoea, vomited matter is yellow, stools are yellow sometimes tinged with bile or blood. Respiration is slow and gasping. These are followed by muscular cramps, mental confusion, cyanosis and collapse. | In chronic poisoning ulcerated sores and eczematous dermatitis (chrome hole) occur. |
| Aluminium | Salts:  Alum – double salts of aluminium and potassium or ammonium (mordant, purification of water) Phosphide (uses as rodenticide). | Burning pain in the mouth, throat and stomach. Vomit is mixed with blood, dyspnoea, rapid pulse, loss of coordination, convulsion and death. | Phosphine is the active constituent, which is produced in the body system when phosphide consumed. |
| Barium | As salts :  Barium chloride nitrate(pyrotechnic), carbonate(rodenti cide) sulphate (for X-ray  examinaition of GI tract), sulphate (depilatory). | Severe abdominal pain, nausea metallic taste, salivation, vomiting, intense thirst, purging, dilation of pupils, dimness of vision, cramps, tremors anuria, convulsion, collapse and death due to respiratory failure. Salts act as cardiac poison and they also paralyse CNS. | Soluble salts are highly poisonous. Among the soluble salts, chlorides are most poisonous. Barium ions are also highly poisonous. Poisoning cases are accidental. |
| Cadmium | As metals: Soft, light white used in welding, electroplating manufacture of electrodes, control rods in atomic piles.  Salt: Cadmium sulphide (coloured glass, paints, plastic). | Increased salivation, nausea, severe vomiting, cramps in the abdomen, diarrohea, collapse but rarely death. | Poisoning may occur from the inhalation of Cadmium dust or fume.  Antidote : BAL. |
| Nickel | Nickel tetracarbonyl (mobile liquid). | Pulmonary oedema. | Poisoning is rare. |

**5.5.1.1 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.

**5.5.1.2 ARSENIC:**

**A. Chemical Test for Arsenic:**

1. **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 inches by 1/4 inch) is placed with one of its ends being fixed on the edge of 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 black deposit on the copper strip volatilizes 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 or 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.

2. **Gutzeit Test:**

The solution obtained from the Wet Digestion process is tested by this method. 1 ml. of the solution is taken into a Gutzeit apparatus, 2 pellets of pure zinc metal are put into it. 5 ml. of dil. sulphuric acid is poured over the contents. The evolved gas is purified by passing over lead acetate paper (to absorb H2S gas) and is reacted finally with mercuric chloride test paper. A yellow stain on the paper indicates the presence of arsenic.

3.  **Marsh’s Test:** (Quantitative)

Electrolytic Marsh Berzelius test is done over conventional zinc-sulphuric acid method for the evolution of nascent hydrogen as these reagents are often contaminated with arsenic etc. Scanty materials like burnt bones, hair and nail peelings containing minute traces of arsenic and for testing the feeble traces of arsenic present as a natural constituent in tissues, the Marsh’s test appears to be the only reliable technique available.

The test is performed in the solution obtained from the wet digestion process. The solution containing arsenic in the pentavalent state is reduced to trivalent state by boiling with pyrogallol solution and sulphurated water. One ml of the test solution is taken into a porcelain basin, mixed with 2 to 3 drops of 0.5% pyrogallol solution and 1.0 ml of saturated sulphurated water (water saturated with SO2) and boiled for 30 minutes. The pentavalent arsenic is reduced to the trivalent state.

A blank control test by taking dilute sulphuric acid in the cathode and anode chambers of the Marsh apparatus by passing a current of approx.4.5 A at 6 volts for 30 minutes. There should be no stain on the Marsh tube at this stage. The test solution containing arsenic in the trivalent stage is then poured into the apparatus and the process is repeated for 30 minutes. A mirror of black metallic lustre is observed at the cooler side of the Marsh tube, if arsenic is present. For quantitative estimation, the control mirrors of arsenic in the Marsh tube are prepared for 0.001, 0.002, 0.006 and 0.008 mg of arsenic trioxide. The mirror obtained with the test solution is compared with these controls and quantity of arsenic is calculated.

To confirm that the mirror deposited in the Marsh tube is only due to arsenic, both the ends of the tube are sealed after replacing hydrogen with air. The tube is then heated slowly on the flame of spirit lamp by keeping the narrower portion of the tube cooler. The mirror disappears and the octahedral crystal of arsenious trioxide gets deposited on the cooler narrower end of the tube which are identified under the high power of the microscope.

**4.**  **Group analysis**

The extracts prepared from the digested materials (dry or wet digestion of biological materials) are subjected to group analysis to detect arsenic by separation.

**1. Tests for Arsenic (V):**

The following tests may be performed with the extracts prepared from the digested materials (dry or wet digestion of biological materials).

1. **Test with Silver Nitrate Solution:**

To 2 ml. of the extract, silver nitrate solution is added. Brownish-red precipitate is formed (distinction from arsenite and phosphate which yield yellow precipitate). The precipitate is soluble in acids and ammonia solution but insoluble in acetic acid.

Alternatively, placing a few drops of test solution in a micro crucible may carry out the test. It is warmed after adding a few drops of concentrated ammonia solution and 10%(v/v) of hydrogen peroxide. It is acidified with acetic acid. On adding 2 drops of silver nitrate solution, the formation of a brownish red precipitate or colouration indicates the presence of As (V).

**B.** **Ammonium Molybdate test:**

To 2 ml. of extract, ammonium molybdate and nitric acid are added in considerable excess. A yellow crystalline precipitate of ammonium arsenomolybdate is obtained on boiling (distinction from arsenites, which give no precipitate, and from phosphates, which yield a precipitate in the cold or upon gentle warming). The precipitate is insoluble in nitric acid but dissolves in ammonia solution and in solutions of caustic alkalis.

**2 Tests for As (III):**

The tests with the extracts may also be performed as in the case of As (V) as stated hereunder. It may be mentioned that As (III) compound are widely used for poisoning.

**A.** Test with Silver Nitrate Solution:

Yellow precipitate of silver arsenite is formed in neutral solution (distinction from arsenate).

**B**. Test with Stannous Chloride and Concentrated Hydrochloric Acid (Bettendorf’s Test):

A drop of test solution is mixed up with 1-2 drops of concentrated ammonia solution in a micro crucible. To this, 2 drops of 10%(v/v) hydrogen peroxide and 2 drops of magnesium sulphate solution are added. It is evaporated slowly and finally heated until fuming ceases. The residue is treated with 1-2 drops of a solution of stannous chloride in concentrated hydrochloric acid and warmed slightly. A brown or black precipitate or colouration is obtained. By this test, arsenic can be detected in presence of antimony.

**5. Test for Organic Arsenicals:**

To 2 ml. of the extract in a test tube, 3 ml. of reagent (stannous chloride + concentrated hydrochloric acid) is added and warmed. A lemon yellow precipitate or colour is observed.

**B. Quantitation:**

**1. Estimation of Arsenic in blood by UV Spectroscopy:**

**Materials:**

**a. Standard Arsenic Solution:**

2.4196 gms. of arsenic trichloride is dissolved in sufficient 1 N hydrochloric acid to produce 1000 ml. This solution contains 1 mg. of arsenic per ml . This is diluted serially with water to produce solutions containing 0.5, 2.0, 5.0 and 10.0 μg of arsenic in 1 ml.

**b. Silver Diethyl dithio carbamate Solution:**

A 0.5% solution of silver diethyldithio carbamate in pyridine is prepared. This reagent should be stored in amber bottles.

**c. Stannous Chloride Reagent (40%):**

40 gms. of stannous chloride dihydrate is dissolved in sufficient hydrochloric acid to produce 100 ml.

**d. Digestion Mixture:**

A mixture containing 3 volumes of nitric acid, 1 volume of sulphuric acid and 1 volume of perchloric acid is prepared.

**Method :**

**1. Sample Preparation:**

5 ml. of blood sample is placed in a 125 ml. flask. 5 ml. of the digestion mixture is added. It is heated gently at first, then at about 150oC. When the solution boils and begins to char, 2 ml. of nitric acid is added. 1 ml. of nitric acid and a few drops of perchloric acid are added continuously until a clear straw coloured solution is obtained. The temperature is maintained until white fumes of sulphur trioxide are evolved and the solution is free from nitric acid. It is cooled. The contents of the flask is transferred quantitatively to a 10 ml. volumetric flask and diluted to volume with water.

**2.** 3 ml. of digested sample is transferred to the arsenic-generating vessel. Sufficient water is added to produce 35 ml. 5 ml. of hydrochloric acid, 2 ml. of a 15% solution of potassium iodide and 0.5 ml. of stannous chloride reagent (40%) are added. The solution is swirled and allowed to stand for 15 minutes. A pad of glass wool moistened with lead acetate solution is inserted into the lower tube of the generating vessel. 3 ml. of silver diethyl dithio carbamate solution is introduced into the absorber tube. 3 g. of granulated zinc is added to the flask. The two parts of the apparatus are assembled. The evolution of arsenic for 1 hour is allowed. Silver diethyl diethyldithio carbamate solution is transferred from the absorbed tube to a 1 cm cell and the absorbance is measured at 540 nm using in the reference cell a blank prepared by treating 5 ml. of water in the same manner.

**3.** The procedure as above is repeated using 5 ml. each of diluted standard solutions. The absorbance of each of the solutions is measured.

**4.** The concentration of arsenic in the sample of blood is determined from the curve obtained by plotting the absorbance of each of standard solutions against the concentration of arsenic.

**2. Determination of Arsenic in urine by atomic absorption spectrophotometry:**

In the method the arsenic generator (as stated in 5.9.1) is connected to atomic absorption spectrophotometer.

**Materials:**

1. **Standard Arsenic Solution:**

As described earlier in 5.9.1.

2. **Stannous Chloride Reagent (20%):**

20 gms. of stannous chloride dihydrate is dissolved in sufficient hydrochloric acid to produce 100 ml.

**Method 1:**

To 25 ml. of urine sample 1 ml. of a 20% solution of potassium iodide and 0.5 ml. of 20% stannous chloride reagent are added, mixed and allowed to stand for 20 minutes. The arsenic generator is isolated from the spectrophotometer and 100 ml. of the sample solution is introduced into the vessel. 0.5 gm of granulated zinc and 1 ml. of hydrochloric acid are added. The two parts of the generator are assembled immediately and stirred for 2 minutes with a magnetic stirrer. The arsenic generator is now connected to the spectrophotometer and the absorbance is recorded at 193.7 nm.

The above procedure is repeated using 25 ml. of each of the diluted standard solutions and 25 ml. of water (blank).

The absorbance of each of standard solution of arsenic is plotted against the concentration of arsenic and the concentration in the unknown sample is known thereafter from the calibration curve (non-linear).

**5.5.1.3 ALUMINIUM**

**A. Chemical Tests for Aluminium:**

**1. Test with Aluminon Reagent (a Saturated solution of Ammonium salt of Aurine tricarboxylic acid):**

To 1 ml. of extract, ammonia solution is added. White gelatinous precipitate appears. The precipitate is taken up in micro test tube. 2M hydrochloric acid is added to dissolve this. 1 ml. of 10 M ammonium acetate and 2 ml. of 0.1 percent aqueous solution of the reagent are added. It is shaken and allowed to stand for 5 minutes. Ammonical ammonium acetate solution is added in excess to decolorize the excess dyestuff and prevent interference of chromium, silica etc. A bright red precipitate or colouration persisting in alkaline solution is obtained.

**2. Test with Alizarin S (0.1% aqueous solution of sodium alizarin sulphonate) reagent:**

1 drop of the extract (which has been treated with just sufficient 1M sodium hydroxide solution to give the tetrahydroxoaluminate ion) is placed on a spot plate. 1 drop of reagent is added. Then acetic acid is added drop-wise until the violet colour just disappears. 1 drop of acid is added in excess when a red precipitate or colouration is observed.

**B. Quantitation**

**1. Determination of aluminium in viscera with 8-hydroxyquinoline**:

**Procedure:**

25 ml. of the extract (obtained from acid digestion) is taken in a 500 ml. beaker. 1 ml. of concentrated hydrochloric acid is added and stirred. It is diluted to 200 ml. 5-6 ml. of oxime reagent (a 10 percent solution in 20 percent acetic acid) and 5 gm. of is added. The beaker is covered with a clock glass and heated on an electric hot plate at 95oC for 2.5 hours. The precipitation is complete when the supernatant liquid, originally greenish yellow, acquires a pale orange-yellow colour. The precipitate is compact and can be filtered easily. It is allowed to cool and the precipitate is filtered through a sintered glass-filtering crucible (porosity No. 3 or No.4). The precipitate is washed with a little hot water and finally with cold water. It is dried at 130oC. The precipitate is weighed as

Al (C9H6ON)3.

**2. Determination of Aluminium in viscera by spectrophotometry**:

**Method:**

a. **Solochrome Cyanine R Solution:**

0.1 gm. of the solid reagent is dissolved in water and diluted to 100 ml. and filtered through a whatman No.541 filter paper, if necessary. This solution should be prepared daily.

b. **Standard Aluminium Solution:**

1.3192 gms. of A. R aluminium potassium sulphate is dissolved in water and diluted to 1000 ml. 1 ml. of this solution ≡ 75 μg. of aluminium.

c. **Buffer Solution (Concentrated):**

27.5 gms. of ammonium acetate and 11.0 gms. of hydrated sodium acetate are dissolved in 100 ml. of water. 1.0 ml. of glacial acetic acid is added and mixed.

d. **Buffer Solution (Dilute):**

To one volume of concentrated buffer solution, 5 volume of water is added and the PH is adjusted to 6.1 by adding acetic acid or sodium hydroxide solution.

**Procedure:**

1. 20 ml. of aliquot is transferred to a 250 ml. beaker. 5 ml. of 5 volume hydrogen peroxide is added and mixed well. The PH of the solution is adjusted to 6 (using either 0.2M sodium hydroxide or 0.2M hydrochloric acid). 5 ml. of Solochrome Cyanine R. solution is added and mixed. 50 ml. of the dilute buffer solution is introduced and diluted to 100 ml. in a volumetric flask. The optical density is measured after 30 minutes with a spectrophotometer at 535 mμ against a reagent blank in a 5 mm. cell.

2. The procedure as above is followed to measure optical density of standard solution of aluminium for construction of calibration curve using 0, 1, 2, 3, 4 and 5 ml. of standard aluminium solution.

3. The amount of aluminium in the unknown sample is measured using the calibration curve.

**5.5.1.4 ANTIMONY**

**A. Chemical Tests for Antimony:**

1. **Reinsch Test:**

The test is performed as stated in case of arsenic. A bluish black deposit on the copper strip indicates the presence of antimony. The stained strip after necessary processing as stated in case of arsenic is heated in the Reinsch tube and the sublimate produced in observed under microscope. Characteristic needle shaped crystals of Sb2O3 are observed.

1. **Micro Test with Stained Copper Strip in Reinsch Test:**

A portion of the stained copper strip obtained from the Reinsch Test is taken in a porcelain tile. The black stain is dissolved by the action of a few drops of dilute hydrochloric acid. The solution thus obtained is spotted on a piece of filter paper. The paper is dried and exposed to hydrogen sulphide. An orange spot indicates the presence of antimony.

1. **Marsh Test:**

The procedure as stated in case of arsenic is followed. The mirror formed on sublimation does not gives the octahedral crystal (distinction from arsenic).

1. **Group Analysis:**

Group analysis is done with the extracts prepared from the digested material (dry or wet digestion of biological materials or blood) to detect antimony by group separation.

1. **Rhodamine B Reagent:**

1 ml. of the extract is taken and made strongly acidic with hydrochloric acid. A little of solid sodium nitrite is added. 1 ml. of 0.01% aqueous solution of Rhodamine B is then added. The bright red colour of the reagent changes to blue.

1. **Phosphomolybdic Reagent:**

1ml of the extract is taken in a semi-micro test tube. 0.5-1 ml. of phosphomolybdic acid reagent is added and heated for a short time. The reagent is reduced to a blue compound, which can be extracted with amyl alcohol.

**5.5.1.5 BARIUM**

**A. Chemical Tests:**

Residue obtained by dry digestion is dissolved in 2 ml of conc. hydrochloric acid, boiled and filtered.

(1) **Flame Test**: A persistence apple green flame is observed.

(2) A portion of the acidified solution is boiled with a few drops of conc. nitric acid, made alkaline with ammonium chloride and ammonium hydroxide solutions, filtered, if necessary and to the clear solution is added an excess of ammonium carbonate solution.- A white precipitate which dissolve in acetic acid indicates the presence of barium.

(3) The precipitate dissolved in acetic acid as obtained in test (2) is divided into three portions.

1. The one portion is added dilute sulphuric acid, which gives a white precipitate of barium sulphate insoluble in con. nitric and hydrochloric acids.
2. To another portion is added a few drops of potassium chromate solution, which produces a yellow precipitate of barium chromate. It is insoluble in dilute acetic acid (distinction from strontium and calcium) but readily soluble in mineral acids.
3. The third portion is evaporated to dryness. The residue dissolved in water. A drop is spotted on a piece of filter paper, dried and to it is poured a drop of the solution of sodium rhodizonate. A brown or reddish brown coloured spot indicates the presence of barium which is insoluble in dilute hydrochloric acid (distinction from strontium). The above test may also be performed on drop-reaction paper.

**B. Quantitation:**

**1. Determination of Barium in viscera**:

**Method:**

100 ml. of the extract (prepared from acid digested material of visceral matter of known weight with a mixture of concentrated nitric acid and concentrated sulphuric acid as described in section 3 of this monograph) is taken in a beaker. After adding a slight excess of hot 1 N sulphuric acid it is heated to boiling slowly and with constant stirring. It is then digested on the steam bath until the precipitate has settled. It is filtered through No. 4 vitreosol filtering crucible and washed with a little water until the acid is removed. (8 or 10 washings are usually necessary). The crucible is dried and heated in an air over at 100- 110oC and then ignited in a muffle furnace for 15 minutes until constant weight is obtained. The percentage of barium is calculated in terms of BaSO4.

**5.5.1.6. BISMUTH**

**Chemical Tests for Bismuth:**

**1. Reinsch Test:**

A gray deposit on the copper strip, which does not sublime on heating in a Reinsch tube, indicates the presence of bismuth.

**2. Micro Test:**

A portion of the stained copper strip from the Reinsch test is taken in a spotted tile. The deposit is dissolved in nitric acid. The solution is evaporated to dryness in two portions. To one portion is added one drop of potassium iodide solution followed by a drop of acidified aqueous cinchonine solution. The formation of orange colour indicates bismuth.

3. The extract obtained after acid digestion is subjected to systematic group analysis for the presence of bismuth.

**5.5.1.7. CADMIUM**

1. **Chemical Tests for Cadmium**

**1. Test with Hydrogen Sulphide:**

Hydrogen sulphide gas is passed through the acidic solution of the extract (0.3N with respect to hydrochloric acid). It gives a yellow precipitate of cadmium sulphide which is soluble in hydrochloric acid, but insoluble in ammonia.

**2. Test with Potassium Cyanide:**

To 1 ml. of extract, potassium cyanide solution is added drop by drop. A white precipitate is formed which dissolves on adding excess of reagent. Hydrogen gas is passed through it when a yellow precipitate is formed.

**3. Test with Dinitro-P-Diphenyl Carbazide (0.1% solution in ethanol):** (1)

1 drop of acidic or neutral or ammoniacal extract is placed on a spot plate. 1 drop of sodium hydroxide (2M) and 1 drop of potassium cyanide solution (10%) are added. Then 1 drop of reagent and 2 drops of formaldehyde solution (40%) are added. A brown precipitate is formed which very rapidly becomes greenish blue.

Test with 4-Nitronapthalene – diazoamino azo-benzene (Cadion 2B)(the reagent is prepared by dissolving 0.02 g of Cadion 2B in 100 ml. of ethanol to which 1 ml. of 2 M potassium hydroxide is added).

1 drop of the reagent is placed on a drop reaction paper. 1 drop of extract (which should be slightly acidified with 2M acetic acid containing a little sodium potassium tartarate). Then, 1 drop of 2M potassium hydroxide solution is added. A bright-pink spot surrounded by a blue circle is produced.

**B. Quantitation**

**Determination of Cadmium in blood by atomic absorption spectrophotometry: (**2)

The following atomic absorption spectrophotometric technique is applicable to blood and involves atomization in a graphite furnace assembly. Blood sample should be anti-coagulated with di-potassium edetate. If heparin is used, a blank containing heparin of the same batch should be carried through the preparative and assay procedure.

**Materials:**

1. **Standard Cadmium Solution:**

2.7442 gms. of cadmium nitrate [Cd(NO3)2, 4H2O] is dissolved in sufficient 1 M nitric acid to produce 1000 ml. The solution contains 1 mg. of cadmium in 1 ml. It is serially diluted with a 0.05% solution of nitric acid to produce solutions containing 0.0005, 0.001, 0.002, 0.004, 0.006, 0.008 and 0.01 μg./ml.

**Method:**

1. To 100 μl of the sample of whole blood 100 μl of a 0.05% solution of nitric acid and 100 μl of a 1% solution of ammonium hydrogen phosphate are added and mixed thoroughly. 20 μl. of the mixture is introduced into the graphite furnace. The drying, ashing and atomization are made. The absorbance is recorded at 228.8 nm.

2. The procedure is repeated using in place of sample, solutions prepared by adding 100 μl of each of the diluted standard solutions to 100 μl quantities of pooled normal blood.

3. The absorbance of each of standard solutions is plotted against the concentrations of cadmium. The intercept is reduced to zero by drawing a parallel line through the origin. The concentration of cadmium in the sample is read off from the calibration curve. The should be linear in the range 0 to 0.01μg./ml.

**5.5.1.8 CHROMIUM**

**Chemical tests for chromium**:

The tests mentioned hereunder are applicable for Cr3+. Chromate and dichromate containing Cr6+ have been covered in the tests for anions.

1. **Test with Diphenylcarbazide (1% solution in alcohol):**

To 1 drop of acidified solution of extract on a spot plate, 2 drops of 0.1M peroxy disulphate solution and 1 drop of 0.1M silver nitrate solution are added. It is allowed to stand for 2-3 minutes. 1 drop of the diphenyl carbazide reagent is added. A violet or red colour is formed.

2. **Test with Chromotropic Acid:**

1 drop of extract is placed in a semi-micro test tube. 1 drop of chromotropic acid solution (saturated solution of chromotropic acid in water) and 1 drop of dilute nitric acid (1 : 1) are added. A red colouration is observed.

**5.5.1.9 COPPER**

**A. Chemical tests for copper:**

The analysis of copper is done in the residue obtained after incineration of the organic material in the muffle furnace. In the presence of copper, the dry residue generally assumes a greenish blue tinge. The residue is dissolved in 5 ml of conc. hydrochloric acid boiled and filtered. The presence of copper is established by performing the following tests in the clear filtrate. 5 ml of the acidic solution is diluted with 2 ml of water, warmed and then to it hydrogen sulphide gas is passed. A brownish black precipitate is obtained. The precipitate is filtered through Whatman filter paper, washed thrice with boiling water and then dissolved in 2 ml of nitric acid. The nitric acid is removed by evaporation and the residue is taken in 1.0 ml of water. This solution is subjected to the following tests.

(1) Two drops of the aqueous solution are taken in a spotted tile and to it is added a few drops of ammonium hydroxide- A blue colour is obtained.

(2) The blue coloured solution obtained in the above test (i) as above is made acidic by adding a few drops of acetic acid and then a few drops of potassium ferrocyanide solution is added to it. A chocolate brown colour is obtained.

(ii) **Spot Test (Feigl’s test**): 2 drops of the aqueous solution is mixed with two drops of dilute zinc nitrate solution and 2 drops of mercury-ammonium thiocyanate reagent. Pink to violet coloured precipitate is obtained.

3) **Test with Rubenic acid**: (7)

The aqueous solution is spotted on a piece of Whatman No.1 filter paper, dried, sprayed with rubeanic acid and then exposed to ammonia vapour. An olive-green coloured spot is obtained.

Limit of identification: 0.006 ug copper

4) **Test with 2.2’-Diquinolyl (Cuproin): (**3, 4)

A drop of the test solution (pH above 3) is treated on a spot plate with several crystals of hydroxylamine hydrochloride and 3 drops of a saturated ethanol solution of cuproin. A purple to pink colour develops according to the amount of copper present.

Limit of identification: 0.05 g of copper.

Limit of dilution: 1:1000,000

5) **Test with Benzoinoxime:** (5)

A drop of the weakly acidic test solution is treated on filter paper with a drop of a 5% alcoholic solution of benzoinoxime and held over ammonia. A green colouration indicates copper.

6) **Test with Ammonium Thiocyanate and o-Tolidine or p-Phenylinediamine**: (6)

A drop of the reagent solution is placed on filter paper, followed by a drop of the neutral or slightly acidic test solution. A light or dark blue stain is formed according to the amount of copper present.

Reagent solution: A solution of 0.1g of o-tolidine and 0.5g of ammonium thiocyanate is in 5 ml of acetone.

Limit of identification: 0.003-µg copper (in 0.015 ml)

Interference: Salts of silver, mercury, iron, cerium, gold and thallium

7) **Test with Rubeanic Acid:**

The aqueous solution is spotted on a piece of No.1 filter paper, dried,

sprayed with rubeanic acid and then exposed to ammonia vapour. An

olive-green coloured spot is obtained.

Limit of identification: 0.006µg copper

8) **Group separation**

By systematic group analysis with extract

1. **Thin Layer Chromatography**

**Method:** A portion of aqueous solution obtained by dry ashing method is spotted on a thin layer plate of silica gel G (thickness 0.2mm) along with a control solution of aqueous copper sulfate (0.001%, w/v) which is then developed to a distance of 10cm with methanol-acetic acid (100:0.3, v/v) in previously saturated TLC chamber. After development, the plate is dried in air and sprayed with alkaline 2-thiobarbituric acid reagent. Bluish green spots appear immediately having hRf as 60.

**Preparation of Alkaline 2-Thiobarbituric Acid Reagent**

It is prepared by dissolving 2-thiobarbituric acid in an aqueous solution of anhydrous sodium carbonate (0.05% w/v). (8).

**B**. **Quantitation**

**1. Determination of Copper in biological materials by iodometry:**

20 gms. of the tissues are incinerated in a muffle furnace. The residue thus obtained is dissolved concentrated hydrochloric acid (10 ml.) and filtered. The residue is washed twice with 10 ml. portions of distilled water. The filtrate is collected and mixed with hydrochloric acid solution. 2 ml. of nitric acid is added and heated just to dryness. The residue is dissolved in 10 ml. of distilled water. 1 gm. of ammonium chloride is added to it followed by an excess of ammonium hydroxide solution and the precipitate is then filtered. The precipitate on the filter paper is washed twice with 5 ml. portions of distilled water and the washings are also mixed with the ammoniacal filtrate. It is then boiled to remove excess of ammonia, cooled and transferred into an iodine flask. 1 ml. of dilute acetic acid is added to it followed by 1 gm. of potassium iodide. The flask is then kept in a dark place for 5 minutes. The content of the flask is then diluted with 10 ml. water through washing of the mouth and stopper of the flask. The liberated iodine is titrated with 0.01 N sodium thiosulphate solution using starch-potassium iodide as indicator.

1 ml. of 0.01 N sodium thiosulphate ≡ 0.6354 mg. of copper

≡ 2.497 mg. of Copper Sulphate.

**2. Spectrophotometric determination of Copper in biological materials using diethyl dithio carbamate:**

10 gm. of tissue material is oxidized by wet digestion method using a mixture of concentrated nitric acid and concentrated sulphuric acid as described earlier (in section-3) to get the toxic metal liberated from organic matrices. 5 ml. a known volume of diluted extract is taken into a stoppered separating funnel. Ammonium hydroxide solution is added to neutralize the solution and then the solution is made alkaline by adding 1 ml. of 6.5 N ammonium hydroxide solution. To it 2 ml. of 10% ammonium citrate and 5 ml. of sodium salt of diethyl dithio-carbamate solution (by dissolving 0.175 gm. of the salt in 100 ml. of copper free distilled water) are added. 10 ml. of redistilled chloroform is added to the separating funnel and shaken for 10 minutes. The two layers appear on settling the separating funnel for some time. The chloroform layer is separated, quickly passed through No. 1 filter paper containing anhydrous sodium sulphate and the absorbance of the chloroform layer is measured at 435 mμ (this is the λmax value obtained on scanning copper-dithio-carbamate complex from 400-700 mμ )

The above procedure is separated for the blank as well as different standard solutions of copper. The absorbance of each of the standard solutions of copper is plotted against the concentration of copper and a calibration curve is prepared. From the curve itself, the concentration of copper in unknown sample is determined.

**3. Determination of Copper in serum and viscera by Atomic Absorption Spectrophotometry:** (9)

**Materials:**

**Standard Copper Solution:**

1 gm. of copper foil is dissolved in a minimum volume of a 50% solution of nitric acid and sufficient of a 1% solution of nitric acid is added to produce 1000 ml. This solution contains 1 mg. of copper in 1 ml. It is diluted serially with water to produce solutions containing 0.5, 1.0, 1.5 and 2.0 μg of copper in 1 ml. It is serially diluted with sodium chloride-potassium chloride solution to produce standard solutions containing 0.5, 1.5 and 2 μg of copper in 1 ml.

**Method:**

1 ml. of the serum sample 1 ml. of concentrated extract obtained from the digested mixture (wet digestion of viscera materials by a mixture of concentrated nitric acid and concentrated sulphuric acid) is taken and diluted with sufficient of a 6% solution of butan-1-01 to produce 10 ml., mixed and aspirated into an oxidizing (blue flame of a mixture of air and acetylene) flame and the absorbance at 324.7 nm. is recorded.

The above procedure is separated with 1 ml. each of the diluted standard solutions and with 1 ml. of sodium chloride – potassium chloride solution (blank).

The absorbance of 1 each of the standard solutions is plotted against the concentration of copper and a calibration curve is prepared (which may be non linear). From the calibration curve, the concentration of copper in unknown sample is determined.

* + - 1. **. MANGANESE**

**Chemical tests for Manganese:**

The tests mentioned hereunder are applicable for Mn2+ ions. Mn7+or Mn6+ has been covered in the tests for anions (permanganate or manganate).

1. **Test with Sodium Bismuthate**:

A drop of extract is placed on a spot plate. 1 drop of concentrated nitric acid and a little sodium bismuthate are added. The purple colour of permanganic acid appears.

2. **Test with Ammonium Peroxydisulphate**:

To 5 drops of extract in a micro crucible, 1 drop of 0.1N silver nitrate solution is added and stirred. A few mg of solid ammonium peroxodisulphate is added and heated gently. The characteristic colour of permanganate appears.

3. **Test with Sodium Phosphate**:

To 0.5 ml. of extract a few drops of sodium phosphate is added. A pink precipitate appears.

**5.5.1.11. MERCURY**

**A. Chemical tests for Mercury:**

(1) **Reinsch Test**:

A silvery shining deposits 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 deposits on the cooler side is viewed under a microscope. Shining round globules of metallic mercury are observed.

2) **Micro Test:**

A portion of the stained copper strip from Reinsch test is taken into a spotted tile. Few drops of conc. nitric acid is added to dissolve the stain on the copper strip. After evaporation, the residue is taken in dilute hydrochloric acid and spotted on a Silica gel G plate (of thickness 0.2mm). The presence of mercury is established by spraying the chromatogram with dithizone.

3) **Test with Diphenylcarbazone**: (10, 11).

A drop of the test solution is placed on a filter paper impregnated with a freshly prepared 1% alcoholic solution of diphenylcabazone. According to the concentration of the mercury, a violet or blue fleck appears.

Limit of identification: 0.1% mercury (with added nitric acid)

Limit of dilution: 1:500,000

4) **Test with Cuprous Iodide:** (12)

One drop of potassium iodide-sodium sulfite solution is placed on a spot plate or a filter paper followed by a drop of copper sulphate solution and finally by means of capillary, a drop of test solution is added. Depending on the quantity, a red or orange colour appears.

Limit of identification: 0.-3 µg of mercury

**Reagents:**

1. Potassium Iodide-Sodium Sulfite Solution:- Dissolve 5 g KI and 20g Na2SO3 7H2O in 100 ml of water.

2) Copper Sulfate Solution: - 5 g CuSO4 5H2O in 100 ml 1N of HCl

5) **Test with Stannous Chloride and Aniline**: (13)

A drop of test solution is placed on filter paper followed by a drop of freshly prepared stannous chloride solution and a drop of aniline. A black to brown colour indicates mercury.

Limit of identification: 1 µg mercury

Limit of dilution: 1:50,000

6) **Group analysis**

The extract obtained after acid digestion is subjected to systematic group analysis by passing H2S gas at 0.3N acidity with respect to HCl. A black precipitate is obtained. The precipitate is insoluble in hot dilute nitric acid, alkali hydroxide but soluble in sodium sulphide.

To the extract as above stannous chloride solution is added in moderate amount. The appearance of silky white precipitate indicates the presence of mercury.

To a few drops of test solution on a spot plate, a small crystal of ammonium thiocyanate is added followed by a little solid cobalt acetate. A blue colour is produced in the presence of mercuric ion.

**B. Quantitation**

1. **Determination of Mercury in biological materials by spectrophotometry:**

**Method:**

**1. Sample Preparation:**

6 gms. of the visceral material is taken into a standard joint 250 ml. round bottom flask. 2.5 ml. of concentrated nitric acid and 2.5 ml. of concentrated sulphuric acid are added to it. It is fitted with a revenue type condenser (ice-cold water circulating through it). The flask is first warmed and then heated gently till foaming ceases. The mixture is boiled gently thereafter for about 4 hours. The flask is then cooled and put in an ice bath. To this mixture 0.05 gms. of potassium permanganate is added and reheated with the same refluxing arrangement. The heating is continued till the permanganate colour is discharged. The addition of 0.5 gm. portions of potassium permanganate and the heating were repeated till at the end permanganate colour persisted even after prolonged heating. The flask fitted with the condenser is cooled and then put in an ice bath and 1 drop of 50% hydroxylamine hydrochloride solution in water is added to discharge the permanganate colour. The condenser is refitted and from the tip of condenser distilled water is added for washing. The mixture is further cooled by keeping the flask for sometime more in ice-bath. The condenser is then removed and contents of flask is filtered through glass wool. The filtrate is collected in a 50 ml. measuring flask. The flask is then washed free of sulphate with distilled water and the glass wool is washed with 1(N) sulphuric acid. The volume of the flask is made with distilled water. The extract thus prepared is taken into a separating funnel. 5 ml. of redistilled chloroform is added. It is shaken for 2 minutes (chloroform layer is discarded). 10 ml. of ice-cold dithizone solution (prepared by dissolving 35 mg. of dithizone in 500 ml. of acid washed redistilled chloroform and then diluting 1 ml. of this solution with 9 ml. of chloroform) is added to the separating funnel and shaken for 5 minutes. The chloroform layer is separated as much as possible and quickly passed through anhydrous sodium sulphate. The absorbance of the chloroform part is read in spectrophotometer at 600 mμ wavelength (A1).

**2.** 5 ml. of this chloroform part is taken into a separating funnel and to it is added 5 ml. of iodine solution (prepared by dissolving 6 gms. of potassium iodide and 2 gms. of potassium hydrogen phthalate in 100 ml. of water. It is shaken for 2 minutes and then the chloroform layer is filtered through anhydrous sodium sulphate). The absorbance of this chloroform solution is again read in spectrophotometer at 600 mμ (A2).

**3.** The difference in the absorbance between A1 and A2 is calculated. The amount of mercury is calculated from the calibration curve prepared by plotting known amounts of mercury against the difference of two absorbance reading as described above.

1. **Determination of Mercury in urine and viscera by cold vapour atomic absorption spectrophotometry:**

Flame atomization is not necessary for the atomic absorption spectrophotometry of mercury. The cold vapour technique described here required a reduction vessel to produce mercury vapour. The vapour is led to a quartz absorption cell within the atomic absorption spectrophotometer. The method described hereunder is applicable to inorganic and organic mercurial compounds.

**Materials:**

1. **Standard Mercury Solutions:**

0.1353 gm of mercuric chloride is dissolved in sufficient 0.1 M hydrochloric acid to produce 100 ml. This solution contains 1 mg. of Hg per ml. This is serially diluted to produce standard solutions of mercury containing 0.5, 0.10 and 0.15μg. of mercury in 1 ml.

2. **Stannous Chloride Solutions:**

A 5% solution of stannous chloride dihydrate in a 25% solution of hydrochloric

acid.

3. **Hydrogen Peroxide Solution (50 volume):**

Strong hydrogen peroxide solution is diluted with equal volume of water.

**Method:**

5 ml. of urine is transferred into a 35 ml. stoppered tube. 1 ml. of hydrochloric acid is mixed and allowed to cool. 10 ml. of a 5% solution of potassium permanganate is added and mixed. This is kept loosely stoppered overnight. The excess of permanganate is removed by adding dropwise hydrogen peroxide solution (50 volume). 0.5 ml. of the stannous chloride solution is transferred to the reduction vessel. The magnetic stirrer and the argon gas flow are turned on and the baseline absorbance is recorded at 253.7 nm. 3 ml. of the prepared extract (from urine) or 5 ml. of the prepared extract by acid digestion of viscera as stated in 5.96 is added and the reduction vessel is closed. It is stirred for exactly 2 minutes and the absorbance at 253.3 nm is recorded in respect of urine and viscera samples.

1. The above procedure is repeated using 5 ml. of each of the diluted standard solutions and 5 ml. of water (blank). The absorbance at 253.7 nm. is recorded.
2. The absorbance of each of the standard solutions is plotted against the concentration of mercury. The concentration of mercury in urine or viscera is obtained from the calibration curve (the curve should be linear for concentrations in the range 0 to 0.1µg).
3. **Determination of Mercury in blood and urine by cold vapour atomic absorption spectrophotometry:**

In this method mercury is isolated by destruction of organic matrices and mercury present in solution is reduced to metallic mercury using sodium borohydride in sodium hydroxide in a reduction vessel. The vapour is led to the cell of the spectrophotometer for determination of concentration.

**Method for Urine Samples:**

1. 2 ml. of urine is mixed with 0.1 ml. of 35% (w/w) nitric acid, 0.2 ml. of 50% (w/w) sulphuric acid and 0.5 ml. of 5% (w/v) potassium permanganate. It is allowed to stand at room temperature for 15 minutes. If the colour of solution is changed from purple to brown then a further 0.5 ml. of the permanganate solution is added, mixed and allowed to stand for a further 15 minutes. This process of adding successive aliquots of permanganate solution and allowing the reaction to proceed is maintained until the purple colour is sustained.

With increasing masses of dissolved organic materials increasing volumes of permanganate solution will be required. After the appearance of permanganate colour to the solution, 0.4 ml. of 2.5% (w/v), potassium persulphate is added and mixed. It is incubated at 85-95oC for at least 90 minutes and allowed to cool. 0.5 ml. each of n-butanol (used to control foaming) and 5% (w/v) hydroxylamine hydrochloride in 3% (w/v) sodium chloride are added and mixed. The total volume is made up to10 ml. with water and mixed well prior to determination. 1 ml. of each of 0.6% sodium boro hydride in 0.6% sodium hydroxide and 18% (w/w) hydrochloric acid and two drop of secondary octyl alcohol are added in a reduction vessel. The magnetic stirrer and the argon gas flow are turned on and the baseline absorbance at 253.7 nm is recorded. 5.0 ml. of the sample solution prepared above is added and the reduction vessel is closed. It is stirred for 2-3 minutes and the absorbance at 253.3 nm is recorded i.e. for urine sample.

2. The above procedure is repeated using 5 ml. each of each of the diluted standard solutions and 5 ml. water (blank). The absorbance at 253.7 nm. is recorded.

3. The absorbance of each of the standard solutions is plotted against the concentration of mercury. The concentration of mercury in urine is plotted against the concentration of mercury. The concentration of mercury in urine is obtained from the calibration curve.

**Method for Blood Samples:**

1. 1 ml. of anti-coagulated whole blood is mixed with approximately 3 ml. of a 5:2:1 mixture of 70% (w/w) nitric acid, 70%(w/w) perchloric acid and 98% (w/w) sulphuric acid in a 10 ml. nominal volume graduated polyethylene test tube or 100 ml. of flask. The digestion mixture is warmed to 40oC for 60 minutes with frequent mixing until the brown fumes of oxides of nitrogen dissipate and the remaining liquid is golden yellow in colour. It is important to control the temperature so that boiling any stronger than could be described as effervescence is precluded. The volume is made up to 5 ml. with water. The solution is mixed thoroughly prior to determination.

2. 1 ml. of each of 0.6% sodium boro hydride in 0.6% sodium hydroxide and 18% (w/w) hydrochloric acid and two drops of secondary octyl alcohol are added in a reduction vessel. The magnetic stirrer and argon gas flow are turned on and the baseline absorbance at 253.7 nm is recorded. 5 ml. of sample solution prepared above is added and the reduction vessel is closed. It is stirred for 2-3 minutes and the absorbance at 253.3 nm is recorded i.e. for blood sample.

3. The absorbance of each of the standard solutions is plotted against the concentration of mercury. The concentration of mercury in blood is plotted against the concentration of mercury. The concentration of mercury in blood is obtained from the calibration curve.

**5.5.1.12 LEAD**

**A. Chemical tests for Lead:**

Lead may be precipitated as lead sulphate during digestion of biological material with a mixture of concentrated nitric acid and concentrated sulphuric acid. The white precipitate of lead sulphate as the insoluble portion may be processed through treatment for insoluble residue under group analysis for detection of lead. However, a portion of lead will pass to the extract prepared from the digested mixture.

(1) To one ml of the test solution is added one ml of dilute hydrochloric acid. A white precipitate is obtained which dissolve on boiling and reappears on cooling.

(2) To one ml of the aqueous test solution is added one drop of dilute nitric acid and one ml of potassium iodide solution. A bright yellow precipitate is obtained. On boiling the content, the precipitate dissolve out and on cooling golden yellow spangles are obtained.

(3) A portion of the solution suspected to contain lead is spotted on a piece of filter paper and dried. To it is added one drop each of dilute aqueous pyridine, and very dilute solution of sodium bicarbonate followed by one drop of 0.1 percent gallocyanine. A violet coloured spot is observed.

(4) Test with Dithizone Solution (2-5 mg. in 100 ml chloroform or Proportionate amount):

1 ml. of extract (neutral or faintly alkaline) is taken in a micro test tube. A few crystals and then 2 drops of dithizone solution are added. It is shaken for 30 seconds. The green colour of the reagent changes to red.

**B. Quantitation**

**1. Determination of Lead in viscera by UV Spectrophotometric method:**

**Materials: Standard Lead Solutions:**

0.16 gms. of lead nitrate is dissolved in sufficient 0.1M nitric acid to produce 100 ml. This solution contains 1 mg. of lead in 1 ml. This is serially diluted to produce solutions containing 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 μg of lead in 1 ml.

**Method:**

i. 1.5 ml. of 10% ammonium acetate solution and 5 ml. of 10% ammonium citrate solution are added to 10 ml. of the total extract obtained after acid-digestion of visceral sample. The mixture is made alkaline by adding ammonium hydroxide solution (Sp. gravity – 0.888). 2.5 ml. of 10% potassium cyanide and 1 ml. of 10% hydroxylamine hydrochloride are added.

This is taken in a 250 ml. separating funnel and extracted with 20 ml. of 0.1% dithizone solution in chloroform by shaking for 2 minutes vigorously. The separating funnel is kept aside for 15 minutes to facilitate separation of two layers. The chloroform layer is separated and taken into another clean separating funnel. It is washed twice with 15 ml. portion of water by shaking each time for 15 seconds only. The aqueous layer is discarded. The chloroform layer which is now free from excess of dithizone is passed through a funnel containing a pad of anhydrous sodium sulphate on filter paper. The clear chloroform layer is collected. The absorption of the chloroform layer is measured at 525 mμ (which is the wavelength of maximum absorption for lead-dithizone complex – obtained by scanning in UV spectrophotometry in the range 200 – 700 nm).

ii. The procedure as above is repeated with 10 ml. of each of the standard solution of lead containing 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 μg of lead per ml. and also with 10 ml. of water (blank).

iii. The absorbance of each of the standard solution is plotted against the concentration of lead. The concentration of lead in viscera is obtained from the calibration curve.

**2. Determination of Lead in blood by flameless atomic absorption spectrophotometry:**

**Method:**

i 100 μl of the blood sample is transferred to a polystyrene test tube. 150 μl of water and 150 μl of 2 M nitric acid are added, mixed vigorously for 30 seconds, centrifuged. The supernatant liquid is transferred into a 1 ml. polyolefin cup. 10 μl of this solution is introduced into the graphite furnace. The absorbance at 217 nm. is measured after drying, ashing and atomizing the extract of ash.

ii. The above procedure is repeated using a series of polystyrene test tubes containing individually of 100 μl of the pooled normal blood and 150 μl of the each of the diluted standard solutions.

iii. The absorbance of each standard solution is plotted against the concentration of lead. The intercept is reduced to zero by drawing a parallel line through the origin. The concentration of lead in blood sample is read off.

**3. Flame Atomization Assay for Lead in blood:** (14)

Standard lead solution: Dissolve 0.16g of lead nitrate in sufficient 0.1M nitric acid to produce 100ml. This solutions contains 1mg of Pb per ml. Dilutions can be made by diluting this solutions with water to produce solutions containing 0.1, 0.2, 0.4, 0.6, 0.8 1.0μg of Pb per ml.

**Method:**

The blood sample, and a quantity of pooled normal blood to be used for preparing the standards should be agitated for at least 30 minutes immediately before transferring to the vessel for atomization.

Transfer 10μl of the blood sample to a 10x5 mm nickel crucible (Delves cup), add 10μl of water, and heat at 1500c for 1minute or until sputtering occurs. Add 20μl of strong hydrogen peroxide solution, and evaporate to dryness at 1500c. Place the cup in the air-acetylene flame and under the nickel absorption tube, and record the absorbance at 217nm. The correct positioning of the cup is crucial.

Repeat the procedure using a series of nickel crucible containing individually 10μl of the pooled normal blood and 10μl of each of diluted standard solution.

Plot the absorbance of each standard solution against the concentration of lead, reduce the intercept to zero by drawing a parallel line through the origin and read off the concentration in the sample.

* + - 1. **NICKEL**

**Chemical tests for Nickel:**

1. **Test with** α**-Nitroso** β**-Napthol (1% solution in ethanol):**

1 drop of faintly acidic solution of the extract is placed on drop reaction paper. 1 drop of reagent is added. A brown precipitate is formed which is soluble in hydrochloric acid.

2. **Test with Dimethyl Glyoxime Reagent (1% solution in ethanol)**:

1 drop of the extract is placed on a drop reaction paper. 1 drop of reagent is added. The paper is hold over ammonia vapour. Alternatively, 1 drop of extract is placed on a spot plate. A drop of reagent and 1 drop of dilute ammonia solution are added. A red spot (in case of test paper) or precipitate (or colouration) is produced.

* + - 1. **THALLIUM**

**A. Chemical tests for Thallium:**

**Sample Preparation from Biological Materials**.

The finely mined visceral materials (10-15 gms.) are made into a fine slurry with arsenic free concentrated hydrochloric acid in a flask. The mixture is heated on a boiling water bath, perchloric acid is added in 0.5 ml. portions within 5 minutes to oxidize the organic matter. (The volume of perchloric acid should not exceed 0.5 ml. to avoid explosion). After the organic matter is dissolved; the solution is cooled in an ice bath and filtered. This is concentrated when it becomes dark. The concentrated extract thus obtained is transferred to a separating funnel and chlorine water is added in excess. The solution is extracted with 50 ml. portion of diethyl ether twice (The aqueous part is rejected). The ethereal extract is evaporated to dryness. The residue is taken up in 15 ml. of distilled water followed by addition of a few drops of concentrated hydrochloric acid and 2 ml. of concentrated sulphuric acid. The solution is heated till the evaporation of sulphuric acid starts. It is cooled. To the solution 30 ml. of ammonium chloride solution(150 g / litre) is added and evaporated to dryness. The residue is dissolved in water. The extract is subjected to the following tests (alternatively, the extract prepared by wet digestion may also be used for the following tests. However, the digestion by using perchloric acid is preferred).

1. **Potassium Chromate Solution:**

To 1 ml. of extract, potassium chromate solution is added. The formation of yellow precipitate indicates thallous ion.

2. **Ammonium Sulphide Solution**:

To 1 ml. of extract, ammonium sulphide solution is added. Black precipitate appears in case of thallous ion.

3. **Potassium Ferrocyanide Solution**:

1 ml. of extract is made alkaline. To this potassium ferrocyanide solution is added. Brown precipitate appears (thallous ion).

4. **Tests for Thallium3+:**

1. No precipitate occurs with potassium chromate solution.
2. Brownish black precipitation occurs on adding potassium iodide solution to the extract.
3. No precipitation occurs with dilute hydrochloric acid.

5. **Crystal Test**:

To a drop of test solution on microscopic slide, 1 drop of dilute hydrochloric acid is added. The white precipitate that is formed shows irregular crosses and clusters of radiating crystals under microscope.

**B. Quantitation**

**1. Determination of thallium in urine and blood by atomic absorption spectrophotometry:**

**Material:**

1. **Standard Thallium Solution:**

1.3034 gms. of Thallous nitrate (TlNO3) is dissolved in sufficient water to produce 1000 ml. This solution contains 1 mg. of thallium per ml. The solution is serially diluted to produce solution containing 0.2, 0.5, 1.0, 2.0 and 4.0 μg of thallium per ml.

2. **Sodium Diethyl Dithio Carbamate:**

A 1% solution of sodium diethyl dithio carbamate in pyridine.

**Method for blood:**

1. 5 ml. of the anti-coagulated blood sample is transferred to a screw capped centrifuge tube. 1 ml. of sodium diethyldithio carbamate solution and 1 ml. of 5% aqueous solution of Triton X –100 (a non-ionic surfactant) are added and mixed thoroughly and allowed to stand for 10 minutes. 3 ml. of methyl isobutyl ketone is added, shaken thoroughly and then centrifuged. The organic layer is aspirated into an oxidizing blue flame (air-acetylene flame) and the absorption is recorded at 276.7 nm.

2. The above procedure is repeated with 5 ml. each of the diluted standard solutions and with 5 ml. of water.

3. The absorbance of each of the standard solutions is plotted against the concentration of thallium. The concentration of thallium in blood is obtained from the calibration curve.

**Method for Urine:** (15)

The method is the same as that given for blood except that the pH of the sample and of the standard solution should be adjusted to 7 to 7.5 before addition of sodium diethyl dithiocarbarmate solution and the addition of non-ionic surfactant is omitted.

**5.5.1.15 ZINC**

**A. Chemical tests for Zinc:**

The residue obtained by dry digestion method is dissolved in 2 ml of conc. hydrochloric acid, boiled and filtered. To 0.5 ml of the filtrate is added a few drops of nitric acid and boiled. The solution is made alkaline by adding ammonium chloride and ammonium hydroxide solutions and then filtered. The clear filtrate is tested for the presence of zinc.

**1. Tests with Hydrogen Sulphide:**

To 1 portion of the ammoniacal solution is passed hydrogen sulphide gas. A white precipitate indicates the presence of zinc.

The precipitate is filtered, washed thrice with 10 ml portions of boiled water and dissolved in 2 ml of dilute hydrochloric acid. The solution is again boiled to remove any traces of remaining H2S gas. To it is added saturated caustic soda solution drop wise. A white precipitate is obtained. On adding excess of alkali and boiling the precipitate dissolves. To this clear solution, hydrogen sulphide gas is again passed- A white precipitate confirms the presence of Zinc.

2. **Test with Potassium Ferrocyanide:**

A portion of the ammoniacal solution is acidified with acetic acid and 0.5 ml of potassium ferrocyanide solution is added to it. A white gelatinous precipitate is obtained. A few drops of bromine water when added to the precipitate produce a yellow colour which on boiling forms a bluish green precipitate.

3. **Ammonium Mercuric Thiocyanate – Copper sulphate Test:**

To 2 drops of acidified (preferably with sulphuric acid) extract placed on a spot plate, 1 drop of 0.25 M copper sulphate solution and 1 drop of ammonium mercuric thiocyanate solution (0.8 gm of mercuric chloride and 0.9 gm of ammonium thiocyanate dissolved in 10 ml of water or proportionate amount). A violet (or blackish purple) precipitate appears.

**B. Quantitation**

**1. Determination of Zinc in biological materials by EDTA by Titration method:**

**Method:**

1. 10 gms. of the visceral materials is cut into small pieces and taken into a silica basin. It is heated on a burner flame to remove the excess of moisture and then kept in a muffle furnace. It is heated for 2 hours at 350oC in the furnace. The incinerated materials in the basin are taken out of the furnace. The residue left in the basin is dissolved in 10 ml. of dilute hydrochloric acid and filtered. 5 ml. of concentrated nitric acid is added to it and heated to dryness. The process is repeated. The residue is then dissolved in 10 ml. of dilute hydrochloric acid. Ammonium hydroxide solution is then added dropwise with shaking until a precipitate just persists on shaking. 2 ml. of acetic acid and 5 ml. of 2 M sodium acetate solution are added with shaking until a reddish colour just appears and persists. It is boiled and then filtered. 1 gm of ammonium chloride is added. This is made alkaline by adding an excess of ammonium hydroxide solution and again filtered. The filtrate is collected in a measuring flask and the volume is made up to 25 ml. with water. 2 ml. of buffer solution (142 ml. of conc. ammonium hydroxide, Sp. gr. 0.88 + 17.5 gms. of ammonium chloride and diluted to 250 ml. with water) is added. This is titrated against .01M EDTA solution using 2 drops of Eriochrome Black T as indicator (0.2 gm. of indicator in a mixture or 15 ml. of triethanolamine and 5 ml. of absolute alcohol). At the end point the colour of the solution is changed from wine red to blue. The following relation determines the amount of zinc.

1 ml. of 0.01 M EDTA Solution ≡ 0.6538 mg. of zinc.

**2. Determination of Zinc in viscera and plasma by Atomic Absorption Spectrophotometry:**

**Materials:**

1. **Standard Zinc Solution:**

2.0846 g. of zinc chloride is dissolved in sufficient 1 M hydrochloric acid to produce 1000 ml. This solution contains 1 mg. of zinc in 1 ml. This solution is diluted serially with sodium chloride – potassium chloride solution to produce solutions containing 0.5, 1.0, 1.5, 2.0 and 2.5 μg. of zinc in 1 ml.

**Method:**

1. 1 ml. of serum sample is diluted to 10 ml. with a 6% solution of butan-1-ol and mixed. It is aspirated into an oxidizing (blue) flame (of air – acetylene) and the absorbance is recorded at 213.9 nm.

2. The procedure as above is repeated with 1 ml. of extract of acid-adjusted visceral material (wet digestion).

3. The procedure as in 1 is repeated with 1 ml. of each standard solutions and 1 ml. of sodium chloride-potassium chloride (blank).

4. The absorbance of each of the standard solutions is plotted against the concentration of zinc. The concentration of zinc in viscera is obtained from the calibration curve. (16)

**5.5.2 CHARACTERISTIC OF SOME TOXIC ANIONS:**

The toxic anions include borate, bromide, chloride, chlorate, cyanide, fluoride, hypochlorite, iodide, nitrate, nitrite, oxalate, bromate, iodate, sulphite, sulphide, thiocyanate, hexacyanoferrate, thiosulphate, chromate, dichromate and manganate. These are mostly available in the form of their sodium and potassium salts. The anions interact with enzyme systems or inhibit the normal functioning of blood by converting hemoglobin to other form.The so-called toxic non- metals are to be considered in the form of their anion when produced by the dissociation of salts in aqueous solution or in other medium. The salts are mainly used in industries viz. dyeing electroplating, calico-printing and in household and special purposes viz. hypochlorite as disinfectant, phosphides as rodenticides. Fluorides are used in toothpastes to prevent decay and nitrites and oxalates in textile processing and nitrates as oxidizer and explosive ingredient and thiosulphate in photography.

The toxic anions are extracted as stated earlier and the extracts are taken up for carrying out chemical tests. The tests for different anions will be covered hereunder.

**5.5.2.1. Tests for Borate**

1. Acidify a portion of the sample with dilute hydrochloric acid and apply the solution to turmeric paper. A brown red colour which intensifies when the paper is dried indicates the presence of borate. Moisten the paper with dil ammonia solution. A green black colour is produced.

2. To a portion of extract equal volume of silver nitrate solution is added. A yellow precipitate appears which is insoluble in 30% (v/v) solution of nitric acid and slightly soluble in dilute ammonia solution.

3. Transfer a portion of the extract to an evaporating basis, add 1 ml. of sulphuric acid and 3 ml of ethanol and ignite. A green border around the flame indicates the presence of borate

**5.5.2.2 Tests for Bromide**

To a portion of the extract, an equal volume of silver nitrate solution is added. A yellow precipitate is observed. The ppt. is insoluble in 30 % v/v solution of nitric acid and slightly soluble in dil. ammonia solution , indicates the presence of bromide.

To a portion of the sample, added 5 ml. of chlorine water and 3 ml. of chloroform. A yellow colour, which is extracted into the chloroform layer, indicates bromide.

**5.5.2.3 Tests for Chloride**

1. **Test with Silver Nitrate solution:**

To a portion of the extract equal volume of silver nitrate solution is added. The formation of a yellow precipitate which is insoluble in dilute nitric acid but soluble in dilute ammonia or potassium cyanide or sodium thiosulphate solution indicates presence of chloride.

2. **Test with Diphenyl Carbazide Reagent:**

To a portion of the extract a small quantity of solid potassium dichromate and 1 ml. of concentrated sulphuric acid are added. The mixture is heated gently. A red colour vapour (of chromyl chloride) evolves which turns diphenyl carbazide spot on filter paper violet (1% diphenyl carbazide solution in alcohol is used).

**5.5.2.4 Tests for Iodide**

1.T**est with Lead Acetate:**

To a test portion of the extract is added a few drops of lead acetate solution. A yellow precipitate is obtained which is soluble in much hot water forming a colourless solution. On cooling golden yellow plates appears.

2. **Tests with Chlorine Water:**

When this reagent is added dropwise to a portion of the extract the colour of the solution becomes brown due to liberation of iodine. On shaking with 1-2 ml. of carbon tetrachloride it dissolves in carbon tetrachloride forming a violet solution, which settles out below the aqueous layer. (free iodine may also be identified by the blue colour formed by starch solution). If excess chlorine water is used the solution becomes colourless (due to oxidation to colourless iodic acid).

3. **Tests with Copper Sulphate Solution:**

On adding a few drops of copper sulphate solution to the extract, brown precipitate appears immediately.

**5.5.2.5** **Tests for Fluoride:**

1. **Tests with Calcium Chloride:**

2 ml. of 0.25% calcium chloride solution is added to 2 ml. of extract. A white gelatinous precipitate is obtained which is insoluble in 30% (v/v) solution of acetic acid but slightly soluble in hydrochloric acid.

2.**Test with Zirconium – Alizarin Reagent:**

On a spot plate 2 drops of alizarin S (0.1% aqueous solution) is mixed with 2 drops of zirconyl chloride solution (0.1 g. of solid dissolved in 20 ml. of concentrated hydrochloric acid and diluted to 100 ml.). To the mixture 2 drops of extract is added. The change of colour to yellow indicates fluoride.

**5.5.2.6. Tests for Chlorate**

**1. Tests with Indigo:**

To ml. of the extract a dilute solution of indigo in concentrated sulphuric acid is added until the latter auquires a pale blue colour. Dilute sulphurous or sodium sulphite solution is then added drop by drop. The blue colour disappears.

**2. Tests with Manganous Sulphate – Phosphoric Acid:**

2 drops of the extract and 2 drops of manganous sulphate-phosphoric acid reagent (prepared by mixing equal volumes of saturated manganous sulphate solution and concentrated phosphoric acid) are placed in a micro crucible, warmed rapidly over a burner and allowed to cool. A violet colouration appears. Very pale colouration may be intensified by adding a drop of 1% alcoholic diphenyl carbazide solution (when a deep violet colour is obtained).

**5.5.2.7 Tests for Bromate**

**1.Test with Silver Nitrate:**

To 5 ml. of the extract 2 ml. of silver nitrate solution is added. A white crystalline precipitate is obtained. The precipitate is soluble in hot water, rapidly soluble in dil. ammonia solution (forming a complex) and sparingly soluble in dil. Nitric acid.

**2.Test with Manganous Sulphate:**

To a few drops of extract in a semi-micro test tube, 2 drops of 0.25 M manganous sulphate acidified with dilute sulphuric acid is added and warmed for 2-3 minutes in a boiling water bath. It is cooled. A few drops of benzidine reagent and a few small crystals of sodium acetate are added. A blue colouration is observed.

**5.5.2.8. Tests for Iodate**

**1.Test with Silver Nitrate Solution:**

2 ml. of silver nitrate solution is added to 5 ml. of extract. A white curdy precipitate is obtained which is readily soluble in dilute ammonia solution but sparingly soluble in dilute nitric acid. If the ammoniacal solution of the precipitate is treated drop wise with sulphurous acid solution light yellow precipitate (of silver iodide) is obtained. This is not dissolved by concentrated ammonia solution (difference from bromate).

**2.Test with Barium Chloride:**

2 ml. of barium chloride solution is added to 5 ml. of extract. A white precipitate is obtained (difference from chlorate) which is soluble in hot water and in dilute nitric acid but insoluble in alcohol (difference from iodide). If the precipitate is well washed and treated with a little sulphurous acid solution and 1-2 ml. of carbon tetrachloride the organic layer is coloured violet and a white precipitate (of barium sulphate) is obtained.

**3. Test with Starch Paper:**

A piece of starch paper is treated successively with a drop of 0.1M potassium thiocyanate solution and a drop of acidic solution of the sample. A blue spot is obtained.

**5.5.2.9 Tests for Hypochlorites**

**1.Test with Potassium Iodide-Starch Paper:**

1 drop of concentrated extract (neutral or weakly alkaline) is placed on potassium iodide-starch paper. A bluish-black colour is produced.

**2.Test with Lead Nitrate:**

2 ml. of extract is boiled with lead nitrate solution. A brown solid is found to separate.

**3.Test with Cobalt Nitrate**

A few drops of cobalt nitrate is added to the 2 ml. of extract. A black precipitate is obtained.

**5.5.2.10.Tests for Cyanide**

**1.Prussian Blue Test:**

5 ml. of a freshly prepared 10% solution of ferrous sulphate in freshly boiled and cooled water is added to 2 ml. of the extract. A greenish precipitate is formed. Hydrochloric acid is added drop by drop to dissolve the precipitate. A blue colouration or precipitate (sometimes precipitate is formed on keeping) indicates the presence of cyanide.

**2. Ferric Thiocyanate Test:**

1ml. of ammonium polysulphide is added to 5 ml. of the extract. The mixture is evaporated to dryness (other than on a water bath should be avoided) 1-2 drops of dilute hydrochloric acid is added. It is allowed to cool. 1-2 drops of 3% ferric chloride solution is added. A red colouration is obtained.

**3. Quantitation covered in section -4**

**5.5.2.11 Tests for Cyanate**

**1. Copper Sulphate – Pyridine Test:**

1 ml. of the extract is added to a dilute solution of copper sulphate to which a few drops of pyridine have been previously added. A lilac – blue precipitate is formed. This is soluble in chloroform with the production of a sapphire-blue solution.

**Alternatively:**

A few drops of pyridine are added to 2-3 drops of a 0.25M solution of copper sulphate. 2 ml. of chloroform is added followed by a few drops of extract of sample. The mixture is shaken briskly. The chloroform layer acquires a blue colour.

**2. Tests with Cobalt Acetate Solution:**

1 ml. of cobalt acetate is added to 2 ml. of concentrated extract. A blue colouration is produced.

**5.5.2.12 Tests for Thiocyanates**

**1. Tests with Ferric Chloride:**

A few drops of extract is placed on a spot plate. 2 drops of cobalt nitrate solution is added. A red precipitate or colouration is produced.

**2. Tests with Cobalt Nitrate:**

1 drop of the extract is mixed with 1 drop of 0.5M solution of cobalt nitrate in a micro-crucible and the mixture is evaporated to dryness. The residue becomes violet in colour, which gradually fades. A few drops of acetone is added. A blue or green colouration is obtained.

**5.5.2.13 Tests for Hexacyanoferrate (Ii)**

**1. Tests with Ferrous Sulphate:**

A few drops of ferrous sulphate solution is added to 2 ml. of extract. A white precipitate is formed.

**2. Tests with Copper Sulphate Solution:**

A few drops of copper sulphate solution is added to 2 ml. of extract. A brown precipitate is formed.

**5.5.2.14 Tests for Hexacyanoferrate (iii)**

**1. Tests with Silver Nitrate:**

2 ml. of silver nitrate solution is added to equal volume of extract. An orange-red precipitate is formed.

**2. Tests with Ferrous Sulphate:**

A few drops of ferrous sulphate solution is added to 2 ml. of extract when a dark-blue precipitate (Prussian Blue) is formed.

**3. Tests with Ferric Chloride:**

A few drops of ferric chloride solution is added to 2 ml. of extract when a brown colouration is produced.

**5.5.2.15** **Tests for Sulphites**

**1. Fuchsine Test:**

1 drop of fuchsine reagent (prepared by dissolving 0.015 g. fuchsine in 100 ml. water) is placed on a spot plate. 1 drop of the neutral solution of extract is added. The magenta colour of the reagent is discharged.

**2. Tests with Lead Nitrate:**

A few drops of lead nitrate solution is added to 2 ml. of extract when a white precipitate is obtained. The precipitate is soluble in dilute nitric acid.

**5.5.2.16 Tests for Thiosulphate**

**1. Tests with Potassium Cyanide:**

2 ml. of extract is made alkaline with sodium hydroxide. A few drops of potassium cyanide is added and boiled for some time and acidified thereafter with hydrochloric acid. A few drops of ferric chloride solution is added when a red colouration is observed.

**2.Test with Ferric Chloride:**

A few drops of ferric chloride solution is added to 2 ml. of the extract when a dark-violet colouration appears. On standing the colour disappears rapidly.

**3.Test with Ethylenediamine Nickel Reagent:**

A few drops of reagent (prepared by treating a little nickel chloride solution with ethylenediamine until a violet colour appears) is added to 2 ml. of a neutral or slightly alkaline solution of the extract. A crystalline violet precipitate is formed.

**5.5.2.17 Tests for Sulphide**

**1. Tests with Sodium Nitroprusside:**

1 drop of the alkaline extract is placed on a spot plate. 1 drop of a 1% solution (freshly prepared) of sodium nitroprusside is added. A violet colouration appears.

**2. Methylene Blue Test:**

1 drop of the extract is placed on a spot plate. 1 drop of concentrated hydrochloric acid is added and mixed. To this a few grains of p-aminodimethyl aniline is added followed by a drop of 0.5M ferric chloride solution. A clear blue colouration appears within 2-3 minutes.

**5.5.2.18 Tests for Sulphate**

**1. Sodium Rhodizonate Test:**

1 drop of barium chloride solution is placed upon filter or drop reaction paper followed by a drop of freshly prepared 0.5% aqueous solution of sodium rhodizonate. The reddish –brown spot on the paper is treated with a drop of the acid or alkaline solution of the extract. The coloured spot disappears.

**2.Test with Barium Chloride:**

To 2 ml. of extract a few drops of dilute hydrochloric acid is added. 2 ml. of barium chloride solution is added to it. A white precipitate is obtained. The precipitate is insoluble in warm dilute hydrochloric acid and in dilute nitric acid but moderately soluble in boiling concentrated hydrochloric acid.

**3.Test with Potassium Permanganate-Barium Sulphate:**

3 drops of extract is taken in a semi-micro centrifuge tube. 2 drops of 0.02M potassium permanganate solution and 1 drop of barium chloride are added. A pink precipitate is obtained. A few drops of 3% hydrogen peroxide solution is added and shake. It is centrifuged. The coloured precipitate is clearly visible.

**5.5.2.19 Tests for Nitrite:**

**1. Tests with Indole:**

A few drops of the extract is placed in a semi-micro test tube. 10 drops of indole reagent (0.15% solution of indole in rectified spirit) and 5 drops of 0.8M sulphuric acid are added. A purplish colouration is observed.

**2. Test with Sulphanilic Acid-α-Napthylamine:**

A few drops of the neutral or acidic solution of the extract is taken on a spot plate. 1 drop of sulphanilic acid (prepared by dissolving 1 gm of sulphanilic acid in 100 ml. of warm 30% acetic acid) followed by 1 drop of α-napthylamine (prepared by boiling 0.3 gm of α-napthylamine with 70 ml. of water, filtering or decanting from the small residue and mixing with 30 ml. of glacial acetic acid) are added. A red colour is formed.

**5.5.2.20 Tests for Nitrate**

**1.Test with Brucine:**

A few drops of test solution is placed on a spot plate. 1 drop of concentrated sulphuric acid and a small crystal of brucine are added. The mixture is stirred with a glass rod. A blood red colour is produced.

**2. Test with Sulphanilic Acid -**α**-Napthylamine Reagent:**

A few drops of the neutral or acetic acid solution of the sample is mixed with 1 drop of sulphanilic acid and 1 drop of α-napthylamine. A few mg. of zinc dust is added. A red colouration indicates nitrate.

**5.5.2.21 Tests for Phosphate**

**1. Tests with Ammonium Molybdate:**

2 ml. of the extract is mixed up with 2 ml. of ammonium molybdate solution in a test tube. A few drops of concentrated nitric acid is added and warmed. The appearance of a canary yellow precipitate is observed.

**2. Tests with Ammonium Molybdate-Benzidine Acetate:**

A drop of acidic solution of extract is placed on a filter paper. 1 drop of ammonium molybdate reagent is added followed by 1 drop of 0.05% benzidine acetate in acetic acid. The paper is exposed in ammonia vapour. A blue stain is formed in the presence of arsenate. Then 1 drop of tartaric acid – ammonium molybdate reagent is used and warming is done before exposure to ammonia vapour (the tartaric acid-ammonium molybdate reagent is prepared by dissolving 0.15 mg. of crystalline tartaric acid in 1 ml. of ammonium molybdate reagent).

**3. Tests with Ammonium Molybdate-Quinine Sulphate Reagent:**

1 ml. of extract is placed in a semi-micro test tube and 1 ml. of reagent (prepared by dissolving 1 gm of finely powdered ammonium molybdate in 5 ml. of water and adding and stirring a solution of 0.025 gm. of quinine sulphate in 20 ml. of concentrated nitric acid) is added. It is warmed gently on a water bath. A yellow precipitate is produced with in a few minutes.

**5.5.2.22 Tests for Oxalate**

**1. Tests with Permanganate:**

2 drops of dilute sulphuric acid and 3 drops of 1% solution of potassium permanganate are added to 1 ml. of the extract. The violet colour disappears.

**2.Test with Calcium Chloride:**

To 1 ml. of the extract 1 ml. of 25% solution of calcium chloride is added. A white coloured precipitate is formed instantaneously. The precipitate is soluble in 30% (v/v) solution of acetic acid but insoluble in dilute hydrochloric acid.

**5.5.2.23 Tests for Chromate**

**1. Tests with Barium Chloride:**

To 2 ml. of the extract 1 ml. of barium chloride solution is added. A pale yellow precipitate is obtained which is insoluble in water and acetic acid but soluble in dilute mineral acids.

**2.Test with Silver Nitrate:**

To 2 ml. of the extract 2 ml. of silver nitrate solution is added. A brownish red precipitate is obtained which is soluble in dilute nitric acid and ammonia solution but insoluble in acetic acid.

**3.Test with Lead Acetate:**

To 2 ml. of the extract a few drops of lead acetate solution is added. A yellow precipitate is obtained which is insoluble in acetic acid but soluble in dilute nitric acid.

**4.Test with Hydrogen Peroxide:**

To 2 ml. of acidic solution of the extract a few drops of hydrogen peroxide is added. A deep blue solution is obtained.

**5.5.2.24 Tests for Dichromate**

**1.Test with Barium Chloride:**

To 2 ml. of the extract 1 ml. of barium chloride solution is added. A pale yellow precipitate is formed in minute amount (distinction from chromate). The precipitate formation becomes quantitative if sodium hydroxide or sodium acetate solution is added.

**2.Test with Silver Nitrate:**

To 2 ml. of the extract 2 ml. of silver nitrate solution is added. A reddish-brown precipitate is formed.

**5.5.3. DETERMINATION OF ANIONS IN BIOLOGICAL MATERIAL**

Out of different anions that may be concentrated to poisoning case, cyanide is commonly encountered. The quantitation of cyanide in blood has already been covered in Section 4. However, quantitation of some anions have been covered in the manual.

* + - 1. **Determination of Cyanide in blood – see section 4 (volatile poisons):**

**5.5.3.2 Determination of Nitrite in urine by spectrophotometry:**

A. **Materials:**

1. **Standard Nitrite Solution:**

1.5 gms. of sodium nitrite is dissolved in sufficient water to produce 1000 ml. This solution contains 1 mg. of nitrite per ml. It is serially diluted with water to produce solutions containing 10, 20, 50 and 100 μg of nitrite per ml.

2. **Naphthylamine Solution:**

0.48 gm. of 1 Naphthylamine is dissolved in sufficient of a 20% (v/v) solution of hydrochloric acid and made upto 100 ml.

3. **Sulphanilic Acid Solution:**

0.6 gm. of sulphanilic acid is dissolved in sufficient of a 20% (v/v) solution of hydrochloric acid and made upto 100 ml.

B. **Method:**

1. 1 ml. of urine is taken in a 50 ml. volumetric flask. In another volumetric flask of same capacity, 1 ml. of water is also added as a blank. The following procedure is carried out with each of the flask.

2. 1 ml. of the sulphanilic acid solution is added, mixed and allowed to stand for 10 minutes. 1 ml. of the 1Naphthylamine solution and 1 ml. of a 16.4% solution of sodium acetate are added. It is diluted to 50 ml. with water, mixed and allowed to stand for 10 minutes. The absorbance of the sample solution is recorded at 510 nm using the blank solution in the reference cell.

3. The procedure is repeated with each of the diluted standard solutions.

4. The absorbance is plotted against concentration to prepare calibration curve. From calibration curve, the amount of nitrite in unknown sample is known (curve should be linear in the range 0-50μg./ ml.).

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SECTION: 6: ANALYSIS OF NEUTRAL POISONS (ORGANIC

NON-VOLATILE)

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

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

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

**6.4 Responsibility**: Gazetted Officers and associated Scientific staff.

**6.5 ORGANOPHOSPHOROUS INSECTICIDES:**

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

**6.5.1 Classification of Organo Phosphorous Insecticides:**

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.

**6.5.1.1 Derivatives of Phosphoric Acids**

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

**TABLE 6.1 : Derivatives of Phosphoric Acids.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl. No.** | **Name** | **Other Names** | **Chemical Name** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12. | Dichlorovos  Naled  Phosphamidon  Phosphinon  Phosdrin  Bidrin  Birlane  Gardona  Dimefox  Mipafox  Avenin  Cyolane | Dichlorfos,  DDVF  DDVP  Bromchlophos  Dibrom.  Demecron | O, O-Dimethyl O – 2, 2  dichlorovinyl  phosphat  O, O – Dimethyl – O – 2, 2  Dichloro –1, 2- dibromomethyl  Phosphate.  O, O – Dimethyl – O [2-chloro – N,  N – dimethyl – carbamoyl] -methylvinyl phosphate.  O, O-Diethyl-O (2, 2-dichloro – 1 – chloroethoxylvinyl phosphate.  O, O – Dimethyl – O – (1-methyl- 2-carbomethoxy-vinyl) phosphate.  3-Dimethoxy phosphinyloxy.  N, N – dimethyl Crotonamide.  2-chloro 1-(2, 4- dichlorophenyl) vinyl diethyl phosphate.  2-chloro-1 (2, 4, 5- trichlorophenyl) vinyl dimethyl phosphate.  Bis-dimethyl flurophosphate,  N,N-di isopropyl phosphorodiamide fluoride.  O, O-Dimethyl-N-(isopropoxy – carbamoyl phosphate).  Diethyl – N-3-dithioanyl - 2- imino-phosphate. |

**6.5.1.2 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 6.2:** Derivatives of Thiophosphoric Acid:

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

**6.5.1.3 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 6.3 :** Derivatives of Dithiophosphoric Acid:

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

* + - 1. **Miscellaneous Organo Phosphorous Compounds:**

**TABLE 6.4 :** Miscellaneous Organo Phosphorous Compounds:

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

**6.6 ANALYSIS OF ORGANO PHOSPHOROUS PESTICIDES** (25)**:**

**6.6.1 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 peticide 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 3.

**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 3.

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.

**6.6.2 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.

**6.6.2.1 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. **Mercurous Nitrate Reagent: (1)**

**Preparation of Reagent :** 1 gm. of mercurous nitrate (Analytical Reagent Grade) is dissolved in 100 ml. of water followed by addition of a few drops of Conc. nitric acid.

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

Colour of Spot: Black.

**Responding Compounds:** Organo-phosphorous and organo dithio phosphorous Insecticides.

II. **Potassium Iodate-Starch Reagent: (13)**

**Preparation of Reagent and Related Solutions:**

1. **Potassium Iodate:** 5 gms. of potassium iodate is dissolved in 100 ml. of distilled water.
2. **Starch Solution:** 1 gm. of soluble starch is dissolved in 100 ml. of boiled water. The solution is cooled thereafter for preservation.

**Reagent Solution**: 25 ml. of A is mixed with 2 ml. of B immediately

before use.

1. **Hydrochloric Acid Solution:** 20 ml. of concentrated hydrochloric acid is diluted to 100 ml. with distilled water.
2. **Ammonia Solution:** 20 ml. of ammonia solution (Sp. gr. 0.88) is diluted to 100 ml. with distilled water.

**Method of Spraying and**

**the Colour of Spot :** The developed plate is sprayed with 20% dilute hydrochloric acid followed by reagent solution. Violet spots appear within 2 minutes. The plate is sprayed again with ammonia solution (20%) when the intensity of colour of spot is increased.

**Responding Compounds :** Sulfur containing organo-phosphorous

insecticides.

III. **Cobalt Acetate – o-Tolidine Reagent: (14)**

**Preparation of Reagent :**

1. Cobalt Acetate Solution: 5 gms. of cobalt acetate is dissolved in 100 ml.

of distilled water.

1. o-Tolidine Reagent: 1 gm. of o-tolidine is dissolved in 100 ml. of 10%

(v/v) acetic acid.

**Method of Spraying and**

**Colour of the 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-tolidine in acetic acid. Blue spots are observed.

**Responding Compounds** **:** Phosphamidon and Endosulfan.

IV. **Tollen’s Reagent (15) :** 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, Propoxur (Baygon), carbaryl (Sevin) and other phenolic compounds.

V. **Griess Reagent. (16)**

**Preparation of Reagent:**   
  
 1. 5% of Sodium Nitrite Solution: 5 gms. of sodium nitrite is dissolved in

10% (v/v) acetic acid solution.

2. **0.1% 1-Napthyl amine Solution:** 0.1 gm of 1-napthylamine is dissolved in 10 ml. of glacial acetic acid and diluted to 100 ml. with distilled water.

3. **5% Stannous Chloride Solution:** 5 gms. of stannous chloride is dissolved in 100 ml. of 50% hydrochloric acid solution (v/v).

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

The developed plate is sprayed with 5% stannous chloride solution. The plate is then heated for 10 minutes at 100oC. It is cooled and sprayed with a freshly prepared 5% sodium nitrite solution followed by 1-napthylamine solution as stated above. Pink-Orange coloured spots are obtained.

**Responding Compounds:**

Methyl Parathion, Ethyl Parathion, Fenitrothion, p-Nitrophenol (a metabolite of methyl and ethyl parathion), 3 Methyl 4-Nitrophenol (a metabolite of Fenitrothion) and also other organic compounds containing nitro group and aromatic amino group.

VI. **Mercuric Nitrate – Potassum Hexacyanoferrate Reagent:**

**Preparation of Reagent.**

1. **0.5% Mercury (II) Nitrate Solution:** 0.5 gm. of mercury (II) nitrate is dissolved in 100 ml. of distilled water.

2. **1% Potassium Hexacyanoferrate Solution:** 1 gm. of potassium hexacyanoferrate in 100 ml. of distilled water.

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

The developed plate is sprayed with 0.5% mercury (II) nitrate solution followed by 1% potassium hexacyanoferrate solution

Bluish green coloured spots are obtained.

**Responding Compounds:** Sulphur containing organo phosphorous compounds.

Selective Detection of Dichlorovos (DDVP) (17).

**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 (18):**

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 of the 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 (19):**

**Reagent Solutions**:

**Chloranil Reagent (0.5 % w/v)** : Dissolve 0.5 g. chloranil in 100 ml acetone.

Anhydrous Sodium Carbonate Solution:- 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

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

**Solvent system:** Chloroform : Acetone (7 : 3)

**Other Chromogenic Reagents for TLC.**

a) Palladium (II) chloride. **(2)**

b) Bromo- fluoresceine - silver nitrate **(3)**

c) Congo red. **(4)**

d) 4-(p-nitrobenzyl) pyridine tetraethylene pentamine **(5)**

**6.6.2.2 Identification and Quantitation of Organophosphorous Pesticides by GLC:** (19)

GC is preferably used for analysis of volatile and thermo stable pesticides. In recent years, capillary columns have almost completely replaced the packed columns owing to their high resolving power which allows the separation of a large number of pesticides with similar physico-chemical characteristic. The most frequently used detectors include ECD, NPD, FPD and MSD. This last one has become popular for pesticides analysis.

The ECD (Electron Capture Detector) has been the detector most used in pesticide residue analysis. It shows a very high sensitivity to polychlorinated hydrocarbons and other halogenated pesticides but its selectivity is rather poor because all kinds of electron attracting functional groups such as nitro groups and aromatic structures also give a response on this detector. OCPs, OPPs and pyrethroid pesticides have been determined with this detector.

NPD (Nitrogen Phosphorous Detector) is used in pesticide residue analysis because of its selectivity for phosphorous and nitrogen containing compounds.

The FPD (Flame Photometric Detector), in phosphorus mode, has frequently been the instrumental technique of choice for the analysis of organophosphorous pesticide.

MSD (Mass Spectral Detector) can be employed to achieve selective detection, by full scan of selective ion monitoring of target pesticides in the presence of the complex matrix. MSD is a highly sensitive and specific technique .The most widely used technique for pesticide residue analysis is MSD with electron impact (EI) ionization. Quantification is usually achieved by the technique of selected ion monitoring (SIM). With this technique, selectivity is also improved.

**Table:** GLC Conditions Related to Organo Phosphorous pesticides.

|  |  |  |  |
| --- | --- | --- | --- |
| GLCCondition 1 | GLC **Condition 2** | GLC **Condition 3** | GLC **Condition 4** |
| Column:5 m x 0.53 mm  (i.d.) OV-17 (50% phenyl siloxane and 50% methyl siloxane) megabore column of 2.0 μm thickness.  Oven Temperature:  Initial : 110oC.  Final : 290oC.  Rate : 10oC / min.  Final Temp.hold: 5min.  Flow rate :  Hydrogen: 3.5 ml./min.  Air : 100 ml./min.  Nitrogen: 7 ml./min.  Detector: NPD. | Column:50 x 0.53 mm. OV-17(50% phenyl poly- siloxane + 50% methyl siloxane) megabore colu-mn of film thickness 2.0μm.  Oven Temperature:  Initial : 110oC.  Final : 270oC.  Rate : 10oC / min.  Final Temp.hold: 5min  Detector NPD  Internal Standard Diltia-zem (Retention time→ 19.81 min.). | Column: SPB-608  30 m x 0.53 mm (i.d.)  of film thickness μm.  Oven temperature .Programming:  150oC (0.5 min.) to 230oC at 6oC / min., holding at 230oC for  2 min, then to 245oC at 3oC / min.  Carrier gas: Helium.  Flow rate: 8 ml./min.  Detector: FPD. | Column: DB 17  1.5 m x 0.53 mm (i.d.)  Column Temperature Programme:  140oC (initial) to 180oC at 10oC / min. and then to 270oC at 6oC / min.  Carrier gas: Nitrogen  Flow rate: 4.5 ml./min.  Detector : FPD. |

**Quantitation:** By peak area method using internal standard.

* + - 1. **Conditions for Mass Spectrometry of Organo-Phosphorous**

**pesticides:**

Ion Source Temperature : 200oC.

EV : 70 ev

Column : 3 ft. 3% OV-17.

Initial temperature : 1 minute hold at 200oC.

Final temperature : 10 minutes hold at 300oC.

Rate : 20oC / min.

**3. Carbamate and Urea Pesticides at 8ppb from Water**

**Conditions**

Sample: 3ml water containing 8ng/ml of each analyte (except: 100ng/ml carbofuron, 80ng/ml propoxur, 20ng/ml carbaryl) in 10% NaCl,  
Direct Injection: 60µlsamplethroughinterfaceSPME,

Fiber: PDMS/DVB, 60µm film, Extraction:immersion,40min,rapidstirring, Desorption: static, 5 min, in acetonitrile: water (65:35), dynamic, valve open during run, Column:SUPELCOSIL™LC-8,15cmx4.6mm,ID,5µmparticles, Mobile Phase: acetonitrile : water(8:82to65:35in9min,hold3min)  
Flow Rate:2.0mL/min  
Temp.:35°C  
Det.: UV, 240nm  
Analyte Data  
A. Methomyl  
B. Oxamyl  
C. Fenuron  
D. Monuron  
E. Carbofuran  
F. Propoxur  
G. Carbaryl (Sevin)  
H. Fluometuron  
I. Diuron  
1. Propham  
2. Siduron  
3. Linuron  
4. Chlorpropham  
5. Barban  
6. Neburon

**4. Chlorinated Pesticides By SPME/GC**

**Conditions**

SPME:100µm PDMS phase fiber immersed in 4mL water (15 min)  
Columns:15m x 0.20mm ID, 0.20µm film   
SPB-5; SPB-608   
Oven Temp.:120°C (1 min) to 180°C at 30°C/min, then to 290°C at 10°C/min  
Carrier: helium, 37cm/sec (set at 120°C)  
Det.: ECD, 300°C  
Inj.:260°C (splitless - closed 3 min)

**Analyte Data**

1. a-BHC  
2. b-BHC  
3. g-BHC  
4. d-BHC  
5. Heptachlor  
6. Aldrin  
7. Heptachlor epoxide  
8. g-Chlordane  
9. Endosulfan I  
10. a-Chlordane  
11. 4,4'-DDE  
12. Dieldrin  
13. Endrin  
14. Endosulfan II  
15. 4,4'-DDD  
16. Endrin aldehyde  
17. Endosulfan sulfate  
18. 4,4'-DDT  
19. Endrin ketone  
20. Methoxychlor

Internal Standard: Decachlorobiphenyl

**5. Chlorinated Pesticides Extracted from Water by SPME**

**Class of Compound: Pesticides**

**Conditions**

Sample: 200ppt each analyte in 2mL water  
SPME Fiber: polydimethylsiloxane, 100µm film  
Extraction: immersion, 15 min (rapid stirring)  
Column: SPB-5, 15m x 0.20mm ID, 0.20µm film  
Oven: 120°C (1 min) to 180°C at 30°C/min, then to 290°C at 10°C/min  
Carrier: helium, 37cm/sec (set at 120°C)   
Det.: ECD, 300°C  
Inj.: splitless (splitter closed 3 min), 260°C

Analyte Data

1. a-BHC  
   2. b-BHC  
   3. g-BHC (Lindane)  
   4. d-BHC  
   5. Heptachlor  
   6. Aldrin  
   7. Heptachlor epoxide  
   8. g-Chlordane  
   9. Endosulfan I  
   10. a-Chlordane  
   11. 4,4'-DDE  
   12. Dieldrin  
   13. Endrin  
   14. Endosulfan II  
   15. 4,4'-DDD  
   16. Endrin aldehyde  
   17. Endosulfan sulfate  
   18. 4,4'-DDT  
   19. Endrin ketone  
   20. Methoxychlor

Internal Standard: Decachlorobiphenyl

**7. Organophosphorus Pesticides Extracted from Water**

Class of Compound: Pesticides  
Technology: GC

**Conditions**

Sample: 50ppb each analyte in 1.5mL saturated salt water, pH 7.2  
SPME Fiber: polydimethylsiloxane, 100µm film  
Extraction: immersion, 20 min (rapid stirring)  
Column: PTE-5, 30m x 0.25mm ID, 0.25µm film   
Oven: 60°C (1 min) to 300°C at 12°C/min, hold 5 min  
Carrier: helium, 30cm/sec   
Det.: MS (m/z = 45-400, 0.6sec/scan)  
Inj.: splitless, 270°C (0.75mm ID injector liner)

Analyte Data

1. O, O, O-Triethylphosphorothioate  
2. Thionazin  
3. Sulfotep  
4. Phorate  
5. Dimethoate  
6. Disulfoton  
7. Methyl parathion  
8. Ethyl parathion  
9. Famphur (Famphos)

**8. Pesticides in Fruits and Vegetables**

Class of Compound: Pesticides  
Technology: GC

**Conditions**

Sample: Homogenize 50g sample in 100ml acetonitrile. Add 10g NaCl. Homogenize 5 min.  
Extraction Tube: ENVI-Carb, 6ml, 500mg   
Extraction: Centrifuge 13ml of acetonitrile layer at high speed for 5 min.

Evaporate 10ml aliquot to 0.5ml under nitrogen at 35°C.Transfer sample to SPE tube. Elute pesticides with 20ml acetonitrile: toluene (3:1). Concentrate to 2ml by rotary evaporation.

Add 2 x 10ml acetone, concentrating material to 2ml after each addition.  
Add 50µl cis-chlordane in acetone (500ng/µl), dilute to 2.5ml with acetone.  
Column: 14% cyanopropylphenyl/86% dimethyl siloxane, 30m x 0.25mm ID, 0.15µm film

Oven: 70°C (2 min) to 130°C at 25°C, to 220°C at 2°C/min, to 280°C at 10°C/min, hold 4.6 min

Carrier: Helium  
Detector: MSD, 285°C  
Injection: 2µl, splitless

Analyte Data

1. Diphenylamine  
2. Methoxychlor

**10. Pesticides by US EPA Method IP-8, TO-10A, ASTM D4861.**

Class of Compounds: Pesticides  
   
**Conditions**

Sample: polyurethane foam (PUF) spiked with 250ng each pesticide  
Sampling Tube: ORBO-1000   
Column: SPB-608, 30m x 0.53mm ID, 0.5µm film  
Cat. No.: 25312  
Oven: 150oC (1 min) to 270oC at 4oC/min  
Carrier: Helium, 7mL/min  
Detector.: ECD  
Injection: 1µL

Analyte Data

1. Dichlorvos  
2. 2,4,5-Trichlorophenol  
3. Pentachlorobenzene  
4. 2,4-D methyl ester  
5. Hexachlorobenzene  
6. a-BHC  
7. Pentachlorophenol  
8. g-BHC (Lindane)  
9. b-BHC  
10. Heptachlor  
11. Chlorothalonil  
12. Aldrin  
13. Ronnel  
14. Chlorpyrifos  
15. Heptachlor epoxide  
16. trans-Nonachlor  
17. Dieldrin  
18. Captan  
19. 4,4'-DDE  
20. 4,4'-DDT  
21. Mirex  
22. Methoxychlor  
23. Octachloronaphthalene (surr. std.)

**1. Multi-residue Analysis of Organophosphorus Insecticides by Ion Trap GC/MS (43)**

**Instrument Conditions**

**Gas Chromatograph**

Column: DB5-ms, 60m. X 0.25mm X 0.25µm

Flow rate: 1.2 ml/min.

Oven Program: 50°C for 2 min., then 20°C/min. to 160°C, then 8°C/min. to 300°C and hold for 1 minute.

Injector program: 50°C for 0.2 min., then program at 180°C/min. to 250°C and hold for 10 minutes.

Relay Program: time 0.0 min. split vent open time 0.2 min. split vent closed

time 1.8 min. split vent open

Transfer Line: 300°C

Injection Volume: 1μl

**Mass Spectrometer**

Mass Range: 60-345 u

Seconds/Scan: 1

Threshold: 0 counts

Filament: 13 Amps

Ion Trap Temp: 250°C

**4. Solid Phase Micro extraction of Organophosphate Insecticides and Analysis by Capillary GC/MS.**

Sample: 35mL in 40mL vial (exposed to 4M NaCl, pH 2)

SPME Fiber: 85µm polyacrylate

Extraction: immersion, 240 min, rapid stirring

Desorption: 5 min, 310°C

Column: Cross-linked 5% phenyl methyl silicon, 30m x 0.25mm ID,

0.25µm film

Oven: 35°C (15 min) to 300°C at 10°C/min, hold 5 min

Carrier: helium, 1mL/min

Det.: MS (EM Voltage: relative +700,

Single ion monitoring

**6.7 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 suicidal poisoning cases. Accidental poisoning cases are also known. The identification and sometimes quantitation are 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 organochloro insecticides are given below.

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

|  |  |  |
| --- | --- | --- |
| **Sl. No.** | Name of Insecticide | Chemical Name |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12. | DDT  BHC  Lindane  Endrin.  Dieldrin  Endosulfan  Heptox  Chlorodan  Toxphene.  Kelthane  Heptachlor  Methoxychlor | 1, 1, 1, Trichloro –2, 2 bis (p-chloro phenyl) ethane.  Gamma isomer of 1, 2, 3, 4, 5, 6 hexachloro cyclohexane (Benzene hexachloride).  Gamma isomer of hexachloro cyclohexane.  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.  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, 7, 8, 9, 10, 10-Hexachloro 1, 5, 5a, 6, 9, 9a-hexahydro 6, 9 methano 2, 4, 3 – benzo oxathiepin – 3- oxide.  1, 4, 5, 6, 7, 8, 9-Heptachloro 3a, 4, 7, 7a-tetrahydro-4, 7 endo-methanonapthalene.  1, 2, 4, 5, 6, 7, 8, 8a-Octachloro 3a, 4, 7, 7a tetrahydro – 4, 7 methano indane.  Chlorinated comphene.  1, 1-Bis (chlorophenyl) 2, 2, 2-trichloro-ethane.  1, 4, 5, 6, 7, 8, 8a –Heptachloro 3a, 4, 7, 7a-tetrahydro 4, 7-methano indane.  2, 2 – Bis-(p-chlorophenyl) 1, 1, 1-trichloro-ethane. |

**6.8 ANALYSIS OF ORGANO CHLORO PESTICIDES:**

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

**6.8.1 Isolation and Stripping of Organochloro Insecticide in Biological and Non-Biological Matrices:**

The extraction, stripping methods and selection of solvent for extraction have already been covered in sections 3 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.

**6.8.2 Detection and Identification of Organo Chloro insecticides:**

**6.8.2.1 TLC Conditions and Spray Reagents for Identification of Organochloro 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 (6, 20).**

**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 110oC.

Bluish green spots are obtained.

**Responding Compounds:**

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

II. **o-Tolidine Reagent:** (5)

**Preparation of Reagent:**

0.5 gm. of o-tolidine is dissolved in 100 ml. of acetone.

**Mode of Spraying:**

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

Bluish green spots are obtained.

**Responding Compounds:**

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

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.

**6.8.2.2 Individual test for some of the Organochloro Pesticides:**

**i. Benzenehexachloride and lindane**

Technical BHC is a mixture of as many as 8 isomeric forms of benzene hexachloride namely alpha (65-70 %), beta (6%-8 %), gamma (12%-15%), Delta (2 %-5%), epsilon (3-7 %) and traces of Eta, theta and zeta isomers. Lindane is approximately 99% of gamma isomer of BHC. The presence of BHC (Technical) is established by separation of its isomer by chromatographic technique.

**a) Colour Tests**:

1. The extracted residue is dissolved in one ml of petroleum ether and taken into a flask. Then solvent is removed by evaporation. 2 ml of purified aniline is added to it and refluxed for one and half an hour vigorously on a boiling water bath, then cool it at room temperature. The cooled aniline mixture is transferred to a 250ml separating funnel with about 50 ml of 3N hydrochloric acid followed by 50 ml distilled water. This is extracted with 20 ml of ether. The ether layer is separated, passed through anhydrous sodium sulphate and evaporated to about 1 ml. and the remaining solvent is removed by blowing a current of dry air. Few drops of Pentane is added to dissolve the residue. To it 10 ml of sulphuric acid and vanadium pentaoxide reagent (0.5 mg of vanadium penta - oxide per 10 ml sulphuric acid) is added. A violet colour is produced. The intensity of the colour produced is compared with that of a control of known amount of BHC treated similarly.

2. The extracted residue containing BHC (or lindane) is treated with dichloromethane dissolved in acetic acid and 0.5 gm zinc power is added to it. The dichlorination takes place forming free benzene which is nitrated with the nitration mixture consisting of 1:1 of fuming nitric acid and concentrated sulphuric acid. After nitration is complete, the mixture is cooled, diluted with ice cold water and then extracted thrice with 10 ml portions of chloroform. The chloroform extract is evaporated to dryness, residue is taken into 5 ml ethyl methyl ketone and to it 5 ml of 10 % NaOH is added. Red colour is formed. The intensity of the colour so obtained can be compared with the standard sample of BHC (or Lindane) treated similarly with the help of a colorimeter.

**b) Thin layer chromatographic method-I:-**

The extracted tissue residue is dissolved in one ml of acetone and 10 ml aliquot of it is spotted on silica gel “G” TLC plate(0.25 mm thick layer). Control spots of known B.H.C. insecticides are also applied on the same TLC plate and the spotted plate is developed in the solvent system petroleum ether: liquid paraffin (9:1v/v) The plate is air dried and then sprayed with o-di aniline chromogenic reagent after air drying the plate is irradiated to long UV light 366nm for 10 mts. Brownish Black coloured spots are observed against off-white background with Rf values 0.41,0.60, 0.66,0.77 and 0.83.

Extracted residue is nitrated with mixture of Conc. HNO3 and Conc. H2So4 and the nitrated product is extracted with chloroform. After evaporation of chloroform the remaining residue is taken in a few drops of cyclohexane and to it is added few drops of 1 % alcoholic KOH, violet colour is obtained slowly changing to brown and finally to yellow. Other chlorinated insecticides do not exhibit this sequence of colour.

A Portion of the residue is treated with sodium metal in the presence of isopropyl alcohol. The product is taken in methyl alcohol and then treated with phenyl azide. After reaction is complete, the mixture is treated with diazotized sulphanilic acid in acidic medium. A red coloured complex is obtained. The red colour solution can be studied under spectrophotometer for absorption spectrum (max is at 515nm).

**c) Thin Layer Chromatographic method-II:**

Extracted residue is dissolved in a small amount of acetone and an aliquot of it is spotted on silica gel g plate (o.225 mm thick layer) control spots of known chlorinated insecticides are also applied on the same TLC plate and the spotted plate is developed in the solvent system n-hexane: Acetone (95:5). Developed plate is air dried and then sprayed with chromogenic reagent (1 % ethanolic AgNO3 + 3 ml Conc. NH4OH). After air drying the plate is irradiated to long U.V. light(366nm) for 10 minutes. Black coloured spot is obtained against white background. Rf value 0.05.

**ii. DDT [1,1,1 trichloro-2,2,bis (P-chloro penyl)ethane]**

**a) Colour Tests**

1. 5 ml of chloro benzene, is added to the small portion of extracted residue and warmed gently ,then filtered 0.1 gm of anhydrous aluminium chloride is then added to it. A red to deep violet colour is obtained depending upon the concentration of DDT.

2. Play of colours- A portion of the extracted residue is taken in one ml of n-hexane and transferred into a glass tube. The tube is kept in boiling water bath for 10 minutes to remove all solvent then is cooled in ice bar in ice bath then 10 ml of a chilled nitrating mixture containing of equal volume of concentrated nitric acid and Sulphuric acid is added to it. The tube is then placed in a hot water bath. The temperature of which is raised gradually up to boiling of water. Heating is continued for one hour. If any carbonaceous material remains more of nitrating mixture is added and heating is continued for further half an hour. After cooling the liquid is diluted with 50 ml. of ice cold water transferred to a separating funnel and extracted thrice with 50 ml of 1% potassium hydroxide solution and then three times with 50 ml portion of distilled water. The chloroform layer is then passed through anhydrous sodium sulphate and evaporated just to dryness. The residue is taken in small amount of chloroform and transferred into two separate spots on a white tile and the chloroform is evaporated off. To one spot is added one drop of 20 % alcoholic potassium hydroxide solution. A play of colour from rose to bright blue to green to yellow is observed in presence of DDT. How ever, the presence of DDE, DDA, DDD and methoxychlor also gives this test. To the other spot tile is added one drop of alcoholic KOH solution followed by the addition of 1 drop of acetone. Bright blue colour changing to bright purple is obtained while gradually changing to grey and ultimately yellow colour is obtained. It is sensitive for DDT.

**b) Identification by TLC:-**

The extracted residue is dissolved in a small amount of acetone and spotted on silica gel ‘G’ TLC plate along with control sample. Spotted plate is developed in the solvent system n-Hexane: Acetone (95:5). Developed plate is air dried and then sprayed with 1 % ethanolic silver nitrate solution containing 5 ml of conc. Ammonium hydroxide or 1 % diphenylamine in alcohol after drying, the plate is irradiated to long U.V. light (366 nm ) for 10 minutes. With silver nitrate reagent two black coloured spots are observed against white background. With diphenylamine reagent green to grey coloured spots are obtained. With Rf values 0.65 and 0.85

**iii Endrin**

**a) Colour tests:**

Small portion of the residue extracted from viscera is dissolved is few drops of n-hexane then few drops of conc. H2SO4 is added to it- a red colour is developed. If fuming Hno3 is added to it –a red colour is developed. If fuming HNO3 is added to it green colour is developed. Extracted residue is nitrated with mixture of conc. HNO3 and conc. H2SO4 and the nitrated product is extracted with chloroform. After evaporation of chloroform the remaining residue is taken in a few drops of cyclo hexane and to it is added few drops of ! % alcoholic KOH, violet colour is obtained slowly changing to brown and finally to yellow. Other chlorinated insecticides do not exhibit this sequence of colour. A portion of the residue is treated with sodium metal in the presence of isopropylacohol. The product is taken in methyl alcohol and then treated with phenyl azide. After reaction is complete, the mixture is treated with diazotized sulphanilic acid in acidic medium. A red colour complex is obtained. The red colour solution can be studied under spectrophotometer for absorption spectrum(max is at 515nm).

**b) Thin Layer Chromatographic method:**

Extracted residue is dissolved in a small amount of acetone and an aliquot of it is spotted on silica gel g plate (0.25 mm thick layer) control spots of known chlorinated insecticides are also applied on the same TLC plate and the spotted plate is developed in the solvent system n-hexane: Acetone (95:5). Developed plate is air dried and then sprayed with chromogenic reagent (1 % ethanolic AgNO3 + 3 ml NH4OH). After air drying the plate is irradiated to long U.V. light (366 nm ) for 10 minutes. Black coloured spot is obtained against white back ground. Rf value 0.05.

**6.8.2.3 Identification and Quantitation of Organochloro Insecticide by GLC:**

Gas-liquid Chromatography (GLC) is also used for identification of organo-chloro compound in biological and non-biological matrices. Prior to the advent of electron capture detector (ECD), GLC had limited utility because of non-availability of specific detector for the purpose and also poor sensitivity of the then existing detectors. Two methods have been described here using electron capture detector.

**Method : 1**

The extract in biological or non-biological materials is purified by passing the extract through dry, partially deactivated alumina and silica columns to remove fat, colour and decomposed proteins etc to have ‘an’ interference free extract.

GLC Condition and Relative Retention Time (RRT) of Organo chloro Insecticides Relative to Aldrin.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Different Chromatographic System** | | | | |  | **RRT Under Different Systems.** | | | |
| System | **Column &**  **Detector** | Stationary **Phase** | **Flow Rate of Mobile Phase-1 ml./min** | **Temp.of column (0oC)** | Compound | **System A** | **System B** | **System C** | **System D** |
| A  B  C  D | Column in all case  (A,B,C & D)  6ft.x ¼” (OD) glass column packed with 80/100 mesh Gaschrom Q  Detector  In all cases  (A,B,C & D)  ECD | 3% OV-1  4% SE-30,  6%  QF-1  10%DC200,  15% QF-1  5%  QF-1 | 70  95  100  70 | 180  190  190  160 | Aldrin  Alpha BHC  Beta BHC  Gamma BHC  Chlordane  P,P’ DDT  Dieldrin  Endrin  Thiodan  Endosulfan  Heptachlor  Kelthane  (Dicofor)  Lindane  Methoxychlor  Toxaphene | 1.00  0.35  0.40  0.47  -  3.50  1.93  -  1.63  -  0.78  -  0.44  5.70  - | 1.00  0.50  0.62  0.70  1.72  3.50  2.32  2.58  1.92  3.05  0.83  -  0.85  -  3.67 | 1.00  0.45  0.58  0.66  0.77,1.57,0.82 “& 1.08  3.50  2.23  2.58  1.89  -  0.81  2.32  0.57  5.46  3.80 | 1.00  0.61  1.00  1.11  2.32  5.22  3.60  4.28  2.80  -  0.86  1.31  0.80  9.2  6.54 |

#### Method : 2

As described in method 1, the purified extract is subjected to GLC as described hereunder along with the analytical data.

**TABLE:** Gas Chromatographic Condition and Relative Retention Time of Organo-chloro Insecticides Relative to Aldrin (RRT).

|  |  |  |
| --- | --- | --- |
| Gas Chromatographic Conditions | Organo-Chloro Compound | **RRT (Aldrin)** |
| Column : 6'x 4 mm (ID) glass column packed with 80/100 mesh  Chrom Q.  Stationary Phase : 15% QF-1  and 10% DC-200 (1 : 1)  Carrier Gas: Nitrogen.  Flow rate: 120 ml./min.  Injection : 225oC.  Column : 200oC  Detector : Electron Capture Detector,  Concentric type, Tritium  source. | Aldrin  Alpha BHC  Beta BHC  Gamma BHC  Chlordane  P,P’ DDT  Dieldrin  Endrin  Endosulfan  Heptachlor  Kelthane (Dicofol)  Methoxychlor  Toxaphene | 1.00  0.46  0.60  0.68  1.72  3.28  2.22  2.55  1.89  0.81  1.31  0.51, 3.23 & 4.8  1.70, 3.03, 3.66 & 4.70. |

**Quantitation :** This may be done by using peak-area calculation method.

**6.9 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.

**6.9.1 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.

**6.9.2 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.

**6.9.3 Detection and Quantitation of Carbamates :**

**6.9.3.1 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 (21)** : 10% Ammoniacal silver nitrate

solution.

Mode of Spraying : Direct spraying with the reagent.

Colour of Spot : Black.

Responding Compounds : Propoxur (Baygon), Carbaryl (Sevin),

Carbofuran Zineb, Dipterex and

other Phenolic compounds, Phosphamidon (Demecron), Nuvan, Fenthion (Dalf, Bay tex), Metasystox.

**2. Diazotised Sulphanilic Acid after Alkaline Hydrolysis (24).**

**Preparation of Reagent:**

a) Sodium Hydroxide Solution (10%, w/v) 10 gms. of sodium hydroxide is dissolved in 100 ml. of distilled water.

b) Diazotised Sulphanilic Acid:

0.45 gms. of sulphanilic acid is dissolved in 4.5 ml. of concentrated hydrochloric acid and diluted to 50 ml. with distilled water. To 10 ml. of this diluted solution 10 ml. of 4.5% aqueous sodium nitrate solution is added. The resulting solution is maintained at 0oC for 15 minutes and an equal volume of 10% aqueous sodium carbonate solution is added just before use.

**Mode of Spraying:**

The developed plate is sprayed with 10% sodium hydroxide solution. Thereafter the plate is sprayed with diazotised sulphanilic acid after 5 minutes.

**Colour of the Spots:** Orange or Violet.

**Responding Compounds:** Propoxur, Carbaryl, Carbofuran, and other phenolic compounds.

**3. Alkaline Fast Blue B Salt (22):**

**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, Carbofuran, other Phenolic compounds.

**4. Phenyl Hydrazine Hydrochloride Reagent for Carbaryl: (8)**

**Preparation of Reagent :** Equal volume of 1% (w/v) aqueous solution of phenyl hydrazine hydrochloride is 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. Ceric Ammonium Nitrate Reagent for Carbaryl: (9,10)**

**Preparation of Reagent:**

a. **1% Ceric Ammonium Nitrate solution :** 1 gm of ceric ammonium nitrate is dissolved in 100 ml. of 20%(v/v) hydrochloric acid.

b. **1% Sodium Nitrite Solution :** 1 gm. of sodium nitrite is dissolved in 100 ml. of distilled water .

c. **10% Sodium Hydroxide Solution:** 10 gms. of sodium hydroxide is dissolved in 100 ml. of distilled water.

**Mode of Spraying:** The developed plate is sprayed with 10% sodium hydroxide solution (c) followed by 1% ceric ammonium nitrate solution (a). A violet spot appears immediately. On spraying with 10% sodium nitrite solution (b), the intensity of colour increases.

**6. 4-Amino-Antipyrine Reagent : (11,23)**

**Preparation of Reagent :**

A. **4-Amino-Antipyrine Reagent:** 1.5 gms of 4-amino-antipyrine, 3 gms. of sodium carbonate and 3 gms. of sodium bicarbonate are dissolved in 100 ml. of distilled water.

B. **Potassium Ferricyanide Solution:** 4 gms. of potassium ferricyanide, 3 gms. of sodium carbonate and 3 gms. of sodium bicarbonate are dissolved in 100 ml. of distilled water.

C. **Sodium Hydroxide Solution :** 5 gms. of sodium hydroxide is dissolved in 100 ml. of distilled water.

**Mode of Spraying**  **:** The developed plate is first sprayed with sodium hydroxide solution followed by 4 aminoantipyrine reagent and potassium ferrocyanide successively.

**Colour of Spot** **:** Red.

**Responding Compounds:** Propoxur, Carbaryl, Carbofuran and other

phenolic compounds.

**6.9.3.2 Gas Chromatographic Conditions for Identification of Carbamate Pesticides:**

Column : 15m X 0.53 mm (i.d.) DB-1 fused silica capillary column.

Carrier gas : Helium.

Flow rate : 10 ml./ min.

Column temperature : Initial : 50oC.

Final : 235oC.

Gradient to

Temperature : 20oC / min.

Duration : 3.5 min.

Detector : NPD.

**6.9.3.3 GC-MS Conditions for Identification of Carbamate Insecticides:**

Column for GC : 25 m. X 0.25 mm (i.d.), BPX-5,

Capillary column.

Carrier gas : Helium.

Flow rate : 1 ml. / min.

Column temperature : Initial temperature : 60oC.

Final temperature : 260oC.

Gradient to

temperature : 25oC./ min.

Duration: 1.0 – 11.0 min.

MS Detector : Finnigan ITD 800 ion-trap detection

system.

**6.9.3.4 HPLC Condition for Identification of Carbamate Insecticides:**

**Condition - 1 :**

Column : 150 mm X 4.6 mm (i.d.), 5 µm C18

column.

Column temperature : 42oC.

Mobile Phase : 18% Methanol in water for 0.5 minute followed by linear gradient

to 70% methanol in water over 28.5 minutes and then 70 to 100% methanol over 1 minute and finally 100% methanol for 10 minutes.

Detector : Fluorescence Detector.

Excitation : 330 nm.

Emission : 465 nm.

**Condition – 2 :**

Column : 250 mm X 4.6 mm (i.d.), 5 µm. 5-

phenyl column.

Column temperature : 25oC.

Flow rate : 1 ml./min.

Mobile Phase : 3% acetonitrile in water for 1

minute then linear gradient to 27%

acetonitrtile in water over 24 minutes then to 37% acetonitrile over 5 minutes, then to 100%

acetonitrile over 10 minutes and 100% acetonitrile for 5 minutes.

Detector : Fluorescence Detector.

Excitation : 330 nm.

Emission : 465 nm.

**1. Carbamate and Urea Pesticides (SPME/HPLC)**

**Conditions**

Sample: 3mL water containing 8ng/mL of each analyte in 10% NaCl  
SPME Fiber: PDMS/DVB, 60µm film  
Extraction: immersion, 40 min, rapid stirring  
Desorption: static, 5 min in acetonitrile: water (65:35); dynamic, valve open during run  
Column: SUPELCOSIL LC-8, 15cm x 4.6mm ID, 5µm particles  
Mobile Phase: acetonitrile: water   
(18:82 to 65:35 in 9 min, hold 3 min)  
Flow Rate: 2.0mL/min  
Temp.: 35°C  
Det.: UV, 240nm

**6.9.3.5 Quantitative determination of Carbamates by Chromatographic method:**

This is done by peak-area method as discussed in the preceding Sections for quantitation by chromatographic methods (GC, HPLC or GC-MS).

**6.10 Simultaneous Identification of Organo Phosphorous, Organo Chloro and Carbamate Insecticides:**

As the three classes of pesticides are extensively used in criminal poisoning cases due to their easy availability, rapid screening, identification and quantitation, are undertaken as deaths by pesticides in criminal cases account for more than 80%. The extract of biological or non-biological matrices may be subjected to the following analysis.

**6.10.1 TLC Identification of Organo Phosphorous, Organo Chloro and Carbamate Insecticide in Forensic Samples:**

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

Solvent systems:

No. 1 : Hexane : Benzene : Methanol : Acetone (50 : 30 : 19 : 1)

No. 2 : Benzene : Chloroform : Methanol : Acetic Acid (70 : 20 : 10 : 1)

No. 3 : Benzene : Hexane : Chloroform : Methanol : Acetic Acid

(50: 30 : 15 : 5 : 1)

**Chromatographic (Spray) Reagents for TLC Identification:**

I. 0.5% Rhodamine B solution in Ethanol.

**Preparation** : 0.5 gms. of Rhodamine B is dissolved in 100 ml.

of ethanol.

**Method of Spraying** : Direct spraying.

**Visualization**  : Under UV light.

**Colour of Spot** : Orange spot.

II. 0.5% Palladium Chloride Solution.

**Preparation**  : 0.5 gms. of palladium chloride is dissolved in 100

ml. 2N hydrochloric acid.

**Mode of Spraying** : Direct Spraying.

**Visualization**  : By naked eye.

**Colour of Spot** : Yellow / Yellow-Brown / Brown.

Table: hRf values of Insecticides.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Insecticide** | **hRf value of Insecticides in Different Solvent Systems.** | | |
| **No. 1** | **No. 2** | **No. 3** |
| Phosphamidon.  Aldrin.  Dieldrin.  Parathion.  Fenitrothion.  Malathion.  Propoxur.  Carbaryl.  Endosulfan.  Methyl Parathion. | 90  61  72  91  45  24  94  79  53 and 81  74 | 65  41  51  40  47  32  93  75  41 and 96  34 | 69  83  57  83  62  35  96  82  94 and 55  55 |

**6.10.2 TLC Identification of Quinalphos, Edifenphos and Carbofuran** (27, 28, 29)**:**

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

Spray Reagent and

Colour of spots etc. : Same as stated in 6.10.1.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name of Insecticide** | **Solvent Systems** | **hRf in Different Solvent System** | | |
| **No. 1** | **No. 2** | **No. 3** |
| Quinalphos  Edifenphos  Carbofuran | No.1: Hexane: Benzene: Chloroform (70:20:10,v/v).  No.2: Hexane: Benzene: Ethyl Acetate: Acetic Acid (60:25:15:0.5,v/v).  No.3: Hexane: Benzene: Methanol: Acetic Acid  ( 70:20:10:0.25,v/v).  No.1: Hexane: Chloroform: Benzene: Acetic Acid (70:20:10:0.25,v/v).  No.2: Benzene: Chloroform: Methanol: Acetic Acid: (60:30:10:0.5,v/v).  No.3: Hexane: Chloroform: Acetone: Acetic Acid (70:30:2:0.25,v/v).  No.1: Hexane : Ethyl Acetate : Chloroform : Acetone: (50:30:10:10, v/v).  No.2: Benzene : Chloroform : Acetone  (60:30:10,v/v).  No.3: Hexane: Benzene: Chloroform: Acetic Acid (60:20:20:0.5,v/v). | 35  21  62 | 74  58  81 | 50  73  55 |

**6.10.3 Micellar TLC for Identification of Some Insecticides (Organo-Phosphorous, Organo-Chloro and Carbamate)** (30) **:**

**Micellar TLC Conditions:**

Plate : Silica gel G (thickness 0.2 mm).

Solvent Systems : No. 1 : Hexane : Benzene : : 60 : 40.

No. 2 : Hexane : Benzene : Petroleum ether (40-60oC) : : : 60 : 30 : 10.

Method : Ascending method of development by two procedures.

**I. Direct Addition Method:**

10 μl of 1% solution of sodium lauryl sulphate is added to the concentrated acetone extract of the sample. The samples are spotted on activated TLC plate for development.

**II. Plate Spraying Method:**

The activated TLC plate is sprayed uniformly by 1% aqueous solution of sodium lauryl sulphate. It is dried and activated again at 110ºC for 5 minutes. The chromatogram is then developed as usual.

The chromatogram is also developed under normal environment (without micellar environment).

Chromogenic (Spray) Reagent for Identification: 0.5% Rhodamine B solution in ethanol (orange spots under UV) or 0.5% palladium chloride solution in 2N hydrochloric acid (naked eye: yellow / yellow-brown/ brown spot) or by a suitable reagent as described earlier. After location of spot the hRf values of samples and controls are calculated for identification thereafter.

**TABLE :** Micellar TLC Versus Conventional TLC Study for Multi-Residue Analysis of Pesticides in Samples.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **HRf Values in Different Solvent Systems** | | | | | |
| **Conventional TLC** | | **Micellar TLC** | | | |
| **Solvent System No. 1** | **Solvent System No. 2** | **Direct Addition Method** | | **Plate Spraying Method** | |
| **Solvent System No.1** | **Solvent System No. 2** | **Solvent System No. 1** | **Solvent System No. 2** |
| 1. Edifenphos  2. Phorate  3. Phhosphamidon  4. Quinalphos  5. Endrin  6. Endosulfan  7.Methyl Parathion  8. Propoxur. | 65.38  35.83  25.53  60.30  33.57 and 67.20  38.46 and  69.23  28.46  47.53 | 58.05  28.46  22.23  52.15  28.05 and  61.57  30.30 and  63.07  23.58  44.61 | 66.72  36.49  29.92  62.15  33.76 and  68.25  38.66 and  69.92  29.20  48.25 | 59.25  29.20  22.48  52.57  28.32 and  62.12  31.12 and  63.92  24.06  45.10 | 69.82  40.15  29.75  63.58  35.52 and  71.25  45.22 and  75.25  33.12  52.35 | 60.85  33.35  26.15  55.72  34.62 and  65.65  32.76 and  66.69  27.54  47.48 |

**6.10.4 Identification of some pesticides (Organo-phosphorous, Organo -chloro and Carbamates) by HPTLC:**

**HPTLC Conditions :**

Plate : Pre-coated HPTLC Plate, Silica gel G 60 F254,

Aluminium.

Format : 10 cm. X 10 cm. / 20 cm. X 10 cm., Thickness –

0.2 mm.

Spotting Volume of

Concentrated Extract

of Sample : 5 μl of concentrated extracts of samples as well as

controls.

Separation Technique : Ascending.

Migration Distance : 6 cm. / 8 cm./ 10 cm. (3 modes)

Mobile Phase : System 1 : Hexane : Tolune : Methanol

(7.0: 2.5: 0.5)

System 2 : Cyclo-Hexane : Iso-Octane : Acetone :

(6.5 : 2.5 : 1.0)

System 3 : Cyclo-Hexane : Iso-Octane : Benzene :

(6.0. 2.0 : 2.0)

Migration Time : 6 cm. : 10 – 12 mins.

8 cm. : 20 – 21 mins.

10 cm. : 28 – 30 mins.

Detection : Normal / U.V.

Densitometric Mode

(CAMAG HPTLC) : Scanning (CAMAG Scanner II)

Absorbance / Reflectance, Wave length : 254 nm.

**TABLE : HPTLC Study for Multi-Residue Analysis of Pesticides in Forensic Samples.**

|  |  |  |
| --- | --- | --- |
| **Mobile Phases** | **Mirgration Distance (in cm.)** | **hRf of Insecticides in Different Mobile Phases** |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Endo-sulfan** | **Phospha-midon** | **Phorate** | **Methyl Para-thion** | **Chlor pyri-phos** | **Propo-xur** | **Quinal-phos** | **Carbo-furan** |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 1 | 10  8  6 | 15 & 88  16 & 87  13 & 86 | 28  19  26 | 85  81  75 | 75  69  66 | 75  74  62 | 2 & 9  20 & 87  17 & 75 | 72  66  58 | 20  15  12 |
| 2 | 10  8  6 | 15 & 85  13 & 81  13 & 83 | 25  19  17 | 85  81  75 | 40  25  28 | 70  62  60 | 58 & 95  38 & 75  41 & 92 | 60  50  49 | 60  60  50 |
| 3 | 10  8  6 | 20 & 55  19 & 55  20 & 55 | 15  14  12 | 67  71  75 | 10  10  8 | 65  69  75 | 50 & 85  44 & 84  41 & 75 | 20  20  20 | 85  80  83 |

#### 6.10.5 Gas Chromatography /GC-MS conditions for Some classes of Pesticides (20, 31)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analysis | Column | Length (m) | Carrier gas flow | Injection | Column  temp. (°C) | Gradient. To temp. | Duration | Detector |
| Organo- phosphate | DB17 | 15 | N2  4.5ml min-1 | Direct  200°C | 140  140  180 | 10°C min-1 to 180°C  6°C min-1 to 270°C | 0.5 min  10.5 min | FPD |
| Carbamate | DB1 | 15 | He  10 ml min-1 | Cool  on-col | 50  50 | 20°C min-1 to 235°C | 0.5 min  3.5 min | NPD |
| Organo-  Chlorine | DB1 | 30 | He  Pressure  7.2 p.s.i. | Splitless  175°C | 100  100  225 | 25°C min-1 to 225°C  2.5°C min-1 to 265°C | 1.0 min  3.0 min | ECD |
| Pyrethroid | DB1 | 30 | He  Pressure  7.2 p.s.i. | Splitless  175°C | 100  100  225 | 25°C min-1 to 225°C  2°C min-1 to 275°C | 1.0 min  9.0 min | ECD |
| Cloralose  (tris deriv) | DB1 | 30 | He  6 ml min-1 | Splitless  175°C | 100  100  225 | 25°C min-1 to 225°C  2°C min-1 to 250°C | 1.0 min  1.5 min | ECD |
| Metaldehyde  (acetaldehyde) | GS-Q | 30 | N2  5 ml min-1 | Splitless  220°C | 110  110 | 5°C min-1 to 130°C | 1.0 min  5.0 min | FID |
| All the above analyses are on 0.53 mm I.D. fused silica capillary columns with nitrogen make-up gas.  Where a pressure is quoted instead of a flow, this is a constant column head pressure (flow 5-10ml min-1). 1 p.s.i. = 6894.76 pa.  FPD = Flame photometric detection: NPD = nitrogen-phosphorous detection; ECD = electron-capture detection: FID = flame ionization detection  **GC-MS confirmation on 0.25 mm ID capillary with Finnigan ITD 800 ion-trap detection (ITD) system:** | | | | | | | | |
| OP.OC.  Pyrethroid | BPX-5 | 25 | He  1 ml min-1 | Splitless  22°C | 60  60 | 25°C min-1 to 260°C | 1.0 min  11.0 min | ITD |
| Carbamate |  | As above |  | 150°C |  | As above |  |  |
| Chloralose |  | As above |  | 250°C |  | As above |  |  |
| Strychninc |  | As above |  | 270°C | 260 | Isothermal | 40.0 min | ITD |
| Aldicarb  As nitrile |  | As above |  | 300°C | 60  60 | 25°C min-1 to 260°C | 4.0 min  3.0 min | ITD |

Columns: DB series and GS-Q are manufactured by J&W (Folsom. CA): BPX-5 by SGE (Ringwood. Australia). Injection volumes: 1μl for ECD. NPD and ITD. 2μl for FPD and 25μl of vapour for metaldehyde analysis using FID. GC instruments: for Organo Phosphorous compounds. Ai93 (Ai Cambridge Ltd., UK). For all others. HP5890 (Hewlett-Packard, Avondale, PA, USA).

**6.11 FUNGICIDES:**

The characteristics of compounds under different classes are presented below.

**TABLE:** **Fungicides under different classes.**

|  |  |  |
| --- | --- | --- |
| **Class** | **Compounds/Formulation/Preparations** | **Salient Features** |
| Sulphur Fungicides  Copper Fungicides.  Mercury Fungicides. | **Inorganic type:**  1. Mixture of lime and lead arsenate.  2. Sulphur lime.  3. Calcium monosulphide.  4. Ammonium polysulphide.  5. Barium sulphide (solbar).  6. Potassium sulphide.  **Inorganic:**  Bordeaux Mixture:  A mixture of copper sulphate and lime in the form of paste.  (Other name : Boville Bordelaise).  Burgandy Mixture (Soda mixture):  1 part of copper sulphate + 1 or 2 parts of crystalline sodium carbonate.  Chestnut Compound:  2 parts of copper sulphate + 11 parts of ammonium carbonate. (well powdered, thoroughly mixed and stored in air-tight containers).  Copper Oxychloride  Copper oxide.  Inorgnic Mercury Compounds:  Mercuric Chloride, Mercurous Chloride.  Organic Mercury Compounds:  Ethyl mercuric chloride, Ethyl mercuric phosphate, Methyl mercuric sulphate, Methyl mercuric cyanide, Cereson –M{N-(ethyl mercuric)-p-toluene sulfona-mide}, Panogen: (methyl mercuric dicya- nodiamine) Agacol (methyl ethyl mer-curic chloride), Uspulan, Germisan, Semesan. | Different theories viz. sulphur vapour theory, sulphur dioxide theory, pentathionic acid theory, hydrogen sulphide theory pro- posed for the action of sulphur as fungicide are now obsolete. The direct action theory is now accepted. According to the theory, sulphur acts as hydrogen acceptor in the metabolic system and disturbs normal hydrogenation and dehydrogenation reaction in the cell. –H2S formed in the fermentation process disturbs the Krebs cycle.  Extraction of inorganic compound is to be done by dry or wet ashing.  These mercuric compounds (organic and inorganic) are very toxic. The toxicity depends on their solubility in water. Soluble salts of mercury such as mercuric chloride are more toxic than the insoluble salts such as mercurous chloride. |

|  |  |  |
| --- | --- | --- |
| **Class** | **Compounds/Formulation/Preparations** | **Salient Features** |
| Miscellaneous Orga- nic Fungicides.  1. Quinone Fungici-  dal Compound.  2. Heterocylic Nitro-  genous compound  3. Organo-Tin  Compounds.  4. Guanidine Moiety  5. Imidazoline  Moiety.  6. Napthaquinone  Moiety.  7. Anthraquinone  Moiety. | Chloranil (2, 3, 5, 6 – tetrachloro 1, 4, - benzoquinone).  Captan :  N-(trichloromethyl thio)-4- cyclohexane 1, 2 – dicarboximide.  Flopet:  N-(trichlorumethylthio) phthalimide.  Brestan:  (triphenyl tin acetate).  Duter :  (triphenyl tin hydroxide).  Dodine :  N-dodecyl guanidine acetate.  Glyodin :  2-Heptadecyl –  2-imidazoline acetate.  Dichlone:  2, 3 dichloro 1, 4 napthaquinone.  Dithianon :  2, 3-dichano-1, 4 dithio-anthraquinone. | Yellow crystalline substance,  insoluble in water but soluble in organic solvents (xylene, chloroform, ether, acetone), unstable to alkalis and moisture LD50 for rat : 4000 mg/kg.  White crystalline substance, M.P. 170oC, practically insoluble in water but slightly soluble in most of organic solvents. In moist condition it is hydrolyzed to tetrahydrophthali- mide.  White crystalline substance, M.P. 177oC, insoluble in water but poorly soluble in organic solvent.  White crystalline substance, M.P. – 124–125oC, slightly soluble in water and most organic solvent LD50 for rats : 125 mg./kg. Marketed as 20% or 60% wettable powder.  White crystalline compound, practically insoluble in water and most organic solvents, M.P. 120oc, L.D50 for rat: 500 mg. / kg.  White crystalline powder, M.P. 136oC, poorly soluble in cold water, moderately in hot water and alcohol but insoluble in organic solvents. Stable to acids and alkalis. It is marketed as 70% wettable power.  Orange coloured powder, practically insoluble in water but soluble in propylene glycol and ethylene dichloride. On prolonged treatment with al-kali it is hydrolysed to aminoethylamide of stearic acid.  Yellow crystalline substance,  insoluble in water but soluble in organic solvents, unstable towards alkali.  LD50 for rat : 1300 mg./ kg.  LD50 for rat : 1015 mg./ kg. |

**6.11.1 Analysis of Fungicides :**

As fungicides may be of organic or inorganic in nature (containing sulphur in organic compounds or copper, mercury in inorganic compounds), the presence of elements viz. sulphur or copper or mercury is to be established or searched prior to the analysis of individual fungicides for their identification. The inorganic fungicides may be water soluble or insoluble and the elements i.e. copper or mercury, if present is isolated by dry or wet ashing of biological materials. Thus, the analysis, of fungicides in forensic samples involve their isolation, test for elements (S or Cu or Hg) and identification.

**6.11.1.1 Isolation of Fungicides in Biological Materials:**

**Isolation of Elemental sulphur:**

Elemental sulphur is isolated from biological and non-biological materials by extraction with carbon disulphide or pyridine. Alternatively, the sample is subjected to preliminary treatment with hydrochloric acid to remove acid –soluble constituents. The residue is then dried at 105oC for several hours (any amorphous sulphur is then converted to crystalline form and becomes soluble in carbon disulphide). The extract thus obtained in each of the above two cases is subjected to identification test for element etc.

**Isolation of Organic Sulphur Fungicides in Biological Material:**

The dithio-carbamates possess the insecticidal and fungicidal properties. These are water soluble compounds (prepared by the reaction between p and s – aliphatic and aromatic amines and alcoholic solution of carbon disulphide). Their extraction in biological materials is normally difficult. However, the following procedures may be conveniently adopted.

1. 50-100 gms. of biological material is added to 50 ml. of ethyl or methyl alcohol in a conical flask and heated gently for about 15 minutes at 60oC. It is cooled and kept aside for 2 hours and then filtered through No. 1 filter paper. The filtrate is concentrated on hot water bath to a minimum volume sufficient for analysis.
2. 50 gms. of biological material is added to 50 ml. of n-butanol and mixed thoroughly. It is heated gently at 60oC on a water bath for 15-20 minutes. The material in the flask is filtered through No. 1 filter paper. The filtrate thus obtained is concentrated under vacuum to a minimum volume sufficient for chemical analysis.

**Isolation of Copper Fungicides in Biological Materials:**

The biological material is subjected to dry ashing or wet digestion as described in Section 3. The residue obtained in dry ashing is dissolved in hydrochloride and subjected to the chemical tests for identification. The final extract obtained in wet digestion is also subjected to the chemical tests etc. for analysis.

**Isolation of Inorganic Mercury Fungicides in Biological Materials:**

20 gms. of biological material is macerated and thoroughly shaken with 50 ml. of distilled water. It is warmed on a hot water bath for 15 minutes at 60oC with frequent stirring. It is filtered. The residue is washed with 10 ml. portion of distilled water twice. The filtrates are combined and centrifuged. The supernatant liquid is concentrated and subjected to chemical tests.

**Isolation of Organic Mercury Fungicides:**

This is done by wet digestion method as described in Section 3. The final extract obtained is subjected to chemical analysis.

**Isolation of Miscellaneous Organic Fungicides in Biological Materials:**

50 gms. of biological material is extracted with diethyl ether in acidic or alkaline condition for extraction of miscellaneous fungicides (dicarboximide, guanidine, imidazoline, naphthaquinone moieties).

**6.11.1.2 Identification of Fungicides:**

The extract obtained in any of the above methods is subjected to chemical tests.

**Test for Sulphur Fungicides:** (18)

1. One drop of clear extract is spotted on freshly prepared black thallium sulphide paper (prepared by dipping or bathing No. 42 filter paper in 5% thallous carbamate or acetate solution for a few minutes and draining off excess liquid. It is then dried by hot air and dipped in a warmed solution of ammonium sulphide at 80oC when deposit of thallous sulphide appears). A brown red or light brown fleck appears at the site of spotting (The rest portion of paper appears perfectly white).

1. 1 drop of test solution is treated with a drop of 2% sodium sulphide solution. 0.5 ml. of acetone is added immediately. A blue or greenish blue colour appears indicating the presence of elemental sulphur.

Limit of identification: 6 µg of sulfur. (Polysulfides interferes)

1. Detection through conversion into hydrogen sulphide (16):A micro test tube is used. A slight amount of sample is mixed with a few mg of benzoin and the mouth of the tube is closed with moist lead acetate paper. The test tube is then plunged into a glycerol bath previously heated to 130oC. The temperature is then raised to 150oC. According to the amount of free sulfur the paper acquires rapidly a black or brown stain.

Limit of identification: 0.5 µg of sulfur.

If free or loosely bounded sulfur is evaporated to dryness, first with a drop of alkali hydroxide and then with a drop of 1% KCN, alkali thiocyanate is formed which, after acidification with dilute H2SO4, shows the familiar ferric thiocyanate colour reaction (blood red colouration).

Limit of identification: 0.5 µg of sulfur.(17)

**Test for Elemental Sulfur**

**a) Test with silver nitrate solution:** (13)

A drop of the test solution is treated with few drops of 1% solution of silver nitrate in dilute nitric acid. The mixture is turns black due to silver sulfide.

**b) Test with Iodine-Azide Solution:**

A drop of the test solution is treated with a few drops of an iodine-azide solution (3g of NaN3 in 100 ml 0.1 N iodine). Brisk evolution of gas through the mixture is observed.

**Tests for Dithiocarbamates** (12)

**Test by Conversion into Cupric Salts Soluble in Organic Solvents:**

A characteristic feature of water-soluble dithiocarbamates and their N-methyl substituted derivatives is, their precipitation as brown cupric salts, which dissolves in water-immiscible organic solvents and turns organic layer red-brown.

Procedure:- A drop of neutral test solution in water is treated with 1 drop of cupric chloride solution in acetic acid (prepared by dissolving 1gm of cupric chloride in 1:1 acetic acid solution, v/v) and shaken with 2ml of chloroform. The reddish-brown colouration of chloroform layer indicates the presence of dithio carbamates and their N-methyl substituted derivative.

Reagent: 1% aqueous solution of CuCl2 mixed with an equal volume of

1:1 acetic acid.

**Test for Copper in Copper Fungicides:**

The extract (from dry ashing or wet digestion) may be subjected to test for Cu2+ as described in Section 5.

**Test for Mercury in Mercury Fungicides:**

Soluble salts of mercury such as mercuric chloride are more toxic than the insoluble salts such as mercurous chloride. It is necessary to distinguish between the soluble and insoluble forms of mercury salts to assess their toxicity. This is done as follows:

**Procedure for detection of water-soluble mercury salts**

5g of the tissues are macerated with 25 ml of distilled water and then filtered through Whatman No.1 filter paper. The residues on the paper are washed twice with 10 ml portion of distilled water. The filtrates are combined, centrifuged and the supernatant liquid is subjected to Reinsch test. A positive finding establishes the presence of soluble salts of Mercury.

The extract (from wet digestion or ashing) may be subjected to tests for Hg2+ as described in Section 5.

**6.12 HERBICIDES:**

The herbicides in use include different classes of chemical compounds viz. phenoxy acids (phenoxy acetic or propionic or butyric acid), substituted ureas, triazines, dinitroaniline, chloroacetamides, thiocarbamates etc. These compounds are often used in combination with benzonitriles, bromoxynil or loxynil to broaden the range of weed control.

**6.12.1 Analysis of Herbicides in biological materials:**

The herbicides are extracted in biological materials by solvent extraction or special method. The purified extracted is then subjected to chemical analysis for identification and quantitation, if necessary.

**6.12.1.1 Extraction and Stripping of Herbicides in Biological materials:**

**Extraction of Phenoxyacids and Benzonitriles:**

The extraction in biological materials is done at acidic PH with medium polar solvents viz. diethyl ether or methylene chloride or ethyl acetate.

**Alternative Method:**

By Solid Phase Extraction:

**Extraction of Urea in Biological Materials:**

This is done by direct solvent extraction with dichloromethane or chloroform.

**Extraction of Triazines in Biological Material:**

This is done by direct solvent extraction with dichloromethane or acetonitrile or ethyl acetate.

**Alternate Method:**

By solid phase extraction using C18 cyclohexyl or XAD resin.

**Extraction of Dinitroaniline, Chloracetamides and Thiocarbamates in Biological Materials:**

This is done by solvent extraction with dichloromethane.

**Stripping:**

1. By clean-up in a Florisil column.
2. By passing through C18 SPE cartridges.
3. By liquid-liquid partition.
4. By column (alumina or Silica gel G or Florisil) chromatography.

**6.12.1.2 TLC and HPTLC detection of Herbicides in Biological Materials:**

The purified extract of the biological materials may be subjected to TLC or HPTLC method using any of the following chromatographic conditions for their detection (by comparing with the hRf of control samples of herbicides).

Table : TLC / HPTLC conditions for detection of Herbicides.

|  |  |  |
| --- | --- | --- |
| **Compound** | **TLC / HPTLC Condition** | **Detection** |
| 1. Altrazine 17.  2.Triazine Herbicide.  3. Semicarbazone  Herbicides.  4.Triazine Herbicides  (Simazine, Atrazine  Ametryne, Prome-  Tryne, Aziprotryne) | TLC Plate: Silica gel G (0.2 mm thickness).  Solvent System: Toluene: Acetone (85: 15).  Development: By ascending method.  Spray Reagent: 4, 4-tetramethyl amino phenyl methane.  Spraying: Direct.  Visualization: Under UV.  TLC Plate: Silica gel G (0.2 mm. thickness).  Solvent system: Toluene: Acetone (9: 1).  or  Methylene Chloride: Acetone  (9 : 1).  or  Chloroform: Ethyl Acetate  (9: 1).  or  Benzene: Acetic Acid (9: 1).  Development: Ascending Method.  Visualization: Under UV or Spraying with o-Tolidine.  TLC Plate: Silica gel G (0.2 mm. thickness).  Solvent System: Benzene: Chloroform: Methanol  (9: 3: 2,).  Chloroform: Methanol  (3 : 1).  Development: Ascending method.  Spray Reagent: 1% Ferric Chloride in n-butanol and 3%  2, 4- dinitro phenyl hydrazine in a mixture of chloroform-methanol (9 : 1).  TLC plate: Aminoplast (a layer of carbamide-  formaldehyde polymer) or cellulose or cellulose acetate.  Solvent system: Cyclo-hexane: acetone (9: 1)  or  Water: Acetone (7 : 3).  or  Water: Methanol (1: 1).  Development: Ascending Method.  Visualization: Under UV. | By Rf value.  As above.  As above.  As above. |

|  |  |  |
| --- | --- | --- |
| **Compound** | **TLC / HPTLC Condition** | **Detection** |
| 5. Altrazine, Crimidine, Simazine, Hexazinone, Desethylatrazine.  6. Herbicides (MCPB,  MCPA, 2, 4D, 1-  Napthyl Acetic acid  2, 4 Dichloro Phe-  noxy Pripionic Acid,  2, 4, 5T, Decamba).  7. Herbicides (Urea  derivatives) (Butu  ron, chlorobromu-  ron, chlorotoluron,  diuron, Fenuron,  Isoproturon, Linu  ron, Monuron, Methabenzithiozuron,  Metoxuron, Bebu-  ron).  8. Triazine Herbicides  (Norazine, Simazine  atrazine, Propazine,  Simetone, Aratone,  Prometone, Desme-  Tryne, Simetryne,  Ametryne, Prome-  Tryne).  9. Urea Herbicides  (Fenuron, Metoxu-  ron, Fluometuron,  Chlortoluron, Me-  Thabenzthiazuron,  Chlorxuron). | HPTLC Plate: Silica gel F (Nano-Dusil-20-UV).  Solvent System: Chloroform: Acetone (9.5 : 0.5).  Development: Ascending method.  Detection and quantitation: By scanning densitometry of fluorescence quenching at 254 nm.  TLC Plate: Silica gel G (0.2 mm. thickness).  Solvent systems: Benzene: Ethyl Acetate (17 : 1).  Or  Toluene : Ethyl acetate  (17 : 1).  Development: Ascending Method.  Detection: Under UV or Derivatization with 4-Bromo  Methol – 7-methoxy coumarin prior to TLC  To give fluorescent spots of separated  derivatised product of herbicides.    Layer : Silica gel GF.  Development: Two dimensional.  Solvent system: First with diethyl ether-toluene (1:3)  Followed by chloroform-nitromethane(1 : 3) in the orthogonal direction.  Visualization : Under UV.  Plate : Silica gel 60F for TLC and HPTLC.  Solvent system : Pentene : Chloroform : Acetonitrile  (50 : 40 : 10v/v).  Development: Ascending Methanol.  Detection: By fluorescence under UV.  Plate: C18 F HPTLC.  Solvent: Methanol: Water  (75 : 25v/v).  Development: By ascending method.  Visualization : By fluorescence quenching. | By fluorescence quenching & comparing hRf value.  By comparing hRf values.  As above.  As above.  As above. |
| 10. Urea Herbicides  (Metoxuron, Chlo  rotoluron, Meto –  bromuron).  11. Atrazine and its  Derivatives. | Plate: Amino-bonded Silica gel F.  Solvent system: Chloroform: Toluene (8 : 2v/v).  Development: Ascending method.  Visualization : By fluorescence quenching.  Plate : Silica gel 60 HPTLC.  Solvent system: Chloroform: Acetone (3 : 2v/v) followed by n-Propanol : n-Butanol : Acetic acid : Water (1 : 1 : 1 : 0.5v/v).  Development: Multiple development.  Visualization : Under UV. | As above.  As above |

**6.12.1.3 Gas Chromatographic Method for Herbicides:**

Various groups of herbicides viz. phenoxy acetic acids, benzonitriles and substituted urea can be subjected to GC only after derivatization viz. alkyl, chloralkyl, silyl or pentafluoro benzyl derivatives. The use of methyl ester derivatives is common (by using diazomethane). Boron trichloride or trifluoride is also used. Alkyl derivatives containing fluorine or chlorine atom is used as derivatising agent to make the derivatives susceptible to ECD viz. pentafluorobenzyl ester (PFB), pentafluorobenzyl bromide (PFBB). The use of diazomethane or trimethyl silyl diazomethane as derivatising agent for benzonitrile is popular. Alkylation of phenyl urea herbicides is carried out by alkyl iodide. Sulphonyl urea herbicides are thermally labile. The derivatization to methyl-derivatives overcomes the problem.

**Column :** Packed column with supports coated with stationary phases of different polarity. Non-polar methyl silicones. DC-200, SE-30, OV-1, OV-101 together with more polar silicones OV-17, OV-210 and OV-225 are often used.

Capillary Columns (WCOT : 12-15 m X 0.2 mm (i.d.) with bonded stationary phases. Capillary column coated with low polarity phases viz. BP-1, HP-1, DB-1, HP-5, DB-5 are widely used (standard columns of different manufactures).

**Detector :** FID has now been replaced by NPD as specific detector for nitrogen containing herbicides viz. triazines, substituted ureas, thiocarbamates. ECD is used for halogenated derivatives of herbicides (chloro and fluoro derivatives). FID is occasionally used for triazines and thiocarbamates.

**Quantitation:** By peak area method.

**6.13 PYRETHROID INSECTICIDES** (26)**:**

The pyrethroids have also emerged as a major class of synthetic organic insecticides since its commercial production in 1976 for agricultural applications. These compounds are comparatively less toxic than other classes in respect of mammalian toxicity. Owing to their availability these compounds are misused in criminal poisoning cases. Thus the analysis of this class of pesticides has become important in the present context.

The chemical name of compounds and their characteristics are given below.

**TABLE: Name and Characteristics of Pyrethroids.**

|  |  |  |
| --- | --- | --- |
| **Name** | **Chemical Name** | **Characteristics** |
| 1. Fenvalerate.  2. Permethrin./ SR/ dichlorovinyl  3. Cyfluthrin | (RS)-α-cyano-3-phenoxy benzyl(RS)-2-(4-chlorophenyl)-3-methyl butyrate.  Or  Cyano - (3-phenoxy phenyl) methyl – 4-chloro-α (1-methyl ethyl) benzeneacetate.  3-phenoxy benzyl (1 RS, 3 RS; 1 RS, 3 )-3- (2, 2-) 2, 2-dimethyl cyclopropane carboxylate.  Or  (3-Phenoxy phenyl) methyl.3-(2,2-dichloro- ethenyl) 2, 2-dimethyl cyclopropane carboxylate.  (RS)-α-cyano-4 fluoro-3-phenoxybenzyl.  (RS)-α-cyano-4 fluro-3-phynoxybenzyl. (1RS)-cis-trans-3(2, 2 dichlorovinyl)  2,2-dimethyl cyclopropane carboxylate. | Viscous yellow or brown liquid, sometimes partly crystalline at room temperature. Very low soluble in water but farely soluble in hexane, methanol.  Non systemic. It is available commercially as emulsion or wettable powder. It may be stable in acid media but hydrolysed in alkaline media also available in combination with other insecticides fenitro-thion, dimethoate etc.  LD50 for rats: 320-400 mg./kg.  Yellow-brown to brown liquid, sometimes tend to crystalline at room temperature. Almost insoluble in water, but fairly soluble in methanol, xylene. Stable to heat, more stable in acid than alkali, also available in combination with other insecticides (dimethoate, mala- thion, pyrethrins etc)  LD50 varies with the  percentage abundances of isomers.  Viscous, partly crystalline, amber oil, M.P.-64oC, M.P. technicalgrade106oC. Practically insoluble in water but soluble in organic solvents thermally stable at room temp., available commercially as  emulsion, wettable powder, also available in combination with other insecticides such as dimethoate, dichlorovos etc.  LD50 for rat = 500 mg./kg |
| 4. Deltamethrin.  5. Fenpropathrin.  6. Bifenthrin. | (S)- α-cyano-3 phenoxy benzyl (IR, 3R)-3-(2,2 dibromovinyl)-2,2 = dimethyl cyclo-propane carboxylate.  Or  (S)- α-cyano-3 phynoxy benzyl (IR)-cis-3-(2,2-dibromovinyl) 2,2 = dimethyl cyclo-propane carboxylate.  (RS)- α-cyano-3 phynoxy benzyl 2,2,3,3- tetramethyl cyclopropane-carboxylate.  Or  Cyano-3 phynoxyphenyl) methyl 2,2,3,3- tetramethyl cyclopropane-carboxylate.  2-Methyl biphenyl-3-yl  methyl (Z)-(1RS, 3RS)-3-(2-chloro-3,3,3 = trifluroprop-1=enyl) 2,2 dimethyl cyclopropane carboxy late.  Or  2-Methyl biphenyl-3-yl methyl (Z)-(1RS, 3RS )-cis-3-(2-chloro-3,3,3 = trifluroprop-1-enyl) 2,2 dimethyl cyclopropane carboxylate. | Colourless crystals, MP -100-120oC, practically insoluble in water but soluble in dioxane, cyclohexanone, dichloromethane, ace-tone, benzene etc. Extremely stable on exposure to air, UV radiation and in sunlight, cis – trans isomers are available  spitting of the ester bond and loss of bromine occurs. More stable in acidic than in alkaline media.  Non systemic, available comm-ercially as emulsions, wettable powders, also available in com-bination with other insecticides (dimethoate, triazophos, endo-sulfan, chlorpyriphos).  LD 50 for ducks: 4640 mg./kg.  Yellow brown solid, M.P. 45-50oC, Practically insoluble in water but soluble in xylene, cy-clohexanone and methanol, decomposed in alkaline condition, exposure of light and air leads to oxidation and loss of activity, commercially available as emulsion and wettable powder, available in combination with other insecticides (fenitrothion, clofe-ntzine.  LD 50 for rats = 70 mg./kg.  Viscous liquid or crystalline or waxy solid, MP-51-66oC,  insoluble in water, slightly soluble in heptane and methanol stable for 2 years at 25oC, decomposes slowly in sunlight. Commercially available as emulsions, wettable powder.  LD 50 for rats = 2000 mg./kg. |

**6.13.1 Analysis of Pyrethroids:**

The analysis involves three major steps viz. extraction of pyrethroids in biological materials, their stripping and identification/quantitation by using TLC, GC and HPLC methods.

**6.13.1.1 Extraction of Pyrethroids:**

**Method 1:** Extraction in Biological and Non Biological Materials by Solvent

Extraction:

A. The extraction is done by solvent extraction using organic solvent viz. hexane, ethyl acetate, solvent ether, benzene or a binary solvent mixture in definite proportion viz. hexane-acetone, hexane-isopropanol, petroleum ether-solvent ether as described earlier in details in case of organo-phosphorous insecticides.

B. Samples of low water content i.e. cereals, grains is homogenized and extracted with binary solvent mixture viz. acetone-hexane (1 : !), hexane-isopropanol (3 : !) or single solvent viz. methanol, acetone or acetonitrile. Pre-soaking of sample with distilled water for 5 minutes may improve the extraction rate.

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

D. Soil samples are extracted with acetone-hexane, methanol, acetone or acetonitrile.

E. Animal tissues (of high lipid content) are homogenized and extracted with acetone-hexane or diethyl ether-light petroleum ether or ethyl acetate in presence of anhydrous sodium or ammonium sulphate.

F. The solvent partition system used in the pyrethroids residue analysis include acetone-hexane, acetone-dichloromethane, acetone-light petroleum, acetonitrile-light petroleum.

**Method 2:** Solid Phase Extraction:

The pyrethroids in human plasma are extracted with a C18 Sep-Pak cartridge. 1 ml. of plasma and 3 ml. of water are applied to C18 Sep-Pak cartridge and eluted with methanol-water (5: 95). Only a few ml. of methanol is needed to elute pyrethroids quantitatively.

**Method 3:** Super Critical Fluid Chromatography:

Carbon dioxide is used as extractant because of its moderate critical temperature (31oC) and pressure (73 atms). Methanol or benzene-isopropanol can be used as organic modifier and is added to adjust the dissolving power of the fluid.

**6.13.1.2 Stripping of Extracted Materials:**

The extract obtained in the foregoing section may be purified (stripping) by any of the following methods.

A. **Liquid-Liquid Partition:**

Liquid-liquid partition is a well established technique for the separation of pesticides from the interferences. When the samples is of animal or plant origin, it can be homogenized with a mixture of a non-polar solvent and a water immiscible solvent viz. hexane-acetone. After the suction filter of the homogenate, the filtrate is partitioned with a polar solvent in the presence of sodium chloride solution. The pyrethroids are partitioned in the organic phase and interferences or co-extractives remained in the aqueous phase.

B. **Adsorption Chromatography:**

It is used as an additional clean up for eliminating the interferences of co-extractives or interferences. The commonly used sorbent materials include viz. Florisil, Silica gel G, alumina. These adsorbents show a polar nature and retain the lipid fraction on elution with organic solvents of low polarity. Thus, the above column materials are suitable for clean up of a polar analyte such pyrethroids. In case of Florisil, the material is to be activated at 130oC for at least 8 hours and then deactivated to an appropriate degree by the addition of 5% water ( if too much water is added, the efficiency in fat retention will be insufficient). Alumina is also effective as a sorbent material. But it is to be activated at 130oC for 3-6 hours. The natural alumina shows a strong retaining activity for fats and lipids (1 gm of alumina may retain about 40 mg of fat.).

**6.13.1.3 Detection of Pyrethroids by TLC:**

The purified extract as described earlier may be subjected to TLC analysis by following the conditions as described below.

**TLC Conditions:**

Plate : Silica gel G (0.25 mm. thickness).

Solvent Systems : No. 1 : Petroleum Ether (60-80oC) : Diethyl

Ether (9 : 1).

No. 2 : Cyclohexane : Toluene (7 : 3)

No. 3 : Cyclohexane : Toluene (6 : 4)

No. 4 : Hexane : Acetone : Acetic Acid

(25 : 25 : 1)

No. 5 : Toluene : Diethyl Ether : Acetic Acid (75 : 25 : 1)

No. 6 : Hexane : Chloroform : Acetic Acid : (9 : 5 : 0.5 )

No. 7 : Hexane : Benzene (4.5 : 5.5).

No. 8 : Benzene

No. 9 : Hexane : Chloroform : Benzene

(4.5 : 0.5 : 5.0)

No.10: Hexane : Chloroform (7 : 3)

TABLE : hRf Values of Pyrethroids and Metabolites in Different Solvent Systems.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **hRf in Different Solvent Systems** | | | | | **Spray Reagent** |
| **No. 1** | **No. 2** | **No. 3** | **No. 4** | **No. 5** |
| Allethrin  Cypermethrin  Cypermethrin  Deltamethrin  Fenvalerate  4-Hydroxy Fenvalerate  Permethrin | 18  35  54  49  45  -  42  -  68  59 | -  11  13  10  -  44  8  -  30  22 | -  -  38  -  -  12  42  -  -  - | -  -  -  -  -  31  78  62  -  - | -  -  -  -  -  -  72  41  -  - | 1. Sodium Hydroxide Phospho-  molybdic Acid- O-Toludine.  2. Bromine followed by 0.1%  O- Toludine. |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **hRf in Different Solvent Systems** | | | | | **Spray Reagent** |
| **No. 6** | **No. 7** | **No. 8** | **No. 9** | **No. 10** |
| Cis-Permethrin  Trans-Permethrin  Cis-permethrin  Trans-Cypermethrin  Deltamethrin  Fenvalerate | 87  82  57  49  -  45 | 86  76  64  56  53  52 | 88  85  83  80  50  80 | 75  67  48  42  82  39 | 79  69  46  37  45  36 | Silver Nitrate |

**6.13.1.4 Gas Chromatographic Conditions for Analysis of Pyrethroids:**

GC is the method of choice for the analysis of pyrethroids residues. In case of halogen containing pyrethroids viz. biphenthrin, cyfluthrin, cypermethrin, deltamethrin etc. ECD is the detector of choice (detection limit nano to pico gm. level). For other pyrethroids having no halogen atom, FID can be used. The stationary phases include OV-1, OV-101, SE-30, SP-2100, DC-200 etc. The purified extract of sample may be subjected to GC. The GC conditions for different pyrethroids are shown below.

|  |  |  |
| --- | --- | --- |
| **Compound** | **Detector** | **GLC Conditions** |
| Abbreviations: P=Packed Column, C=Fused Capillary Column  Tc=Column Temperature, Td =Detector Temperature, Ti =Inlet Temp. |
| Allethrin  Biphenthrin  Cyhalothrin | FID  ECD  ECD | P2, 2; 1 m x 3 mm. (i.d.), 0.28% OV-17+3% OV, 210 Chromosorb  WHP 80/100, Tc: 190oC, Td : 250oC.  C, 30 m x 0.25 mm. (i.d.), DB1, Tc : 60oC (15oC/min.) Ti : 250oC,  Td : 300oC.  C, 25 m x 0.32 mm.(i.d.), OV–1701, Tc : 6oC (1 min)–280oC(40oC/min).  C, 15 m x 0.37 mm (i.d.), DB1 (1.5μm), Ti : 190oC, Tc : 220oC, Td= 270oC. |
| Cypermethrin,  Fenvalerate.  Cypermethrin,  Fenvalerate.  Cypermethrin.  Cypermethrin,  Fenvalerate,  Permethrin.  Cypermethrin,  Permethrin. | ECD  ECD  ECD  ECD  ECD | C, 25 m x 0.3 mm (i.d.), HP cross linked methyl.  C, 25 m x 0.3 mm (i.d.), SE 54 (0.5 μm) or Cpsil 8, Tc : 40oC (2 min) –  20oC (1 min.); 180oC (1oC / min.) – 250oC.  P, 5 m x 3 mm. (i.d.), 5% OV 101/GCO, Ti : 270oC, Td : 300oC, Tc:240oC.  P, 1.8 m x 0.2 mm. (i.d), 1.5% OV101 or 2% OV-201/GCO, 100/210 mesh  Tc : 210oC.  C, 30 m x 0.25 mm. (i.d.), DB1,(0.25 μm), Ti : 250oC, Td : 270oC, Tc: 269oC (2oC/min.), (10oC/min.) – 180oC, 4oC/min – 240oC, 25 min. |
| Cypermethrin,  Fenvalerate.  Fenvalerate,  Cypermethrin,  Deltamethrin.  Fenvalerate,  Cypermethrin,  Deltamethrin | NPD  ECD  ECD | C, 15 m x 0.53 mm. (i.d.), SPB 608 (0.5 μm), Ti : 220oC, Td : 220oC, Tc:150oC - (10oC/min.) – 220oC (10 min.).  P, 0.5 m x 1.75 mm (i.d.), 3% SE-30 / GCQ 100 / 120 mesh.  P, 0.9 m x 2 mm. (i.d.), 1.5% SP 2201 / supelcoport 100 / 120 mesh.  C, 30 m x 0.53 mm. (i.d.), Supelco CPB 20(0.5 μm ), Tc : 150oC (1 min.) – 4oC / min. to 220oC (25 min.). |

**6.13.1.5 HPLC Analysis of Pyrethroids:**

The analysis of pyrethroids in forensic samples by HPLC has become popular due to development of HPLC in attaining performance by using high performance column, new detector, optimum methods etc. The HPLC method allow quantitative determination of non-volatile and thermally labile compounds without derivatization and much clean-up of samples and also permit resolution of diastereo isomers and enantiomers of pyrethroid. The reverse phase (RP) columns are now used extensively due to their wider separation ability and lower solvent cost. Non-polar components viz. pyrethrins and pyrethroids are strongly retained by RP columns. In normal phase, bonded or other; the mobile phase is hydrophilic and the least polar compounds elute first.

Ultra-violet detection (UV) is the most popular (range 200 – 350 nm.). the fluorescence detection shows a higher sensitivity compare to UV.

**Table: HPLC Conditions for Analysis of Pyrethroids.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Compounds** | **Column** | **Solvent System** | **Flow Rate** | **Detector** |
| 1. Cypermethrin  2. Cypermethrin  3. Deltamethrin  4. Fenvalerate  5. Fluvanilate  6. Permethrin,  Fenvalerate,  Deltametrhrin,  Phenothin.  7. Permethrin,  Fenvalerate,  Deltametrhrin,  Bioresmethrin,  Piperonylbutoxide.  8. Permethrin  9. Permethrin,  Deltametrhrin,  Fenvalerate,  Bioresmethrin  Phenothrin. | 200 x 4.6 mm (i.d.),5μm Hypersil  200 x 4.5 mm (i.d.)  5 μm Partisil-5.  150 x 7 mm (i.d.) Lichrosorb, RP – 8.  100 x 7 mm (i.d)10 μm Lichrosorb Si-60.  300 x 3.9 mm (i.d), 10 μm Porasil  150 x 4.6 mm (i.d.),  Novapak C18 RP – HPLC  300 x 3.9 mm (i.d),  Bondapak C18.  Spherisorb  150 x 4.6 mm (i.d.),  Whatmon Patrisil  150 x 3.9 mm (i.d.),  Novapak C18 | Hexane-30% Water Standard  Dichloro Methane(85:15).  Light Petroleum + 0.1% Acetic acid, Dioxane or Ethanol (99 : 1) or (95 : 5).  Hexane-Dipropyl Ether (93 : 7).  Or  Acetonitrile-1% H2SO4 (70 : 30)  2.5% Ethyl Acetate in Hexane.  Acetonitrile – Water (8 : 2)  Methanol-Water (4 : 1)  Methanol-Water (4 : 1) or  2,2,4- Trimethyl Pentane  -Propan –2-01 (9 : 1).  Methanol-Water (65 : 35).  75% aqueous Acetonitrile. | 15ml./ min.  1.6 ml./ min  1.17 ml/min  1.33 ml/min  …………..  1.5 ml/min.  2.5 ml/min  1 ml./min.  1 ml./ min.  1 ml./ min. | UV(212 nm.)  UV(212 nm.)  UV(230 nm.)  UV(230 nm.)  UV  UV.  UV.  UV(235 nm.)  UV(206 nm.)  UV(254, 280 nm.)  UV(225 nm.) |

**6.14 MS DATA OF PESTICIDES:**

**Eight Peak Index of EI Spectra arranged in increasing order of Major Peak**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Drug** | **Formula** | **Mol. Wt** | **Eight most intense peaks (decreasing order)** | | | | | | | |
| 1 | Aldicarb | C7H14SN2O2 | 190 | 41 | 86 | 58 | 85 | 87 | 44 | 55 | 76 |
| 2 | Dichlorophenoxyacetic Acid (2,4-D) | C8H6Cl2O3 | 221 | 43 | 57 | 41 | 55 | 71 | 69 | 56 | 42 |
| 3 | Atrazine | C8H14ClN5 | 216 | 43 | 58 | 44 | 200 | 68 | 215 | 41 | 42 |
| 4 | Aldrin | C12H8Cl6 | 365 | 66 | 91 | 79 | 263 | 65 | 101 | 261 | 265 |
| 5 | Endrin | C12H8Cl6O | 381 | 67 | 81 | 263 | 36 | 79 | 82 | 261 | 265 |
| 6 | Phorate | C6H17PS3O2 | 260 | 75 | 47 | 121 | 97 | 65 | 45 | 93 | 79 |
| 7 | Dieldrin | C12H8Cl6O | 381 | 79 | 82 | 81 | 263 | 77 | 108 | 265 | 80 |
| 8 | Chlorfenvinphos | C13H14Cl3O4P | 358 | 81 | 267 | 109 | 269 | 323 | 91 | 170 | 173 |
| 9 | Nicotine | C10H14N2 | 162 | 84 | 133 | 42 | 162 | 161 | 105 | 77 | 119 |
| 10 | Dimethoate | C5H12NO3PS2 | 229 | 87 | 93 | 125 | 58 | 47 | 63 | 79 | 42 |
| 11 | Pyrethirn II | C22H28O5 | 372 | 91 | 133 | 161 | 117 | 107 | 160 | 105 | 115 |
| 12 | Parathion | C10H14NO5PS | 291 | 97 | 109 | 291 | 139 | 125 | 137 | 155 | 123 |
| 13 | Chlorpyrifos | C9H11C13NO3PS | 350 | 97 | 195 | 189 | 65 | 47 | 314 | 201 | 225 |
| 14 | Dichlofenthion | C10H13Cl2O3PS | 315 | 97 | 279 | 223 | 109 | 162 | 251 | 65 | 281 |
| 15 | Heptachlor | C10H5Cl7 | 373 | 100 | 272 | 274 | 270 | 237 | 102 | 65 | 276 |
| 16 | Parathion-methyl | C8H10NO5PS | 263 | 109 | 125 | 263 | 79 | 63 | 93 | 47 | 247 |
| 17 | Dichlorvos | C4H7Cl2O4P | 220 | 109 | 185 | 79 | 187 | 165 | 47 | 220 | 110 |
| 18 | Propoxur | C11H15NO3 | 209 | 110 | 152 | 43 | 58 | 41 | 111 | 81 | 64 |
| 19 | Pyrethrin I | C21H28O3 | 328 | 123 | 43 | 91 | 81 | 105 | 55 | 133 | 77 |
| 20 | Allethrin | C19H26O3 | 302 | 123 | 79 | 43 | 81 | 91 | 136 | 107 | 55 |
| 21 | Malathion | `C10H19O6PS2 | 330 | 125 | 93 | 127 | 173 | 158 | 99 | 55 | 79 |
| 22 | Fenitrothion | C9H12NO5PS | 277 | 125 | 109 | 79 | 47 | 63 | 93 | 51 | 277 |
| 23 | Monocrotophos | C7H14NO5P | 203 | 127 | 67 | 97 | 109 | 58 | 192 | 43 | 193 |
| 24 | Mevinphos | C7H13O6P | 224 | 127 | 192 | 109 | 67 | 43 | 193 | 39 | 79 |
| 25 | Diazinon | C12H21N2O3PS | 304 | 137 | 179 | 152 | 93 | 153 | 189 | 97 | 43 |
| 26 | Dicofol | C17H9Cl4O | 370 | 139 | 111 | 141 | 75 | 83 | 151 | 113 | 253 |
| 27 | Carbaryl | C12H11NO2 | 201 | 144 | 115 | 116 | 57 | 58 | 63 | 145 | 89 |
| 28 | Isoproturon | C12H18N2O | 206 | 146 | 72 | 44 | 128 | 45 | 161 | 42 | 147 |
| 29 | Cypermethrin | C22H19Cl2NO3 | 416 | 163 | 181 | 165 | 91 | 77 | 51 | 127 | 115 |
| 30 | Carbofuran | C12H15NO3 | 221 | 164 | 149 | 41 | 58 | 131 | 122 | 51 | 123 |
| 31 | Methiocarb | C11H15NO2S | 225 | 168 | 153 | 45 | 109 | 91 | 58 | 57 | 169 |
| 32 | Lindan | C6H6Cl6 | 291 | 181 | 183 | 109 | 219 | 111 | 217 | 51 | 221 |
| 33 | Permethrin | C21H20Cl2O3 | 290 | 183 | 163 | 165 | 44 | 184 | 91 | 77 | 127 |
| 34 | Endosulfan | C9H6Cl6O35 | 407 | 195 | 36 | 237 | 41 | 241 | 75 | 239 | 170 |
| 35 | Dienphane (DDT) | C14H9Cl5 | 354 | 235 | 237 | 165 | 236 | 75 | 239 | 82 | 199 |
| 36 | Fenthion | C10H15PS2O | 278 | 278 | 125 | 109 | 169 | 93 | 153 | 63 | 47 |

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**SECTION – 7: ANALYSIS OF BASIC DRUGS / POISONS**

**(ORGANIC NON-VOLATILES)**

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

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

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

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

**7.5 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 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 Arrest Poisons/Drugs:**

These 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, in clinical toxicological cases blood and urine constitute 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.

**7.6 EXTRACTION OF DRUGS** :

The procedures for extraction have already been covered in Section:3.

**7.7 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 often a particular or important group has been elaborated to meet the requirement. The methods are applied to sample after their extraction, if necessary.

**7.7.1 Screening Tests:**

A. To the residue of extract is added a few drops of Mayer’s reagent (prepared by dissolving 1.357 g of mercuric chloride and 5 g of pot. Iodide in 100 ml of water). A white or gelatinous white precipitate indicates the presence of basic drugs.

B. To another portion of the above residue a few drops of phosphotungstic acid is added. A red precipitate indicates the presence of basic drugs.

**7.7.2 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’s |
| Morphine | Violet | Bright orange yellow | Dark reddish brown |
| Heroin | Reddish purple | Pale yellow | Reddish brown |
| Codeine | Dark violet | Greenish yellow | Olive green |

**7.7.3 Special colour tests performed with extract for basic drugs.**

|  |  |  |
| --- | --- | --- |
| Compound | **Reagent** | **Colour observed** |
| Strychnine | (K2Cr2O7 + conc. sulphuric acid)  A crystal of potassium dichromate is drawn by means of glass rod through the sulphuric acid. | A play of colour is observed –  momentary blue changing to a beautiful violet colour which gradually change to reddish purple, red or orange and finally to yellow. The colour is discharged by stannous chloride but reappears on adding conc. nitric acid. |
| Brucine | Conc. nitric acid. | Red colour. |

**7.7.4** **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: 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 solution + 45 ml. of 20% (w/v) solution of perchloric acid  + 50 ml. of 50% (v/v) solution 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. of water with stirring. | Violet / Blue-violet / Brown violet / Grey violet. | Alkaloids. |
| Koppanyi- Zwikker  Test. | 1% solution of cobalt nitrate in ethanol. After adding above reagent 0.1 ml. of pyrrolidine is added. | Violet. | Sulphonamides |
| Forrest Reagent. | Mixing together equal volumes of a 0.2% solution of potassium dichromate; a 30% (v/v) solution of sulphuric acid, a 20% solution of perchloric acid and a 50% solution of nitric acid. | Red / Violet red / Brown red / Orange / Pink orange / Red Orange / Brown.  Blue | Phenothiazines  Imipramine and related compounds. |
| Marquis Reagent | 1 volume of formalin + 9 vols of concentrated  sulphuric acid. | Yellow / Orange | Benzodiazepines |
| Cobalt thiocyanate. | A: 2.5 gms. of cobalt thiocyanate in 100 ml of water.  B: 16% HCl | Blue colour on adding 1 drop each of A and B. | Methaqualone PCP, Cocaine Heroin. |
| Antimony  Pentachloride | . By passing chlorine gas through dried (by P2O5) and molten antimony trichloride and dissolving it in chloroform. | Green brown /  Brown turning to black violet | Betamethasone  Dexamethasone  Digitoxigenin,  Digoxigenin, Digitoxin, digoxin, Lanatoside C |
| Copper  Sulphate | 1% solution of copper sulphate | Orange brown/Green  Brown/violet brown | Sulfonamides |
| Mercurous Nitrate | By adding solid sodium bicarbonate to a saturated solution of mercurous nitrate. A precipitate forms which changes to a biscuit colour (should be used freshly). | A dark grey or black colour | Sulfonamides with in additional ring. |
| Dragendorff’s Reagent | 1 gm of bismuth subnitrate is dissolved in 3 ml. of 10M of hydrochloric acid. It is diluted to 20 ml. 1 gm of potassium iodide is dissolved in it. 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. |

**Table:** Special Colour Tests For Different Drugs Of Abuse:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Reagent** | **Compound** | **Colour Observed in Cobalt Thio- cyanate Test** | **Colour observed in Fisher Morris Tests** | |
|  |  |  | **Aqueous layer** | **Chloroform layer** |
| 1. Cobalt Thiocyanate Test Reagent A: 16% Hydrochloric  acid  Reagent B: 2.5 gms.of Cobalt  Thiocyanate in 100 ml. of water.  1 drop of each of A and B are added. | Methaqualone  Mecloqualone  Cocaine  Phencyclidine  Caffeine | Blue  Blue  Blue  Blue  Pink | No colour  No colour  Faint green  Tinged  No colour | Yellow  Yellow  Faint green.  Tinged.  No colour. |
| 2. Fisher Morris Test.  Reagent A: Concentrated formic acid.  Reagent B: 5% aqueous sodium nitrite solution.  5 drops of A and 3 drops of  B are added to sample. After 2 minutes, 15 drops of  chloroform are added and colour of layers are observed. | Heroin  Diphene hydramine  Diazepam | Blue  Blue  Blur green | Faint Blue  Yellow  Faint yellow | Yellow  No colour.  Faint yellow |

**7.7.5 Thin Layer Chromatography Of Some Common Basic Drugs**

**7.7.5.1 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 | : | 1. | Iodoplatinate Solution (for alkaloids) – Violet / Blue violet / Brown violet / Grey violet spots. |
|  |  | 2. | Dragendorff’s Reagent (alkaloids & benzodiazepines) – Orange Spots. |
|  |  | 3. | FPN Reagent (for Phenothiazines) - Orange red / Violet red / Brown red / Orange / Red orange / Pink orange / Blue / Violet / Red Brown spot. |

Samples : Extract of samples along with control

drugs and alkaloids.

**Table: Rf values of Some Common Basic Drugs:**

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Name of Alkaloid and Basic Drug** | **hRf (Rf X 100) Value.** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15. | Atropine  Imipramine  Quinine  Nicotine  Caffeine  Codeine  Morphine  Chlorpromazine  Nicotinic Acid  Papaverine  Chlordiazepoxide  Cocaine  Strychnine  Brucine  Promazine. | 18  48  50  54  32  33  37  49  58  61  62  65  38  49  44 |

**Additional TLC Data of Basic Drugs**

Plate: Silica gel G (0.2mm)

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl. No.** | **Solvent (v/v)** | **Reference compounds (2mg/ml each)** | **hRf** |
| 1. | Chloroform  : Acetone (80 : 20 | Paracetamol  Clonazepam  Secobarbital  Methylphenobarbital | 15  32  55  70 |
| **2.** | Ethylacetate | Sulfathiazole  Phenacetin  Salcylamide  Secobarbital | 20  38  55  68 |
| 3. | Chloroform : Methanol (90 :10) | Hydrochlorothiazide  Sulfafurazole  Phenacetin  Prazepam | 11  33  52  72 |
| 4a. | Ethylacetate : Methanol –: Conc. Ammonia (85 :10 :5) | Sulfadimidine  Hydrochlorthiazide  Temaepam  Prazepam | 13  34  63  81 |
| 4b. | Ethylacetate : Methanol : Conc. Ammonia (85 :10 : 5) | Morphine  Codeine  Hydroxyzine  Trimipramine | 20  35  53  80 |
| 5. | Methanol | Codeine  Trimipramine  Hyddroxyzine  Diazepam | 20  36  56  82 |
| 6. | Methanol : n-Butanol (60 :40) | Codeine  Diphenhydramine  Quinine  Diazepam | 22  48  65  85 |
| 7. | Methanol : Conc.  Ammonia (100 :1.5) | Atropine  Codeine  Chlorprothixene  Diazepam | 18  33  56  75 |
| 8. | Cyclohexane : Toluene : Diethylamine (75 :15 :10) | Codeine  Desipramine  Prazepam  Trimipramine | 6  20  36  62 |
| 9. | Chloroform : Methanol (90 :10) | Desipramine  Physostigmine  Trimipramine  Lidocaine | 11  36  54  71 |
| 10. | Acetone | Amitriptyline  Procaine  Papaverine | 15  30  47 |

* + - 1. **TLC Conditions And Data For Screening Of Some Amphetamines, Other Stimulants And Anorectics:**

Plate : Silica gel G (0.2 mm thickness)

Development : By ascending technique.

Solvent Systems : No. 1 – Methanol **:** Ammonia **: :** 100 **:** 15

No. 2.- Cyclohexane **:** Toluene **:** Diethyl Amine **: : :** 75 **:** 15 **:** 10

No. 3 – Chloroform **:** Methanol **: :** 90 **:** 10

Spray Reagent : Dragendorff’s Spray : Yellow / Orange / Red orange /

Brown orange spot.

Acidified iodoplatinate Spray : Violet / Blue violet / Grey violet / Brown violet spot.

**TABLE :** hRf Values of Some Amphetamine, Stimulants etc.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Serial**  **No.** | **Compound** | **hRf Value in Solvent Systems** | | |
| **No. 1** | **No.2** | **No.3** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16. | Amphetamine  Benzphetamine  Chlorphentermine  Ephedrine  Hydroxyamphetamine  Lobeline  Mephentermine  Mescaline  Methoxyamphetamine  Methylamphetamine  Methylenedioxyamphetamine  Methyphenidate  Phenmetrazine  Phenylephrine  Pseudoephedrine  Trimethoxyamphetamine | 43  73  44  30  35  61  25  20  73  31  39  57  50  33  33  33 | 15  67  18  05  02  17  34  04  36  28  17  34  14  01  54  08 | 09  70  17  05  02  35  08  10  77  13  12  14  21  01  04  11 |

**7.7.5.3 TLC Conditions And Data For Screening Of Some Antidepressants:**

The TLC condition as stated above in section 7.7.5.2. will hold good. The Rf values are given below.

**Table: hRf Values of Some Antidepressants.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Serial**  **No.** | **Compound** | **hRf in Solvent Systems** | | |
| **No. 1** | **No.2** | **No.3** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14. | Amitriptyline  Butriptyline  Clomipramine  Desipramine  Imipramine  Iprindole  Improniazid  Nialamide  Nomifensinc  Opipramol  Phenelzine  Protriptylene  Tafenacin  Trimipramine | 51  59  51  26  48  47  69  70  56  54  77  19  45  59 | 55  61  54  20  49  49  01  02  08  06  37  17  25  62 | 32  48  34  11  23  24  23  25  29  22  12  07  21  54 |

**7.7.5.4 TLC Conditions And Data For Screening Of Some Antihistamines:**

The TLC conditions as in 7.7.5.2. (amphetamines etc.). The Rf values are presented hereunder.

**Table: hRf Values of Some Antihistamines.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Serial**  **No.** | **Compound** | **hRf in Solvent Systems** | | |
| **No. 1** | **No.2** | **No.3** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9. | Antazoline  Bromodiphenhydramine  Chlorpheniramine  Cyclizine  Doxylamine  Promethazine  Propiomazine  Pheneramine  Trimeprazine | 31  49  45  57  48  50  55  45  58 | 07  40  33  49  41  37  34  35  55 | 07  43  18  41  10  35  42  15  39 |

**7.7.5.5 TLC Conditions And Data For Screening Of Some Local Anesthetics:**

The TLC conditions are same as stated in 7.7.5.2. The Rf Values are presented hereunder.

**Table :** hRf Values of Local Anesthetics.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Serial**  **No.** | **Compound** | **hRf in Solvent Systems** | | |
| **No. 1** | **No.2** | **No.3** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12. | Amethocaine  Benzocaine  Bupivacaine  Butacaine  Butanilicaine  Chlorprocaine  Cocaine  Lignocaine  Oxybuprocaine  Procaine  Propoxycaine  Proxymetcaine | 57  67  69  71  76  59  65  70  62  54  58  62 | 15  06  42  09  14  05  47  35  23  06  03  26 | 32  57  73  30  54  23  47  73  41  31  33  41 |

**7.7.5.6 TLC conditions and data for screening of some narcotic analgesics:**

The TLC conditions are same as stated in 7.7.5.2. The Rf values are presented hereunder.

**TABLE:** Rf Values of Narcotic Analgesics.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Serial**  **No.** | **Compound** | **hRf Value in Solvent Systems** | | |
| **No. 1** | **No.2** | **No.3** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16.  17. | Apomorphine  Benzylmorphine  Buprenorphine  Codeine  Dextropropoxyphene  Diamorphine  Dihjdrocodeine  Etorphine  Fentanyl  Hydrocodone  Hydromorphone  Methadone  6 Monoacetyl morphine  Morphine  Papaverine  Pethidine  Thebaine | 83  41  76  33  68  47  26  73  70  25  23  48  46  37  61  52  45 | 00  06  09  06  59  15  08  09  45  04  03  61  09  00  08  37  23 | 21  23  68  18  55  38  13  61  74  20  09  20  19  09  65  34  17 |

* + - 1. **TLC Conditions and Data for Screening of Some Phenothiazines and Other**

**Tranquilisers**:

Plate : Silica gel G (0.2 mm thickness).

TLC Solvent Systems : 3 Solvent Systems (No. 1, 2 and 3 as

stated in section

(Ref TLC Data of Some Amphetamines

Development : By ascending technique.

Spray Reagent: FPN Spray : Red / Brown-red spot.

**TABLE :** hRf Values of Some Phenothiazines and Other Tranquilisers.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sl.No. | **Compound** | HRf Value in Solvent Systems | | |
|  |  | No.1 | No.2 | No.3 |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16.  17.  18.  19.  20.  21.  22.  23 | Acepromazine  Acetophenazine  Benactyzine  Benzoctamine  Chlorpromazine  Chlorprothixene  Dimethothiazine  Ethopropazine  Fluopromazine  Fluphenazine  Mesoridazine  Methotrimeprazine  Pecazine  Piperacetazine  Prochlorperazine  Promazine  Promethazine  Thiopropazate  Thioproperazine  Thioridazine  Trifluoperazine  Trimeprazine  Trimetozine | 48  53  66  59  66  56  56  67  54  63  38  57  53  56  49  44  50  61  46  48  53  58  61 | 26  03  40  57  01  51  13  64  49  06  03  49  46  06  33  41  37  35  07  43  33  55  11 | 24  25  53  52  63  51  48  47  35  23  06  38  44  19  37  30  35  53  34  30  30  39  72 |

**7.7.5.8 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: hRf Values of Some Benzodiazepines**

|  |  |  |
| --- | --- | --- |
| **Sl. No.** | **Compound** | **hRf Value in Solvent Systems** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **No. 1** | **No. 2** | **No. 3.** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16.  17. | Bromazepam  Chlordiazepoxide  Clobazam  Clonazepam  Demaxepam  Diazepam  Flunitrazepam  Flurazepam  Ketazolam  Lorazepam  Medazepam  Nitrazepam  Nordazepam  Oxazepam  Prazepam  Temazepam  Triazolam | 13  10  53  35  15  58  54  03  45  23  56  35  34  22  64  51  05 | 62  51  73  60  44  77  77  74  73  45  79  59  67  44  81  63  45 | 20  11  49  45  22  48  48  03  45  39  40  45  45  37  55  47  02 |

**7.7.5.9 TLC Conditions and Data for Screening of Some Sulfonamides**

Plate : Silica gel G (0.2 mm. thickness)

Development : By ascending technique.

Solvent systems : No. 1 : Methanol : Ammonia :::100 :1.5

No. 2 : Chloroform : n-Butanol : Petroleum Ether

:::1:1:1

No. 3 : Cyclohexane : Acetone : Acetic Acid

:::40 : 50 : 10

Spray Reagent : 1. Spraying with 0.25% sodium nitrite and 0.5 N

hydrochloric acid solution followed by 5%

α- naphthol solution in 2N sodium hydroxide.

Orange red spot is observed.

2. Spraying with 0.25% sodium nitrite and 0.5N

hydrochloride acid followed by 1% alcohol

solution of N-1-napthyl-ethylene-

diamine dihydrochloride. Pink spot is observed.

**Table : Rf Values of Sulfonamides in Solvent Systems.**

|  |  |  |
| --- | --- | --- |
| **Sl. No.** | **Compound** | **hRf Value in Solvent Systems** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **No. 1** | **No. 2** | **No. 3.** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12. | Sulphathiazole  Sulphapyridine  Sulphadiazine  Sulphadimidine  Sulphaguanidine  Sulphacetamide  Sulphamerazine  Sulphanilamide  Sulphafurazole  Sulphamethizole  Sulphaphenazole  Sulphasomidine | 66  67  64  62  65  70  65  67  65  65  69  64 | 41  37  39  52  15  31  44  43  51  -  77  19 | 38  54  -  56  28  49  -  43  55  -  -  33 |

**7.7.5.10 TLC Conditions and Data for Screening of Some Cardiac Glycosides:**

Plate : Silica gel G (0.2 mm. thickness)

Development : By ascending technique.

Solvent systems : Benzene : Ethanol : : 7 : 3

Spray Reagent : 1. P-Anisaldehyde Reagent (prepared by

dissolving 0.5 ml. in 50 ml. of hydrochloric

acid) – Blue spot is formed (Quabain gives

yellow spot).

2. Perchloric Acid (prepared by adding 15 ml. of

72%, w/w acid to water to produce 100 ml.).

Fluorescence spots (blue / red / yellow green)

are observed.

**Table:** hRf Values of Some Cardiac Glycosides:

|  |  |  |
| --- | --- | --- |
| **Sl. No.** | **Compound** | **hRf Value** |
| 1.  2.  3.  4.  5.  6. | Acetyldigitoxin  Deslanoside  Digitoxin  Digoxin  Lanatoside C  Quabain | 82  27  72  62  36  09 |

**7.7.5.11 TLC Conditions and Data for Screening of Some Ergot Alkaloids:**

Plate : Silica gel G (0.2 mm thickness) dipped in

or sprayed With 0.1M potassium hydroxide in methanol and dried.

Development : By ascending technique.

Solvent System : Acetone.

Spray Reagent : 5% Napthaquinone sulphonate Solution.

After spraying with sulphonate solution,

The plate is then sprayed with 10% v/v

solution of hydrochloric acid and heating at

110oC for 20 minutes. Red-violet spots on

a light pink background is observed.

**Table:** hRf Values of Some Ergot Alkaloids:

|  |  |  |
| --- | --- | --- |
| **Sl. No.** | **Compound** | **hRf Value** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9. | Co-dergocrine  Dihydroergotamine  Ergometrine  Ergotamine  Ergotoxine  Lysergamide  Lysergide  Methyl ergometrine  Methysergide | 29  14  08  23  48  06  24  12  12 |

**7.7.5.12 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 / Brownish

Orange/ Reddish Orange/spot.

**Table: hRf Values of Some Common Drugs of Abuse:**

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

**7.7.6 UV Spectrophotometry**

* + - 1. **UV Absorption Data of Some Common Basic Drugs:**

The purified extract (obtained after extraction of samples including biological materials) is dissolved in methanol and scanned in a UV Spectrophotometer along with control substance (Pure). The absorptions maxima have been shown in table as hereunder. There may be some differences due to presence of lipid materials in the extract.

**Table: UV absorption Data of Some Common Basic Drugs.**

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Name of the Compound** | **Absorption Maxima, λmax in methanol**  **(in nm.)** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16  17  18 | Cocaine  Cinchonine  Chlordiazepoxide  Papaverine  Quinidine  Promazine  Strychnine  Hyoscine  Hyoscyamine  Nicotine  Nicotinamide  Brucine  Caffeine  Codeine  Papaverine  Morphine  Diazepam  Nitrazepam | 233  235  246  250  251  254  257  258  259  261  265  273  285  236  285  242  280  258 |

**7.7.6.2 UV Absorption Data of important Basic Drugs:**

**Table: UV Absorption Data of Some Important Basic Drugs (Anti Depressants):**

|  |  |  |  |
| --- | --- | --- | --- |
| **Serial**  **No.** | **Compound** | **Absorption Maxima (nm) in** | |
| **0.5N H2SO4** | **0.5N NaOH** |
| 1.  2.  3.  4.  5. | Amitriptylene  Desimipramine  Dorepin  Imipramine  Nortriptyline | 238  251  292  250  239 | 238  251  -  253  239 |

**7.7.6.3 UV Absorption Data of Some Benzodiazepines:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Serial**  **No.** | **Compound** | **Absorption Maxima (nm) in** | | |
| **0.5N H2SO4** | **0.5N NaOH** | **Ethanol** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16.  17.  18.  19.  20. | Alprazolam  Bromazepam  Camazepam  Chlordiazepoxide  Clobazam  Clonazepam  Clorezepic Acid  Diazepam  Flunitrazepam  Flurazepam  Ketazolam  Loprazolam  Lorazepam  Lormetazepam  Medazepam  Nitrazepam  Nordazepam  Oxazepam  Prazepam  Temazepam | 260  239,345  -  246,308  230, 289  273  273, 287  242, 284, 368  -  236, 284  -  -  -  231, 311  253  289  238, 283, 361  234, 280  240, 285, 360  237, 284,358 | -  237, 348  -  262  286  -  -  -  -  231, 312  -  -  -  -  -  -  240, 340  233, 344  -  231, 313 | -  233, 320  231, 325  -  -  245, 309  -  -  252, 308  -  242  330  230, 316  -  -  280  -  230, 315  -  230, 314 |

**7.7.6.4 UV Data of Sulpha Drugs:**

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Name of the Compound** | **Absorption Maxima (nm) in Ethanol.** |
| 1.  2.  3.  4.  5.  6.  7. | Sulphathiazole  Sulphapyridine  Sulphadiazine  Sulphaguanidine  Sulphamerazine  Sulphanilamide  Sulphaphenazole | 258  244  240  259  244  263  267 |

**7.7.7 Gas Chromatography:**

**7.7.7.1 General Screening and Identification of Some Common Basic Drugs by Gas Chromatography:**

A wide range of basic drugs including alkaloids may be screened or detected by gas chromatography (sample concentration ≈ 1 μg / ml.). The presence of one nitrogen atom present in basic drugs will produce a signal in an alkali flame ionization detector. The method of extraction of drugs and detector selected should ensure minimum interference from other compounds which do not contain nitrogen.

**Gas Chromatographic Conditions :**

Column : 2.5% of dimethyl dichloro silicone treated and acid washed .2m X 4 mm. (internal diameter) glass column. The support should be deactivated by treating with KOH. If this is not done, acidic drugs (barbiturate) may also be eluted.

Column temperature : 100 – 300oC,

Carrier gas, flow rate : Nitrogen, 45 ml./min.

Reference Compound : Compounds having even number of carbon

atom and containing N – atom.

Retention indices : Shown in table hereunder.

**Table:** Retention Indices (Gas Chromatography) of Alkaloids and Basic Drugs.

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Name of the Compound** | **Retention Indices** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16 | Nicotine  Nicotinamide  Caffeine  Cocaine  Atropine  Hyoscine  Codeine  Diazepam  Morphine  Chlorpromazine  Nitrazepam  Quinine  Quinidine  Papaverine  Strychnine  Brucine | 1346  1346  1810  2187  2199  2303  2363  2425  2454  2487  2750  2798  2798  2825  3280  3310 |

**7.7.7.2 General Screening and Identification of Stimulants, Amphetamines Etc. by Gas Chromatography:**

**Chromatographic Conditions:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **System** | **Column** | **Column Temperature** | **Carrier Gas** | **Reference Compound** |
| 1. | 2.5% SE-30 on 80-100 mesh  Chromosorb G (acid washed and dimethyl dichloro silane treated), 2m x 4mm.(i.d), glass column(su-pport should be fully deactivated). | 100-300oC, Temperature best suitable =  Retention index  10 | Nitrogen,  45 ml./min. | n-Alkane with even number of carbon atoms. |
| 2. | 10% Apiezon L and 2% KOH on 80-100 mesh Chromosorb W HP, 2m x 3mm i.d. glass column. | 170-260oC, held for  8 minutes. | Nitrogen,  30 ml./ min. | N-Alkanes with even number of carbon atom or retention time rela – tive to diphenylamine. |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 3. | 3% OV on 80-100 mesh Chromosorb W HP, 2m x 3 mm. i.d. glass column. | At 170oC for 2 minutes and then programmed at 16oC per minute to 270oC and held for 8 minutes. | Nitrogen,  30 ml./min. | N-Alkanes with even numbers of carbon atom. |

**Table: Retention Indices (Gas Chromatography) of Amphetamines and Other Stimulants.**

|  |  |  |
| --- | --- | --- |
| **Serial**  **No.** | **Name of the Compound** | **Retention Indices in Different Systems** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **No. 1** | **No.2** | **No.3** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16.  17. | Amphetamine  Benzphetamine  Caffeine  Chlorphentrmine  Dimethylamphetamine  Ephedrine  Hydroxyamphetamine  Mescaline  Methoxyamphetamine  Methylamphetamine  Methylenedioxyamphetamine  Methylephedrine  Methyl Phenidate  Phenmetrazine  Phentermine  Pseudoephedrine  Trimethoxyamphetamine | 1123  1855  1810  1342  1236  1363  1320  1688  1385  1176  1472  1400  1737  1431  1147  1354  1748 | 1134  1895  1862  1410  1261  1386  -  -  -  1200  -  1440  -  1473  1182  1399  - | 1536  2172  2376  1725  1429  1467  -  -  -  1722  -  1480  2200  1873  1450  1543  - |

**7.7.7.3 Screening and Identification of Antidepressants by Gas Chromatography:**

**Chromatographic Conditions:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **System** | **Column** | **Column Temperature** | **Carrier Gas** | **Reference Compound** |
| 1. | Same as System No.1 stated under 7.7.7.1. |  |  |  |
| 2. | 3% Poly A 103 on 80-100 mesh Chromosorb W HP, 1 m x 4 mm. i.d. glass column. | 200oC | Nitrogen,  60 ml./ min. | n-alkanes with an even number of carbon atoms. |

**Table: Retention Indices (Gas Chromatography) of Antidepressants Related to GLC.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Serial**  **No.** | **Name of the Compound** | **Retention Indices in Different Systems** | |
| **System 1** | **System 2** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10. | Amitriptyline  Butriptyline  Clomipramine  Desipramine  Doxepin  Iproniazid  Nortriptyline  Noxiptyline  Protriptyline  Trimipramine | 2196  2181  2406  2242  2217  1593  2210  2267  2261  2201 | 2510  2465  2795  -  2570  -  -  -  2590  2505 |

**7.7.7.4 Screening and Identification of Antihistamines by Gas Chromatography:**

**Chromatographic Conditions.**

**System Conditions**

1. Same as system No. 1 stated under 7.7.7.1.
2. As above.
3. As above.
4. Same as system No. 2 stated under 7.7.7.1.

(Gas Chromatography)

**Table: Retention Indices of Antihistamines Related to GLC.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Serial**  **No.** | **Name of the Compound** | **Retention Indices in Different Systems** | | | |
| **No. 1** | **No. 2** | **No. 3** | **No. 4** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9. | Bromopheniramine  Chlorpheniramine  Cyclizine  Diphenylhydramine  Diphenylpyraline  Pheniramine  Promethazine  Propiomazine  Trimeprazine | 2096  2002  2020  1873  2099  1804  2259  2738  2309 | 2159  2038  2081  1872  2136  1826  -  -  - | 2457  2586  2348  2378  2447  -  2546  -  2646 | 2470  2335  2320  2105  2405  2100  2675  -  2715 |

**7.7.7.5 Screening and identification of Local Anesthetics:**

**Chromatographic Condition:**

Same as System No. 1 stated under 7.7.7.1. (Gas Chromatography of Stimulants).

**Table:** Retention Indices (Gas Chromatography) of Local Anesthetics.

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Name of the Compound** | **Retention Indices** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13. | Amethocaine  Benzocaine  Butacaine  Chlorprocaine  Cocaine  Hexylcaine  Oxethezaine  Oxybuprocaine  Pramoxine  Prilocaine  Procaine  Propoxycaine  Proxymetacaine | 2219  1555  2457  2229  2187  1965  2525  2471  2281  1825  2018  2335  2323 |

**7.7.7.6 Screening and Identification of Narcotic Analgesics by GC:**

**Chromatographic Conditions:**

3 Systems (No.1, 2 and 3) as Stated Under 7.7.7.1. related to Stimulants.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **System** | **Column** | **Column Temperature** | **Carrier Gas** | **Reference Compound** |
| 1 | 2.5% SE – 30 on 80 - 100 mesh Chromosorb G(acid washed and dimethyl dichloro silane treated), 2m x 4 mm i.d. glass column (Support should be fully deactivated) | 100 – 300oC temperature best suitable  retention =  = index  10 | Nitrogen,  45 ml./min. | n-alkanes with an even number of carbon atoms. |
| 2 | 2.5% OV – 17 on 80 - 100 mesh Chromosorb G(acid washed and dimethyl dichloro silane treated), 2m x 4 mm i.d. glass column (Support should be fully deactivated) | As above | As above | As above |

**Table: Retention Indices (Gas Chromatography) R of Narcotic analgesics.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Serial**  **No.** | **Name of the Compound** | **Retention Indices in Different Systems** | | |
| **No. 1** | **No.2** | **No.3** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16. | Acetyl Codeine  Apomorphine  Codeine  Dextromorpamide  Dextropropoxyphene  Diamorphine  Dihydrocodeine  Dihydromorphine  Hydromorphine  6-Monoacetylmorphine  Morphine  Nalorphine  Norcodeine  Normorphine  Papaverine  Pethidine | 2510  2530  2376  2940  2188  2614  2363  2451  2467  2537  2454  2577  2388  2438  2825  1751 | -  -  1600  -  1938  -  -  -  -  -  -  -  -  -  -  1753 | -  -  2681  3625  2173  -  2702  2504  -  -  2542  -  -  -  -  2025 |

**7.7.7.7 General Screening and Identification of Some Benzodiazepines by Gas Chromatography:**

**Table: Retention Indices (Gas Chromatography) of Benzodiazepines.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Serial**  **No.** | **Name of the Compound** | **Retention Indices in Different Systems** | |
| **No. 1** | **No. 2** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16.  17.  18.  19. | Bromazepam  Chlordiazepoxide  Clobazam  Clonazepam  Clozapine  Demaxepam  Diazepam  Flunitrazepam  Flurazepam  Ketazolam  Lorazepam  Lormetazepam  Medazepam  Nitrazepam  Nordazepam  Oxazepam  Prazepam  Temazepam  Triazolam | 2663  2453, 2530, 2799  2694  2885  2967  2529  2425  2645  2785  2425  2402  2674  2226  2750  2496  2336  2641  2633  2965 | 3280  3065  3174  3600  3455  3043  2940  3190  3220  2940  2910  -  2620  3450  3041  2803  3145  3125  - |

**7.7.7.8 General screening and identification of some Phenothiazines by Gas Chromatography:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **System** | **Column** | **Column Temperature** | **Carrier Gas** | **Reference Compound** |
| 1 | 2.5% SE – 30 on 80 - 100 mesh Chromosorb G(acid washed and dimethyl dichloro silane treated), 2m x 4 mm i.d. glass column (Support should be fully deactivated) | 100 – 300oC temperature best suitable  retention =  = index  10 | Nitrogen,  45 ml./min. | n-alkanes with an even number of carbon atoms. |
| 2 | 3% Poly A 103 on 80-100 mesh Chromosorb W HP, 1 m x 4 mm. i.d. glass column. | 200ºC | Nitrogen at 60ml/min. | -do- |

**Table: Retention Indices (Gas Chromatography) of Phenothiazines:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Serial**  **No.** | **Name of the Compound** | **Retention Indices in Different Systems** | |
| **No. 1** | **No. 2** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16.  17.  18.  19.  20.  21.  22.  23. | Acepromazine  Azacyclonol  Benzoctamine  Benactyzine  Carphenazine  Chlormezanone  Chlorpromazine  Chlorprothixene  Dimethothiazine  Ethopropazine  Fluopromazine  Hydroxyzine  Methdilazine  Pericyazine  Phenothiazine  Prochlorperazine  Promazine  Promethazine  Propiomazine  Thiethylperazine  Thioridazine  Trifluoperazine  Trimerazine | 2694  2243  2082  2248  3590  2238  2486  2487  3078  2357  2211  2849  2467  2207  -  2954  2316  2339  2738  3247  3114  2683  - | 3230  -  2445  -  -  -  2940  2910  -  2775  2550  -  -  -  2845  -  2745  2675  -  -  -  3050  2715 |

**7.7.7.9 General Screening and Identification of Some Sulphonamides by Gas Chromatography:**

The sulphonamides are separated as their methyl derivative (by injecting the samples mixed with 0.2M trimethyl anilinium hydroxide in methanol) as per the following chromatographic condition.

Column : 5% OV-17 on 80-100 mesh Gas-

Chrom Q, 1.5m X 2 mm. i.d. glass

column.

Column Temperature : 250ºC.

Carrier Gas : Nitrogen at 30 ml. / min.

Reference Compound : Griseofulvin.

**Table: Retention Time of Methyl Derivatives of Sulfonamides Relative to Griseofulvin.**

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Name of the Compound** | **Relative Retention Time**  **(RRT)** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14. | Glymidine  Sulfamerazine  Sulphacetamide  Sulphadiazine  Sulphadimidine  Sulphafurazole  Sulphamethizole  Sulphamethoxazole  Sulphamethoxydiazine  Sulphamethoxy pyridazine  Sulphamoxole  Sulphaphenazole  Sulphapyridine  Sulphathiazole | 0.53  0.69  0.16  0.66  0.71  0.42  0.98  0.40  1.38  0.93  0.40  1.71  0.47  0.49 |

**N.B.:** Detectors used in the Screening of Drugs in the Preceding Method:

The response of flame ionization detector is dependent on the number of carbon atoms, but sensitivity decreases if O or N is present. For drugs alkali flame ionisation detector is used. Electron capture detector is also sensitive for benzodiazepines. In general FID is normally used.

**7.7.7.10 Gas Chromatography of Benzodiazepines of Interest:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Conditions** | | **Packed Column** | **Capillary Column** |
| 1. | Column | 2 m x 4mm i.d., glass column  Packing: Chromosorb WHP. | Fused silica, chemically bonded and cross linked methyl silicone viz., BP1, DB-1 or equivalent, 25m x 0.25 (i.d), film thickness of 0.25μm. |
| 2. | Column temperature | Programmed from 200-280oC at 16oC/min. | 250oC |
| 3. | Injector/Detector Temperature | 290oC | 275oC |
| 4. | Carrier Gas | Nitrogen | Nitrogen |
| 5. | Sampling | Solution of standard and samples in methanol are injected in each case |  |

**Table: Retention Indices of Benzodiazepines.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Serial**  **No.** | **Name of the Compound** | **Retention Indices in Different Systems** | |
| **Packed Column (SE-30/OV-1)** | **Capillary Column (BP-1)** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16.  17.  18.  19.  20.  21.  22.  23.  24.  25.  26. | Alprazolam  Bromazepam  Camazepam  Chlordiazepoxide  Clobazam  Clorezepic Acid  Clotiazepam  Cloxazolam  Delorezepam  Diazepam  Estazolam  Flunitrazepam  Flurazepam  Halazepam  Haloxazolam  Triazolam  Ketazolam  Loprazolam  Lorazepam  Lormetazepam  Medazepam  Nitrazepam  Nordazepam  Oxazepam  Prazepam  Tetrazepam | 3050  2633  2954  2799 (2453, 2530)  2694  2885  2420  2405  2650  2425  2955  2645  2785  2335  2620  3130  2425  Does not elute  2402  2674  2226  2750  2456  2336  2641  2640 | 2936  2626  2954  2815 (2531, 2646)  2582  2833  2485  2344 (2604)  2537  2471  2897  2633  2795  2314  2634 (2518, 2272)  3025  2475  2448  2703 (2946)  2287  2755  2522  2374  2676  2463 |

**7.7.7.11 Quantitation of Basic Drugs by Gas Chromatography:**

Quantitative work usually requires some form of sample preparation to isolate the drug from the bulk of material for interference free analysis. For the purpose, some degree of concentration or dilution and purification are required. These steps or processes will certainly invite some degree of analytical error. A further difficulty may be caused by the non-reproducibility of injected volumes. It is necessary to compensate for these errors. This is done by comparing the response of the unknown with the response of an added internal standard. The internal standard should be added as early as possible in the course of analysis and should have chromatographic properties as close as possible to the drug preferably with a longer retention time. It is often possible to obtain unmarked analogues of drugs or compounds specially synthesized for use as internal standards.

But, the internal standard will not behave exactly as the drug and careful control of variables such as PH is necessary. If a derivative is to be prepared, the standard should also be derivatisable. An inappropriate internal standard can seriously affect precision. If a mass spectrometer is used as the detector, then the ideal internal standard is a 13C analogue of the drug.

Calibration should include points of higher and lower concentration than the sample and quality assessment of samples should be included as appropriate concentrations in frequently run assays. Peak measurement maybe peak height or by the peak area obtained by integration. The plot of the ratio of peak height (or area) of the drug to internal standard versus concentrations will be a straight line with most detector.

**7.7.8 High Performance Liquid Chromatography**

**7.7.8.1 Screening and identification of Amphetamines and Other Stimulants by HPLC:** (1,2)

**Chromatographic Conditions:**

|  |  |  |
| --- | --- | --- |
| **System** | **Column** | **Eluent** |
| No. 1 | Silica (Spherisorb S5W, 5μm, 12.5 cm. X 4.9 mm i.d.) | A solution containing 1.175 g (0.01M) of ammonium perchlorate in 1000 ml. of methanol with adjustment of PH at 6.7 by adding 1 ml. of 0.1M sodium hydroxide in methanol. |
| No. 2 | ODS – Silica (ODS-Hypersil,  5 μm, 25 cm X 5 mm. i.d.) | A solution containing 19.66 gms. (0.2M) of phosphoric acid and 7.314 gms (0.1M) of diethylamine in 1000 ml. of a 10% (v/v) solution of methanol with adjustment of PH at 3.15 by addition sodium hydroxide solution. |
| No. 3. | Silica (Spherisorb, 5 μm, 25 cm. X 5mm. i.d.) | Methanol : ammonium nitrate buffer solution (90 : 10). The buffer is prepared by adding 94 ml. of strong ammonia solution and 21.5 ml. of nitric acid to 884 ml. of water with adjustment of PH at 10 by adding strong ammonia solution. |

**Table: K1 Values (HPLC) of Different Amphetamines and Other Stimulants.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Serial**  **No.** | **Name of the Compound** | **K1 Value in Different Systems** | | |
| **No. 1** | **No.2** | **No.3** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14. | Amphetamine  Benzphetamine  Chlorphentermine  Dimethylamphetamine  DOM  Ephedrine  Hydroxyamphetamine  Mephentermine  Methoxyamphetamine  Mescaline  Methylamphetamine  Methylphenidate  Phenylephrine  Pseudoephedrine | 0.9  1.2  0.9  -  -  1.0  -  1.5  -  1.3  2.0  1.7  1.3  1.2 | 8.48  -  -  11.08  -  5.68  2.24  -  14.95  16.82  10.52  -  -  5.90 | 0.98  0.15  0.82  1.89  1.13  1.79  1.11  2.48  -  2.17  2.07  0.36  1.64  1.77 |

**7.7.8.2 Screening and Identification of Antidepressants by HPLC:** (3)

**Chromatographic Conditions:**

|  |  |  |
| --- | --- | --- |
| **System** | **Column** | **Eluent** |
| No. 1 | Same as No. in 7.7.8.1. (HPLC of amphetamines etc.) |  |
| No. 2 | ODS – Silica (ODS-Hypersil,  5 μm, 16 cm X 5 mm. i.d.) | Acetonitrile: Phosphate buffer (PH=3.0) (30:70). The buffer is prepared by adding 0.6 ml. of nonylamine to 1000 ml. of 0.01 M sodium dihydrogen phosphate (1.1998 g / lit.) with adjustment of PH at 3.0 by adding phosphoric acid. |

**Table: K1 Value (HPLC) of Antidepressants in Different System.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Serial**  **No.** | **Name of the Compound** | **K1 Value in Different Systems** | |
| **No. 1** | **No.2** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11. | Amitriptylene  Butriptylene  Clomimipramine  Desimipramine  Imipramine  Nortriptylene  Oxypertine  Trimipramine  Viloxazine  Noxiptylene  Protriptylene | 3.3  2.7  3.4  2.1  4.2  2.0  0.7  2.7  2.7  -  2.1 | 5.42  7.33  9.92  3.60  4.17  4.58  1.33  6.17  0.17  1.63  3.60 |

**7.7.8.3 Screening and Identification of Antihistamine by HPLC:**

**Chromatographic Conditions:**

System No. 1 as stated in 7.7.8.1. (HPLC of amphetamines etc.)

**Table: K1 Value (HPLC) of Antihistamines.**

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Name of the Compound** | **K1 Value** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16.  17. | Antazoline  Bromopheniramine  Buclizine  Chlorcyclizine  Chlorpheniramine  Cyclizine  Cyproheptadine  Dimethothiazine  Meclozine  Mepyramine  Methapyrilene  Phenindamine  Pheniramine  Promethazine  Pyrrobutamine  Tolpropamine  Triprolidine | 1.8  4.1  0.7  2.3  3.9  2.9  3.2  2.1  0.7  3.9  4.1  2.5  4.1  5.0  2.8  2.9  3.2 |

**7.7.8.4 Screening and Identification of Local Anesthetics by HPLC:** (6)

**Chromatographic Condition:**

|  |  |  |
| --- | --- | --- |
| **System** | **Column** | **Eluent** |
| No. 1 | Same as system No. 1 as stated under 7.7.8.1 (amphetamine) | Same as system No. 1 as stated under 7.7.8.1 (amphetamine) |
| No. 2 | ODS-Silica (ODS – Hypersil 5 μm, 16 cm x 5 mm i.d.). | Methanol : Water: 1% (v/v) solution of phosphoric acid : Hexylamine (30:70:100:1.4). |
| No. 3 | Same as above. | Methanol : 1% (v/v) solution of Phos- phoric acid : Hexylamine (100 : 100 : 1.4) |

**Table:** K1 Values (HPLC) of Antihistamines in Different Systems.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Serial**  **No.** | **Name of the Compound** | **K1 Value in Different Systems** | | |
| **No. 1** | **No.2** | **No.3** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16.  17.  18. | Amethocaine  Benzocaine  Benzylecgonine  Bupivacaine  Butanilicaine  Chlorprocaine  Cocaine  Cyclomethycaine  Dimethisoquin  Lignocaine  Mepivacaine  Oxybuprocaine  Piperocaine  Pramoxine  Pilocaine  Procaine  Propoxycaine  Proxymetacaine | 2.0  0.1  0.9  (tailing peak)  0.9  -  -  2.8  -  2.2  0.6  0.9  -  -  0.6  1.0  1.9  -  2.1 | 16.25  20.06  5.68  7.19  4.42  0.24  2.68  -  -  0.79  1.09  16.25  4.59  -  1.38  0.00  1.09  1.38 | 1.33  1.61  -  0.86  -  -  -  10.31  11.24  -  -  0.86  -  2.48  -  -  -  - |

**7.7.8.5 Screening and Identification of Benzodiazepines by HPLC:** (4,5)

**Chromatographic Conditions:**

|  |  |  |
| --- | --- | --- |
| **System** | **Column** | **Eluent** |
| No. 1 | ODS-Silica (ODS – Hypersil,  5 μm, 20 cm x 5 mm i.d.). | Methanol : Water : Phosphate Buffer  (55 : 25 : 20).  The buffer is prepared by dissolving 11.038 gms. (0.092M) of sodium dihydrogen phosphate and 1.136 gms (0.008M) of disodium hydrogen phosphate in sufficient water to produce 1000 ml. |
| No. 2 | As above. | Methanol : Water: Phosphate Buffer (as above) in the ratio 70 : 10 : 20. |
| No. 3 | Silica (Spherisorb, 5 μm,  25 cm x 5 mm. i.d.). | Methanol to which 100 μl of perchloric acid per litre has been added. |

**Table: K1 Values (HPLC) of Some Benzodiazepines:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Serial**  **No.** | **Name of the Compound** | **K1 Value in Different Systems** | | |
| **No. 1** | **No.2** | **No.3** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16.  17.  18.  19. | Alprazolam  Bromazepam  Clobazam  Chlordiazepoxide  Clonazepam  Demoxepam  Diazepam  Flunitrazepam  Flurazepam  Ketazolam  Lorazepam  Medazepam  Midazolam  Nitrazepam  Nordazepam  Oxazepam  Prazepam  Temazepam  Triazolam | 4.70  2.32  3.91  6.41  2.85  2.42  9.47  3.15  -  12.81  4.60  -  9.75  2.96  8.00  4.62  -  5.68  4.38 | -  -  -  -  -  -  2.29  -  3.19  2.45  -  7.05  2.10  -  -  -  4.60  -  - | 2.79  2.99  0.03  2.87  0.35  0.03  2.49  0.47  6.50  0.04  0.14  4.44  5.90  1.49  1.99  0.73  2.19  0.60  1.83 |

**7.7.8.6 Screening and Identification of Some Phenothiazines by HPLC:**

**Chromatographic Conditions:**

Column : Silica (Spherisorb S5W, 5μm, 12.5 cm. X 4.9 mm.

i.d.)

Eluent : A solution containing 1.175 gms. (0.01M) of

ammonium perchlorate in 1000 ml.of methanol.

The PH of the solution is adjusted to PH 6.7 by the addition of 1 ml.of 0.1M sodium hydroxide in methanol.

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Name of the Compound** | **K1 Value** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16.  17.  18.  19.  20. | Acetophenazine  Butaperazine  Carphenazine  Chlorpromazine  Diethazine  Dimethothiazine  Fluopromazine  Fluphenazine  Mesoridazine  Methotrimeprazine  Metopimazine  Pericyazine  Perphenazine  Prochlorperazine  Propiomazine  Thiethylperazine  Thioproperazine  Thioridazine  Trifluoperazine  Trimeprazine | 1.9  3.4  1.7  4.1  3.4  2.1  2.7  1.2  6.0  3.2  1.4  1.3  1.9  3.9  2.1  3.8  4.1  5.2  3.0  3.1 |

**7.7.8.7 Screening and Identification of Sulfonamides by HPLC:** (7)

**Chromatographic Conditions:**

Column : Silica (Sphersorb, 5μm, 25 cm. X 4 mm.

i.d.)

Eluent : Cyclohexane : Ethanol : Acetic Acid

(85.7 : 11.4 : 2.9)

**Table: K1 Values (HPLC) of Some Sulfonamides.**

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Compound** | **K1 Value** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14. | Sulfadoxine  Sulfamerazine  Sulfaquinoxaline  Sulphacetamide  Sulphachlorpyridazine  Sulphadiazine  Sulphadimidine  Sulphafurazole  Sulphamethoxazole  Sulphamethoxydiazine  Sulphamoxole  Sulphapnilamide  Sulphapyridine  Sulphathiazole | 4.4  8.1  4.8  7.7  3.3  8.7  7.1  6.0  4.8  8.2  12.6  8.9  3.8  13.4 |

**7.7.8.8 Screening and Identification of Some Cardiac Glycosides by HPLC:**

**Chromatographic Conditions:**

Column : Silica (Lichrosorb SI60, 10μm, 25 cm. X

4 mm. i.d.)

Eluent : Cyclohexane : Ethanol : Acetic Acid

(60 : 9 : 1)

**Table: K1 Values (HPLC) for Some Cardiac Glycosides**

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Compound** | **K1 Value** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10. | Digitoxigenin  Digitoxin  Digoxigenin  Digixin  Gitaloxin  Gitoxigenin  Gitoxin  Lanatoside A  Lanatoside B  Lanatoside C | 2.0  5.4  4.5  11.3  6.8  3.7  8.6  17.9  31.8  39.5 |

**7.7.8.9 Screening and Identification of Ergot Alkaloids by HPLC:**

**Chromatographic Conditions:**

|  |  |  |
| --- | --- | --- |
| **System** | **Column** | **Eluent** |
| No. 1 | Silica (Spherisorb S5W, 5 μm, 12.5 cm. X 4.9 mm i.d.) | A solution containing 1.175 gm. (0.01M) of ammonium perchlorate in 1000 ml. of methanol with adjustment of PH at 6.7 by adding 1 ml. of 0.1M sodium hydroxide in methanol. |
| No. 2 | ODS – Silica (ODS-Hypersil,  5 μm, 10 cm X 5 mm. i.d.) | Methanol : Phosphate buffer : : 60 : 40. The phosphate buffer is prepared by dissolving 3.43 gms. (0.022M) of sodium dihydrogen phosphate and 10.03 (0.028M) of disodium hydrogen phosphate in sufficient water to produce 1000 ml. |

**TABLE: K1 Values (HPLC) of Ergot Alkaloids:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Serial**  **No.** | **Compound** | **K1 Value in Different Systems** | |
| **No. 1** | **No.2** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14. | Dihydroergocristine  Hydroergotamine  Dihydroergotamine  Ergocornine  Ergocristine  Ergocryptine  Ergometrine  Ergosine  Ergosinine  Ergotamine  Lysergide  Methylergometrine  Methylsergide  Lysergamide | -  -  0.6  0.4  0.3  0.4  0.4  0.3  0.3  0.4  0.7  0.4  0.4  0.5 | 18.3  15.9  11.4  10.2  17.3  15.2  0.50  7.08  17.7  9.58  1.83  0.83  2.33  0.33 |

**N.B. :** Detector used in the HPLC Screening of Drugs.

UV detector is used for routine analysis of drugs. Many organic compounds absorb at 254nm. and hence a fixed wavelength detector has many uses. However, a variable wavelength detector can be invaluable to increase the sensitivity of detection by using the wavelength of maximum absorption. This can also increase the selectivity of detection by enhancing the peak of interest relative to interfering peaks. Most drugs show some absorption at a very low wavelength (220nm or less), but as selectivity is low such detection wavelength should only be used to enhance sensitivity in the analysis of sample said to contain a particular drug.

**7.7.8.10 Quantitation of Drugs by HPLC:**

The methods of quantitative analysis are essentially those inherited from gas chromatography. Peak height or peak area can be measured either manually or with electronic devices. Peak height measurements have the advantage of simplicity but are sensitive to changes in peak shape. Peak areas measurements should always be used where peaks are broad and tailing.

For a given system, a calibration curve must be constructed for each compound to be analysed because the detector response to each will be different. The curve of peak height (or area) against drug concentration can then be used to quantify the unknown sample. Such external calibration require careful control of injection volumes and valve injection should be used.

However, external calibration is still susceptible to errors arising from fluctuations in column performance and the internal standard technique gives better precision. This requires the addition of a fixed amount of a substance (internal standard) to the sample before injection. Quantitation is carried out using a peak height (or area) ratios of drug to internal standard. For the analysis of drugs in complex samples, the internal standard should be added to the mixture before extraction to compensate for extraction losses.

**7.7.9 Prelude to Chemical Analysis of Basic Drugs:**

**Informatics:**

Like other classes of poison the following informations are required to be known for speedy disposal of cases.

1. The history with mentioning of name of drug, if possible.
2. P.M. Report in a detailed form.
3. If strips of tablets or pills in containers, pharmaceutical preparation, injection samples are sent for analysis, the active constituent, are to be known from label or from B.P., I.P. or USP. The active constituents are to be separated following B.P. or I.P. or USP methods to check up some physical properties viz. m.p., solubility, colour etc.

The Proprietary names as well as chemical name of some of the drugs are given below. However, it’s a very partial presentation on drugs of forensic importance.

**Table: Brand Name and Chemical Name of Some Basic Drugs.**

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Chemical Name** | **Proprietary / Brand Name** |
| 1.  2.  3.  4.  5.  6.  7.  8.  9.  10.  11.  12.  13.  14.  15.  16. | Chlordiazepoxide  Clonazepam  Clozapine  Diazepam  Lorazepam  Nitrazepam  Oxazepam  Methaqualone  Pethidine  Pheniramine  Diphenylhydramine  Benzocaine  Alprazolam  Midazepam  Lysergamide  Mecloqualone | Amixide, Libotrip, Librium, Lim-bival, Normaxin.  Lonazep, Rivotril, Clonopin.  Sizopin.  Anxol, Calmpose, Camrelease TR, Depsonil-DZ, Elcion-CR, Lori Placidon, Vallium.  Ativan, Larpose, Trapen.  Nitavan, Nitravet, Nitrasun  Serepax.  Mequin, Normi-Nox, Quaalude  Isonipecaine, Meperidine  Inhiston  Benzhydramine  Anaesthesium, Anaesthamine,  Ethoforme  Xanax.  Hypnovel.  Ergine, Lysergic Acid Amide Nabarene. |

**7.7.10 Extraction and clean up of Basic Drugs in samples:**

1. In Tablets, Pharmaceutical Preparation and Injection:

As stated earlier, the active constituents are to be separated following the procedures mentioned in B.P. / I.P./ USP. Alternatively, the separation may be done as per procedures mentioned under Section 3.

1. Extraction of Basic Drugs in Biological and Non-Biological Matrices:

These are to be done as detailed in Section 3.

1. Stripping of active constituents extracted:

These are to be done as detailed in Section 3.

1. Analysis:
   1. The rapid screening methods viz. colour tests, UV Spectra, TLC are followed as detailed in this Section to locate the class and compound.

In case of analysis of tablets, the physical parameter, viz. colour, solubility, M.P. etc. furnish clues prior to analysis.

* 1. The confirmation is made by any of the methods viz. TLC, GLC or HPLC.
  2. The quantitation is done by HPLC or GLC as and when required by peak area or peak height calculation method using added internal standard or otherwise.

Sometime the basic drugs viz. phenothiazines and benzodiazepines may be partially extracted in acid-ether fraction. In case of quantitation, this fraction is to be taken to avoid error.

**7.7.11 Description and Analyticals Related to Some Basic Drugs:**

**Table: Characteristics and Analysis of Basic Drugs of Different Classes.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name and Characteristics** | **Colour Test** | | **TLC Data for Identification** | **GC / HPLC Data for Identification.** |
| **Reagent** | **Colour** |
| 1. Chlordiazepoxide (Benzodiazepine).  Soluble in: Ethanol, ether and chloroform. Insoluble in water. | Marquis Test. | Yellow. | Solvent System I :  Benzene : Acetone : Ammonium Hydroxide : : : 50 : 50 : 1.5.  Solvent System II:  Methanol : Ammonium Hydroxide : : 100 : 1.5.  Spray Reagents:   1. Dragendorff’s Reagent. 2. Acidified Iodoplatinate. | Described earlier. |
| 2. Chlonazepam (Benzodiazepine)  Soluble in: Ethanol and ether.  Insoluble in: Water. Sparingly Soluble in: Acetone, Chloroform. | Formaldehyde –Sulphuric acid. | Orange. | As above. | Described earlier. |
| 3. Diazepam (Benzodiazepine). | Dragendorff’s reagent  FPN reagent Marquis reagent. | Orange.  Yellow.  Yellow. | As above. | Described earlier. |
| 4. Lorazepam (Benzodiazepine.  Soluble in Ethanol, acetone, glacial acetic acid.  Insoluble in: Water.  Sparingly Soluble in  Chloroform, ether. | Formaldehyde  -Sulphuric acid.  Marquis test  Sulphuric acid. | Orange.  Yellow.  Yellow. | As above. | Described earlier. |
| 5. Nitrazepam (Benzodiazepine).  Soluble in: Ethanol,  Chloroform, ether.  Insoluble in: Water. | Formaldehyde  -Sulphuric acid. | Orange. | As above. | Described earlier. |
| 6. Oxazepam (Benzodiazepine.  Soluble in: Ethanol,  Chloroform, ether, dioxan  Insoluble in: Water. | Formaldehyde.  - Sulphuric acid. | Orange. | As above. | Described earlier. |
| 7.Pheniramine (antihistamine).  State: Yellowish oily liquid.  Soluble in: Ethanol, chloroform & ether.  Insoluble in Water.  Salts are soluble in water. | Libermann’s Test. | Red Orange. | Plate : Silica gel G  Solvent Systems:  No. 1  Benzene : Acetone : Ammonia  : : : 50 : 50 : 1.5.  No. 2.  Methanol : Ammonia : : 100:1.5.  Spray reagents:  1.Dragendorff’s reagent  2.Acidified Iodoplatinate. | Given earlier. |
| 8.Promethazine (antihistamine).  State: Hydrochloride  Exists as white or faintly yellow crystalline solid of m.p. 222oC. | Formaldehyde  -Sulphuric acid.  Forrest  Reagent  FPN reagent  Mandelin’s  Reagent.  Marquis Reagent. | Blue violet.  Red.  Orange.  Green-violet  Violet. | Plate : Silica gel G Solvent Systems.  No. 1.  Methanol : Ammonia : : 100:1.5.  No. 2.  Benzene : Acetone : Ammonia  : : : 50 : 50 : 1.5.  No. 3.  Chloroform : Methanol : : 90:10. | Given earlier. |
| 9. Amphetamines (CNS -  Stimulant). | Marquis Reagent.  Mandelin’s Reagent.  Frohde’s Reagent. | Brown Orange.  Green(dark- ens rapidly)  Brown yellow. | Plate : Silica gel G  Solvent Systems.  n-Hexane: Acetone : Ammonium Hydroxide : : : 80 : 20 : 1.  Spray reagent : Iodoplatinate Spray (violet –brown spot). | Given earlier.  GC-given earlier & also in section 8. |
| 10.Glutethimide (Depressant).  Highly Soluble in : Fats and oil.  Soluble in: Water. | Koppayani  -Zwikker Test  Libermann’s  Test.  Mercurous nitrate Test. | Violet.  Red Orange.  Black. | Plate : Silica gel G  Solvent Systems:  No. 1.  Ethyl acetate : Methanol : 25% : Ammonia : : : 85 : 10 : 5.  No. 2.  Chloroform : Acetone : : 80 : 20.  Isopropyl Alcohol : Chloroform  : Ammonia : : : 9 : 9 : 2.  Spray reagent :  Mercuric chloride. Diphenyl carbazone spray reagent (violet spot). | G.C: Given earlier  HPLC  Column – 12.5 X 4.5 mm (i.d.).  Alkyl silica Column (SAS – Hypersil).  Eluent:  Acetonitrile :  Tetrabutyl ammonium phosphate (0.005M, PH=4.5)  : : 20 : 80.  K1 Value  7.97  Special Point:  Abs. Maxima in ethanol at wave lengths(nm): 252, 258 and 264. |

**1. Analgesics**.

**Class of Compound:** Analgesics

**Conditions**

Column: SUPELCOSIL LC-ABZ, 5cm x 4.6mm ID, 5µm particles   
Mobile Phase: acetonitrile: 25mM KH2PO4, pH 2.3 with phosphoric acid (20:80)  
Flow Rate: 2mL/min  
Det.: UV, 230nm  
Inj.: 5µL mobile phase, analyte quantities shown

**Analyte Data**

1. Dextromethorphan  
2. Acetylsalicylic acid

**2. Cardiac Drugs**

**Conditions**

Column: SUPELCOSIL ABZ+Plus, 15cm x 4.6mm ID, 5µm  
Mobile Phase: A = acetonitrile   
B = 25mM KH2PO4 (pH 3.0)  
Flow Rate: 2mL/min  
Det.: UV, 220nm  
Inj.: 20µL methanol:water, 50:50 (30µg/µL pindolol, diltiazem, 100µg/mL   
dipyridamole, 50µg/mL other analytes)

**Analyte Data**

1. Procainamide  
2. Pindolol  
3. Oxprenolol  
4. Dipyridamole  
5. Diltiazem  
6. Verapamil  
7. Digoxin  
8. Lidoflazine  
9. Flunarizine  
10. Nifedipine

**3. Analgesics, Narcotic**.

**Class of Compound**: Analgesics, Narcotic  
**Technology**: HPLC

**Conditions**

Column: SUPELCOSIL LC-ABZ, 15cm x 4.6mm ID, 5µm particles   
Mobile Phase: acetonitrile:25mM KH2PO4, pH 7.5 (35:65)  
Flow Rate: 1.5mL/min  
Det.: UV, 254nm   
Inj.: 10µL water, analyte quantities shown

**Analyte Data**

1. Codeine (10µg/mL)  
2. Oxycodone (50µg/mL)  
3. Meperidine (200µg/mL)

**4. Sedatives, Barbiturates in Serum**

**Class of Compound**: Sedatives- Barbiturates in Serum

**Conditions**

Column: Hisep, 15cm x 4.6mm ID, 5µm particles   
Mobile Phase: 50mM KH2PO4/5mM sodium decylsulfate, pH 6.6  
Flow Rate: 1mL/min  
Temp.: 35°C  
Det.: UV, 220nm  
Inj.: 20µL spiked human plasma containing 10µg/mL each compound (direct injection)

**Analyte Data**

1. Aprobarbital  
2. Hexobarbital  
3. Cyclobarbital  
4. Amobarbital  
5. Phenobarbital  
6. Mephobarbital  
7. Secobarbital

**5. Aspirin and Related Compounds**.

**Class of Compound**: Aspirin and Related Compounds

**Conditions**

Column: SUPELCOGEL TPR-100, 15cm x 4.6mm, 5µm particles   
Mobile Phase: acetonitrile:water, 30:70 + 0.1% TFA  
Flow Rate: 1.0mL/min  
Temp.: ambient  
Det.: UV, 234nm  
Inj.: 10µL  
**Analyte Data**

1. 4-Hydroxybenzoic acid  
2. Acetylsalicylic acid  
3. p-Acetophenetidide  
4. Salicylic acid

**6. Antibacterials, Septra Solution**.

**Class of Compound** : Anti-bacterials: Septra Solution  
   
**Conditions**

Column: SUPELCOSIL LC-18-DB, 25cm x 4.6mm ID, 5µm particles  
Cat. No.: 58355-U  
Mobile Phase: acetonitrile:water, 80:20 + 0.1% triethylamine (pH 5.9)  
Flow Rate: 2mL/min  
Temp.: 25°C  
Det.: UV, 254nm  
Inj.: 25µL mobile phase

**Analyte Data**

1. Trimethoprim (64µg/mL)  
2. Sulfamethoxazole (320µg/mL)  
3. Formulation Excipient

**7. Antipsychotic Drugs**.  
**Class of Compound**: Antipsychotic Drugs  
   
**Conditions**

Column: SUPELCOSIL LC-Si, 25cm x 4.6mm ID, 5µm particles   
Mobile Phase: heptane: methylene chloride: methanol, (70:20:10) + 0.002% triethylamine  
Flow Rate: 2mL/min  
Det.: UV, 263nm  
Inj.: 100µL

**Analyte Data**

1. Thioridazine (6.2ng)  
2. Sulforidazine (12.5ng)  
3. Mesoridazine (12.5ng)

**8. Anti-Inflammatory Drugs and Diuretics in Serum**.

**General Description**

Gradient Program  
Time (min) %A  
0 20  
5 30  
15 20

**Class of Compound**: Anti-Inflammatory Drugs and Diuretics in Serum  
**Conditions**

Column: Hisep, 15cm x 4.6mm ID, 5µm particles  
Mobile Phase: A = acetonitrile  
B = 180mM ammonium acetate, pH 5.4  
Flow Rate: 2mL/min  
Temp.: 30°C  
Det.: UV, 267nm  
Inj.: 10µL horse serum containing 5µg/mL hydrochlorothiazide, 10µg/mL each other drug

**Analyte Data**

1. Hydrochlorothiazide  
2. Oxyphenbutazone  
3. Furosemide  
4. Phenylbutazone

**9. Diazepam and Warfarin**   
**Conditions**

Column: SUPELCOSIL LC-ABZ, 5cm x 4.6mm ID, 5µm particles   
Mobile Phase: acetonitrile:25mM KH2PO4, pH 2.3 with phosphoric acid (40:60)  
Flow Rate: 2mL/min  
Det.: UV, 230nm   
Inj.: 5µL mobile phase, analyte quantities shown

**Analyte Data**

1. Diazepam (3µg/mL)  
2. Warfarin (20µg/mL)

**References:**

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**SECTION – 8: ANALYSIS OF ACIDIC (ORGANIC NON**

**VOLATILE) POISONS/ DRUGS**

**8.1 Title**: Analysis of acidic (organic non-volatile) poisons/ drugs

**8.2 Scope:** Analysis of acidic organic non volatile poisons / drugs in

crime exhibits by different analytical methods.

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

* 1. **Responsibilities:** Gazetted officers and other scientific staff
  2. **ACIDIC POISONS/DRUGS:**

**Acidic Drugs:**

Drugs which are acidic in nature are called acidic drugs. These drugs readily reacts 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.

**8.5.1 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.

8.5.1.1 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.

**8.5.1.1.1 Extraction of Barbiturates in Biological and Non - Biological Matrices:**

This is achieved according to methods described in Section-3. 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.

**8.5.1.1.2 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 100ml of absolute alcohol 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.

2. **Zwikker’s Test:**

The residue of extract is taken up in chloroform. To 1 ml. of chloroform extract, 2-3 drops of 0.5 ml. of 5% pyridine in chloroform is added and shaken. The colour of chloroform layer becomes purple. Then 1 drop of glacial acetic acid is added. If the colour of chloroform layer changes from purple to weak blue, the presence of non-thiobarbiturate is indicated.

If chloroform layer becomes green after adding pyridine in chloroform, the presence of thiobarbiturate is indicated. This green colour changes to light green on adding acetic acid.

**8.5.1.1.3 Screening of Barbiturates by UV Spectrophotometer:**

Barbiturates show almost same absorption maxima under a definite condition of acid or alkali etc. The appearance of absorption maxima is an indication for the presence of barbiturate.

|  |  |  |
| --- | --- | --- |
| Compound | **Absorption Maxima (in nm) 0.05 M Borax Buffer (pH=9.2)** | **Absorption Maxima**  **(in nm) in 1M NaOH.** |
| 1. Allobarbital. 2. Barbital. 3. Butobarbital. 4. Butabital. 5. Cyclobarbital. 6. Pentobarbital. 7. Phenobarbital. 8. Secbutabarbital. | 241  239  239  240  239  239  239  239 | 256  254  254  255  256  255  254  254 |

**Additional Data on UV Spectroscopy of Barbiturates Etc:**

1. All barbiturates show absorption maxima at 240 nm in 0.05 M borax buffer (PH=10, prepared by dissolving 19.07 gms. of borax in water and diluting it to 1000 ml.).

2. Barbiturates in general (other than N-substituted compounds) show absorption maxima at 255 nm in 2.5N sodium hydroxide.

1. Characteristic absorption maxima for some compounds

Vinyl barbital in aqueous alkali = 247 nm.

Glutithimide in ethanol = 252, 258 and 264 nm.

Meprobamate = shows no absorption in the range 230-360 nm.

4. Thiobarbiturates show absorption maxima at 290 nm and 239 nm in 2 N sulphuric acid.

**8.5.1.1.4 Screening of Barbiturates by I.R. Spectroscopy:**

The data as presented hereunder may be profitably explored if the samples are obtained as tablets or their remnants. The extracted purified material may be subjected to I.R. spectroscopy (using KBr. Disc). In case of biological materials, there may be interference due to extraneous materials viz. traces of fat and protein etc. in spite of best efforts for purifying the material. The I.R. bands may be varied and complex.

**Table: Principal I. R. Peaks of Some Barbiturates.**

|  |  |
| --- | --- |
| **Compound** | **Principal Peaks (Wave No. in Cm-1)** |
| Allobarbital.  Barbital.  Butobarbital.  Butabarbital.  Cyclobarbital.  Pentobarbital.  Phenobarbital.  Secbutabarbital.  Vinylbarbital.  Glutithimide.  Meprobamate.  Thiopentone. | 1687, 1315, 925, 1219, 847, 1640.  1680, 1720, 1767, 1320, 1245, 875.  1696, 1727, 1760, 1242, 850, 1215.  1690, 1720, 1740, 1310, 1290, 1200.  1693, 1725, 1745, 1300, 1210, 830.  1685, 1719, 1744, 1315, 1218, 845.  1712, 1684, 1670, 1770, 1310, 1300.  1675, 1760, 1317, 1303, 1230, 853.  1692, 1730, 1750, 1318, 1220, 1630.  1686, 1710, 1200, 1270, 1281, 704.  1688, 1069, 1090, 1590, 1310, 1140.  1670, 1540, 1300, 1170, 1735, 1220. |

**8.5.1.1.5 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 (2.5: 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 batch 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 toxic hazards.

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

2. **Zwikker’s Reagent:**

**Preparation of Reagent:**

40 ml. of 10% solution of copper sulphate is mixed with 10 ml. of pyridine. It is made up to 100 ml. by adding water.

**Colour of Spots:** Pink spots are observed in case of non-thio barbiturates and green spots for barbiturates.

3. **Mercurous Nitrate:** A saturated solution of mercurous nitrate containing a few drops of conc. nitric acid.

**Colour of Spots:** Black spots are observed for barbiturates.

|  |  |  |  |
| --- | --- | --- | --- |
| **Compound (Barbiturate)** | **hRf in Solvent Systems** | | |
| **No. 1** | **No. 2** | **No. 3** |
| Allobarbital.  Amobarbital.  Barbital.  Butabital.  Butobarbital.  Cyclobarbital.  Methyl Phenobarbital.  Pentobarbital.  Phenobarbital.  Secbutabarbital.  Secobarbital.  Vinylbital.  Glutithimide.  Meprobamate.  Thiopentone. | 31  40  33  44  39  35  41  44  29  44  42  40  78  69  77 | 50  52  41  54  50  50  70  55  47  50  55  38  63  60  49 | 53  74  51  67  68  59  72  76  38  69  -  56  -  -  80 |

**8.5.1.1.6 Identification of Barbiturates by GLC:**

**Table: Gas Chromatographic Conditions and Data on Barbiturates.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Analyticals on GC** | | | **Compound** | **Retention Indices** | | |
| **Conditions** | **GC Method** | | **Packed SE-30 (Derivatized)** | **SE-30 Capillary Column** | **SE-54 Capillary Column** |
| **Without Derivatization** | **Derivatized with Trimethyl Ammo -nium Hydroxide (0.2M) in Methanol** |
| 1. Column  etc.  2. Carrier  gas and  flow  rate.  3. Column  tempera-  ture.  4. Injector / Detector  tempera-  ture.  5. Internal  standard.  6. Internal / Standard/  Drug standard concentration. | SE-30 or SE-54 glass capillary.  Nitrogen, 1ml./ min.  Isothermal at  200oC or pro-grammed from 200-260oC at 4oC/min.  275oC.  n-Alkanes.  1 mg./ml. | 6’x 2-4 mm (i.d.) glass column with 3% SE-30 on 80-100 mesh Chromosorb G WP.  Nitrogen, 45-50 ml./ min.  190 – 200oC.  220oC.  n-Alkanes.  1 mg./ml. | Allobarbital.  Amobarbital.  Barbital.  Butalbital.  Butobarbital.  Cyclobarbital.  Methyl-  Phenobarbital.  Pentobarbital.  Phenobarbital.  Secbutabarbital.  Secobarbital.  Vinylbital. | 1491  1600  1415  1553  1557  1850  1832  1632  1831  1564  1670  1629 | 1575  1695  1465  1642  1642  1946  1875  1719  1934  1635  1770  1712 | 1629  1751  1519  1698  1695  2026  1650  1778  2012  1692  1827  1774 |

**8.5.1.1.7 Identification of Barbiturates by HPLC:**

**Table:** Data on HPLC of Barbiturates.

|  |  |  |  |
| --- | --- | --- | --- |
| **Conditions** | **HPLC Conditions** | **Compound** | **Capacity Ratio (K' Value)** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Method 1** | **Method 2** |  | **Method 1** | **Method 2** | |
| **Mobile Phase A** | **Mobile Phase B** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 1. Column  packing  etc.  2. Mobile  Phase.  3. Flow rate  4. Detector. | 250 mm x 4.6 mm(i.d.) Octa-decyl silica HPLC grade, 5 micrometer (Sphersorb 5 ODS-2 or  equivalent).  Acetonitrile : Water :30: :70.  0.9 ml./ min.  UV at 220 and 254 nm. | 150 mm x 4.6 mm (i.d.) Octadecyl silica HPLC grade, 5 micrometer (ODS –Hypersil or  equivalent).  Mobile Phase A.  0.1M Sodium dihy-drogen phosphate buffer : Methanol : : 60 : 40.  Mobile Phase B.  0.1M Sodium dihy-drogen phosphate buffer : Methanol with adjustment of PH at 8.5 by : : 60:40.  2 ml./ min.  UV at 216 and 254 nm. | Allobarbital.  Amobarbital.  Barbital.  Butalbital.  Butobarbital.  Cyclobarbital.  Methyl-  Phenobarbital.  Pentobarbital.  Phenobarbital.  Secbutabarbital.  Secobarbital.  Vinylbital. | 1.35  4.86  0.60  2.90  2.56  2.56  5.72  4.63  1.94  2.24  6.81  4.80 | 2.46  10.19  1.11  6.17  5.43  5.25  7.27  10.96  3.09  4.89  16.28  10.40 | 1.37  7.05  0.63  3.48  3.42  2.61  3.84  8.07  1.23  3.32  11.47  7.05 |

**8.5.1.1.8 Quantitation of Barbiturates in Samples:** (2,3,4,5, 6,7)

This may be conveniently done by peak area calculation in the chromatogram in GC or HPLC.

**8.5.2 Salicylates:**

**8.5.2.1 Salicylic Acid and Salicylates in Biological Materials:**

A. **Extraction of Salicylic Acid:**

50 gms. of biological materials (viscera) or 5 ml. of serum or blood or 20 ml. of urine is subjected to Stas-Otto process. The acid ether extract should contain salicylic acid (Stas-Otto process-Refer Section-3).

B. **Extraction of Salicylates:**

50 gms. of biological materials (viscera) or 5 ml. of serum or blood or 20 ml. of urine is subjected to extraction by warm absolute alcohol. The alcoholic solution is filtered. The filtrate is dehydrated with anhydrous sodium sulphate and then evaporated to dryness. The residue is extracted with ether in portions. The combined ether extract is evaporated to dryness. The residue is kept reserved for different tests etc.

**8.5.2.2 Test with Urine Samples or Extract of Biological Materials:**

1. To 1 ml. of concentrated extract of biological material or urine 3 drops of ferric chloride solution is added. A violet colour indicates the presence of salicylic acid.

2. To 0.5 ml. of concentrated extract of biological materials or urine or plasma, 4.5 ml. of 0.55% ferric nitrate solution in 0.04N nitric acid is added. A purple colour (proportioned to the concentration of salicylate) is obtained.

**N.B.** Salicylates including aspirin are to be hydrolysed to salicylic acid by boiling with a few drops of 1 N hydrochloric acid, cooling and neutralizing before carrying out the above tests.

3. **McNally’s Test:**

To the residue of extract of biological materials a few drops of acetone and 1-2 ml. of water are added. 2 drops of 0.5% copper sulphate solution in 10% acetic acid is added followed by a pinch of solid sodium nitrite. It is shaken and heated gradually to boiling and maintained in boiling condition for a few minutes. A red colour is formed if salicylates and salicylic acid are present.

**8.5.2.3 Identification of salicylic acid and salicylates by TLC:**

Plate: Plates coated with Silica gel GF254 (0.2 mm. thickness)

Method: Ascending method of Development.

**Table: TLC Data of Salicylic acid and Salicylates.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Solvent** | **Visualization** | **Compound** | **hRf Value** |
| Glacial acetic acid:  Benzene : Ether : Methanol ( 9 : 60 : 30 : 1). | By U.V. Light. | Aspirin  Salicylic acid.  Genistic acid. | 50  57  34 |

**8.5.2.4 Identification of Salicylic Acid and Salicylates by UV Spectroscopy.**

**Table: UV Data of Salicylic Acid and Salicylates.**

|  |  |
| --- | --- |
| **Compound** | **Absorption of Maxima at Wavelength (nm).** |

|  |  |  |
| --- | --- | --- |
|  | **In 0.5N Sodium Hydroxide Solution** | **In 0.1N Sulphuric Acid solution** |

|  |  |  |
| --- | --- | --- |
| Salicylic Acid  Aspirin  Salicylamide | 260, 300   * -   242, 328 | 302  229  276 |

**8.5.2.5 Identification of Salicylic acid and Salicylates by GLC:**

**Table: Chromatographic (GC) Conditions and Data on Salicylic Acid and Salicylates.**

|  |  |  |
| --- | --- | --- |
| **GLC Conditions** | **Compound** | **RRT Relative to Barbitone** |
| 1. Column 10% Apiezon-L on 80-100 mesh  Chromosorb W.  2. Carrier Gas : Nitrogen.  3. Flow rate : 50 ml./ min.  4. Column temperature : 210oC.  5. Detector : FID.  Hydrogen flow rate : 50 ml./ min.  Air flow rate : 30 ml./ min. | Aspirin.  Salicylic acid.  Salicylamide. | 0.36  0.54  1.04 |

Other methods like FTIR and GC-MS can be used for identification of salicylates.

**8.5.2.6 Mass Spectrum peaks of common Salicylates:** (8)

Principal peaks at m/z

Acetyl salicylic acid 120, 43, 138, 92, 121, 39, 64, 63

Salicylic acid 120, 92, 138, 64, 39, 63, 121, 65

Salicyluric acid 121, 120, 69, 92, 195, 39, 93, 45.

Salicylamide 120, 92, 137, 65, 121, 39, 64, 53.

**8.5.2.7 HPLC conditions and Data on Common Salicylates** (9)

|  |  |  |
| --- | --- | --- |
| **HPLC Conditions** | **Compound** | **K1 values** |
| 1. Column: octadecylsilica gel (ODS – Hypersil ,5 μm 16cm X 5mm i.d)   1. Eluent – a). Isopropyl alcohol: Formic acid: 0.1 M Potassium dihydrogenphosphate (13.61gm/litre) :::540:1:1000   b). Isopropyl alcohol: Formic acid: 0.1 M Potassium dihydrogen phosphate (13.61gm/litre) (176:1:1000). | Salicylic acid | 0.7  4.6 |
| 1. Column: octadecylsilica gel (ODS – Hypersil ,5 μm 16cm X 5mm i.d)   1. Eluent – a). Isopropyl alcohol: formic acid: 0.1 M Potassium dihydrogenphosphate (13.61gm/litre) :::540:1:1000   b). Isopropyl alcohol: Formic acid: 0.1 M Potassium dihydrogen phosphate (13.61gm/litre) :::176:1:1000. | Acetyl salicylic acid | 0.5  2.7 |

**HPLC Conditions for Acetyl Salicylic Acid and Salicylic Acid** (10)

**Acetyl Salicylic Acid**

Column – Octadecylsilyl silica gel (5μm)

Mobile Phase – Phosphoric acid: Acetonitrile: Water (2:400: 600)

Flow rate – 1ml/min

Detector – UV at 237nm.

**Salicylic Acid**

Column – Octadecylsilyl silica gel (5μm)

Mobile Phase – Glacial acetic acid: Methanol: Water (1:40:60)

Flow rate – 0.5ml/min

Detector – UV at 270nm

N.B: For detail refer British Pharmacopoeia method given under corresponding monograph

**8.5.3 Phenols**

Phenols of forensic importance include phenol (carbolic acid – covered in the section of organic volatiles), cresols and resorcinol. These are disinfectants in nature.

**8.5.3.1 Isolation of Phenols in Biological Materials:**

Carbolic acid is isolated as volatile by acid-distillation for volatiles. Other phenols are isolated by acid-ether extract in Stas-Otto process. This is evaporated to dryness and subjected to chemical analysis.

**8.5.3.2 Screening of Phenols by Colour Tests:**

The dried residue of extract is subjected to chemical tests by different reagents.

**Table: Tests for Phenols.**

|  |  |  |
| --- | --- | --- |
| **Reagents** | **Procedure** | **Colour Observed for Different Phenols.** |
| 1. Ferric Chloride Test: | 2-3 drops of ferric chloride is added to the residue of extract. | Blue Colour- Phenol, Para chloro phenol.  Blue Violet Colour –Sallol, Salicylic acid, Salicylamide. |
| 2. Libermann’s Test: Reagent: 1 gm. of potassium nitrite is dissolved in sulphuric acid to produce 10 ml. | To the residue of extract 2-3 drops of the reagent is added. | Blue red colour- Phenol.  Violet colour – Resorcinol.  Green colour-Alpha naphthol Beta-naphthol.  Brown to black-Cresols,  p-Amino-phenol. |
| 3. Folin – Ciocalteau Reagent: Stock Solution: 100 gms. of sodium  tungstate and 25 gms. of sodium molybdate are dissolved in 800 ml. of water. 50 ml. of phosphoric acid and 100ml of hydrochloric acid are added. The mixture is refluxed for 10 hours. 4-6 drops of bromine is added. It is allowed to stand for 2 hours and then boiled for 15 minutes to remove excess of bromine, cooled, filtered and then diluted to 1000 ml. The stock solution is diluted with water (1 : 2) before use.  The stock solution should be preserved at 4oC and is stable for 2 months. The colour of solution is yellow. If it turns green it should not be used. | 1 ml. of diluted reagent is added to the residue of the extract. It is then made alkaline with 2 M sodium hydroxide. | A blue colour is formed indicating presence of phenol. |

**N.B.** Other tests have been described under carbolic acid (section:4 Volatile Poisons).

**8.5.3.3 Differentiation of m- Cresol and o-Cresol by Colour Test:**

The test is applicable to solid samples as remnants.

**Procedure:**

A little amount of sample is heated with an equal quantity of phthalic anhydride and a few drops of concentrated sulphuric acid until the mixture assumes orange colour. The mixture is cooled. A few drops of water is added. The solution assumes a red colour (in case of o-cresol) or a blue violet colour (in case of m-cresol).

**8.5.3.4 Alternate screening tests for Phenols:**

The dried residue of extract of biological material is solubilized in 25 ml. of chloroform. A little solid potassium hydroxide is added and warmed. The various colours are obtained for phenol: Carbolic acid-red, Napthols-Blue, Thymol-Dark red, Pyrocatechin-Yellow brown, Resorcinol-Red, Hydroquinone – Yellow red or Reddish brown and Pyrogallol – Reddish yellow.

**8.5.3.4.1 Screening of Phenols by UV Spectrophotometry:**

**Table: UV Spectrophotometric Data of Phenols.**

|  |  |  |
| --- | --- | --- |
| **Compound** | **Absorption Maxima at Wave Lengths (nm) in** | |
| **Ethanol** | **0.1 N NaOH** |
| Phenol  Cresol.  β-Naphthol | 272, 194.6  275, 163 or  279, 145.  226, 274, 326, 285, 230, 322, 120, 330,140. | 286, 274.  239 and 194 or  290 and 251.  …………… |

**8.5.3.4.2** **Gas Chromatographic Conditions for Identification of Phenols.**

Column : 2 m. x 4 mm (i.d.) glass column packed

with 2.5% SE-30 on 80 – 100 mesh.

Chromosorb G (Acid washed and

dimethyl dichloro silane treated). The

support should be fully deactivated.

Column temperature : 100 – 150oC

Carrier gas : Nitrogen.

Flow rate : 45 ml./ min.

**8.5.3.4.3 Quantitation of Phenol by G.C:**

By Peak area calculation as described earlier.

**8.6 MS DATA OF DRUGS**

**Eight Peak Index of EI Spectra**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Drug** | **Formula** | **Mol. Wt** | **Eight most intense peaks (decreasing order)** | | | | | | | |
| 1 | Methadone | C21H27NO | 309 | 72 | 28 | 165 | 42 | 180 | 178 | 179 | 91 |
| 2 | Cocaine | C17H21NO4 | 303 | 82 | 28 | 182 | 83 | 77 | 42 | 94 | 105 |
| 3 | Chloral hydrate metabolite | C2H3Cl3O | 148 | 31 | 49 | 77 | 113 | 82 | 51 | 115 | 29 |
| 4 | Barbital 1,2 (or 4) demethyl derivative |  | 212 | 184 | 40 | 169 | 140 | 126 | 183 | 44 | 55 |
| 5 | Secobarbital | C12H18N2O3 | 238 | 41 | 168 | 167 | 43 | 39 | 55 | 97 | 124 |
| 6 | Allobarbital | C10H12N2O3 | 208 | 167 | 41 | 124 | 80 | 39 | 32 | 28 | 166 |
| 7 | Nicotine | C10H14N2 | 162 | 84 | 42 | 133 | 162 | 161 | 51 | 119 | 65 |
| 8 | Amphetamine | C9H13N | 135 | 44 | 91 | 45 | 65 | 42 | 43 | 41 | 51 |
| 9 | Strychnine | C21H22N2O2 | 334 | 334 | 44 | 120 | 77 | 41 | 107 | 55 | 144 |
| 10 | Pantazocine | C19H27NO | 285 | 217 | 55 | 70 | 110 | 202 | 270 | 69 | 285 |
| 11 | Meprobamate | C9H18N2O4 | 218 | 83 | 55 | 71 | 96 | 114 | 144 | 62 | 56 |
| 12 | Diphenhydramine | C17H21NO | 255 | 58 | 73 | 167 | 165 | 45 | 44 | 168 | 42 |
| 13 | Chlorpromazine | C17H19ClN2S | 318 | 58 | 86 | 318 | 85 | 272 | 320 | 42 | 232 |
| 14 | Flurazepam | C21H23ClFN3O | 387 | 86 | 58 | 30 | 57 | 99 | 87 | 71 | 43 |
| 15 | Chloroquine | C18H26ClN3 | 319 | 86 | 58 | 73 | 87 | 319 | 41 | 99 | 245 |
| 16 | Phenobarbital | C12H12N2O3 | 232 | 204 | 63 | 146 | 232 | 117 | 143 | 174 | 89 |
| 17 | Promethazine | C17H20N2S | 284 | 72 | 73 | 84 | 118 | 213 | 180 | 119 | 285 |
| 18 | Methaqualone MTB 4 TMS | C19H22N2O2Si | 338 | 73 | 323 | 338 | 321 | 143 | 45 | 77 | 154 |
| 19 | Oxazepam | C15H11ClN2O2 | 286 | 77 | 205 | 233 | 239 | 267 | 51 | 268 | 75 |
| 20 | Benzyl alcohol | C7H8O | 108 | 79 | 108 | 107 | 77 | 51 | 50 | 39 | 91 |
| 21 | Phencyclidine | C17H25N | 243 | 200 | 91 | 84 | 28 | 242 | 243 | 15 | 129 |
| 22 | Salicylic acid | C7H6O3 | 138 | 92 | 120 | 39 | 138 | 64 | 63 | 65 | 38 |
| 23 | Methyl salicylate | C8H8O3 | 152 | 120 | 92 | 152 | 121 | 65 | 64 | 93 | 63 |
| 24 | Phenol | C6H6O | 94 | 94 | 66 | 39 | 65 | 40 | 95 | 38 | 55 |
| 25 | Camphor | C10H16O | 152 | 126 | 95 | 41 | 81 | 39 | 69 | 108 | 83 |
| 26 | Benzyl alcohol | C7H8O | 108 | 79 | 108 | 107 | 77 | 51 | 50 | 39 | 91 |
| 27 | Caffeine | C8H10N4O2 | 194 | 194 | 109 | 55 | 67 | 82 | 15 | 18 | 42 |
| 28 | Glutethimide | C13H15NO2 | 217 | 117 | 189 | 132 | 115 | 91 | 160 | 77 | 39 |
| 29 | Pentobarbital | C11H18N2O3 | 226 | 156 | 141 | 43 | 41 | 157 | 55 | 71 | 39 |
| 30 | Morphine | C17H19NO3 | 285 | 285 | 162 | 42 | 28 | 44 | 31 | 215 | 70 |
| 31 | Codeine | C18H21NO3 | 299 | 299 | 162 | 229 | 42 | 214 | 300 | 124 | 188 |
| 32 | Secobarbital | C12H18N2O3 | 238 | 168 | 167 | 41 | 43 | 124 | 97 | 169 | 195 |
| 33 | Phenothiazine | C12H9NS | 199 | 199 | 167 | 198 | 166 | 99 | 154 | 69 | 77 |
| 34 | Secobarbital | C12H18N2O3 | 238 | 41 | 168 | 167 | 43 | 39 | 55 | 97 | 124 |
| 35 | Chlorpheniramine | C16H19ClN2 | 274 | 203 | 58 | 205 | 72 | 204 | 167 | 202 | 168 |
| 36 | Oxazepam | C15H11ClN2O2 | 286 | 77 | 205 | 233 | 239 | 267 | 51 | 268 | 75 |
| 37 | Hexobarbital | C12H16N2O3 | 236 | 221 | 81 | 28 | 79 | 157 | 80 | 27 | 155 |
| 38 | DDT | C14H9C15 | 352 | 235 | 237 | 165 | 212 | 246 | 75 | 176 | 36 |
| 39 | Diazepam MTB1 | C15H11ClN2O | 270 | 242 | 270 | 269 | 241 | 243 | 271 | 244 | 103 |
| 40 | Diazepam | C16H13ClN2O | 284 | 256 | 283 | 255 | 284 | 257 | 258 | 285 | 165 |
| 41 | Diazepam | C16H13ClN2O | 284 | 256 | 283 | 284 | 255 | 257 | 258 | 285 | 222 |
| 42 | Chlordiazepoxide | C16H14ClN3O | 299 | 282 | 283 | 28 | 284 | 299 | 241 | 247 | 77 |
| 43 | Chlordiazepoxide | C16H14ClN3O | 299 | 282 | 283 | 284 | 299 | 285 | 77 | 56 | 41 |
| 44 | Thebaine | C19H21NO3 | 311 | 311 | 296 | 42 | 44 | 255 | 312 | 310 | 253 |
| 45 | Tetrahydrocannabinol | C21H30O2 | 314 | 314 | 299 | 231 | 271 | 43 | 41 | 315 | 243 |
| 46 | Papaverine | C20H21NO4 | 339 | 324 | 338 | 339 | 308 | 293 | 325 | 220 | 340 |
| 47 | Heroin | C21H23NO5 | 369 | 327 | 369 | 268 | 43 | 310 | 215 | 42 | 204 |
| 48 | Psedomorphine | C34H36N2O6 | 622 | 30 | 44 | 42 | 568 | 31 | 58 | 27 | 81 |
| 49 | Methanol | CH4O | 32 | 31 | 32 | 29 | 30 | 28 | 33 | 34 | 27 |
| 50 | Ethylene glycol | C2H6O2 | 62 | 31 | 33 | 29 | 32 | 43 | 28 | 27 | 42 |
| 51 | Ethanol | C2H5OH | 46 | 31 | 45 | 29 | 27 | 46 | 43 | 30 | 42 |
| 52 | Triclofos sodium |  | 251 | 31 | 49 | 77 | 29 | 113 | 51 | 48 | 115 |
| 53 | 3-hydroxyquinal barbitone |  | - | 41 | 45 | 43 | 168 | 39 | 70 | 69 | 167 |
| 54 | ▲9 – THC-11-oic acid | C22H30O4 | 358 | 41 | 229 | 43 | 329 | 69 | 344 | 29 | 283 |
| 55 | Barbituric acid | C4H4N2O3 | 128 | 42 | 128 | 85 | 43 | 44 | 41 | 70 | 69 |
| 56 | Trifluperidol |  | - | 42 | 271 | 258 | 123 | 56 | 83 | 240 | 95 |
| 57 | Digitoxin | C41H64O13 | 765 | 43 | 29 | 39 | 41 | 45 | 57 | 68 | 58 |
| 58 | Isosorbide Dinitrate | C6H8N2O8 | 236 | 43 | 31 | 29 | 61 | 60 | 85 | 73 | 44 |
| 59 | Ergotixine | C31H39N5O5 | 561 | 43 | 70 | 29 | 71 | 27 | 154 | 41 | 267 |
| 60 | Trimethoxy amphetamine | C12H19NO3 | 225 | 43 | 182 | 167 | 225 | 181 | 183 | 151 | 142 |
| 61 | Adrenaline | C9H13NO3 | 183 | 44 | 42 | 124 | 165 | 163 | 123 | 93 | 65 |
| 62 | Amphetamine | C9H13N | 135 | 44 | 91 | 40 | 42 | 65 | 45 | 39 | 43 |
| 63 | Norpriptyline | C19H21N | 263 | 44 | 202 | 45 | 220 | 218 | 215 | 91 | - |
| 64 | Etorphine | C25H33NO4 | 411 | 44 | 215 | 411 | 324 | 45 | 164 | 42 | 216 |
| 65 | Pentazocine | C19H27NO | 285 | 45 | 217 | 70 | 41 | 69 | 110 | 285 | 202 |
| 66 | Pentaerythritol tetranitrate | C5H8N4O12 | 316 | 46 | 76 | 57 | 55 | 56 | 60 | 47 | 97 |
| 67 | Buprenorphine | C29H41NO4 | 467 | 55 | 378 | 43 | 29 | 57 | 410 | 379 | 84 |
| 68 | Psilocybin | C12H17N2O4P | 284 | 58 | 42 | 30 | 51 | 204 | 146 | 77 | 44 |
| 69 | Acetylcholine | C7H16ClNO2 | 181 | 58 | 43 | 57 | 149 | 71 | 42 | 41 | 55 |
| 70 | Amitriptyline | C20H23N | 277 | 58 | 59 | 202 | 42 | 203 | 214 | 217 | - |
| 71 | Normethadone | C20H25NO | 295 | 58 | 72 | 29 | 42 | 71 | 57 | 59 | 224 |
| 72 | Diphenhydramine | C17H21NO | 255 | 58 | 73 | 45 | 167 | 165 | 166 | 44 | 152 |
| 73 | Pseudoephedrine | C10H15NO | 165 | 58 | 77 | 59 | 56 | 51 | 42 | 105 | 91 |
| 74 | Chlorpromazine | C17H19ClN2S | 319 | 58 | 86 | 318 | 85 | 320 | 272 | 319 | 273 |
| 75 | Ephedrine | C10H15NO | 174 | 58 | 146 | 56 | 105 | 77 | 42 | 106 | 40 |
| 76 | Psilocin | C12H16N2O | 204 | 58 | 204 | 59 | 42 | 30 | 146 | 77 | 44 |
| 77 | Imipramine | C19H24N2 | 280 | 58 | 235 | 85 | 234 | 236 | 195 | 193 | 208 |
| 78 | Amodiaquine | C20H22ClN3O | 355 | 58 | 282 | 30 | 284 | 355 | 73 | 283 | 44 |
| 79 | Trifluomeprazine | C19H21F3N2S | 366 | 58 | 366 | 59 | 100 | 266 | 284 | 84 | 44 |
| 80 | Dextromethorphan | C18H25NO | 271 | 59 | 271 | 150 | 270 | 31 | 214 | 42 | 171 |
| 81 | Captopril | C9H15NO3S | 217 | 70 | 41 | 69 | 75 | 114 | 42 | 217 | 68 |
| 82 | Amyl nitrite | C5H11NO2 | 117 | 70 | 43 | 55 | 41 | 57 | 42 | 71 | - |
| 83 | Loxapine | C18H18ClN3O | 228 | 70 | 83 | 42 | 257 | 193 | 56 | 228 | 104 |
| 84 | Bromhexine | C14H20Br2N2 | 376 | 70 | 112 | 293 | 264 | 44 | 42 | 305 | 41 |
| 85 | Pethidine | C15H21NO2 | 247 | 71 | 70 | 44 | 57 | 42 | 247 | 43 | 246 |
| 86 | Pethidinic Acid |  | - | 71 | 70 | 57 | 43 | 219 | 42 | 218 | 44 |
| 87 | Atenolol | C14H22N2O3 | 266 | 72 | 30 | 56 | 98 | 43 | 107 | 41 | 73 |
| 88 | Metoprolol | C15H25NO3 | 267 | 72 | 30 | 107 | 56 | 45 | 41 | 44 | 43 |
| 89 | Isoprenaline | C11H17NO3 | 211 | 72 | 44 | 43 | 124 | 123 | 30 | 42 | 41 |
| 90 | Propranolol | C16H21NO2 | 259 | 72 | 56 | 30 | 43 | 98 | 115 | 144 | 41 |
| 91 | Methadone | C21H27NO | 310 | 72 | 73 | 91 | 293 | 223 | 165 | 85 | 71 |
| 92 | Promethazine | C17H20N2S | 284 | 72 | 73 | 284 | 198 | 213 | 199 | 180 | 56 |
| 93 | Digoxin | C41H64O14 | 781 | 73 | 58 | 57 | 43 | 41 | 39 | 29 | 45 |
| 94 | Valproic acid | C8H16O2 | 144 | 73 | 102 | 41 | 57 | 43 | 27 | 55 | 29 |
| 95 | Menthol | C10H20O | 156 | 81 | 95 | 71 | 41 | 67 | 55 | 138 | 123 |
| 96 | Ecgonine | C9H15NO3 | 185 | 82 | 97 | 42 | 83 | 96 | 57 | 94 | 55 |
| 97 | Minoxidil | C9H15N5O | 209 | 84 | 209 | 67 | 43 | 110 | 41 | 192 | 164 |
| 98 | Chloroquine | C18H26ClN3 | 320 | 86 | 58 | 319 | 87 | 73 | 247 | 245 | 112 |
| 99 | Lignocaine | C14H22N2O | 234 | 86 | 87 | 58 | 44 | 72 | 42 | 120 | 85 |
| 100 | Fluorazepam | C21H23ClFN3O | 388 | 86 | 87 | 99 | 58 | 84 | 387 | 315 | 56 |
| 101 | Procaine | C13H20N2O2 | 236 | 86 | 99 | 120 | 58 | 87 | 30 | 92 | 71 |
| 102 | Hyoscine | C17H21NO4 | 303 | 94 | 138 | 42 | 108 | 136 | 41 | 96 | 97 |
| 103 | Pilocarpine | C11H16N2O2 | 208 | 95 | 96 | 42 | 109 | 41 | 208 | 54 | 39 |
| 104 | Clidinium Bromide | C22H26BrNO3 | 432 | 105 | 77 | 96 | 183 | 51 | 42 | 182 | 94 |
| 105 | Aconitine |  | 646 | 105 | 554 | 540 | 43 | 45 | 77 | 31 | 29 |
| 106 | Paracetamol | C8H9NO2 | 151 | 109 | 151 | 43 | 80 | 108 | 81 | 53 | 52 |
| 107 | Trofluoperazine | C21H24F3H3S | 408 | 113 | 70 | 407 | 43 | 141 | 42 | 127 | 71 |
| 108 | Disulfiram | C10H20N2S4 | 297 | 116 | 88 | 29 | 44 | 60 | 148 | 56 | 27 |
| 109 | Atropine | C17H23NO3 | 289 | 124 | 82 | 94 | 83 | 42 | 96 | 103 | 67 |
| 110 | Ergotamine | C33H35N5O5 | 582 | 125 | 44 | 70 | 91 | 41 | 40 | 244 | 153 |
| 111 | Quinidine | C20H24N2O2 | 360 | 136 | 81 | 322 | 188 | 55 | 42 | 41 | 172 |
| 112 |  |  | 226 | 141 | 156 | 43 | 41 | 157 | 55 | 39 | 98 |
| 113 | Barbitone | C8H12N2O3 | 184 | 156 | 141 | 55 | 155 | 98 | 39 | 82 | 43 |
| 114 | Amylobarbitone | C11H18N2O3 | 226 | 156 | 141 | 157 | 41 | 55 | 142 | 98 | 39 |
| 115 | Quinalbarbitone | C12H18N2O3 | 238 | 167 | 168 | 41 | 43 | 97 | 124 | 99 | 55 |
| 116 | Thiopentone | C11H18N2O2S | 242 | 172 | 157 | 173 | 43 | 41 | 55 | 69 | 71 |
| 117 | Carbamazepine | C15H12N2O | 236 | 193 | 192 | 236 | 191 | 194 | 165 | 190 | 237 |
| 118 | Caffeine | C8H10N4O2 | 194 | 194 | 109 | 55 | 67 | 82 | 195 | 42 | 110 |
| 119 | Phencylcidine | C17H25N | 243 | 200 | 91 | 243 | 242 | 84 | 186 | 161 | 201 |
| 120 | Phenobarbitone | C12H12N2O3 | 232 | 204 | 117 | 146 | 161 | 77 | 103 | 115 | 118 |
| 121 | 4-hydroxy phenobarbitone |  |  | 219 | 248 | 148 | 220 | 120 | 218 | 133 | 65 |
| 122 | Noscapine | C22H23NO2 | 413 | 220 | 221 | 205 | 147 | 42 | 193 | 77 | 118 |
| 123 | Heptabarbitone | C13H18N2O3 | 250 | 221 | 43 | 78 | 93 | 80 | 41 | 141 | 39 |
| 124 | Ergometrine | C19H23N3O2 | 325 | 221 | 72 | 325 | 54 | 196 | 55 | 207 | 181 |
| 125 | Hexobarbitone | C12H16N2O3 | 236 | 221 | 81 | 157 | 80 | 79 | 155 | 41 | 77 |
| 126 | ∆8 – THC | C21H30O2 | 314 | 221 | 314 | 248 | 261 | 193 | 236 | 222 | 315 |
| 127 | Haloperiodol | C21H23ClFNO2 | 376 | 224 | 42 | 237 | 226 | 123 | 206 | 239 | 56 |
| 128 | Methaqualone | C16H14N2O | 250 | 235 | 250 | 91 | 233 | 236 | 65 | 76 | 132 |
| 129 | Bromazepam | C14H10BrN3O | 316 | 236 | 317 | 315 | 288 | 316 | 286 | 208 | 78 |
| 130 | Fentanyl | C22H28N2O | 337 | 245 | 146 | 42 | 189 | 44 | 105 | 29 | 43 |
| 131 | Oxazepam | C15H11ClN2O2 | 288 | 257 | 77 | 268 | 239 | 205 | 267 | 233 | 259 |
| 132 | Apomorphine | C17H17NO2 | 267 | 266 | 267 | 224 | 220 | 268 | 44 | 250 | 248 |
| 133 | Lysergamide | C16H17N3O | 267 | 267 | 221 | 207 | 180 | 223 | 154 | 196 | 268 |
| 134 | Lysergic Acid | C16H16N2O2 | 268 | 268 | 224 | 154 | 180 | 207 | 223 | 192 | 179 |
| 135 | 8α-Hydroxy-∆9-THC |  |  | 271 | 43 | 41 | 311 | 295 | 312 | 297 | 91 |
| 136 | 8α-Hydroxy-∆9-THC |  |  | 271 | 43 | 295 | 41 | 297 | 29 | 330 | 272 |
| 137 | Normorphine | C16H17NO3 | 271 | 271 | 81 | 150 | 201 | 148 | 110 | 272 | 82 |
| 138 | Temazepam | C16H13ClN2O2 | 301 | 271 | 273 | 300 | 272 | 256 | 77 | 255 | 257 |
| 139 | Nitrazepam | C15H11N3O3 | 281 | 280 | 253 | 281 | 206 | 234 | 252 | 254 | 264 |
| 140 | Clonazepam | C15H10ClN3O3 | 316 | 280 | 314 | 315 | 286 | 234 | 288 | 316 | 240 |
| 141 | Chlordiazepoxide | C16H14ClN3O | 300 | 282 | 299 | 284 | 283 | 241 | 56 | 301 | 253 |
| 142 | Morphine | C17H19NO3 | 303 | 285 | 162 | 42 | 215 | 286 | 124 | 44 | 284 |
| 143 | Flunitrazepam | C16H12FN3O3 | 313 | 285 | 312 | 313 | 286 | 266 | 238 | 294 | 284 |
| 144 | Lorazepam | C15H10C12N2O2 | 321 | 291 | 239 | 274 | 293 | 75 | 302 | 276 | 138 |
| 145 | Cannabinol | C21H26O2 | 310 | 295 | 296 | 238 | 310 | 119 | 43 | 251 | 239 |
| 146 | Codeine | C18H21NO3 | 317 | 299 | 42 | 162 | 124 | 229 | 59 | 300 | 69 |
| 147 | ∆9 – THC | C21H30O2 | 314 | 299 | 231 | 314 | 43 | 41 | 295 | 55 | 271 |
| 148 | Alprazolam | C17H13CLN4 | 309 | 308 | 279 | 204 | 273 | 77 | 307 | 310 | 309 |
| 149 | Thebaine | C19H21NO3 | 311 | 311 | 255 | 42 | 44 | 296 | 310 | 312 | 174 |
| 150 | Lysergide | C20H25N3O | 323 | 323 | 221 | 181 | 222 | 207 | 72 | 223 | 324 |
| 151 | 6-Monoacetyl-morphine | C19H21NO4 | 327 | 327 | 268 | 42 | 43 | 215 | 44 | 328 | 269 |
| 152 | Nifedipine | C17H18N2O6 | 346 | 329 | 284 | 224 | 268 | 330 | 285 | 225 | 270 |
| 153 | Acetylcodeine | C20H23NO4 | 341 | 341 | 282 | 229 | 42 | 43 | 59 | 342 | 204 |
| 154 | Reserpine | C33H40N2O9 | 608 | 608 | 606 | 195 | 609 | 395 | 397 | 212 | 396 |
| 155 | Dextropropoxyphene | C22H29NO2 | 339 | 58 | 202 | 203 | 59 | 215 | 189 | 216 | 191 |
| 156 | Tramadol HCl | C16H25NO2 | 263 | 58 | 263 | 135 | 77 | 264 | 150 | 121 | 218 |
| 157 | Melatonin | C13H16N2O2 | 232 | 160 | 173 | 145 | 117 | 158 | 174 | 232 | 161 |
| 158 | Zopiclone | C17H17ClN6O3 | 388 | 245 | 143 | 217 | 248 | 99 | 46 | 139 | 56 |
| 159 | Fluphenazine | C22H26F3N3OS | 437 | 42 | 280 | 56 | 143 | 70 | 113 | 72 | 437 |
| 160 | Hydroxyzine | C21H22ClN2O2 | 374 | 201 | 18 | 166 | 299 | 132 | 271 | 203 | 374 |
| 161 | Busplion | C21H31N6O2 | 385 | 177 | 271 | 268 | 148 | 122 | 123 | 108 | 388 |
| 162 | Trihexyphenidyl | C20H31NO | 301 | 98 | 55 | 105 | 55 | 77 | 218 | 192 | 171 |
| 163 | Midazolam | C18H13ClFN3 | 325 | 310 | 312 | 325 | 163 | 311 | 222 | 142 | 111 |
| 164 | Clozapine | C18H19ClN4 | 326 | 243 | 256 | 70 | 192 | 227 | 245 | 326 | 99 |
| 165 | Pimozide | C28H27F2N3O | 461 | 230 | 231 | 42 | 109 | 187 | 82 | 134 | 461 |
| 166 | Thioridazine | C21H26N2S2 | 370 | 98 | 270 | 126 | 243 | 281 | 309 | 70 | 42 |
| 167 | Trifluoperidol | C22H23F4NO2 | 409 | 42 | 271 | 258 | 123 | 56 | 240 | 165 | 83 |
| 168 | Clomipramine | C19H23ClN2 | 314 | 58 | 85 | 269 | 268 | 314 | 242 | 130 | 164 |
| 169 | Amoxapine | C17H16ClN3O | 313 | 245 | 193 | 56 | 257 | 247 | 228 | 164 | 313 |
| 170 | Mianserin | C18H2ON2 | 264 | 193 | 264 | 43 | 72 | 71 | 42 | 220 | 249 |
| 171 | Imepramine | C19H24N2 | 280 | 235 | 58 | 85 | 234 | 280 | 195 | 130 | 35 |
| 172 | Fluoxetine | C17H18F3 | 309 | 44 | 309 | 251 | 104 | 148 | 164 | 183 | 233 |
| 173 | Dothiepin | C19H21NS | 295 | 58 | 40 | 236 | 202 | 235 | 201 | 165 | 293 |
| 174 | Trimiprimine | C20H26N2 | 294 | 58 | 249 | 208 | 234 | 99 | 193 | 84 | 294 |

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SECTION–9:ANALYSIS OF PLANT POISONS

(NON-VOLATILE ORGANIC)

**9.1 Title:** Analysis of plant poisons (organic non-volatile)

**9.2 Scope:** Poisonous plants and their constituents

**9.3 Purpose:** Systematic analysis of plant poisons.

**9.4 Responsibility:** Gazetted Officers and connected Scientific staff.

9.5 CLASSIFICATION OF PLANT POISONS & THEIR CHARACTERISTICS

The active constituents of plants that exert toxic effects are organic compounds and non-volatile in nature (exception nicotine is steam volatile). Synthetic derivatives of morphine, cocaine, lysergic acid are very potential for abuse. These synthetic compounds fall under the class “Drugs of abuse”. Today, drugs of abuse include designer drugs and heir synthesis is tried also in clandestine laboratories.

**9.5.1 Plant poisons under different classes:**

|  |  |  |
| --- | --- | --- |
| **Sl. No.** | **Class** | **Example** |
| 1.  2.  3.  4. | Neurotic  Spinal  Cerebral  Cardiac | Papaver somniferum Linn  Strychnos nux vomica Linn.  i) Cannabis sativa Linn., ii) Erythroxylon coca Linn., iii) Atropa belladonna Linn., iv) Dhatura fastuosa Linn.  i) Nicotiana tabacum Linn., ii) Aconitum napellus Linn., iii) Digitalis purpurea Linn. |
| 5.  6. | Irritant  Miscellaneous | i) Abrus precatorius Linn.  ii) Calotropis gigantea (Linn) R. Br. Ex. Ait  Calotropis procera (Ait) R. Br. ex.  iii) Cytisus laburnum Linn  iv) Taxus baccata Linn.  v) Croton tiglium Linn.  vi) Argemone mexicana Linn.  vii) Gloriosa superba Linn.  i) Cyanogenetic glycosides.  ii) Ergot.  iii) Oleander (Glycoside) |

**9.5.2** **Characteristics of poisonous plants under different classes:**

**A. Characteristic of Neurotic Plant Poisons**.

|  |  |  |  |
| --- | --- | --- | --- |
| **Poisonous Plant** | **Active Constituents (Active Principle)** | **Active Constituent Present in Different Parts of Plants Etc.** | Other Characteristics |
| Papaver somniferum Linn(N.O. Papaveraceae)  Common name :  opinion Poppy, white Poppy, Poppy and Carnation Poppy. | Active Constituents:  25 alkaloids in different varieties of plants exist in combination with three acids viz. meconic, lactic and sulphuric. Other ingredients include three neutral principles–meconin, meconism and opinion as well as pectin, glucose, mucilarges caoutchoue, wax and odorous fatty and colouring matter.  % of majors alkaloid present: Morphine-10 - 20%, Narcotine-0.75 - 10%, Papaverine- 0.5 - 1.0%, Thebaine-0.2 - 1.0%, Codeine-0.2 - 0.8%, Narceine-0.1 – 0.5%. | Opium alkaloid is obtained in air-dried milky exude or incising the unripe capsules.  Traces of morphine are present in ripe and dry poppy capsules (Posto Ka doda).  Poppy seeds are innocuous and used as food and sprinkled over some Indian sweets. A bland oil known as Poppy seed oil is obtained from the seeds. | Symptoms of Poisoning: 3 stages: Excitement, Stupor (depression), Narcosis (deep coma).  Unusual Symptoms: Vomiting, Purging and Convulsions.  Codeine Phosphate is used in cough syrup.  Alkaloids other than morphine in opium are not so active.  Morphine is synergic with alcohol.  Two proprietary medicines containing opium or morphine are Chlorodyne and Sydenham’s Laudanum.  Opium is served in drink Kasoomba or for smoking (modak, chandu or opium dross) in festive occasions and social gathering as an age old custom. |

**B. Spinal Plant Poison.**

Characteristics of Strychnos nux vomica Linn.

|  |  |  |  |
| --- | --- | --- | --- |
| **Poisonous Plant** | **Active Constituents (Active Principle)** | **Active Constituent Present in Different Parts of plants Etc.** | Other Characteristics |
| Strychnos nux vomica Linn Belongs to N.O. Loganiacease.  Familiar Names in India:  Kuchila, Kuchla. | Alkaloids:  Strychnine (C21H22O2N)  Brucine-(C23H26O4N2)  together with strychnicigasuric or caffeotannic acid and traces of glucoside named logamin | Active constituents are found in seeds of ripe fruits.  Bark, wood and leaves contain brucine but no strychnine. | Strychnine is used as respiratory stimulant, rodenticide and vermin killer. Symptoms: Choking sensation in the throat & stiffness of neck, increased CNS activity, convulsion, cyanosis, blood stained froth, contraction of respiratory muscles. |

**C. Characteristics of Cerebral Plant Poisons:**

I. Cannabis sativa Linn.

|  |  |  |
| --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principle)** | **Active Constituent Present in Different Parts of plants.** |
| Cannabis sativa Linn or Cannabis indica  Common name: Indian Hemp, Marijuana. | Contains several active constituents in resins viz. cannabinol, cannabidiol, cannabidiolic acid and tetrahydrocannabinol (THC). THC exists in isomeric forms. Out of different forms, Δ9 THC is the most active. | Active constituents are found in leaves and fruiting tops of female plant, also in dried leaves and fruiting shoots of both male and female plant and in crude resin collected from leaves and flowering tops of plant, used in various forms viz Ganja, Bhang, Charas and Majur. |

II. Characteristics of Erythroxylon coca Linn.

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principle)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Erythroxylon  coca Linn  Common name:  Coca belongs to N.O. Erythroxylaceae. | Cocaine (alkaloid) | Leaves. | Synthetic form:  Cocaine hydrochloride (used as local anaesthetic in ophthalmic practice and in dental and minor operative surgery) Cocaine hydrochloride is colourless and exists as acicular crystals. It is soluble in water, chloroform and glycerine. |

III. Characteristics of Atropa belladonna Linn.

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principle)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Atropa belladonna  Common name:  Deadly nightshade.  Belongs to N.O. Solanaceae | Atropine, (alkaloid) C17H23NO3, crystallises in odourless and colourless prismatic needles and has a bitter taste. It also contains two alkaloids-hyoscine and belladonine. | All parts including seeds. | Atropine is highly soluble in ether, alcohol and chloroform. It can be split by strong acids and alkalis into tropine and tropic acid.  The synthetic form is atropine sulphate. It exists as colourless crystals or white powder, soluble in water and alcohol and used as antidote for organophosphorous and carbamate pesticides. |

IV. Characteristics of Dhatura fastuosa Linn.

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principle)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Dhatura fastuosa Linn Common name: Dhatura.  Exists in two different varieties viz.  Dhatura alba-A, white flowered plant (Safed Dhatura) and Dhatura Niger, a black or rather deep purple flowered plant (Kala Dhatura). Both these varieties grow commonly on waste land all over India, and have bell shaped flowers, can have more or less spherical fruits which are covered with sharp spinous projections and contain yellowish brown seeds. Dhatura stramonium (thorn apples) grows at Himalaya. | Hyoscine, hyoscyamine and atropine. | All parts of the plant are poisonous but the seeds and fruits are considered to be the most toxic. | The seeds are normally used as stupefying agent.  The leaves and seeds are also used for pharmacopoeial preparations viz. Stramonium and Stramonii pulvis. |

**D. Characteristics of Cardiac Plant Poisons:**

I. Characteristics of Nicotiana tabaccum Linn.

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principles)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Nicotiana tabaccum Linn  Common names: Tabacco, Tambaku.  Belongs to N.O. Solanaceae | Nicotine – (alkaloid)  0.6-8% and anabasin in combination with malic and citric acid, volatile, hygroscopic, brownish, burning acrid taste with penetrating and disagreeable smell.  Nicotianine (alkaloid) - Known as tobacco camphor. | Leaves. | Used for smoking or as snuff. It is chewed with lime alone or with lime and pan. The leaves are used for manufacturing cigars or cigarettes. |

II. Characteristics of Aconitum napellus Linn.

|  |  |  |  |
| --- | --- | --- | --- |
| Description of Plant | **Active Constituents (Active Principles)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Aconitum napellus Linn. Common name: Aconite, Monk’s Hood, Wolfsbane, Blue Rocket, Mitha Zahar, Dudhia Bish, Bikh.  Different species grow in the temperate Himalayan region.  Aconitum Chasman – thum(chief source of aconite-known as bikh or bish in Kolkata).  Belongs to N.O. Ranunculaceae | Different alkaloids combined with aconitic acid:  Alkaloids present: Aconitine (acetyl ben-zoyl aconine), picraco-nitine(benzoyl-aconine) bikhaconitine, aconine and other alkaloids such as indaconitine chief constituent: Aconine C34N49NO22 | Root (main) and other parts of plant. | Aconitine first stimulates and then paralyses the peripheral terminations of the sensory and secretory nerves, CNS, myo-cardium skeletal and smooth nerves.  It has a bitter sweet taste severe burning and tingling of the lips, tongue, mouth and throat followed by numbness and anaesthesia of the parts occur.  Aconitum heterophyllum is, a non-poisonous variety of aconite and known as atis.  It is used for criminal and suicidal poisoning, added to liquor to increase intoxication, also used for cattle poisoning and arrow head poison. |

III. Characteristics of Digitalis purpurea Linn.

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| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principle)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Digitalis Purpurea Linn  Belongs to N.O. Scrophulariaceae | Several glycosides are the active constituents viz.,  Digitoxin, Digitalin, Digitalien & Digitonin. | Root, leaves, seeds etc.  Digoxin(glycoside) is obtained in the leaves of Digitalis lantana. It occurs as colourless crystals, having no odour. | Symptoms are gastrointestinal at first and later followed by action on heart. Poisoning is accidental and also rare |

**E. Characteristics of Irritant Plant Poisons:**

I. Characteristics of Abrus precatorius Linn;

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principles)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Abrus Precatorius Linn.  Common names:  Jequirity, Indian Liquorice, Gunchi or Rati.  Belongs to N.O. Leguminosae | Abrin – a glycoside (α-methyl – amino-β-3- indolypropionic acid) and acts as a toxalbumin  Also contains glycyrrhizin.  Arbin loses its activity when boiled. | Seeds (mainly)  Root and stem may contain glycyrrhizin. | Seeds are used in criminal poisoning and for destroying cattle. The seeds are mixed with dhatura, opium and onion and a small quantity of spirit to form a paste, which is made into spikes or sui and then hardened in the sun.  Cooked seeds are harmless. Arbin acts as a toxalbumin and its action resemble those of viperine snake-bite with intense local symptom and hemorrhages followed by drowsiness. It causes agglutination of red blood corpuscles. |

II. Characteristics of Calotropis gigantea / Procera Linn.

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| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principles)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Calotropis gigantea or procera Linn.  Common names:  Madar, Akdo, Aak,  Calatropis gigantea,  has purple flowers.  Calotropis procera has white flowers.  Both resemble each other in chemical and physiological action.  Belongs to N.O. Asclepiadaceae | Uscharin, Calotoxin and Calacin. | Milky juice from fresh leaves, stalks root. The juice on keeping and heating coagulates. The serum left is highly toxic due to  gigantin– a white crystalline substance of molecular formula C24H36O4. | It acts locally as an irritant, but after absorption acts as a cerebro-spinal poison. When applied to the skin, madar juice acts as a local irritant producing redness, inflammation and vesication. It also irritates eye. When administered internally, it acts as a gastrointestinal irritant and also as a cerebro-spinal poison. When used in the form of snuff, the powder madar root may cause death.  Leaves are used as a poultice over the abdomen in colic. The tincture is used in dysentry. The milky juice is used as vesicants, as a depilatory and as a remedy for chronic skin affections. It is also used by tanners for removing hairs and destroys the offensive odour of fresh leather.  It is used in criminal abortion (abortion sticks) and by snake charmer to control unruly snakes (root of calatropis procera is used). |

III. Characteristics of Cystisus laburnum Linn.

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principles)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Cystisus laburnum Linn  Common name: Laburnum.  The plant has the most nauseous and disagreeable odour and taste.  Belongs to N.O. Leguminosae | Cystisine (alkaloid).  well known as Sophorine or Baptitoxin. | Wood, bark, pods, seeds. | Accidental poisoning cases among children are known.  Burning, dysphagias, abdominal pain, thirst, purging, giddiness, irregular pulse and coma are the common symptoms.  It is the chief ingredient in Australian or Persian insect powder. |

IV. Characteristics of Texus baccata Linn.

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principles)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Texus baccata Linn.  Common name: Yew, Birmi, Tnuno, Gallu, Geli and Bhirmi. | Taxine and Tosicatin (alkaloids). | Leaves and berries. | Symptoms include giddiness, dilated pupils, vomitting, purging, pain in abdomen, irregular pulse, laboured breathing, convulsion and collapse.  It is used as cattle poison and abortifacient. Accidental poisoning among children are known. |

V. Characteristics of Croton tiglium Linn.

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principles)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Croton tiglium Linn.  Common names: Croton tiglium, Jamal-gota or Naepala.  Belongs to N.O. Euphobiaceae | Crotin (a toxalbumin similar to ricin) and Crotonoside (glycoside). | Seeds. | On contact with skin, it produces burning, redness and vesication. When swallowed it acts as a gastrointestinal irritant. |

VI. Characteristics of Argemone mexicana Linn.

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principles)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Argemone Mexicana Linn.  Common names:  Yellow Mexicana Poppy, Prickly, Poloy, Darudi, Satyanasi, Pila Dhatura.  It has prickly oblong or elliptic capsules containing numerical spherical seeds resembling mustard seeds.  Belongs to N.O. Papaveraceae | Sanguirine, Dihydrosanguirine, Berberine and Protopine | Seeds | Oil extracted from seeds is known as argemone oil. It is added to mustard oil as an adulterant.  Dihydrosanguirine is more toxic.  Active constituents pro-duce odema of legs, breathlessness, diarrhoea.  Dropsy i.e. hair loss, pigmentation of skin and non-tender hepatomegaly in epidemic form may occur. |

VII. Characteristics of Gloriosa superba Linn.

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principles)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Glorisa Superba Linn.  Common names: Karihari, Khadiyang.  Belongs to N.O. Liliaceae | Superbine (a glycoside) and cochicine | Root (root is juicy, tuberous and flattened or cylindrical). | Symptoms include burning and numbness in mouth, throat, nausea, violent vomiting, ataxia, convulsion and collapse. |

**F. Characteristics of Miscellaneous Plant Poisons:**

I. Characteristics of Cyanogenetic Glycosides.

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principles)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Cyanogenetic glycosides are formed in certain plants, vegetables and fruits viz. Bitter almonds linseed plant, flower, kadvi jowar plant, millet, oilseeds, beans, bamboo shoots etc. | Cyanogenetic glycosides, produced from structurally related amino acids viz., aldoximes and nitriles by a 5-step pathway.  HCN is produced which is the potent toxicant. | Plants, vegetables, fruits, beans etc. | Shows the characteristic of poisoning by HCN. |

II. Characteristics of Ergot.

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principles)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| Ergot is sclerotium (compact mycelium or spawn) of the parasitic fungus clavicep purp – purea attacking the grains of several plants such as rye, oats, wheat, barley and bajra in wet season and ill drained soil. | Ergotoxin, Ergotamine, Ergometrine  (Alkaloid) | Seeds of different plants viz. rye, oats, wheat, barley, bajra. | Used for stimulating action on uterus, treatment of migraine, and abortificient. Synthetic: as free base or salts of tartaric and malic acid.  Symptoms include rapid pulse, confusion, convulsion, unconsciousness, renal failure and respiratory failure in high doses. |

III. Characteristics of Oleander.

|  |  |  |  |
| --- | --- | --- | --- |
| **Description of Plant** | **Active Constituents (Active Principles)** | **Parts of plants containing Active Constituent** | Other Characteristics |
| 1.Cerebra thevetia or thevetia nerifolia.  Common name: Pila (yellow) Kaner.  It has yellow bell shaped flower and a green globular fruit containing a single nut, light brown and triangular.  Belongs to N.O. Apocynaceae  2. Cerbera odollan (Dabur) white flower.  3. Nerium odorum white flower. | Thevetin, Thevetoxin, Cerberin (all glycosides).  Cerberin and cerebro –side.  Nerin. | Milky juice which exudes from all parts of plant seeds and roots.  Seed and root.  All parts. | Symptoms include burning pain in mouth and dryness of throat, numb-ness of tongue, vomiting, dizziness, dilated pupils and fainting etc.  Criminal poisoning occurs frequently. Seeds and roots are mixed with oil on water for criminal abortion, also used as cattle poison (through ear of coin or chapatti).  Symptoms include vomiting, purging collapse, heart failure.  Digitalis like action. |

**9.6 EXTRACTION AND STRIPPING OF PLANT POISONS IN MATRICES:**

The step is important for analysis as the detection and identification by different methods viz., classical and instrumental is solely dependent on the efficiency of extraction, which is dependent on a number of factors viz., analytical requirement, nature of sample and nature of poison (whether alkaloid or glycoside). The plant materials viz. root, stem, leaves and seeds (non-biological matrices) are required to be extracted. The details of methods of extraction have already been covered. For extraction of opium of Indian origin, special extraction methods are to be followed for two ingredients viz. meconic acid and porphyroxine. Special methods have also been described for Nicotiana tabacum Linn, cyanogenetic glycoside. The stripping methods as described in Section 3 are to be followed depending on matrices.

**9.6.1 General methods of extraction of Plant poisons:**

|  |  |  |
| --- | --- | --- |
| **Sl. No.** | **Matrices** | **Methodology** |
| 1.  2.  3.  4.  5. | Plant materials viz. leaves, stem, barks, seeds etc.  Mustard oil adulterated with argemone oil  Food and food products, milk, tea, pharmaceutical preparations, water, cereals etc  Biological Materials viz. viscera, stomach wash, stomach contents etc.  Blood. | Dried, crushed and extracted by direct solvent extraction or by continuous solvent extraction after de-fatting by petroleum ether in case of seeds.  By extracting oil with conc. Hydrochloric acid on a water bath  By Stas-Otto process or Ammonium sulphate method or micellar method  By Stas-Otto Process or Ammonium sulphate method or micellar method.  After deproteinization extraction by Stas-Otto method or micellar method |

* + 1. **Methods of Extraction of Different Plant Poison in Biological Material**

**Plant Poison Fraction in Stas – Otto method**

Papaver somniferum Linn. Basic – Chloroform extract

Strychnos nux vomica Linn. As above

Canabis sativa Linn. Acid ether extraction

Erythroxylon coca Linn. Basic – chloroform extract

Atropa belladonna As above

Datura fastuosa Linn. As above

Aconitum napellus Linn. As above

Digitalis purpurea Linn. Acid ether extract

Abrus precatorious Linn. As above

Calotropis gigantea or Procera As above

Cystisus laburnum Basic chloroform extract

Taxus baccata Linn. As above

Croton tiglium Linn. Acid – ether or chloroform extract

Ergot Alkaline – ether – chloroform (3:1) extract

Oleander Acid – ether extract

**9.6.3 Special method:**

1. **Extraction of Nicotine**.

By alkaline-steam distillation (as described in volatile poisons).

2. **Extraction of Cyanogenetic glycosides**.

By acid steam distillation.

3. **Extraction of Active constituents of Strychnos nux vomica Linn and**

**their Separation**:

The plant contains two active constituents viz. strychnine and brucine. The active constituents are extracted together in the basic chloroform extract. As the presence of brucine interferes with the tests for strychnine, it is essential to separate strychnine from brucine.

**Separation of Strychnine from Brucine:**

The chloroform extract of basic fraction in Stas-Otto process is dissolved in 2 ml. of dilute sulphuric acid, followed by 2 drops of concentrated nitric acid. It is allowed to stand for 30-60 minutes at 15-20oC, thereafter made strongly alkaline by sodium hydroxide and extracted several times with chloroform. The extract is washed. It is now free from brucine and fit to carry out test for strychnine.

4.  **Extraction of meconic acid and porphyroxine in opium:**

Morphine and meconic acid are the two important constituents of opium and the presence of these two components is an indication of opium poisoning. Porphyroxine is a characteristic constituent of opium. The two special components i.e. meconic acid and porphyroxine are to be extracted for their identification (C19H22NO4).

**a) Extraction of Meconic Acid in Biological Materials:**

200 gms. of finely minced viscera or stomach contents is subjected to Stas-Otto process (as described in Section 3). The acid aqueous solution contains meconic acid. A portion of it is treated with a slight excess of 10% basic lead acetate solution when a precipitate of lead meconate appears. It is heated on a water bath for 5 minutes, allowed to settle and filtered. The precipitate is washed with warm distilled water. The washed precipitate is suspended in about 10 ml. of water and the suspension is saturated with hydrogen sulphide gas. A black precipitate of lead sulphide is formed and free meconic acid is liberated. It is filtered. The filtrate is concentrated to a small volume so that all traces of H2S disappear. It is filtered again. The filtrate contains meconic acid. The other portion of acid extract is kept reserved for extraction of porphyroxine and morphine.

**b) Extraction of Porphyroxine:**

The remaining portion of the acid solution as stated above is taken. It is rendered alkaline with ammonia and then extracted with ether instead of chloroform. The ether extract contains the alkaline porphyroxine. It is kept reserved for detection of porphyroxine. The alkaline aqueous solution is now ready for extraction of morphine.

**9.7 TESTS FOR ACTIVE CONSTITUENTS OF PLANT POISONS:**

The purified extracts of samples i.e. plant materials and other materials (biological and non-biological) are subjected to analysis by different methods, viz., colour and crystal tests, UV and IR spectroscopy, TLC, GLC and HPLC.

The colour tests are performed with different reagents. These are applied to the purified extracts of plant materials (leaves / bark, seeds, stem etc.) and biological and non-biological matrices related to crime.

**9.7.1 Tests for Opium Alkaloids:**

1. **Frohde’s Test:**

To the dried residue of extract, a few drops of Frohde’s reagent is added. A violet colour changing to green and finally blue is observed.

2. **Marquis Test:**

To the dried residue of extract in a porcelain basin (flat type), 1 drop of Marquis reagent is added. A purple red colour is produced which changes to violet and finally blue.

3. **Husemann’s Test:**

To a little dried extract in a porcelain basin, 2-3 drops of conc. sulphuric acid is added and heated on a water bath for 30 minutes over a small flame for a few minutes until white fumes appear. A reddish or reddish-brown or black colour appears. It is cooled. One drop of conc. nitric acid and a crystal of potassium nitrate is added. A reddish violet colour appears which immediately changes to blood red and then to reddish yellow and finally fades away.

4. **Urotropine Test:**

To a little dried extract in a porcelain basin, a few drops of aqueous solution of urotropine is added and warmed slightly. A purple colour changing to blue and then green is observed.

**9.7.2 Tests for Porphyroxine and Meconic acid:**

1. **Tests for Porphyroxine:**

2 drops of acetic acid solution of the residue of extract is taken in a porcelain basin followed by addition of a few drops of dilute hydrochloric acid. It is warmed over a low flame. A pink or rose red colour is produced.

2**. Tests for Meconic Acid:**

A few drops of neutral solution of ferric chloride is added to 1-2 ml. of an aqueous extract (extract obtained after separation of meconic acid). A blood red colour is produced which is not destroyed by boiling or by adding hydrochloric acid or mercuric chloride solution.

**9.7.3 Colour tests for Strychnine:**

1. **Mandelin’s Test:**

To the brucine-free residue, one drop of Mandelin’s reagent (1% solution of ammonium vanadate in conc. sulphuric acid). A deep violet-blue or deep purple colour appears which finally changes to yellow on long standing.

**Separation of Strychnine from Brucine:**

The chloroform extract (basic fraction) containing strychnine and brucine is evaporated to dryness. 2 ml. of dilute sulphuric acid is added followed by 2 drops of conc. nitric acid. It is allowed to stand for 30-60 minutes at 10-20oC, thereafter made alkaline with NaOH solution and extracted several times with chloroform. The chloroform extract is combined, washed and evaporated to dryness. The residue does not contain brucine.

2. **Play of Colours Test:**

To the brucine-free residue of extract in a porcelain dish, 1 drop of pure conc. sulphuric acid is added. No colouration is observed. A crystal of potassium dichromate is drawn by a glass rod through sulphuric acid. A play of colours is observed – first a momentary blue changing to a violet colour which gradually changes to reddish purple, red or orange and finally to yellow.

**9.7.4 Colour Tests for Brucine:**

1. **Nitric Acid Test:**

To the dried residue of the extract, one drop of conc. nitric acid is added. A blood red colour appears which changes to reddish yellow and finally to pure yellow. To this a few drops of stannous chloride solution is added. An intense purple colour develops which is destroyed by adding a few drops of conc. HNO3.

2. **Vitali’s Test:**

To a little dried residue of the extract in a porcelain dish, 1-2 drops of fuming or conc. nitric acid is added. It is evaporated to dryness on a water bath. It is allowed to cool. A few drops of freshly prepared alcoholic caustic potash solution is added. A fine violet colour is produced which changes to orange red or red and then disappears. On adding a few drops of excess alcoholic caustic potash solution, the colour reappears.

**9.7.5 Colour Test for Cannabis sativa Linn:**

1. **Fast Blue B Test:**

A small amount of residue of the extract is placed in a test tube and a very small amount solid Fast blue B reagent (solid Fast blue B: anhydrous sodium sulphate : : 2.5 : 100) is added. One ml. of chloroform is then added and shaken. It is kept for two minutes. The chloroform layer becomes purple red in colour.

2. **Duquenois-Levine Test:**

A small amount of residue of the extract is placed in a test tube. It is shaken with 2 ml. of a reagent containing acetaldehyde and vanillin (5 drops of acetaldehyde and 0.4 gm. of vanillin dissolved in 20 ml. of 95% ethanol) for one minute. 2 ml. of conc. hydrochloric acid is added, shaken and allowed to stand for 10 minutes. If a colour develops, 2 ml. of chloroform is added. The lower (chloroform) layer becomes violet.

**9.7.6 Colour Test for Cocaine:**

1. **Scott Test:**

The residue of the extract is taken in a test tube. 5 drops of 2% cobalt thiocyanate solution (prepared by dissolving 2 gms. of cobalt thiocyanate in water and diluted with 96% glycerine in the proportion water : glycerine: : 1 : 1). The mixture is shaken and a blue colour develops at once which indicates the presence of cocaine. (methaqualone also gives positive reaction).

If a blue colour develops, one drop of conc. hydrochloric acid is added. The blue colour disappears and a clear pink colour appears. If a blue colour does not disappear, one drop of conc. hydrochloric acid is added. Then a few drops of chloroform is added and shaken. The chloroform layer becomes intense blue.

2. **Gold Chloride Test:**

One drop of 5% gold chloride solution in water is added to a few drops of extract. A precipitate is formed which appears as delicate rosette or long rod shaped crystal under microscope. The precipitate is also insoluble in dilute hydrochloric acid.

**9.7.7 Colour Test for Atropa belladonna**:

1. **Vitali’s Test:**

A portion of residue of the extract is treated with a few drops of fuming nitric acid in a porcelain basin. It is evaporated to dryness on a water bath. The residue is cooled and moistened with a few drops of freshly prepared alcoholic caustic potash solution when a violet colour is produced, which soon changes to red and finally disappears. The colour may be made to reappear by adding more alcoholic caustic potash solution.

2. **Gerrard’s Test:**

1-2 ml. of 2% mercuric chloride solution in 50% of alcohol is added to a portion of residue of the extract. A red colour develops immediately. hyoscyamine produces a yellow colour which becomes red on burning, while hyocine does not produce any change of colour.

**9.7.8 Colour test for Dhatura fastuosa Linn**:

1. **Vitali’s Test:**

A portion of residue of the extract is treated as above when violet colour is produced which immediately changes to red and then disappears. On adding a few drops of alcoholic KOH, the colour reappears.

**9.7.9 Colour Test for Nicotiana tabacum Linn:**

1. **Mayer’s Reagent:**

(Potassium mercury iodide prepared by dissolving 1.357 gm. of mercuric chloride and 5 gms. of potassium iodide in 100 ml. of water).

The dried residue of extract is acidified with acetic acid followed by addition of 2 drops of reagent. A white or yellowish precipitate is obtained.

2. **Phosphomolybdic Acid:**

To the dried residue of the extract, a few drops of phosphomolybdic acid is added and warmed. A yellowish white precipitate is obtained.

3. **Silicotungstic Acid:**

To the dried residue of the extract, a few drops of silicotungstic acid are added. A white precipitate is obtained.

**9.7.10 Colour test for Aconitum napellus Linn:**

1. **Palet’s Reaction:**

To the purified extract of the residue, a few drops of a mixture consisting of 2.5 gm. syrupy phosphoric acid and 0.1 gm of sodium molybdate is added and heated over a small flame until vapours appear. A violet colour develops.

2. **Alvarez Reaction:**

To the purified residue in a porcelain dish, 5-10 drops of pure bromine is added and evaporated to dryness on a water bath. 1-2 ml. of conc. nitric acid is added and evaporated to dryness (a few drops of bromine is to be added if nitric acid loses its colour). To the yellow oxidation product, 1 ml. of saturated alcoholic solution of sodium hydroxide is added and again evaporated to dryness. A red or brown residue is obtained. It is allowed to cool. 5-6 drops of a 10% copper sulphate solution is added. A green colour develops.

**9.7.11 Colour Test for Digitalis purpurea Linn:**

1. **Tests with Antimony Pentachloride:**

The dried residue of the extract is taken in a small porcelain basin. To this 5 drops of a 1 : 1 mixture of ethanol and chloroform is added and stirred. The organic solvent extract is spotted on No.1 filter paper in varying amounts and sprayed with a 10% solution of antimony pentachloride in chloroform. The development of yellow colour changing to purple is observed. On warming the spotted paper in hot air for 5 minutes, the colour of spot changes to black.

**9.7.12 Colour Test for Abrus precatorius Linn:**

1. **Fast Blue B-Potassium Hydroxide Test:**

To the dried residue of the extract in a porcelain basin, a few drops of 5% ethanolic solution of Fast Blue B salt is added followed by 2 drops of aqueous KOH solution. A red to orange colour is observed.

2. **Marquis Reagent:**

To the dried residue of extract, 2 drops of Marquis reagent (prepared by mixing 1 volume of formalin or formaldehyde solution with 9 volumes of concentrated sulphuric acid) is added. A pink colour is formed.

3. **Van Urk Reagent:**

To the dried residue of the extract, one drop of Van Urk Reagent (prepared by dissolving 1 gm. of p-amino benzaldehyde in 100 ml. ethanol and adding 10 ml. of hydrochloric acid). A green colour changing to blue is observed.

4. **Special Test (Agglutination Test):**

Two drops of the aqueous solution of residue of the extract are added to 2 ml. of defibrinated blood (undiluted) in a small test tube. The red blood corpuscles agglutinate into a mass like that of sealing wax.

**9.7.13 Colour Test for Calotropis gigantea / Calotropis procera**

1. **Conc. Hydrochloric Acid:**

To a small portion of residue of the extract, a few drops of conc. hydrochloric acid is added and slightly warmed. A greenish-blue colour is formed.

2. **Conc. Sulphuric Acid:**

To a small portion of residue of the extract, a few drops of conc. sulphuric acid are added. A pink to purple colour develops after a few minutes.

3. **Frohde’s Reagent:**

To a small portion of residue of the extract, 2 drops of Frohde’s reagent is added. A deep green colour changing to blue and finally to green colour develops.

**9.7.14 Colour Test for Cystisus laburnum:**

1. **Conc. Sulphuric Acid:**

To a small portion of residue of the extract, a few drops of conc. sulphuric acid are added. A yellow colour is formed.

2. **Conc. Nitric + Conc. Sulphuric Acid:**

To a small portion of residue of the extract, a few drops of a mixture of conc. nitric acid and conc. sulphuric acid are added. A yellow colour is formed.

3. **Ferric Chloride Solution:**

To a small portion of residue of the extract, a few drops of ferric chloride solution are added. A blood red colour is formed which disappears on adding hydrogen peroxide. On further heating, it assumes a blue colour.

**9.7.15 Colour Test for Taxus baccata Linn:**

1. **Conc. Sulphuric Acid:**

To the residue of extract, one drop of conc. sulphuric acid is added. A violet colour is produced which disappears on addition of water.

2. **Nitric Acid:**

The solution of residue of extract is prepared in sulphuric acid using a few drops of acid. To the acid solution in small quantity, one drop of conc. nitric acid is added. A rose-red color is formed.

3. **Molybdic Acid in Sulphuric Acid:**

To the residue of extract, two drops of molybdic acid in conc. sulphuric acid are added. A rose-red colour is produced.

**9.7.16 Colour Test for Croton tiglium Linn:**

1. 2 ml. of concentrated extract of residue in ethanol is added to an equal volume of 40% sodium hydroxide solution in a small test tube. A brownish red or reddish violet ring is developed at the junction of the two liquids. (The colour formation is rapid by warming).

2. 2 ml. of concentrated ether extract of residue is taken in a porcelain basin and the solvent is evaporated off. To the residue, an alcoholic solution of 1% solution of p-dimethyl amino benzaldehyde in rectified spirit acidified with 1 ml. of conc. sulphuric acid, is added drop by drop. A transient red colour is observed. On evaporating to dryness on hot water bath, the residue becomes brownish red to purple in colour which changes to pale blue on adding an excess of reagent.

**9.7.17 Colour Test for Argemone mexicana:**

1. **Nitric Acid:**

A few drops of oil are mixed with an equal volume of concentrated nitric acid, when a crimson orange colour appears.

2. **Cupric Acetate:**

When 1 ml. of glacial acetic acid and 2 ml. of cupric acetate solution are added to 5 ml. of oil sample in a test tube and then boiled on a water bath for 15 minutes, greenish discoloration occurs.

3. **Ferric Chloride:**

2 ml. of conc. hydrochloric acid is added to 4 ml. of oil, mixed thoroughly and warmed on a boiling water bath for 4-5 minutes. 1 ml. of ferric chloride solution (prepared by dissolving 10 gms. of fresh ferric chloride in 10 ml. of conc. hydrochloric acid and making up the volume to 100 ml. by distilled water) is added and again heated on a water bath for 10 minutes. A precipitate of reddish brown colour appears. The precipitate is acicular or needle shaped when observed under microscope.

**9.7.18 Colour Test for Gloriosa superba Linn:**

1. **Conc. Sulphuric Acid:**

To a portion of residue of the extract in a porcelain basin, a few drops of conc. sulphuric acid are added. A yellow colour appears.

2. **Con Sulphuric Acid + Potassium Nitrate:**

To a portion of residue of extract in a porcelain basin, 2 drops of conc. sulphuric acid and a few specks of potassium nitrate crystals are added. A violet colour changing to red appears.

3. **Conc. Nitric Acid:**

To a portion of the extract, 1 drop of conc. nitric acid is added. A deep violet colour with a yellow tinge at the margin appears.

**9.7.19 Colour Test for Cyanogenic Glycosides:**

1. Plant material or biological material is subjected to steam distillation in acid medium for release of HCN gas. The distillate is collected in N/10 solution of sodium hydroxide. The distillate thus obtained may be subjected to tests for cyanides as described earlier.

2. **Tests with Picrate Paper:**

A portion of plant or biological material is taken in a test tube and few drops of water and toluene are added. The tube is then firmly corked with a moistened picrate paper suspended from the cork (paper is prepared by dipping in 0.05M aqueous solution of picric acid, neutralized with NaHCO3 and then filtered). The test tube is then placed in incubator at 40oC for 2 hrs. A colour change from yellow to reddish brown occurs due to enzymatic release of HCN gas.

**9.7.20 Colour Test for Ergot:**

1. **Marquis Reagent:**

To a portion of dried residue of the extract, one drop of reagent is added. A brown colour develops.

2. **Vitali’s Test:**

Procedure for the test has been given earlier. A play of colours from dull orange to yellow and then purple is observed with the residue of the extract.

3. **Mandelin’s Reagent:**

To a portion of the dried residue of the extract, one drop of reagent is added. A purple brown colour develops.

4. **Frohde’s Reagent:**

To a portion of dried residue of the extract, 1 ml. of reagent (prepared by dissolving 0.5 gm. of ammonium molybdate in 100 ml. of water) is added. A colour change from deep green to red, grey and finally blue is observed.

5. **Fluorescence:**

Blue fluorescence of ethanolic solution of residue of extract is observed under UV light.

**9.7.21 Colour Test for Oleander:**

1. **Keller’s Test:**

The residue of the extract is dissolved in 1 ml. of glacial acetic acid containing 5% ferric sulphate and the solution is layered over conc. sulphuric acid containing 0.05% ferric sulphate. An immediate crimson colour in the sulphuric acid layer and a green colour in the acetic acid layer develop if nerin is present. In case thevetin is present, an immediate blue colour in the acetic acid layer and a mauve colour in the H2SO4 layer appear. (The extract should be pure and active constituents should be in sufficient quantity for the test.).

2. The purified acid ether extract of the sample is taken in a porcelain crucible. One drop of conc. sulphuric or phosphoric acid is added. It is warmed on a water bath. An immediate pink colour appears if nerin is present and a yellowish brown colour slowly changing to pink is obtained if thevetin is present. (For the test, extract should be pure and the active constituents should be in sufficient quantity.).

**9.8 MICRO CRYSTAL TESTS FOR SCREENING OF PLANT POISONS:**

**9.8.1 Crystal Tests:**

The tests are performed with different reagents on the residue of extracts. The crystals formed show definite geometry, characteristic to a particular plant poison. Thus, the tests may be used for general screening. There are some special microscopic tests and crystal tests, which will be covered separately.

**Table: Reagent and Pattern of Crystals.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl. No.** | **Plant Poison** | **Reagent** | **Type of Crystal** |
| 1.  2.  3.  4.  5. | Morphine  Strychnine  Nicotiana tabacum Linn  Aconitum napellus Linn.  Cystisus laburnum | A – 5% Mercuric chloride  B – Potassium mercuric iodide.  C – 5% Potassium iodide.  A – Gold chloride.  B – Potassium mercuric iodide.  C – Mercuric chloride.  Potassium tri-iodide  Gold chloride.  A – Potassium mercuric iodide.  B – Potassium tri-iodide. | Tufts  Needles  Orange plates.  Feathery rosettes.  Wedge shaped curved crystal  Curved ferns.  Nail shaped crystal.  Amorphous precipitate formed. If recrystallised with alcohol shows rectangular prisms or golden yellow needles.  Fans of needle.  Short rods. |

**9.8.2 Specific Crystal Tests for Plant Poisons:**

**9.8.2.1 Crystal Test for Brucine:**

2 drops of acetic acid is added to dried residue of the extract. The acetic acid solution of extract is taken into the cavity slide and evaporated to dryness. The dried residue is dissolved in one drop of alcohol by rubbing with the help of small glass rod and one drop of methyl iodide is added. It is examined under microscope after 10 minutes, rosette shaped crystals are observed.

**9.8.2.2 Crystal Test for Cocaine:**

To the dried residue of the extract or sample, a few drops of saturated solution of alum and one drop of saturated potassium permanganate are added successively with stirring. It is placed on a microscopic slide and covered with cover slip. Under microscope, characteristic rectangular pink coloured crystals are observed.

**9.8.2.3 Crystal Test for Aconitum napellus Linn:**

After undertaking preparative TLC, the scrapped material around spot is taken up in acetone and the extract is evaporated to dryness. One drop of .01 N hydrochloric acid is added followed by 2 drops of 5% sodium carbonate solution. Crystals having shape of rosettes are observed.

**9.8.2.4 Microscopic Test for Dhatura fastuosa Linn:**

The dried residue of extract of vomit or stomach contents or viscera is taken up on microscopic slide with 1 drop of glycerine. A characteristic structure similar to eyelids is seen.

**9.8.2.5 Microscopic examination for Bhang, Ganja, Charas and Modak:**

The above form of cannabis sativa in crude form, if examined under microscope retort shaped, short, unicellular hair containing a cystolith at the root is found in fair number along with the long ordinary hair which possesses no cystolith. In some cases cystolith may be found detached from the tapering end of the hair. The material is taken in 1 drop of dilute caustic soda solution and then examined under microscope. The ganja hair appears smooth while the hair found in charas shows warty surface. In case of modak, ghee, sugar is removed by repeated washing with petroleum ether, alcohol and hot water and then examined. In case of ganja or charas mixed with tobacco it is treated with chloral hydrate solution (50 gms. in 20 ml. water) in a test tube. It is boiled for a few minutes when finer particles will float. 1 drop of liquid containing floating material is examined under microscope.

**9.9 ANALYSIS OF PLANT POISONS:**

The purified extracts of samples i.e. plant materials and other materials (biological and non-biological) are subjected to analysis by different methods viz. colour and crystal tests, UV and IR Spectroscopy, TLC, GLC and HPLC.

**9.9.1 UV and IR data of Plant poisons:**

**Table: UV & IR data of Plant Poisons**

|  |  |  |
| --- | --- | --- |
| **Plant Poison** | **UV Data** | **IR Data** |
| **λmax in nm in diverse media** | **Principal Peaks of sample in KBr. Disc. In Wave No.(Cm-1)** |
| Morphine  Strychnine.  Brucine.  Cannabis sativa Linn.  Cocaine.  Atropine.  Hyoscine.  Hyoscyamine  Aconitine.  Digitoxin.  Digoxin | Aqu. acid : 285  Aqu. alkali : 298  Aqu. acid : 254  Aqu. alkali : 255, 278.  Aqu. acid : 265, 306  Aqu. alkali : 266  Different constituents in ethanol.  Cannabidiol – 278  Cannabinol - 285  Δ9 THC - 278  Δ9 Tetra hydro  cannabidiolic acid – 278, 283.  Aqu. acid - 233  Aqu. acid - 252, 258, 264  No alkaline shift.  Aqu. acid - 251, 257, 263  Aqu. Acid – 252, 258, 264  Aqu. acid - 234, 275  ----------------------------------  --------------------------------- | 805, 1243, 1118, 945  1086. 833.  1664, 764, 1050, 1110, 1282.  775.  1500, 1660, 1280,  1195, 1120, 1212.  1585, 1630, 1020, 1210, 1240, 1050  1620, 1050, 1580, 1030, 1120, 1228  1580, 1030, 1620, 1180, 1080, 1260  1580, 1040, 1620, 1180, 1130, 1050  1710, 1738, 1275, 1110, 712, 1037  1720, 1035, 1153, 1163, 1063, 1204.  1730, 853, 1166, 736, 705, 1047  1490, 1210, 1140, 810, 1020, 835  1092, 1273, 1713, 1235, 710, 1020  1072, 1058, 1010, 1740, 1168, 990  ( as bromide of sample)  1075, 1769, 1055, 1020, 1160, 1110  (as bromide of sample). |

* + 1. **Table: TLC (Silica gel G) Data and Conditions for Identification of Plant**

**poisons.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Solvent Systems** | | **Spray Reagent and Colour of Spot.** | **Plant Poison** | | | **hRf in solvent** | | | |
| **No. 1** | | | **No. 2** |
| No. 1  Carbon tetrachloride : n-Butanol : Methanol : 6 N ammonia : : : : 40:30:3:2.  No. 2:  Xylene :Butanone: Methanol Diethylamine : : : : 40:40:6:2 | | 1. Dragendorff’s  reagent:  Colour of Spot – Orange /Red orange/Brown orange.  2. Acidified potassium iodoplatinate : Colour of Spot- Violet/Blue violet/ Grey or Brown violet. | Active constituents of Papaver somniferum Linn  Narcotine  Papaverine  Thebaine  Cocaine  Morphine  Narcine. | | | 90  85  80  60  40  25 | | | 74  70  56  37  15  02 |
| No. 1.:Methanol : Ammonia : : 100 : 1.5  No. 2:  Cyclohexane : Chloroform : Diethylamine: : :50 : 40 :10. | | As above. | Active constituents of strychnos nuxvomica Linn  Strychnine  Brucine | | | 22  28 | | | 38  18 |
| No. 1: Benzene : n-Hexane : Diethylamine : : :25 : 10 :1  No. 2: n-Hexane : Dichloro-methane : Acetone::: 84:6:60 | | Fast Blue B (by dissolving 15 mg. of salt in 20 ml. of 0.1N sodium hydroxide solution).  Colour of Spot- Violet/ Bluish violet. | Active constituents of Cannabis sativa Linn.  Cannabidiol  Tetrahydrocannabinol  Cannabinol | | | 45  35  25 | | | 60  63  54 |
| No.1: Chloroform : Dioxane  : Ethyl Acetate : 29% Ammonia : : : : 25 : 60 : 10 : 15.  No.2: Methanol : 29% Ammonia : : 100 : 1.5 | | 1. Dragendorff’s reagent  Colour Spot: Orange/ Red orange/ Brown.  2. Acidified iodoplatimate reagent:  Colour of spot- Violet/ Blue or Grey or Brown violet. | Active constituents of Erythroxylon Coca Linn:  Cocaine | | | 81 | | | 59 |
| No. 1: Chloroform : Tetra-  hydrofuran : Diethylamine :::  80:10:10.  No. 2: Chloroform : Diethy-  lamine : : 90 : 10. | 1. Acidified potassium iodoplatimate reagent: Colour of Spot -  Violet/Blue or Grey or Brown violet.  2. 5% Cupric acetate in 20% acetic acid :  Colour of Spot: Yellow fluorescent spot. | | | Active constituents of Atropa belladonna:  Atropine/Hyocyamine  Homatropine  Scopolamine | | | | 39  43  52 | 45  53  65 |
| No. 1: Methanol : Water : :  70 : 3.  No.2: Cyclohexane : chloroform : Diethylamine : : : 50 : 40 : 10. | Acidified iodoplatinate reagent:  Colour of Spot–Violet/ Blue or Grey or Brown violet | | | Active constituents of Dhatura fastuosa Linn  Atropine  Hyoscyamine  Hyocine (Scopolamine) | | | | 33  48  40 | 31  27  39 |
| No. 1: Methanol : Ammonic  : : 100 : 1.5.  No. 2: Cyclohexane : Benzene : Diethylamine : : 75 : 15 : 10. | 1. Dragendorff’s reagent:  Colour of Spot – Orange/Red orange/ Brown orange.  2. Acidified potassium iodoplatimate reagent:  Colour of Spot–Violet/  Blur or Grey or Brown violet. | | | Active constitueut of Nicotania tabacum Linn:  Nicotine | | | | 57 | 53. |
| No. 1: Methanol : Water :  70 : 30.  No. 2: Cyclohexane : Chlo-  roform : Diethyl amine  : : : 30 : 70 : 0.5  (Stationary phase for  No. 2: Aluminium oxide G). | As above. | | | Aconitum napellus Linn | | | | 20 | 65 |
| No. 1: Benzene : Ethanol  : : 70 : 30.  No. 2: Hexane : Benzene  : Methanol ::: 60:30:10 | 1. Perchloric acid Solution Spray : 10%(v/v)  Colour of Spot – fluorescing spot under UV light.  2. 5% Solution of  p-anisaldehyde in ethanol-After spraying the plate is to be heated at 100oC for 5 minutes.  Colour of Spot-Blue. | | | Active constituents of Digitalis purpurea Linn.  Digitoxin  Digoxin | | | | 72  62 | 62  50 |
| No. 1: Chloroform : Diethyl  formamide : Ethanol  : : : 70 : 10 : 20.  No. 2: Iso-propanol : Chloroform : Methanol : Water:::: 50:20:25:5. | | Van Urk reagent  prepared by dissolving 1 gm. of p-dimethyl amino benzaldehyde in 100 ml. of ethanol and adding 10 ml. of hydrochloric acid.  Colour of Spot- Blue/ Bluish green/Greenish blue/yellowish brown/ yellow/brown. | | | Active constituents in Abrus Precatorius Linn. | | 3  9  13  35  (4 spots) | | 5  17  29  51  69  96  (six spots) |
| No. 1: Chloroform : Diethyl  formamide : Ethanol  : : : 70 : 10 : 20.  No. 2: Chloroform : Ethanol  : : 70 :25. | | 0.5% Fast Blue B solution in 50% ethanol and again spraying with 0.1N aqueous KOH.  Colour of Spot- Violet/ Bluish violet. | | | Active constituents of Calatropis gigantean or Calatropis procera Linn:  Calotoxin  Calcatine  Uscharine | | 85  95  78 | | 60  85  68 |
| No. 1: Chloroform : Methanol : :  95 : 5.  No. 2: Chloroform : Ethanol  : : 90 : 10. | | 5% Dimethyl amino benzaldehyde in ethanol followed by addition of 10 ml. of conc. sulphuric acid.  Colour of Spot:  Light blue coloured spot. | | | Active constituents of Ergot:  Ergometrine  Ergotamine  Ergosine  Ergometrinine  Ergocristine  Ergocornine  Ergocryptine  Ergotaminine  Ergosinine  Ergocristinine  Ergocornine  Ergocryptinine | | 3  13  13  -  28  28  28  34  34  45  45  45 | | 17  51  51  -  69  69  69  75  75  81  81  81 |

***NOTE:*** *The hRf values may vary with experimental conditions.*

**9.9.3 Identification of Plant poisons by Gas Chromatography:**

The purified extract of active constituents in plant materials and biological materials or preferably the extracts obtained from preparative TLC (by extracting the scrapped portion of chromatogram of interest i.e. area around the spot by organic solvent) are subjected to GLC as mentioned hereunder.

**Table: GLC Data of Active constituents of Plants Poisons.** (1,2,3,4,5,6,7,8)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Plant Poison** | **Gas Chromatographic Conditions** | | | | | **Retention Time (RT) or Relative Retention Time (RRT in minute) of Active Constituents(in bracket)** |
| **Column and packing Material Etc.** | **Carried Gas and Flow Rate** | **Column Tem-perature (inoC)** | **Detector** | |
| Papaver Somniferum Linn1 | 3'X 1/8"(O.D.) Spiral glass packed with 100/120 mesh silanized Gas Chrom P.  Stationary Phase:  1% High efficiency – 8B  Sample volume:3-5 μl. | Nitrogen  Set 1:  60.0 ml./min.  Set 2:  54.5 ml./min. | 220oC | FID. | | R.R.T.(Codeine)  Set 1:  Codeine (1.00)  Thebaine (1.75)  Morphine (4.03)  Papaverine (8.59)  Set 2:  Codeine (1.00)  Thebaine (1.42)  Morphine (3.0)  Papaverine (6.5)  Narcotine (11.8) |
| Strychnos nuxvomica Linn2 | 6'X 4 mm (ID)  Glass column, packed with 2-3 /100 SE – 30 on Chromosorb W, 80 / 100 mesh.  Sample volume: 1-3 μl. | Nitrogen  43.0 ml./min. | 220oC | Argon Ionization  Detector | | Strychnine (25.9)  Brucine (80.0) |
| Cannabis  Sativa Linn 3 | 6'X 4 mm (ID) Glass column, packed with 3% OV-17 on Chromosorb W HP, 80/100 mesh. | Nitrogen  60 ml./min. | Column temp.:  240oC  Detector & Injection temp.: Both 270oC | FID. | | Cannabidiol (6.0)  THC (8.0)  Cannabinol (11.0)  n-triacontane (14.0). |
| Erythroxylon Coca Linn4 | 6'X 4 mm (ID) packed with 3% OV-1 on Chromosorb W HP, 80/100 mesh. | Nitrogen  50 ml./min. | Injection Temp: 210oC  Detector Temp: 275oC | FID | | Cocaine (4.5) |
| Atropa Belladona5 | 2ft. X 4 mm (ID)  Glass column, packed with 3% OV-1 on Chromosorb W HP, 80/100 mesh. | Nitrogen  25 ml./min. | Injection, column & Detector Temp:  All 250oC | FID | | Homatropine (1.3),  Atropine / Hyoscyamine (2.0)  Scopolamine (3.2) |
| Dhatura fastuosa Linn | 2ft. X 4 mm (ID)  Glass column, packed with 3% OV-1 on Chromosorb W HP, 80/100 mesh. | Nitrogen  25 ml./min | Injection, column & Detector Temp:  All 250oC | | FID | Homatropine (1.3),  Atropine / Hyoscyamine (2.0)  Scopolamine (3.2) | |
| Nicotiana tabacum Linn | 5'X 1/8" (I.D.) stainless steel column packed with 5% SE-30 on 60-80 mesh chromosorb WAW | Nitrogen  30.7 ml./ min. | 230oC | | FID | Nicotine (0.08) relative to codeine (RRT). |
| Aconitine  napellus Linn | 5'X 1/8" (I.D.) stainless steel column packed with 60-80 mesh Chromosorb W | Nitrogen  30.7 ml./ min. | Oven Temp:  230oC or  250oC or  270oC  Injection Temp:  30oC above oven temp. | | FID | Retention Time  At 230oC-Aconitine (10.6)  250oC –Aconitine (5.1)  270oC-Aconitine (3.0)  RRT relative to Codeine  230oC–Aconitine (1.08)  250oC – Aconitine (1.06)  270oC – Aconitine (1.00) |
| Digitalis Purpurea Linn | 12' X 4 mm I.D. Glass column packed with 0.75% SE-30 coated on 100-140 mesh siliconized Gas Chrom P. | Argon  104 ml./min. | Column Temp:  228oC  Detector Temp: 220oC | | Tritium Argon Detector | Tetramethyl Silane Derivative (TMS) of samples used:  Ref maker Cholestane (6.2)  Digitoxigenin – TMS –(37.5)  Digoxigenin – TMS (47.5) |
| Cystisus laburnum | 6'X 4 mm (ID)  Glass column, packed with 2-3 /100 SE – 30 on Chromosorb W, 80 / 100 mesh. | Nitrogen  45 ml./min. | 204oC | | Argon  Ionization  Detector | Cystisine (5.1) |

**9.9.4 Identification of Plant poisons by HPLC:**

The purified extract of active constituents in plant or biological materials or other matrices or preferably the extracts obtained from preparative TLC (by extracting the scrapped portion of chromatogram of interest i.e. area around the spot by organic solvent) is subjected to HPLC as mentioned hereunder.

**Table: HPLC Data on Active Constituents of Plant Poisons.** (9,10,11,12,13,14,15)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Plant Poison** | **HPLC Conditions** | | | | | **Retention Time (RT in minute) of Active Constituents (in bracket).** |
| **Column** | **Mobile Phase** | **Flow Rate(ml.**  **min-1)** | **Internal Standard** | **Detector** |
| Papaver Somniferum Linn.1 | Alumina 250 X 4.6 mm.(I.D) with Spherisorb and shandon C18 4.5 X 2.1 mm (I.D.). | Methanol : Acetonitrile : Citrate–0.01 TMS (Tetra methyl ammonium hydroxide) buffer, PH=6.0 (28 : 17 : 55). | 1 |  | UV detector  λ = 260 mm fluorescence  λ = 260/400 nm. | Meconic Acid (2.05)  Papaverine = (3.61)  Thebaine = (12.16)  Codeine = (12.23)  Morphine= (14.35). |
| Strychnos nux vomica Linn.2 | Normal Phase, 10μm Silica gel, 250 X 2.6 mm (I.D.). | Conc. ammonium hydroxide (28-30% NH3): Methanol (0.75:99.25, v/v). | 1.1 | Quinine (10μg./ml) | UV detector  λ = 254 mm | Internal Standard(2.2)  Strychnine (4.8) |
| Cannabis Sativa Linn.3 | 250 mm X 4.6 mm (I.D.) Octadecyl Silica (medium load of C18 on partisil 5) | 0.02 N Sulphuric acid : Methanol (20 : 80). | 2 | di-n-octyl phthalate (13 μg/ml) | UV detector  λ = 220 nm  or λ=254nm | Internal  standard (19.0)  Cannabidiol (2.5)  Cannabidiolic  Acid (3.5)  Cannabinol (5.0)  Tetrahydro  Cannabinol (6.0)  Cannabinolic  acid (12.0)  Tetrahydro cannabinolic acid (14.0) |
| Erythro – xylon Coca Linn4 | Reverse phase supelcosil 5 μm, LC-8, 250 X 4.6 mm (I.D.). | Acetonitrile: Tetrahydrofuran: 0.1% Tri – ethylamine in water (v/v) (40 : 10 : 50). | 1 | - | UV detector  λ = 254 nm. | Cocaine (6.5). |
| Atropa be- lladona5 | Reverse phase, Lichrosorb C8,  250 X 4 mm (I.D). | Acetonitrile: Aqueous sodium dodecyl sulphate  (30 : 70). | 2.5 | - | UV detector  λ = 220 nm | Atropine (5.50)  Scopolamine (17.42) |
| Nicotiana tabacum Linn.6 | Normal Phase Microsorb- Si, 15 X 4.6 mm (I.D.). | Dioxane : Iso-Propanol :Ammonium hydroxide (80 : 0.3 : 0.4). | 1 | Cotinine | UV detector  λ = 254 nm. | Internal  standard (2.10).  Nicotine (2.75). |
| Digitalis Purpurea Linn.7 | Reverse Phase, Nucleosil C18 (10 mμ). 250 X 4 mm (I.D.). | Acetonitrile  &  Acetonitrile  : water (20:80). | 1  Gradient flow | - | UV detector  λ = 220 nm | Digoxigenin (6.4)  Digoxin (11.5)  Digixigenin (18.9)  Digitoxin (24.1). |

**Quantitation:** It is done by external or internal standard method, as the standard procedure in HPLC.

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**SECTION – 10: ANALYSIS OF MISCELLANEOUS POISONS**

**10.1 Title:** Analysis of miscellaneous poisons

**10.2 Scope:** Analysis of miscellaneous poisons in crime exhibits

**10.3 Purpose:** To identify the miscellaneous poisons in crime exhibits.

**10.4 Responsibilities:** Gazetted Officers and other scientific staff

**10.5 MISCELLANEOUS POISONS:**

These poisons may be organic or inorganic, volatile or non-volatile and of animal, plant origin or toxins produced thereof, or synthetic type, acids and alkalis or derivatives of phenols or phosphides (widely used as rodenticide). The list of miscellaneous poisons discussed below .

1. Insect and Animal Toxins.
2. Mechanical Poisons.
3. Acids and Alkalis.
4. Phosphides.
5. Chemical warfare agents

However, the poisoning cases by plant poisons are very common even today. Before the advent of synthetic insecticides, plant poisons cover a major area in the spectrum of poisons. The other important poisons also include acids, phosphides and phenols.

**10.5.1 Insect and Animal Toxins:**

Venomous or poisonous animals (excluding birds) and insects are capable to deliver toxins during biting or stinging. They produce the toxins in highly developed secretory glands or group of cells or the by-product of metabolism. On the contrary, poisonous animals or insects whose tissues are toxic in part or full do not possess any poisons delivery system. Poisoning occurs by ingestion only. Thus, all venomous animals are poisonous but the reverse is not always true.

These toxins vary considerably in their chemistry and toxicology. Venoms, for instance, may be composed of proteins of both large and small molecular weight including polypeptides and enzymes. They may be amines, lipids, steroids, amino polysaccharides, quinones, 5-HTglycosides or other substances. Among all of poisonous insect and animals, cantharides, snakes and scorpions are of medico-legal importance.

10.5.1.1 Cantharides (Spanish fly):

**Active Principle:**

The active principle is cantharidin, the anhydride or lactone of cantharidic acid, which is a colourless crystalline solid. It is freely soluble in alcohol, chloroform, acetone and fixed oils.

Cantharidin is a powerful vesicant and may be administered in the form of powdered beetles or the tincture or active principle. It is also used as an aphrodisiac or an abortificient or a counter irritant to the skin in the blistering plaster or a promoter in the growth of hair. Cantharidin is a nephrotoxic or kidney poison and is readily absorbed from all surfaces including the skin.

10 mg. of Cantharidin or 1.5 gms. of powder cantharides is found to be a fatal dose. The Indian fly (beetle) yielding cantharidin is known as Mylabris cichorii (Teleni Makhi) occurs abundantly in the rainy season in certain parts of northern India and Kashmir.

**I. Analysis of Cantharides:**

The materials suspected to contain cantharides is extracted for the active constituents and identification thereafter.

a) Isolation of Cantharide in Samples:

The material is shaken with chloroform. The chloroform layer is separated and evaporated to dryness. The residue thus obtained is subjected to analysis.

b) Test:

1 drop of warm olive oil is added to the residue of extract and rubbed gently to take the residue into oil drop. The oil drops, thus obtained is applied to the skin of a rabbit. After a few hours a blister is produced on the skin.

**c) Identification of Cantharides in the extract of sample by GC, IR and MS:**

**Table: GC, IR and MS Data of Cantharide.**

|  |  |  |
| --- | --- | --- |
| **GC Conditions** | **Wave Number of Principal Peaks in IR (cm-1)** | **M / Z of Principal Peaks in MS.** |
| Column: 2 m. x 4 mm (i.d.) glass column packed with 2.5% SE-30 on 80 – 100 mesh Chromosorb G (Acid washed and dimethyl chloro silane treated). The support should be fully deactivated.  Carrier Gas and Flow rate: Nitrogen and 45 ml./ min.  Column temperature: 165ºC isothermal.  Reference compound:  n -Alkalines with even no. of carbon atom. | 1242, 962, 900, 1786, 1852 and 1002. | 128, 96, 70, 39, 41, 27, 42, 29. |

**10.5.1.2 Snakes (Ophidia):**

There are about 2500 species of snakes in the world predominantly in the warm climates. Of the 216 species of snakes found in India, only 25 species are poisonous. The poisonous snakes normally belong to two families viz. colubridae or colubrine snakes (King cobra, Krait and sea snakes) and viperiadae or viper snakes (Russel’s viper and Echis carinata or saw scaled viper).

**Mode of Ejection and Transmission of Venom:**

In poisonous snakes, the poison is transmitted through the hollow teeth known as fangs, which are the modified upper marginal teeth. These fangs are connected to the poison gland (venom gland) by means of a duct. The venom gland in poisonous snake is a modified parotid salivary gland, which secretes and stores the toxic saliva called venom. When the snake bites, muscles compress the venom gland and force the venom to come out of the fangs.

**Active Constituents of Venom:**

The venoms of snakes are complex mixtures containing a number of toxic substances and enzymes. In fresh state, it is a clear transparent, amber tinted fluid and dries into a yellow granular mass that retains its activity for many years. The venoms contain toxalbumins and several toxic principles such as fibrinolysins, proteolysins, neurotoxins, haemolysins, thromboplastins (predominant in viper venom), agglutinins, cardiotoxins and enzymes viz. cholinestearase (predominant in colubrine snake venoms), coagulase and hyaluronidase etc. In addition to these, snake venoms contain inorganic substances viz. sodium, potassium, calcium, magnesium and small amounts of metals like zinc, iron, cobalt, manganese and nickel. A toxalbumin is a toxic protein, which causes agglutination and lysis of the red cells even in great dilution. It is antigenic in nature and capable of producing specific antitoxin (antibody) when injected into the body.

**Action of Venoms:**

The venoms of various poisonous snakes have different actions on the body e.g. Elapid (Cobra, King Cobra, common krait, banded krait and the coral) venom is neurotoxic. Viper (pit viper and pitless viper) venom is mainly vasculotoxic and sea snakes venom mycotic.

Neurotoxic venom causes muscular weakness of the legs and paralysis involving the muscles and respiration. The neurotoxins act primarily on the motor nerve cells and their action resembles curare. The neurotoxins of cobra venom produce both convulsions and paralysis whereas krait venom causes only muscular paralysis. Local symptoms at the site of bite are minimum as compared to those caused by vasculotoxic venom.

Vasculotoxic venom produces enzymatic destruction of cell walls and coagulation disorders. As a result, the endothelium of blood cells is destroyed, the red cells are lysed and the other tissue cells are destroyed. Blood from such patients fail to clot normally even after addition of thrombin because of the extremely low level of fibrinogen. Locally, there is oozing of haemolytic blood, severe swelling of the bite-area and spreading cellulites. Haemorrhages from external orifices are seen.

Mycotoxic venom produces generalized muscular pain followed by myoglobinuria after 3 to 5 hours ending in respiratory failure in fatal cases.

**Fatal Dose:**

15 mg. of dried cobra venom, 14 mg. of the viper venom, 6 mg. of krait venom and 8 mg. of saw scaled viper venom are fatal. The amount of dried cobra venom ejected in one bite is about 200-350 mg. The viper yields 150-200 mg. the krait about 22 mg. and the saw scaled viper about 25 mg.

**Antivenin:**

The antivenin is used for the treatment of snakebite cases. It neutralizes the circulating toxins only and not the toxin fixed in the tissues. Specific antivenin is prepared by hyper-immunizing horses against the venom of a particular snake while polyvalent antivenin is prepared hyper-immunizing horses against the venoms of four common poisonous snakes viz. cobra, common krait, Russel’s viper and saw scaled viper. The strength of the polyvalent antivenin is such that 1 ml. will neutralise 20 MLD of king cobra venom. Specific anti-venom is most valuable when the identity of the snake is known.

I. Analysis of snake venoms:

The analysis of snake venom is somehow different as routine chemical tests are not applicable. The skin portion around the bite area may be a source of venom for examination of its constituents by special tests viz. test for cholinesterase or thromboplastin in venom, toxicity test, clot quality test, preciptin test, gel-diffusion test and immuno-assays.

1. **Test for Cholinesterase and Thromboplastin in and around bite**

**area:**

The aqueous washings from the bite area may tested for the activity of cholinesterase (in case of bite by colubrine snake) and also thromboplastin (in case of bite by viper snake).

B. **Toxicity Test:**

This test is a physiological test for confirmation of snakebite.

**Procedure:**

The aqueous washings of the bite area or the serum of the victim is injected into fowls with or without antivenin. The death of the fowl after injection of serum without antivenin and the survival of the fowl after injection with antivenin after a few hours confirms the snakebite.

C. **Clot Quality Test for Snake Venom:**

A capillary tube filled with blood taken from a finger prick of the victim is kept horizontal for 30 minutes at room temperature. It is then raised vertically. Non-clotted blood due to consumption of fibrinogen or defibrination runs out of its own accord or can easily be blown out. This test is very useful in the diagnosis of viper bite compared to the test for coagulation viz. the prothrombin time, which is markedly reduced in a viper bite.

**N.B.** Clot quality test should be carried out at the time of prick without delay.

D. **Precipitin Test for Snake Venom:**

The test depends upon the fact that when a foreign protein is injected into an animal, certain specific antibodies known as preciptin are formed in that animal’s blood which have the capacity to precipitate the specific foreign protein. The specific foreign protein, which causes the production of the preciptin is known as preciptinogen or antigen.

**Test Procedure:**

The aqueous wash or wash by isotonic solution of the bite area or the serum of the victim is allowed to come in contact with antivenin (the specific antivenin when the snake is identified or polyvalent antivenin when the snake is not identified). A reaction between the two is then shown by the formation of a cloudy precipitate at the line or plane of contact. This is sensitive for a very low dilution of protein (1 in 1,00,000). This test can be done in small test tubes.

The preciptin test may be done by new methods, which have several advantages over the tube method viz. (1) very small quantity of the extract and antivenin are required. (2) perfectly clear extract is not required.

E. **Gel Diffusion Test:**

The test is carried out on the thin layer of agar solution on a glass plate or petridish. Small hole are punched out in the dried layer in a particular manner i.e. One in the center and three or four round the circumference of a circle of about 2.5 cm. in diameter. The wash from the bite area or serum of the victim (diluted with isotonic saline solution) is placed in the central hole and the antivenin in the other holes. Diffusion occurs if the reaction is positive and a line of precipitation appear. This reaction is completed after an overnight diffusion.

F. **Immuno – Assays:**

These methods have recently been introduced. It is based on antibody-antigen reaction which is very sensitive and specific. A labelled antigen or antibody is employed for the purpose viz. a radioisotope.

Other methods are based on the use of diverse traces in different ways viz. an enzyme (in enzyme immuno-assay, EIA), or erythrocyte (haemagglutination inhibition assay), a bacteriophage (viroimmuno assay), a fluorescent group and a stable free radical (FRAT, Spin immuno-assay). Among the methods, enzymes are found to be most effective as these exert catalytic action i.e. act as a stamplifier and catalyze the formation of more than 105 product molecules per minute. Labelled component of an antibody-antigen reaction binds to its complementary binding site and competes with unlabelled component. The concentration of the labelled component is measured by assaying enzyme activity or radioactivity, which is proportional to the concentration of antigen (protein) present in the sample.

The commonly used types of enzyme immuno-assay are homogeneous enzyme immuno assay or enzyme multiplied immuno-assay (EMIT). Another type in which the antigen or antibody is linked to a solid-state carrier is known as enzyme linked immunosorbent assay (ELISA). The above two techniques are simple, rapid and do not require pre-treatment of biological sample. ELISA technique is expected to serve the purpose of FSL for detection of snake venom in blood or washing of the bite area.

G.  **Other Test:**

The aqueous extract (washings) of bite area may also be tested for the presence of inorganic substance viz. sodium, potassium, calcium and magnesium by spot tests and atomic absorption spectrophotometer and also zinc, iron, cobalt, manganese and nickel.

II. Samples to be collected in case of Snake Bite:

1. The aqueous washing of bite area on a clear cotton swab (control sample of cotton to be preserved also).

B. Blood serum or blood of victim.

C. The visceral tissues may be sent for examination, if necessary to confirm the presence of other poison (viscera is not suitable for detection of venom).

10.5.1.3 Scorpions:

There are about 500 species of venomous scorpions. But only a few of these are dangerous to human. Scorpions have a crab-like appearance with a long fleshy, five-segmented tail like post abdomen ending in a broad sac and prominent hollow string which communicates by means of a duct with the venom secreting gland. The sting conveys venom into the wound made by it. The venom of scorpion consists of a mixture of toxalbumins which exert haemolytic and neurotoxic action and the death is normally anaphylactic in nature. Its toxicity is worse than that of snakes but fortunately a small quantity is injected only by it. The death due to scorpion stings is rarely found compared to snake bite.

In case of fatal consequence, all the tests except the clot quality test (applicable in vasculotoxic venom) under snake venom in the preceding section may be tried for the presence of venom in the washing of sting area or bloom of the victim.

10.5.2 Mechanical Poisons:

Mechanical poisons are not poisons in the true sense, as these are not absorbed in the biological systems. But these produce symptoms of irritants because of mechanical action of their sharp angular edges and points thereby causing irritation of stomach and bowels due to contact. These poisons viz. powdered glass, pins, needles, glass fibers, chopped animal hair etc. are thus included in Section 328 of IPC. Out of these materials, powdered glass has some medico-legal importance. Glass powder is used for destroying cattle i.e. homicidal purposes, but rarely found in suicidal poisoning. It does not produce the desired effect, if it entangled in the mucous or food in the stomach. Similarly, it will not have any effects if it is well powdered.

Glass is a non-crystalline substance. Chemically ordinary glass is mostly sodium calcium silicate. Heat resistant glass (borosilicate glass or borosil) containing about eighty percent silica and some boron oxide. Optical glass contains comparatively larger amount of lead, barium and zinc oxide. Coloured glass contains oxides of various metals known to give coloured compounds. Sum-glass contains rare earths as additional elements.

**I. Detection in Stomach Contents / Mucous section / Faecal matter:**

A. Glass fragments picked up from stomach contents or materials adhered to the tenacious mucous section or faecal matters are washed with water and then with ether. Alternatively, the contents of the bowl or the ejection can be destroyed by concentrated hydrochloric acid and potassium chlorate. The organic matter passes in to the solution and the glass is left as fragments. In suspected homicidal poisoning by glass, the organs removed should not be preserved in glass container. The particles are then viewed by naked eye, magnifying glass and then under microscope (glass fragment should appear as transparent and amorphous particles). The picked up particles are melted in a spatula on flame. If the molten material is touched with a thick platinum wire and the wire is pulled up. A glass thread is formed.

B. **By Refractive Index Determination:**

This is very important in case of glass and determined by immersion method: In this method glass fragment is placed in a mixture of two immiscible liquids (one with higher and the other with lower refractive index) is used. One of them is gradually added till the glass in the liquid mixture becomes invisible. The refractive index of that mixture of liquid is then determined by Abbe Refractometer. The refractive index thus obtained will be the refractive index of glass.

C. **Spectroscopic Analysis:**

Glass can be identified by emission spectrographic analysis which

indicates its elemental composition.

1. **SEM – EDX Method: (Scanning Electron Microscope with Energy Dispersive X-ray Analysis):**

Primary electrons having higher energy (accelerated by high voltage of about 15 KV to 50 KV) are made to fall on the particles under analysis. The X-rays emitted are then detected by X-ray detector. This X-ray detector measures the energy in each photon of X-ray radiation and this quanta of energy can be stored electronically. A histogram of number of quanta versus the energy can be drawn. From this histogram elemental analysis of particles under examination can be determined.

**10.5.3 ACIDS**

Poisoning cases by acids include mineral acids (hydrochloric, nitric and sulphuric acid), carbolic, oxalic and acetic acid. Suicidal poisoning cases with the acids (mainly mineral acids) are common compared to homicidal cases. Accidental poisoning by oxalic acid and acetic acid are known. The common sign and symptom in case of acids include corrosion of mucous membrane, lips, tongue, stains of specific colour on skin, burning pain, salivation, convulsion, vomiting, ulceration etc. Perforation or contraction of stomach may occur specially in case of sulphuric acid. The action of acetic acid is milder. However, oxalic acid is the most potent as it combines with calcium ion in blood to form calcium oxalate thereby hindering acutely the functioning of blood. The characteristics of acids are given below. The same in respect of carbolic acid have already been covered in section 4. The biological material for analysis should never be preserved with saturated sodium chloride solution.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Name of acid | Source/uses | Properties | Sign and symptoms | Other characteristics |
| Hydrochloric acid  Other names: Muriatic acid, sprits of salts | Source: Industries, laboratory and house hold (for cleaning)  0.2 – 0.5% acid is found in stomach | Liquid (faint or dark yellow in case of commercial acids) having intensely irritating odour, is volatile, and freely soluble in water | Less active than nitric or sulphuric acid. Usually no staining occur on skin or mucous membrane, but reddish brown stain appear on dark clothes. Symptoms in acute cases: salivation, ulceration, nausea, vomiting, paralysis of limbs, coryza, corneal ulcer etc. Fumes cause irritation of air passage and glottic spasm occurs in chronic poisoning. | Mucous membrane acted by acids are ash – grey or black in colour,  perforation is rare. |
| Nitric acid.  Other names: Aqua fortis, Red spirit of nitre | Source Industries (explosives and dyes), laboratory, goldsmith’s work place (for dissolving and cleaning ornaments) | Pure acid is colorless (giving off fumes in contact with air). Commercial acid varies in colour (yellow to deep red) due to presence of nitrogen dioxide | Lips, tongue, teeth, mucous membrane get softened and assumes white turning to yellow colour (due to xanthoproteic acid), stains on skin, cloth are yellow in colour. Symptoms in acute cases: Severe pain in mouth, throat, stomach, nausea, vomiting, diarrhoea, thirst, weak pulse, convulsions, collapse and death. | Corrosion of membrane, skin, softening of abdomen, perforation is not common. Inhalation causes lachrymation and burning in throat. |
| Sulphuric acid  Other names: Oil of vitriol, battery acid | Source  Industries: (organic chemicals, drug and pharmaceutical, fertilizer, industries etc.) battery industries, sulphate of indigo (1 part of indigo in 9 parts of acid) | Colourless, heavy hygroscopic oily liquid, emits no fumes in contact with air. | Chars and blackens skin, cloth and any organic matter. A reddish brown stain is formed. Tissue dehydration occurs. Symptoms in acute cases: Brown scars on lips and mouth. Severe pain and gastro-intestinal irritation, nausea, vomiting, convulsion and collapse. | Carbonization of skin and tissue. Charred and black stomach, perforation is common. |
| Acetic acid Other name: Wood acid | In nature as esters in many plants. In pyroligneous acid (liquid fraction) in wood distillation produced by oxidation of alcohol by acetobactor aceti | Colourless liquid having penetrating odour, soluble in water and organic solvent | Symptoms in acute cases: Burns on skin, mouth and esophagus, severe gastro-intestinal corrosion, abdominal pain, rapid and then weak pulse, shallow breathing, convulsion, collapse, shock and death. | Odour of vinegar in stomach. Perforation does not occur. No staining occurs. |
| Oxalic acid  Other name: Acid of sugar | Source: sodium or potassium salts used as bleaching, cleaning agents, stain remover, metal polishes. Available in some vegetables. | Colourless solid crystalline having bitter acidic taste, soluble in water, alcohol and sparingly soluble in ether. | Symptoms in acute cases: Burning pain in mouth, stomach and throat, extreme thirst, swelling of tongue, nausea, vomiting, diarrhoea, dilated pupils, dyspnea, tremors, coma, death. | Black appearance of stomach contents. |

**10.5.3.1** **Extraction of Hydrochloric Acid in Biological Materials and its Identification and Quantitation:**

**A. Extraction:**

The liquid materials viz. stomach wash may be tested directly after repeated filtration, but if the result is not very decisive, distillation is necessary. The solid material should be mixed with water and distilled as described below:

A portion of the material (tissue properly minced, stomach contents, vomit, stomach wash etc.) is distilled and the distillate is collected in two fractions – the first fraction consists mainly of water and the last fraction consists of water and hydrochloric acid. The distillation in such cases is to be continued till the contents of the flask are almost dry so that the free hydrochloric acid may be distilled completely. The last few ml. of the distillate being usually the pure acid.

It may be noted that hydrochloric acid does not begin to distil until its concentration is about 10 percent. If so, for instance 100ml. of a solution containing 1 percent of hydrochloric acid is distilled, the first 85 – 90 ml. of the distillate contains, if at all, only traces of the acid while the last fraction would contain the whole of the acid which may be submitted to the tests for identification.

**B. Tests for Hydrochloric Acid**

1. **Gunzberg’s test:** A few drops of Gunzberg’s reagent (prepared by dissolving 2gms. of phloroglucinol and 1gm. of vanillin in 100ml. of 95% alcohol) is taken in a porcelain dish; evaporated to dryness over a small flame and cooled. A glass rod together with the material to be tested is added to the dried reagent. It is warmed gently. A purplish red colour develops in the presence of free hydrochloric acid.

2. **Silver nitrate test:** A few drops of silver nitrate solution is added to 2ml. of the sample. A curdy white precipitate is obtained which is soluble in ammonia or potassium cyanide and insoluble in strong nitric acid even on boiling (HCN also gives a white precipitate under the same conditions but it is soluble in nitric acid on heating).

**C. Quantitative Determination of Hydrochloric Acid and Chlorides**

The material (Biological materials) is weighed and extracted with warm water for an hour or two with frequent shaking. It is filtered into a measuring flask and the residue is washed till the filtrate is free from chlorides as indicated to a drop of AgNO3 solution. The volume is made up by adding distilled water. The steps as stated below are followed.

**Procedure (a):** An aliquot part (about one third of the total bulk) is taken and neutralized (if it is acid in reaction due to presence of free HCl) with sodium carbonate to fix up the free HCl (volatile) as sodium chloride. It is evaporated to dryness in a platinum or porcelain crucible; incinerated to complete carbonization organic matter which are always present in such filtrate, by breaking up with glass rod the lumps of calcined mass formed during incineration. The carbonized residue is extracted with small portion of distilled water till the filtrate is free from chloride. It is acidified with nitric acid and silver nitrate solution is added in slight excess, which converts all soluble chlorides into insoluble silver chloride. It is filtered and the residue (AgCl) is washed till it is acid free. It is dried and weighed to constant weight. The constant weight of AgCl thus obtained represents the weight of free HCl and chloride taken together.

**Procedure (b):** Another portion of the filtrate is taken (exactly the same volume as taken in (a) as above) and evaporated to dryness without previous neutralization (free HCl and other volatile acid, if present volatilized off). It is incinerated and processed therefore in the same manner as in (a). The weight of AgCl represents only the soluble chloride. If this is deducted from the value obtained in (a) in the preceding step would represent the amount of free HCl.

The above method has also been modified as free HCl and chlorides may be retained by tissues and other organic matters in the form of acid-albumin leading to erroneous result (as result obtained indicate free HCl plus acid albumin). The procedure has been modified as under (c).

**Procedure (c):** the third portion of filtrate [same volume as for (a) or (b)] is taken and evaporated to dryness on the steam bath and left it there for an hour or more (free HCl is expelled leaving acid albumin and chlorides in the residue). Excess of sodium carbonate solution is added and mixed thoroughly with the residue and evaporated to dryness. This is incinerated to complete carbonization.

The experiments (a), (b) and (c) would give the results as Free HCl = (a) – (c), chloride = (b) and acid albumin = (c) – (b). It may be added that the extra precaution for accuracy is necessary in biochemical but not in chemical analysis.

**Calculation for the above:** The weight of dried AgCl if multiplied by the factor 0.2543 gives the weight of anhydrous HCl, by the factor 0.8 gives the weight of concentrated HCl (B.P), which contains 31.9 percent of anhydrous HCl and by the factor 0.4075, gives the weight of soluble chloride calculated as NaCl.

**10.5.3.2** **Extraction of Nitric Acid in Biological Materials and its Identification and Quantitation:**

1. **Extraction of Nitric Acid/Nitrates in Biological materials:**

If a free acid is indicated by test, the tissues, stomach contents etc. are extracted with distilled water on the steam bath for some time and filtered. The filtrate is neutralized with slaked lime or calcium carbonate and evaporated to dryness. This is then extracted with cold 95 percent alcohol in which calcium and sodium nitrates are soluble. The alcoholic extract is filtered and again evaporated to dryness. The residue is dissolved in distilled water. The aqueous solution is filtered and evaporated to dryness. The residue is dissolved in a mixture of alcohol and ether (1:1) in which only calcium nitrate is soluble. It is transferred to a well-corked flask, kept overnight, filtered and washed with alcohol – ether mixture. The filtrate is evaporated to dryness. The residue of calcium nitrate is dissolved in a small amount of water and tested for nitrates.

**B. Test for Nitric acid and Nitrates:**

1. **Brucine Test:** 1 drop of test solution is taken in a porcelain dish. 1 drops of conc. Sulphuric acid followed by a small crystal of brucine. A blood red colour is produced if nitrate is present.

2. **Ferrous Sulphate Test:** A few drops of test solution is taken in a porcelain dish. 2 drops of conc. sulphuric acid and a crystal of ferrous sulphate are added when a dark brown colour develops round the crystal (the test may be carried out in a test tube when the concentration of nitrate is high). A brown ring is formed at the junction of ferrous sulphate solution and conc. sulphuric acid.

3. **Diphenylamine Test:** A few drops of diphenylamine reagent (prepared by dissolving 1 gms. Of diphenylamine in 5ml. of dilute sulphuric acid and making up the volume to 100ml.) is added to 1ml. of test solution when a blue colour develops at the junction (the test is not specific as interference by oxidant permanganate, chromate may occur).

**C. Quantitative Determination of Nitrate and Nitric Acid**

**Procedure**: A weighed amount of finely minced viscera or stomach contents or vomit is extracted with distilled water for a few hours on the steam bath. It is filtered and the residue is thoroughly washed with water. The filtrate is transferred to a flask. An excess of freshly prepared stannous chloride solution (prepared by dissolving 16gms. Of pure granulated tin in 60 gms. of pure HCl) is added and the mixture is boiled for one hour. During the process, the nitric acid and nitrate are reduced to ammonia, which combines with HCl to form ammonium chloride. The mixture is transferred to a porcelain basin and evaporated on the steam bath to dryness. The residue is dissolved in distilled water, filtered and washed. The filtrate is transferred to a flask and rendered alkaline with strong solution of sodium hydroxide (40%) and distilled. The distillate is collected in a conical flask containing a known amount of N/10 sulphuric acid with a few drops of an indicator viz. phenolphthalein. It is titrated with N/10 sodium hydroxide solution. The amount of ammonia obtained is multiplied by 3.7 to get the quantity of nitric acid in the weighed amount of the material taken.

**N.B.** 1. The presence of large quantity of organic matter from tissue or food material may prevent successful extraction of nitrates or their reduction to ammonia for quantitative determination. In such conditions the use of a dialyzer for separation of nitrates in pure form from the mass of organic matters may be helpful.

2. The skin stained yellow with nitric acid or blisters from the skin in acid throwing cases may also be examined for detection of nitrate by a dialyzer.

**10.5.3.3 Extraction of Sulphuric Acid and Soluble Sulphate in Biological Materials**

**and its Identification and Quantitation.**

**A. Extraction of Sulphuric Acid and Soluble Sulphate in Biological Materials.**

1. Finely minced viscera or stomach contents is weighed and extracted with distilled water on the steam bath for a few hours and left overnight. It is filtered and the residue is washed with water till free from sulphate. The filtrate thus collected is concentrated to about 200ml. and transferred to a volumetric flask with rinsing. The volume is made up to 250ml. with distilled water (extract A).

2. 100ml. of extract of A is evaporated to dryness on a steam bath. The residue consists of free H2SO4 and other salts with some organic matter. The free HCl of the stomach and any volatile organic acids originally present in the material are all volatilized at this stage. Sometimes, phosphoric acid is produced by the action of sulphuric acid on phosphates of tissues and foodstuff and it is retained along with the sulphuric acid in the residue. The residue is treated with a mixture of cold alcohol and ether (1:1). It is filtered and insoluble matter (consisting of sulphates and other salts) washed with alcohol – ether mixture till the filtrate is acid free. The filtrate is evaporated on the steam bath and alcohol and ether are expelled completely. The residue is only a syrupy substance (consisting of free sulphuric acid and traces of ethyl hydrogen sulphate and phosphoric acid). 30ml. of water is added. The solution is then heated to boiling for hydrolytic decomposition of ethyl hydrogen sulphate into sulphuric acid and alcohol. The latter is expelled by boiling. This acid solution (extract B) is now ready for detection of free H2SO4 and determination of its quantity. It is divided into two equal portion, one for qualitative test for free H2SO4 and the other for its volumetric or gravimetric determination.

The remaining portion (50ml.) of the filtrate (extract C) from the volumetric flask (extract A) is tested for determination of the total soluble sulphates (free and combined H2SO4) present in the material. By deducting the total amount of free H2SO4 i.e. extract B from the result of extract (c), the amount of sulphates would be obtained.

**B. Test for free mineral acid**

1. **Test with Methyl Violet:** A few drops of aqueous solution of methyl violet (0.01%, w/v) is added to 2ml. of the extract. A greenish blue colour is produced (organic acids do not produce any change in colour).

2. **Test with Congo Red Paper:** the red colour changes to deep blue (organic acids produce a violet colour).

**C. Test for Sulphuric Acid**

1. **Test with Barium chloride:** A few drops of barium chloride solution are added to 2ml. of extract. A white precipitate is formed, which is insoluble in HCl or HNO3 (free acid and sulphates respond to this test).

2. 25ml. of extract is taken in a test tube fitted with a bent tube (bent twice at right angles) and concentrated by boiling. A small piece of copper turning is added and boiled again. The gas liberated, if any is passed through a weak solution of potassium permanganate (0.1%, w/v). The pink colour of the solution is gradually discharged or fades away.

**D. Quantitative Determination of Sulphuric Acid and Soluble Sulphates**

The extracts B and C are measured out and transferred to two beakers acidulated with nitric acid and heated to boiling. Barium chloride solution is added drop by drop till complete precipitation of barium sulphate occurs. These are cooled, filtered and washed till free from sulphate. The residues are dried and ignited. As there are some organic matters in the solution particularly in extract C, there would be slight reduction of sulphate to sulphide which should be reconverted to sulphate in the usual way. The weight of barium sulphate in extract B represents the amount of free H2SO4 and the weight of barium sulphate in extract C represents the total amount of free and combined H2SO4. The weight of barium sulphate is multiplied by 0.4201 and the result will give the weight of H2SO4 in the quantity of the solution examined or multiplication by 0.3420 will give the weight of H2SO4 or sulphate calculated as SO3. The amount of free H2SO4 present in the extract B can also be determined by titration with a standard alkali. But as H3PO4 and in some cases traces of lactic acid are likely to be present, the gravimetric method is preferred.

**10.5.3.4** **Extraction of Oxalic Acid and Oxalates in Biological Materials and its Identification and Quantitation.**

**A. Extraction of Oxalic Acid and Oxalates in Biological Materials**

In case of poisoning, the oxalic acid may be present as (1) free acid, (2) as soluble oxalates, (3) as insoluble oxalates formed as a result of the administration of antidotes. It may be found in the stomach contents, stomach wash and vomit mostly as free acid, in the liver and kidneys and other tissues mostly as soluble oxalates and in the urine mostly as insoluble oxalates. The acid in the three different forms may be isolated by the following method.

**Procedure:** The minced visceral material is mixed with 3 – 4 times of distilled water, digested on a water bath for an hour with frequent shaking and filtered. The filtrate (extract A) will contain the free acid and its soluble salt and the residue B will contain the insoluble oxalates. The filtrate A is evaporated to dryness on a steam bath, cooled and excess of absolute alcohol is added and digested for about an hour for complete solution of the free acid. It is filtered. The alcoholic filtrate will contain the free acid and the residue (alcohol insoluble) will contain the soluble oxalates. The filtrate is evaporated to dryness and dissolved in a small quantity of distilled water. The aqueous solution now contains the free acid.

The alcohol insoluble residue containing the soluble oxalates is digested with alcohol acidified with HCl to liberate oxalic acid. It is filtered and evaporated to dryness. It is then dissolved in distilled water. The aqueous solution now contains the soluble oxalates in the form of free oxalic acid.

The water insoluble residue (portion B) is taken in a beaker and mixed up with 3 – 4 volumes of water. A few ml. of sodium carbonate solution is added to make it distinctly alkaline. It is then boiled for 2 hours to convert the insoluble oxalate to their soluble form, cooled and filtered. The filtrate is evaporated to dryness. To the filtrate alcohol is added (3 – 4 volumes). It is acidified with HCl and digested for about an hour, filtered. The filtrate is evaporated to dryness and the residue is dissolved in distilled water. The aqueous solution contains the insoluble oxalates which has been converted into the soluble form and then into oxalic acid.

**N.B.** The acid – ether extract may also contain oxalic acid. The extract may be tested for oxalic acid.

**B. Identification Tests for Oxalic Acid and Oxalates.**

1. **Test with Lime Water:** To 2ml. of extract, a few drops of lime water is added. A white precipitate insoluble in acetic acid and ammonia solution but soluble in dil. HCl or HNO3 appears.

2. **Test with Calcium Chloride:** 2ml. of extract is neutralized with ammonia solution. A few drops of calcium chloride solution is added. A white precipitate is formed.

3. **Test with dil. KMNO4 Solution:** To 5ml. of extract, a few drops of dil. potassium permanganate solution (0.1%, w/v) is added, acidified with dil. sulphuric acid and warmed. The colour of the solution is discharged.

4. **Test with Silver Nitrate Solution:** A few drops of silver nitrate solution is added to 2ml. of the extract. A white precipitate soluble in ammonia is formed.

**C. Quantitation of Oxalic Acid in Viscera**

The weighed amount of finely minced viscera (about 100gms.) or stomach contents or vomit is mixed up with 3 – 4 volumes of alcohol and acidified with HCl. It is digested in the cold for 2 hours and filtered through flutted filter paper. The residue is washed with alcohol. The combined alcoholic extract is collected. 20ml. of water is added to the filtrate (to prevent the formation of ethyl oxalate during evaporation) and evaporated on the steam bath to expel the alcohol completely. It is filtered and transferred to the filtrate (which is about 20ml. all) to a separating funnel and extracted thrice with 20ml. portion of ether. The combined ethereal extract is filtered through a dry filter paper. The filtrate is collected and evaporated to dryness. The residue is dissolved in 2-3 ml. of distilled water and made distinctly alkali with ammonia solution. 10% calcium chloride solution is added till complete precipitation of calcium oxalate occurs. It is acidified with acetic acid and kept for 24 hours in a covered beaker. It is filtered. The residue is washed till free from acid, dried and ignited in the normal manner to convert calcium oxalate into calcium oxide and weighed as CaO. The weight of CaO multiplied by 2.25 gives the weight of crystalline oxalic acid (C2H2O4, 2H2O) present in the material. If the weight of CaO is multiplied by 1.64, the weight of anhydrous oxalic acid is obtained.

**10.5.3.5** **Extraction of Acetic Acid in Biological Materials and its Identification and Quantitation**

The extraction is carried by acid steam distillation. The distillate is subjected to test for acetic acid.

**Tests for Identification**

1. 0.1ml. of the test solution is mixed with 1 drop of a 5% solution of lanthanum nitrate on a spot plate. 1 drop of 0.01N iodine solution is added followed by a drop of 1N ammonia solution. A blue or brown ring develops around the drop of ammonia in a few minutes.

2. 1ml. of test solution is neutralized by heating with calcium carbonate, 1 drop of ferric chloride solution is added. A red brown colour is formed.

**Quantitation:** By standard procedure for acetic acid.

**10.5.3.6** **Extraction of Carbolic Acid in Biological Materials and its Identification and Quantitation.**

The isolation is done as stated earlier in section 4. The identification etc. has also been described in the same section.

**10.5.4** **ALKALIES**

The poisoning by alkalies is now rare. It may only occur by accident. The poisons in this category includes mainly sodium, potassium and ammonium hydroxide. These are corrosives due to their solvent action on protein, their saponification on lipids and their ability to extract water from the tissues. The sign and symptoms include softening of tissue, swelling of mucous membrane, corrosion, vomiting, convulsion etc.

* + - 1. **Extraction of Sodium hydroxide and Potassium hydroxide in Biological Material and their Identification and Quantitation.**

A. The visceral material is digested with 3 or 4 volumes of distilled water on a steam bath and filtered. The filtrate is evaporated to dryness. The residue is extracted with hot alcohol which dissolves the hydroxides but not the carbonates and other salts. The alcoholic solution is evaporated to dryness and dissolved in a small volume of warm water. The solution would contain the hydroxides. The residue contains the carbonate (formed by conversion) is also dissolved in water. Both the solutions may now be tested for sodium and potassium. The combined extract is subjected to chemical tests.

B. **Identification Tests for Potassium.**

1. **Flame Test:** The characteristic violet flame (best seen through a cobalt glass) appears.

2. **Test with Perchloric Acid:** The test solution is neutralized with HCl. A few drops of aqueous solution of perchloric acid (1:1) is added to 2ml. of neutral solution. A white crystalline precipitate appears.

3. **Test with Platinic Chloride:** The test solution is neutralized with HCl. A few drops of chloroplatinic acid solution (prepared by dissolving 2.6gms. of hydrated chloroplatinic acid H2[PtCl6].6H2O in 10ml. of water). A yellow crystalline precipitate of potassium chloroplatinate is formed, which is insoluble in 80% alcohol.

4. **Cobalti Nitrate Test:** The test solution is acidified with acetic acid. A few drops of freshly prepared solution of sodium cobalti nitrate (0.25gms. in 2ml.) are added. A yellow crystalline precipitate of potassium cobalti nitrate is formed.

5. **Test with Zinc Uranyl Acetate:** 2 drops of neutral test solution is added to 8 drops of zinc uranyl acetate (prepared by dissolving uranyl zinc acetate in appropriate volume of water or 2 M acetic acid) upon a black water glass. It is stirred. A yellow cloudiness or precipitate appears.

6. **Test with Potassium Pyro - antimonate:** The test solution is neutralized by potassium carbonate. An excess of the aqueous solution of reagent is added and allowed to stand for some time. A white crystalline precipitate appears.

C. **Quantitative Determination**

**Potassium:** By gravimetry. Potassium is precipitated as potassium chloroplatinate. The weight of chloroplatinate is multiplied by the factor 0.1155 to give the amount of KOH present in weighed amount of biological material.

**Sodium:** It is rarely required

**10.5.4.2** **Isolation of Ammonia in Biological Materials and its Identification and Quantitation**

A. **Isolation:**

As ammonium hydroxide is volatile. It is quickly disappeared from stomach contents and other materials. It is first isolated by steam distillation without delay. The residue left in the distillation flask is made alkaline and again distilled. The distillate is collected in a minimum volume of water and subjected to the following tests etc.

B. **Test for Identification:**

1. **With Nessler’s Reagent:** A yellow or brown colouration appears.

2. **With Chloroplatinic Acid:** 2ml. of test solution is neutralized with HCl and a few drops of chloroplatinic acid is added. A yellow crystalline precipitate is formed which is soluble in 80% alcohol.

3. **Test with Mercurous Nitrate:** A few drops of mercurous nitrate is added to 2ml. of test solution. A black precipitate is formed.

C. **Quantitation:**

Not required (as result will be faulty)

**10.5.5 Phosphides**

Phosphides (specially of zinc and aluminium) are extensively used as rodenticides. These compounds are also used in criminal poisoning. The active constituent is phosphine gas, which is produced due to action of acid in stomach. This has already been covered as phosphine under section 4. In fact, phosphine is isolated in biological materials by treatment with acid in phosphide poisoning cases and identified by tests (already covered in section 4).

**Quantitation:**

The biological material is subjected to wet-ashing for isolation of Zn2+ or Al3+. The estimation may be done as described in section 5. The estimation of phosphine has also been covered in section 4.

**SECTION – 11: INSTRUMENTAL ANALYSIS IN TOXICOLOGY**

**11.1 Title:** Instrumental Analysis in Toxicology

**11.2 Scope:** Modern instrumental techniques and their applications in

toxicology.

**11.3 Purpose:** To identify and analyse the poisons using modern analytical methods.

**11.4 Responsibility:** Gazetted Officers and other scientific staff

**11.5 Atomic Absorption Spectroscopy:**

The absorption of energy by ground state atoms in the gaseous state, forms the basis of atomic absorption spectroscopy. When a solution containing metallic species is introduced into a flame, the vapors of metallic species are produced in the atomic state. Some of the metal atoms may be raised to an energy level sufficiently high to emit the characteristic radiation of the metal. But a large percentage of the metal atoms will remain in the non-emitting ground state. These ground state atoms of a particular element are receptive of light radiation of their own specific resonance wavelength. Thus, when a light of this specific wavelength is applied, part of that light will be absorbed by the atom of the element and the absorption will be proportional to the population of the atoms in the flame. Thus in atomic absorption spectroscopy, one determines the amount of light absorbed. Mathematically, the total amount of light absorbed may be given by the expression.

At ν, the total amount of light absorbed = [πe2/mc] N f

Where,

e – Charge of the electron of mass m

c – Velocity of light

N – Total number of atoms that can absorb light of frequency

characteristic to the atom at frequency in the light path

f – The oscillator strength or ability for each atom to absorb at frequency ν

m- Mass of the electron

As π, e, m and c are constants; the above equation can be simplified as

Total amount of light absorbed = constant x N f

In quantitative analysis, a series of standard samples of that element which is to be determined quantitatively is aspirated into the burner and percentage absorption measured. Then the absorbance values are plotted against. concentration. If a linear calibration curve is obtained, the slope of the calibration curve can be obtained and then the concentration of unknown solution can be calculated by using the equation A = mC

Where, A – absorbance, m – slope and C – concentration.

AAS is an important technique for the determination of trace metals in biological materials like Cu, Au, Pb, Ni, Zn, Cr, As, Hg, Ag etc in poisoning cases.

**11.5.1 Special features of Atomic Absorption Spectroscopy**

* The technique is specific due to use of hollow cathode lamp
* Accuracy in case of trace quantity
* Advantage in case of certain elements
* The principles of measurement are straightforward and well understood.
* The technology is relatively inexpensive and the equipment is relatively easy to use.
* The technique is well-suited to the measurement of gold, gold pathfinders and base metals
* There are relatively few matrix and other interference effects
* Sample throughput is high as each measurement require only a few seconds when the instrument is calibrated.
* The technique is applicable over a wide range of concentrations for most elements.

**11.5.2 Limitations of Atomic Absorption Spectroscopy**

* All measurements are made following chemical dissolution of the element of interest. Therefore the measurement can only be as profitable depending on simplicity of sample preparation or digestion.
* AAS is a sequential (one element at a time) analytical technique. It is not cost effective for multi-element analysis at a time.
* Occasionally interferences from other elements or chemical species can reduce atomization and depress absorbance, thereby reducing sensitivity.
* Some elements such as Li, Na, K, Rb and Cs ionize rather easily, again reducing atomization and complicating the measurement technique.

|  |  |  |
| --- | --- | --- |
| **Table 11.1: AAS Wavelength data for Elements** | | |
| **Element** | **Symbol** | **Wavelength (nm)** |
| Selenium | Se | 196.0 |
| Boron | B | 249.7 |
| Mercury | Hg | 253.6 |
| Tungsten | W | 255.1 |
| Manganese | Mn | 279.5 |
| Lead | Pb | 283.3 |
| Gallium | Ga | 287.4 |
| Barium | Ba | 553.6 |
| Rubidium | Rb | 780.0 |
| Sodium | Na | 589 |
| Magnesium | Mg | 285 |
| Arsenic | As | 193 |
| Aluminum | Al | 309 |
| Iron | Fe | 248 |
| Chromium | Cr | 357 |
| Copper | Cu | 324 |
| Zinc | Zn | 214 |
| Calcium | Ca | 422 |
| Cadmium | Cd | 229 |
| Gold | Au | 243 |
| Lithium | Li | 671 |
| Thallium | Tl | 277 |

**11.6 CAPILLARY ELECTROPHORESIS** (12, 13)**:**

Capillary electrophoresis is the most efficient separation technique available for the analysis of both macro and micro molecule. Capillary electrophoresis (CE) is electrophoresis performed in a capillary tube. The transformation of conventional electrophoresis to modern CE was revolutionized by the production of inexpensive narrow-bore capillaries for gas chromatography (GC) and the development of HPLC. The basic instrumental set-up consists of a high voltage power supply (0 to 30KV), a fused silica (SiO2) capillary, two buffer reservoirs, two electrodes, and an on–column detector. Sample injection is accomplished by temporarily replacing one of the buffer reservoirs with a sample vial. A specific amount of sample is introduced by controlling either the injection voltage or the injection pressure. The unprecedented resolution of CE is a consequence of the technique’s extremely high efficiency. The separation efficiency of CE and other high resolution techniques such as chromatography and field flow fractionation is modeled by the Van Deemter equation.

Electrophoresis is the movement of electrically charged particles or molecules in a conducting liquid medium, usually aqueous, under the influence of an electrical field.

In electrophoresis, the compounds move toward either the anode or cathode because of the influence of an electric field. In the absence of any other influences, the compounds will theoretically travel through the conducting medium as zones that do not diffuse or spread out. If the zones diffuse, they would blend together and will not be separated. Zone electrophoresis refers to the migration of molecules as zones, which do not undergo zone spreading due to diffusion.

Electrophoresis can be conducted in free solution or in a solution containing a nonconductive medium, such as a gel. Usually, the non-conducting medium is immersed in a conductive aqueous buffer solution, and an electric field is applied across the medium. Media that have been used include paper, cellulose acetate, membranes, and agarose or polyacrylamide gels. When gel is used, the technique is referred to as slab gel electrophoresis. When polyacrylamide is used, the technique is referred to polyacrylamide gel electrophoresis (PAGE). Similarly, it is referred to as paper or cellulose acetate electrophoresis when those media are used. Electrophoresis in a medium has been mainly used for separation of relatively larger biomolecules such as proteins and nucleic acids.

While the use of a medium does decrease zone spreading caused by convection, there are some problems associated with electrophoresis in a medium compared to free solution electrophoresis. There may be interactions, such as adsorption of the solutes with the medium, which can cause zone spreading and there may be channels in the medium, which will also cause zone spreading. Gel electrophoresis is relatively slow and labour intensive compared to capillary electrophoresis.

In recent past, the capillary electrophoresis is emerged as a promising technique in forensic science. In forensic science, the Capillary Electrophoresis is used for the analysis of illicit drugs, gun shot residues, explosives, opiates and drugs in biological materials like urine, blood, viscera and other biological fluids.

**11.6.1 Instrumentation and Overview**:

A capillary electrophoresis system is conceptually simple. The main components are a sample vial, source and destination vials, capillary, detector, high-voltage power supply, and a data out put and handling device, such as an integrator or computer.

Filling the source vial, capillary performs electrophoresis, and destination vials with an electrolyte, usually an aqueous buffer solution. The capillary inlet is placed into a sample vial, the sample is introduced, then the capillary inlet is placed back into the source vial, and an electric field is applied between the source and destination vials. As the solutes migrate through the capillary, the detector detects the same and its output is sent to an integrator or computer. The output is displayed as an electropherogram, which is a plot of detector response versus time.

**11.6.2 Modes of Capillary Electrophoresis:**

It is an optimized instrumental evolution of slab gel electrophoresis in which electrophoretic or electrokinetic separation is carried out in tiny capillaries generally made of fused silica under high electric field.

The versatility of capillary electrophoresis is greatly enhanced due to the availability of different modes. The most often used modes of capillary electrophoresis are capillary zone electrophoresis (CZE), micellar electro kinetic capillary chromatography (MEKC), capillary gel electrophoresis (CGE), capillary iso-electrofocussing (CIEF) and capillary isotachophoresis (CITP).

1. **Capillary Zone Electrophoresis (CZE):**

In capillary zone electrophoresis, electrophoretic separation is based onto plain electrophoretic mechanism in free solution.

Capillary Zone electrophoresis (CZE) is also referred to as free solution capillary electrophoresis (FSCE). It is most widely used method since it is applicable to separation of anions and cations, not the neutrals, in the same run and relatively simple. In this mode, the capillary is filled with a buffer of constant composition, and the source and destination vials are filled with the same buffer.

A sample is injected into the capillary, which is filled with a buffer, and when a voltage is applied, the solutes migrate through the capillary as zones. Solutes are separated as they move through the capillary due to differences in their rates of migration, which are dependent on their electrophoresis mobilities. Electro osmotic flow facilitates movement of the solutes through the capillary from the anode to the cathode. The order of elution in CZE is cations, neutrals and anions. Neutral compounds are not separated from each other but they are separated from ions, whereas ions are separated on the basis of their charge to size ratios. CZE can be used to separate almost any ionized compounds that are soluble in a buffer. Even water insoluble compounds have been separated by CZE with non-aqueous buffers. Application in forensic toxicological analysis include drugs in hair and urine or seized samples.

**2. Micellar Electrokinetic Capillary Chromatography (MEKC):**

Capillary electrophoretic separation is achieved by this method in which separation is based on the hydrophoretic interaction (partitioning) of the analytes with micelles of surfactants added to the buffer which act as a pseudo-stationary phase, being the mobile phase for electro-osmotic flow.

The development of MEKC was a major advancement in capillary electrophoresis since it provided a method for separation of electrically neutral compounds. This mode of electrophoresis is based on the partitioning of solutes between micelles added and the run buffer. i.e. two phases: aqueous and micellar, both of which move. In MEKC, a buffer solution that contains micelles of surfactants is used as the run buffer. When a surfactant is present in solution at a concentration higher than its critical micelle concentration (CMC), it forms micelles, which are aggregations of individual surfactant molecules. Depending on the surfactant, a micelle is made up of different numbers of detergent molecules. Micelles are generally spherical in shape and form such that the hydrophilic groups of the detergent molecules are on the outside of the micelle, toward the aqueous buffer. The hydrophobic hydrocarbon molecules are in the center of the micelle, away from the aqueous buffer. When water insoluble hydrophobic compound is added to an aqueous solution that contains micelles, it partitions into the hydrophobic portions of the micelles. It can be considered to the solubilized by the micelles. Conversely, if a water-soluble hydrophilic compound is added to the solution, it will not partition into the micelles since it is not soluble in them. A compound of intermediate water solubility will partition between the aqueous solution and the micelles, depending on the hydrophobicity of the compound. Hydrophobic water-soluble molecules spend all the time in the buffer since they do not partition into the micelles at all. They are carried through the capillary at the rate of the electro osmotic flow and are the first to elute. Very hydrophobic molecules that are totally solubilized by the micelles spend all of the time in the micelles, and so are carried through the capillary at the same rate as the micelles. MEKC can separate not only neutral molecules, but also ionic molecules i.e. much versatility in separation is achieved for molecular mixtures. The method is used for analysis of narcotic drugs, psychotropic substances.

**3. Capillary Gel Electrophoresis:**

Traditional slab gel electrophoresis has been used to separate large biomolecules on the basis of their sizes. Capillary gel electrophoresis (CGE), which combines the principles of slab gel electrophoresis with the instrumentation and small diameter capillaries of CZE. Capillaries dissipate heat better than slab gels, and so higher electric fields can be used, giving faster separations. Resolution and efficiency in CGE are comparable to those obtained in CZE with efficiencies of 106 - 107 theoretical plates per meter. CGE provides faster, more efficient separations than slab gel electrophoresis. Also, unlike slab gel electrophoresis, CGE facilitates on-line detection and quantitation. In CGE, the capillary is filled with a gel, which is usually either a polyacrylamide or bisacrylamide cross linked polymer or a linear, non cross linked polyacrylamide polymer. There are pores within these gels, and as charged solutes migrate through a gel-filled capillary, they are separated by a molecular sieving mechanism on the basis of their sizes. Small molecules are able to pass through the pores and elute first, whereas larger molecules are retarded by the gel and elute later. Usually, the capillary wall is treated to eliminate electro osmotic flow so the gel will not be extruded from the capillary. Solutes move through the capillary due to electrophoresis, and are separated by the sieving mechanism of the gel.

In CGE, the solutes are separated on the basis of size, so it is well suited for the analysis of charged molecules that vary in size, but not in their charge-to-size ratios, regardless of their chain lengths, so the charge-to-size ratio remains about the same. The method is applicable in the analysis of drugs and psychotropic substances.

**4. Capillary Isoelectric Focusing (CIEF):**

Capillary isoelectric focusing (CIEF) is a focusing type of capillary electrophoresis in which the solutes, usually proteins, are separated on the basis of differences in their isoelectric points, i.e. at characteristic pH. A capillary is filled with a solution of ampholytes and proteins, and an electric filed is applied, creating a pH gradient in the capillary. Proteins focus into narrow zones within the capillary due to differences in their pH. The solution in the capillary is then forced past the detector to generate the electropherogram. The vial that contains the cathode is filled with a catholyte, a basic solution, and the vial that contains the anode is filled with an analyte, an acidic solution. Phosphoric acid, approximately 0.02M, is often used as the analyte and 0.02M sodium hydroxide as the catholyte. Sodium hydroxide solutions should not be exposed to air as they absorb carbon dioxide, which prevents stable focused zones.

Filling the capillary with a mixture of ampholytes and the sample performs CIEF. When a voltage is applied, the samples and ampholytes migrate through the capillary until they reach a point where they are uncharged, that is, where the pH of the solution is equal to the pI of the sample. At this time, a steady state is attained, which is indicated by a decrease in the current to a stable value. If the samples diffuse out of the ones where it is neutral, it moves into an area of different pH, and acquires a charge, which causes it to migrate back into the original zone where it is neutral. In this way, samples are focused into very narrow zones, giving high-efficiency separations. The more ampholytes with different pH values are there in the solution, the narrower the zones, and the narrower the resulting peaks. The capillaries are treated so that there is no electro osmotic flow, ensuring that the movement of samples is solely on the basis of differences in their pH. Electro osmotic flow would prevent the formation of stable focused zones. Also, capillaries are treated to prevent adsorption of sample onto the capillary wall. Coating the capillary wall with polyacrylamide or methyl cellulose can eliminate Electro osmotic flow. After the samples are focused, the sample zones and the ampholytes are then mobilized, i.e., they are pushed through the capillary and detector to obtain an electropherogram. Since the ampholytes are also pushed through the detector, it is desirable that they should not be detected . There is no electro osmotic flow, any neutral compounds that may be present in the sample will not move much in the capillary during focusing, but they will go through the detector during mobilization. This method is used in the analysis of drugs and psychotropic substances.

**5. Capillary Isotachophoresis:**

This is a moving boundary technique in which the sample is sandwiched between two buffers. The output in CITP is different from the other modes in that the zone widths are proportional to the amount of ions in the sample. CITP is sometimes used as a sample pre-concentration step. Anions and cations are usually not separated in the same run.

**Capillary column:**

The capillary column is a key element of the CE separation. Fused silica is by far the most frequently used material, although columns have been made of Teflon and borosilicate glass. The widespread use of fused silica is due to its intrinsic properties, which include transparency over a wide range of the electromagnetic spectrum and a high thermal conductance. Fused silica is also easy to manufacture into capillaries with diameters of a few micrometers. Many reports describe the covalent attachment of silanes with neutral or hydrophilic substituents to the inner wall of the capillary in order to reduce electro osmotic flow and prevent adsorption of the analyte; coatings also tend to stabilize the pH.

An uncoated fused silica capillary is prepared for its first use in electrophoresis by rinsing it with 10 to 15 column volumes of 0.1M NaOH followed by 10 to 15 column volumes of water and 5 to 10 column volumes of the separation buffer. For a coated capillary, the preparation procedure is the same except that 0.1M NaOH is replaced with methanol.

By adding a cationic surfactant, such as cetyl trimethyl ammonium bromide (CTAB), to the separation buffer, the direction of electro osmotic flow can be reversed. The positively charged group on one end of CTAB interacts with the negatively charged silanol groups on the capillary surface, while the hydrocarbon tail points away from the surface. A second layer of CTAB orients itself in the opposite direction so that the hydrophilic tails form a non polar layer. This surfactant bi layer adheres tightly to the wall of the capillary and effectively reverses the charge of the wall from negative to positive, resulting in reversed electro osmotic flow. This procedure is also known as dynamic coating.

**11.6.3 Sample Injection Techniques:**

In capillary electrophoresis, samples can be injected by hydrodynamic, also called hydrostatic, or electrokinetic, also called electro migration, injection. The discussion of sample injection will be limited to the CZE, MEKC, because in CIEF, the capillary is completely filled with sample solution, and in CITP, a sample volume of up to 50% of the capillary may be injected.

**1. Hydrodynamic Injection:**

Poiseuille’s law determines what will be the plug length (PLUG) for the injected volume for capillary using a given pressure difference (injection pressure, P), duration of that pressure difference (t), viscosity of the fluid (η) and the dimensions of the capillary (d = diameter, L = length).

P.t = PLUG . 3200. η.L/d2

P in mbar, t in sec. PLUG in mm, d in μ, η in centipoises (CP), L in cm.

By the above formula PLUG will be calculated.

Injection volume (v) = πr2. PLUG where r is radius of capillary

**2. Electrokinetic Injection:**

Electrokinetic injection is made by placing the capillary and the anode into the source vial and applying a voltage for a given period of time. After the sample is introduced, the anode and capillary are placed back into the sourcevial, an electric field is applied, and electrophoresis proceeds. When the polarity of the voltage is reversed, as in the case of electro osmotic flow reversal, the cathode is placed in the sample vial. The quantity injected Qinj, is given by

**Qinj = V. Π. ctr2 (μEP + μEOF)/L**

Where V is the voltage, c is the sample concentration, t is the time duration the voltage is applied, r is the capillary radius, μEP is the electrophoretic mobility of the solute, and μEOF is the electro osmotic mobility. Neutral solutes are pulled into the capillary by just the electro osmotic flow since μEP is zero for them**.**

**11.6.4 Application of Capillary Electrophoresis:**

**1. Analysis of Methamphetamine Seizures by Capillary Zone Electrophoresis** (1)

Buffer: 15mM Beta-cyclodextrin, 300mM NaH2PO4

Capillary: Fused silica uncoated capillary (64.5cm x 50μm)

Voltage: 20KV

Temperature: 30oC

Detection: UV detection by diode array at 200nm

Internal standard: Phentermine

**2. Analysis of Opiate Drugs in Hair by Capillary Zone Electrophoresis** (2)

Samples analyzed in hair: Morphine, codeine, 6- monoacetylmorphine, acetyl codeine and heroin.

Buffer: 0.1M sodium phosphate, pH2.5, with 40% ethylene glycol

Capillary: Fused silica uncoated capillary.

Voltage: 20KV

Temperature: 25oC

Detection: UV detection by diode array at 214nm, scan: 190-400 nm

**3. Determination of Heroin Metabolites in Human Urine by using Capillary Zone Electrophoresis** (3)

Samples analyzed in human urine: Morphine, codeine, 6- monoacetylmorphine, or morphine.

Buffer: 50mM Sodium phosphate and 0.015M betacylcodextrins (pH 6.0).

Capillary: Fused silica uncoated capillary (77cm x 50μm).

Voltage: 20 KV

Temperature: 25oC

Detection: UV detection by diode array at 214nm.

**4. Determination of Lysergic Acid Diethyl Amide (LSD) in Blood by Capillary Electrophoresis** (4)

Samples Analyzed in blood: LSD, nor-LSD, iso-LSD and iso-nor-LSD Buffer: Citrate – Methanol buffer, pH = 4.0

Capillary: Fused silica uncoated capillary.

Voltage: 20/25 KV

Temperature: 25oC

Injection mode: Electro kinetic mode (10s, 10KV)

Detection: Fluorescence detection at λex = 325nm and λem = 435nm

Internal standard: methylergometrine

**5. Analysis of Forensic Drugs by Micellar Electro Kinetic Capillary Chromatography** (5)

Samples Analyzed: Caffeine, morphine, barbital, pentobarbital, codeine, nalorphine, lidocaine, procaine, heroin, flunitrazepam, acetylcodeine, papaverine, amphetamine, narcotine, cocaine, diazepam, tetracaine, narceine, 6-monoacetylmorphine, acetylcodeine and thebaine.

Buffer: 25mM Borate – 20% Methanol – 100mM Sodium dodecyl sulphate, pH = 9.24.

Capillary: Bare silica capillary (50μm I.D).

Voltage: 25KV

Temperature: 25oC

Injection mode: Hydro dynamic ( pressure injection)

Detection: UV

**6. Gum Opium samples: (6)**

Capillary: Fused silica

Buffer: 100-mM (Sodium Acetate/acetic acid) : Methanol ::: 70:30, pH = 3.1 .

Detector: UV at scanning wavelength 200-300 nm, Quantitation at 224 nm.

Temperature: 25oC

Voltage: 15kV

Injection mode: Hydro dynamic ( pressure injection) 1 sec @ 300 mbar.

pH: 3.1

Injection volume: 4 nl.

**11.7 Inductively Coupled Plasma**

**Principle**: Inductively Coupled Plasma (ICP) are generated from radio frequency magnetic fields induced by a water or air-cooled copper coil looped around a quartz tube. The radio-frequency magnetic field oscillates at 27.12 or 40.68 MHz, at incident powers varying from 0.5 to 2.5KW. Higher powers are usually applied when organic solvents are aspirated. Argon gas flows through a torch, which consists of three concentric tubes usually constructed from fused silica. The plasma is initiated by sending the argon stream with electrons provided from a Tesla Coil. The electrons, detached from the argon atom, collide with argon atoms and populate the coil region with positive and negative charges. As a result of the magnetic field, the particles flow in a closed annular path. Due to the conductance of the gases in the coil regions the charged particles are heated by inductive coupling to a temperature equaling ionization of the argon gas occurs, resulting in the formation of the ICP. The plasma impedance is monitored along with the data of the tube grid current, grid voltage, plate current and voltage to control the plasma power. Since, there is no directly wired connection between the plasma and the energy source, it is called inductively coupled plasma (ICP). In order to prevent possible short-circuiting as well as meltdown, the plasma must be insulated from the rest of the instrument. Insulation is achieved by the concurrent flow of gases through the system. Three gases flow through the system – the outer gas, intermediate gas and inner or carrier gas. The outer gas is typically argon or nitrogen. The outer gas has been demonstrated to serve several purposes including maintaining the plasma, stabilizing the position of the plasma and thermally isolating the plasma from the outer tube. Argon is commonly used for both the intermediate gas and inner or carrier gas, which convey the sample to the plasma

Better multielement limits of detection are obtained due to more efficient coupling. Efficiency and background reduction when high-frequency radio-frequency generators are employed at 40.68 against 27.12 MHz. The temperature of the plasma varies from 5000 to 10000K, depending on the power, gas flow rate, composition, coupling efficiency and the temperature in an ICP vary over the length of the plasma plume, with the hottest part with in the annular region. The core of the plasma varies in temperature from 10000K near the base to about 6000 K at a distance of 25mm above the load coil.

**Components of an ICP**

**ICP Torches**

Torches, through which ICPs are generated, consist of three concentric quartz tubes having optimized intervals between them so that gas consumption is kept to a minimum, and it emerges at a high velocity. The support gas, termed the cooling gas, is delivered at flow rates varying from 6 to 20-l/min. The torch is designed so that the plasma gas flows tangentially thus cooling the outer quartz tube and preventing it from melting.

**Plasma Gases:**

The plasma is usually supported by argon gas, but mixed gases consists of argon and oxygen. For the analysis of hydrocarbons and metallic elements, argon-nitrogen, argon-hydrogen and helium have been used to enhance particle-plasma interaction by increasing plasma temperatures and the residence time of the excited species. Argon gas is used for the high excitation temperatures of about 7000K and provided for efficient atomization and minimum chemical interference. Due to its high ionization potential of 15.75 eV, almost all elements in the periodic table can be determined, and the emission spectrum of argon is very simple and the potential for spectral line interference is significant.

**Nebulizer:**

The role of the nebulizer is to produce fine and homogeneous droplets for transportation to the plasma i.e., to convert bulk liquid samples into droplets to feed into the thermal source is called nebulizer. This aerosol is transported via an aerosol carrier gas using a carrier flow ranging from 0.2 to 1.5 ml/min to a spray chamber, which permits only the smallest aerosol particles of less than 5μm to pass onto the plasma.

**Sample Injection:**

Liquid samples are most commonly flown into the atomization source as a mist. The amount of liquid sample and the size of the droplets that feed into the plasma depend on the (i) design of the nebulizer, (ii) gas flow rate, (iii) viscosity of the liquid, (iv) density of the liquid and (v) surface tension of the liquid.

Normally in ICP-AES system, the sample is a liquid, which will be pumped peristaltically through a flexible capillary tube to a pneumatic nebulizer. Large droplets formed in the aerosol are excluded in the spray chamber and aerosol particles of less than 5μm are transported with the aerosol carrier gas in central channel. Desolvation, vaporization, atomization, ionization and excitation through which emitted radiation is transmitted to sequential emission spectrometers, measures emission lines intensities on a one-by-one basis or simultaneous emission spectrometers, measure the intensities of emission lines for a large number of lines simultaneously. Multi-element analysis implies the measurement of 50-70 elements in less than 15 min.

**Calibration and linear response**

Inductively coupled plasma –atomic emission spectroscopy is characterized by very wide linear ranges of signals versus concentration responses of more than five orders magnitude, allowing major, minor and trace elements to be determined in a single sample preparation without dilution or matrix modification.

A qualitative analysis reveals the presence of elements in a sample. Several analytical lines can be employed to avoid the uncertainty due to spectral line interferences.

**11.7.1 Quantitative Analysis**

In atomic emission spectroscopy methods, single wavelength light is selected to measure the changes in intensity. The emission values vary depending upon the concentration of the analyte at that wavelength of element of interest. To perform quantitative analysis, the sample is introduced into the plasma and the intensity of radiation is measured at the pertinent wavelength. The concentration of the metal in the sample is then calculated by one of two methods, viz. the use of calibration curves, and the standard addition method.

Calibration curves are prepared by making up standard solutions of known concentrations similar to those expected in the sample.

**11.7.2 Applications:**

**I. Metals in Urine by ICP-AES (as on metals covered)** (7)

Indication of exposure to metals**:** Exposure to the following metals or their compounds: aluminum, barium, cadmium, chromium, copper, iron, lead, manganese, molybdenum, nickel, platinum, silver, strontium, tin, titanium, and zinc.

Synonyms**:** vary according to the compound

Sample specimen**:** Urine

Volume:50 to 200 mL in polyethylene bottle

Preservative**:** 5.0 mL conc. HNO3 added after collection

Shipment**:** frozen in dry ice

Stability**:** not established

Controls**:** collect at least 3 urine specimens from unexposed workers

Measurement Technique: Inductively-coupled Argon Plasma -Atomic Emission Spectroscopy (ICP-AES)

Analyte:Any of the above elements either alone or in mixture

Extraction Media**:** polydithiocarbamate resin

Final Solution:4% HNO3 +1% HClO4; (5 ml)

Wavelength:depends upon element; Table 11.2

Background Correction**:** spectral wavelength shift

Calibration:Elements in 4% HNO3 + 1% HClO4

Quality Control:Spiked urines; corrected for creatinine

Range:0.25 to 200 µg per sample

Estimated Load:0.1 µg per sample

Precision (S r):Table 11.2

Accuracy**:** Table 11.2

Applicability**:** This method measures urine concentrations of metals and particularly useful for workers exposed to several metals simultaneously. This is a simultaneous, multielemental analysis, but is not compound specific.

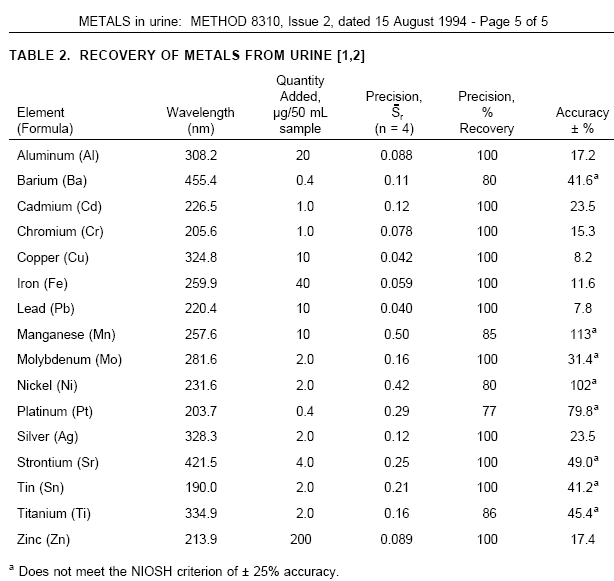
Interferences: Spectral interferences are the primary interferences encountered in ICP-AES analysis. These are minimized by judicious wavelength selection and inter element correction factors. Background corrections are also made.

1. **Other Methods:** Measurement technique similar to that of method 7,8

**Evaluation of method:** (10 - 15)

Recovery of these 16 metals from spiked urine samples are shown in Table –1 (recoveries ranged from 77 to 100%). The precisions determined for the various elements are also given in Table 11.2.

**Table 11.2: Recovery of metals from urine** (8, 9)

****

**3. Elements in Blood or Tissue by ICP-AES as an Indicator of exposure**

Elements or their compounds of interest: Antimony, cadmium, chromium, cobalt, copper, iron, lanthanum, lead, lithium, magnesium, manganese, molybdenum, nickel, platinum, silver, strontium, thallium, vanadium, zinc and zirconium.

Synonyms: Vary according to compound.

Biological Specimen: blood or tissue

Volume: 10 ml (blood) or 1 g (tissue)

Preservative: Heparin (blood); none for tissue

Shipment: Frozen for blood and "wet" tissue; routine for "dry" tissue

Stability: Not established

Controls: Collect at least 3 blood specimens from unexposed workers

Measurement method: Inductively-coupled argon plasma atomic emission spectroscopy (ICP-AES)

Analyte: Elements above

Digestion acid: HNO3: HClO4: H2SO4 (3:1:1 v/v)

Final solution: 10% H2SO4; 10 ml (blood), 5 ml (tissue)

Wavelength: Varies with element; table 11.3

Background correction: Spectral wavelength shift

Calibration: elements in 10% H 2SO4 or yttrium internal standard

Quality control: Spiked blood or tissue; reference materials

Range: 10 to 10,000 µg/100 g blood; 2 to 2000 µg/g tissue

Estimated load: 1 µg/100 g blood; 0.2 µg/g tissue

Precision (s r): table 11.3

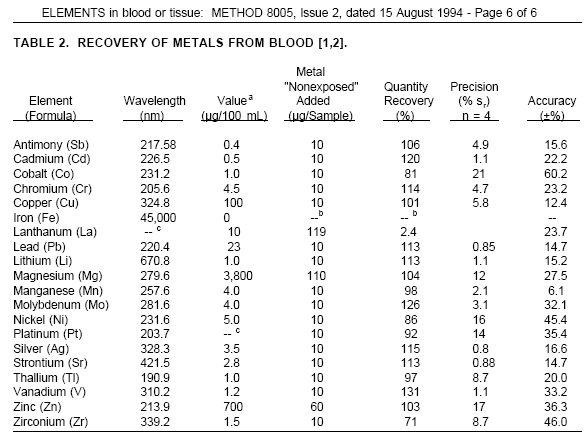
Accuracy: table 11.3

Applicability: This method is useful for monitoring the blood of workers exposed to several metals simultaneously. This is a simultaneous multielemental analysis, but is not compound-specific.

Interferences: Spectral interferences are sometimes encountered. These are minimized by judicious wavelength selection and inter element correction factors. Background corrections (spectral wavelength shift) are also made.

1. **4. Other Methods: A** measurement technique similar to that of method

**Table 11.3: Recovery of metals from Blood** (16)

****

**11.8 ICP-MS**

Quantitative determination of metals in clinical samples is being used as a diagnostic tool in cases involving poisoning and for diagnostics in numerous diseases. Inductively coupled plasma mass spectrometry (ICP-MS) offers multielemental capabilities for the determination of sub-ppb (sub-ng/mL) levels of trace metals.

|  |  |
| --- | --- |
| Table I : Operational Parameters | |
| Plasma gas flow rate | 15.0 L/min |
| Aux. gas flow rate | 1.0 L/min |
| Carrier gas flow rate | 1.2 L/min |
| RF Power | 1300W |
| Nebulizer | Babington or Concentric |
| Spray chamber | glass, double pass |
| Spray chamber temp. | 2°C |
| Sample uptake rate | 0.2-0.4 mL/min |
| Sample Cone | Nickel |
| Skimmer Cone | Nickel |
| Sampling Depth | 6.6 mm |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Table II. Analysis of Certified Whole-Blood Sample | | | | | |
| Analyte | Internal Standard | Meas. Conc. (µg/L) | Expected (µg/L) | Dilution Factor Conc. (µg/L) | Sample Preparation |
| Arsenic | Ge | 46.4 | 50 | 50 | nitric |
| Arsenic | Ge | 93.3 | 100 | 50 | nitric |
| Arsenic | Ge | 143 | 130-195 | 50 | nitric |
| Arsenic | Ge | 22 | 17-26 | 50 | nitric |
| Cadmium | Rh | 21 | 18-22 | 50 | TMAH |
| Lead | Tb | 94 | 92-125 | 50 | TMAH |
| Lead | Tb | 270 | 254-343 | 50 | TMAH |
| Lead | Tb | 170 | 170-240 | 50 | TMAH |
| Mercury | Tb | 10 | 10 | 50 | TMAH |
| Mercury | Tb | 41 | 50 | 50 | TMAH |
| Mercury | Tm | 20.2 | 22 | 20 | nitric |
| Mercury | Tm | 9.6 | 10 | 20 | nitric |
| Mercury | Tm | 5.9 | 6 | 20 | nitric |

* 1. **Chromatographic methods**

It is a separation technique for molecular mixture wherein separation is achieved by the relative affinity or interaction of solutes with stationary (adsorbent) phase and mobile phase and equilibrium attained thereof.

* + 1. **Paper chromatography**
    2. **Thin layer chromatography**

**11.9.3 High Performance Thin Layer Chromatography**

1. **Introduction:**

**Principle:**

In Thin layer chromatography, constituents in a molecular mixture are separated on thin uniform surface of solid stationary phase coated on glass/aluminium/plastic plate with the help of moving liquid phase. The mobile liquid phase migrates (ascends) on solid stationary phase by capillary action carrying the different analyte molecules of a mixture with different speed depending upon the their affinity towards stationary phase. In this process, the constituents (analytes) are separated into fractions. After development of the chromatogram to a certain distance, the thin layer chromatography plate is removed and mobile phase is evaporated. The track is scanned in a densitometer with a light beam in visible or ultraviolet range of the spectrum. Depending upon the mode of scanning absorbance or fluorescence is measured by diffuse reflectance.

Efficiency of separation can be expressed in terms of number of theoretical plates associated with a given system and is expressed by n= L/h where n= number of theoretical plates, L = length of the TLC plates, h= height equivalent to a theoretical plate. The performance in TLC is improved by decrease of h and dependant on variables viz. viscosity, temperature and linear velocity of mobile phase and particle size of stationary phase. These variables change according to Van Demeter equation.

**III. Equipment of HPTLC:**

**A. TLC Plates:**

**Normal Phase TLC plates:**

In normal phase TLC plate, analytes are primarily separated according to their polarity and their solubility for the mobile phase. The chemical constituents in the normal phase TLC plates are silicic acid with gypsum as a binder to increase the mechanical strength of silica gel. In order to aid the detection of the samples, often an insoluble fluorescent indicator is often incorporated into the plates (silica gel GF254). When the plates are placed under UV lamp and read under 254 nm, the base of the plate glows green except where analytes absorb the radiation. In HPTLC plates, the performance in separation is markedly enhanced by using plastic material as binder to adsorbents of more finer grain size. The plates may be of glass or aluminium.

**Reversed phase TLC plates:**

Because of the interest in reversed phase plates HPLC, pre-coated reverse phase TLC plates are available with C18, C8, C2 and phenyl groups chemically bonded on to a silica support to produce stationary phases.

**HPTLC Plates:**

HPTLC plates have the layer of stationary phase particles of size 3-7 μm. with a thickness of 20mm. Due to small size of the particles and higher surface area, HPTLC plates show less lateral spreading and hence the concentration in the spot is higher, which facilitates separation faster with smaller migration distance. Thus, small HPTLC plates may be used effectively. Reverse phase and plates with fluorescence indicator of diverse specification are now available.

**B. Sample Application:**

Sample is applied either manually using capillary tube in the form of spots or automatic TLC sampler in the form of narrow bands using spray technique. Narrow bands at starting zone ensure the highest resolution.

**C. Development Chamber:**

After application of sample plate is developed in flat bottom, Twin trough horizontal or automatic developing chamber. It requires much less volume of solvent (10ml) and saturation time is much less. Thus the analysis is speedy and cost effective.

**D. Automated Multiple Development (AMD):-**

It is a special method for analysis of multiple samples having lower and near overlapping Rf. The advantage of multiple developments is that it is helpful in improving the separation of analytes with low Rf values. In the AMD procedure fractions are focused into narrow bands with typical peak width of about 1 mm so that on available separation distance of 80mm on a HPTLC plate, upto 40 components can be resolved. (i.e., with baseline separation).

**E. Densitometer (Scanner):**

For densitometric evaluation of a HPTLC chromatogram, its tracks are scanned with a light beam in the form a slit for selection in length and width. Diffused reflected light is measured with photosensor (photo multiplier).

**V. Applications of HPTLC:**

**A. Qualitative Analysis:**

1. Rf values are measured and compared with the control samples. To avoid the use of decimals in these values, it may be multiplied by 100 and the values are then referred to as hRf.

2. In-situ spectra (Absorbance Vs Wavelength) of the analyte and standard reference material are recorded and compared.

**B. Quantitative Analysis:**

For quantification, the difference between optical signal from the sample free background and that from a sample zone (fractions) are correlated with the amount of the respective fractions of calibration standards chromatographed on the same plate. Densitometric scanning measurement can be made in absorbance or fluorescence mode by measuring the diffused reflected light. Alternative to classical densitometry, planar chromatography can be evaluated by video technology. Additional operations such as pre and post chromatographic derivatization can be performed as and when required.

For quantitative analysis in absorbance mode, Lambert-Beer’s law is applied.

A or (log I/I0) = abc

,

where a=absorption coefficient,

b= cell path length

c=concentration

A=Absorbance.

HPTLC find application in the analysis of drugs in biological materials specially blood and urine. A few application with their analytical conditions are presented below.

**1. Detection and Quantitative Determination of Furosemide (Lasix®) in Urine**

**Sample preparation**

- Adjust 10 ml urine sample with sulfuric acid (10% aqueous) to pH 2 and extract with 50 ml of diethyl ether.

- Separate organic phase, dry with anhydrous sodium sulfate, filter through a cotton ball and evaporate to dryness in water bath at 60°C under normal pressure (For simultaneous testing for the diuretic chlortalidone (hygrotone) the aqueous phase can be extracted in alkaline medium and processed further).

- Dissolve residue in 0.2 ml methanol.

**Standard Solutions**

Extract 50 ml of urine as described above (of a person who has not received Lasix). Dissolve the residue in 1 ml of methanol i.e. acidic blind extract.

**Stock Solution:** Dissolve 10 mg furosemide in methanol to a volume of 100 ml (10 ml = 1 mg). Into 5 V-shaped vials, pipette 20, 40, 60, 80 and 100 µL stock solution and evaporate to dryness in a stream of nitrogen.

Dissolve the residue in 100 ml acidic blind extract related to urine.

The standard prepared are as follows:

S1 = 0.20 mg/l, S2 = 0.40 mg/l, S3 = 0.80 mg/l, S4 = 1.20 mg/l, S5 = 1.60 mg/l

**Layer:** HPTLC plates, Merck silica gel 60 F254, 20x10 cm\*

**Sample Application**

The sample along with controls are applied on HPTLC plate with 7 mm bands, track distance 3 mm, distance from left edge 12 mm, distance from lower edge 5 mm, delivery rate 8 s/ml for 18 applications per plate side\*.

Recommended application pattern of standards for the quantitative determination in doping analysis and drug monitoring (for doping control screening, considerably less standards are required, e.g. S1 and S3):

**Application Pattern:**

B U1 S1 U2 S2 U3 B U4 ... B = blind extract, U = unknown, S = standard

6 6 6 6 6 6 6 6... µl/track

**Chromatography**

In Horizontal developing chamber 20cm x10 cm\*, in saturated solvent configuration of ethyl acetate: methanol: NH3 (85:10:5,v/v);

*R*f of furosemide : 0.28.

**Application to Sample Analysis**

The chromatographic procedure depends on the purpose of analysis as stated hereunder.

For doping control qualitative identification is required in all cases which is done by post chromatographic derivatization. The samples that appear as red coloured spot or zone on HPTLC plate at respective critical area are subjected to chromatographic separation as a second plate. For result verification spectra comparison of spectra of the underivatized fraction is carried out for quantitation determination. In this way two independent detection/ quantitation results are obtained. For drug monitoring densitometric evaluation without prior derivatization will suffice.

The HPTLC conditions are described below

**Post Chromatographic Derivatization**

- HPTLC plate : Silica gel G-60 F254, E Merck, 20cm X 10cm

- Expose plate to HCl gas in a chamber for 10 min.

- Dry for 5 min at 120°C and let it cool to room temperature.

- Spray with NaNO2 solution (10%, aqueous).

- Then spray with HCl solution (10%, aqueous), dry the plate with a hair dryer in a stream of cold air.

- Overspray with 0.5% aqueous solution of amidosulfonic acid ammonium a gel Merck 60 F254, 20x10 cm.

The plate is now ready for sample application.

**Sample application**

Apply different quantities of the analyte with Automatic TLC Sampler III at a track distance of 5 mm, distance of 8mm from lower edge, distance of 15 mm from left edge.

**Use of control Samples:** These are used in variable amounts

5 10 20 40 60 ng Phenobarbital/spot (ç mg/l)

2 4 8 16 24 ng Primidone/spot (ç mg/l)

2 4 8 16 24 ng PEMA/spot (ç mg/l)

S = standard, U = sample

\* As internal standard for volume correction, 20 ml of a 0.02% methanolic solution of mephobarbital can be added.

**Chromatographic Development**

In a twin trough Chamber of dimension 20cm x 10 cm, the plates are developed with a mixture of dichloromethane: ethyl acetate: methanol: concentrated ammonium hydroxide (18:16:3:1,v/v).

**Densitometric Evaluation**

Densitometric evaluation is done by scanning at 215 nm.

**6. Application to Other Anticonvulsants**

Sample preparation, application and chromatography as described above may be used in the determination of other anticonvulsants listed below with minor changes. Since most anticonvulsants absorb strongly between 200 and 220 nm, adequate signals are achieved with quantities between 25 and 250 ng applied to the plate and measured at 215 nm. Relative standard deviations between ± 3% and ± 6% are achieved, which exceeds reported routine precision of GC or HPLC methods.

***R*F of Common Anticonvulsants and corresponding Internal standards (if necessary)**

**Anticonvulsant** **Rf** **Internal Standard**

Phenobarbital 0.20 Talbutal, mephobarbital

Phenytoin 0.23 Talbutal, mephobarbital

Primidone 0.27 Mephobarbital

Barbital 0.28 Talbutal, mephobarbital

2-ethyl-2-phenylmalonamid 0.37 Mephobarbital

Butethal 0.38 Phenobarbital, mephobarbital

Talbutal 0.40 mephobarbital

Amobarbital 0.41 Phenobarbital, mephobarbital

Pentobarbital 0.42 Phenobarbital

Secobarbital 0.43 Phenobarbital

Carbamazepine 0.44 Nordiazepam

Mephobarbital 0.45 Talbutal

**11.9.4 GAS CHROMATOGRAPHY**:

**Introduction**

Gas Chromatography involves separation of a mixture of volatiles by partitioning between a solid or liquid stationary phase and a gaseous mobile phase and find diverse application in toxicological analysis viz alcohols, volatile poisons, drugs, plant poisons.

Gas chromatography - specifically gas-liquid chromatography - involves a sample being vapourised and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column contains a liquid stationary phase, which is adsorbed onto the surface of an inert solid. On passing through the column volatile components impart different affinities to stationary and mobile phases and separated in fraction.

There are two types of gas chromatography viz- gas-solid and gas-liquid chromatography. In gas- solid chromatography stationary phase is made of solid adsorbent. In gas-liquid chromatography, the stationary phase consists of a solid adsorbent on which liquid material is sorbed.

**Instrumental components**

**Carrier Gas**

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependant upon the type of detector used. The carrier gas system also contains a molecular sieve to remove water and other impurities. In order to perform accurate and reproducible GC, it is necessary to maintain a constant carrier gas flow. Under isothermal conditions, simple pressure control, is adequate for packed or capillary columns. Flow control is highly desirable , if not essential, during temperature programming with packed columns and can be used with advantage on on-column injection to capillary column.

**Sample Injection Port**

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapour. Slow injection of large samples causes band broadening and loss of resolution. The most common injection method is one where a microsyringe is used to inject sample through a rubber septum into a flash vapouriser port at the head of the column. The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample. For packed columns, sample size ranges from tenths of a microliter up to 20 microliters. Capillary columns, on the other hand requires much less sample typically around 10-3 ml. For capillary GC, split/splitless injection is used. Split injection involves injection of a fraction of total sample volume. In splitless, it occurs fully. The injector can be used in one of two modes; split or splitless. The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vapourises to form a mixture of carrier gas, vapourised solvent and vapourised solutes. A proportion of this mixture passes onto the column, but most exits through the split outlet.

The septum purge outlet prevents septum bleed components from entering the column. In splitless injection, total volume of sample passes through the column. Split method may prevent column overloading, but the fraction that reaches the column may not be representative of the original sample as it is a flash vaporization technique. Low injection volumes are suitable for WCOT column as compared to packed column.

Split injection is used for volatile compounds, or for diluting the samples. It is used in headspace method to reduce injection time. In forensic toxicology, split injection is largely used for analysis of medications, drugs of abuse and powders. For toxicological analysis of biological samples, splitless injection is preferred while the split technique is most beneficial for samples containing compounds at high concentrations. Splitless injection may be either on-column or off-column.

**Columns**

There are two general types of column, *packed* and *capillary* (also known as *open tubular*). Packed columns contain a finely divided, inert, solid support material (commonly based on *diatomaceous earth*) coated with liquid stationary phase. Most packed columns are 1.5 - 10m in length and have an internal diameter of 2 - 4mm.

Capillary columns have an internal diameter of a few tenths of a millimeter. These may be one of two types; *wall-coated open tubular* (WCOT) or *support-coated open tubular* (SCOT). Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase. In support-coated columns, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

In 1979, a new type of WCOT column known as the Fused Silica Open Tubular (FSOT) column was innovated. These have much thinner walls than the glass capillary columns, and are given strength by the polyimide coating. These columns are flexible and can be wound into coils. These columns are advantageous in their physical strength, flexibility and low reactivity. Some examples of stationary phases are given below.

Table: Some examples of stationary phases in Gas Liquid Chromatography

|  |  |
| --- | --- |
| Supporting material used | Separated Compounds |
| Apiezon L (Hydrocarbon grease) | Barbiturates, amphetamine |
| SE-30, OV-1, OV-101 (dimethyl silicone polymer) | Separation on the basis of molecular weight |
| Apolane-87 (high temperature non-chiral hydrocarbon phase) | Many drugs |
| Carbowax 20 M (polyethylene glycol) | Alkaloids, basic drugs, amphetamine |
| OV-17 (Phenyl methyl silicone, moderately polar silicone phase) | Many drugs |
| XE-60(Cyanoethyl silicone), OV-225 (Cyclo propyl phenylmethyl silicone) | steroids |
| Polyesters | Fatty acid esters, barbiturates |
| Polyamides (Poly A 103) | Barbiturates, tertiary amine tricyclic antidepressants |
| Chirasil- Val | Optical enantiomers (amino acids, polar drugs) |
| Mixed phases | Anticonvulsant drugs |
| Molecular sieve (4A, 5A, 13X) | Inorganic gases, CO2, N2, CO in blood |
| Silica gel | Inorganic gases, CO2, N2, CO, H2 |
| Chromosorb / Porapack | Fatty acids, amines, alcohol |
| Tenax | Trapping of volatile substances |
| Carbopak B and C | Hydrocarbons C1to C10, ethanol in blood, substances abused by “glue sniffers”, ethylene glycols in blood. |

**Column temperature**

This is one of the controlling factors in gas chromatographic separation. For precise work, column temperature must be controlled to within one tenths of a degree. The optimum column temperature is dependant upon the boiling point of the sample. As a rule of thumb, a temperature slightly above the average boiling point of the sample results in an elution time of 2 - 30 minutes. Minimal temperatures give good resolution, but increase elution times. If a sample has a wide boiling range, then temperature programming can be useful. The column temperature is increased (either continuously or in steps) as separation proceeds. A programming of temperature control is followed now a days.

**Detectors**

There are many detectors, which can be used in gas chromatography. Different detectors will give different types of selectivity. A *non-selective* detector responds to all compounds except the carrier gas, a *selective detector* responds to a range of compounds with a common physical or chemical property and a *specific detector* responds to a particular class / compounds under the class. Detectors can also be grouped into *concentration dependant detectors* and *mass flow dependant detectors*. The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not usually destroy the sample Dilution with make-up gas will lower the detectors response. Mass flow dependant detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector. The response of a mass flow dependant detector is unaffected by make-up gas. The summary on characteristics of detector are presented hereunder in tabular form.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Detector | Type | Support gases | Selectivity | Detectable  Limit | Dynamic range |
| Flame ionization (FID) | Mass flow | Hydrogen and air | Most organic compounds. | 100 pg | 107 |
| Thermal conductivity (TCD) | Concentration | Reference | Universal | 1 ng | 107 |
| Electron capture (ECD) | Concentration | Make-up | Halides, nitrates, nitriles, peroxides, anhydrides, organometallics | 50 fg | 105 |
| Nitrogen-phosphorus | Mass flow | Hydrogen and air | Nitrogen, phosphorus | 10 pg | 106 |
| Flame photometric (FPD) | Mass flow | Hydrogen and air possibly oxygen | Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium | 100 pg | 103 |
| Photo-ionization (PID) | Concentration | Make-up | Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics | 2 pg | 107 |
| Hall electrolytic conductivity | Mass flow | Hydrogen, oxygen | Halide, nitrogen, nitrosamine, sulphur |  |  |

The effluent from the column is mixed with hydrogen and air, and ignited. Organic compounds burning in the flame produce ions and electrons, which can conduct electricity through the flame. A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame. The current resulting from the pyrolysis of any organic compounds is measured. FID is mass sensitive rather than concentration sensitive; which gives the advantage that changes in mobile phase flow rate do not affect the detector's response. The FID is a useful general detector for the analysis of organic compounds; it has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but unfortunately it destroys the sample.

**11.9.4.1 Applications**

**1. Amphetamines Extracted from Urine by SPME**

Sample: 1mL urine + 100µg of each analyte + 5µg methamphetamine-d5 + 0.7g K2CO3 in 12mL vial

SPME Fiber: polydimethylsiloxane, 100µm film

Extraction: headspace, 5 min, 80°C (sample incubated 20 min)

Desorption: 3 min, 250°C

Column: poly(dimethylsiloxane), 15m x 0.53mm ID, 2.0µm film (equivalent to SPB-1 phase)

Oven temperature: 110°C

Carrier: nitrogen, 25mL/min

Detector.: FID, at 250°C

Inj.: splitless, 250°C

**2. Amphetamines in Urine**

**Conditions**

Sample: 1ml urine + 0.7g K2CO3 in 20ml headspace vial,   
equilibrated at 80°C, 30 min  
SPME Fiber: Polydimethylsiloxane, 100µm film  
Extraction: Headspace, 3-5min, 80°C

Derivatization: Methyl bis-trifluoroacetamide(headspace,0.5min,ambient)  
Desorption:1min, 270°C

Column: Methylsiloxane, 12.5m x 0.2mm ID, .033µmfilm,

Oven: 60°C(1min)to140°C(4min)at30°C/min, then to 276°C at 20°c/min, 4 min, Detector: MS, fullscan,

Injection: splitless(closed1min),270°C

**3. Cocaine Extracted from Urine by SPME**

**Class of Compound:** Alkaloids

**Conditions**

Sample: 0.5mL of urine (250ng of each analyte and 20µL 2.5% NaF) in 1mL vial  
SPME Fiber: Polydimethylsiloxane, 100µm film  
 Extraction: Immersion, 30 min  
Desorption: 3 min, 240°C  
Column: Polydimethylsiloxane, 30m x 0.32mm ID, 0.25µm film (equivalent to SPB-1 phase, column Cat. No. 24044)  
Oven: 120°C to 280°C at 10°C/min  
Carrier: Helium, 3mL/min  
Detector: NPD, 280°C  
Injection: Splitless (splitter closed 1 min), 240°C

**4. Tricyclic Antidepressants Extracted from Urine by SPME**

Class of Compound: Antidepressants, Tricyclic  
GC Conditions:

Sample: 1ml urine (1µg each analyte ± 50µl 5M NaOH) in 7.5ml vial  
SPME Fiber: Polydimethylsiloxane, 100µm film  
 Extraction: Headspace, 15 min, 100°C (sample incubated 30 min)  
Desorption: 3 min, 280°C  
Column: Polydimethylsiloxane, 30m x 0.32mm ID, 0.25µm film (equivalent to SPB-1 column, Cat. No. 24044)  
Oven: 100°C to 300°C at 20°C/min  
Carrier: Helium, 3cm/sec  
Detector: FID, 280°C  
Injection: Splitless (splitter closed 1 min), 280°C

**11.9.5 Gas Chromatography/Mass Spectroscopy**

It is one of the hyphenated technique combining two techniques wherein GC that separates chemical mixtures and a very sensitive detector (the MS) with a data collector (the computer component) which identify the mass ions which separate. The two instruments are joined by interface.

Once the sample solution is introduced into the GC inlet it is vaporized immediately because of the high temperature (250 degrees C) and swept onto the column by the carrier gas and the components are separated which pass through the MS for identification of mass ions. Thus separation and identification cum quantification are achieved simultaneously.

Carrier gas (usually Helium).

The sample with carrier gas flow through the column experiencing the normal separation processes. As the various sample components emerge from the column opening, they flow into the capillary column interface. This device is the connection between the GC column and the MS. Some interfaces are separators and concentrate the sample via removal of the helium carrier. The sample then enters the ionization chamber. Two potential methods exist for ion production. The most frequently used method of ionization in the toxicology laboratory is the electron impact (EI). The occasionally used alternative is chemical ionization (CI). For electron impact ionization, a collimated beam of electrons impinge the sample molecules causing the loss of an electron from the molecule. A molecule with one electron missing is represented by M+ and is called the molecular ion (or parent ion). When the resulting peak from this ion is seen in a mass spectrum, it gives the molecular weight of the compound. Chemical ionization begins with ionization of methane (or other gas), creating a radical, which in turn will impact the sample molecule to produce MH+ molecular ions. Some of the molecular ions fragment into smaller daughter ions and neutral fragments. Both positive and negative ions are formed but only positively charged species will be detected.

Less fragmentation occurs with CI than with EI, hence CI yields less information about the detailed structure of a molecule, but does yield the molecular ion; sometimes the molecular ion can not be detected by the EI method, hence the two methods complement one another. Once ionized, a small positive potential is used to repel the positive ions out of the ionization chamber.

The next component is a mass analyzer (filter), which separates the positively charged particles according to their mass. Several types of separating techniques exist viz quadrupole filters, ion traps, magnetic deflection, time-of-flight, radio frequency, cyclotron resonance and focussing to name a few. The most common are quadrupoles and ion traps. After the ions are separated according to their masses, they enter a detector and then on to an amplifier to boost the signal.

The detector sends information to the computer, which acts as a "clearing house". It records all the data produced converts the electrical impulses into visual displays and hard copy displays.  The computer also drives the mass spectrometer.

Identification of a compound based on its mass spectrum relies on the fact that every compound has a unique fragmentation pattern. Even isomers can be differentiated by the experienced operator. Generally, more informations are generated than can possibly be used.  A library of known mass spectra which may be several thousand compounds in size is stored on the computer and may be searched using computer algorithms to assist the analyst in identifying the unknown. It is important to incorporate all other available structural information (chemical, spectral, sample history) into the interpretation wherever appropriate.

The ultimate goal is accurate identification of a compound, which can be facilitated by the utilization of the GC -MS.

Gas chromatography-mass spectrometry (GC - MS) combines the fine separating power of GC with the uniquely powerful detection capabilities of MS. GC -MS with capillary column separation and electron (impact) ionization (EI) is available within the Mass Spectrometry Facility.

This powerful technique is particularly suitable for the analysis of mixtures of volatile and low relative molecular mass compounds (< 800) such as hydrocarbons, fragrances, essential oils and relatively non-polar drugs. Chemical derivatisation, e.g. trimethylsilylation, can often be employed to increase the volatility of compounds containing polar functional groups (-OH, -COOH, -NH2, etc) thereby extending the range of suitable analytes to such compounds as steroids, polar drugs, prostaglandins, bile acids, organic acids, amino acids and small peptides.

**A GC -MS Analysis usually consist of the following steps:**

**Sample preparation**

Samples such as water, soil, urine, blood plasma, etc., have to be subjected to a "clean-up" procedure prior to analysis in order to extract and concentrate the more volatile, low molecular mass components. Extraction can be performed by organic solvents or by solid phase extraction (SPE).

**Derivatisation**

Where considered necessary, the extract can be derivatized with a choice of special reagents. For example, thermally labile and polar carboxylic acids groups can be methylated with diazomethane or trimethylsilated with a variety of commercially available compounds.

**Injection**

Typically, a solution of the analyte mixture (1μl, containing perhaps several hundred nanograms of material) is injected on to the GC column via a heated injection port. As the port is normally held at 250-300 C to facilitate vaporisation, the GC -MS technique might be less suitable for the analysis of thermally labile components that can decompose prior to separation. Derivatisation can offer some protection, but the analyst should be well aware of such dangers.

**GC separation**

Following volatilization in the heated injector, the mixture is pushed by a pressurized carrier gas (usually helium) through the GC column, which is heated in an oven. The column has an inner coating of a special liquid (a silicone). The separation is based on the fact that different compounds dissolve in different extents in this liquid and move through the column at different rates depending on their partitioning between the stationary liquid and the mobile carrier gas. The degree of separation of the components depends on many factors including the nature of the sample, carrier gas type and flow rate, column type, dimensions and stationary phase, and the temperature of the column as described earlier. The column temperature can be kept constant (isothermal) or may be programmed ("ramped") to increase at a predetermined rate, usually linear.

**MS Detection**

The detection method currently available as a facility is the electron ionisation (EI). The MS detector can operate in 2 different modes: scanning and selected ion monitoring (SIM).

**Scanning**

The scanning mode provides a fairly reproducible mass spectral fragmentation pattern (fingerprinting). Mass spectra are recorded (scanned) at regular intervals (typically 0.5 - 1 per second) during the GC separation and stored in the instrument data system for subsequent qualitative or quantitative evaluation. From such patterns, it is often possible to deduce structural features (mass spectral interpretation) but this requires experience and can be very time-consuming particularly for a complex mixture containing hundreds of components.

Such "fingerprints" can also be compared with those stored in a standard database (mass spectral library) and several important databases that are currently available as the facility to assist in problem solving. Although considerable care must be exercised in interpreting the results of such comparisons, this is often a useful technique in helping to identify unknown or quickly eliminate known compounds for further consideration and interpretation. Although library searching is a very useful and time saving technique, it is important to remember that such searches do not identify compounds.

The additional measurement of the GC retention index related to the time it takes a component for traveling through the column provides an additional parameter and is becoming increasingly important in the identification. Software is also available as a facility to assist in the measurements. Quantitative work can be performed by integration of selected ion chromatographic peaks. Software is available for this purpose.

**Selected Ion Monitoring (SIM)**

SIM is much more sensitive technique for trace quantitative analysis. Here, instead of scanning a whole spectrum, only a few ions are detected during the GC separation. This can result in as much as a 500-fold increase in sensitivity at the expense of specificity. Stable isotope-labeled internal standards can be employed for the purpose. Again, software is also available for such analysis. Depending on the analyte, low picogram to nanogram amounts can be measured using this powerful technique.

**11.9.5.1 Applications to Forensic samples**

**1. GC –MS -MS Analysis for Anabolic Steroids in Urine for Dope Testing** (17):

**Instrument Conditions**

**Gas Chromatograph**

Column: HP-1 25m x 0.22mm (i.d) x 0.11µm film

Flow Rate: 1 mL/min.

Oven Temperature Program: 120°C hold 1.6 sec, 50°C/min until 200°C, then 2°C/min until 245°C, then 25°C/min until 300°C, and hold for 5 min.

Injector Temperature: 280°C

Transfer Line Temperature: 300°C

Injection Mode: Splitless, hold for 1 min.

Injection Volume: 2 µl

**Mass Spectrometer**

Mass Range: 130-600

Scan Rate: 2 scan/sec

Background Mass: 45 m/z

Ion Trap Temperature: 220°C

AM Voltage: 4 volts

RF level: 100 m/z

Filament: 60 µAmps

Multiplier: Auto tune + 100 V

Target: Auto tune

**Mass Spectrometer (MS -MS)**

Filament: 90 µAmps

Multiplier: Auto tune + 200 V

Target: 5000

Column:15 m Chrompack CP-Sil 24 column.

**2. Determination of Marijuana (THC) Metabolite in Urine with the GC -MS (Zelda Penton Varian GC)**

Gas Chromatograph

|  |  |
| --- | --- |
| Injector Temperature | 2800C |
| Split State | Initial split 1:50, time 0, off; time 0.7 min split 1:50 |
| Carrier Gas | Helium at 1.0 mL/min (EFC constant flow mode) |
| Column | 15 m x 0.25 mm coated with 0.25 µm CP-Sil 24 |
| Column Temperature | 130 °C, 0 min hold, 20 °C/min to 320 °C, hold 0.5 min (Total time 10.0 min) |
| Mass Spec Temperatures | Trap 220 °C, transfer line 280 °C, manifold 50 °C |
| Auto Sampler Injection Parameters | Pre-injection flushes: twice with ethyl acetate, twice with toluene  Five fill strokes of 3.0 µL, fill speed 1 µL/sec  Inject 1.0 µL sample with 1.0 µL air plug, injection speed 50 µL/sec  Pre-injection delay and post-injection delay each 0.5 sec  Post-injection flushes: thrice with ethyl acetate. |

**11.9.6 High Performance Liquid Chromatography**

HPLC is a separation, detection and quantitation method for mainly organic compounds based on the differential attraction of analytes between a liquid phase (pumped through a column) and a solid phase (packed within the column). HPLC instrumentation includes a pump, injector, column, detector and recorder or data system. The heart of the system is the column where separation occurs. Since the stationary phase is composed of micrometer size porous particles, a high pressure pump is required to move the mobile phase through the column. The chromatographic process begins by injecting the solute onto the top of the column. Separation of components occurs as the analytes and mobile phase are pumped through the column. Eventually, each component elutes from the column as a narrow band (or peak) on the recorder. Detection of the eluting components is important, and this can be either selective or universal, depending upon the detector used. The response of the detector to each component is displayed on a chart recorder or computer screen To collect, store and analyze the chromatographic data, computers, integrators, and other data processing equipment are frequently used.

**Functional Description of the Instrument**

**Mobile Phase reservoir and filtering**

The most common type of solvent reservoir is a glass bottle with teflon tubing and filters to connect to the pump inlet and the purge gas (helium) used to remove dissolved air.

**Solvent Delivery System:-**

High-pressure pumps are needed to force solvents through packed stationary phase beds. Flow rate stability is an important feature of pump. An additional feature found on the more elaborate pumps is the external electronic control. Although it adds to the expense of the pump, external electronic control is a very desirable feature when automation or electronically controlled gradients are to be run. It is desirable to have an integrated degassing system either helium purging, or vacuum degassing.

**Columns**

Typical LC columns are 10, 15 and 25 cm in length and are fitted with extremely small diameter (3, 5 or 10 mm) particles. The internal diameter of the columns is usually 4 or 4.6 mm; this is considered to be the best compromise among sample capacity, mobile phase consumption, speed and resolution. However, if pure substances are to be collected (preparative scale), larger diameter columns may be needed.

**Detectors**

Today, optical detectors are used most frequently in liquid chromatographic systems. These detectors pass a beam of light through the flowing column effluent as it passes through a low volume (~ 10 ml) flowcell. The variations in light intensity caused by UV absorption, fluorescence emission, or change in refractive index (depending on the type of detector used) from the sample components passing through the cell, are monitored as changes in the output voltage. These voltage changes are recorded on a strip chart recorder and frequently are fed into an integrator or computer to provide retention time and peak area data.

The most commonly used detector in LC is the ultraviolet absorption detector. A variable wavelength detector of this type, capable of monitoring from 190 to 460-600 nm, will be found suitable for the detection of the majority samples.

Other detectors in common use include: Refractive index (RI), Fluorescence (FL), electrochemical (EC) conjductivity, Photo diode array and mass-spectrometric (MS) detectors. The RI detector is universal but also the less sensitive one. FL and EC detectors are quite sensitive (up to 10-15 mole) but also quite selective. The MS detector is the most powerful one for the identification.

**Injectors**

Sample introduction can be accomplished manually or by autosamplers. In liquid chromatography, liquid samples may be injected directly and solid samples need to be dissolved in an appropriate solvent, preferably mobile phase. It is essential to remove particles from the sample by filtering, or centrifuging since continuous injections of particulate material will eventually cause blockage of injection devices or columns.

**Data station**

The data station consists of a PC, an integrator/printer that in addition to revealing the chromatograms, stores and monitors various parameters related to the system. The PC selected should be compatible to the basic system.

**11.10 UV VISIBLE SPECTROSCOPY:**

Absorption spectrometry in the UV-Vis regions is considered to be one of the oldest physical methods used for quantitative analysis and structural elucidation.

The laws of absorption of radiant energy with which the analyst is concerned involve the relationship between the magnitude of absorption and the quantity of absorbing material present in solution. These laws are applicable to absorption of energy in any region of the spectrum.

**Bouger – Lamber’s Law:**

It states that the proportion of light absorbed by a transparent medium is independent of the intensity o the incident light, and that each successive layer of the medium absorbs an equal fraction of the light passing through it. This can be expressed mathematically:

I = Io x e-∝t or loge (Io/I) = αt or log (Io/I) = Kl

Where

Io – the intensity of the incident light

I – the intensity of the transmitted light

t – the thickness of the layer in centimeters

α - the absorption coefficient of the medium

K – constant depending only on the medium examined and is known as extinction coefficient.

**Beer’s law:**

The absorption coefficients α and K contain no concentration factor and Beer’s law deals with this variable. It states that the light absorption is proportional to the number of molecules of absorbing substance through which the light passed. If the absorbing substance is dissolved in a transparent medium, the absorption of the solution will be proportional to its molecular concentration.

**Beer – Lambert’s law:**

The combined law may be expressed as

Log (Io/I) = ξcl

Where

ξ - molecular extinction coefficient

c – concentration in gram moles per liter.

The validity of the combined law should be tested for each system, which is being quantitatively investigated. It is simply necessary to prepare a series of known concentrations of the substance being measured. The wavelength at which maximum absorption occurs is determined and the absorbance at this wavelength is determined for each of the solution of known concentration is determined. The values may be plotted against concentrations or the molecular extinction coefficient may be attributed to a deviation from Beer’s law. The curve shows linearity. From the Beer’s curve the concentration of unknown may be conveniently determined and the quantity of active substance present in the sample thereafter. Beer’s law is a limiting law for dilute solutions.

**11.10.1 Derivative Spectrophotometry** (23, 24):

In derivative Spectrophotometry the absorbance (*A*) of a sample is differentiated with respect to wavelength (λ) to generate the first, second, or higher order derivates

|  |  |  |
| --- | --- | --- |
| *A*=f(λ)  Recorder | d*A*/Dλ=f’(λ)  first derivative | d2A/dλ2=f”( λ), etc  Second derivative |

Derivative spectra often yield a characteristic profile, where subtle changes of gradient and curvature in the normal (zero order) spectrum are observed as distinctive bipolar features (Fig.1). This type of spectrometry is applicable for two or more substances having nearly equal values of λmax.

## 11.10.2 Qualitative Applications in Structural Elucidation

Specific identification of a compound can rarely be made on the basis of spectral evidence alone. Often the spectrum serves as confirmatory evidence of identity in support of other analytical data. Spectra are recorded in aqueous acidic, basic, and in ethanolic or methanolic solution. The wavelengths of the principal peaks and the corresponding absorptivity values are noted for each solvent system by comparison with data tabulated in ascending wavelength order for a number of compounds with absorbing properties.

Spectral shifts are among the most useful diagnostic features in drug molecules possessing ionisable groups. A marked bathochromic shift in alkaline solution is observed not only for most of the phenolic drugs such as the phenolic estrogens, but also in the case of hydroxypyridines, ketones, benzodiazepines, pyridones, and nitro-compounds. Similarly shift may occur both in alkaline and acidic medium for cyclic urieids, i.e. barbiturates.

### 11.11 Infra-red Spectrophotometry:

Infra-red Spectrophotometry is the study of the reflected, absorbed, or transmitted radiant energy in that region of the electromagnetic spectrum from wavelength (λ = 0.8 to 500μm). A more commonly used mode of measurement is the frequency (the number of waves per unit length) than wavelength. This is expressed in wave numbers( cm-1). The infrared spectrum is usually divided into three regions, 12 500 to 4000 cm-1 (0.8 to 2.5 μm) (near infra-red), 4000 to 400 cm-1 (2.5 to 25μm) (medium infra-red) and 400 to 20 cm-1 (25 to 500 μm) (far infra-red). Only the mid infrared region (usually referred to simply as ‘infra-red’) is considered here because it is the region, which is widely used in the analysis of drugs and pesticides.

When a molecule is subjected to infra-red radiation, transitions take place between rotational and vibrational energy levels in the ground electronic state. These transitions give rise to an absorption spectrum characteristic of the compound. This is in contrast to ultraviolet radiation, which also causes transitions between rotational and vibrational energy levels, but because of its greater energy these transitions occur at higher energy states. In the infra-red, absorption occurs only where a change of the dipole moment of a molecule can take place. This means that diatomic molecules without dipoles, such as hydrogen, nitrogen and oxygen, do not absorb in the infra-red. Total symmetry about an absorption band will eliminate certain absorptions, e.g. the symmetrical ethane molecule gives no-carbon-carbon stretching absorption bands. Similarly the stretching of bands in functional groups may occur at different wave numbers which have characteristic values. The value gives an indication for the presence of functional group and nature of bonds.

Three main types of absorption occur, namely fundamental, overtone, and combination. Fundamental bands are the primary absorption bands for each mode of vibration, overtone bands occur at multiples of the fundamental band wavelength, and combination bands occur at wavelengths which are the sum or difference of two or more fundamental bands. Overtone and combination bands are usually much weaker than fundamental bands.

Particular bonds or functional groups in a molecule have specific absorption bands at given wave numbers. Changes in the wave number of a band have been correlated with changes in either the structural environment or the physical state of the molecule. These correlations form the basis of qualitative analytical work in infra-red Spectrophotometry. However, many bands in the complex region from 1600 to 400 cm-1, which is usually referred to as the ‘fingerprint region’, are still of unconfirmed origin. A proportion of the bands are characteristic of the molecule as a whole and cannot be assigned to particular functional groups.

**11.12 Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF)** (18-22)

the technique find applications in the analysis of metals in soil and sediments and environmental samples of air and water. However, attempt to extend the methods to environmental samples under different projects met success only with soil samples. The following table represents the limit of detection of different metals by EDXRF. Volatile metals (Hg, As and Se) in soils may be analysed for screening by EDXRF

|  |  |
| --- | --- |
| Element | Limit of detection(ppm) |
| Antimony | 10 |
| Arsenic | 10 |
| Barium | 10 |
| Cadmium | 10 |
| Chromium | 20 |
| Copper | 10 |
| Lead | 10 |
| Manganese | 10 |
| Mercury | 10 |
| Nickel | 10 |
| Selenium | 10 |
| Silver | 10 |
| Thallium | 10 |
| Zinc | 10 |

1. **Performance and Advantage**

**A. Technology Benefits**

The EDXRF technique is rapid, non-destructive requiring only sample preparation by microwave digestion or pulverization of sample. As it does not require drastic reagent or additional waste stream, this is a safe technique.

**B. Faster Analysis Time**

The entire method is electronic data based and very fast.

1. **Low detection limit**

This is evident from table

* The steps in the operation of instrument include calibration with elemental copper ( to correct signal output and processing of peak offset) as per NIST soil standard, checking of calibration standard (using NIST standard as unknown for a certified value 1-15%); sample preparation, analysis of spectrum in EDXRF for quantification.

**Application of EDXRF to Analysis of Volatile Metals**

Mercury, arsenic, and selenium are considered volatile elements and some care must be taken in their analysis, especially in the case of mercury. EDXRF in conjunction with the fundamental parameters software is an excellent method for rapid screening of mercury-contaminated soils. Certified soil reference standards for mercury are not readily available, so the quantitation for mercury (atomic number 80) is performed by using the calibration data for lead (atomic number 82). This quantitation is possible due to the proximity of the elements to each other on the periodic table of the elements and is referred to as a ‘standardless’ calibration. The spectra for each element is easily distinguishable but the mass absorption coefficients for these elements are nearly the same (2.291 for Hg, 2.389 for Pb).

**Interferences of Elements**

**Effect of Background radiation:**

Like many analytical methods, EDXRF suffers from a few limitations and interferences. Due to the production of a continuum of Xrays by the Xray tube, scattered Xrays are produced by interaction with the rhodium anode, air, moisture, sample, and silicon/lithium detector. There is a constant level of radiation (bremsstrahlung) emitted from these interactions that can obscure elements, especially low Z elements. The use of primary filters can aid in reducing the background radiation and allow for better signal to noise ratio in the energy zones of interest. Because of the attenuation of produced Xrays as they interact with air in the sample chamber, low Z elements sensitivity (elements between sodium and phosphorus) is diminished. Detection of these compounds can be enhanced if a vacuum source is connected to the analytical chamber.

There are two elements that are typically masked by high concentration elements; high levels of Iron’s Ka at 6.398 KeV mask Cobalt’s Ka line at 6.924 KeV. A more environmentally significant interference occurs between arsenic and lead. The lead La1 line at 10.550 KeV overlaps the position of the arsenic Ka line at 10.530 KeV, which causes the FP software to attempt to calculate arsenic from the Kb line. This quantitation is usually unsuccessful when the arsenic/lead concentration ratio is below 0.046. Due to this interference, the arsenic detection limit is set at 10% of the lead concentration for samples with high lead content.

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METALS in urine: METHOD 8310, Issue 2, dated 15 August 1994 - Page 3 of 5 complete (200 watts at 1 to 2 torr, or manufacturer's recommendations).

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Section – 12: ANNEXURES

12.1. MEDICO LEGAL AUTOPSY (Postmortem Examination)

An autopsy is an examination performed by a qualified and authorized medical officer to meet with certain requirements of law. The examination should be meticulous, complete and routinely record all positive findings and important negative ones. From the data, the doctor should furnish a factual and objective report keeping in mind that he may have to explain his finding & opinions in the court of law . The evidentiary materials should be properly preserved. The objectives of PM examination are as follows.

1. To determine the identity of a person
2. To determine the cause of death, whether natural or unnatural
3. If the death is unnatural, whether by suicide, homicide or accident and if homicide, whether any evidence is left on the victim
4. To find out the time since death
5. To determine the age to know about live birth and viability etc. in the new born child
6. In the cases of skeletal remains, to determine the source whether

human or animal, the probable cause of death and time elapsed since the death.

**Rules for PM Examination:**

1. It must be done only after obtaining dead body challan requisition from police, magistrate or coroner to conduct PM examination.
2. An inquest report with the available history of the case and circumstance under which the body was recovered. It should also contain the recorded opinion of the witnesses and the police officer regarding injuries, cause of death and indication of suspected foul play, if any.
3. The autopsy must be done in day light as far as possible. But some states have issued special orders to carry out PM at nighttime, if urgently required. However, it must be done without delay.
4. The body must be identified by the escorting police. In medico - legal postmortems especially in unidentified bodies, it is necessary to note all particulars which help to establish the identity with reference to race, religion, sex, age, social status, weight, dental formula, congenital features, acquired peculiarities etc. Photographs should be taken by police before sending for PM and fingertips of the dead body be preserved for finger print examination by expert. Since this procedure requires mutilation, it is desirable to take both plain and rolled finger print impression. In criminal cases identification should never be omitted.
5. In dowry deaths, where accused is prosecuted under 498 A and 304 B IPC, the PM is conducted by a panel of doctors on request from a magistrate but one of them need not be a lady doctor.
6. As per Sec. 174(3) of Cr.P.C. where the body cannot be transported or likely to lose evidences, the doctor is supposed to conduct the PM examination on the spot. A doctor can also demand for visit of scene of crime and can consult toxicological or clinical expert before carrying out the PM if he feels beneficial. There are certain disadvantages in not visiting the scene of crime e.g. fresh abrasion may be produced during shifting of the body, fresh bloodstains may form due to disarrangement of the cloths. Fresh tears may result in cloths from rough handling. The existing rigor mortis may be broken partially and new position may show inappropriate rigidity and lividity which may add confusion. In suspected homicidal deaths and especially deaths in hit and run cases, the utmost care should be taken in shifting the body from crime scenes. In such cases, it is advisable to shift the body by wrapping in a plastic sheet so that the loose physical evidences like paints, chips and glass particles etc. may not fall.

In India, PM is conducted at taluka places, district places and medical colleges. All these three places have a lot of differences in facilities provided in mortuaries, their maintenance and training provided to the medical officers. Ideally, every mortuary should have some basic facilities, healthy and clean atmosphere, sufficient equipments for conducting PM, proper light facilities, staff etc. and this work should be given importance.

1. If the inquest does not include all the injuries, the doctor must call the IO and arrange for entry of all the injuries. If the IO refuses, the doctor can inform his higher officers or magistrate and arrange for fresh inquest. It is advisable for a second inquest to be held preferably by a magistrate. It is always beneficial to document the injuries by photography.
2. Earlier PM examination was not conducted on deceased of certain diseases like tetanus and rabies for fear of spread of disease. The death certificate was issued based on clinical diagnosis. But now a day, the doctor must do the PM examination if the police insists.
3. In medico legal autopsy if everything is normal, the doctor should make a routine practice to send visceral organs, blood and other body fluids for chemical analysis to rule out poisoning, suspected tissues for Histopathological examination to rule out microscopic lesions and blood from fresh body for blood culture by taking blood from heart under aseptic precautions for microbiological investigations, smear from infected area and smear from brain to rule out meningitis, falciparum malaria etc.
4. The doctor can only allow a stranger to witness a PM examination if he is the Investigating Officer of that case, a magistrate or a person with written orders by the magistrate or the students under training with relevant orders. But cannot disclose the facts of the case to the trainees.
5. A doctor can collect case sheet, investigation reports, if necessary to study these before going for PM examination.
6. In normal circumstances, a doctor can refuse to conduct PM examination after sunset and if the case is not in his jurisdiction. But in most of the states, PM examination is allowed to be conducted out during night under sufficient light and but the doctor cannot refuse on the grounds of jurisdiction if insisted upon by the IO or the magistrate under abnormal circumstances. A lady doctor is not exempted from conducting PM examination on pregnancy grounds.
7. A list of all articles and evidences removed from body should be made e.g. clothes, jewellery; bullet removed from body viscera taken etc. They should be labeled and mentioned in report and handed over to the police constable after obtaining a receipt.

12.2 MEDICO LEGAL AUTOPSY – A PROCEDURAL OUTLINE

There are various procedures for autopsy, yet it is the skill and expertise of the expert that matter most. To some extent, the details vary with the object in view and the type of death. Thus, the procedure in a case of drowning must necessarily differ from that in which the cause of death is suspected poisoning. There is no short cut in PM examination. Autopsy must be complete. All the major cavities must be opened and all the organs be examined. The general scheme is recommended for a beginner which is normally modified depending upon the experience of doctor, facilities available and the situation. The general scheme is described under the following heads, which is merely a guideline of the procedural heads. The points are elaborated, wherever felt necessary.

The general scheme of the procedure may be described as (1) External Examination (2) Internal Examination, (3) Medico-legal procedure in poisoning, preservation, packing, labeling and forwarding of the visceral samples and other evidences recovered from the body (4) Post mortem examination report and opinion.

**12.2.1 External Examination:**

1. **Clothings**: Examine the clothings for the marks of violence, cuts, tears, gun shot marks and the stains of vomit, saliva, blood or any other material. Air dry under shed, if wet and pack them for further examination in FSL, if felt necessary for the investigation of the case.
2. Marks of identification especially in unknown bodies: General description is given by describing the race, sex, age, stature, features, scars, color, and distribution of hair on the body, tattoo marks, teeth, colour of the iris, bony framework, deformities, scars, moles, lymph node enlargements and occupation characteristics etc. Photographs of the body from different angles to identify the body include scale to document injuries and fingerprints should be taken.
3. Record the rectal and environmental temperature and time.
4. Condition of the body as regards weight, muscularity, stoutness, height, and approximate age. The approximate age is determined from general appearance, teeth, ossification of bones, closure of skull sutures, lipping of lumber vertebrae and joints of extremities, osteophytic outgrowths on the inter vertebral discs, ossification of laryngeal and costal cartilage etc.
5. Description of the injuries, the core of a medico legal postmortem report is really the evidence as regards cause of death mainly in violence and poisoning. Therefore, all the injuries are numbered and summarized to provide an overview of the entire injury pattern, where there is doubt whether an injury was inflicted before or after death, the tissues are taken for histological and/or histochemical examination. Similarly, any evidence of poisoning is recorded. If there is any disease, it is recorded along with its role for the cause of death. Most of the times, it is beneficial to wash the body and examine it again. This defines some lesions observed by bloodstains, injection marks, the presence of surgical emphysema, skin disease or oedema of legs, dropsy, icterus etc.
6. Examination of the back is done and a record of bruises or postmortem lividity is made. Cut through the skin deep into muscles, if necessary to check deep bruises, spinal deformity, decubitus ulcers, sacral oedema etc.
7. Flex the joints to determine if rigor mortis is present and if so, its extent.
8. Note the incidence, extent, color and degree of fixation of PM lividity. Differentiate patches of PM lividity on dependent parts from bruise by making an incision to verify the presence of extravagated blood in bruise.
9. The extent of decomposition is noted. Samples of insects, eggs, maggots or pupae formed are taken for laboratory examination. They are useful in finding the time of death and some times cause of death in very advanced stage of decomposition where only skeletal remains are left. The maggots also serve as a useful substitute for finding the blood group of the person in decomposed bodies.
10. Radiological examination is necessary in suspected cases of fractures and firearm injuries to document radio-opaque foreign bodies and sometimes for identification and age determination purposes.
11. In the case of newly born infant where the question of live birth and viability is to be determined, it is required to note the shape, whether flat or arched; the presence of centers of ossification in the calcaneum indicates 20th week , the talus 28th week , and the lower end of femur 36th week; and the condition of the umbilical cord.
12. The time since death is estimated based on the rectal temperature, the presence or absence of rigor mortis, the incidence, extent, colour and degree of fixation of post mortem lividity, and the presence, character and extent of putrefaction.

**12.2.2 Internal Examination:**

If there is a fatal wound leading to one of the cavities, the cavity is opened first, otherwise, the head is opened first followed by thorax and abdomen. Every organ in the cavities must always be examined, but the spinal cord is examined in the last.

**Skull and Brain:**

A transverse incision of scalp from mastoid to mastoid over the vertex is made by a scalpel cutting the full thickness of the scalp. After reflecting the flaps anteriorly up to the orbits and posteriorly below the occipital protuberance, the inner surface of the scalp is examined for petechial haemorrhages, or oedema. Note any fracture or separation of sutures after the temporal muscles have been removed. If present, record its dimensions and contour.

To remove the skullcap, a circular cut is made with a saw round the cranium a little above the eyebrow ridges. The removal of skullcap is facilitated by gently inserting and twisting the chisel at various places through the cut. Care should be taken not to produce any PM fracture or extending the existing ones, and to avoid any damage to the meninges and brain during this procedure. Its inner surface is then examined for fracture of the inner plate, and any extradural haemorrhage on the external surface of the brain. The longitudinal venous sinuses is examined for evidences of laceration or thrombosis.

Cut the dura along the line of severed skull cap and put it gently from front to back while cutting falx cerebri and examine for subdural or subarachnoid haemorrhage or the presence of pus, also note the effect on the shape of convolution. Insert four fingers of the left hand between the frontal lobes and the skull. Draw them backwards and cut with the right hand the nerves and vessels as they come out of the skull. Cut the tentorium along the border of the petrous bone. Cut the cervical cord, cervical nerves and vertebral arteries as far below as possible. Support the brain throughout with left hand. Remove the brain along with the cerebellum. Weigh and transfer to a large clean dish for examining its upper as well as under surface including circule of willis for the evidence of any injury, effusion of blood, coup/countercoup injury, inflammatory products, embolism of arteries, aneurysm, morbid growths. Areas of petechial haemorrhages on the brain are examined for fat embolism. Separate the cerebellum at the pons transversely just below the cerebral peduncles. Cut the brain serial coronal sections about 1 cm apart or cut obliquely at the intracerebral fissure exposing basal ganglia, lateral ventricles and white matter, and examine for thickness of grey matter, haemorrhage or other abnormality. Shrinkage of gray matter is common in chronic alcoholics. Cerebral fat emboli, obstructing the small vessels of the brain complete or may be visible to the naked eye as punctate haemorrhages in the white matter. Petechial haemorrhages in the white matter are commonly found in death from anaphylactic shock. In head injury, oedema is seen in the white matter around or deep to contusions, lacerations, or ischaemic lesions. If there are wounds of the brain, successive sections parallel to the wounded surface are made till the whole depth of the wound is revealed. Cut the cerebellum through the vermis to expose the fourth ventricles. Make on oblique cut through each hemisphere to expose the dentate nucleus. Examine for any disease, injury or haemorrhage. The dura matter should lastly be removed from the base of the skull to examine for the presence of fractures.

**Spine and Spinal Cord:**

Normally, spinal cord is not required to be examined in routine except in the cases of local injuries, sudden death following trauma without apparent local injury, death from convulsions, and if specially requested by the IO. For examining spine and spinal cord, make a midline incision from the base of the skull to sacrum. Remove the paraspinal muscles and fascie from the spinous process and laminae. Perform a laminectomy by sawing through the entire length of spine on each side of the spinous processes. The laminae of the firs cervical vertebra should not be severed, otherwise the head will move too freely on the spine. Remove the spinous processes and attached laminae en masse. Examine the dura for inflammation, haemorrhage, suppuration or tumour. Cut the spinal nerves from below upwards as they pass through the spinal foramina. Separate the spinal cord at the foramen magnum carefully, lift it from vertebral column, and place it on table. Cut the cord transversely at several places and examine for any pathological condition such as softening, inflammation or haemorrhage.

Examine the vertebral column with special reference to fracture of the odontoid process and cervical vertebrae. Haemorrhage under the prevertebral fascia is taken as an indication of whiplash injury or fracture of cervical vertebrae which should be specially looked for.

**Neck and Thorax**:

Before examining the thorax, both the cavities, the thorax and the abdomen should be opened by making a longitudinal incision from above the thyroid cartilage to the pubic symphysis avoiding keeping of any wound or the umbilicus in the line of incision.

In infants, the incision is carried a little to the left of the umbilicus. The integument, fascia and muscles are now reflected and examined for extravasation of blood in their inner surface. Abdominal cavity is examined before opening the thoracic cavity to prevent any fluid or blood trickling down from the thorax into the abdomen. The viscera are first examined in their original place and then individually. The abdominal muscles are severed along the subcostal margin. The abdominal and pelvic cavities are then carefully examined for the position of viscera, abnormal contents before anything is disturbed. It should also be noted if there is any collection of blood serum, pus or faaecal matter in the cavity.

For the examination of thorax, the ribs and sternum are first examined for the evidence of any fracture. The cavity of the thorax is then opened by dividing the ribs at their cartilages and the sternum at the sternoclavicular junctions with the costatome and lifting up the sternum separating it from the under line parts without injuring them. The pleural cavities are examined for the presence of adhesions, foreign bodies or a fluid of bloody nature. The pericardium is opened and examined for any adhesions between its two surfaces or if there is any abnormal quantity of fluid present in its cavity. Normally about 20 to 40 ml of serum is found in the pericardial sac. The condition of the chambers of the heart is examined by opening them insitu. The lungs and heart are removed from the cavity and after noting the condition of the lungs for oedema, emphysema, elasticity, atelectasis etc. they are cut open for evidence of disease, congestion, injury, petechial emboli, thrombus etc., and the bronchi is examined for the presence of pus or any other foreign body. The isolated heart is studied as regard its size and weight after sectioning. The chambers of the heart are examined for valvular disease, and the condition of air or pulmonary artery embolism should be looked for. The coronary arteries are cut serially across the lumen at several points and examined for obstruction or thrombosis in their lumen. The aorta is examined for calcareous degeneration or aneurysm.

After removing the viscera, the thoracic cavity is examined for any evidence of fracture, disease, deformity or injury.

Before examining the neck, it is of worth to have a look at mouth cavity for injuries on the inside of lips, checks, and bruising and hyperplasia of gums, especially important in the cases of strangulation, suffocation and epilepsy. In the cases of unidentified bodies it is always beneficial to make dental chart. To examine the structures of the neck an incision is made from the chin to the upper part of the sternum after pushing the head backwards and placing a block beneath the neck, detach the whole viscera of the neck, viz., hyoid, epiglottis, thyroid, larynx, trachea and upper part of oesophagus to the level of subclavian arteries along with the tongue. The tongue is examined for the evidence of bruises, laceration, teeth bite marks etc. While reflecting the skin of the neck, look for any effusion or trauma. After reflecting the soft parts on each side of the middle of the larynx, both the trachea and the oesophagus are removed and examined by cutting them open from their posterior surface. The interior of the oesophagus is examined for evidence of congestion, inflammation or ulceration of its mucus membrane and the presence of foreign body, tumor etc. The larynx and trachea are examined for the presence of froth, any obstructive foreign body, food, vomitted material, oedema and their mucous membrane is examined for congestion or inflammation. Any fracture of the laryngeal cartilages, tracheal rings or hyoid bone should be noted.

**Abdomen**:

At the time of routine incision, the peritoneum is examined for the evidence of adhesions, congestion, inflammation or exudation of lymph or pus. The abdominal and pelvic cavities are then examined for the presence of serous, bloody fluid or gastric contents. Now abdominal organs are removed and examined individually.

1. **Spleen**: Grasp the diaphragmatic surface of the spleen and take it off from the tail of the pancreas. The size, colour, consistence and the condition of its capsule are noted. In the case of the rupture of the spleen, its size and position should be described. The normal size of the spleen in the adult measures 12x8x4 cm.
2. **Stomach**: Normally, the stomach is examined by making a cut along the greater curvature while insitu for the contents as regards their quantity and quality and the degree of their digestibility. But in the suspected cases of poisoning, the stomach is removed after tying a double ligature 3 to 5 cms above the cardiac end and at the pyloric end. It is then opened along the greater curvature in a clean dish. An examination of mucous membrane is made and its appearance is noted. If anything is adhered to it, it should be picked up separately for chemical analysis. The gastric contents should be measured and examination be made as regards to the state of digestion, color, smell and character regarding smoothness or roughness.
3. **Intestine**: Both the small and large intestines are removed by cutting the mesentery and freeing other attachments after ligaturing at both ends and is cut longitudinally along the mesenteric border to examine the inner surface for the presence of congestion, inflammation, erosions, ulcers, perforation and any other lesion. In cases of suspected poisoning, the contents should be sent for chemical analysis.
4. **Liver**: The size, weight, smoothness or roughness of the liver are noted. Normally the liver measures 26cm x18cm x10cm. If there is any injury, its nature and dimensions is noted. The liver is cut open at several places by deep incisions and the color, consistence and blood supply of its tissues are carefully marked. The pathological processes like abcess, new growth or amyloid degeneration are marked. The gall bladder is opened and the presence of bile stones if any and the character and quantity of the bile are noted. Examine the portal vein and hepatic artery for any abnormality.
5. **Pancreas**: The pancreas is normally removed along with the duodenum. A partial cut is made across the tail of the pancreas carefully to expose the pancreatic duct in the middle of the cut surface and traced to its entrance into the duodenum. The pancreas is also examined for fat necrosis by a series of cuts at right angles to its axis. It is very important to examine pancreas in the cases of sudden death.
6. **Kidney**: The color, size and weight of the kidneys are noted. Normally the size of the kidney is 12cm x7cm x4cm. Its capsule is examined as to whether it is adherent or strips off easily. The kidney is held firmly between thumb and fingers and then cut longitudinally through the convex border to the helium and opened like two halves of a book. The cut surface is examined for any pathological process such as nephritis and degenerated changes. The renal pelvis is examined for calculi and evidence of inflammation. Adrenal is removed and examined after sectioning for any haemorrhage.
7. **Urinary bladder**: It is drawn backwards from the symphysis pubis and incised on its anterior wall and urine is removed and preserved for chemical analysis in suspected poisoning. The bladder is examined for congestion, haemorrhage, and inflammation or ulceration of its mucous membrane.
8. **Prostate and Testes**: They arte taken out and sectioned for examination. The prostate is examined for enlargement or malignancy in those cases where there has been a history suggestive of such disorder of the organ. The testes and epididymis are cut longitudinally and examined for evidence of any disease or injury, especially ecchymosis if deaths due to squeezing of testes are alleged.
9. **Female genitalia**: The ovaries, fallopian tubes and uterus as whole mass are removed from the pelvis. Each organ is separated. The uterus is examined for its size, shape, weight and any abnormality. The normal size of the nulliparous organ is 7.5cm x 5cm x 2.5cm but the size and weight vary considerably during pregnancy or when there is any tumor. It is cut longitudinally to expose the endometrium, the thickness of the uterine wall and examine the contents, if any, fluid, foreign body, or foetus. If the uterus contains a foetus, its age is assessed. The ovaries are cut longitudinally and examined for corpora lutea. The fallopian tubes are cut across at intervals to examine potency. The vagina is examined for marks of injury, presence of foreign body, condition of the mucous membrane, presence of rugae, and the condition and type of hymen. Any fluid if present in the vaginal canal may be aspirated for the determination of acid phosphatase or creatine phosphokinase, blood group substance and spermatozoa. The condition of cervix and any marks of instrumental injury are noted.

**Skeleton:**

A detailed inspection of skeleton is required especially in the cases of violence and accidental cases for any deformity or injuries of vertebrae, ribs, pelvis, long bones etc. Dissect the soft tissues and muscles from the surface in order to examine the vertebral bodies for fracture, dislocation, compression or haemorrhage. Haemorrhage under the prevertebral fascia should be taken as an indication of whiplash injury or fracture of cervical vertebrae. The fracture of the odontoid process of the axis may sometimes be overlooked. When such injury is suspected,remove the whole of the cervical vertebrae as a block from the base of the skull to the upper thoracic inlet, the soft tissues being dissected away. The ribs can be examined for fracture by stripping the pleurae from inside and even cutting the intercostals muscles. The presence of blood in soft tissues of the pelvis indicates pelvic fracture which requires a careful search. In case of long bone fractures, the distance from the heel to the fracture site should be measured. Bone marrow can be obtained from sternum, upper end of femoral shaft or vertebral body where a rectangle of cortex is cut and the marrow scooped out.

**12.2.3 Examination of Decomposed Bodies, Mutilated Bodies, Fragmentary Remains and Bones**.

Decomposed bodies are bodies, which show putrefactive changes in varying degree depending upon the time passed since death. Identification, cause of death and time since death are specially looked for at such autopsies.

A mutilated body is one which is deprived of a limb, or a part, or disfigured. In this condition, the soft tissues, muscles and skin may be still attached to the bones. Fragmentary remains include only fragments of the body, such as head, neck, trunk or limb. In such type of cases, medical officer should draw his opinion based on very care examination of the available parts to discover the evidence of crime. He should specially look for the information like; source, whether human or animal, if parts belong to human, belong to the same individual, age, sex, stature, race, identity, special features, cause of death and time since death.

The scheme for the examination of bones is the same as that for mutilated bodies. Anatomist, dentist, anthropologist and radiologist may be consulted if required and opinion may be drawn on the above aspects.

**Disposal of the body:**

The medical officer should see that the dead body is properly sutured and washed. Its appearance should be such that it does not hurt the sentiments of the relatives. The body is handed over to the police under a receipt for further disposal.

# 12.3 Medico legal procedures in poisoning

It should always be remembered that except in corrosive poisons, the pathological findings are rarely conclusive; hence an appropriate portion of viscera will have to be submitted for chemical analysis. It is therefore vital to collect and preserve the relevant material in the right way and should preferably be kept in cold storage. The selection of visceral tissues to be preserved for chemical analysis is based on the history of poisoning or its distribution in various organs and tissues. The detection and distribution of a poison or drug in different body fluids and tissues is of great importance: because

i) There are various routes of entry of poisons into the body viz., by internal route e.g. by mouth or rectum, peritoneal e.g. by injection either subcutaneous, intramuscular, intravenous, intraperitoneal, inhalation, by external application on wounds or skin, introducing into natural orifices such as rectum, vagina, urethra, nose, eye etc. and by sublingual route.

ii) The poison might have passed from one organ.

iii) The poison might have been introduced into one organ like stomach after death to mark a homicidal act.

Secondly, after death, the supply of energy from metabolic processes is dramatically reduced, the integrity of the different compartments within the body breaks down at different rates, complex molecules tend to breakdown into their simpler subunits and move down concentration gradient that were maintained in life by the expenditure of metabolic energy. Obviously, these processes do not occur at once. Thus, for a variable length of time after death, the analysis of appropriate samples may yield useful information about the metabolic state of the poison in the period just before death. Once death takes place, many drugs are released from their binding sites in tissues as pH decreases on death and as the processes of autolysis proceed. These phenomena can make the interpretation of drug concentration after death less than straight forward.

Ideally, the decision to take viscera samples for toxicological or biochemical analysis should be made before conducting the postmortem examination by studying the police report, death report and the treatment report. In the cases, where cause of death is not apparent at PM, it becomes necessary to take tissue samples for toxicological analysis. Ideally, all the stomach contents should be submitted. In some circumstances samples of small bowel content should also be submitted for toxicological analysis. Normally, about 100gms. of individual tissue samples are recommended to be preserved viz. brain, adipose tissue, liver and kidney. The amount of urine obtained in bladder should also be preserved as a routine practice. The doctor should have suitable containers and materials in hand for preservation of viscera. He should ensure that the chain of evidence is maintained properly labeled and submitted to laboratory without delay. In difficult cases, whenever necessary, the expert should be consulted.

**12.4 COLLECTION OF VISCERA**

Since, the poisons or drugs after absorption into the system pass through the liver and spleen and are excreted in urine through kidney, hence, the following materials should be routinely preserved in all cases irrespective of the nature of poison.

1. Stomach and whole of its contents or stomach wash whichever is available and one feet of proximal part of small intestine along with its contents. It is preferable to send these two organs separately.
2. 100 gms of liver in pieces, preferably the portion containing gall bladder and its contents, half of each kidney.
3. Blood about 50 ml obtained from the femoral artery or vein by percutaneous puncture with a wide bore needle. It is never advisable to collect spilled blood or blood from body cavities.
4. Spleen - half in adult and whole in children.
5. 100 ml of urine or the amount available in bladder.

**Additional materials:**

1. In case of injected poison, injection site skin, subcutaneous tissues along with needle tract weighing about 100 gms should be collected. Similar material from opposite area is also taken as a control in separate container.
2. In case of inhaled poison like carbon monoxide, coal gas, hydrocyanic acid, chloroform or other anesthetic drugs etc; lung tissues, brain and blood from the cavity of the heart should be preserved.
3. Bile should be taken in the case of narcotic drugs, cocaine and paracetamol poisoning etc.
4. Shaft of long bones (8-10 cms of femur), a tuft of head hair, finger and toe nails and some muscles should be taken in suspected cases of chronic poisoning by heavy metals like arsenic, lead, antimony etc. In cases of prolonged use of drugs e.g. barbiturates; hair & finger nails are useful.
5. In fatal cases of suspected criminal abortion, the genital organs together with the bladder and rectum and foreign bodies should be preserved.
6. Blood from peripheral vein, lung tissue and a cerebrospinal fluid should be preserved in a suspected case of poisoning by alcohol. In alcohol poisoning, blood should never be collected from heart, pleural or abdominal cavities as it always gives higher results due to proximity of stomach and seepage. The blood from heart or body cavities may be taken for grouping.
7. The heart, portion of brain and spinal cord should be preserved if poisoning by nux vomica or strychnine is suspected. Brain and urine should be preserved in suspected cases of poisoning by barbiturates, opium or anesthetics.
8. Feaces may sometimes be useful especially if porphyria is suspected.
9. Urine should be collected for catecholamines estimation in a suspected case of hypothermia.
10. In highly purified bodies, larvae, maggots, pupa and other entomological samples should be preserved.
11. When the body is partially skeletonised and it is not possible to have soft tissues from any part of body, it may be possible to have soft tissues from the foot because, to some extent foot wear protects the advancement of the putrification. Bone marrow from long bones in skeletonised bodies may also serve the excellent purpose of toxicological analysis.
12. In embalmed bodies, the vitreous humour usually remains uncontaminated by the process and may serve the purpose of analysing urea, creatinine (biochemical) and ethyl alcohol. For toxicological analysis, skeletal muscles and bone marrow are the only materials available in such cases.
13. In the cases of deaths by drowning where the study of diatoms is required, then the spleen, rather than bone marrow may be the most useful material to preserve. Control sample of the water in which the body was recovered should also be taken in separate container. Care should be taken to ensure that the control sample do not contaminate the spleen or bone marrow during the collection or transportation. Spleen tissue may be useful for DNA analysis.
14. Cerebro spinal fluid may be taken in suspected case of alcohol poisoning.
15. If the death is suspected due to inborn error of metabolism, the advice of local reference laboratory should be sought as to what samples are required.
16. Fatty tissues from abdominal wall or perinephric region in the cases of pesticides.
17. About 2.5 cm square from the affected skin area and similar portion from opposite area as control in cases of corrosive poisons.
18. Soil samples from above, beneath and sides of the dead body and control soil samples from some distance away should be taken in cases of exhumed skeletalised dead bodies.

**Technique for Collection of Additional Material:**

**A) Blood:**

a) When biochemical analysis is required to be carried out on serum, the blood sample taken at postmortem is centrifuged as soon as possible. If red cells are required in the investigations like haemoglobinopathies or glycated haemoglobin, then red cells should be taken separately; and if not required, then dispose off. The samples should be sent in frozen condition.

b) Blood samples are best obtained from the femoral artery or vein by percutaneous puncture using 50 ml syringe with a wide bore needle. Blood should not be collected by being milked from a limb. This process can endanger changes in drug concentration. Cardiac blood and blood from paracolic gutter is not suitable for quantitative toxicological analysis. The paracolic gutter blood may be contaminated with gut contents, urine or other body fluids. Try to collect about 50 ml of blood.

c) Blood for alcohol estimation in living should be taken from peripheral vein.

**B) Urine:**

Urine in dead may be collected by direct puncture with needle and syringe of the exposed bladder after the abdomen has been opened. It may also be obtained by insertion of urethral catheter before starting the PM.

**C) Cerebro-Spinal fluid:**

It should be obtained by cisternal puncture or by aspirating with pasteur pipette from the base after reflecting the frontal lobes. The maximum possible amount should be withdrawn,

**D) Bone and Bone marrow:**

8 to 10 cms. portions of the shaft of femur should be taken. The required amount of bone marrow should be withdrawn from the sternum or femur.

**E) Hair:**

About 10 gm or less, if available. The head and pubic hair should be plucked out along with the roots, and not by shaving.

**F) Muscles:**

About 10 gms. Wedge of thigh or chest muscles are collected before the abdomen is opened. 100 to 200 mg. of muscles can be the ideal tissue for DNA extraction.

**G) Nails:**

All the nail should be removed entirely and collected in separate clean envelops.

**H) Skin:**

A piece of at least 2.5 cms square from the affected area in the case of corrosives or skin applications and from thigh or back in suspected metal poisoning. If there is a puncture like injection or animal bite, the whole needle track or bite mark along with the surrounding tissues with 5 cm. square skin should be excised. Control specimen should be taken from similar of the opposite side of the body and preserved separately as control. In firearm injury cases, the affected portion of skin around entrance and exit should be excised and sent.

**I)** In case of poisoning in living patients, vomit, stomach wash, blood and urine are collected.

**J)** The articles collected from scenes of crime in poisoning cases are vomits, purged materials, urine or fecal stains, cloths, bed sheets and covers, medicines and their empty containers or poisons used by the deceased. Remains of food and drinks, containers of food or drinks, cooking utensils etc and the solids and liquids contained in the traps of wash basin. While sending stained clothes, surrounding unstained portion should be sent as control.

**Preservation, packing & forwarding of viscera**

In order to avoid putrefaction of viscera samples, they are taken in clean wide mouthed containers properly. They should be preserved and packed in a suitable container to avoid any breakage during transportation. The samples should be sent at the earliest possible to the forensic laboratory.

The stomach with its contents should be preserved in one wide mouth glass bottle, small intestine with its contents in another and liver, spleen and kidney should be preserved in the third. When additional material is required, it should be preserved separately.

a) In all cases of poisoning including carbolic acid but excluding other acids, saturated solution of common salt should be used. The sufficient quantity of the preservative should be added so as to cover the visceral material.

b) In cases where poisoning by acids is suspected, except carbolic acid, rectified spirit should be used. Denatured alcohol or formalin should never be used while preserving the samples for toxicological analysis.

**A) BLOOD:**

i) When blood is taken for the estimation of volatile poisons viz. various alcohols, ethers, chloroform, hydrocyanic acid etc. and if the analysis is to be taken up immediately, then it should be sent as such in air tight container under frozen condition without adding any preservative.

ii) Post mortem blood taken in suspected cases of poisoning including alcohols but excluding flouride, carbon monoxide and oxalic acid, if immediate analysis is not possible, it should be preserved with fluoride preservative to reach concentration of 1.5% by weight.

iii) Blood for alcohol estimation in living should be preserved with 10 mg sodium fluoride (enzyme inhibitor) and 30 mg of potassium oxalate (anticoagulant) for 10 ml of blood.

iv) The liquid blood when taken for grouping should be preserved with an equal quantity of 5% (w/v) solution of sodium citrate in water containing 0.25% v/v formalin or the PM blood for grouping may be soaked in a starch free clean cotton bandage cloth piece folded several times from heart chambers or body cavity. The piece should be air dried under shade and then packed in a paper packet. This can be an ideal method for the preservation of blood stain from crime scene, body on cloths for DNA test. To avoid bacterial decomposition, airtight containers or polythene bags should never be used for packing such exhibits.

v) In the suspected case of poisoning by carbon monoxide, a layer of 1-2 cms of liquid paraffin should be added immediately over the blood sample to avoid exposure to atmospheric oxygen.

vi) In case of oxalic acid and ethylene glycol poisoning 30mg of sodium citrate should be used for 10 ml of blood and in the case of fluoride poisoning, 10 mg of sodium nitrite should be used. 2-3 ml of intravenous blood is thoroughly mixed with anticoagulant by shaking in a sterile glass container, sealed and forwarded to laboratory in ice-box without any delay.

Heparin and EDTA should never be used as anticoagulants because they interfere with the methanol detection.

vii) The blood serum or the vitreous humour, when biochemical analysis is required, should be sent in frozen conditions.

viii) 0.5% w/v of EDTA or heparin (anticoagulant) preservation of liquid blood is preferred when taken for DNA test.

**B)** **URINE**: If the urine is taken for alcohol analysis, then it should be preserved with 30 mg. of phenylmercuric nitrate per 10ml of urine or by adding a few grains of thymol. For toxicological analysis, the urine may be preserved by adding 2.5 ml conc. HCl drop wise and shaking for 100 ml of urine or it should be acidified to attain a pH of less than 2.0 by adding conc. HCl dropwise. This preservation is also applicable, if the urine sample is taken for biochemical analysis i.e. the determination of the concentration of catecholamines in deaths due to hypothermia. For routine toxicological analysis, urine may be preserved by making it saturated by adding sufficient amount of sodium chloride crystals or it may be preserved by adding equal amount of rectified spirit.

**C)** The tissues taken for histopathological examination may be preserved in 10% formalin. 100 to 200 mg. of tissue taken for DNA extraction may be preserved in 20% solution of DMSO (Dimethyl sulphoxide) saturated with sodium chloride.

**D)** The stomach contents, vomit samples or the food materials taken for bacteriological examination (food poisoning), should never be preserved. The samples are to be sent in frozen condition.

**E)**  The vomitus, fecal matter or any other body fluid like saliva or seminal stains collected for toxicological analysis or biological examination from crime scene, body or clothes should be air dried under shade and sent to the laboratory. The saliva or seminal stains for DNA tests may be preserved by drying as described earlier in case blood stains. Such samples should never be packed in airtight containers or dried under direct sunlight. After packing, the samples should be kept at room temperature. The samples of medicines or poisons recovered from scene of crime or from the possession of the deceased or from any other place should be sent as such in the same original form without adding any preservative. They are required to be sent in airtight and leak proof containers.

**F)** Highly purified bodies, larvae, maggots and other entomological samples may be preserved by refrigeration for toxicological analysis. They should not be preserved in any fixative. They serve as useful substitute for viscera and blood group identification. For entomological examination larvae is put alive to boiling absolute alcohol and then preserved in 50% ethyl alcohol. This technique keeps the larvae in extended state.

**G)** In case of the suspected death due to rabies, 1-2 cm cube of hippocampus, cerebral cortex, cerebellum and medulla should be preserved in 50% glycerol saline for negri bodies and virus isolation.

The stomach and intestine are opened before they are preserved. The liver and kidney are cut into small pieces to ensure penetration of the preservative. The bottle should not be filled up with the preservative, but it is necessary that the viscera should completely immerse under preservative. As an additional precaution, some preservative (25-50 gm) of sodium chloride or a layer of about 2 cm of rectified spirit (as the case may be) should be added.

**Submission of Samples to the Laboratory:**

The samples taken in sterile and suitable containers should be well stoppered to avoid any leakage and then tied with a string or adhesive tape to resist pressure if developed by gases in the container. Each container should be sealed with sealing wax with a personal authenticated seal. The seal should remain intact and its impression should be legible. A facsimile seal impression should be sent along with the PM or in the cases of stomach wash, blood or samples taken from living persons, it should be sent along with the forwarding letter. If the articles are sealed by the IO, the same procedure should be adopted and the legible facsimile seal impression should be sent along with the forwarding letter. Each bottle should be properly labelled and the label should bear the name of the deceased, PM No. or the case registration number, the name, age, sex of the deceased, approximate quantity of the organ, the date, time and place of autopsy, followed by the signature, rubber stamp indicating place and designation of the doctor. About 25ml of preservative should also be taken, sealed and sent separately for analysis to rule out any poison being present as a contaminant. The sealed bottles are then put in viscera box with padded compartments into which bottles snugly fit. The box is then locked, the key of the box and the specimen of the seal used are put in a separate sealed envelope. The sealed box and the envelope are then handed over to police constable after taking a receipt to deliver those to the forensic laboratory.

**The Important Documents which should be submitted along with the Viscera box are as follows.**

* + 1. A copy of the panchnama and FIR if available, brief facts of the case submitted by police and case sheet viz., name, sex and age of the deceased and time at which PM examination was carried out, an account of final illness and a list of drugs to which the deceased had access.
    2. A copy of the autopsy report with detailed observations and findings. It should also clearly mention the specific name of the poison or drug for the detection, estimation for which the viscera is sent and also mention if any additional analysis is required. It there is any previous analysis done, reference should also be mentioned.
    3. A forwarding letter by the IO requesting to examine the submitted sample and informing the findings to the medical officer and indicating the viscera box is transported by the constable authorized to do so.
    4. An authority letter from a magistrate or relevant officer for chemical examiner to examine the viscera.
    5. If possible the statement of the witness relating the symptoms developed and the behaviour of the deceased before death. This is not mandatory but can save the time and material of the expert.

Once, the viscera samples are preserved for chemical analysis but not sent for analysis, they should be destroyed with the assent of the Magistrate or if the investigating officer informs that the case has been closed or if further analysis is not required.

**12.5 POST MORTEM EXAMINATION REPORT AND OPINION**

It is a signed document containing a written record of post mortem findings and can be used as proof for the cause of death, manner of death and time since death. It help the reader visualize what has been seen at autopsy and how the conclusions have been drawn. Accordingly, it must contain all the positive and not all but important negative findings. It should be an accurate, complete and objective record of all autopsy finding from which legally valid conclusions can be drawn. It should be written in a simple language without using technical terms whenever possible.

The report should state the authority ordering the post mortem, name of the deceased, the date, place and the time the body was received, the date, place and time of commencing and completion of postmortem. The name of the person or the means by which the body was identified. An overall account of external examination, a list of clothings and their conditions, detailed description of injuries including their age. The injuries should be numbered and summarized to provide an overview of the entire injury pattern. This is followed by the internal examination. It contains a detailed description of stomach and its contents and general condition of other organs. If special studies like microscopy and radiograpy are performed, the findings are recorded. Any disease, if present, should be described along with its note in the cause of death. Details of samples, tissues and organs removed and preserved are also recorded along with the name of the preservative used.

This is followed by conclusion as to the cause of death, manner of death and time since death based on autopsy findings. There should be no confusion between the cause and manner of death. The cause of death, means the condition or disease that brought the end of life. The manner of death must fall within any one of the categories viz., [1] natural [2]suicide [3] homicide [4] accident [5] undetermined. The opinion regarding the cause and manner of death should be concise and clear, without which the report has no legal value, i.e., [1] cause of death; meningitis; manner of death: natural [2] cause of death: gun shot injury in the forehead, self inflicted; manner of death: suicide [3] cause of death: asphyxia by throtting; manner of death: homicide [4] cause of death: shock and haemorrhage due to multiple fractures, run over by a truck; manner of death: accident [5] cause of death: indeterminate – no disease, no injury, no poisoning; manner of death: undetermined. The time since death can be determined from body temperature, rigor mortis, PM lividity, progress of putrefaction, state of digestion of food etc. Opinion regarding the cause of death should not include any non-medical facts or discussions. If the opinion is based on the statement of the police, history of the case or due to natural causes, the fact should be mentioned in the report. Other conditions contributing the death should also be mentioned. The opinion is given in the form of a certificate by filling in all its columns. This should be followed by the signature, qualifications and designation of the medical officer.

The postmortem certificate is issued within 24 hours after conducting the postmortem. In cases of poisoning, decomposed bodies or where the cause of death requires further examination such as chemical, histopathological or microbiological investigations, the opinion as to the cause of death is reserved pending such examination or the probable cause of death can be given and final cause of death may be given after receiving the CA or HP reports. In some cases, in spite of a thorough post mortem examination, chemical analysis, histopathologic or microbiological examination, the cause of death cannot be arrived at. Under such circumstances, the medical officer is justified in mentioning the cause and manner of death as undetermined. In such type of cases, the investigating officer may still proceed depending on the circumstantial or physical evidences. In poisoning cases with a positive history, some times, negative analytical findings may be obtained from chemical analyser because of many reasons like [1] the poison may have been vomited out, excreted, neutralized, metabolized, detoxified or the poison is in such small amount which may not be detected by the available methods of chemical analysis, [2] the absorbed poison is not evenly distributed in various organs which varies with different poisons, [3] the distribution of poison may also vary with the mode of administration, [4] if the victim has been treated, the medication may alter the poisonous substance and makes its detection difficult, [5] for the reasons not known, in certain deaths due to intravenous narcotism, sometimes, the victims is found with the needle in the vein, no narcotic is found, [6] delayed deaths, [7] adding irrelevant preservatives in the tissues, or preservation of irrelevant tissues etc; [8] decomposed tissues and [9] if the analysis fails to test the suspected poison. Beside considering such limiting factors, non detection of poisons in PM tissues need not necessarily be interpreted as no poisoning has been occurred. Besides, there are some standard opinions which can be given in various types of cases.

In the cases of snake bites, the doctor should see the fang marks, rule out other causes of death, study the case history where treatment is given and study the circumstantial evidence or information given by the police. In the cases of snake bites, the ‘no poison’ given by the toxicologist rules out the presence of other poisons in the tissues than snake venom. Because, it is not possible to detect the venom by chemical analysis as the venom is destroyed very fast. In the cases of snake bite, the organs or the tissues should not be sent for histopathology as there will be no pathogenic findings.

The doctor must submit the PM report to the investigating officer and a copy on demand to the judiciary department. If the lawyer or relative of deceased demands PM report, as in practice, the doctor can direct them to police. The police can issue a copy of the report after filing the case in court. In some states, the relatives of victims can have a copy of the PM reports from medical officer after depositing the prescribed fee in his office.

**Some standard opinions:**

i) In the cases of snakebites, neurotoxic venom which primarily acts on the motor nerve cells e.g. cobra, krait and other/similar snakes. The neurotoxins of cobra venom produce both convulsions and paralysis while krait venom causes muscular paralysis only. Hence, opinion can be given as “on perusal of post mortem examination, case history and circumstantial evidence, it is opined that the death is due to respiratory failure due to snake bite”.

ii) In the case of viper and other similar types of snakes which have haemolytic venom produces enzymatic destruction of cell walls and coagulation disorders. Other functional disturbances are related to the involved organ e.g. convulsions from haemorrhage in the brain. Hence, if death is due to bleeding, opinion may be given as below.

1. “Death is due to shock and haemorrhage as a result of snake bite”.
2. If there is haemorrhage in brain stem, “death is due to coma as a result of brain stem haemorrhage due to snake bite”.

iii) In drug reaction death:

“Death is due to respiratory failure secondary to laryngeal oedema as a result of drug reaction (anaphylaxis)”.

iv) Traffic accidents or assault cases:

“Death is due to shock and haemorrhage as a result of injuries sustained in accident or assault.

v) Poisoning case:

On the basis of case history, PM examination and the toxicological examination report from forensic science laboratory, “death is due to respiratory failure/cardiorespiratory failure as a result of consumption of organophosphorus compound/organo chloro compound/carbamate compound/barbiturate/alcoholacid/metal poisoning /cardiac glycocide”. Instead of giving class, the doctor should give the exact name of the compound responsible for causing death.

vi) Electrocution:

1. When electricity passes through heart “Death is due to syncope as a result of ventricular fibrillation due to electrocution”.
2. When electricity passes through brain stem “Death is due to respiratory failure (respiration centre of the brain) as a result of electrocution”.

vii) Railway accidents:

1. When there is multiple crush injuries “Death is due to shock and haemorrhage as a result of crush injuries”
2. When there is decapitation “Death is instantaneous as a result of decapitation”.

viii) Natural death e.g., blocking of heart functioning or disease:

1. “on perusal of case history or hospital records, PM examination and histopathology report/microbiology report/biochemical analysis report, the death is due to syncope as a result of myocardial infarction secondary to occlusion of right coronary artery due to arterioscleroses/ embolism/ thrombosis”. Mention the relevant cause for occlusion.
2. On perusal of case history, PM examination and histopathology report/microbiology report, I am of the opinion that the death is due to respiration failure as a result of lobar pneumonia”.

The above standard opinions are just for the knowledge or new comers and the experts working in the field of toxicology or forensic medicines. The style of framing an opinion varies with experience of different experts.

The important points to be remembered at the time of conducting post mortem examination.

i) The doctor should not comment about the “nutritional state” of decomposed body.

ii) The doctor must not comment on “congestion” of organs in early state of decomposition because all the internal tissues show reddish-brown discolouration.

iii) PM staining should not be confused for contusion.

iv) The heart looks hypertrophied because of rigor-mortis and one cannot comment whether heart has stopped in diastole or systole.

v) Rigor-mortis is independent of nervous system, hence it can be seen in paralysed limb and amputed limb.

vi) PM staining over posterior surface of heart must not be mistaken for myocardial infarction changes.

vii) PM staining over posterior surface of lungs must not be mistaken for pneumonia.

In the following cases the doctor must look for the corroboratory suggestive evidences along with the post-mortem findings.

* 1. Epilepsy: Many times, a person dies of asphyxia in an epileptic death. But at autopsy apart from asphyxial signs, there may not have points suggestive of an epileptic attack. In such cases, one should look for the findings like bitten tongue, hypertrophied gums if patient is on phenantion sodium, for foreign body in brain, depressed fracture, scarring of brain, refer hospital records. The urine, blood or organs should be analysed for anti-epileptic drugs.
  2. Bronchial Asthama: The autopsy findings are hypertrophic emphysema of lungs and brown atrophy of heart. Hence, the doctor must concentrate on the history and relevant hospital records and the record of chronic use of anti-asthmatic drugs.
  3. Myocardial infarction death: In such death, the doctor should look for hospital records and laboratory investigations like ECG, enzyme studies (SGOT, SGPT, CPK etc), for arteriosclerotic changes of aorta, valves and coronaries; for coronary narrowing, occlusion, thrombus in coronary etc. If patient dies within six to twelve hours after myocardial infarction, no histopathological changes are seen in the heart. Sometimes, coronary may be within the normal limits at autopsy. In such cases, the death was due to acute coronary spasm. In absence of any marked changes in heart and coronaries, one should conclude the cause of death on clinical diagnosis and hospital record.
  4. Vaginal inhibition deaths: Such death is so sudden that there is no time for the body to react. It can be a result of sudden fear and even by a trivial trauma. There shall not be any specific autopsy findings. The case is concluded as vaginal inhibition death based on the history and circumstantial evidence ruling out other causes of death.

Likewise, no specific post-mortem findings are seen in deaths from ventricular fibrillation due to violent blunt force to chest, electrocution and cerebral concussion deaths. Such cases are also concluded based on the circumstantial evidences ruling out other causes of death.

**12.6 ANALYTICAL ASPECTS OF TOXICOLOGICAL EVIDENCES:**

Analytical toxicology is a branch of forensic science which deals with the extraction, isolation, detection and estimation of various types of drugs and poisons or their metabolites in biological and other materials in low concentration. The direct evidence of poisoning is its detection in various tissues obtained at autopsy or detected in urine, blood and gastric lavage taken in living patients. The detection of a poison in various tissues is the evidence of its absorption. But, this does not apply in the cases of ingestion of corrosive poisons like mineral acids and alkalies, the concentration of which can be determined only on the site of application or on the gastro intestinal contents. In chemical analysis, many basic mistakes may happen due to by inexpert use of techniques, desired cleanliness and use of sub-standard chemicals and equipments. Hence, toxicological analysis should always be undertaken by experienced and properly trained analyst. The analysis is desirable to be carried out in well cleaned, standardized and calibrated equipments and chemicals of highest purity should be used.

The common poisons met within India are insecticides or pesticides, poisonous plants, alcohols and sedatives. Poisoning is mainly indicated by crime scene investigation, prescriptions, medications, history of the case, autopsy findings or negative finding i.e., absence of any disease or sudden deaths etc.

When a specific poison is suspected, direct analysis can be done. This will either confirm the suspicion or rule out the poison. If such analysis is undertaken, the test applied should be specific and the poison confirmed by various techniques and the other substances, which behave similarly should be excluded. In some cases, where no information about the identity of poison is available, the analyst’s approach will vary depending on his knowledge and experience i.e., if a person dies after several days of hospitalization, it would be pointless to do the analysis for volatile toxic substances or most of the organic drugs. During the period, these substances would have been eliminated by the body or completely metabolized. The only toxic substances which could be detected after such a long time are usually metallic poisons like arsenic or other poisons having cumulative effect or some fat soluble substances. When the effort of the analyst fails to detect the substance by direct test, he should proceed systematically and make use of protocol for analysis by dividing the substances into various groups and utilize the various extraction, purification and identification procedures.

A systematic analysis includes:

* + 1. Preliminary examination
    2. Isolation of the poisons
    3. Identification
    4. Quantitation

The details of the above are given separately.

**Toxicological Analysis of Decomposed Materials**

Decomposed viscera leads to many problems of toxicological analysis and difficulties in the interpretation of results. In decomposed bodies, the tissues are softened and liquified by autolysis and bacterial actions release carbohydrates, fats and proteins which are extracted with the extracted materials in abundant amounts and complicates the process of analysis leading to serious difficulties in the interpretation of results. Hence, suitable cleanup procedures should always be applied to remove these materials from the extracts. An analyst should always keep the following points in mind at the time of interpretation of results of analysis of tissues taken from decomposed bodies.

1. Some of the substances undergo chemical changes along with the decomposition of tissues. Hence, such substances are not identifiable by chemical tests e.g. chloral hydrate, sodium nitrite, cocaine, aconite, atropine etc.
2. Some substances are formed in the tissues by decomposition which give similar chemical reaction to those obtained from drugs or chemicals such as neurin, muscarin and mydalein. These reactions may misguide the analyst.
3. Volatile substances are lost as a result of decomposition and cyanide, ethyl alcohol, ketones and sulphides may be formed from normal tissue components. Although the cyanide so formed is always in trace amounts, but the alcohol produced by advanced decomposition may be from 50-100 mg per cent.
4. Despite the decomposition, many toxic substances are still identifiable, e.g. carbon monoxide, cyanide, flouride, barbiturates, phosphates, insecticides/ pesticides, hyosine, strychnine, thevetin, cerberin, nicotine, many other drugs and metallic poisons.

**Toxicological report and Interpretation of the Toxicological Evidence**

A toxicological report should be straightforward in a simple non-scientific language. It is suggested that the analyst should confirm his findings by three different independent and uncorrelated parameters. Whenever possible, three entirely different analytical techniques should be used i.e. colour tests, chromatography (TLC, GLC or HPLC etc.), and spectroscopy (IR or UV).The actual choice of parameters or techniques are left to the discretion of the chemist. Method should be selected in view of the poison involved, resources available and the standard of proof acceptable in the prosecution system within which the chemist works. More complicated and highly sensitive techniques should be avoided in routine analysis. These techniques should be used only when essentially required i.e. when the poison is suspected to be present in a very low concentrations and other less sensitive and simpler techniques fail to detect it. The more complex methods are needed only for certain forensic requirements such as comparison of samples or the development of typology. Secondly, it becomes difficult to explain these techniques in the court of law/ in a non - technical way.

The report of toxicologist usually states those substances which have been found and sometimes their concentrations. However, the toxicologist should have a detailed record of his work including the substances for which negative results were obtained. The record should also include the details of various steps of the procedures, the methodology used, the dates of the work and the test results. For proper interpretation of an analytical result, the analyst requires the proper knowledge about pharmacokinetics and metabolism of drugs and poisons and the technique of reporting as below.

(1) Despite the history of poisoning, poison is not detected in the material. There are several reasons for this apparently contradictory analytical findings:

i) The poison may have been vomited out, excreted, neutralised, metabolised, detoxified or the poison is in such a small quantity that it cannot be detected by the available methods of chemical analysis or the quantity present or extracted is below the detection limits.

ii) The absorbed poison, might have not evenly distributed in various organs which varies with different poisons and also with its mode of administration e.g. blood levels are higher than liver levels when the poison is administered by intravenous route and reverse generally applies when the poison is administered orally.

iii) Sometimes, the amount of material received may be small, but the analyst has to look for all possible types of poisons.

iv) The detection of highly potent toxic i.e. low LD substances is quite difficult.

v) The interference of proteins, fats and decomposition products sometimes make the detection difficult or rather impossible.

vi) The treatment given to the victim may alter the poisonous substance and make its detection difficult or even impossible.

vii) The poison is also not detected if irrelevant tissues are taken or if the tissues are wrongly preserved.

viii) If tissues are sent unpreserved and analysis is taken up after long time, the poison present in the tissues might have decomposed and remains undetected.

ix) For reasons not yet known in deaths due to intravenous narcotism and anesthetics, sometimes even when the victim is found with the needle, with syringe attached still in vein or material is taken and sent with all precautions, no narcotic substance or anaesthetic material is detected considering such limiting factors, non-detection of poison in PM tissues should not necessarily be interpreted as ‘no poison’ having been involved.

(2) The isolated material may be different than the suspected to be consumed. Because certain compounds are altered in the body by biochemical process e.g. detection of phenobarbitone when primidone has been taken and the finding of oxazepam when diazepam has been taken.

(3) Morphine is detected from liver, urine and bile when the person has consumed diacetyl morphine (Heroin). Because the metabolic process degrade the heroin to morphine. So, if the heroin is detected at needle sight, it would be justifiable to say that morphine in the tissue was derived from the usage of heroin.

(4) The metabolic process degrade a small amount of absorbed codeine (methyl morphine) to morphine. Therefore, detection of high concentration of codeine and low concentration of morphine suggests that the origin of morphine is probably codeine.

(5) The findings of high conc. of morphine and a small amount of codeine indicates that the morphine was due to the use of either morphine or heroin along with codeine.

(6) The presence of quinine, methaqualone or any other common adulterant in bile and/ or urine may suggest the possibility of heroin or brown sugar addiction. But this finding should be supported by other circumstantial evidences.

(7) The reaction of a therapeutic dose of a substance may range from slight effect to signs of over dosage. Therefore the so called lethal dose can not be fixed in any mathematical sense. As such, a person can survive after consuming a fatal dose where as a non lethal high doses sometimes results in death. Such results are due to personal variation in response to each individual. Age, weight, sex, state of health, route of administration and the association of other synergic substances also play important part. The lethal dose is the dose that kills in the case in question. It may be quite small in those who are seriously ill or who show idiosyncrasy or allergy to the poison or drug. Such a dose may hardly have any effect in those who are habituated or addicted to the particular substance. What is usually implied by the term “minimum lethal dose” is the smallest dose that has been recorded as fatal to a healthy person of that age. It is normally about ten times the maximum pharmaceutical dose.

(8) Every quantitative measurement has a certain degree of uncertainty and as a result the figure quoted without a probable error can be misleading.

(9) While reporting results, the term “average” should not be used without serious consideration to any individual situation e.g. such terms can only be used if all samples lose equal amount of drugs with negligible variations after preservation. But in actual practice, some samples lose more and some hardly lose any amount. Legally, if the term is average, if used, indicates that every sample loses the same amount,

(10) Normal values are averages of many observations. Therefore, when a substance that is normally not present in the body is isolated, it indicates exposure to the substance. But when a substance that is normally present in the body is isolated, it becomes necessary to consider a range of values before interpreting the result as exposure to the toxic levels of this substance i.e. quantitative analysis is required in such cases.

(11) There are many poisons like proteinous poisons, once absorbed are rather impossible to extract and detect from tissues by chemical methods of analysis. Similarly, the extraction of water soluble compounds is very difficult.

It is the responsibility of the medical officer to determine the cause of death and the time elapsed since death. The toxicologist can only state that a potentially toxic substance was taken and also estimate the quantity taken. If this is in the range of a toxic or lethal dose and if all other clinical and pathological findings fit, then it can be said that poisoning is the cause of death. It is often possible to give a rough estimate of the likely survival time after exposure to fatal amount of various toxic materials.

**12.7 LAW RELATING TO POISONS:**

A Poison may be defined as a substance which, when administered, inhaled or swallowed is capable of acting deleteriously on the body. Thus, there is no boundary between a medicine and a poison, a medicine in a toxic dose is a poison and a poison in small dose may be a medicine. In law, the real difference between a medicine and a poison is the intention with which it is given. Section 284 IPC lays down the punishment for careless handling of poisonous substances, while sections 299, 304 A, 324, 320 and 328 deal with offences relating to administration of such substances. In law, there is no difference between the murder by poison and other means. The punishment can be imprisonment up to ten years and also fine. The negligent conduct with respect to poison resulting in damage to a person is punishable with imprisonment of six months or with fine up to Rs.1000 or both.

**Sale of Poisons:**

1. The sale of poisons is controlled by the Poisons Act, the Drugs Act and the Pharmacy Act.

2. The Poison Act (12 of 1919) was designated to regulate the import, possession and sale of poison.

3. The Dangerous Drugs Act (2 of 1930) was passed to regulate the import and sale of narcotic drugs, especially those derived from opium, Indian hemp and coca leaf.

4. The Drug Act (23 of 1940) was passed to regulate the import, manufacture and sale of drugs like:

i) Patent medicines

ii) Vaccines, sera, toxins and other biological products

iii) Vitamins and hormones

Also other drugs used for internal or external administration to human beings or animals.

The Act was amended in 1955 to bring within insecticides, disinfectants and contraceptives and further amended by Drugs (Amendment) Act, 1962 (21 of 1962) to include cosmetics. The Act has been further amended by Drugs (Amendment) Act, 1964 (13 of 1964) to include Ayurvedic and Unani drugs.

5. The Drugs and Cosmetics Rules, 1945: Under this Act, the rules were framed to regulate import of drugs, functions and procedures of the central drugs laboratory, appointment of licensing authorities and manufacture, distribution and sale of drugs under these rules. The drugs are classified into certain schedules and regulations are laid down for their storage, display, sale, dispensing, labeling, prescribing etc. The drugs are classified as below:

Schedule C drugs – Biological and special products

Schedule E drugs – Poisons

Schedule F drugs – Vaccines and sera.

Schedule G drugs – Hormone preparations

Schedule H drugs – To be sold only on the prescription of a

registered medical practitioner.

Schedule J drugs – List of diseases for the cure of which no drug should be advertised.

Schedule L drugs – Antibiotics, antihistamines and chemotherapeutic agents.

The Pharmacy Act, 1948: Regulates pharmacy profession and state pharmacy council.

6. The Drug Control Act, 1950: Regulates, control, sale, supply and distribution of drugs etc.

7. The Drugs and Magic Remedies Act, 1954: Bans the offending advertisements, aims at preventing medication etc.

8. Narcotic Drugs and Psychotropic Substances Act, 1985: This Act consolidates and amends the existing laws relating to narcotics drugs, drugs of abuse etc.

**Medico Legal Aspects of Drugs and Poisons:**

* 1. The pharmacist must record in a prescription register the particulars of drugs supplied on doctors prescription i.e. serial number of entry and date of supply, name and address of prescriber, name and address of patient, name of drug, quantity, name of manufacturer and expiry date, signature of the pharmacist.
  2. Schedule H and L drugs are to be sold on prescription by a registered doctor.
  3. The doctor should take care in treating poisoned patient and should assist the police.
  4. He should collect relevant specimens. Failure to collect specimens are punishable under section 201 IPC.
  5. As a private practitioner, maintain professional secrecy and when sure, accidental and suicidal poisoning may not needed to be informed. But all specimens should be collected . If the patient becomes serious , arrangement should be made to record dying declaration and PM examination , if patient dies. If the police approaches the doctor for information , he must reveal under section 175 Cr.P.C. If he conceals information then such an act is punishable under section 193 I.P.C.
  6. As per section 39 of Cr. P.C; a private doctor must inform immediately a homicidal poisoning case. Failure to inform is punishable under 176 I.P.C.

* 1. A Government doctor must inform all cases of poisoning, may be accidental, suicidal or homicidal.

**12.8 APPENDICES**

**No.1 SPECIMEN LETTER**

**Specimen letter requesting for authorisation from Magistrate/Police Officer for chemical analysis of viscera**

Letter No.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Hospital

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Dated \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

To

The Sub/Circle Inspector of Police

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sir,

Sub: Chemical analysis of material objects – Regarding.

Ref: 1. Crime No. of the case \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

2. Postmortem No./I.P.No.(or other reference) \_\_\_\_\_\_\_\_\_\_\_\_\_

Material objects were collected from the above referred case and are kept under safe custody. Chemical analysis of the articles is necessary to arrive at a final opinion. I request you to kindly give me the authorisation and depute a police constable for the transmission of the material objects to the Chemical Examiner.

Yours faithfully,

Name \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Designation \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

N.B:

1. In some states the autopsy surgeons (Medical Officers) are authorized to sent the exhibits directly to FSL.
2. In some states the exhibits are forwarded by the law enforcing agencies through the Judicial authority.
3. In some states the above format is applicable.
4. Or the exhibits may be forwarded by any of the above modes.
5. Whatever be the case there should be proper authorization by the Judicial authority or any competent authority as per rule of the state.
6. **No.2**

**Specimen label to be affixed to containers of viscera/body fluids**

**for chemical analysis**

I. Hospital \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Place \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Postmortem No. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Dated

Name of the deceased \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Age \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ sex\_\_\_\_\_\_\_\_\_

Nature of Specimen - Stomach and part of intestine with contents/or any other part

as applicable (to be stated)

Preservative used - Rectified spirit/saturated saline

Case reference:

Signature of M.O. with date

II. Hospital \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Place \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Postmortem No. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Dated

Name of the deceased \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Age \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Sex\_\_\_\_\_\_\_

Nature of Specimen - Part of liver and one kidney or half of each kidney/ or any

other portion as applicable (to be stated)

Preservative used - Rectified spirit/saturated saline

Case reference:

Signature of M.O. with date

III. Hospital \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Place \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Date \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Postmortem No. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Name of the deceased \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Age \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Sex\_\_\_\_\_\_\_\_\_

Nature of Specimen - Blood

Preservative used - Sodium fluoride

Case reference:

Signature of M.O. with date

IV. Hospital \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Place \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Date \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Postmortem No. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Name of the deceased \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Age \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Sex\_\_\_\_\_\_

Nature of Specimen - Urine

Preservative used - Sodium fluoride

Case reference

Signature of M.O. with date

**No.3**

**Specimen letter from Medical Officer to the chemical examiner requesting for chemical analysis to be done in a particular case**

Letter No./ Memo No.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Dated\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Hospital

To

The Chemical Examiner to the Government \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sir,

I am forwarding herewith the material objects mentioned below through (name) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ P.C. No.\_\_\_\_\_\_\_\_\_\_\_\_\_\_ for chemical examination and certificate. The relevant details of the case are given below. I request you to kindly analyse the materials and forward the report to me at the earliest.

Yours faithfully,

Signature :

Name and Designation:

Date-

**Relevant particulars**

Postmortem No. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Dated \_\_\_\_\_\_\_\_\_\_\_\_

Name of the deceased \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Age \_\_\_\_\_\_\_\_\_\_\_Sex\_\_\_\_\_\_\_\_

Crime No.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ of \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ P.S.

Bottle No. 1. Stomach with contents and part of intestine with contents

2. Part of liver and kidney

3. Blood

1. Urine
2. Sample of the preservative used (rectified spirit/saturated saline)

**Mode of packing Specimen Seal**

(here enter the details)

**Information furnished by the Police**

(here enter a brief history of the case)

**Clinical history and treatment given**

(if the deceased was under treatment prior to death, time of admission, time of death, details of drugs given and the diagnosis etc., should be entered or copy of the bed ticket may be enclosed)

**Postmortem appearance**

(detailed information of the postmortem appearance, especially findings related to the stomach and intestines and their contents, changes in the mucosa etc., should be furnished).

**Examination required**

(Here enter the type of analysis required, whether it is qualitative or quantitative and also the nature of the poisoning suspected).

**Priority required or not**

(if it is homicide or if the case is pending forinvestigation, priority can be requested).

Enclosure : (A copy of the label)

Sample of the seal: (here a sample of the lac seal should be affixed)

**No.4**

**Specimen forwarding letter to be sent by the Medical Officer to the Director of a Forensic Science Laboratory in connection with examination of trace evidence (hair, stains etc.,) in a particular case**

**C E R T I F I C A T E**

Certified that the Director, Forensic Science Laboratory \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ is the authority to examine the exhibits sent to him in connection with the case of State versus \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ u/s \_\_\_\_\_\_\_\_\_\_\_\_ of \_\_\_\_\_\_\_\_\_ police station and if necessary to make them to pieces or remove portions for the purpose of the said examination.

Place \_\_\_\_\_\_\_\_\_\_\_\_\_\_ Signature and designation of

Date \_\_\_\_\_\_\_\_\_\_\_\_\_\_ the forwarding authority

Seal \_\_\_\_\_\_\_\_\_\_\_\_\_\_

**I. Nature of crime**

**II. List of articles sent for examination**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sl  No | Description of exhibits | How, when and by whom found | Source of exhibits | Remarks |

**III. Nature of examination required**

Forwarded to :

The Director

State Forensic Science Laboratory

Specimen seal impression Signature of forwarding authority

Designation of forwarding authority

### No.5

# P.M.EXAMINATION REPORT

Observation and points to be covered.

1. P.M. No dated \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

2. Brief history (specially covering points viz. time of death, exhibits collected at the scene of crime, if any; profession of deceased, whether meal/drinks/water taken or not, when last found etc.)

3. Description of viscera (portions collected)

1. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
2. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
3. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
4. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Viscera of -------------------------- age-------sex------------

In case of animal viscera: Name of animal--------------, colour------- , age-------

4. Name of preservative used \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

5. Weight of parcel / Exhibit \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

6. Copy of label & lac seal (specimen to be affixed)

7. Mode and date of dispatch.

Information on animal \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Name \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Age \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Colour \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_, etc.

8. Appearance of body \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

9. Sign of decomposition \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

10. Wounds and Bruises \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Position\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Character\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Size \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

11. State of natural orfices \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Give details\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

(Eyes, ears, nostrils, ex. Ejection, salivation, frothing, blood, if any, mouth, vagina.)

12. State of limb\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

13. Rigor mortis \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

14. Position of: the following organ / parts

Hands \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Eyelids \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Pupils \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Mouth \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Tongue \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Pleura \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

15. Heart : Shape & appearance

16. Lungs \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ colour,

Consistence \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

17. Position of larynx.

18. Condition of liver, spleen, kidneys, intestine (Condition of wall, congestion, if any)

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

19.Stomach ( Description of colour, smell of contents to be given)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

20. Generative organs \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

21. Condition of spinal Cord \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

22. Condition of Head \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

23. Fractures and dislocations \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

24. Details of injury and disease. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

25. Opinion as to the cause of death/ Health.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Signature of Autopsy surgeon

Date :

**12.9 Major Metabolites of Poisons and Instrumentation used in the Detection and Estimation of various Poisons**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl**  **No** | **Poison** | **Metabolite** | **Instrumentation method used** |
|  | **I. INSECTICIDES** | | |
| 1. | Parathion | p-nitrophenol | U.V. spectrometry in n. Hexane max = 274 mu |
| 2. | Malathion | Malathion mono acid | U.V. spectrometry of hydrolysed product after complexing with Cu+2  max = 418 mu |
| 3. | Dimethoate (Rogor) | Hydrolysed product | T.L.C. |
| 4. | Diazinon | 1.Diethyl phospho-  rothionic acid.  1.Diethyl phosphoric  acid. | U.V. spectrometry of hydrolysed product pyrimidinol, whose  max in 95% EtoH is 272 mu |
| 5. | Endrin | --- | U.V. spectrometry max = 224 mu |
| 6. | Carbaryl (Sevin) | --- | U.V. spectrometry of hydrolysed product diazotised with p-nitro  aniline in alkaline medium max = about 590 mu |
| 7. | Isopropoxur (Baygon) | --- | U.V. spectrometry max = about 500 mu |
|  | **II. VOLATILE POISONS** | | |
| 1. | Ethyl alcohol | Acetic acid, carbon  dioxide water | Gas Chromatograph/Volumetry |
| 2. | Methyl alcohol | Formaldehyde,Formic acid, carbon dioxide, water | 1.Gas Chromatograph  2.U.V spectrometry The oxidised product formaldehyde forms a violet complex with chromotropic acid whose absorption max is 570 mu |
| 3. | Chloral hydrate | Trichloroethanol | Colorimetry, Gas Chromatography |
| 4. | Cyanide as hydrocyanic  acid | --- | Volumetric |
|  | **III. PLANT POISONS** | | |
| 1. | Oleander | Cerberin hydroxyl derivative  Theretoxin hydroxyl derivative  Nerifolin hydroxyl derivative  Folineum hydroxyl derivative  Thevetin hydroxyl derivative | Thin layer chromatography |
| 2. | Madar | Hydroxy uscharin  Uscherin glucoronic acid  Calactin(Hydroxy derivative)  Calotropin  Gigantin | Thin layer chromatography |
| 3. | Oduvan | --- | Thin layer chromatography |
| 4. | Strychnus Nuxvomica |  | Thin layer chromatography |
| 5. | Atropa Belladona | Atropine glucuromide  Tropic acid glucuromide  Esters of tropine and tropic acid | Thin layer chromatography |
|  | **IV. DRUGS**  **1. barbiturates** | | |
| 1. | Phenobarbitone | p-hydroxy phenobarbitone | 1. U.V. abs in 2N NH4OH  max = 240 mu which shifts on changing pH  2. T.L.C. |
| 2. | Heptabarbitone | Cyclopentenone | 1. U.V. abs in 2N NH4OH  max = 240 mu which shifts on changing pH  2. T.L.C. |
| 3. | Quinalbarbitone | Hydroxyquinol-barbitone | 1. U.V. abs in 2N NH4OH  max = 240 mu which shifts on changing pH  2. T.L.C. |
| 4. | Pentobarbitone | Hydroxy pento-barbitone | 1. U.V. abs in 2N NH4OH  max = 240 mu which shifts on changing pH  2. T.L.C. |
| 5. | Butobarbitone | Hydroxy buto-barbitone | 1. U.V. abs in 2N NH4OH  max = 240 mu which shifts on changing pH  2. T.L.C. |
| 6. | Amylobarbitone | Hydroxy amylo-barbitone | 1. U.V. abs in 2N NH4OH  max = 240 mu which shifts on changing pH  2. T.L.C. |
|  | **2. BENZODIAZEPINES** | | |
| 1. | Diazepam | Oxazepam | 1. U.V. abs in 2N HCl. Max = 242 mu & 287 mu  2. T.L.C., Dragendorff spray |
| 2. | Chlorazepam | Lactum derivative oxazepam | 1. U.V. abs in 0.1N NaOH. Max = 243 mu  2. T.L.C., Iodoplatinate spray |
| 3. | Oxazepam | Glucuronide conjugate | 1. U.V. abs in ethanol, Max = 230 mu  2. T.L.C., Dragendorff spray |
| 4. | Nitrazepam | 7-amino metabolite and 7-acetylamino metabolite | 1. U.V. abs in ethanol, Max = 218 mu & 260 mu  2. T.L.C., Dragendorff spray |
|  | **3. PHENOTHAZINES** | | |
| 1. | Chlorpromazine | Mostly 7-hydroxy derivative | 1. U.V.abs in 0.1N H2SO4 max=255mu  1.T.L.C.:1. F.P.N reagent  2.Iodoplatmate spray  3.Sulphuric acid/EtOH spray |
| 2. | Promethazine | Promethazine sulphoxide | U.V. Abs in EtOH/water (1:1) mix, /EtOH  Max = 252 mu  T.L.C.: 1. FPN Spray  2.Iodoplatinate spray  3.Sulphuric acid-EtOH spray |
| 3. | Trifluoperazine | --- | 1. U.V. abs in 0.01N HCl Max = 256 mu  2. T.L.C.= 1.F.P.N. Spray  2.Iodoplatinate spray  3.Sulphuric acid/EtOH spray |
|  | **V. TOXIC CATIONS** | | |
| 1. | Arsenic | --- | Spectrography, ICP-AAS  A.A.S  Volumetric |
| 2. | Mercury | --- | 1.Spectrography  2.A.A.S  3.Volumetric  4. ICP-AAS |
| 3. | Copper | --- | 1.Spectrography  2.A.A.S  3.Volumetric  4. ICP-AAS |
|  | **VI. TOXIC ANIONS** | | |
| 1. | Phosphide (as zinc or aluminium phosphide) | --- | Visible spectrophotometry |
| 2. | Nitrite (as sodium nitrite) | --- | Visible spectrophotometry; Ion Chromatography |
| 3. | Chlorate (as potassium chlorate) | --- | Volumetric, Ion Chromatography |