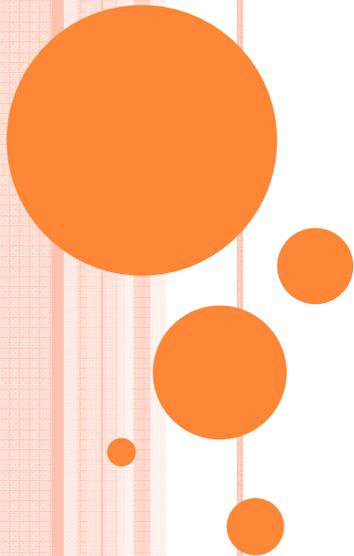


# **ANALYTICAL TOXICOLOGY**

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**2009**



# INTRODUCTION

- For many years, **Toxicology or the science of poisons and poisoning**, was considered to be no more than a branch of forensic science and criminology.
- Nowadays, it is clear that study of applied toxicology in its various forms- **clinical, occupational, forensic, nutritional, veterinary, and environmental toxicology**- is important to the continued development of life.
- Accidental or suicidal poisoning, the abuse of narcotic and psychotropic drugs, the dangerous habit of glue-sniffing, and drug abuse in sport (“doping”) have increased enormously during recent years.



- **Analytical toxicology** services which provide **support for diagnosis, prognosis, and management of poisoning.**
- So, analytical toxicology is planned to provide the means for **detecting, identifying, and quantifying toxic substances or poisons** which are likely to be encountered in these situations, and to assist in interpreting the analytical results.



- Analytical toxicology is important for use in hospital and toxicological laboratories, but also in numerous other analytical establishments- including hospital, anti doping, and industrial quality control laboratories, and clinical laboratories engaged in drugs and poisons investigations.
- In developed countries, analytical toxicology may be provided by a specialized laboratory attached to a clinical toxicology unit, by a hospital biochemistry laboratory, an pharmaceutical analysis ( or drugs analysis ) unit, a department of forensic medicine, or a government health or forensic science laboratory.



- Analytical toxicology is different with forensic toxicology. Forensic toxicology covers any application of the science and study of poisons to the elucidation of questions that occur in judicial proceedings. The subject is usually associated with work for the police, the coroner, and the criminal law courts.
- However, the analysis and identification of “controlled” drugs and poisons are all aspects of forensic toxicology.
- Accidental self-poisoning and attempted suicide cases are generally the responsibility of the clinical toxicologist, analytical toxicologist, or hospital biochemist, working in conjunction with a poison control centre.
- A small proportion of these cases is referred to the forensic toxicologist, either because of an allegation of malicious poisoning, or because the patient dies and a coroner’s inquest is ordered.



- Poison or toxic substance is defined as a chemical substance harmful to living organism, it is obvious that “harmfulness” is not a property that can be measured by any chemical method of analysis.
- Toxicity is a biological concept, usually determined by some form of bioassay.
- The most commonly used in assessment of toxicity is the measurement of short term lethally or acute toxicity.
- For given substance this involves determining the median concentration which is lethal to a fixed proportion, usually 50%, of a test population of organisms after continuous exposure for fixed time, usually 48 or 96hrs ( $LC_{50}$  or  $LD_{50}$ ). A small  $LD_{50}$  should be indicated a highly toxic substance.
- Others assessment of toxicity include a chronic toxicity test at long-term and assessment of teratogenic, carcinogenic, and mutagenic effects.

- The ultimate objective of any analytical toxicologist is attempt to provide answers to questions that may arise during investigations.
- **The traditional questions which must be answered are:**
  - Has this person been poisoned ?.
  - If the result is positive, What was the nature of the poison?.
  - How was it administered?
  - Was it dangerous or lethal amount?.



# CURRICULA

- **Introduction**
- **The clinical aspect of analytical toxicology**
- **Classification of Poisons and Group test**
- **Collection of samples** (biological samples, food and drink, tablets and capsules, and syringes).
- **Extraction and Pretreatment of samples,**
- **Analytical requirements:**, Laboratory management, Analytical probing, Rapid detection for poison.
- **Final Semester Examination (UAS I)**



# BIBLIOGRAPHY

- Flanagan R.J., Braithwaite R.A., Brown S.S., Widdop B., and de Wolff F.A., “**Basic analytical toxicology**”, World Health Organization, Geneva, 1995.
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## 2. CLINICAL ASPECTS

- Analytical toxicologist can play a useful role in management of patients poisoned with drugs or other chemicals.
- The analyst must have basic knowledge of emergency medicine and intensive care, and must be able to communicate with clinician.
- A good understanding of pharmacology, toxicology, some knowledge of active compounds metabolism, and the use of antidote are desirable.



# 2.1. DIAGNOSIS OF ACUTE POISONING

## 2.1.1. Establishing a diagnosis.

- In the case of a comatose patients, the circumstances in which the patient was found and whether any tablet bottles or other containers were present can be important.
- If the patients is awake, he/she should be questioned about the presence of poisons in the home or workplace.
- The patient's past medical history, occupation, and hobbies may also be relevant, since they may indicate possible access to specific poisons.



- Physical examination of the patient may indicate the poison or class of poison involved. For example, the combination of pinpoint pupils, hypersalivation, incontinence and respiratory depression suggest poisoning with a cholinesterase inhibitor such as an organophosphorus pesticide.
- Many drugs have similar effects on the body, while some clinical features may be the results of secondary effects.
- Diagnoses other than poisoning must also be considered. For example, coma can be caused by cerebrovascular accident or uncontrolled diabetes.
- The availability of the results of biochemical and haematological test are important in these circumstances.
- Poisoning with certain compounds may be misdiagnosed especially if the patient presents in the later stages of episode. Example: cardiorespiratory arrest by cyanide.



## 2.1.2. CLASSIFICATION OF COMA

- Coma or loss of consciousness is common in acute poisoning, especially if central nervous system (CNS) depressants are involved.
- A simple system, the Edinburgh scale is often used to classify the depth or grade of coma of poisoned patient. This system has the advantage that the severity of an episode can be easily described in conversation with laboratory staff and with poisons information services that may be consulted for advice.



# CLASSIFICATION OF COMA USING THE EDINBURG SCALE

Grade of Coma	Clinical features
1	Patient drowsy but responds to verbal commands
2	Patient unconscious but responds to minimal stimuli (for example: shaking, shouting)
3	Patient unconscious and responds only to painful stimuli (for example: rubbing the sternum)
4	Patients unconscious with no responds to any stimuli

## 2.2. TREATMENT OF ACUTE POISONING

- If the poison has been inhaled, the patient should first be removed from the contaminated environment.
- If skin contamination has occurred, contaminated clothing, should be removed and the skin washed with an appropriate fluid usually water.
- If the poison has been ingested, gastric aspiration and lavage are often performed (in adult). In children, emesis can be induced by oral administration of ipecac syrup, to minimize the risk of continued absorption.
- The poison can be minimized by oral administration of activated charcoal.
- Most patients can be treated successfully using supportive care.



- **Antidote/protective agents.**

Specific therapeutic procedures such as antidotal therapy is sometime indicated. But analytical toxicological results may be required before some treatments are commenced because they are not without risk to the patients.

Antidotes or protective agents are only available for a limited number of poisons.



# • ACTIVE ELIMINATION THERAPY

There are four main methods of enhancing elimination of the poison from systemic circulation:

1. Repeated oral administration of activated charcoal
2. Forced diuresis with alteration of urine pH
3. Peritoneal dialysis and haemodialysis
4. Haemoperfusion



## 2.3. THE ROLE OF THE ANALYTICAL TOXICOLOGY

- Most poisoned patients can be treated successfully without any contribution from the laboratory other than routine clinical biochemistry and haematology.
- Toxicological analysis can play a useful role if the diagnosis is in doubt, the administration of antidotes or protective agents is contemplated or the use of active elimination therapy is being considered.
- The analyst's dealing with case of poisoning are usually into pre-analytical, analytical, and post analytical phase.
- Test for any poisons that the patient is thought to have taken and for which specific therapy is available will normally be given priority over coma screening.



# 3. CLASSIFICATION OF POISONS

- Drugs and poisons can be classified **pharmacologically** ( antidiabetic, anticonvulsant, etc, or by **chemical structure** (alkaloids, barbiturates, phenothiazines, opiates, sulfonamides, benzodiazepines, etc).
- However, for analytical purposes it is more useful to classify poisons **according to the methods use for extraction.**
- **Seven major** groups are usually considered:



- **Group 1: Gaseous substances**, isolated by diffusion or distillation.
- **Group 2: Volatile substances**, isolated by diffusion or distillation.
- **Group 3: Drugs or non-volatile substances**, isolated by solvent extraction.
- **Group 4: Pesticides**, isolated by solvent extraction.
- **Group 5: Metallic poisons**, isolated by ashing or by wet oxidation of organic matter.
- **Group 6: Toxic anions**, isolated by dialysis or extraction.
- **Group 7: Miscellaneous poisons** which require special extraction techniques such as ion-exchange columns, formation of derivatives or ion-pairs, freeze-drying and continuous extraction with a polar solvent. The most common are cannabinoids, digoxin, and insulin.



# GROUP 1 POISONS: GASES

- The presence of this group of poisons is usually indicated by circumstances of the incident.
- For examples: Some compounds which are gases at normal temperature and pressure can be detected by head-space gas chromatography. The gas chromatography can detect butane, ethane, methane, nitrous oxide, propane, and low boiling point fluorocarbons.
- Only two poisons require routine exclusion, these are cyanide and carbon monoxide.



# GROUP 2 POISONS: VOLATILE COMPOUNDS

- Rapid onset of symptoms, followed by serious illness or death is the most valuable clue to the presence of this groups.
- This groups includes alcohols ( methanol, ethanol, etc), aldehydes, ketones, benzene, toluene, acetone, halogenated hydrocarbons and the "glue-sniffing" compounds.



# GROUP 3 POISONS: DRUGS (SOLVENT SOLUBLE)

- This group includes : analgesics, opiates or synthetic narcotics, sedatives and hypnotics, stimulants, antidepressants, etc.
- In general, postmortem stomach contents, blood, and urine samples may be analyzed. Analysis can also be performed for drug overdose, for suspicious deaths or criminal cases.
- Extraction can be carried out by using Stas-Otto Methods, or general solvent extraction, modified by a few additional clean-up procedures.



# GROUP 4 POISONS: PESTICIDES

- Pesticides include insecticides, fungicides, herbicides, rodenticides, nematocides, molluscides, and acaricides.
- Pesticides cover a wide variety of substances which is used to destroy undesirable life forms.
- The common names approved by the British Standards Institution (BSI) and the International Organization for Standardization (ISO) have been used for convenience and brevity. IUPAC names can be obtained from the Pesticides Manual.



# GROUP 5 POISONS: METALLIC POISONS

- Metallic poisons should be checked as a matter of routine if vomiting and diarrhoea are noted as symptoms.
- The commonest poisons are: arsenic, antimony, lead, cadmium, lithium, mercury, thallium, chromium, selenium, etc.
- The Reinsch test will detect only seven of twenty potentially toxic metals.



# GROUP 6 POISONS : ANIONS

- Most commonly-encountered anions may be found in biological fluids, albeit at low concentrations.
- Possible sources for excessive concentrations of some anions of toxicological significance are: borate, bromide, chlorate, cyanide, fluoride, hypochlorite, iodide/iodine, nitrate, nitrite, oxalate, phosphate, sulfate, sulfide, and thiocyanate.
- Qualitative test for anions may be performed on stomach washing, gastric contents, or vomit.



# 4. COLLECTION, AND STORAGE OF SPECIMENS

- Before starting an analysis, it is important to obtain information about the patient (medical, social and occupational history, treatment given, and the results of laboratory or other investigation, etc).
- It is also important to be aware of the time that elapsed between ingestion or exposure and the collection of samples, since this may influence the interpretation of results.
- All relevant information about patient should be recorded in the request form.



- Specimens sent for analysis must be clearly labelled with the patient's full name, the date and time of collection, and the nature of the specimen.
- Containers of volatile materials should be packaged separately from biological specimen to avoid the possibility of cross-contamination.
- All biological specimen should be stored at 4°C prior to analysis. If possible any specimen remaining after the analysis should be kept at 4°C for 3-4 weeks, in case further analysis are required.
- In many case (e.g. if the patient dies) any specimen remaining should be kept at -20°C until investigation of the incident has been concluded.



# 4.1. URINE

- Urine is useful for screening test as it is often available in large volume and usually contains higher concentration of drugs and poisons than blood.
- A 50 ml sample is sufficient for comprehensive series of tests, and no preservative should be added.
- The sample should be collected as soon as possible, ideally before any drug therapy (e.g. diuretics, tranquillisers, tricyclic antidepressants) have been administered.
- If the sample is obtained by catheterization, it may be contaminated with the catheter lubricant which contains lignocaine.
- If syrup of ipecac has been given to induce emesis, there is a possibility of emetine being present in the urine.



## 4.2. STOMACH CONTENTS

- The samples include vomit, gastric aspirate, or stomach washings.
- At least 50 ml of sample is required to carry out a wide range of tests, and no preservative should be added.
- If it is obtained soon after the overdose was taken it may be possible to recognise the presence of undegraded tablets or capsules.
- An immediate clue may be obtained by detecting the characteristic odour and smell of certain compounds.
- This can be a very variable sample and additional procedure such as homogenization followed by filtration or centrifugation may be required to obtain a fluid amenable to analysis.



## 4.3. BLOOD (PLASMA OR SERUM)

- Blood is normally reserved for quantitative assay, but for some poison such as carbon monoxide and cyanide whole blood has to be used for qualitative tests.
- Samples which should be taken on admission of the patients are 10 ml of heparinised blood, 2 ml fluoridated blood, and 10 ml of blood without preservative or anticoagulant.
- The use of disinfectant swabs containing alcohols, and of heparin containing phenolic preservative should be avoided.
- The sample should be dispensed with care to avoid haemolysis. Any drugs administered for treatment of the poisoning must be reported to the laboratory.



## 4.4. SCENE RESIDUES

- It is important that all bottles or other containers and other suspect materials found with or near the patients (scene residues, including food and drink) are retained for analysis if necessary since they may be related to the poisoning episode.
- There is always the possibility that the original contents of containers have been discarded and replaced either with innocuous material or with more noxious ingredients such as acid, bleach or pesticides.
- Note that it is always best analyse biological specimens in the first instance if possible.



## 4.5. PHYSICAL EXAMINATION

- High concentration of some drugs or metabolites can impart characteristic colour to urine.
- Deferoxamine or methylen blue gives in treatment may colour red or blue respectively.
- Strong smelling poisons such as camphor, ethchlorvynol and methyl salicylate can some times be recognized in urine since they are excreted in part unchanged.
- Sulfonamides may give rise to yellow or greenish brown crystals in neutral or alkaline urine.
- Colorless crystal of calcium oxalate form at neutral pH after ingestion of ethylene glycol.



# SOME POSSIBLE CAUSE OF COLORED URINE

Color of Urine	Possible caused
Brown or black	Nitrobenzene, phenols, rhubarb
Yellow or orange	Cascara, fluorescein, nitrofurantoin, senna
Wine red or brown	Aloin, phenothiazines, quinin,
Blue or green	Amitriptyline, indometacine, phenols



# CHARACTERISTIC SMELL ASSOCIATED WITH POISONS

Smell	Possible cause
Bitter almonds	Cyanide
Fruity	Alcohols, esters
Garlic	Arsenic, phosphorus
Mothballs	Camphor
Pears	Chloral hydrat
Petrol	Petroleum distillate
Phenolic	Disinfectants, phenols
State tobacco	Nicotine
Shoe polish	Nitrobenzene
Sweet	Choloroform and other halogenated hydrocarbons



## 5. DIRECT TEST ON URINE, STOMACH CONTENTS AND BLOOD (OVERDOSE)

- The 9 qualitative test described are based on simple color reaction and cover a number of important drugs and other poisons.
- Other test such Reinsch test for metallic poisons (arsenic, mercury, antimony, etc) are not appeared in this chapter.
- Test for salicylate is best performed on urine rather than stomach contents or scene residues. Test for chlorates and other oxidizing agents and for ferrous or ferric iron can only be carried out on stomach contents or scene residues



## 5.1. SALICYLIC ACID (INCLUDING ASPIRIN)

- Add 100  $\mu\text{l}$  of Trinder's reagent (a mixture of 40 g mercuric chloride in 850 ml of water and 120 ml 1 N hydrochloric acid , and 40 g of hydrated ferric nitrate, diluted to 1 L with water) to 2 ml of urine and mix for 5 seconds: Violet color indicates the presence of salicylate.
- If only stomach contents or scene residues are available, hydrolyse by heating with 0,5 N HCl on boiling water bath for 2 minutes and neutralize with 0,5N NaOH before performing the test.



## 5.2. PHENOTHIAZINES

- Add 1 ml of PFN reagent (a mixture of 5 ml of aqueous ferric chloride solution (50 g/L), 45 ml of aqueous perchloric acid (200 g/kg) and 50 ml of aqueous nitric acid (500 ml/L), to 1 ml of sample and mix for 5 seconds: Color ranging from pink to red, orange, violet, or blue suggest the presence of phenothiazines.
- Positive results should be confirmed by thin layer chromatography (TLC)



## 5.3. IMIPRAMINE AND RELATED COMPOUNDS.

- Add 1 ml of Forrest reagent (a mixture of 25 ml of aqueous potassium dichromate (2 g/L), 25 ml of aqueous sulfuric acid (300 ml/L), 25 ml of aqueous perchloric acid (200 g/Kg) and 25 ml aqueous nitric acid (500 ml/L) to 0,5 ml of sample and mix for 5 seconds: A yellow-green color deepening through dark green to blue indicates the presence of imipramine or related compounds.
- Positive results should be confirmed by TLC.



## 5.4. TRICHLORO COMPOUNDS

- Add 1 ml of sodium hydroxide solution (5 M) and 1 ml of pyridine ( Fujiwara test) to 1 ml of urine or sample, mix carefully and heat in a boiling water bath for 2 minutes. An intensive red/purple in the pyridine layer indicate ingestion of trichloro compounds ( including chloral hydrate, chloroform, dichloralphenazone, and trichloroethylene).
- A blank urine or water and solution of trichloroacetic acid should be tested at the same time.



## 5.5. PARACETAMOL, AND PHENACETIN

- Add 0.5 ml of concentrated HCl to 0.5 ml of sample, heat in a boiling water bath for 10 minutes and cool. Add 1 ml of aqueous o-cresol solution (10 g/L) to 0.2 ml of hydrolysate, add 2 ml ammonium hydroxide solution (4 M) and mix for 5 seconds. A strong blue to blue black color which forms immediately, indicates the presence of paracetamol or phenacetine ( o-cresol/ammonia test).
- The test is very sensitive and can detect in therapeutic concentration.
- If a positive result is obtained in this test, carry out a quantitative assay for paracetamol on plasma or serum.



## 5.6. PARAQUAT AND DIQUAT

- Add 0.5 ml of aqueous ammonium hydroxide (2M) to 1 ml of the test sample mix for 5 seconds and add about 20 mg of solid sodium dithionite. A strong blue to blue-black color indicates paraquat or diquat gives a yellow-green color but paraquat may also be present.
- If the color fades on continued agitation in air and is restored by adding further sodium dithionite paraquat or diquat is confirmed.
- Confirmation of the severity of the poisoning is obtained by measuring the plasma- paraquat concentration.



## 5.7. ETHANOL AND ALDEHYDES

- Place 1 ml of urine in the test tube; apply 1 drop of 2.5% potassium dichromate in 50% sulfuric acid to a strip of glass fiber filter paper, insert the paper in the neck of the tube. Lightly stopper the tube and place in a boiling water bath for 2 minutes. A change color from orange to green indicates the presence of volatile reducing substance including ethanol, aldehydes, etc.
- If a positive result is obtained, carry out a quantitative assay of for ethanol on blood.



## 5.8. CHLORATES AND OXIDIZING AGENTS

- Carefully add 0.5 ml of diphenylamine (10 g/L) in concentrated sulfuric acid to 0.5 ml of filtered stomach contents or scene residues. A strong blue color which develops rapidly indicates the presence of oxidizing agent such as chlorates, and ethchlorvynol ( red coloration) (Diphenylamine Test)
- The test is specific and sufficiently sensitive to detect therapeutic concentration.



## 5.9. FERROUS-FERRIC IRON

- To 50  $\mu\text{L}$  of filtered stomach contents or scene residues add 100  $\mu\text{L}$  of aqueous HCl (2M) and 50  $\mu\text{L}$  of aqueous potassium ferricyanide solution (2M). A deep blue precipitation with potassium ferricyanide or potassium ferrocyanide indicates the presence of ferrous or ferric iron.
- If positive result is obtained, carry out a quantitative assay for iron on serum.



## 6. OTHER DIRECT TEST ON BLOOD

### 6.1. **Glucose.**

Measure the blood glucose by a standard method or approximately by “Dextrostix” or similar method. Hypoglycaemia is a feature of overdose with insulin, hypoglycaemic agents, ethanol, and paracetamol (liver damage).

### 6.2. **Urea.**

Measure the blood urea by standard method, or approximately by the “Urastrat” method to exclude uraemia.

### 6.3. **Carbon monoxide.**

Dilute sample of the blood (1 in 20) with 0.01 M ammonia and compare the color with a sample of normal blood treated similarly. A pinkish tint suggest the presence of carboxyhaemoglobin.



## 6.4. Organophosphorus Compounds.

To each of two tube add 3 ml of dithiobisnitro - benzoic acid solution and 0.1 ml of 5% acetyl thiocholine iodide solution; to the first tube add 20  $\mu$ L of normal serum and to the second tube add 20  $\mu$ L of sample serum and allow to stand for 2 minutes. Any significant difference in color between the tubes is suggestive of the presence of an organophosphorus compound or other cholinesterase inhibitor.



## 7. REINSCH TEST

- To 15 ml of the sample of body fluid or homogenised tissue extract, add 3 ml of HCl and immerse in the liquid a 5 mm X 10 mm **copper strip** or spiral of copper wire formed by winding the wire around the glass rod ten times; heat gently for 2 hours. Remove the metal, wash gently with water and examine the surface.
- A silver deposit indicate the presence of **mercury**.
- A shiny black deposit which is insoluble in potassium cyanide solution is given by **bismuth**.
- A dull black deposit which is soluble in potassium cyanide solution is given by **arsenic**,
- A purple deposit which is insoluble in potassium cyanide solution is given by **antimony**.
- Dark deposit may indicate **selenium or tellurium**.



# EXTRACTION OF URINE

- To 10 ml of urine + phosphoric acid or tartaric acid to adjust pH 3, extract with 2 X 30 ml portions of ether. Combine the ether extracts and wash with 5 ml of water.
- Retain the aqueous layer for later extraction.
- Extract the ethereal layer with 5 ml of saturated sodium bicarbonate solution and retain the aqueous layer for possible examination for the presence of salicylate or strong acid- Fraction A-1).
- Extract the ethereal layer with 5 ml of 0.5 M sodium hydroxide and retain the aqueous extract for barbiturates or weakly acid (Fraction A-2).
- Wash the ethereal solution with water, discard the washing, dry the solution with anhydrous sodium sulfate, and evaporate to dryness. The residue may contain neutral drugs (Fraction A-3N)



- To aqueous solution retained after the first extraction, add sufficient dilute ammonia solution to adjust pH 8, extract with 2 X 10 ml portions of chloroform, combine the extracts.
- Wash the extracts with water, add a little tartaric acid to prevent the loss of volatile bases, and evaporate to dryness. The residue may contain basic drugs (Basic compounds- Fraction B).
- To aqueous layer after extraction of Fraction B add hydrochloric acid heat at 100°C for 30 minutes, cool. Extract with 2 X 10 ml portions of ether. Reserve the aqueous layer.
- Wash the combined ether extracts with 5 ml of M sodium hydroxide, and evaporate to dryness. The residue may contain benzophenones (Fraction BE).



- Adjust the reserved aqueous solution to pH 9 with addition of ammonia solution.
- Extract with a mixture of ethyl acetate and isopropyl alcohol (9:1). Combine the extract.
- Evaporate the solvent layer to dryness.
- The residue may contain Opiates ( Fraction O).

.Extraction system is called the Stass-Otto method of Extraction.

This method can be used for extraction of Stomach Contents, and also for extraction of scene residues including food and drink.



# EXTRACTION OF BLOOD, SERUM, OR PLASMA.

- To 4 ml of sample add 2 ml of phosphate buffer (pH 7.4), and 40 ml of chloroform, shake vigorously, add 2 g of anhydrous sodium sulfate and shake to produce a solid cake.
- Decant the chloroform extract through a filter, extract the cake again with a further 20 ml of chloroform, and combine the chloroform extracts.
- Retain the sodium sulfate cake.
- Extract the chloroform solution with saturated sodium carbonate solution. The aqueous layer may contain strong acid (Fraction A-1)
- To the chloroform extract add 8 ml of 0.5M sodium hydroxide, shake for 2 minutes and centrifuge. The aqueous layer may contain weakly acid (Fraction A-2)
- Wash the chloroform extract with water, discard the washing. Dry the chloroform extract. The extract may contain neutral drugs (Fraction A-3N).



- If sufficient, to 4 ml of original sample add dilute ammonia solution to make alkaline.
- Extract with 2 X 10-ml portions of chloroform. Dry the chloroform extract with anhydrous sodium sulfate,
- Evaporate the extract to dryness. The residue may contain basic drugs (Fraction B).
- If there is not sufficient of the original sample for extraction. The retained sodium sulfate cake after extraction of acid compounds is make to alkaline with addition of dilute ammonia solution. Extract with 2 X 10 ml portions of chloroform.
- Dry the chloroform extract with anhydrous sodium sulfate, and evaporate to dryness. The residue may contain basic drugs (Fraction B).



# DRUGS COMMONLY TAKEN IN OVERDOSES

- **Fraction A (Strong Acids):**

Salicylates, Phenytoin.

- **Fraction A-2 (Weakly Acids):**

Barbiturates, Chlorpropamide, Glutethimide, Paracetamol, Phenylbutazone, and Phenytoin.

- **Fraction A-3 (Neutral Drugs):**

Caffeine, Carbromal, Chlordiazepoxide, Chlormethiazole, Ethchlorvynol, Flurazepam, Glutethimide, Lorazepam, Meprobamate, Methaqualone, Methyprylone, Nitrazepam, Phenazone, Temazepam, Theophylline



- Fraction B (Basic Drugs)

Amitriptyline, Amphetamine, Caffeine, Chlordiazepoxide, Chlormethiazole, Chlorpromazine, Clomipramine, Desipramine, Dextropropyphene, Diazepam, Dihydrocodein, Dothiepin, Doxepin, Ergot alkaloids, Flurazepam, Imipramine, Lorazepam, Maprotiline, Methadone, Methaqualone, Mianserin, Morphine, Nitrazepam, Orphanedrine, Oxprenolol, Phenezine, Propanolol, Quinine, Temazepam, Theophylline, Thioridazine, Trimipramine.

- Fraction BE (Benzophenones): Benzodiazepines

- Fraction O (Opiates):

Codeine, Morphine, etc.



# EXAMINATION OF FRACTION A, B, O

- Ultraviolet Spectrophotometry
- Chromatography:
  - Thin-layer Chromatography (TLC)
  - Gas Chromatography (GC)
  - High Performance Liquid Chromatography (HPLC).
- Radioimmunochemistry.



# TLC FOR BASIC DRUGS

SYSTEM OF TLC	PLATE	MOBILE PHASE
System TA	Silica gel G, 250 $\mu\text{m}$ thickness, dipped in or spray with 0.1M KOH in methanol, and dried	Methanol: strong ammonia solution (100 : 1,5)
System TB	The same plates as System TA	Cyclohexane: toluene: diethylamine (75:15:10)
System TC	The same plates as System TA	Chloroform: methanol (90: 10)



# TLC FOR ACIDS AND NEUTRAL DRUGS

SYSTEM TLC	PLATES	MOBILE PHASE
System TD	Silica gel G, 250 $\mu\text{m}$	Chloroform : Acetone (4 : 1)
System TE	Same plates as System TD	Ethyl acetate : methanol: strong ammonia solution (85 : 10 : 5)
System TF	Same plates as System TD	Ethyl acetate



# TLC VISUALIZATION REAGENTS

- Mercurous nitrate spray for acid extract, which gives white spots with a grey centre on a darker background with barbiturates, glutethimide
- Ferric chloride spray for phenols, which gives blue or violet spots.
- Van Urk reagent spray which gives yellow to orange spots with sulfonamide, meprobamate and indole compounds.
- Acidified iodoplatinate solution, which gives purple, blue, or brown spots with acids, basic and neutral drugs



- Mandelin's reagent, which gives colors spots ranging from blue and green to orange and red with a variety of basic compounds.
- FPN reagent, which gives red or brown-red spots with phenothiazines, and blue spots with dibenzapines.
- Ninhydrin spray which gives violet or pink spots with primary amines, and yellow spots with secondary amines.
- Dragendorff reagents spray, which gives yellow, orange, red-orange, or brown-orange spots with tertiary alkaloids.
- Marquis reagent spray, which gives black, blue, or violet with alkaloids related to morphine.



# RF VALUES

- Rf values for drugs taken in overdoses will be found in drug monographs and in the indexes to analytical data.
- See table no 15:



**Thank You**

