

Analytical Aspects with Brief Overview of Depressants

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Abstract

Depressants are drugs that slow down the functions of the central nervous system (CNS). These drugs are used to reduce anxiety and insomnia without drowsiness. The depressants cause relaxed feeling if used in small quantity but cause unconsciousness, vomiting and even death if taken in high quantity. It affects concentration and coordination of a person by slowing down his/her ability to respond in unexpected situations. These drugs are also attributed for their physiological and psychological effects, eventually in large dose it become lethal. The different

physical and chemical features of some very often used depressants are discussed in this manuscript.

Keyword: Depressant, TLC, UV spectroscopy, HPLC, GLC etc.

Introduction

The classical depressants are hypnotics (which induce sleep), most antianxiety medicine (diazepam or valium), muscle spasm prevent seizure, but these drugs rapidly develop dependence and tolerance which finally leads to coma and death, so use of these drugs is highly unsafe. The different group of depressants includes benzodiazepines, barbiturates, barbiturate-like substances and alcohols. Further several related compounds, including kava and gamma-hydroxybutyric acid (GHB) resemble effects those of the depressants. All depressants have the potential for being misused, and many prescription drugs are widely used for drug abuse. Street names of depressant drugs are Barb, Benzo, Downers, Georgia home boy, Grievous bodily harm, Liquid X, Nerve pills, Reds, Yellows, Rophies, Tranks, GHB, Phennies, R2, Roofies, Rophies, Tranks etc. Depressants are taken in the form of Pills, Syrup and injectable liquid. It is basically classified as:

1. Barbiturates
2. Benzodiazepines
3. Barbiturate like substances
4. Alcohols

Barbiturates

Barbiturates, which are derived from barbituric acid, are marked as sedative, hypnotic and depressant, which act on CNS and depress its activity. It was prepared first time by Adolf von Baeyer by condensation of melonic acid ester (diethylmelonoate) and urea in presence of sodium ethoxide in ethanol. He named the compound 'barbiturate' combining the names of St. Barbara and urea. Barbiturates are a group of synthetically developed drug intended for therapeutic purposes. After 40 years two German scientists, Emil Hermann Fischer and Joseph von mering, synthesize a

new drug from barbituric acid and named it 'barbitone'. They found that barbitone was a useful hypnotic drug which induces sleep in both animal and humans. In 1912, a second barbiturate named as 'phenobarbitone' was discovered.

In 1960 two compounds named as Librium and valium introduced in market, with passage of time numerous barbiturates were synthesized and introduced in the market and this caused significant increase in cases of poisoning.

These drugs are rapidly used for drug abuse and produce dependence, tolerance in addict. If these drugs are taken in large amount become lethal. Combination of these drugs with even small quantity of alcohol causes very high intoxication. Naming of barbiturates in United states, usually follow the suffix 'al' for example Phenobarbital, however in Britain, it usually end with 'one' for example Phenobarbitone.

The possibility of two different substituents at methine ($-\text{CH}_2-$) carbon in the structure (fig 1) yields a number of barbituric acid derivatives (barbiturates) with different potency.

Classification of barbiturates on the basis of time of action

1. Ultra Short acting drugs: Its short course of action is attributed to be used in anesthesia. Such type of drugs has an immediate action that lasts for a very short duration, eg, Thiopental (Pentothal), Methohexital (Brevital) etc.
2. Short Acting Drugs: These are used as sedatives and impart relatively fast action (10-15 minutes) and short duration of action (3-4 hrs), eg. Pentobarbital (Nebutal), Secobarbital (Seconal) etc.
3. Intermediate acting drugs: These are generally used to induce sleep. The action starts slow (45-60 minutes) and lasts for an intermediate duration (6-8 hrs.) eg. Amobarbital (Amyta),

Butabarbital (Butisol) etc.

4. Long acting drugs: These are generally used in seizures. The action starts slow (30-60 minutes) and lasts longer (10-16 hrs.), eg. Phenobarbital (Luminal), Chloral hydrate, Paraldehyde etc.
5. Carbamate: It is a pesticide which produces depressant like symptoms on administration, eg. Meprobamate (Miltown, Euanil) etc.

Different properties of some common barbiturates

1. Phenobarbitone

Trade name	: Luminal, Phenobarbital
Street name	: Purple Hearts, Goof Balls
IUPAC Name	: 5-Ethyl-5-phenylbarbituric acid
Synonyms	: Phenobarbituric acid, Phenylethylbarbituric acid, 5-phenyl-5-ethylbarbituric acid, Phenylethylmalonylurea
Duration of action	: Long
Relative abuse potential	: Low, Half-life 2-4 days, detection window upto 3 weeks
Mode of administration	: IV and intra muscular injection, oral
Appearance	: White, crystalline plates with three different phases, clear transparent in liquid form
Therapeutic use	: Anticonvulsant, Sedative
Molecular Formula	: $C_{12}H_{12}N_2O_3$
Molecular Mass	: 254.24
Solubility	: Slightly soluble in water (1 g/L); insoluble in benzene; aqueous alkali hydroxides, carbonates, diethyl ether and ethanol

Chemical structure : Fig 1

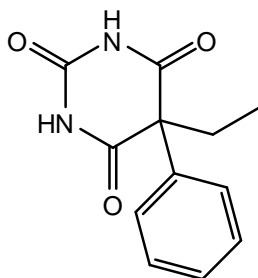


Fig. 1 Chemical Structure of Phenobarbitone

2. Phenobarbitone sodium

Trade name	: Gardenal Sodium, Luminal Sodium, Phenobal Sodium, Linasen, PBS.
Street name	: Purple Hearts, Goof Balls
IUPAC Name	: 5-Ethyl-5-phenylbarbituric acid, sodium salt
Synonyms	: 5-Ethyl-5-phenylbarbituric acid sodium, Phenobarbital sodium, Phenobarbitone sodium, sodium ethylphenylbarbiturate.
Duration of action	: Long
Relative abuse potential	: Low
Mode of administration	: Orally and IV injections
Appearance	: Slightly hygroscopic crystals or white powder
Therapeutic use	: Anticonvulsant
Molecular Formula	: $C_{12}H_{11}N_2NaO_3$
Molecular Mass	: 254.22 g/mol
Solubility	: Very soluble in water (1 kg/L) and ethanol, insoluble in chloroform and diethyl ether

Chemical structure : Fig 2

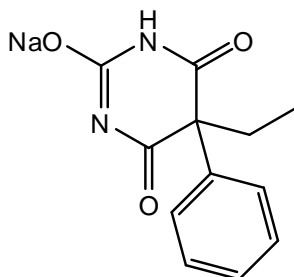


Fig. 2 Chemical Structure of Phenobarbitone Sodium

3. Methylphenobarbitone

Trade name	: Prominal, Mebaral, Phenmiton, Phenmitone
IUPAC Name	: 5-Ethyl-1-methyl-5-phenyl-1,3-diazinane-2,4,6-trione
Synonyms	: 1-Methylphenobarbital, 5-Ethyl-1-methyl- 5-phenylbarbituric acid phenemal, Mebaral, Mebhobarbital, Mebhobarbitone, Metiphenobarbital, Metiphenobarbitale.
Duration of action	: Long, half-life is 11-67 hr
Relative abuse potential	: Low
Mode of administration	: Orally and IV injections
Appearance	: Solid, whitish in color
Threptic use	: Anticonvulsant, Hypnotic, Sedative, Use in epilepsy
Molecular Formula	: C ₁₃ H ₁₄ N ₂ O ₃
Molecular Mass	: 246.26
Solubility	: Slightly soluble in water (0.17mg/ml)

Chemical structure : Fig 3

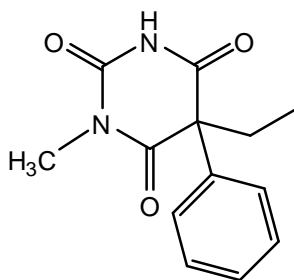


Fig. 3 Chemical Structure of Methylphenobarbitone

4. Quinalbarbitone sodium

Trade name	: Seconal Sodium, Secobarbital,
Street name	: Red Birds, Red Devils, Lilly, F-40's, Pink Ladies
IUPAC Name	: 5-(pentan-2-yl)-5-(prop-2-en-1-yl)-1,3-diazinane-2,4,6-trione
Synonyms	: 5-(1-Methylbutyl)-5-(2-propenyl)-2,4,6-(1H,3H,5H)-pyrimidinetrione, 5-allyl-5-(1-methylbutyl) barbituric acid, Secobarbitalum
Duration of action	: Short, half-life 22-29 days, detection window 2-10 days
Relative abuse potential	: High, cause allergic reaction
Mode of administration	: Orally, Rectal and IV injections
Appearance: Red capsules	: white and colorless, bitter powder
Therapeutic use	: Hypnotic, anesthetic
Molecular Formula	: $C_{12}H_{17}N_2NaO_3$
Molecular Mass	: 260.27
Solubility	: Freely soluble in Water & Methanol and insoluble in chloroform, ethanol

Chemical structure : Fig 4

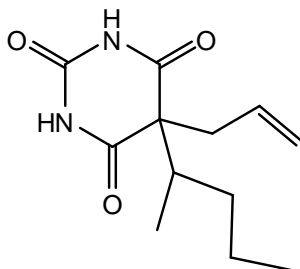


Fig. 4 Chemical structure of Quinalbarbitone

5. Cyclobarbitone

Trade name	: Phanodorm, Ciclobarbitol, Cyclobarbitol, Dormamed, Hexemal, Zylhexenenyla ethyl barbitursäure, Ciclobarbitale, cyclobarbitol sodium, Hexodorm, Neoclinal, Hexemalcalcium
IUPAC name	: 5-(cyclohexen-1-yl)-5-ethyl-1,3-diazinane-2,4,6-trione
Synonyms	: 2,4,6-(1H,3H,5H)-Pyrimidinetrione-5-(1-cyclohexen-1-yl)-5-ethyl-5-(1-cyclohexen-1-yl)-5-ethylbarbituric acid, Adorm
Duration of action	: Short
Relative abuse potential	: Moderate
Mode of administration	: Orally, Rectal and IV injections
Appearance	: Calcium salt is yellowish white powder with bitter taste
Therapeutic use	: Hypnotic, Sedative
Molecular Formula	: $C_{12}H_{16}N_2O_3$
Molecular Mass	: 236.27
Solubility	: Sparingly soluble in water

Chemical structure : Fig 5

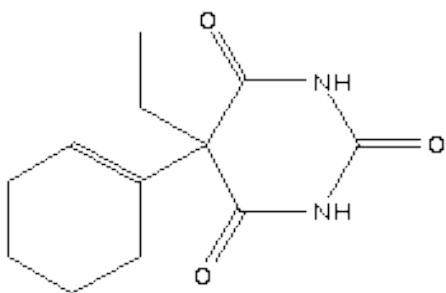


Fig. 5 Chemical structure of Cyclobarbitone

6. Butobarbitone

Trade name	: Soneryl, Neonal, sonabarb
IUPAC name	: 5-butyl-5-ethyl-1,3-diazinane-2,4,6-trione
Synonyms	: 5-butyl-5-ethylbarbituric acid, butobarbital, Butethal, Butobarbital sodium salt,
Duration of action	: Intermediate
Relative abuse potential	: Moderate
Mode of administration	: Orally, Rectal and IV injections
Appearance	: crystalline white powder with odour
Therapeutic use	: Hypnotic,
Molecular Formula	: $C_{10}H_{16}N_2O_3$
Molecular Mass	: 212.24 g/mol
Solubility	: soluble in water at 20 °C is 4.9 g/l

Chemical structure : Fig 6

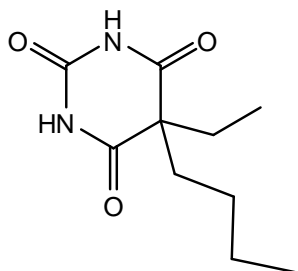


Fig. 6 Chemical Structure of Butobarbitone

7. Amylobarbitone

Trade name	: Amytal, Amobarbital, pentymalum
Street Name	: Amy's, Birds, Blue Angels, Blue Birds, Blues, Blue Bullets, Clouds, Blue Devils, Blue Dolls, Blue Heavens, Downers, Blue Velvets
IUPAC name	: 5-ethyl-5-(3-methylbutyl)-1,3-diazinane-2,4,6-trione
Duration of action	: It is intermediate acting drug having its half-life 15 to 40 hr depends on dose and detection window
Relative abuse potential	: High
Mode of administration	: Orally and intra-ventricular injection
Appearance	: crystalline white powder with bitter in taste, no odour
Therapeutic use	: Hypnotic short term treatment of insomnia, reduces anxiety & induce sedation
Molecular Formula	: $C_{10}H_{18}N_2O_3$
Molecular Mass	: 226.27 g/mol
Solubility	: 603 mg/L (at 25 °C) in water, freely soluble in benzene

Chemical structure : Fig:-7

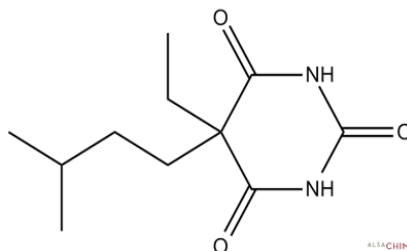


Fig. 7 Chemical structure of Amylobarbitone

- 8. Thiopentone sodium:** This drug is also commonly known for its “truth serum”. It is used during interrogation because it weakens the capacity of taking decision to the subject. It is among a list of drugs which has a heightened risk of causing significant harm in patient, when used in error.

Trade name	: Intraval, Sodium pentothal, Pentothal, Thiopentone, Methohexital, Thiopental
IUPAC name	: 5-ethyl-4,6-dioxo-5-pentan-2-yl-1H-pyrimidine-2-thiolate
Synonyms	: Bomathal, Nesdonal, Penthiobarbital, Pentothal Sodico, Sodipental, Thiomebumal, Thionembutal, Nycomed, Thiopentobarbital, Trapanal, Thiomebumal, Sodium, Hypnostan, Leopental, Nesdonal, Ravonal, Tiopental sodico.
Duration of action	: Ultra-short acting
Relative abuse potential	: Half-life 6-46 hrs. Detection window 2-10 days
Mode of administration	: Orally and intra-ventricular injections.
Appearance	: White powder, bitter in taste and garlic like smell
Threptic use	: Anesthetic

Molecular Formula	: $C_{11}H_{17}N_2NaO_2S$
Molecular Mass	: 264.31 g/mol
Solubility	: 96 mg/L in water at 25 °C
Threptic use	: Anesthetics, Hypnotics and Sedatives
Chemical structure	: Fig 8

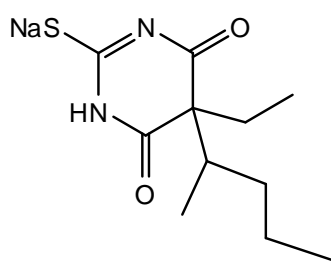


Fig. 8 Chemical Structure of Thiopental Sodium

9. Pentobarbital:

Trade name	: Nebutal, Nembutal, Mebubarbital, Mebumal, Ethaminal
Street name	: Yellow Jacket, Mexican Yellows, Abbots, Nembies
IUPAC name	: 5-ethyl-5-pentan-2-yl-1,3-diazinane-2,4,6-trione
Synonyms	: Diabutal, Etaminal, Ethaminal, Mebubarbital, Mebumal, Monosodium Salt Pentobarbital, Nembutal, Pentobarbitone,
Duration of action	: Short-acting, Half-life 35-48 hr. detection window 2-10 days
Relative abuse potential	: High, also used in physician assisted suicide
Mode of administration	: Orally and intra-ventricular injections, intramuscular
Appearance	: Odorless and colorless

Molecular Formula	: $C_{11}H_{18}N_2O_3$
Molecular Mass	: 226.27 g/mol
Solubility	: In water, 679 mg/L at 25 °C, very slightly soluble in water and carbon tetrachloride; very soluble in acetone and methyl alcohol
Therapeutic use	: Anticonvulsant, sedative hypnotic
Chemical structure	: Fig 9

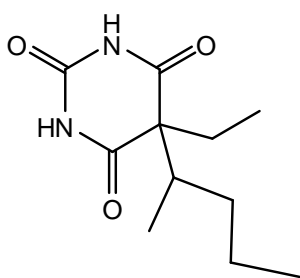


Fig. 9 Chemical Structure of Pentobarbital

Therapeutic use of Barbiturates: It is used as anticonvulsant-sedative in lower doses and to hypnosis, anesthesia in higher doses. Clinical use of barbiturates varies according to their time of action, short acting and intermediate acting barbiturates are given as sedative or tranquilizer due to their hypnotic or sleep inducing effect. Long acting barbiturates are more often used in case of epilepsy (convulsion). Phenobarbitone is oldest anticonvulsant drug still in use, and its first clinical use is reported in 1912. It is found more effective in epilepsy than bromide drugs.

The barbiturates like amylobarbitol used in treatment of anxiety, and some other formulations are used for treatment of insomnia like valium, Librium. Multidrug therapies are also given for various problems like stress, tension, duodenal ulcer, asthma, bronchitis and psychiatric treatments.

Sign & Symptoms of Barbiturates

1. Sign & symptoms of intoxication with low dose produce sleepiness, decreased alertness, slurred speech, muscular incoordination and drowsiness.

Table 1: Therapeutic, toxic and lethal doses of the barbiturates

S.No	Name of Drug	Therapeutic dose (µg/ml)	Toxic dose (µg/ml)	Lethal dose (µg/ml)
1.	Long acting	10-40	40-60	> 60
2.	Intermediate acting	1-5	10-30	> 30
3.	Short acting	1	7	10
4.	Phenobarbital	10-40	40-60	> 80
5.	Pentobarbital	1-3	> 5	10-169
6.	Secobarbital	1-22	> 3	5-52
7.	Amobarbital	1-5	10-30	13-96
8.	Thiopental	1-40	> 7	10-400

2. Sign & symptoms of intoxication with high dose are decreased activity of central nervous system followed by decrease in rate of heart beat, blood pressure falls, and respiratory depression, impair judgement, emotional disturbance, depression, memory loss and confusion, develop personality defect, and often behave like children and create suicide tendency.
3. Sometimes very high doses of barbiturates damage the tissue of central nervous system & brain.
4. In case of pregnant women barbiturate doses effect new born in many ways like development abnormalities, effect on brain processing etc.
5. Mild withdrawal symptoms of barbiturates are insomnia, restlessness, disturb dreaming, irritability, depression, apprehension, mild tremors, loss of appetite, piloerection (standing of hairs from end).
6. Intermediate withdrawal symptoms of barbiturates are severe tremors, muscle rigidity, impaired motor activity, and retching, vomiting and significant weight loss.

7. Severe withdrawal symptoms of barbiturates are very severe tremors, extreme anxiety, delirium, hallucination, convulsion, hyperthermia, unusually high fever.
8. Withdrawal symptoms depend on several factors like tolerance, frequency of dose administration and drug action time.
9. Factors affecting the drug action are the amount of dose taken, route of administration, physiological activities of different individuals, health condition of abuser or patient.

Tolerance: Tolerance which involves a decrease in response to a drug when taken repeatedly with constant amount of dose. In other word if a person is taking a drug continuously, he found that effect of drug decreases with time, because to increase tolerance of body toward that drug, while to get same effect he increases dose which is called dependence.

Toxicity: Barbiturates are especially more dangerous when abused with alcohol, opiates, and benzodiazepines because the action of barbiturates enhances when taken in combination of these drugs. Since these drugs act on same receptors in the body on which barbiturates act and therefore potentiate the effect of the barbiturates, many drug abusers combine multiple drugs this becomes a dangerous combination leading to increased sedation, impaired motor coordination, suppressed breathing and other adverse effects that may potentially be lethal or may cause death.

Symptoms of overdose: It consist of drowsiness, slurred speech, nystagmus, hypotension, ataxia, respiratory depression, CNS depression, hypothermia, cutaneous bullae, coma, cardio respiratory arrest and death. Delayed deaths usually arise from acute renal failure, pneumonia, acute lung injury, cerebral edema, and multi organ system failure.

Window of Detection in Saliva & Urine

1. Window of detection in Saliva is 0-36 hr. after oral administration.

2. Window of detection in Urine is 1-3 days after administration. Except in case of phenobarbitone there is window of detection 1-21 days.

Analysis of barbiturates

1. By colour test:

- (i) Dille-koppanyi test: Sample along with control is taken in spotting plate, 3 drops of Dille-koppanyi reagent (preparation is given later on) is added, development of purple color confirm the presence of barbiturates.
- (ii) Cobalt-acetate test: In a test tube two ml of extract is dissolved in methanol and then poured few drops of cobalt acetate reagent to it, violet colour develops which confirms presence of barbiturates.
- (iii) Mercuric sulphate test: Two ml of extract is dissolved in chloroform in a test tube, and then added 0.5 ml of solution 1 followed by addition of 0.5 ml of solution 2. Appearance of violet colour shows presence of barbiturates.
- (iv) Zwikker's Test: In the 1 ml chloroform extract of residue added 2-3 drops 0.5 ml solution 1 and shaken, followed by addition of 1 drop of solution 2 in it, if the colour in chloroform layer changes purple to weak blue non thiobarbiturates are confirmed. If chloroform layer becomes green after adding pyridine in chloroform, which changes to light green after adding glacial acetic acid, barbiturates are confirmed.
- (v) Test for barbituric acid: Take small of solid depressant drug or a drop of liquid solution of drug on spot plate, add 1 drop each of solution 1 and 2, depending upon amount of barbituric acid present in formulation, more or less intense red-violet color develops. The red and violet colors appear due to formation of alkali salt of violuric acid.

(vi) Test with oxamide or ethyl oxamate: Few drop of test solution or small amount of solid sample placed in a micro test tube along with excess of oxamate and then dry it on bath that has been preheated to 140-160 °C. Yellow or red color appears which shows presence of barbituric and thiobarbituric acid. Derivatives of barbituric acid which have no free CH₂ group in the 5-position don't give above color reaction.

(vii) Test with sulfadiazine: Take small amount of test solution of barbituric acid and Thiobarbituric acid in a micro test tube and treated with several drop of aqueous solution of sulfadiazine, the mixture is warmed in boiling water. A yellow or red color appears due to presence of barbituric acid & thiobarbituric acid. Yellow or Red color developed due to formation of polymethine dyes, which fluoresces in characteristic manner in UV light. Now a drop of solution is placed on filter paper, a colored stain appears and under UV light it shows blue-green color for barbituric acid or orange to yellow color for thiobarbituric acid. Derivatives of barbituric acid which have no free CH₂ group in the 5-position don't give above color reaction.

(viii) Test with Pyridylpyridinium dichloride: Take sample in a test tube, evaporate it and dried residue taken in micro test tube, add 1-2 drops of solution 1 followed by addition 1-2 drops of solution 2 and the mixture is then heated for 2-3 min. at 120 °C in a glycerol bath, on cooling it gives red color for barbituric acid and blue color for thiobarbituric acid.

Some detection methods of common drugs

1. Allobarbital

- Mercurous nitrate test - Two ml of extract is taken in a test tube and then few drops of mercurous nitrate solution is added, appearance of black colour confirms presence of Allobarbital.
- Vanillin test - Two ml of extract is taken in a test tube and then few drops of vanillin reagent is added, brown colour is observed which indicates presence of Allobarbital.

2. Amobarbital

- Mercurous nitrate test - Two ml of extract is taken in a test tube, few drops of mercurous nitrate solution is added to it, greyish color is observed which indicates presence of Amobarbital.

3. Pentobarbital

- Mercurous nitrate test - Two ml of extract is taken in a test tube. Added few drops of mercurous nitrate solution to it, grey colour is observed which indicates presence of Allobarbital.
- Vanillin test - Two ml of extract is taken in a test tube, added few drops of vanillin reagent, violet colour is observed which indicates presence of Allobarbital.

4. Phenobarbital

- Liebermann's test –Two ml of extract is taken in a test tube then few drops of Liebermann's reagent is added to it, red or orange colour is observed which indicates presence of Phenobarbital.
- Mercuric Sulphate test – Two ml of extract dissolved in Chloroform is taken in a test tube, followed by addition of 0.5ml each of solution 1, and solution 2, bluish-violet color is observed which shows presence of Phenobarbital.

5. Secbutabarbital

- Vanillin test - Two ml of extract is taken in a test tube, added few drops of vanillin reagent to it, orange colour is observed which indicates presence of secbutabarbital.

6. Thialbarbital

- Vanillin test - Two ml of extract is taken in a test tube, added few drops of vanillin reagent, appearance of brown to orange colour indicates presence of Thialbarbital.

- Palladium chloride test - Two ml of extract taken in test tube, added 1 ml of palladium chloride solution, and heated solution gently for two minutes, orange to yellow colour is observed which indicates the presence of Thialbarbital.

7. Thiopental

- Vanillin test - Two ml of extract is taken in a test tube, added few drops of vanillin reagent, brown colour is observed which indicates presence of Thiopental.
- Palladium chloride test - Two ml of extract taken in a test tube and added 1 ml of palladium chloride solution to it, then heated the solution gently for 2 minutes, orange colour is observed which indicates the presence of Thiopental.

2. By UV Spectrophotometer

Barbiturates show almost same absorption maxima under a definite condition of acid or alkali. The appearance of absorption maxima is an indication for the presence of barbiturates.

Table 2. UV Absorption maxima of different depressants

S.No.	Compound Name	Absorption maxima, 0.05 M Borax Buffer (pH = 9.2)	Absorption maxima in 1M NaOH aqueous
1.	Allobarbital	241	256
2.	Butabarbital	239	254
3.	Pentobarbital	239	255
4.	Phenobarbital	239	254
5.	Secbutabarbital	239	254

- All reading of absorption maxima are taken in nm.
- 0.05 M borax buffer prepared by dissolving 19.07 gm borax in 1000 ml of water.

- Barbiturates in general (except N-substituted compounds) show absorption maxima at 255 nm in 2.5 N NaOH solution.
- Thiobarbiturates show absorption maxima at 290 nm and 239 nm in 2 N Sulphuric acid.
- Meprobamate does not show any absorption in the range 230-360 nm.

3. By Thin Layer Chromatography (TLC): This technique is also very helpful in detecting barbiturates. It is detected by colour of spots and R_f values in a specified solvent.

- Plate: Silica Gel G, 0.2 mm thickness of stationary phase coating.
- Development: Ascending type.
- Solvent system

System 1 – Ethyl acetate: Methanol: 25% Ammonia (85:10:05).

System 2 – Chloroform: Acetone (80:20).

System 3 – Isopropyl Alcohol: Chloroform: Ammonia (45:45:10)

System 4 – Benzene: Acetic acid (90:10).

System 5 – Dioxane: Benzene: Ammonium hydroxide (LR) (20:75:05)

- Visualization Agent
 - First observe plate under UV light at 254 nm. Then expose plate to concentrated ammonia vapours and observe plate again under UV light at 254 nm.
 - Mercuric chloride diphenylcarbazone reagent: Spray plate with the reagent, blue violet spot on a pink background indicates the presence of barbiturates.
 - 0.2% aqueous potassium permanganate solution spray
 - Mercurous nitrate reagent:- Spray reagent on the plate, black spots are observed which

- indicate presence of barbiturates.
- Zwikker's reagent:- spray reagent on the plate pink spot is observed in case of non-thiobarbiturates and green spot is observed in case of barbiturates.

4. By Gas Liquid Chromatography

(i) Without derivatization

- Packed Column- The analysis of underivatized barbiturates is not recommended because of their decomposition at high temperature and presence of mild steel component of test tube. Thus it is necessary to derivatize barbiturates before inject into GC.
- Capillary column– In case of capillary column, there is no such high temperature required for separation of mixture. Therefore there is no decomposition of barbiturates further since column is made up of inert fused silica very high temperature is restricted.

(ii) With derivatization

Barbiturates are injected into GC after derivatization which avoid decomposition of drug into column. Derivatization is done with the help of derivatization reagents.

Table 3. Packed and capillary column specifications for detecting depressants

S.No.	Condition of GC	Without derivatization (capillary column)	With derivatization (packed column)
1.	Column	25m×0.35mm×0.52µm. Internal diameter, made up of fused silica, chemically bonded and cross linked methyl silicone or methylphenylsilicone coated inside with liquid phase such as OV 1, SE 30, and SE-54.	180cm×4 mm internal diameter of column, made up of glass or steel or Teflon etc. and stationary phase filled into column containing 3% SE-30 liquid phase coated on 80/100 mesh chromosorb G HP.
2.	Carrier gas	Nitrogen	Nitrogen
3.	Flow rate	1 ml/min.	40-45 ml/min.
4.	Split ratio	20:1	-----.
5.	Column temp.	Isothermal at 200 °C Programmed 200-260 °C, at 4 °C/min.	Isothermal 190-200 °C
6.	Injector temp	275 °C	220 °C
7.	Detector temp.	275 °C	220 °C
8.	Detector used	Flame ionization detector	Flame ionization detector
9.	Internal standard	N- alkane	N-alkane
10.	Internal standard/ drug standard concentration	1 mg/ml.	1 mg/ml.
11.	Derivatizing agent	-----.	Trimethyl anilinium hydroxide 0.2 M in methanol.

5. By High Performance Liquid Chromatography

Table 4. HPLC specifications for detecting depressants

S.No.	Condition	Method 1(normal phase)	Method 2 (reverse phase)
1.	Column	250mm×4.6mm	150 mm × 4.6 mm
2.	Packing material	Octadecyl-silica HPLC grade, 5µm (Sperisorb 5 ODS-2 or equivalent)	Octadecyl-silica HPLC grade, 5µm (Sperisorb 5 ODS-Hypersil or equivalent)
3.	Mobile phase	Acetonitrile: water (70:30)	0.1M sodium dihydrogen phosphate buffer: methanol (60:40), pH 3.5 adjusted with phosphoric acid and pH 8.5 adjusted with NaOH Sol ⁿ .
4.	Flow rate	0.9 ml/min	2.0 ml/min.
5.	Detection	UV at 220 nm,254 nm	UV at 216 and 254 nm
6.	Injection volume	1-5 µl by syringe or loop injector	1-5 µl by syringe or loop injector
7.	Quantitation	By peak area and external standard method	By peak area and external standard method

Preparation of reagents:

1. Dille-koppanyi Test

Reagent 1: 0.2 gm cobalt acetate dissolved in 100 ml of methanol and 0.2 ml of glacial acetic acid is added into it.

Reagent 2: 5 ml of isopropylamine is added in 95 ml of methanol.

2. Cobalt acetate test

Solution 1: Prepare 1 % solution of dry cobalt acetate in water.

Solution 2: Prepare 5 % solution of isopropylamine in water.

3. Mercuric Sulphate test

Solution 1: Prepare 0.5 % solution of mercuric sulphate in water

Solution 2: Prepare 0.1 % solution of diphenyl carbazone in water

4. Zwikker' test

Solution 1: Prepare 5% pyridine into chloroform.

Solution 2: Glacial acetic acid

5. Mercurous Nitrate reagent

A freshly prepared saturated solution of mercurous nitrate in water.

6. Vanillin reagent

Solution 1: Take 1gm vanillin dissolved in 50 ml ethanol

Solution 2: Take 1ml 1M KOH dissolved in 100 ml ethanol

7. Liebermann's reagent

1 gm of Potassium nitrite dissolved in 10 ml of sulphuric acid.

8. Mercuric sulphate test

Solution 1: Prepare 0.5 % solution of mercuric sulphate in water.

Solution 2: Prepare 0.1 % solution of diphenyl carbazone in water.

9. Test for barbituric acid

Solution 1: Take 2N acetic acid.

Solution 2; Take saturated aqueous sodium nitrite solution.

10. Test with Pyridylpyridinium dichloride

Solution 1: Take Dimethylformamide.

Solution 2: Prepare 1% solution of Pyridylpyridinium dichloride dissolved in dimethylformamide.

11. Mercuric chloride diphenylcarbazone reagent

Solution 1: Diphenylcarbazone solution is prepared by 0.1 gm. of diphenyl carbazone in 50 ml of ethanol.

Solution 2: Mercuric chloride solution is prepared by dissolving 0.1 gm mercuric chloride in 50 ml of ethanol.

Spray reagent is prepared by mixing of solution 1 and solution 2 in equal volume just before the spraying. Solution 1 always prepared fresh for spraying.

12. Zwikker's reagent

40 ml 10% solution of copper sulphate is mixed with 10 ml of pyridine and make up to 100 ml.

13. Mercurous Nitrate

A saturated solution of mercurous nitrate containing a few drop of conc. Nitric acid added into it.

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