ANALYTICAL TOXICOLOGY

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Urine

Urine is useful for screening tests as it is often available in large volumes and usually contains higher concentrations of drugs or other poisons than blood. The presence of metabolites may sometimes assist identification if chromatographic techniques are used. A 50-ml specimen from an adult, collected in a sealed, sterile container, is sufficient for most purposes; no preservative should be added. The sample should be obtained as soon as possible, ideally before any drug therapy is initiated.

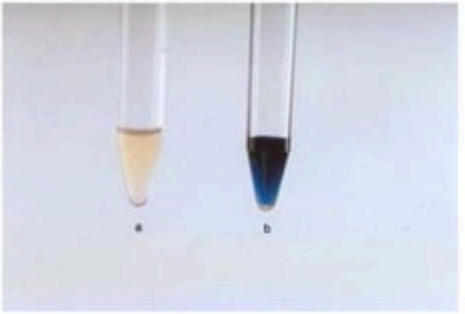
Stomach contents

Stomach contents may include vomit, gastric aspirate and stomach washings — it is important to obtain the first sample of washings, since later samples may be very dilute. A volume of at least 20 ml is required to carry out a wide range of tests; no preservative should be added. This can be a very variable sample and additional procedures such as homogenization followed by filtration and/or centrifugation may be required to produce a fluid amenable to analysis.

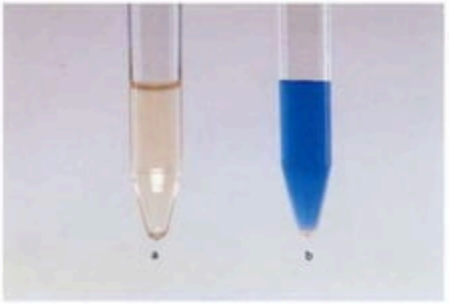
Blood

Blood (plasma or serum) is normally reserved for quantitative assays but for some poisons, such as carbon monoxide and cyanide, whole blood has to be used for qualitative tests. For adults, a 10-ml sample should be collected in a heparinized tube on admission.

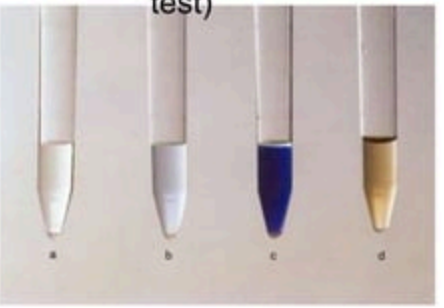
Phenothiazines
(FPN test)



Imiparamine
(Forrest
test)

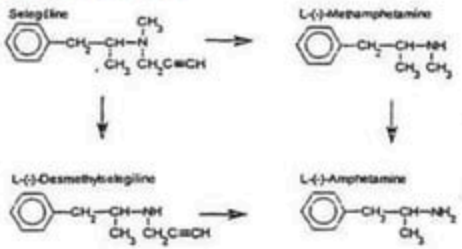


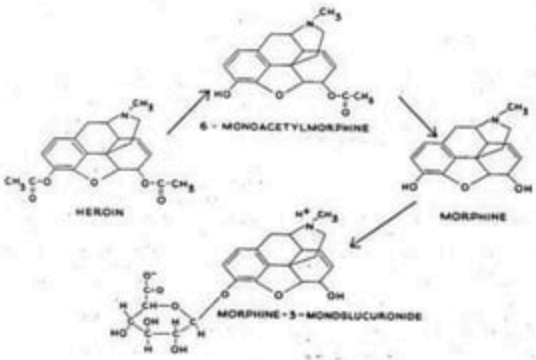
Paracetmol and
phenacetin
(ammonia test)

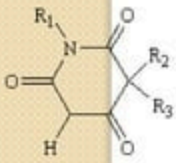


Paraquat and diquat
(dithionite test)

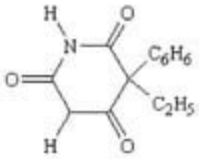
Analysis of Metabolites of Drug



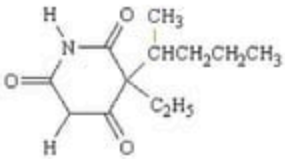




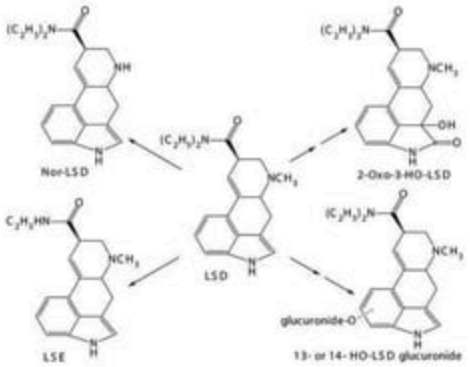
Barbituates



Phenobarbital



Pentobarbital



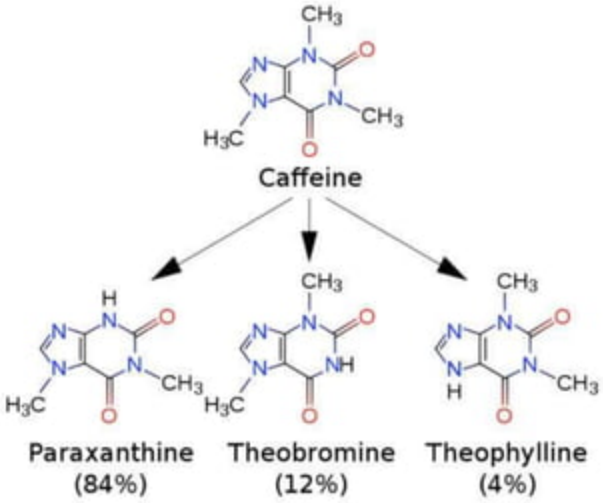
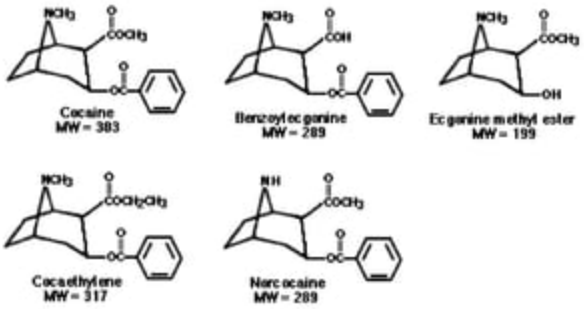


Table 15-4
Urinary Metabolites of Benzodiazepines

Generic name	Brand name	Urinary metabolite
Oxazepam	Serax	Oxazepam
Temazepam	Restoril	Temazepam, Oxazepam
Chlordiazepoxide	Librium	Nordiazepam, Oxazepam
Diazepam	Valium	Diazepam, Nordiazepam, Oxazepam, Temazepam
Prazepam	Centrax, Verstran	Nordiazepam, Oxazepam
Clorazepate	Tranxene	Nordiazepam, Oxazepam
Medazepam	Nobrium	Nordiazepam, Oxazepam, Temazepam
Alprazolam	Xanax	α-Hydroxyalprazolam
Clonazepam	Klonopin	Aminoclonazepam, Clonazepam



Sample Treatment

- Protein Precipitation (PP)
- It is accomplished by using organic solvent (typically acetonitrile or methanol) or an acid (typically perchloric or trichloroacetic acid). It is followed by centrifugation to separate proteins from liquid supernatant.
- Liquid-liquid extraction (LLE)
- To obtain a sensitive analysis for a complex biological media (plasma, urine) liquid-liquid extraction (LLE).
- LLE is in general simpler also less expensive and flexible as several samples may be prepared in parallels.
- Solid-phase extraction (SPE)
- Higher recoveries, no problems with emulsions, less solvent consumption and a smaller sample volume requirement.
- Sample treatment with high speed and feasibility for treatment of numerous samples at one time is possible.

Metabolite identification

- *Full scan*
- *Precursor ion and constant neutral loss scan*
- **Product ion scan**
- **MRM**

Metabolite Determination

- LC-MS/MS
- *Reversed phase chromatography*
- Reversed phase chromatography is most widely used technique in analysis of drugs and their metabolites due to its extensive application to most small molecules which are separated by their degree of hydrophobic interaction with the stationary phase.
- An increased polarity of the metabolite decreased retention on the stationary phase.
- For polar metabolites short chain bonded phases, such as C_8, phenyl or cyano are more appropriate. Add ion-paring reagent into mobile phase.
- *Ultra-high performance liquid chromatography (UHPLC)*
- For fast analyses using sub-2μm particle column dimensions are typically 50x2 mm. An additional benefit of UHPLC is the low consumption of mobile phase, where it saves at least 80% compared to HPLC.
- Advantages as enhanced separation efficiency, short analysis time and high detection sensitivity make UHPLC coupled with MS/MS an even more powerful analytical support in pharmacokinetic studies.

GC-MS

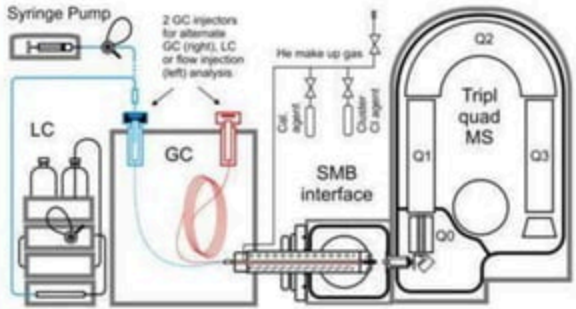
- The GC-MS analysis of polar compounds, such as metabolites, from biological matrices requires analytes extraction into a volatile organic solvent.
- time-consuming sample preparation including derivatization to become stable, volatile and amenable to the ionization technique.

Capillary electrophoresis (CE)

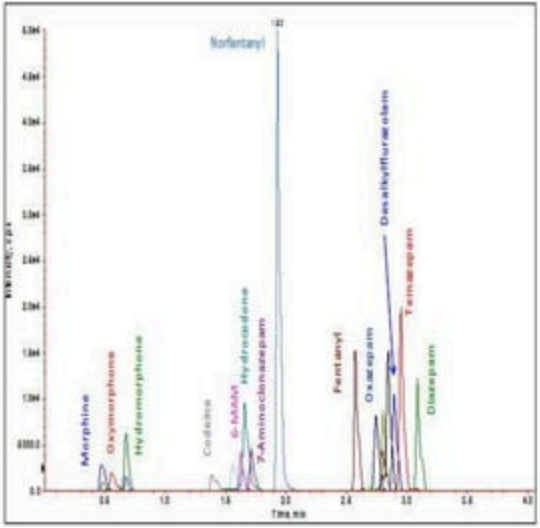
- CE in many instances can have distinct advantages over HPLC in terms of simplicity, rapid method development, solvent saving and minimal sample requirement [10-30 nL injected] making this technique very interesting for rapid and practical analyses in the biomedical field.
- But have less sensitivity.

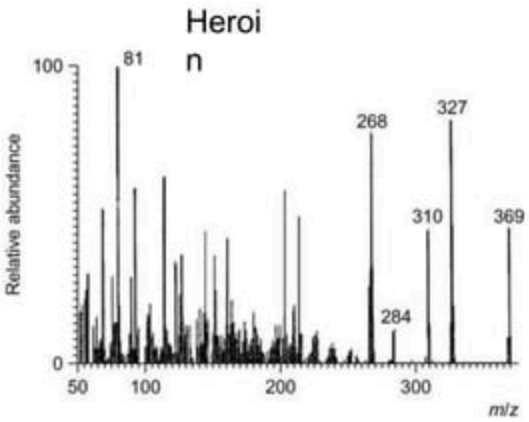
Mass spectrometry

- Separate ion according to m/z ratio.
- Currently, the QQQ using single or multiple reaction monitoring is most often used for quantitative analysis of metabolites.
- SIM suffers from insufficient selectivity in comparison with MRM.
- And also much lower sensitivity.
- IT and TOF analyzers are also used for metabolite determination or use combined with QQQ (Qtrap, Q-TOF).


Syringe Pump
2 GC injectors for alternate GC (right), LC or flow injection (left) analysis
He make up gas
LC
GC
Col. ligand
Cooler Cl ligand
SMB interface
Q2
Tripl quad MS
Q1
Q3
Q0


HPLC
MS/MS

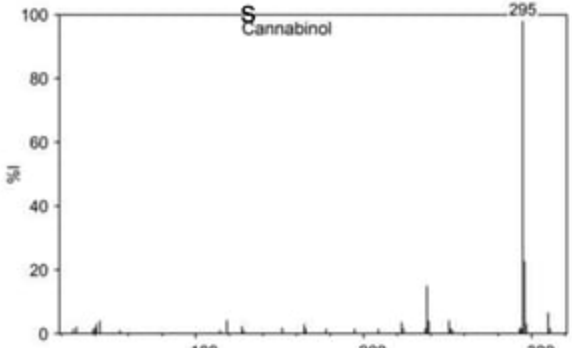

Norfentanyl
Desalkylflurazolam
Temazepam
Fentanyl
Oxazepam
Diazepam
Hydrocodone
7-Aminoclonazepam
Morphine
Oxymorphone
Hydromorphone
Codeine
6-MAM
Time, min


Heroin
Relative abundance
100
0
81
268
327
310
369
284
50
100
200
300
m/z

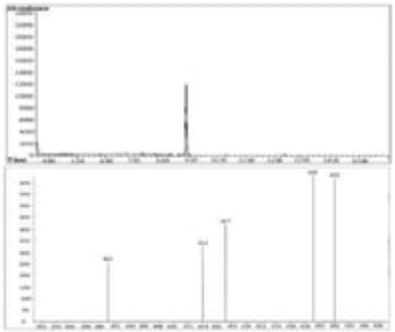
Cocaine

Fig. 3 Total ion chromatogram electron impact ionization mass spectrum of cocaine (molecular weight: 303).

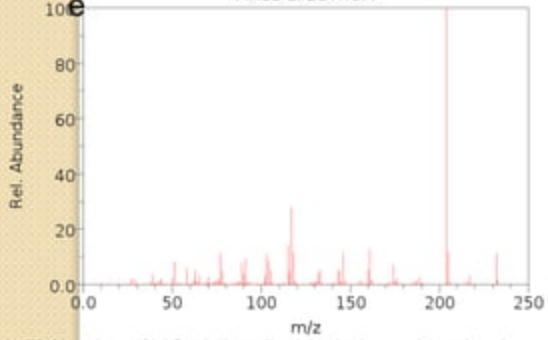
Cannabis



Morphine



Barbiturate



NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

Thin layer chromatography

Acid mixture extract A

10 ml urine sample

1 ml of dil HCL And 10 ml $CHCl_3$

Shake for 5 min

For 10 min

Then lower organic layer to tapered glass tube

Evaporate extract at 60^0 C

Basic mixture extract B

10 ml urine sample

2 ml NH_4Cl and 10ml $CHCl_3$: propan-2-ol

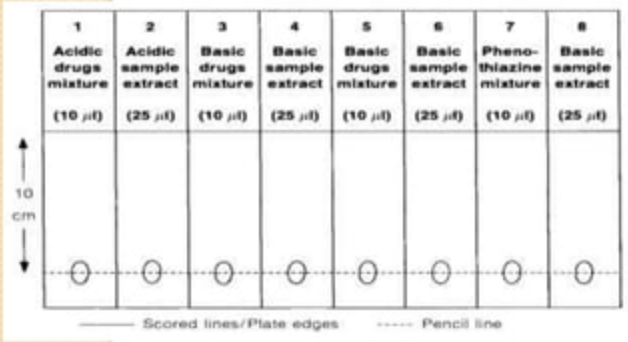
Shake for 5 min

For 10 min

Then lower organic layer to tapered glass tube

0.5 ml methanolic HCL

3. Purification of extracts of stomach contents
   (a) Prior to the solvent evaporation stage, add 5 ml of aqueous sodium hydroxide solution to extract A, and 5 ml of aqueous hydrochloric acid to extract B.
   (b) Shake on a mechanical shaker for 5 minutes, centrifuge in a bench centrifuge for 10 minutes and discard both organic layers.
   (c) Add 5 ml of aqueous hydrochloric acid to the aqueous residue from extract A, and 5 ml of ammonium chloride buffer to the aqueous residue from extract B, and re-extract into chloroform or chloroform : propan-2-ol as in methods 1 and 2 above.

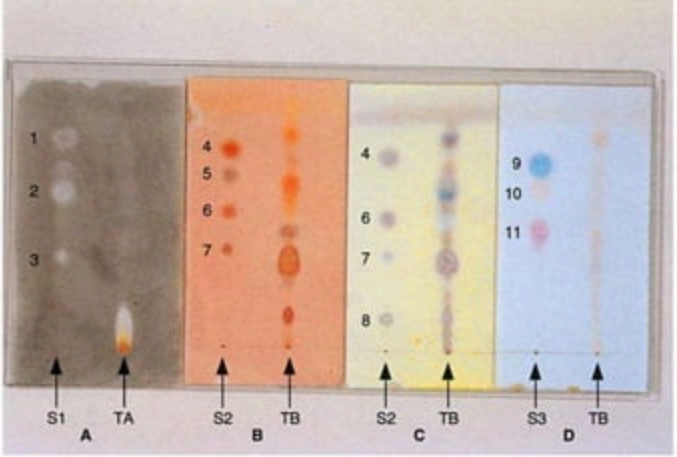


Standards

All 1 g/l in chloroform:

1. Acidic drugs mixture (amobarbital, mefenamic acid, phenobarbital, theophylline).
2. Basic drugs mixture (amitriptyline, codeine, nicotine, nortriptyline).
3. Phenothiazine mixture (perphenazine, trifluoperazine, thioridazine).

TLC visualization reagent-
Mercurous nitrate
Acidified indoplatinate
FPN reagent
Marquis reagent



Codeine and methadone

TLC visualization reagent

A Mercurous nitrate
B Acidified indoplatinate
C Mandelin reagent
D Sulfuric acid

1 Amobarbital
2 Phenobarbital
3 Theophylline
4 Amitriptyline
5 Nicotine
6 Nortriptyline
7 Codeine
8 Mefanamic acid
9 Thioridazine
10 Trifluroperazine
11 perphenazine

Analysis of stomach content

Clinical interpretation

Arsenic

Acute poising of Arsenic causes abdominal pain, vomiting, bloody diarrhoea, massive haemolysis.

Mercury

Mercury vapors cause stomatitis, increased salivation, metallic taste, diarrhoea, pneumonitis, renal failure.

Mercury salts may cause gastric pain, vomiting.

Reinsch Test

Qualitative test

Cu mesh clean in HNO_3

Rinse the Cu with D.W.

Add 10 ml conc. HCl and 20 ml test solution

For one hour

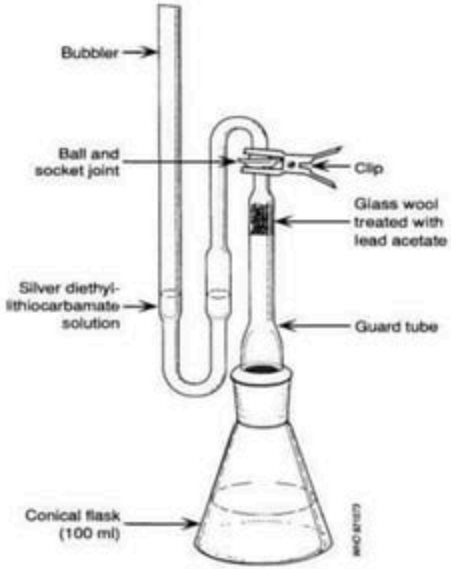
Fume cupboard

Cool and wash with D.W.

Results
Dull black- Arsenic
Silvery- Mercury

Modified Gutzeit apparatus

Quantitative test



Clean and dry with acetone
Glass wool treated with Pb acetate
Add 3 ml silver diethyldithiocarbamate
Into bubbler
Add 2 g KI and 50 ml sample and swirl until dissolved. Then add 2 ml $SnCl_2$ and 10 ml conc. HCl
Add 10g granular zinc and quickly connect with bubbler. Kept for 45 min at RT. Absorbance of sol at 540 nm against blank and calculate arsenic concentration by previously prepared calibration graph.

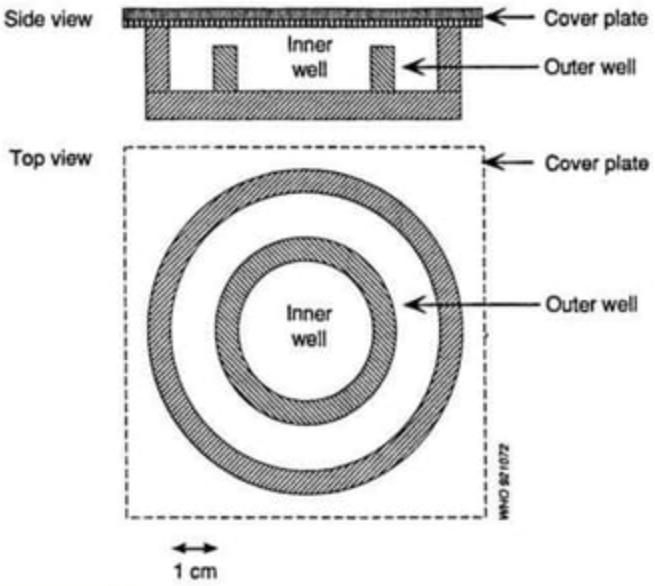
Cyanide

- Qualitative test
- Dissolve 1ml of sample in 2 ml NaOH. Add 2 ml ferrous sulphate and add sufficient HCl to dissolve ferrous hydroxide precipitate.
- Blue color indicates presence of cyanide.

Quantitative test

Method
Take three microdiffusion cells and add to each of the centre wells
1 0.5 ml of p-nitrobenzaldehyde solution
2 0.5 ml of o-dinitrobenzene solution
3 0.5 ml of sodium hydroxide solution

Fig. 1. Conway microdiffusion apparatus



2. To the outer wells add 0.1 ml of:
   — purified water (cell 1);
   — potassium cyanide solution (cell 2);
   — test blood specimen (cell 3).
3. To each outer well add 0.5 ml of purified water and, on the opposite side of the outer well, 1.0 ml of dilute sulfuric acid.
4. Seal each well using silicone grease, and carefully mix the components of the outer wells.
5. Incubate at room temperature for 20 minutes and then add 1 ml of aqueous methanol (1:1) to the centre wells.
6. Transfer the contents of the centre wells to 5.0-ml volumetric flasks and make up to volume with aqueous methanol (1:1).

Results

The red coloration obtained with cyanide-containing solutions is stable for about 15 minutes. Measure the absorbance of the solutions from cells 2 and 3 at 560 nm against the purified water blank (cell 1.

Assess the cyanide ion concentration in the sample by comparison with the reading obtained from the standard.

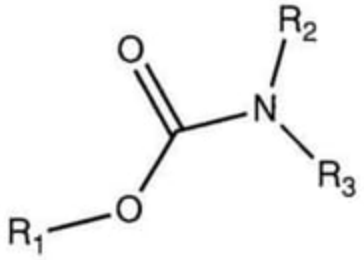
Sensitivity

Cyanide, 0.5 mg/l.

Clinical interpretation

Causes ataxia, headache, anaxiety, confusion, coma, metabolic acidosis, collapse, respiratory arrest.

Carbamate pesticide



Clinical interpretation

Exposure to carbamates may cause anorexia, abdominal pain, nausea, vomiting, diarrhoea, lacrimation, increased salivation, sweating, anxiety, ataxia and acute pulmonary oedema. Antidotal therapy with atropine may be indicated, but pralidoxime should not be used.

Acidify 1 ml sample by 0.5 ml dil. HCl & extract with 4 ml $CHCl_3$

Shake for 5 min.

For 5 min.

Remove upper aq. Layer and filter $CHCl_3$ extract by phase separating filter paper

Evaporate for 40^0 C

Dissolve residue in 1 ml methanol

Apply 0.1 ml furfuraldehyde

Expose paper for conc. HCl fumes for 5 min.

Carbamate gives black spot

Organochlorine

Clinical interpretation

Features of poisoning with organochlorine pesticides include vomiting, weakness and numbness of the extremities, apprehension, excitement, diarrhoea and muscular tremor, with convulsions and respiratory depression in severe cases. Treatment is symptomatic and supportive.

Extract 10 ml of sample with 5 ml petroleum ether

Allow for 5 min . Take upper layer & extract with second 5 ml PE

Combine ether extract & wash with DW , NAOH

Add 5 g sodium sulphate

Reconstitute extract in 100 μl of methanol

Spot the 20 μl sample on plate

Develop chromatogram using cyclohexane

Spray $KMnO_4$ & 2-amino ethanol

Heat plate at 100^0c for 20 min

Allow to cool & spray $AgNO_3$ reagent

Expose d to UV for 15 min

Give brown, black spots.

lindane 09
dicophane 26
heptachlor 34
aldrin 41

Organophosphorous

Adjust the ph 7 of 10 ml of sample with $NaHCo_3$ ➡ Extract 10 ml of sample with 5 ml MTB Ether ➡ Allow for 5 min . Take upper layer & extract with second 5 ml MTB Ether ➡

➡

⬇

Follow same procedure as in OC ⬅ Spray the plate 4-(p-nitro benzyl)pyridine ⬅

⬅ Allow to cool & spray Acetone: tetraethylen epentamine ⬅ Give purple spot

110^0C for 30 min

dimethoate	11
methidathion	40
malathion	42
dioxathion	47
propetamphos	49
bromophos	54
chlorpyrifos	58

Clinical interpretation-
May cause broncorrhoea, Respiratory distress, nausea, muscle weakness , paralysis

ANALYTICAL TOXICOLOGY REQUEST

To: [Insert laboratory name, address and telephone no.]

Discuss special requirements BEFORE sending samples.

Doctor (PLEASE PRINT):

Telephone/bleep no:

Hospital address
for report:

Signed: Date:

Patient: Other names:

Age/date of birth: Sex:

Consultant: Ward:

Reference no:

Sample type	Date	Time
Blood (10 ml heparinized)		
Urine (50 ml; catheter yes/no)		
Stomach contents (50 ml)		
Other (give details)		

Date/time of admission:

Date/time of ingestion or exposure:

Drugs prescribed or used in treatment:

Drugs/poisons claimed or suspected:

Clinical details/investigation required/priority:

Reference

www.who.int/ipcs/publications/training.../**analytical_toxicology.pdf**

jat.oxfordjournals.org/

http://dx.doi.org/10.5772/51676

en.**wikipedia**.org/**wiki**/Journal_of_**Analytical_Toxicology**

THANK YOU