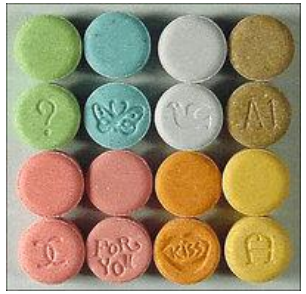
Psychoactive Substances

Dr. Farzaneh Jokar

Psychoactive Compounds

Amphetamines

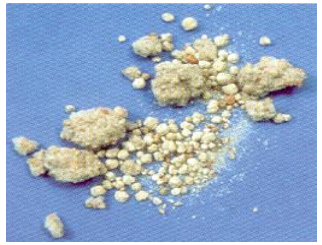
MDMA

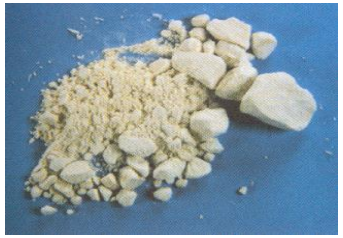


Cocaine

Cannabis

Heroin/opiates





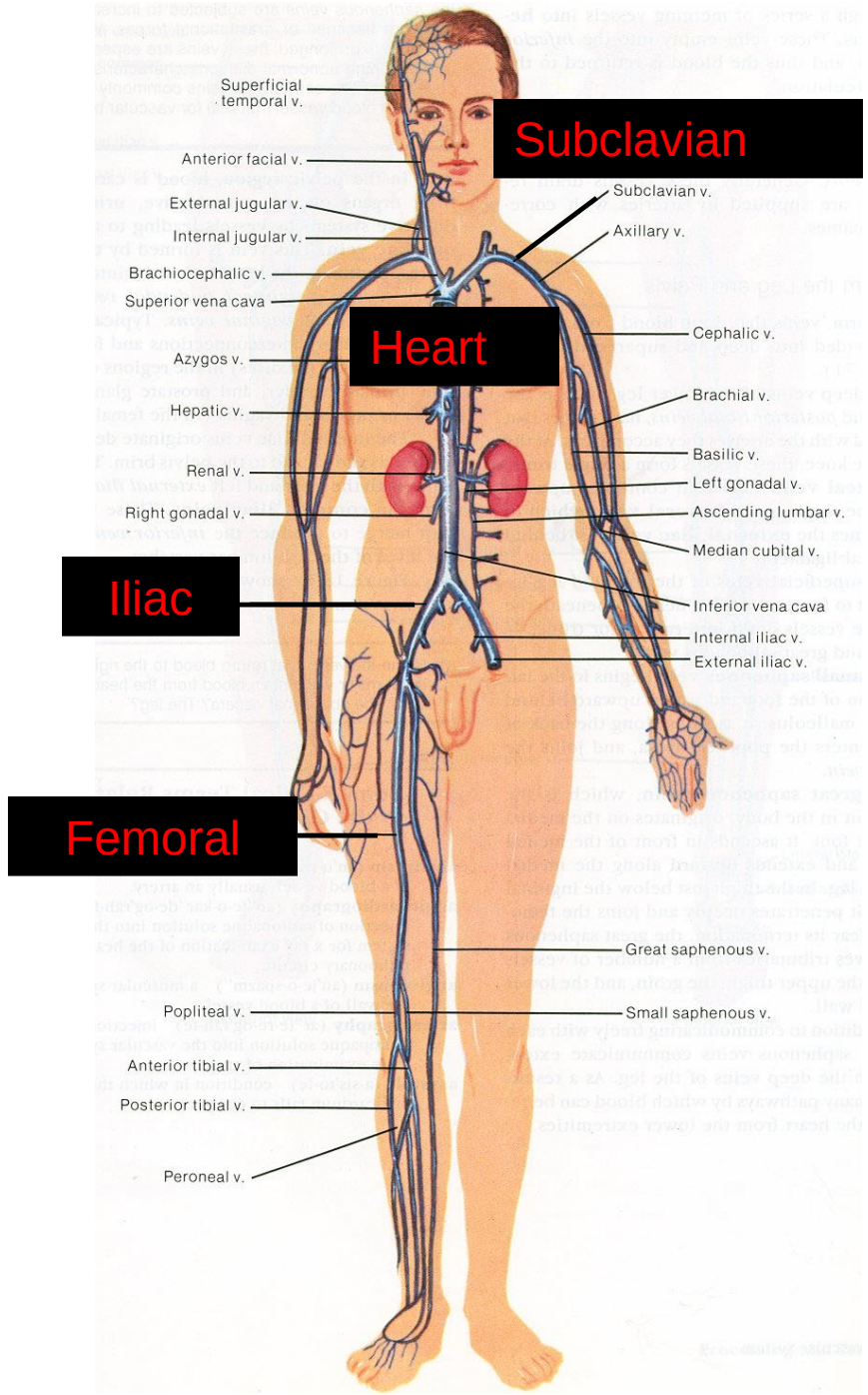
Biological Samples for Psychoactive Drugs Analysis

Typical autopsy specimens

- Blood
- Urine
- Hair
- Saliva
- Sweat

Blood

- Whole blood is the most complex fluid to be analyzed.
- Plasma is containing soluble proteins, dissolved fats and salts, and suspended cells.
- Serum is similar to plasma except for the presence of soluble factors that originate the coagulation phenomena.
- Serum and plasma have large amounts of proteins

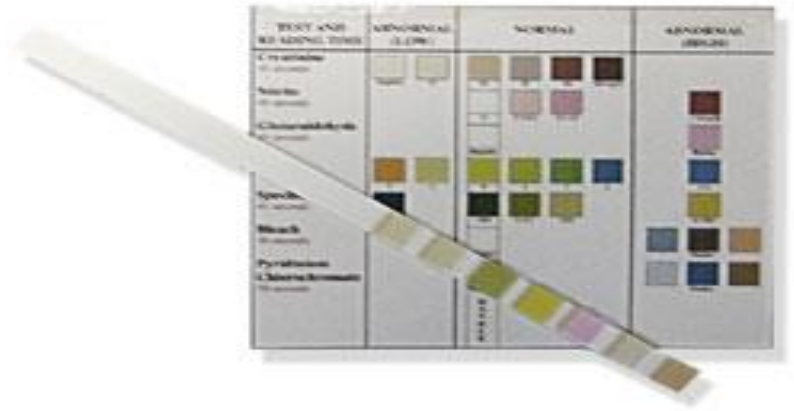


- **Central sites**
  - ***Heart***
- **Peripheral sites**
  - ***Femoral***
  - ***Iliac***
  - ***Subclavian***
- **Other sites**
  - ***Head blood***
  - ***Hematoma blood***

Peripheral blood

- Peripheral blood concentration have been shown to be more reliable for toxicological analysis than the conventional heart blood.
- In all suspected poisoning deaths or in all cases of unknown causes of death a femoral blood specimen should be collected

Urine Testing: The Most Commonly Used Drug Test



Urine

- Urine specimen is of great value even in small amount especially in screening of substance of abuse.
- urine can be collected by catheter or suprapubic puncture with 5-10 ml syringe and needle, body in supine position.

Urine

- Advantages:
- The accumulation of drugs and metabolites in urine results in high concentrations facilitating detection of drug use or exposure.
- It is free of proteins and lipids, therefore can be analyzed either directly by immunoassay.
- Urine analysis reveal recent exposure (alternative for blood).

Advantages of Oral Fluid (Saliva) analysis

- Ease of collection.
- Real time values.
- Elimination of adulteration and substitution possibilities.
- Chain of Custody.
- Drugs detected at low concentrations.

Saliva test

- Oral fluid based testing most closely mimics results found with blood and is preferable for detecting on-the-job drug use or in post-accident. applications, in this case because the degree of intoxication can be approximated based on the amount of substance in the blood.

Saliva test

- **Detection in saliva tests begins immediately upon use:**

  Marijuana and Hashish (THC): 1h after ingestion, up to 1 day.

  Cocaine (including crack): From time of ingestion up to 2 to 3 days.

  Opiates: From time of ingestion up to 2 to 3 days.

  Methamphetamine and Ecstasy (MDMA,"Crank,"Ice"): From time of ingestion up to 2 to 3 days.

Saliva PH

- Human saliva normally has a lower PH than human plasma.

  Plasma ratio for basic drugs are greater than unity.

  Plasma ratio for acidic drugs are less than unity.

Average oral fluid to blood concentration ratios for selected drugs

Drug (type)	Average oral fluid to blood concentration ratio
Alcohol (ethanol)	1.07
Barbiturates	0.3
Buprenorphine	1
Codeine (basic)	4
Methamphetamine (basic)	2
MDMA (basic)	7
Cocaine (basic)	3
Diazepam (acidic)	0.01-0.02
Methadone (basic)	1.6
Morphine (basic)	0.8
Δ9-Tetrahydrocannabinol (neutral)	1.2

Opiates

- The major metabolite found in saliva after heroin use is **6-MAM** (saliva plasma ratio of 6)
- The heroin parent drug is found in saliva for up to 24h after having smocked and up to 60min after injection.
- Morphine appears in saliva after the administration of morphine.
- Codeine is found in saliva with saliva:plasma ratio of 3.3.

Cocaine

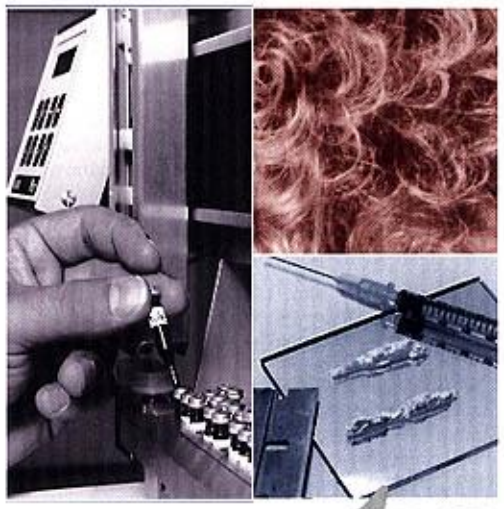
- Cocaine parent drug is the major analyte found in saliva after cocaine use.
- Benzoylecgonine and ecgoninemethyl ester appear in saliva within 15 min after cocaine use at concentration similar to those found in blood .

Amphetamines

- Parent drugs rather than metabolites are found in saliva.
- The saliva:plasma ratio for ampheteamine is 2.76 and for methamphetamine is 3.98.
- The proposed US workplace screening cut-off in saliva is 50 ng/ml amphetamine, MDMA, MDA or methamphetamine.

Cannabis

- Cannabinoids are excreted in only **trace amounts** in saliva.
- Cannabinoids in saliva often result from residuals left in the mouth during ingestion or smoking of marijuana products.
- For this reason concentrations are highest immediately after smoking and decline rapidly over the first 2 to 4h.
- In contrast cannabinoids may not appear in urine or sweat for several hours after smoking.



Hair Testing for drugs of abuse

hair testing

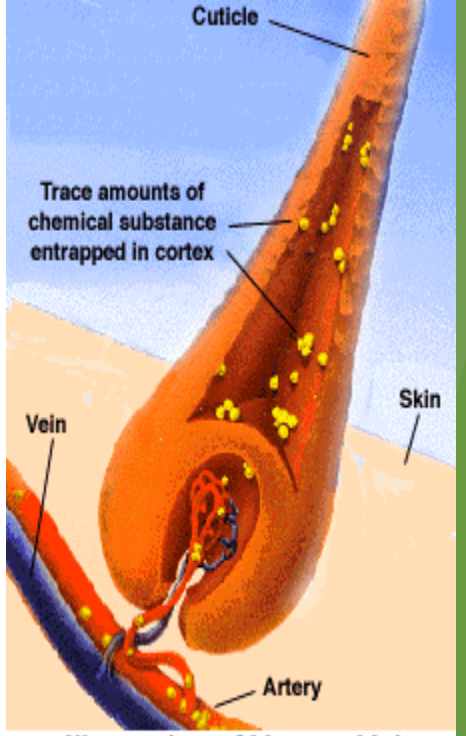


Illustration of Human Hair

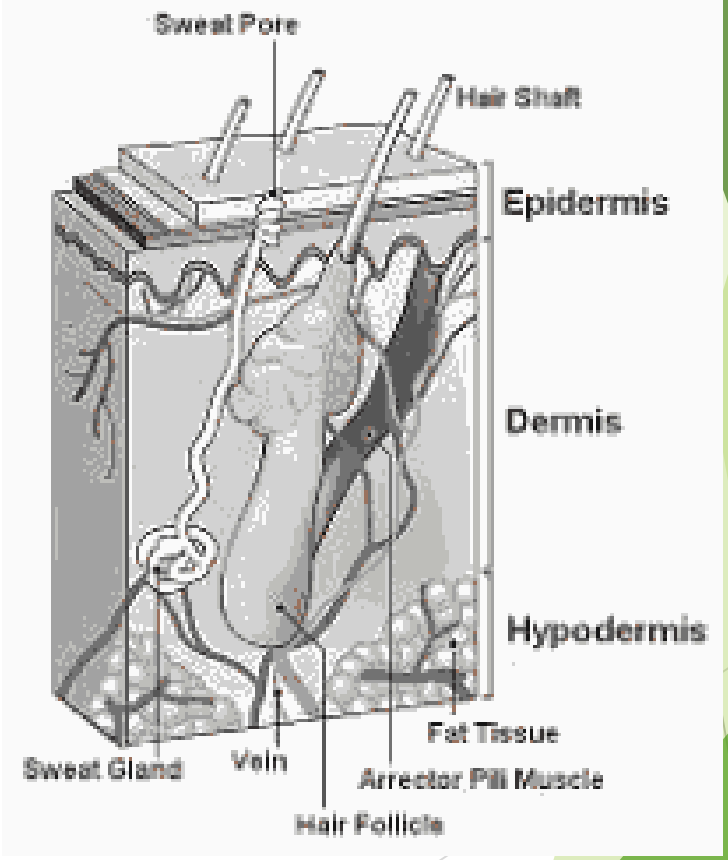
Hair Analysis

A new wave in drug testing.

A breakthrough in the forensic science field.

Drugs incorporation in to hair

- From the blood during hair formation
- From sweat and sebum.
- From external environment.



Drug Stability

- These drug deposits are not washed or flushed out of the hair and do not diminish over time, but are released only upon the destruction of the hair fiber.
- Drug use can thus be detected months or even years following ingestion, depending upon the length of the sample collected.

Advantages of hair analysis

- Longer window of detection, months instead of 48-72 hours.
- Eliminates the need for random testing.
- 5-10 times more sensitive test than urinalysis, and proven in a court of law.
- Easier to collect, store and transport.
- Differentiates between on-off incidents and addictive behavior.

Specimen collection

Decontamination procedures

- Technical false-positive are caused by errors in the collection, processing and analysis of specimens.

- **Wash step**

  The agents used in washing are detergents (shampoo), surgical scrubbing solutions, surfactants, phosphate buffer and organic solvents (methanol, acetone, dichloromethane, ethanol).

Decontamination

- Detection of metabolites in hair that can not be explained by hydrolysis or environmental exposure (cone et al. 1991).

- Cocaethylen and norcocaine are only formed when cocaine is metabolized.

Drug solubilisation

- The hair sample can be pulverized in a ball-mill prior to testing, cutting to segments or the entire hair dissolved.

Notes

- **The alkaline hydrolysis** of hair is not suitable for chemically unstable compounds such as **cocaine, 6-MAM, benzoylecgonine,** which are hydrolyzed under alkaline strong treatment.
- The samples can be incubated in 0.1M HCl overnight, or 0.6M HCl for 30min at 120^0 .
- **The organic solvent** incubation is the simplest method.

  Its involves placing hair samples in methanol and then in an ultrasound bath at 45^0 for hours.

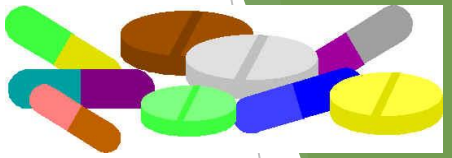
Sweat

- This specimen is mostly constituted by water (99%),
- and sodium chloride is the most concentrated solute.
- The mechanisms of incorporation
- of drugs into this fluid include passive diffusion from blood.
- There is a strong correlation between the pKa of a substance and the amount found in the sweat

Sweat Testing

- Advantages
  - Convenient & less invasive method for monitoring drug use
  - Window of detection ≥ urine testing (dependent upon drug class)
  - Presence of parent drug (heroin, 6AM)
  - Difficult to adulterate specimen

Tablets, powders and syringes



- These samples should be packed by a suitable shield to avoid injury. These items may be particularly useful in deaths in medical personal or drug addicts who may use agents which are difficult to detect once they have entered the body.

Psychoactive Analysis

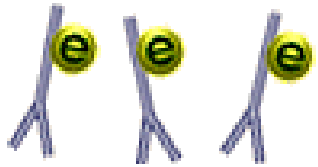
- Screening tests
  - Immunoassay
  - TLC
  - HPLC, GC, GC-MS

Immunoassay

Immunoassay

- An immunoassay is a test that uses antibody and antigen complexes as a means of generating a measurable result.

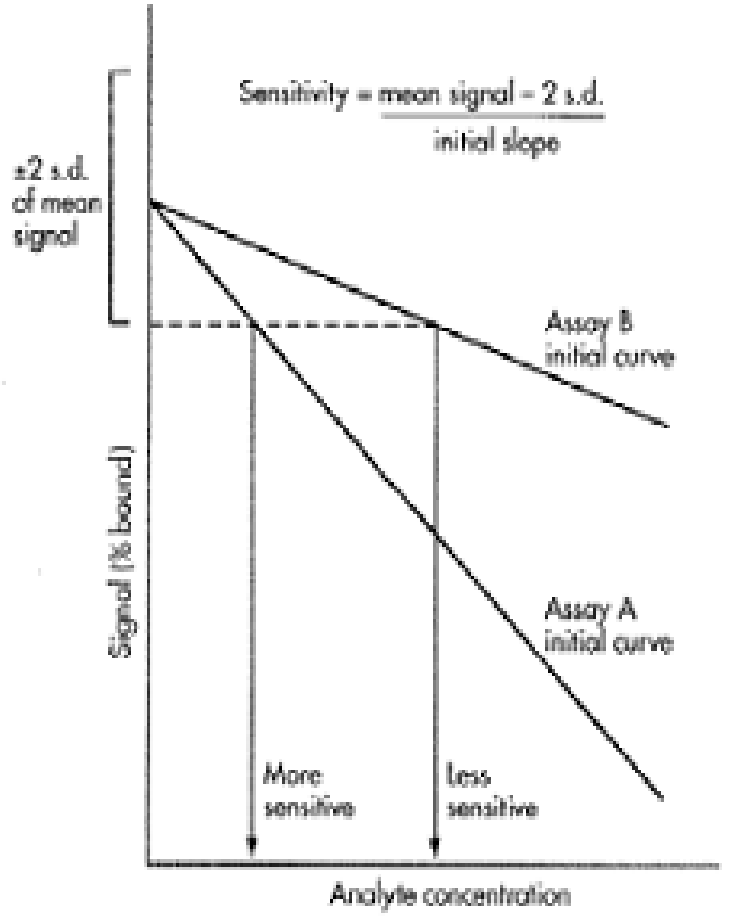
- An antibody/antigen complex is also known as an immune-complex.



Determinants of sensitivity

- **Sensitivity** - minimal concentration that can be reliably estimated.

- **Functional sensitivity** - lowest concentration in the assay for which the coefficient of variation (CV) is less than 20%.
- Depends on:
  - Increase in signal.
  - Decrease in background noise (nonspecific binding).

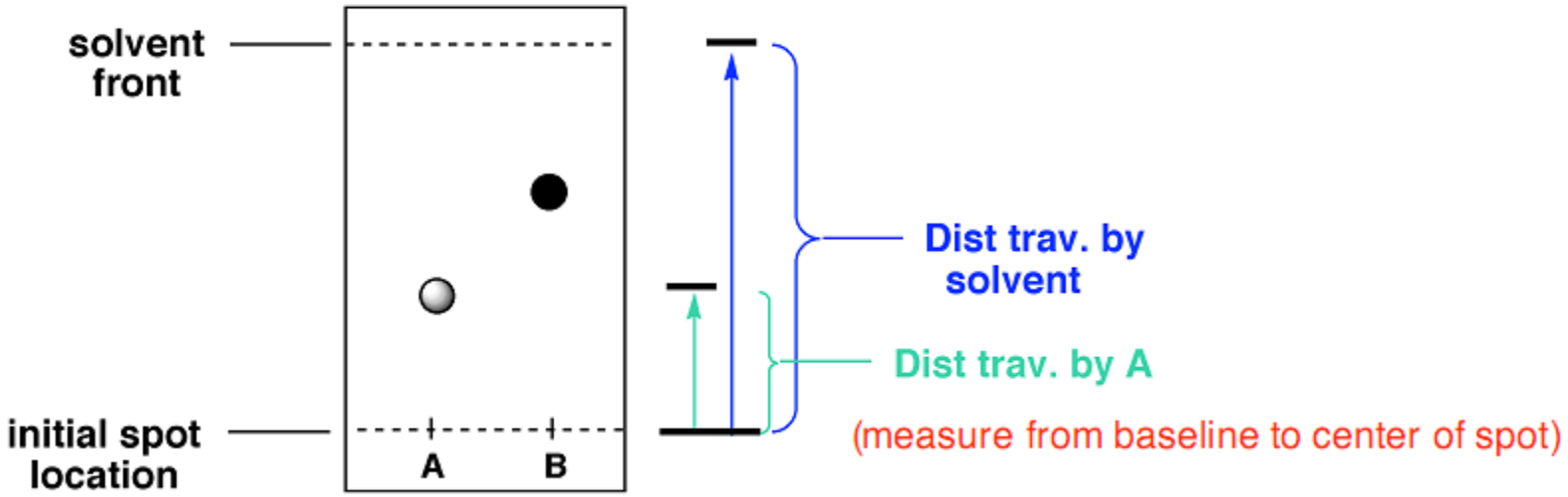


Cross reactivity

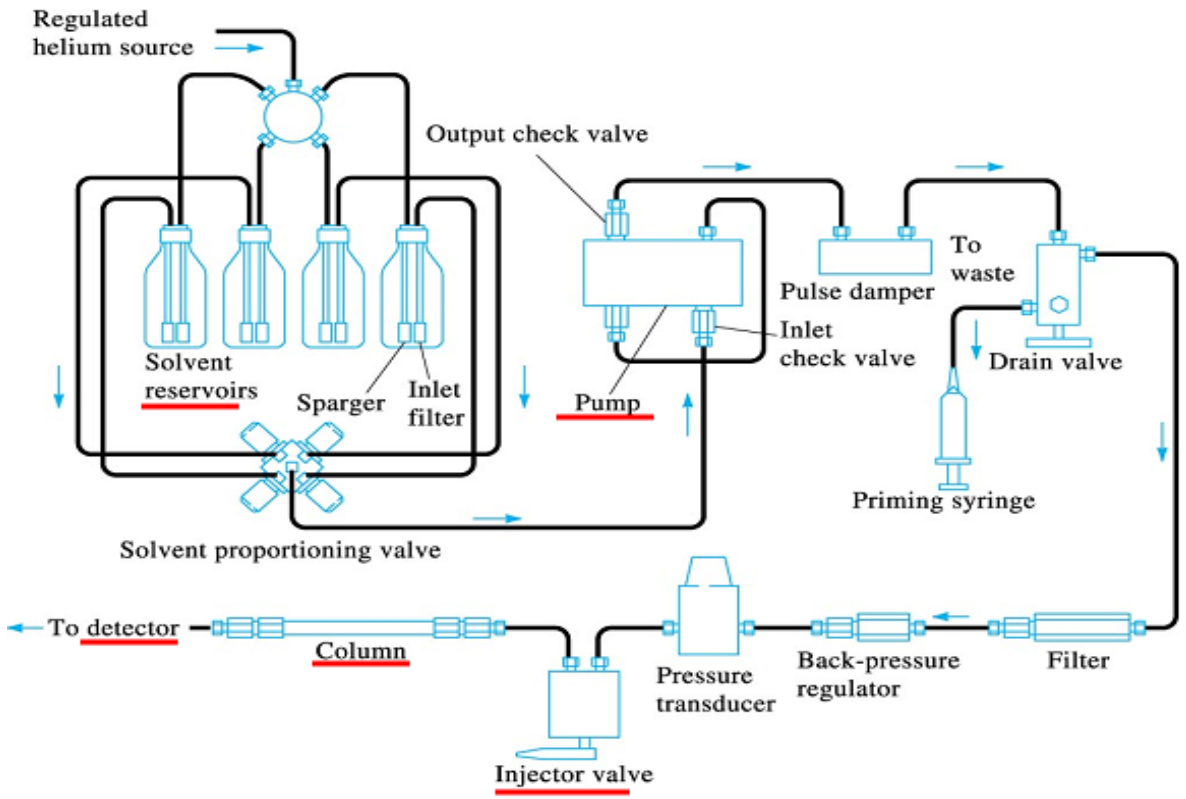
- Many drugs share the same core structure as the drug of abuse.
- Structural unrelated medications may have 3D conformation that possess sufficient binding to certain antibodies:
  - Ranitidine with MA
  - Rifampicine and ofloxacin with opiates
  - Thioridazine with PCP

TLC

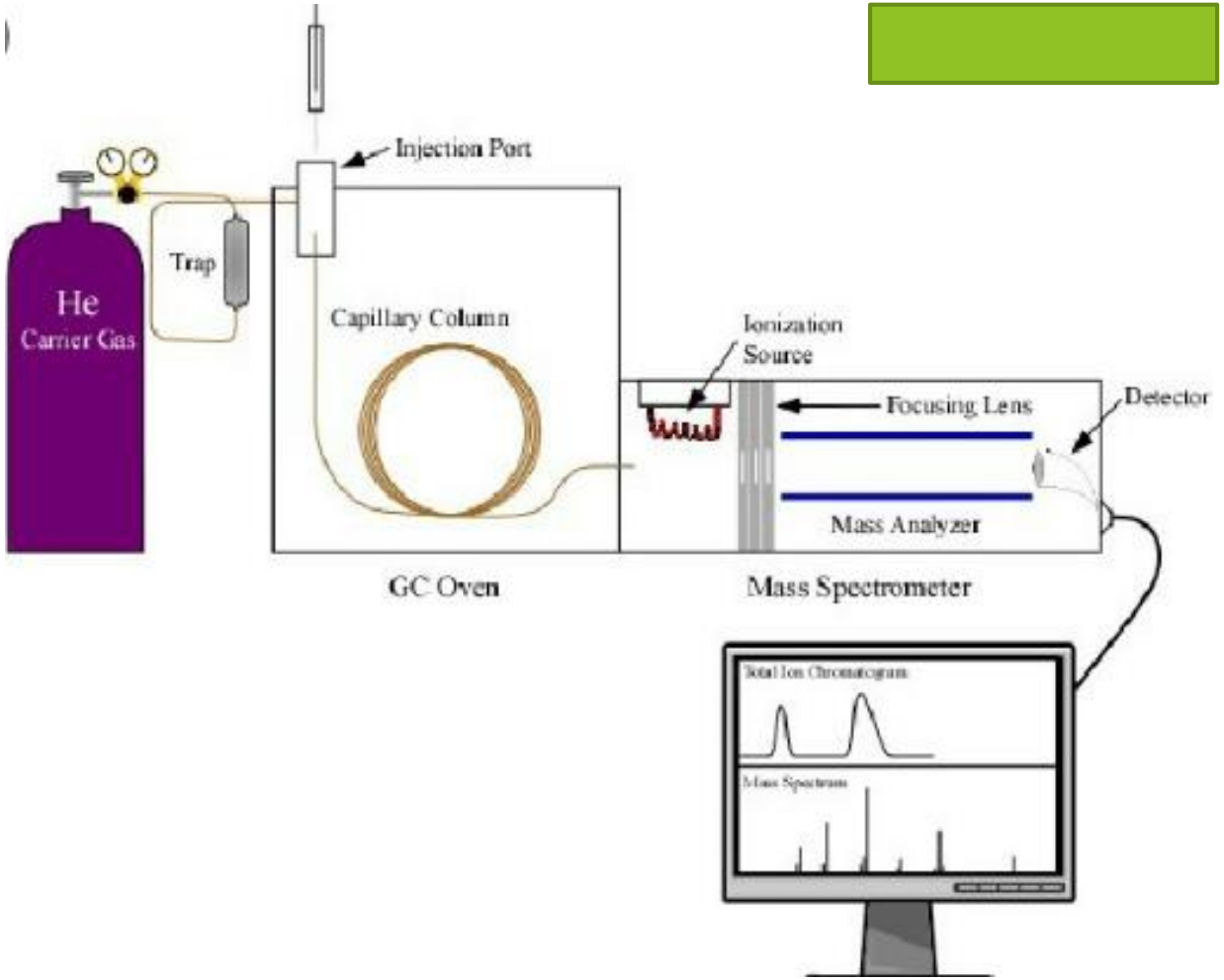
$$R_f = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent}}$$



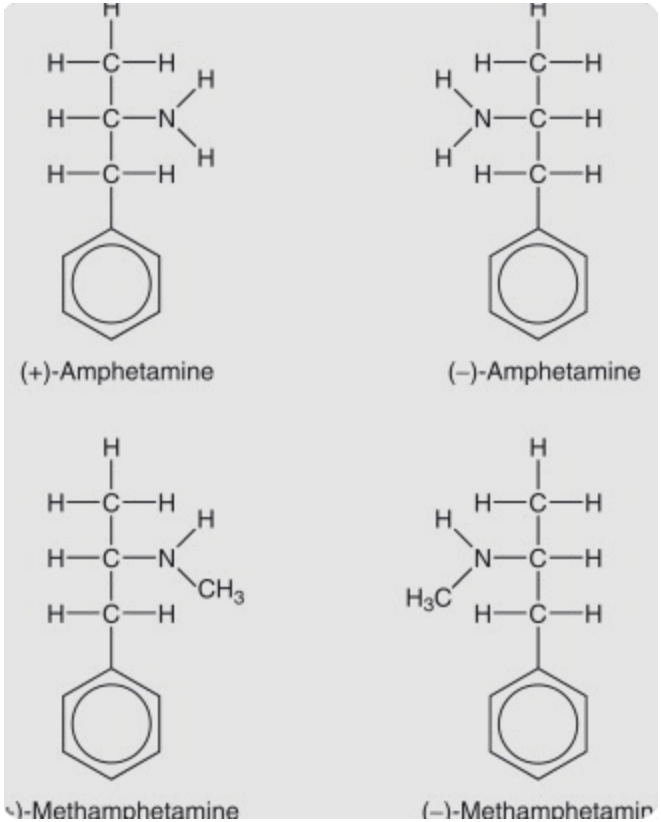
HPLC

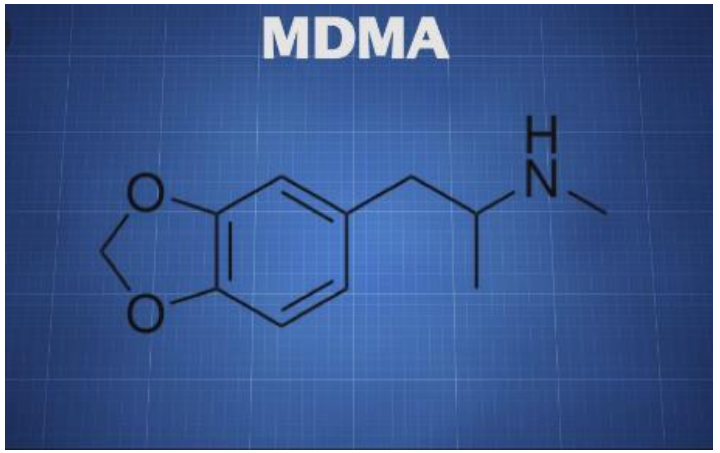

Regulated
helium source
Output check valve
Solvent
reservoirs
Sparger
Inlet
filter
Pump
Pulse damper
Inlet
check valve
To
waste
Drain valve
Priming syringe
Solvent proportioning valve
To detector
Column
Pressure
transducer
Back-pressure
regulator
Filter
Injector valve

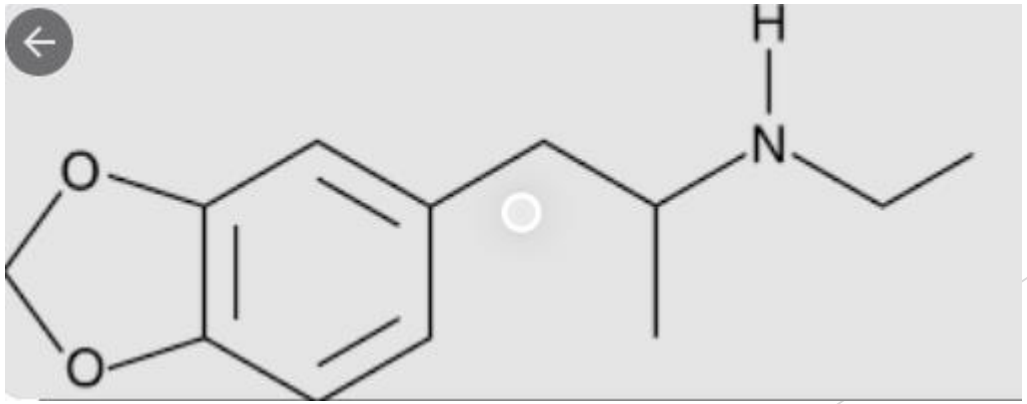
GC-MS


Injection Port
He
Carrier Gas
Trap
Capillary Column
Ionization
Source
Focusing Lens
Detector
Mass Analyzer
GC Oven
Mass Spectrometer

Amphetamines



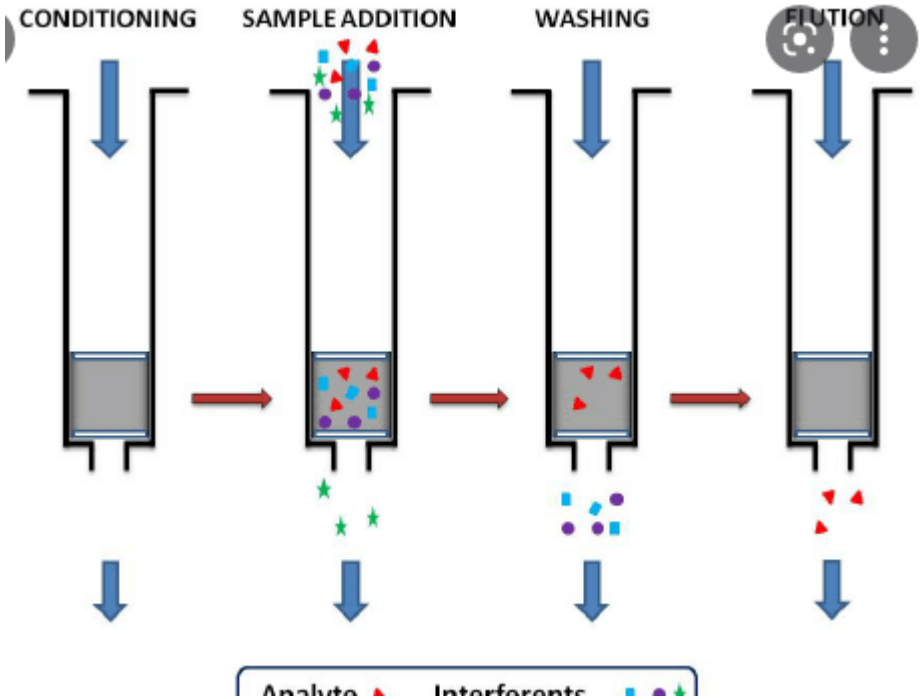




Analysis

- Screening: Immunoassay (+ or -)
- Extraction
  - LLE (liquid-liquid extraction)
  - SPE (solid phase extraction)
  - Derivatization (TMS, PFBA, Acetic acid anhyd)
  - Analysis
  - GC-MS, LC-MS

SPE

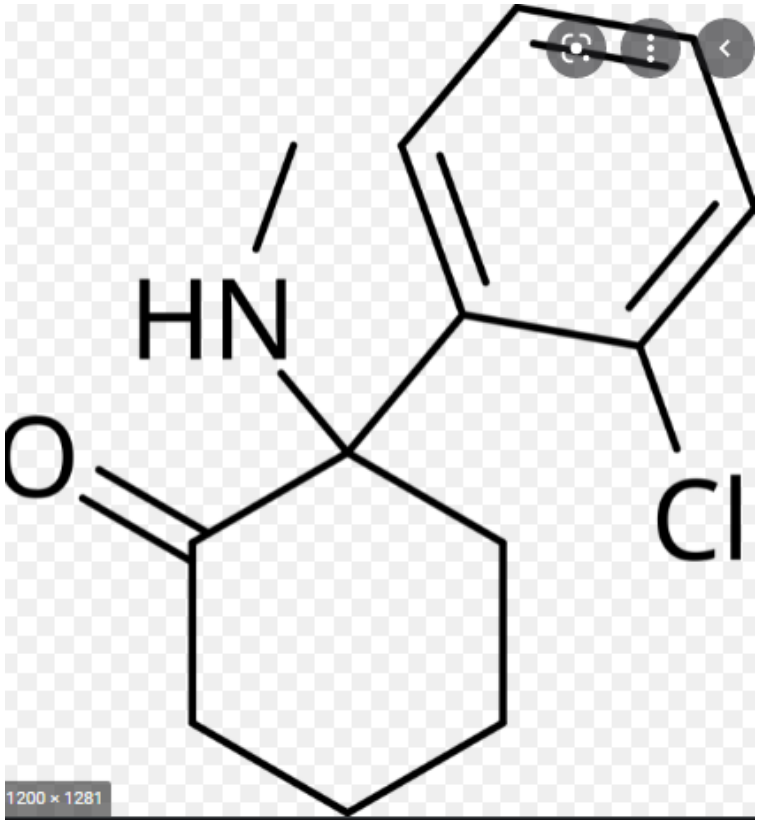


Cannabinoids

Analysis

- Screening : Immunoassay (+ or -)
- Extraction
  - Alkaline Hydrolysis
  - LLE
  - SPE (High recovery)
- Derivatization : PFBA
- Confirmation: GC-MS, LC-MS

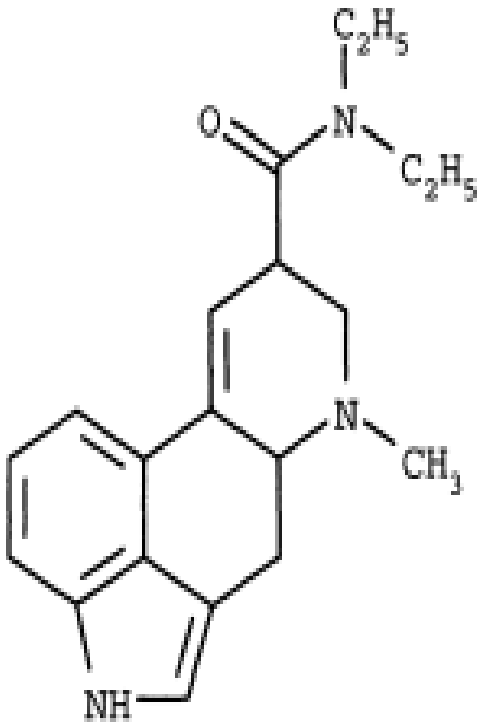
Ketamin



Ketamine Analysis

- Screening
- Extraction
  - LLE
  - SPE
- Analysis
  - GC-MS

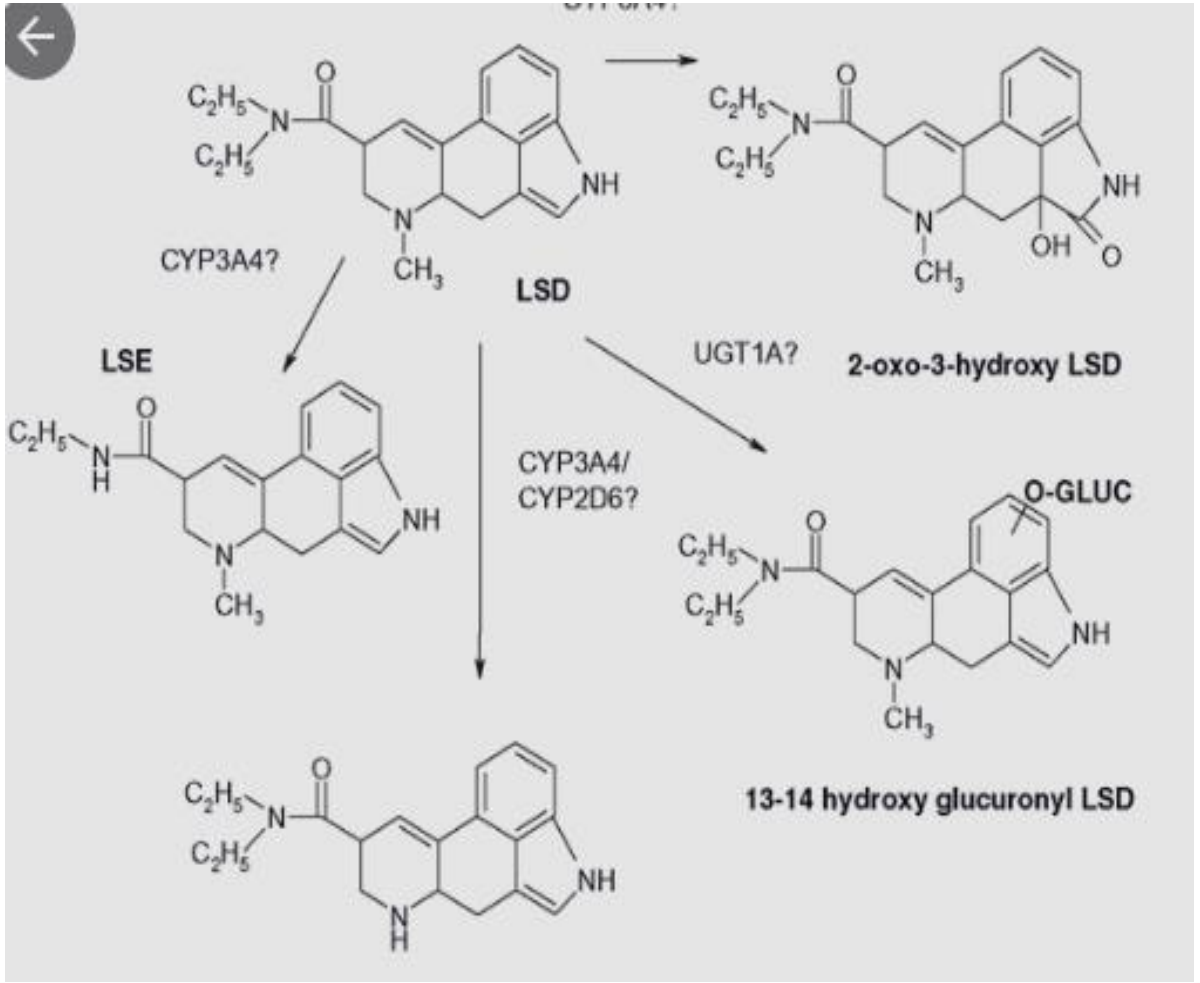
LSD



(+)-Lysergide (LSD),
d-Lysergic acid diethylamide
$C_{20}H_{25}N_3O$
M.W. = 323.4
PKa : 7.8

LSD tartrate
$(C_{20}H_{25}N_3O)_2C_4H_6O_6 \bullet 2CH_3OH$
M.W. = 860.9

LSD


CYP3A4?
LSD
LSE
UGT1A?
2-oxo-3-hydroxy LSD
CYP3A4/
CYP2D6?
O-GLUC
13-14 hydroxy glucuronyl LSD

LSD Analysis

Sample preparation [36]

- Blood, serum or urine (1-3 ml) are diluted to 20 ml with borate buffer pH 9.5 (5 g $Na_2B_4O_7.10\ H_2O$ in 1 L). The determinations are carried out with external standardisation.
- Apply the diluted specimen to an Extrelut® (or equivalent) column.
- Elute the column with dichloromethane-isopropanol (85:15).
- Evaporate to dryness.
- Reconstitute the residue with 200 L methanol.
- Inject 10 µl in the HPLC system.

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Sample preparation

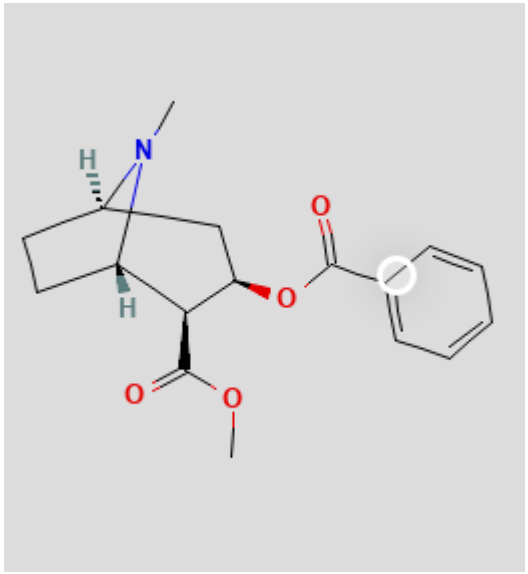
At a pH > 8.0 LSD can be efficiently extracted from urine or serum samples with a relatively non-polar solvent such as n-butyl chloride[15, 26]. Additional cleanup can be achieved by back-extraction of the basic LSD into a phosphate buffer (pH 4.5), and then re-extraction into n-butyl chloride after addition of ammonium hydroxide to the aqueous layer[28].

Solid-phase extraction methods have also been used for isolation of LSD from biological matrices[25, 38, 39, 40]. Most of the solid-phase extractions employ an adsorbent possessing both hydrophobic and cationic characteristics. After the biological specimen is adjusted to a pH of 5 to 6, it is added to the extraction column, and after washing the column with a dilute acid and methanol, the LSD is eluted with an organic solvent such as ethyl acetate containing 2 to 4% ammonium hydroxide.

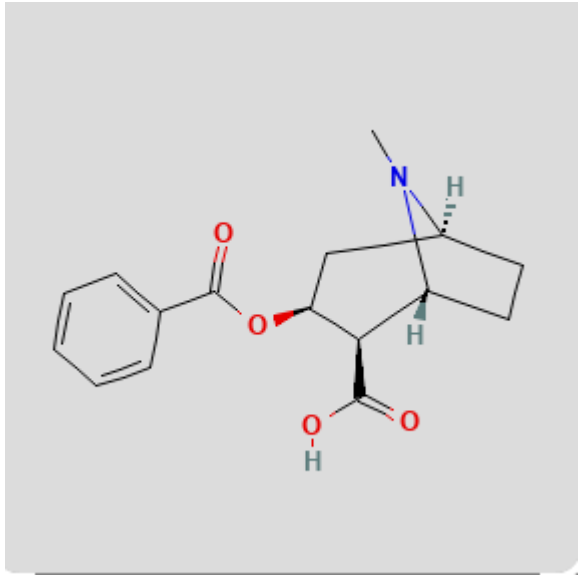
Immunoaffinity resins can achieve very selective extraction of LSD and some of its metabolites from biological samples[25, 41, 42].

Derivatisation

Trimethylsilylation of the indole nitrogen is the derivatisation most often used for GC-MS analysis of LSD. Underivatised LSD can be gas chromatographed, but sensitivity is generally severely limited due to adsorptive losses during the chromatographic process. The specific trimethylsilylating reagents used include bis (trimethylsilyl)trifluoroacetamide (BSTFA)[26, 28] and N-methyl(trimethylsilyl)- trifluoroacetamide (MSTFA) in pyridine (1:1 v/v)[15].



Cocaine



Benzoylecgonine

Cocaine

- Screening
- Extraction
  - SPE
  - Derivatization (PFPA)
- Detection
  - GC-MS