Medicinal products acting on cholinergic system

Medicinal products acting on cholinergic system are classified into cholinomimetics (ChM) and cholinolytics (ChL). M-ChM are products which directly or indirectly can show acetylcholine-like effects. Pilocarpine hydrochloride is M-ChM drug.

**Pilocarpinum hydrochloridum (Pilocarpine hydrochloride)**

\[
\text{d-cis-ethyl-β-(1-methylimidazolil-5-methyl)-butyrolactone}
\]

*Pilocarpine* is an alkaloid, which can be obtained from African plant known as Pilocarpus Jaborandi. Pilocarpine is an imidazolin derivative, which have 2 nitrogen heteroatoms in the molecule. Nitrogen heteroatom in the 1st position is “pirolic”, (its electron couple takes part in double bond formation and included in aromatic ring) is connected with hydrogen atom which gives to a molecule weak acidic property (can be easily separated from the molecule). Nitrogen heteroatom in the 3rd position is “pyridinic” and gives to a molecule basic property.

Pilocarpine is used in medicine in the form of Pilocarpine Hydrochloride, which is a colorless crystalline hygroscopic powder. It is an optically active compound. It is quite photolabile and undergoes isomerization under influence of light by forming isopilocarpine, which has not pharmacological activity.

**Identification methods**
Chlorine ion detection by AgNO₃ lead to AgCl formation.

Butyrolactone existence « \( \text{O} \text{C} \) » in the structure of the molecule is identified by hydroxamate formation reaction: Pilocarpin interacts with hydroxylamine in basic environment (NH₂OH/NaOH) and forms hydroxamic acid which then with iron (III) chloride in acidic environment (FeCl₃/HCl) lead to a reddish-violet iron hydroxamate formation.

This reaction also is typical for lactones \( \text{O} \text{C} \text{N} \), lactams \( \text{O} \text{C} \text{N} \), amides \( \text{O} \text{C} \text{N} \), imides \( \text{O} \text{C} \text{N} \).

Pilocarpine gives specific reaction with sodium nitroprusside \( \text{Na}_2[\text{Fe(CN)}_5\text{NO}]·2\text{H}_2\text{O} \) (lactone ring detection), a cherry-like color is formed in the basic media (NaOH), which is not disappeared with the addition of hydrochloric acid solution (HCl). Pilocarpine quantity detection by photo colorimetric method is based on this reaction.

Pilocarpine like other alkaloids interacts with general alkaloid precipitating reagents (Dragendorff, Mayer, prcnic acid, Nessler's reagent (K₂[HgI₄]) by forming insoluble compounds in water.

Pilocarpine due to tertiary nitrogen atom interacts with a 2% citric acid (C₆H₈O₇) and acetic acid anhydride (CH₃CO)₂O by forming red color.

Khelch reaction is typical for pilocarpine identification, which is based on a complex salts formation after interaction with chromeperoxide (CrO₅). The following reagents are included in this reaction: sulfuric acid (H₂SO₄), potassium bichromate (K₂Cr₂O₇), hydrogen peroxide (H₂O₂) and chloroform (CHCl₃). In the result nadchrome acid and chromperoxide are formed. Chromperoxide with pilocarpine forms bluish-violet complex and is dissolved in the chlorophorm.

**Quantity detection methods**
Quantitatively pilocarpine hydrochloride can be detected by:
- Acid-base titration in non-aqueous media (media is the acetic acid or formic acid, titrant is a 0.1M HClO₄). Mercury acetate should be added in order to prevent dissociation of halogens.
- Neutralization: alkalimetry is used in the aqua-alcoholic media: hydrochloric acid is titrated by 0.1M NaOH solution, indicator is a phenolphthaleine.

\[
\text{C}_{11}\text{H}_{16}\text{O}_2\text{N}_2 \cdot \text{HCl} + \text{NaOH} \rightarrow \text{C}_{11}\text{H}_{16}\text{O}_2\text{N}_2 + \text{NaCl} + \text{H}_2\text{O}
\]

- Iodometry (in the presence of NaCl, pH=6) after removing the polyiodide precipitate. The extra quantity of iodine is titreted by sodium thiosulphate, indicator is a starch.

\[
\text{I}_2 + 2\text{Na}_2\text{S}_2\text{O}_3 = 2\text{NaI} + \text{Na}_2\text{S}_4\text{O}_6
\]

- Physicochemical methods (UV spectrophotometry, IR-spectroscopy, photo-colorimetric method etc.).

**Pilocarpine** is used in the form of eye drops: 1-2% solutions or eye ointment: 1-5%, for the treatment of glaucoma.

### Cholinolytics

Cholinolytics are agents which are able to prevent acetylcholine interaction with choline-receptors. M-cholinolytic natural alkaloid salts are: atropine sulphate, scopolamine bromide. Synthetic alkaloid salts are: Homatropine hydrobromide, diphenyltropine hydrochloride (tropacine) and tropodiphene hydrochloride (tropaphen) are.

<table>
<thead>
<tr>
<th>Atropine Sulfate-</th>
<th><img src="image" alt="Atropine Sulfate" /></th>
<th>Odorless, white crystalline powder M.T. 188–194°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scopolamine Hydrobromide -</td>
<td><img src="image" alt="Scopolamine Hydrobromide" /></td>
<td>Odorless, colorless, crystalline powder M.T. 193–197°C</td>
</tr>
<tr>
<td>Compound</td>
<td>Chemical Structure</td>
<td>Properties</td>
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<td>--------------------------------</td>
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</tr>
<tr>
<td>Homatropine Hydrobromide</td>
<td><img src="image1" alt="Homatropine" /></td>
<td>White crystalline powder M.T. 210-214°C</td>
</tr>
<tr>
<td>Diphenyltropine hydrochloride</td>
<td><img src="image2" alt="Diphenyltropine" /></td>
<td>White or weak nude crystalline powder M.T. 212-216°C</td>
</tr>
<tr>
<td>Tropodifene hydrochloride</td>
<td><img src="image3" alt="Tropodifene" /></td>
<td>Odorless, white or weak nude crystalline powder M.T. 190-197°C</td>
</tr>
</tbody>
</table>

All compounds are tropin alcohol derivatives, except scopolamine which is a scopin alcohol derivative and is differed from tropin derivatives due to the presence of an oxygen bridge in the 6th and 7 positions.

**Atropine synthesis**: Atropine and scopolamine can be obtained from raw plant materials in their basic forms by using organic solutions: dichlorine ethane C\(_2\)H\(_4\)Cl\(_2\), benzene C\(_6\)H\(_6\) and kerosene. Then by adding sodium hydroxide L-hyosciamine converts to a racemic mixture (with the left rotator L-hyoscyamine and D- hyoscyamine) and atropine is formed (activity of atropine is conditioned by L-hyoscyamine). Racemization speed and degree depends on the pH of media, temperature, solvents.
character, etc. In the pH 3,0-4,0 atropine is stable as an ether. Afterwards by removing D-hyoscyamine scopolamine is obtained.

Atropine synthesis is carried out by using siccine acid dialdehyde, methyl amine and acetone.

\[
\text{siccine acid dialdehyde} \quad \text{methyl amine} \quad \text{acetone} 
\]

\[
\text{tropinone} \quad \text{tropine} 
\]

\[
\text{atropine} \quad \text{atropine sulphate} 
\]

**Physical properties.** Tropane alkaloids and its synthetic analogues are easily soluble in water and ethanol. Atropine sulphate practically insoluble in chloroform. Scopolamine hydrobromide is poorly soluble in chloroform, while homatropine hydrobromide is soluble better, but biphenyl-tropin and tropodifene hydrochloride are easily soluble there. Natural alkaloids are differed from synthetic alkaloids based on their solubility in the chloroform. In some conditions atropine undergoes dehydratation by forming apoatropine, which then undergoes dimerization and lead to a formation of
beladonin and isotropic acid. Apoatropine is considered as an impermissible mixture and its quantity is controlled by pharmacopeia.

**Identity.** Tropane derivatives identity and quantity are determined by gas-liquid chromatography, IR, UV-spectrophotometric and HPLC methods. Alkaloids can be determined also by chemical reactions: hydrolysis, nitration, oxidation, neutralization due to tertiary nitrogen, complex ether group, phenyl radical and inorganic acid.

The Vitali-Morin reaction is used for tropine alcohol alkaloids (atropine sulphate, diphenyltropine and tropadiphen hydrochlorides) and scopine alcohol alcaloid (scopolamine hydrobromide) identity. The reaction is based on their hydrolysis, nitration and released acids oxidation (HNO₃, KOH alcoholic solution). When potassium hydroxide alcohol solution and acetone is added to the residue, a violet colored solution is formed, which has a quinoid structure.

Diphenyl acetic acid which is formed due to diphenyltropine hydrochloride (tropacine) hydrolysis contacting two aromatic rings is nitrated by nitric acid afterwards due to interaction with potassium hydroxide alcoholic solution leads to a formation of a compound having o-quinoid structure.

Homatropine hydrobromide does not give the Vitali-Morin reaction due to which it differs from other derivatives.
It is conditioned by hydroxyl group in the 4th position which is very sensible to oxidation and under nitric acid influence it is oxidized up to ketone. In order to get a positive Vitali-Morin reaction, homatropine should be acetylated with acetic acid anhydride for blocking alcoholic group.

- Tropane derivatives can be also differed by precipitating (general alkaloid) reagents such as: picric acid solution, Dragendorff reagent, phosphor molibdenic acid and etc.
- With iodine solution leads to a poly-iodide formation,
- Interacts with Mark reagent,
- Basic form is formed as a precipitate with alkali solution and the precipitate is identified by melting temperature
- Atropine solution is heated with sulfuric acid (H$_2$SO$_4$) in the presence of potassium dichromate (K$_2$Cr$_2$O$_7$) due to which bitter almond smell is felt (conditioned by benzaldehyde),

- Atropine sulphate and scopolamine hydrobromide with p-dimethylaminobenzaldehyde and concentric sulfuric acid form products with raspberry color.
- Atropine sulphate and scopolamine hydrobromide with betta naphthol and concentric sulfuric acid form green colored products and fluorescence.
- Scopolamine hydrobromide is oxidized by ammonium molybdate (NH$_4$)$_2$MoO$_4$ in the presence of HCl forming colored products.
- Basic form of homatropine due to interaction with the alcoholic solution of mercury chloride (II) HgCl$_2$ (t°C) leads to a colored product formation.
- Similar oxidation reactions can also give homatropine and scopolamine hydrobromide.
Tropaphen hydrochloride is identification by hydroxamate formation reaction is used too: it interacts with hydroxyl amine basic solution afterwards with 10% iron chloride (II) acidic solution.

- Acetoxy group CH₃-C(=O)-O-, included in the structure of Tropodiphene (is differed from diphenyltropine) is determined by ethyl acetate formation which has a typical smell. Tropodiphene is hydrolyzed in a basic environment by forming sodium acetate, tropin alcohol and α-phenyl-β-(p-acetoxyphenyl)-propionic acid. After addition of ethyl alcohol ethyl acetate is formed in the acidic environment.

- Chloride in hydrochloride forms (Diphenyltropine and tropodiphene hydrochloride) is identified by AgNO₃ solution.
- Atropine sulphate can be detected by BaCl₂ solution.
- Hydrobromides (scopolamine, homatropine) give a positive reaction towards bromide ions and are determined by copper sulphate and conc. sulfuric acid.

**Tropane derivatives quantitative analysis** is realized by non-aqueous titration method. Titrant is 0.1M HClO₄, media is non-aqueous acetic acid (indicator is a crystalline violet). Hydrochlorides titration (tropacine, tropaphen), Hydrobromides (scopolamine, homatropine) is realized in the presence of mercury acetate, which prevents halogen-ion dissociation. For example:
Atropine sulphate quantitative analysis is realized without adding mercury acetate because sulfuric acid in the presence of acetic acid acts as an one-base acid.

\[
\begin{align*}
\text{HClO}_4 + \text{CH}_3\text{COOH} & \rightarrow \text{ClO}_4^- + \text{CH}_3\text{COOH}_2^+ \\
\text{CH}_3\text{COOH}_2^+ + \text{CH}_3\text{COO}^- & \rightarrow 2\text{CH}_3\text{COOH}
\end{align*}
\]

- Tropane derivatives can be determined by neutralization method in the aqua-alcoholic media in the presence of chloroform. Tropane basic form which is formed during titration is dissolved in chlorophorm (indicator is phenolphthalein).
- Scopolamine hydrobromide can be detected by the argentometric method in the presence of acetic acid (indicator is bromine phenol blue).
- Atropine sulfate, homotropine, scopolamine hydrobromides can be detected by extraction-photometric method
- The same preparations are detected by reverse iodometric method (polyiodides formation reaction is used) after acidifying with hydrochloric acid.
- Photocholorimetry method is used too.
- Extraction-photometric method: Methyl orange with tropane derivatives forms yellow colored ionic compounds which then are extracted by chlorophorm solution and detected in 420-425 region.

**Anticholinesterase medicinal product**

Indirect cholinergic products also known as products which are able to inhibit acetyl cholinesterase, as a result of which neurotransmitter is accumulated. Some authors classify anti-CHE drugs into reverse and non reverse products. Physostigmine, pyridostigmine, neostigmine (proserine) Galanthamine are included in the first group. Pirofos, isoflurofate, phosphor-organic compounds are included in the second group.
There are 2 main groups of anticholinesterase drugs: carbamates and phosphor-organic compounds.

**Carbamates:** Phystostigmine compound belongs to carbamates and it represents meta-amino phenol derivative.

![Chemical structure of phystostigmine](image)

It is a white, odorless, crystalline powder that poorly is dissolved in water, but easily is dissolved in alcohol. As an alkaloid it is very labile toward temperature, humidity, basics. It undergoes to basic hydrolysis’ (NaOH) by forming methylamine, sodium carbonate, and heterocyclic eseroline condensed with phenol.

![Chemical reaction of phystostigmine](image)

It was proved that eseroline is physiologically inactive, so can be concluded that phystostigmine biological activity is conditioned by methyl-uretane group. Eseroline is oxidized by forming a red-rubreserine, which then form eserine-blue and eserine-brown which are inactive. Phystostigmine solution is stable in pH=6. Phystostigmine usage is limited in the medicine because of its side effects. Nowadays Proserine is widely used in the medicine (neostigmine methyl sulphate), pyridostigmine.
These compounds don’t have disadvantages typical to physostigmine, as they have quaternary nitrogen and its positive charge doesn’t allow to penetrate BBB and posses side effects on CNS. Also they are stable as they have dimethyl carbamate group instead of a methyl-carbamate group. Neostigmine easily is dissolved in water, ethanol and chloroform, but it doesn’t dissolved in ethers. Neostigmine has 2 specific properties: quaternary nitrogen is approximately 4.7 Å far from ether group. Quaternary centers are directly connected with aromatic ring which decreases the number of conformations that can be formed. Solutions are stable and can be sterilized by boiling.

**Identity** is detected by:

- IR specteroscopy
- Iodometry: adding iodine solution into neostigmine methyl-sulfate aqueous solution a brown polyiodide precipitate is formed.
- Dimethylcarbamoiil and sulfur groups are detected by heating preparation in aqueous bath with 30% sodium hydroxide solution (NaOH).
Released dimethylamine can be detected by typical smell and a by litmus paper (red color is changed to blue). Sulphate ion is detected by a barium chloride solution. Formed meta-dimethylaminophenol can be detected by diazotized sulfanilic acid, for getting the azo-compound (azo-dye) which has a reddish-orange color.

Neostigmine methyl sulfate **quantitative analysis** can be realized by Keldal method in Keldal tube acting by 30% sodium hydroxide solution and released dimethylamine rectify in boric acid solution. Dimethylamine tetrahydroxiborate is formed, which is titrated by 0.1M HCl. Methyl red is used as an indicator.
Neostigmine quantitatively can be detected by iodometry (due to polyiodide formation reaction). Titrant is 0.1M iodine solution. Neostigmine methyl-sulphate quantity analyses can be also realized through spectrophotometry or extraction-photometry.

Nowadays Neostigmine and Pyridostigmine have a large usage in medicine.

Tacrine and donepezil, which don’t have carbamate part, are classified as non-classic anticholinesterase drugs. Tacrine has a large usage in Alzheimer disease treatment. 20% of threatening patients have been recovery, but its usage is also limited due to hepatotoxicity. It is non-selective toward both acetylcholinesterase and butyrilcholinesterase.

Donepezil is another non-classical anticholinesterase drug, which was recently found and used for Alzheimer disease and dementia. It is approximately 570-1250 times selective to the Acetylcholinesterase than Butirrilcholinesterase and has more sensibility toward central A-ch than the peripheral A-ch. In contrast to tacrine, donepezil has a greater influence on the central Ach: it has a longer effectiveness and less hepatotoxicity.
1. Butirolactone ring in pilocarpine molecule is identified by the following reagent
   a. Salicylic acid solution
   b. FeCl₃ solution
   c. NH₂OH/NaOH, FeCl₃/HCl
   d. Hydrochloric acid

2. Atropine sulphate can not be identified by
   a. BaCl₂ solution
   b. Picrinic acid
   c. K₂Cr₂O₇, H₂SO₄, t°
   d. NaNO₂/HCl solution

3. Which of the following methods are used for tropane derivatives quantity detection?
   1. Acid base titration in non aqueous environment (glacial acetic acid)
   2. Neutralization (water-alcoholic environment, chlorophorm)
   3. Nitritometry
   4. Complexometry
      a) 2.3.  b) 1.2.  c) 3.4.  d) 1.3.

4. Ezerolin which is released after physostigmine basic hydrolysis is oxidized by forming
   a. Benzoic acid
   b. Rubreserine red
   c. Dimethylamine
   d. Benzaldehyde

5. Physostigmine according to its chemical structure depends on carbamates (uretanes) group due to the following group
   a. Methyluretane
   b. Phenole
   c. Imidazole
   d. Thyazole
Drugs acting on adrenergic system Adrenomimetics (phenyl-alkylamines)

Ephedrine and its diastereoisomer pseudoephedrine are included in this group, which are obtained from plant Ephedra. “Ephedra monospersma” type contains the largest quantity of ephedrine. Ephedrine is released from raw material by distillation. Ephedrine and pseudoephedrine can be differed from each other by oxalates re-crystallization (pseudoephedrine oxalate dissolves well in water).

1-eritro-2-methyl-amino-1-phenylpropanol-1-hydrochloride

The biosynthesis method is; sugar fermentation in the presence of benzaldehyde.

Benzaldehyde → l-phenyl-acetyl-ketone → base-l-ephedrine → ephedrine hydrochloride

**Physical properties**

Ephedrine hydrochloride and dephedrine are white or colorless crystalline substances which are well soluble in water and are practically insoluble in ether. However, ephedrine hydrochloride is soluble in ethanol but dephedrine is easily soluble there. Both ephedrine hydrochloride and dephedrine are differed from each other by their melting points and specific angle rotation.

**Identification methods**

- Spectrophotometric method. 0,05% water solution of ephedrine gives specific absorptions in UV-region.
- Ephedrine is oxidized by oxidizers, (HNO₃, H₂SO₄, H₂O₂, K₂Cr₂O₇), e.g. in heating condition by potassium hexacyanoferrate K₃[Fe(CN)₆], as a result of oxidation, benzaldehyde is formed which can be detected by organoleptic test (it has typical almond smell).
- Ephedrine forms a blue characteristic complex with copper sulfate (CuSO₄) in the presence of sodium hydroxide (NaOH). After shaking the reaction mixture with ether a reddish-violet color is appeared, but aqueous layer keeps its blue color.
Ephedrine can be differed from other arylalkylamines by its interaction with Phosphomolybdic acid \( \text{H}_3\text{PMo}_{12}\text{O}_{40} \). A typical yellow color is formed, which is dissolved in an ammonia solution.

Ephedrine similar to amino acids, amino alcohols and amino phenols interacts with ninhydrin in basic media, dark-violet color is formed.

Chloride ion is detected in ephedrine hydrochloride by \( \text{AgNO}_3 \).

Base of Ephedrine is soluble in water, thus precipitate is not formed when a base solution is added in its hydrochloride solution. Due to these property it can be differed from other alkaloid salts.

**Quantitative analysis**

Acid-basic titration in a non-aqueous media, using formic acid and acetic acid as a media. This combination of solvents inhibits dissociation of HCl, which allows us to carry out the titration without using mercury acetate. The titrant is \( \text{HClO}_4 \), indicator is a crystalline violet. SPh (pharmacopea) suggests an acid-base titration with glacial acetic acid media and mercury acetate.

\[
\begin{align*}
\text{OH} & \quad \text{N}^+ \\
\text{CH}_3 & \quad \text{H} \\
\text{CH}_3 & \quad \text{Cl}^- + \text{(CH}_3\text{CO})_2\text{O} + \text{HClO}_4 \quad \text{HCCOH} \rightarrow \text{OH} & \quad \text{N}^+ \\
\text{CH}_3 & \quad \text{H} \\
\text{CH}_3 & \quad \text{ClO}_4^- + \text{CH}_3\text{COCl} + \text{CH}_3\text{COOH}
\end{align*}
\]

Alkalimetry: neutralization according to HCl, titrant is sodium hydroxide(\( \text{NaOH} \)) and indicator is phenolphthalein.

Argentometry (according to chlorine ion)-Volhard method. \( \text{AgNO}_3 \) is used as a reagent and its surplus quantity is titrated by ammonia rodanite solution, indicator is iron ammonia alum.

Periodatometry; which is based on ephedrine oxidative property with potassium periodate in weak basic media, indicator is starch solution.

Photo-colorimetric method, which is based on complex color intensity detection formed in result of ephedrine and \( \text{CuSO}_4 \) interaction. Color reaction with ninhydrine is used as well.

Spectrophotometric method. UV-spectrosopy in visible reagion.

Extraction-photometric method, which is based on ionic residues and dyes interaction (methyl-orange, bromthymol blue):

Ephedrine is kept according to B-list, in well-closed vials, protected from light, in cold and dry place. Ephedrine is an adrenomimetic agent, so it possesses a vasoconstrictor and bronchodilator effect. It is prescribed for bronchial asthma and allergic diseases.
Catecholamines

Adrenaline structure is based on synthesis of several products, which at first was obtained from extract of animal's gland. The compounds, which are formed due to bio-synthesis carrying out in the organism, are called catecholamines. They are representatives of biogenic-amines and derivatives of pyrocatechin (chatecholes).

Synthesis of Noradrenaline which occurs in the organism under enzymes influence.

The catecholamine receptors exist in almost all the organs of the human body. They regulate a variety of physiological functions and they especially enhance heart beat strength and frequency, decrease the peripheral veins resistance, stimulate glycogenolysis, as well lipolysis and protein metabolism. Hence, they are widely used in medicine as a medicaments influencing on adrenergic system.

Catecholamines general structure

As a drugs Dopamine (dofamine), epinephrine (Adrenalin), epinephrine hydrotartrate and norepinephrine hydrotartrate (noradrenalin) are used.

Natural source of adrenaline and corticosteroids is pigs and cattle gland. Gland consists of 2 layers—cortical and nuclear. In the nuclear layer adrenaline synthesis is carried out, but in the cortical layer, approximately 40 different hormones are produced, which are known as corticosteroids. Hormones are extracted from raw material by using 80% ethyl alcohol. Proteins undergo denaturation, after which alcohol is excreted from the extract. Removing of the fat from the remaining aqueous solution is realized by petroleic ether, afterwards the solution must be acidified and dichlorethan must be added as well. The removing of other substances from the solution is carried out with lead acetate. The remained adrenaline is removed from mixture by preliminary converting it into adrenaline-base.
(by the help of ammonia solution), than the same must be done with adrenaline bitartrate (by vinegar acid). This process is repeated several times.

Adrenaline extraction was suggested by Russian scientist Cibulsky, after which chemical structure was confirmed by synthesis. Nowadays there are many synthesis methods.

Synthesis: Dopamine is obtained due to interaction of HCl with homoveratryl-amine hydrochloride (100-115°C).

\[
\begin{align*}
\text{H}_2\text{C} & \text{-C-O-} & \text{NH}_2 \cdot \text{HCl} & \xrightarrow{\text{HCl}} & \text{H}_2\text{C} & \text{-C-O-} & \text{NH}_2 \cdot \text{HCl} \\
\text{H}_2\text{C} & \text{-C-O-} & & & & & \\
\end{align*}
\]

Adrenalin synthesis is realized from pyrocatechin and chloroacetic acid chlorine anhydride interaction.

\[
\begin{align*}
\text{Pyrocatechin} & \xrightarrow{\text{Cl-CH}_2\text{Cl}} & \text{chloromethyl 3,4-dioxyphenylketon} & \xrightarrow{\text{CH}_2\text{NH}_2} & \text{adrenalon} & \xrightarrow{\text{[H] Ni}} & \text{adrenalin} \\
\end{align*}
\]

Norepinephrine synthesis is carried out using pyrocatechol or 1,2-dihydroxybenzene, which is subjected to formaldehydation, cyanation and hydration.

\[
\begin{align*}
\text{HO} & \xrightarrow{\text{H}_2\text{C}=\text{O}} & \text{HO} & \xrightarrow{\text{KCN}} & \text{HO} & \xrightarrow{[\text{H}]} & \text{HO} \\
\end{align*}
\]

2-(3,4-dioxy-phenyl)-ethyl amine hydrochloride Dopamine (Dopamine)

I-1-(3',4'-dioxyphenyl)-2-methyl amino ethanol Adrenalin (Epinephrine)

I-1-(3',4'-dioxy phenyl)-2-methylamino ethanol hydrochloride Adrenalin hydrotartrate
I-1-(3',4'-dioxy phenyl)-2-aminoethanol hydrotartrate  Noradrenaline hydrotartrate

**Physical properties**

Catecholamines are white crystalline substances and can have grey or yellow shade. There are two asymmetric carbon atoms in epinephrine and norepinephrine molecules, due to which they have isomers. As a result of synthesis, the obtained racemate mixture is separate by using vinegar acid. Left rotating epinephrine and norepinephrine hydrotartrate are used in the medicine which are approximately 12 times more active than right rotators. SPh controls optical isomers content in epinephrine and in bitartrate and nor epinephrine bitartrate. Epinephrine bitartrate optically active isomer is separated by vinegar acid. Epinephrine (basic form) is poorly soluble in water, moderate in 0.1M hydrochloric acid and is practically insoluble in ethanol and chloroform. Drugs, which are in the form of salts are easily soluble in water and are practically insoluble in ether and chloroform. Dopamine, epinephrine and epinephrine bitartrate are less or very less soluble in ethanol. Being phenols, they all are soluble in basic solutions and can form phenolates. Phenolic hydroxyles give molecule acidic properties. The primary amino group in dopamine and nor-epinephrine structure as well as the secondary amino group in the epinephrine structure gives basic properties to all 3 molecules, which allows formation of salts.

**Identity**

- SPh suggests IR spectroscopy.
- UV- spectroscopy.
  - Cathecholamines give colored reaction:
    - with α-nitrozo β-naphthol (bright red color).
    - with ninhydrine it forms a yellow color.
    - Some precipitating (general alkaloid) reagents can be used for differentiation
  - Dopamine with 4-aminoantipyrine forms red color product.
  - Epinephrine and norepinephrine bitartrate like phenols give azo conjugation reaction with diazonium salt forming colored azo compound.
  - Epinephrine forms a reddish-violet color by heating up to 60°C in the presence of both potassium iodate (KIO₃) and diluted orthophosphoric acid (H₃PO₄) solutions. This reaction is typical to diatom phenols.
  - SPh suggests general colored reactions with an iron chloride (III) solution FeCl₃. Dopamine, epinephrine and epinephrine bitartrate form a emerald-green color with this reagent, which then changed to cherry-red color by adding one drop ammonia solution. The change of media pH leads to change of complex composition.
Specific reaction of epinephrine is the interaction with reagent which is prepared by rubbing mercury yellow oxide (HgO) and potassium thiocyanate (KSCN) in dilute nitric acid media. Heating up to boiling temperature purple red color or red color is formed.

Similar to other phenols, catecholamines are oxidized by oxygen in the air, forming colored products. There are some chemical reactions, which are based on their oxidation:

- Epinephrine and nor epinephrine can be differed from each other by 0,1M iodine solution I₂ oxidation reaction in 2 buffer solutions: when pH is 3,56 and 6,5 area. Epinephrine in both 3,56 and 6,5 pH values can form adrenochrom, which has a dark-red color.

![Epinephrine and nor epinephrine comparison](image)

Epinephrine (adrenalin) Adrenochrom

Color is kept in pH 3,56 case and also in case of addition 0,1M sodium thiosulphate. Nor-epinephrine forms noradrenochrom (reddish-violet), only in 6,5 pH conditions.

![Epinephrine and nor epinephrine comparison](image)

Nor-epinephrine (Nor-adrenalin) Nor-adrenochrom

- Epinephrine and nor-Epinephrine can reduce silver (Ag) from silver nitrate ammonia solution.
- It gives positive reaction with the Fehling solution (KNa[Cu(C₄H₄O₆)₂])(reduce Cu, Cu₂O is formed).
- Colored reaction of 1,2-dinitrobenzene is based on oxidation of the product in the basic media.

1,2-di nitrobenzol reduction is occurred till a formation of o-quinoid structure compound with bluish-violet.
Epinephrine with base solution (a few amount) forms yellow-green fluorescence as a result of epinephrine oxidation (adrenolutin is formed).

In hydrochlorides, chlorine ion is detected by using AgNO3 (silver nitrate).

Tartrate ion is detected by the following consequential reactions: dehydratation, then oxidation by conc. H2SO4, in heating conditions and in the presence of resorcinol. At first, glyoxal and formic acid are formed.

Formic acid is condensed with 3 mol resorcinol. As a result aurine dye is formed with quinoid structure.

The acceptable quantity of homoveratrylamine (precursor of Dopamine) is checked for dopamine high quality, which shouldn’t exceed 0.8%. It is checked by Thin-layer chromatography method.

For epinephrine and norepinephrine quality the appropriate acceptable quantities of adrenalone and noradrenalone are checked by optical density in 0.01M hydrochloric acid solution in 310nm region.

Norepinephrine quantity also is detected in epinephrine and its bitartrate. For detection colored reaction is used with 1,2-naphthoquinon-4-potassium sulfonate by using chromatography sylfol sheet. For quantity detection high performance liquid chromatographic method is used (acceptable quantity should be not more than 0.0018%), as a mobile phase ethanol-water (85:15) is used.
Quantitative analysis

- Epinephrine, norepinephrine and their bitartrates are detected by acid-base titration in non-aqueous media (media is glacial acetic acid, titrant is 0.1M HClO₄, indicator is crystalline violet). Mercury acetate should be added to prevent dissociation in case of hydrochlorides.
- Epinephrine, norepinephrine and their bitartrates can be detected by UV-spectrophotometry.

These medicinal products are kept in well closed vials, protected from light in cold and dry places. For stabilization of epinephrine and norepinephrine bitartrates injection solutions, 0.1% of sodium metabisulfite must be added.

Adrenaline and noradrenaline synthetic analogues

The first synthetic analogue of noradrenaline and adrenaline was izadrine (isopropil noradrenaline), which was synthesized in 1936 and in contrast to adrenaline doesn’t show a vasoconstrictor effect. Izadrine is a non selective β-adrenomimetic, and used as productive broncholytic and cardio tonic agent. Other analogues were synthesized such as phenoterol, salbutamol and terbutaline.

Synthetic analogues general formula

![Synthetic analogues general formula](image)

In industry isadrin is obtained from chlorine acetic acid and pyrocatechin.
Physical properties

Catecholamines synthetic analogues are white crystalline powders. Isoprenaline hydrochloride is dissolved in water and ethanol. All mentioned compounds are not dissolved in ether practically.

Identification reactions:
- All mentioned preparations are identified by IR and UV spectrophotometry.
- Isadrin (isoprenaline hydrochloride) due to interaction with FeCl₃ forms emerald-green color which turns into cherry-red color by adding ammonia solution.
- Salbutamol forms reddish-violet color with FeCl₃.
- Isadrin (isoprenaline hydrochloride) forms violet sediment with HNO₃ solution.
- Isadrin (isoprenaline hydrochloride) gives azo-conjugation reactions similar other phenols.
Isadrin (isoprenalin hydrochloride) gives colored products due to interaction with the following reagents:

- α-nitrozo-β-naphtol /grey red/
- ninhydrin /yellow/
- potassium iodine in acidic environment /cherry-red /
- phosphorous-molybdate acid /green/

Isadrin (isoprenalin hydrochloride) turns into raspberry color after interaction with chloramine NH₂Cl, and due to interaction with 4-aminoantipyrin it turns into red color.

Isadrin (isoprenalin hydrochloride) forms orange color due to interaction with cerium sulfate Ce(SO₄)₂.

Isadrin can be differed from other aril-alkyl amines having natural origin due to formed colored products. For example with copper ions in the presence of sodium hydroxide it forms complex compound which is differed by its green color from the complex compounds formed from ephedrine and mezatone.

Isadrin is differed from ephedrine by interaction with phosphorus-wolframic acid. With the mentioned reagent isadrin forms white sediment which then turns into brown.

Isadrin is differed from nor-epinephrine due to oxidation reaction with iodine solution (reaction is carried out in the presence of 0,1M HCl which brings to a pH regulation, after which sodium thiosulfate Na₂S₂O₃ is added which leads to a discoloring of iodine solution and it turns into pink color.

For salbutamol determination it should interact with 2% sodium tetra borate (B₄Na₂O₇), 3% 4-amino-antipyrine, hexacyanoferrate K₃[Fe(CN)₆] (III) solutions which should be mixed with chloroform CHCl₃. Chloroform layer turns into reddish-orange.

Quantitative determination

- Acid base titration in non aqueous media; media is glacial acetic acid, titrant HClO₄, indicator crystalline violet. In case of hydrochloride form titration is carried out in the presence of mercury acetate.
- UV, IR spectroscopy and photoelectrocolorimetric methods based on colored reactiones.

Synthetic catecholamines are easily oxidized from light and air oxigen, so they should be kept in well closed vials in dry and cool place.

Hydroxypropanole amine derivatives

Preparations of this group are classified into adreno-blockators. They contain a hydroxyl-amino-propanol group in the molecule. Propranolol hydrochloride (anaprine), atenolol, timolol maleate and fluoxetine hydrochloride are preparations from this group. Latter phenoxy-propanol-amine derivatives were synthesized which are differed from other representatives according to aliphatic amine structure.
The one most widely used product is propranolol hydrochloride, which is synthesized according to the following mechanism.

**Physical properties**

Hydroxypropanolamine derivatives are white or almost white crystalline, odorless compounds. Anapriline and thimolol maleate are soluble in water and ethanol, atenolol is less soluble in water and...
ethanol. Fluoxetine hydrochloride is dissolved poorly in water, but well soluble in ethanol and methanol.

Atenolol is soluble in chloroform, while anapriline is less soluble. Thymolol maleate is moderately soluble in chloroform and is practically insoluble in ether. Anapriline aqueous solutions have opalescence which disappears in case of acidification with 2-3 drops of mineral acids.

**Identity**
- IR, UV-spectrophotometry are the main identification methods.
- Anapriline (propranolol hydrochloride) can be identified according to the melting temperature of its base form (92-97°C) which is obtained in the result of interaction of initial compound with sodium hydroxide. The base form is separated by ether, then ether is evaporated by distillation.
- Anapriline and Fluoxetine solutions give positive reaction toward chlorides by AgNO₃.
- Malonic acid is detected in thymolol maleate. Thymolol base is released by ether in basic media, after which water phase is boiled with bromine water. Then resorcinol and sulfuric acid are added to the obtained mezo dibrom siccine acid solution. After heating in an aqueous bath for 15 minutes it forms a bluish-black color.

**Quantitative analysis methods**
- According to SPh anapriline quantitative analysis method is carried out by acid-base titration in non-aqueous media (media is glacial acetic acid, titrant is a 0.1M HClO₄, indicator is crystalline violet). In case of HCl form mercury acetate is added.
- In titration of thymolol maleate and atenolol the equivalence point is detected by the potenciometric method using glassy, platine and calomel electrodes.
- The quantity of these compounds in their drug forms is detected by HPLC.

Propranolol hydrochloride and atenolol are long-term selective cardio-selective adrenoblockers. They are used as antianginal, hypotensive and anti-arrhythmic agents. Propranolol hydrochloride is used for the treatment of angina pectoralis, also in arrhythmias and hypotonia. It is produced in 0,01g and 0,04 g tablets and 1 and 5ml 0,1% solution. Thymolol maleate is non cardio-selective (non selective) β-adrenoblockator and is an agent which is used in medicine for glaucoma treatment.

**Control tests**
1. Choose the methods and reagents for ephedrine hydrochloride identification
   1. UV spectrophotometry
   2. potassium hexacyanoferrate (III) solution
   3. ninhydrin, OH⁻
   4. sodium carbonate solution
   a/ 1.2.3     b/1.2.4     c/1.3.4    d/2.3.4

2. Which method is not used for ephedrine hydrochloride quantity analysis?
   a) acid-base titration in non aqueous media / mixture of formic acid and acetic acid anhydride/
   b) neutralization /alkalimetry/
   c) argentometry
   d) acidometry

3. Choose the reagents which are used for both adrenaline and noradrenaline identification:
   1. FeCl₃ solution
   2. α-nitrozo-β-naphthol solution
   3. ninhydrin, OH⁻
   4. AgNO₃, NH₄OH solution
   a/ 1.2.3   b/2.3.4   c/1.2.4   d/ all mentioned

4. Which reagent is used to differ adrenaline from noradrenaline
   a) FeCl₃ solution
   b) 0.1M iodine solution /in corresponding pH value/
   c) ninhydrine, OH⁻
   d) Fehling’s reagent

5. Choose the methods and reagents for both dopamine and epinephrine identification
   1. IR-spectrophotometry
   2. UV-spectrophotometry
   3. ninhydrine, OH⁻
   4. α-nitrozo-β-naphthol solution
   a/1.2.3.4     b/2.3.4     c/1.3.4    d/1.2.4

**CNS STIMULANTS**

CNS stimulants stimulate both the cerebral (especially that of the cortex) functions and facilitate inter-neuronal transmission of impulses, which leads to psycho-motor activity improvement. The function of brain and memory integration is increased, due to the effect of CNS stimulators. And vice versa, a decrease of appetite, fatigue and sleep need is observed. These compounds in high doses, possess
analeptic activity, therefore increase both the pulmonary ventilation and restore the normal reflexes, for example, after anesthesia.

**Methylxanthine derivatives**

Methylxanthine derivatives form a large group of psychomotor stimulators. From methylxanthine derivatives, caffeine, theophylline and theobromine are used in medicine. These compounds are alkaloids, which are contained in tea leaves, in the seeds of coffee and cacao and in the nuts of cola plant. From the earliest times humans have been using aqueous extracts of parts of some plants, containing caffeine, theophylline (“divine leaf”), and theobromine (“divine food”) for their CNS stimulating effects.

Caffeine was isolated from the plant in its native form, in 1820 by Range and its first synthesis was carried out in 1897 by Fischer and by Traube in 1900-1904. Since then several derivatives of caffeine and theophylline have been used in medicine as psychomotor stimulants.

Xanthene ring represents 2,6 dihydroxypurine, which is subjected to lactam-lactim tautomerism.

![Chemical structure of Purine alkaloids](image)

Purine alkaloids can be obtained either natural sources or by synthetic ways.

Natural sources are used for getting purine alkaloids from waste products of tea production, which contain up to 3% caffeine. Synthesis is carried out by reverse extraction method, and caffeine is obtained by re-crystallization from cold extract solutions.

Caffeine obtainment by synthetic method is more accessible and economical. Nowadays the most important method of xanthenes synthesizing is realized from uric acid (C₅H₄N₄O₃) and formamide solution (CH₃NO). Caffeine and theobromine are formed due to Xanthine methylation. Thus, caffeine is formed when dimethylsulphate is added into xanthene in pH 8-9 conditions. Theobromine is formed in the presence of KOH and methanol at 60-70°C.

The other synthetic method for purine alkaloids synthesis is a Traube synthesis method, where dialkyl-urine and cyan acetic acid are used as precursors.

![Synthetic methods of uric acid and xanthine](image)

**Physical properties of xanthine derivatives**

<table>
<thead>
<tr>
<th>Chemical substance</th>
<th>Chemical structure</th>
<th>Prescription</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td><img src="image" alt="Caffeine structure" /></td>
<td>It is a white, needle-like crystal or is a white crystalline powder. M.t. 235-238°C. It dissolves slowly in the cold</td>
</tr>
</tbody>
</table>
Theobromine

It is a white odorless crystalline powder. It practically insoluble in the cold water (1:300), poorly soluble in the hot.

Theophylline

It is a white or almost white odorless crystalline powder. M.t. 270-274°C. It is poorly soluble in the cold water (1:120), and easily soluble in the hot water.

Identity

- Acid-basic properties.

  Caffeine is Brensted base, which has basic properties due to nitrogen atom in 9th position. Theophylline and theobromine have shown amphoteric properties in contrast to caffeine. Basic properties are depended on nitrogen atom in 9th position. Acidic properties are depended on active hydrogen atom for theophylline in 7th position and for theobromine in 1st position. Due to amphoteric properties theobromine and theophilline dissolve in both acids and dilute bases solutions.

  Due to caffeine basic properties it interacts with:
  - Alkaloid reagents (colored complexes are formed, but it doesn’t interact with Mayer reagent K₂HgI₄)
  - Mineral acids (forms unstable salts which are easily hydrolized)

  Theophiline and theobromine due to their acidic properties interact with:
  - Basics (NaOH, KOH) they form soluble salts then with heavy metals (Co²⁺, Cu²⁺, Hg²⁺, Ag⁺) they form insoluble compounds.

Theobromine
Caffeine, theophylline and theobromine are differed from each other by the interaction with cobalt chloride (CoCl₂). Thus, theobromine forms a dark bluish precipitate, theophylline forms a pink precipitate with the same reagent and caffeine doesn’t interact with cobalt chloride (it has not acidic properties).

Theophylline is formed a transparent gelatinous mass due to interaction with silver nitrate AgNO₃, and theobromine brown gelatinous mass due to interaction with the same reagent.

Xantine derivatives (caffeine, theophylline and theobromine) can interact with iodine solution by forming per-iodide. This reaction is also used for their quantitative analysis.

Murrexide test reaction is general reaction for xanthene derivatives, which is based on purine ring degradation under the oxidizers influence (bromine water, hydrogen peroxide, nitric acid) which then leads to a formation of alloxane and dialuronic acid methyl derivatives. These products with ammonia can formed red ammonium salt (tetramethylpurpuric acid ammonia salt).
- **Basic hydrolysis reaction.** Caffeine is unstable in basic media at pH=9 or higher conditions and leads to a broken down of a molecule and forms caffeine dicarbonic acid, which is latter broken down as well and lead to a formation of caffeidin and its appropriate carbonate. Caffeidine is an pharmacological antagonist of caffeine and can cause side effects.

- Theophylline is broken down in the same way by forming theophyllidine, which can be identified through the azo-dye formation reaction with diazonium salts. In case of theobromine and caffeine azo-dye formation reaction is negative due to CH₃ group at 7th position.
Theophylline can be identified by 2,6-dichlorochinon chlorimide in the presence of borate buffer. A blue merocyaninic acid dye is formed.

\[ \text{H}_3\text{C} - \text{N} - \text{C} - \text{H}_3 + \text{Cl} - \text{N} - \text{N} - \text{Cl} \rightarrow \text{H}_3\text{C} - \text{N} - \text{C} - \text{N} - \text{Cl} - \text{N} - \text{Cl} \]

Purine derivatives can give appropriate absorptions in UV and IR regions.

General identification reaction is interaction with HgCl\(_2\) which leads to a formation of complex compound with equivalent quantities. For example caffeine leads to the formation of C\(_9\)H\(_{10}\)N\(_4\)O\(_2\) x HgCl\(_2\) compound. The reaction is used also for quantitative determination by indirect complexometric method.

**QUANTITATIVE ANALYSIS**

- **Acid base titration in non-aqueous media**

  Caffeine, due to its basic properties, can be detected by acid-base titration in non-aqueous media. The media is acetic acid anhydride, titrant is HClO\(_4\) and crystalline violet is used as an indicator.

  For theobromine and theophylline detection dimethylformamide is used as a media, potassium or sodium methylate as a titrant and phenol red as an indicator.

- **Argentometry. Volhard method**—theophylline and theobromine are interacted with the addition of silver nitrate. Insoluble salts are formed, titrant is NH\(_4\)SCN, indicator is iron ammonia alum.

- **Conjugation of argentometry and acid base titration method.** With silver nitrate solution silver salts and nitric acid are formed. Then nitric acid is titrated by 0.1M NaOH solution, indicator phenol red.
Cerimetric method. For caffeine, theophylline and theobromine quantity cerimetric method is used as well. Titrant is cerium sulphate, which as a oxidizer forms alloxanes with purine alkaloids. In interaction with caffeine and theophylline 1,3-dimethylalloxane is formed, and in case of theobromine 3-methyl-alloxane is formed.

Iodometry. Caffeine content can be detected by iodometry. The surplus quantity of iodine is titrated by sodium thiosulfate, indicator starch.

Physicochemical methods UV-spectroscopy, HPLC, gas-liquid-chromatography.

USAGE. Caffeine is used in the combination with analgesic drugs, such as pentalgine, sedalgine and caffetine.

OTHER DERIVATIVES

Euphylline and diprophylline are widely used in medicine. They are obtained from theophylline derivatives. Caffeine sodium benzoate is synthesized from caffeine.
It contains 58-62% caffeine and 38-40% sodium benzoate.

**Identification methods**
- All identification reactions typical to Caffeine can be realized due to caffeine residue.
- The presence of sodium ion is detected by zinc-uranyl-acetate (ZnUO₂(CH₃COO)₄) or by flame color (yellow color).
- Benzoate ion is identified with FeCl₃ and gives a yellow color.

**Diprophylline**

- Typical identification method is the interaction with potassium hydrosulfate (KHSO₄), nitropruside in piperidin media. Diprophylline undergoes dehydration by KHSO₄, leading to aldehydes formation. Then aldehydes interact with nitropruside in piperidine media and form blue colored compound. The blue color is turned into pink color by adding 2-3 drops of NaOH solution.
- Boiling diplphylline with NaOH solution ammonia is released which is identified by litmus paper or by smell.

**Euphylline (aminophilline)**

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Preparation contains 14-18% ethylene-diamine.

- **Ethylene diamine** containing in the euphylline molecule is detected by CuSO₄. A reddish-violet color is formed.

Euphylline and diprophylline possess spasmolytic and diuretic activity.

**Control tests**

1. Due to acidic properties theophylline interacts with the following reagents
   1. NaOH
   2. AgNO₃
   3. Co(NO₃)₂
   4. H₂O₂
   a/ 1.2.3  b/ 2.3  c/2.3.4  d/1.3

2. Choose the reagents which are used for xantine derivatives general identification method
   a) H₂O₂, H⁺, NH₃ /murexide sample reaction/
   b) AgNO₃, NaOH solutions
   c) Co(NO₃)₂, NaOH solutions
   d) NaOH, CuSO₄ solutions

3. Ethylen diamine in euphylline molecule is detected by
   a) NaOH solution
   b) CuSO₄ solution
   c) NaNO₂ solution
   d) bromine water
4. Caffeine, theobromine and theophylline are differed from each other by
   a) CoCl₂ solution
   b) murexide sample reaction
   c) picrinic acid solution
   d) phosphormolibdenic acid solution

5. Which method is not used for caffeine quantity detection?
   a) argentometry
   b) acid base titration in non aqueous media /glacial acetic acid/
   c) iodometry
   d) UV-spectrophotometry

---

**Somnolents**

Somnolents are barbituric acid (pirimidine 2,4,6-trion) derivatives. Pyrimidine is a 6-membered heterocyclic compound with 2 nitrogen atoms located in the 1ˢᵗ and 3ʳᵈ positions. Barbituric acid derivatives or cyclic ureids in comparison with acyclic ureids are condensation products of urea and malonic acid.

![Diagram of urea and malonic acid reaction](image)

Due to condensation closed cyclic system with 2 nitrogen atoms (1ˢᵗ and 3ʳᵈ positions) is formed, thus barbiturates are classified into pyrimidine derivatives. The somnolent effect of barbiturates was discovered in the 20ʰ century by Fisher and Mering. The first somnolents were synthesized by Fisher in 1904.

Barbiturates are first generation somnolents, and except their somnolent effect, they have the inhibitory influence on skeleton muscles, smooth muscles and heat muscle activity. Barbiturates can have different CNS inhibitory effect, which depends on both dosage and administration way, and can be used as relaxants, somnolents, anticonvulsants or as a general anesthetics. Nowadays the usage of
Barbiturates (as a relaxant and somnolent) has been limited, due to their high toxicity. They inhibit the CNS and can activate liver enzymes. Additionally they can cause tolerance and dependence.

2 tautomer forms are typical for barbituric acid derivatives: lactam-lactim, keto-enolic. Tautomerism is an isomers conversation into each other conditioned by active hydrogen movement and electron density distribution. If 2 hydrogens in the 5th position are substituted with other groups, so the molecule looses it’s keto-enolic tautomerism.

![Tautomerism diagram]

Lactim or aci-form is responsible for acidic properties. Thus, in basic environment they can interact with metals ions and can form salts. Barbiturates acidity depends on the quantity of substitutes, so 5,5-bisubstituted barbituric acid and 1,5,5-trisubstituted barbituric acid are weak acids and can easily form salts.

Barbituric acid derivatives medicinal forms can be divided into 2 groups: barbiturates (lactam form) and barbiturates sodium salts (lactim form).

Barbiturates are: barbital, phenobarbital, benzobarbital (benzonal) and sodium salts are: barbital-sodium, hexobarbital-sodium (hexenal), thiopental-sodium, which are differed from each other by R1, R2, R3 radicals.

<table>
<thead>
<tr>
<th>Barbital</th>
<th>White odourless crystalline powder</th>
<th>M.T. 189-192°C</th>
</tr>
</thead>
</table>
| ![Barbital structure](https://example.com/barbital.png) | ![White odourless crystalline powder](https://example.com/powder.png) | }
| **Phenobarbital** | ![Phenobarbital structure](image) | White odourless crystalline powder  
M.T. 175-179°C |
| **Benzobarbital** | ![Benzobarbital structure](image) | White crystalline powder  
M.T. 134-137°C |
| **Hexobarbital Sodium** | ![Hexobarbital Sodium structure](image) | White powder and hygroscopic |
| **Thiopental Sodium** | ![Thiopental Sodium structure](image) | Yellow or yellow green crystalline powder with special smell and hygroscopic |

**Synthesis:** synthesis of barbituric acid derivatives is carried out in 2 pathways. In the first phase malonic acid diethyl ether is obtained, in second phase the obtained ether is condensed with urine (in the presence of sodium alcoholate and in alcohol media).

![Chemical structures](image)

Benzyl chloride is used as a precursor for phenobarbital synthesis. Phenyl-ethyl malonic acid diethyl ether is synthesized and latter is condensed with urine.
Benzobarbital is formed from interaction of Phenobarbital with benzoyl chloride.

For the synthesis of barbiturates sodium salts barbiturates must be dissolved in equal quantity of sodium hydroxide alcoholic solution, then sodium salt is precipitated by ether.

Barbiturates are badly soluble in water. Their alcoholic or aqueous solutions have an acidic reaction. Barbiturates sodium salts are easily soluble in water and alcohol and their water solutions have a basic reaction.

**Identity**

- Barbiturates can be identified by IR and UV-scopy.

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Barbiturates can be identified also by chemical reactions: complex formation with heavy metals, neutralization with sodium salts, sodium ion and functional groups identification, oxidation and melting with caustic alkali.

Barbituric acid derivatives can form insoluble salts with silver, mercury, copper and cobalt ions. This reaction is formed only with the ionized form of barbiturate, so barbiturate acid form (lactam form) should be turned into ionic form (salt or lactim form) by the interaction with sodium hydrocarbonate or carbonate solution. During salt-type formation the negative charge is delocalized in more energetically suitable form, that leads to a formation of ambident ion.

- All barbiturates and their sodium salts can form bluish-violet colored complexes with cobalt ions in the presence of CaCl₂.
- Mercury complex salt is formed by the interaction of 0.1 M mercury (II) nitrate solution in the presence of acetic acid sodium.

But the interaction with copper ions leads to a formation of complexes with different colors which gives chance to differ them from each other.

Barbiturates with silver ions can form mono-substituted (soluble in water) and bi-substituted (insoluble in water) silver salts. Firstly, in the presence of Na₂CO₃ sodium salts are formed, which interact with silver nitrate leading to a formation of mono-substituted silver salt. With the addition of AgNO₃ the mono-substituted silver salt is turned into a bi-substituted silver salt form, which is released as a precipitate.
Sodium-hexobarbital and benzobarbital, which have a substitute in the 1<sup>st</sup> position can form only mono-substituted silver salts.

Barbiturates general property is their ability to undergo hydrolytic splitting under different conditions. In soft conditions, (keeping long time in humid place) amid groups splitting is possible in 1-2 and 1-6 positions.

In hard conditions (melting barbiturates with caustic alkalis) whole molecule destruction is carried out. The following substances are formed: ammonia (is detected by smell or litmus paper), sodium carbonate Na<sub>2</sub>CO<sub>3</sub> and di alkyl acetic acid sodium salt.

Adding HCl surplus quantity at the reaction products CO<sub>2</sub> is released and fatty acid smell is felt. Barbiturates and their sodium salts can form condensation products with formaldehyde or p-dimethyl amino-benzaldehyde in the presence of conc. H<sub>2</sub>SO<sub>4</sub>.
Choosing the appropriate aldehyde and conditions, colored compounds can be obtained, which then can be used for separate derivatives identity. For example, if vanillin is used as a reagent in the presence of conc. H₂SO₄ after boiling a cherry color is formed, which then turns into a bluish-violet in color. This is typical for **Phenobarbital**.

Barbiturates can be identified by colored reactions based on oxidation typical to that of pyrimidine derivatives. Several oxidizers can be used, conc. H₂SO₄ and potassium bichromate gives green color.

Melting barbiturates with resorcinol and conc. H₂SO₄ in the addition of sodium hydroxide solution lead to green fluorescence.

**Partial reactions**
Barbituric acid sodium salts can be detected through a neutralization by adding dilute HCl. Water insoluble barbiturate is released which is identified by melting temperature.

Sodium ion can be detected by flame color and zinc-uranyl acetate.

Barbiturates are differed from each other according to functional groups located in the 1st and 5th positions. Thus, phenyl radical of Phenobarbital is detected by a nitrozo-compound formation (cherry like azo dye). This reaction is carried out when conc. HNO₃ and H₂SO₄ are added. Yellow color formation is conditioned by Phenobarbital meta-nitro derivative formation. This reaction also can give benzobarbital (benzonal). In meta-nitro phenobarbital molecule the nitro group is reduced (Zn, HCl) into amine group. Then diazotation and azo conjugation reactions (thill the formation of cherry like azo dye) is carried out by sodium nitrite in the presence of HCl and β-naphthol basic solution.
For benzonal, a specific reaction is the interaction with iron chloride. With the addition of sodium hydroxide, benzoate ion is formed which with iron ions can give a yellowish-pink precipitate.

- Sulfur atom in thiopental sodium molecule is detected by heating with both sodium hydroxide and lead acetate. This reaction leads to a formation of lead-sulfide (black precipitate).

\[
\text{Na}_2\text{S} + \text{Pb(CH}_3\text{COO)}_2 \rightarrow \text{PbS} + 2\text{CH}_3\text{COONa}
\]

- Hexenal, which contains cyclic hexen is able to discolor bromine water.

Quality control. Depending on barbiturates chemical properties and synthesis methods their quality can be changed. For quality the solution transparency is checked. Transparency can be changed due to humidity and interaction with carbon oxide (in the air). As a result insoluble compounds are formed giving muddiness to the solution.

Transparency of barbiturates acid form is checked by dissolving it in a sodium carbonate (a soluble sodium salt is formed).
For barbital quality and purity the content of ethyl barbituric acid should be checked, in case of phenobarbital phenyl barbituric acid quantity must be checked. These substances have strong acidic reaction, than their appropriate barbiturates, and their quantity is detected by checking pH with potentiometric method.

**Quantitative analysis**

- Barbiturate acidic forms or lactam forms quantitatively are detected by acid-base titration in non aqueous media (media is dimethyl formamide). Titrant is 0.1 M sodium hydroxide in methanol and benzene. Indicator is thymol blue.

  ![Chemical Reaction 1](image1)

- The quantity of barbituric acid derivatives are detected by the combined method of mercurimetry and complexonometry. Barbiturates acid forms are dissolved in ethanol, and sodium salts are dissolved in water. Then sodium acetate 10% solution and 0,1M mercury (II) nitrate solution surplus quantity are added. Mercury barbiturate precipitate is formed which is filtrated and surplus quantity of mercury (II) nitrate in the filtrate is detected by complexonometric method (titrant is trilon B, indicator is xylenol orange or hexamethylenetetramine).

  ![Chemical Reaction 2](image2)
Barbiturates sodium salts quantitative analysis is carried out by acidimetric method (titrant is 0.1M HCl, indicator is methyl orange). The solution must be titrated up to a formation of a pink color.

Barbiturate quantity can be also detected by argentometry based on mono- and di-substituted silver salts formation.

Barbiturates and their sodium salts can quantitatively be detected by gravimetric method.

Thiopental-sodium quantitatively can be detected by iodine-chlorometric method, which is based on sulfur oxidation by iodine monochloride.

Hexenal can be detected by bromatometry.

HPLC, spectrophotometry and other physicochemical methods are used.
Control tests

1. The initial products of barbiturates synthesis are
   1. malonic acid
   2. pyrimidine
   3. urea
   4. malonic acid derivatives
   a/ 1.3          b/2.3        c/1.2           d/3.4

2. Choose the product which is not formed in result of barbiturates melting with NaOH
   a) pyridine
   b) ammonia
   c) dialkylacetic acid sodium salt
   d) sodium carbonate

3. Barbiturates quantity /acidic form/ is detected by the following method
   a) acid-base titration in non aqueous media /dimethylformamide/
   b) acid-base titration in non aqueous media /glassial acetic acid/
   c) Keldal method
   d) nitritometry

4. Choose the reagent by which hexenal- sodium can be differed from other barbiturates
   a) melting with caustic alkali
   b) bromine water
   c) Na₂CO₃, AgNO₃ solutions
   d) zinc uranilacetate

5. Choose the same method for quantity detection both barbiturates acidic and salts forms
   1. neutralization /acidometry/
   2. bromatometry
   3. mercurimetry combined with complexonometry
Benzodiazepines

Benzodiazepines are used as a somnolent (can be classified as II generation), but possess also tranquilizer, anesthetic, anti conscious and miorelaxant effects. 1,4-benzodiazepine bicyclic system is included in benzodiazepines structure. Compounds of this group contain phenyl radical next to C5 and represent 5-phenyl-3H-1,4-benzodiazepine (for example chlozepid) and 1,2-dihydro-3H-1,4-benzodiazepine-2-on (for example sibazon, nitrazepam, nozepam, phenazepam, etc.) derivative.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Physical Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxazepam</td>
<td><img src="image1" alt="Oxazepam Structure" /></td>
<td>Odorless, white crystalline powder</td>
</tr>
<tr>
<td>Phenazepam</td>
<td><img src="image2" alt="Phenazepam Structure" /></td>
<td>Odorless, white or light yellowish crystalline powder M.T. 225-230°C</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td><img src="image3" alt="Nitrazepam Structure" /></td>
<td>White or light yellowish crystalline powder M.T. 225-230°C</td>
</tr>
</tbody>
</table>

4. argentometry

a/ 1.2  b/ 3.4  c/ 1.4  d/ 2.3
<table>
<thead>
<tr>
<th>Compound</th>
<th>Odorless, white or light yellowish crystalline powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td><img src="image" alt="Diazepam structure" /></td>
</tr>
<tr>
<td>Medazepam</td>
<td><img src="image" alt="Medazepam structure" /></td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td><img src="image" alt="Chlordiazepoxide structure" /></td>
</tr>
</tbody>
</table>

**General physical properties**

Preparations of this group have light yellowish color. All of them are poorly soluble or almost insoluble in water, which is conditioned by an azomethine fragment, (they are Schiff base) and they are hydrophobic compounds. The whole cycle of benzodiazepine, with phenyl radical, carbonyl group and other radicals, is responsible for absorption of light in IR and UV region. These properties are used for identity.

**Synthesis**

For example nitrazepam is synthesized from 2-amino-5-nitrobenzophenon and the bromine acetic acid bromine anhydrate interaction, in the presence of ammonia.
Chemical properties and quality analysis

1,4-benzodiazepine group chemical structure features give a chance to classify their properties and reactions according to

a) acid-basic properties
b) oxidation reactions
c) hydrolytic splitting and hydrolysis products further detection
d) covalently connected halogen atom detection
e) partial reactions

**Acid-base properties.** Chlozepide and mezapame possess expressed basic properties.

Nitrazepam, phenazepam, nozepam are amphoteric. Azometine fragment \( \text{R} - \text{N} - \text{R}^3 \) give basic properties to the molecule, but due to acidic properties, they can have both lactim-lactam and keto-enolic tautomerism (which is carried out due to methylene group active hydrogen). Due to acidic properties these compounds are soluble in base solutions and form insoluble complex with heavy metals salts (Co\(^{2+}\)).

**Oxidation reactions.** Benzodiazepins easily oxidizing property in different conditions is explained due to partial hydration of benzodiazepine cycle. As oxidizers, potassium permanganate, \( \text{H}_2\text{SO}_4 \), \( \text{K}_2\text{Cr}_2\text{O}_7 \), \( \text{HClO}_4 \) and other regents are used. For example, phenazepam in the mixture of chloroform, ethanol \( \text{HClO}_4 \) forms yellow-green oxidation product with green fluorescence.
**Hydrolytic splitting with hydrolysis products formation.** The complete destruction of molecule is carried out in hard conditions (melting with alkalis). Ammonia (in case of oxazepam) and methylamine (in case of diazepam) are released from amide group, which can be detected by litmus paper and smell.

In acidic hydrolysis amide and azometine groups are decomposed leading to a formation of benzodiazepine yellow colored derivatives having absorption in UV region.

Pramary aromatic amine group, which is formed after benzodiazepine acidic hydrolysis, is identified by azo-dye formation (which is conditioned by diazotation and azo conjugation reactions). Diazotation reaction is used for benzodiazepines quantity detection by nitritometry method.

[Diagram of diazepam and oxazepam with NaOH and NH₂-C₃H₅ and NH₃]
Those derivatives, which have not substitute in the first position: oxazepam, nitrazepam and phenazepam, can give diazotation reaction after hydrolysis. Derivatives having substitutes (diazepam) after hydrolysis are turned into benzophenon colored derivatives. Diazepam forms 2-methylamina-5-chlorbenzophenon, which has yellow color.

Pyrolysis reactions. Heating preparations an alloy is formed with green color, which is not changed its color even after addition of ethanol (C₂H₅OH), independent from media pH. Phenazepam is the exception, which forms a violet melting mass and color is changed depending on the media’s pH. Adding both ethanol and NaOH the color turns into bluish-violet color, but after adding dilute H₂SO₄ bluish-green color is formed, which then turns into yellow. It gives a chance to differ phenazepam from other derivatives.

Benzodiazepines identity can be detected by UV spectroscopy.
Benzodiazepines contain tertiary nitrogen, thus can be identified by general alkaloid reagents (Dragendorf, potassium tetraiodine bismuth), Bushard (iodine solution in potassium iodide, picrinic acid), precipitates are formed which are ionic associates. For example after addition of ammonia Reineke salt (NH₄[Cr(NH₃)₂(NCS)₄]) at diazepame which is dissolved in dilute HCl solution a pink precipitate is formed, which is dissolved in acetone. Benzodiazepins can form a yellow colored compounds through the interaction with picric acid.

Chlorine (oxazepam, diazepam) and bromine atoms (phenazepam) detection is carried out after mineralization by silver nitrate solution. Halogen atoms can be detected also by

a) Breinshtain sample test. The testing compound powder is put on copper wire, which then is keeping on the flame, it should give green color flame. The color is conditioned by a copper halogen formation.

b) burning with oxygen in a flask and using a sodium hydroxide solution as an absorbent liquid. Then obtained solution is acidified with sulfuric acid and detection reactions for chlorides and bromides are carried out.

Oxazepam can be differed from other derivatives due to amidocarbonilic part in its molecule. Heating oxazepam alcoholic solution with concentric phosphoric acid, formaldehyde is formed. After fuschian sulfuric acid addition in the presence of H₂SO₃ compound with quinoid structure is released. Obtained compound has a violet color.

Nitrazepam can be hydrated by zinc powder in the HCl presence. Hydrolysis is carried out and nitro group is reduced up to amino group forming 2,5-diaminabenzophenon. 2 amino groups can form dark-red bis-azo compound with the both sodium-nitrit hydrochloric and β-naphtol basic solutions.
Boiling phenazepam solution in the presence of NaOH ammonia is released, which is identified by litmus paper. After acidifying solutions with HCl the filtrate can give positive reaction toward bromide ion.

Diazepam identity can be detected by ninhydrin reaction. The latter must be boiled with ethanol which leads to a formation of light blue color, which is easily turned into red one by the addition of CuSO₄.

The presence of other mixtures can be detected by thin layer chromatography.

**Quantitative analysis:** Benzodiazepines quantitative analysis is realized by non aqueous titration, using as solvent formic acid with acetic acid anhydride or glacial acetic acid. Titrant is 0.1M HClO₄, indicator is crystalline violet.

Spectrophotometry, photocolormetry. Nowadays widely HPLC is used.

**Control tests**
1. Benzodiazepines acid-basic properties are conditioned by
1. carbonil group
2. lactam-lactim tautomerization
3. keto-enol tautomerization
4. azometine group
a/ 1.3  b/ 2.3.4  c/ 3.4  d/ 1.2

2. Choose the benzodiazepines which form azo-dye after acidic hydrolysis
1. oxazepam
2. phenazepam
3. nitrazepam
4. diazepam
a/ all mentioned  b/ 1.2  c/ 1.2.3  d/ 2.3

3. Choose nitrazepam identification reactions
1. pyrolis
2. diazotation
3. precipitate formation reaction
4. mineralization
a/ 1.2.3.4  b/1.2.3  c/1.2  d/2.4

4. Benzodiazepine quantity is detected by
1. UV spectrophotometry
2. acid-base titration in non aqueous media /glacial acetic acid/
3. HPLC
4. iodometry
a/ 1.2  b/ 2.3  c/ 1.4  d/ 1.2.3

5. Choose the products which are formed in result of diazepam hydrolytic splitting reaction
a) methylamine
b) ammonia
c) chlorine
d) benzene

**Antipsychotic drugs**
Antipsychotics, known also as neuroleptics are tranquilizers they can cause calmness. They are used in the treatment of patients with psychotic disorders of thought and behavior, and in order to relief severe emotional tension, especially schizophrenia. These drugs are differed from somnolents and sedative remedies, as they don’t cause analgesia and don’t depress the biological centers of the brain. These derivatives don’t cause physical and psychical dependence. The major neuroleptics except antipsychotic activity possess both the sedative and tranquilizer activity, and also antihistaminic, cholinolytic and hypothermal activity. These drugs prolong the influence of sedatives, somnolents and analgesics as well as alcohol effect.

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Chemical Structure</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promazine hydrochloride – /Ptopazine/</td>
<td><img src="image" alt="Promazine Hydrochloride" /></td>
<td>Odorless, white crystalline powder M.T. 177-181°C</td>
</tr>
<tr>
<td>Promethazine hydrochloride – /Diprazine/</td>
<td><img src="image" alt="Promethazine Hydrochloride" /></td>
<td>Odorless, white crystalline powder M.T. 221-225°C</td>
</tr>
<tr>
<td>Chlorpromazine hydrochloride – /Aminazine/</td>
<td><img src="image" alt="Chlorpromazine Hydrochloride" /></td>
<td>White crystalline, hygroscopic powder M.T. 195-198°C</td>
</tr>
<tr>
<td>Levomepromazine – /Tizercine/</td>
<td><img src="image" alt="Levomepromazine" /></td>
<td>White-yellowish, hygroscopic powder. Unstable under air oxygen and light</td>
</tr>
<tr>
<td>Trifluoperazine hydrochloride – /Trifthazine/</td>
<td><img src="image" alt="Trifluoperazine Hydrochloride" /></td>
<td>White or yellow-green colored, odorless, crystalline powder M.T. 232-240°C</td>
</tr>
</tbody>
</table>
General physical properties
These derivatives are odorless, white crystalline powders with different shadows. They are easily soluble in the water, because they are in hydrochloride form. The water solutions have an acidic reaction: in alkyl-derivatives case pH=3-4, and acyl-derivatives case pH=4-6.

General structure of alkyl-derivatives

Acyl-derivatives

Moracyzine (Ethmosine)

Ethacysine

Synthesis
Phenothiazine derivatives synthesis is completed by 3 pathways

- Phenothiazine ring synthesis
- Alkyl or acyl radical synthesis
- Connection of alkyl or acyl radical to phenothiazine ring at 10th position, and hydrochloride formation.

Phenothiazine ring synthesis
Alkyl radical synthesis

Connection of alkyl radical and phenothiazine ring
Connection of acyl radical to phenothiazine ring

For ethmosine synthesis β-chlorine-propionil chloride is connected at ortho position of phenothiazine ring, and then with morpholine ring.

Chemical properties and identity

- **acid-basic properties**

  As these preparations are salts of strong mineral acids and nitrogen containing organic base, the interaction of aqueous solutions with weak base leads to formation of basic forms as sediments and can be detected by melting temperature.
After removing the precipitate chlorine ions can be detected in solution by AgNO₃ and HNO₃. White precipitate is formed.

\[
\text{NH}_4\text{Cl} + \text{AgNO}_3 \rightarrow \text{AgCl} + \text{NH}_4\text{NO}_3
\]

Beside this the released basic forms contain III nitrogen atom and can interact with alkaloid reagents forming water insoluble complexes with typical melting temperature and crystals structure.

With malonic acid anhydride C₃H₂O₃, acetone C₃H₆O solution a yellowish-orange color is formed, which can give absorptions in UV region.

With heavy metals salts they can form a colored complexes (Fe³⁺, Co²⁺, Hg²⁺).

In phenothiazine derivatives sulfur atom can be detected after mineralization by Na₂CO₃ and KNO₃ mixture. Sulphate ion is formed which is identified by barium chloride solution.

Phenothiazine derivatives with sodium cobalt nitrite in the presence of acetic acid anhydride and heating conditions form red colored product. Thifluoperazine in the same conditions forms green color.

**Red-ox properties**

Phenothiazine derivatives have strong reductive properties. In international pharmacopeias, different oxidizing reagents are used, such as Br₂, K₂Cr₂O₇ (FPh), conc. H₂SO₄ (BPh), FeCl₃ solution, cerium (IV) sulfate (Japan pharmacopeia). Color is formed in the result of oxidation (S-oxide or S,S-dioxide) and is conditioned by the character of carbon atom radical and does not depend on the nature of oxidizing agent.

**Reactions typical to separate preparations**

Amide group in derivatives of the antiarrhythmic agents can be detected by hydroxamate reaction.
Fluorine atom can be detected by burning in oxygen media. The obtained fluorine interacts with zirconium alizarin reagent which has red color (alizarin and zirconium nitrate complex). A red color turns into yellow (free alizarin is released). Fluorine also is determined by CaCl₂ and iron thiocyanate [Fe(SCN)₆]³⁻.

Urethane group in ethmozine and ethacisine molecule after hydrolysis can be detected according to released ethanol identity by iodoform formation reaction.

Quantitative analysis methods

Pharmacopeia suggests acid-base titration in non-aqueous media in the presence of mercury acetate, titrant is HClO₄, indicator crystalline violet. For promazine and aminazine as a solvent acetone is used, for diprazine the mixture of acetic acid and formic acid is used too.

Neutralization according to hydrochloride.

Gravimetric method (weight method or preparation basic form or complex compound formed with alkaloid reagents)

Cerimetric method, titrant is cerium sulfate solution.

Indirect complexonometric method.

Iodometry (based on poly-iodate fomrtaion) for chlorpromazine hydrochloride

Bromatometry for aminazine

Iodine chlorometry for promazine and chlorpromazine hydrochloride
Control tests

1. Which properties show phenothiazine derivatives from redox properties?
   a) oxidizing
   b) reductive
   c) acidic
   d) basic

2. Which method is not used for phenothiazine derivatives quantity detection?
   a) acid base titration /in glacial acetic acid media/
   b) alkalimetry
   c) gravimetry
   d) nitritometry
3. Phenothiazine derivatives are oxidized by
   1. Ce(SO₄)₂ solution
   2. K₂Cr₂O₇, H⁺ solution
   3. bromine water
   4. H₂SO₄/conc./
   a/1.2 b/all c/2.3.4 d/3.4

4. Oxidation of phenothiazine derivatives leads to formation of
   1. sulfide ion
   2. S-oxyde
   3. S.S-dioxyde
   4. SO₂
   a/ 2.3 b/ 1.2 c/ 2.3.4 d/ 3.4

5. Bromatometry is used for quantity detection of
   a) aminazine
   b) propazine
   c) diprazine
   d) ethmosine

Local anesthetic drugs
Para-amino benzoic acid derivatives

Preparations are benzocaine (anesthesin), procaine hydrochloride (novocain), tetracain hydrochloride (dicain).

<table>
<thead>
<tr>
<th>Benzocaine (Anesthesin)</th>
<th><img src="image" alt="Benzocaine" /></th>
<th>Odorless white crystalline powder M.T. 89-92°C:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procaine hydrochloride (Novocain hydrochloride)</td>
<td><img src="image" alt="Procaine" /></td>
<td>Odorless white crystalline powder M.T. 154-158°C:</td>
</tr>
</tbody>
</table>
**Synthesis:** para-nitro benzoic acid is the precursor for all this substances is. Para-nitro benzoic acid is obtained from para-nitrotoluene oxidation.

Anesthesin is synthesized by etherification of para-nitro benzoic acid with ethyl alcohol: para-nitro benzoic acid ester is obtained which then is reduced by iron in acetic acid media.

The most simple way of Novocain synthesis is Anesthesin per-etherification with $\beta$ – diethylaminoethanol in sodium alcoholate media.

**Physical properties:** Para-aminobenzoic acid derivatives are white crystalline, odorless powders. Anesthesin is distinguished by bad solubility in water in contrast to others. It is weak base; its salts are unstable and are easily hydrolyzed. Novocain, dicain, which are hydrochlorides are well soluble in water. All preparations are well dissolved in alcohol. Anesthesin is easily dissolved in both chloroform and ether. Novocaine, dicaine are badly dissolved in chloroform and almost unsolved in ether. Thus, anesthesin can be differed from other para-aminobenzoic acid derivatives by its physical properties.

**Identity**

- Diazotation and azodye formation reactions: this is a general reaction, due to primary aromatic amine group, consequently this reactions can give novocain and anesthesin. As a reagents
sodium nitrite in acidic environment and \( \beta \)-naphthol in basic environment are used. In the result of these reactions azo-dye is formed.

Dicain doesn’t give this reaction because it contains secondary aromatic amine group. Thus, dicain can be differed from other derivatives in this group by this reaction.

- Due to primary aromatic amine benzocaine and procaine hydrochloride with \( 2,4 \)-dinitrochlorobenzene in basic and heating conditions form yellow-orange colored quinone compound.

- Anesthesin, dicain and novocain as esters interact with hydroxylamine in basic media and then with iron III chloride in acidic media and form colored iron hydroxamates.
For hydrochlorides (novocain and dicain) it is typical chlorine ions detection reaction. The same compounds with ammonia hydroxide solution form basic form of preparations as precipitate with specific melting temperature.

Preparations can be identified by some alkaloid precipitating reagents (picrinic acid, mercury chloride, phosphormolibdenic acid etc.),

Drugs identification can also be realized by halogenation reaction. As other primary aromatic amines, dicain, novocain can form di-bromine or di-iodine derivatives (sediments).

Primary aromatic amine containing drugs with sodium hydroxide alcoholic solution and chlorophorm solution form isonitriles with unpleasant smell.

Primary aromatic amine containing derivatives with p-dimethylaminobenzaldehyde form yellow or orange Shif's base.

Partial reactions are based on color or water insoluble compounds formation. For Anesthesin such reaction is a soaping reaction in the presence of caustic alkali. The formed ethyl alcohol can be determined by iodoform formation reaction. Novocain and dicain also form soaping products, but iodoform formation reaction is negative for them.
➢ P-aminobenzoic acid derivatives are oxidized by forming colored or colorless products:
  □ Anesthesin rapidly is oxidized under 5% chloramine solution in acidic media by forming a reddish-orange product, which is separated by ether. Anesthesin with the mixture of concentric nitric acid and sulfuric acid forms yellowish-green color, which is turned into red color by adding water and sodium hydroxide solution.
  □ Procaine is oxidized by perhydrol and concentric sulfuric acid and forms violet colored product. Procaine with the mixture of concentric nitric acid and sulfuric acid in heating condition forms reddish-orange color.
  □ The specific reaction for tetracaine hydrochloride is the oxidation by potassium iodate in phosphoric acid environment and heating condition. Violet color is formed. Benzocaine and procain hydrochloride don’t give this reaction. This reaction is used for tetracaine hydrochloride quantity detection by photocolorimetric method.

➢ Dicain containing secondary amine group forms blood-red colored product heating with concentric nitric acid and potassium hydroxide alcoholic solution (Vital-Morene reaction). The reaction is based on dicain nitration and further formation of orto-quinoid compounds of potassium salts. Novocain and anesthesin don’t give this reaction.

➢ Dicaine is differed from other local anesthetics by N-nitrozo compound formation. After basic hydrolysis and further acidification p-buthylaminobenzoic acid is formed (white precipitate) which is dissolved in HCl. Then sodium nitrite solution is added and N-nitrozo compound precipitate is formed.
Benzocain with iodine solution in acidic media (without heating) forms poliiodide brown precipitate.

**Quantitative analysis**

Quantitative determination is mainly realized by nitritometric method. Anesthesin, novocain and other primary aromatic amines form diazonium salts, but dicain forms nitrozo compound like other secondary amines. Titration should be carried out slowly, because the time is needed for diazonium salts formation.

Equivalence point in dicain, novocain titration is detected due to external indicators, for example iodine-starch paper. At equivalence point iodine is released on the iodine-starch paper, which is dyed starch blue.

$$2\text{KI} + 2\text{NaNO}_2 + 2\text{H}_2\text{SO}_4 \rightarrow \text{I}_2 + 2\text{NO} + \text{K}_2\text{SO}_4 + \text{Na}_2\text{SO}_4 + 2\text{H}_2\text{O}$$
Equivalence point of para-amino benzoic acid derivatives in nitritometric titration also can be detected by potentiometry or by using internal indicators: neutral red, mixture of tropeolin 00 and methylen blue, etc.

- Preparations, which represent hydrochlorides (novocain, novocainamid, dicain) quantitatively can be analyzed according to connected hydrochloric acid neutralization in chloroform presence.

- It is also possible to determine by argentometry according to chloride ion (Volhard method).

\[
\text{Cl}^- + \text{AgNO}_3 \rightarrow \text{AgCl} + \text{NO}_3^- \\
\text{AgNO}_3 + \text{NH}_4\text{NCS} \rightarrow \text{AgNCS} + \text{NH}_4\text{NO}_3
\]

- titrant

\[
3\text{NH}_4\text{NCS} + \text{FeNH}_4(\text{SO}_4)_2 \rightarrow \text{Fe(SCN)}_3 + 2(\text{NH}_4)_2\text{SO}_4
\]

- indicator

- Anesthesin and novocain quantitatively can also be detected by bromatometry and iodine-chlorometry in the presence of potassium iodide. The separated iodine is titrated through sodium thiosulfate.

\[
\text{KBrO}_3 + 5\text{HBr} + 6\text{HCl} \rightarrow 3\text{Br}_2 + 6\text{KCl} + 3\text{H}_2\text{O}
\]

- KI + Br₂ \rightarrow I₂ + KBr

- indicator: starch solution

\[
\text{I}_2 + \text{Na}_2\text{S}_2\text{O}_3 \rightarrow \text{NaI} + \text{Na}_2\text{S}_4\text{O}_6
\]
Acid base titration in non aqueous media is also used. Environment is glacial acetic acid or formic acid or acetic acid anhydride, titrant is 0,1M HClO₄.

Spectrophotometry, extraction photometry and photocolorometry are used too.

Procaine and tetracaine hydrochlorides quantity can be determined by indirect complexometry.

Anesthesin, novocain and dicain are used as local anesthetics. Anesthesin is prescribed in 5-10% ointments, oily solutions, suppositories, also in 0.25-0.3g tablets and powders. Novocain has a wide usage for both infiltration and spine anesthesia in 0,25-0,5% aqueous solution form. Dicain is rather stronger than novocain, and approximately 10 times more toxic than novocain, that is the reason why it is classified in the A list of drugs and is used in ophthalmology for superficial anesthesia in 0,5-2% solution form, also in 0.3% solution form for peridural anesthesia.

Local anesthetics represent ethers of aromatic acids and amino alcohols. Further some compounds were observed, where amino alcohol residue was converted into amino acidic, and aromatic acidic residue into alcoholic. From these compounds, amides are widely used in the medicine, which are more stable and are hardly submitted to hydrolysis. They don’t cause a cross sensitivity with benzoic acid derivatives. Lidocain (xylocain) has a wide usage, from this group derivative’s, which removes novocain usage.

**Lidocaine hydrochloride**

![Lidocaine hydrochloride structure](image)

Lidocain is well dissolved in water. It excels novocain by anesthetic activity, strength and duration. In contrast to novocain, lidocain can be used for superficial anesthesia, because it doesn’t excite tissues. Solutions are stable and even in long-term boiling with strong acids and basics don’t decay. Lidocain toxicity depends on its concentration. Lidocain toxic symptoms are giddiness, low blood pressure, concussion are appeared seldom. Allergic reactions are also very seldom noticeable.

Lidocain is synthesized by the following way 2,6-xylidin is condensed with chlorine-acetyl-chloride (in the presence of sodium acetate). N-chlorine-acetyl-2,6-xylidine is formed, which interacting with diethylamine forms lidocain.
Identity

- 2,6-dimethylaniline a precursor, which is formed due to lidocain pyrolysis also from the acidic or basic hydrolysis, forms an azo-dye (diazotation and azo-conjugation reactions).

- Lidocain hydrochloride can be determined by silver nitrate solution, due to chloride ion.
- Gas liquid chromatography also is used.

Quantitative analysis

- It is realized by neutralization in the aqua-alcohol media, sodium hydroxide solution is titrant, indicator is a phenolphthalein.
- It is also determined through argentometry, due to chloride ion (Volhard method).
- Acid base titration in formic acid and acetic acid anhydridie mixture, titrant is HClO₄, indicator is crystalline violet.

Trimecain hydrochloride
Trimecain is also considered to dimethylphenylacetamide derivative and synthesis is realized similar to lidocain, but instead of 2,6-dimethylanilin 2,4,6 trimethylaniline is used.

Trimecain is a white or weak yellow color crystalline powder, with 139-142° melting temperature. It is easily dissolved in the water, ethanol and chloroform. It is practically insoluble in the ether.

**Identity**

- Precursor, (row substance) 2,4,-trimethylanyline can form an azo-dye (diazotation and azo-conjugation reaction), which is received from trimecain pyrolysis, the latter can also be form due to acidic and basic hydrolysis.
Trimecain determination is also realized by micro-crystallization reaction formation, which is carried out on object glass, mixing one drop preparation with 0,1N potassium bichromate and sulfuric acid. After 5-10min, the needle-shaped crystals are formed, which are gathered in clusters.

- Chloride ion is detected through the silver nitrate.
- UV-spectroscopy.
- Trimecain is oxidized in the mixture of copper sulphate and concentric sulfuric acid, heating up to 155-160°C, and after cooling and adding ammonia solution a blue color is observed, with the reddish-pink fluorescence.

**Quantitative analysis**

- Quantitatively is detected by non-aqueous titration method in the mixture of formic and acetic anhydrate acids. Chloric acid is a titrant, indicator crystalline violet.
- Neutralization method in the aqua-alcoholic solution, titrant is a 0,1M sodium hydroxide solution, indicator is a phenolphthalein.

- Atgentometry due to chloride ion.
- UV-spectroscopy.
- Extraction photometry.

**Usage and storage.** All above mentioned drugs are kept in the well closed vials, in dry please against light and in the room temperature.

0,25-0,5% solutions are used for infiltration and 1-2% solutions for peridural anesthesia.

**Control tests**

1. Choose the drugs of p-aminobenzoic acid ester which form azo dye
   - anesthesin
   - dicaine
   - novocaine
   - aminazine
   a/ 1.3  b/ 2.3  c/ 3.4  d/ 1.2.3

2. Anesthesin forms iron hydroxamate due to
   a) primary amino group
   b) ester group
   c) aromatic ring
   d) secondary amine

3. Anesthesin and novocain are determined by the following reactions
   1. diazotation and azo conjugation
   2. hydroxamate sample formation
   3. soaping
4. pyrolisis
a/ 1.2  b/ 2.3  c/ 1.2.3  d/ 3.4

4. 2,6-dimethylanilin which is formed from lidocain pyrolisis can be determined the following reactions and reagents
1. diazotation and azo conjugation
2. NaNO₂, HCl, β-naphthol, NaOH
3. silver nitrate solution
4. alkali solution
a/ 1.2.3  b/ 1.2  c/ 3.4  d/ 1.3.4

5. Argentometry method for trimecain hydrochloride is implemented due to
   a) chloride ion
   b) primary amine
   c) aromatic ring
   d) hydrochloric acid

NON STEROIDAL ANTIIMMFLAMMATORY DRUGS

This group of analgesics according to their anti inflammatory (also by analgesic and anti fever) properties comes near to the steroidal hormonal preparations; cortisone, hydrocortisone, dexamethasone, etc.

Salicylates are considered as the best drugs of this group. Free form of salicylic acid has been found in both the Cassia acutifolia plant leaves and chamomile flowers in the nature. Salicylic acid was synthesized in 1860 and acetyl salicylic acid in 1869. Nowadays salicylic acid industrial synthesis is carried out by Kolbe-Shmidt method; dry phenol and sodium hydroxide in equal quantities are used, in autoclave (130°C; 4.5-5.0 atm.).

\[
\text{C}_6\text{H}_5\text{OH} + \text{NaOH} \xrightarrow{t=130^\circ\text{C}P=4.5\text{atm.}} \text{C}_6\text{H}_5\text{ONa} \xrightarrow{\text{CO}_2} \text{C}_6\text{H}_5\text{COONa} \xrightarrow{\text{H}^+} \text{C}_6\text{H}_5\text{COOH}
\]

**Analyze methods**
Identity

- Salicylic acid with iron (III) chloride forms a violet colored iron (III) monosalicylate (pH=2-3). The color is disappears by the addition of hydrochloric acid. In pH=3-8 condition red colored iron (III) disalicylate is formed and in pH=8-10 yellow colored trisalicylate is released. The formed complexes are destructed by mineral acids, colors are disappeared and white precipitate of salicylic acid is formed.

\[
\text{Salicylic acid} + \text{FeCl}_3 \rightarrow [\text{Iron (III) monosalicylate}] + 2\text{HCl}
\]

Salicylate ion interacts also with other heavy metals salts. Whit silver nitrate solution white precipitate is formed.

- Salicylic acid forms a pink aurinic-dye due to interaction with formaldehyde in the presence of sulfuric acid (Mark reagent).

- Salicylic acid melting or heating with the crystals of organic salts (sodium citrate or acetate) or Na₂CO₃ or conc. H₂SO₄ decarboxylation is carried out and phenol (smell is appeared) and dioxide are formed, which passing through the lime water (Ca(OH)₂) and forms opalescence.

\[
\text{CO}_2 + \text{Ca(OH)}_2 \rightarrow \text{CaCO}_3 \\
\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3
\]

- Salicylic acid is determined by melting temperature 156-161°C:
- Sodium-salicylate identification is also realized due to sodium ion: by flame color and zinc-uranyl acetate.
- For sodium salicylate identification it is neutralized by hydrochloric acid and salicylic acid precipitate is formed, which is determined by melting temperature /156-161°C/ and above mentioned reactions.
- Sodium salicylate due to phenolic hydroxyl group interacts with iron (III) chloride leading to reddish violet iron salicylate. With copper sulfate solution green colored copper salicylate is formed.
- Salicylic acid and sodium salicylate with concentric sulfuric acid and methanol in heating condition form methyl salicylate with specific odor.
- Sodium salicylate in drug forms is determined by IR spectroscopy.

### Quantity detection

Quantitatively salicylic acid is detected by alkalinmetry, titrant is 0,1M sodium hydroxide solution, in the ethanol media, indicator is phenolphthalein.

\[
\begin{align*}
\text{COOH} + 2\text{NaOH} & \rightarrow \text{COONa} + 2\text{H}_2\text{O} \\
\end{align*}
\]

- Sodium-salicylate is detected by neutralization (acidimetric method). Solution is titrated by hydrochloric acid in the ether media, by the presence of indicators mixture: methyl orange and methyl blue.

\[
\begin{align*}
\text{COONa} + \text{HCl} & \rightarrow \text{COOH} + \text{NaCl} \\
\end{align*}
\]

- Salicylic acid and sodium salicylic acid quantitatively can also be detected by bromatometry.

\[
\begin{align*}
\text{KBrO}_3 + 5\text{KBr} + 3\text{H}_2\text{SO}_4 & \rightarrow 3\text{Br}_2 + 3\text{K}_2\text{SO}_4 + 3\text{H}_2\text{O} \\
\text{COOH} + 3\text{Br}_2 & \rightarrow \text{Br} \quad \text{Br} \quad \text{Br} \quad \text{Br} \quad \text{Br} \quad \text{OH} + 3\text{HBr} + \text{CO}_2 \\
\end{align*}
\]

Bromine surplus is detected by iodometry

\[
\begin{align*}
\text{Br}_2 + 2\text{KI} & \rightarrow \text{I}_2 + 2\text{KBr} \\
\text{I}_2 + 2\text{Na}_2\text{S}_2\text{O}_3 & \rightarrow 2\text{NaI} + \text{Na}_2\text{S}_4\text{O}_6 \\
\end{align*}
\]

Iodchlorometry also is used for quantity detection analysis.
The surplus of ICl is detected by iodometry

\[
\text{ICl} + \text{KI} \rightarrow \text{I}_2 + \text{KCl}
\]

\[
\text{I}_2 + 2\text{Na}_2\text{S}_2\text{O}_3 \rightarrow 2\text{NaI} + \text{Na}_2\text{S}_4\text{O}_6
\]

Quantitatively sodium salicylate is detected by the acid-base titration in the glacial acetic acid media, indicator is a crystalline violet and titrant 0,1M HClO₄.

Salicylic acid derivatives are:

- Sodium salicylate
- Methyl salicylate
- Acetyl salicylate (aspirin)
- Salicylamide
- Phenyl salicylate (salol)
- Salazat

The aim of salicylic acid derivatives synthesis was to get the forms of preparations which must be stable in the stomach acidic media, which must not influence on the mucous membrane and the molecule must be hydrolyzed in the basic media of intestines. As a result, salicylic acid must be formed having antiseptic, anti fever and anti inflammatory effect. This principle is known as the Nencky Salol principle. Splitting products are partially excreted from the organism from its kidneys and by disinfecting urinary ways. Due to this principle some drugs are covered with Salol, so they could reach the intestines and for enhancement of efficiency.
Aspirin has a weak acidic taste: acetic acid has a typical smell, it is an odorless, white crystalline powder or colorless needle-like crystals. It is melted in 135°C. It is stable in dry air, but it is hydrolysed from humidity into salicylic acid and acetic acid. It is less soluble in the water (1:300), easily soluble in alcohol (1:5). It decays in alkali and carbonic acids solutions.

**Synthesis:** Acetysalicylic acid is synthesized by heating the mixture of salicylic acid, acetic acid anhydride and concentric sulfuric acid.
Identification reactions

- IR spectrophotometry
- Hydrolysis products of acetylsalicylic acid (in the basic media) are identified

\[
\text{\begin{align*}
\text{O} & \quad \text{Na} & \quad \text{Na} & \quad \text{O} \\
\text{O} & \quad \text{CH}_3 & \quad \text{O} & \quad \text{CH}_3 \\
\end{align*}} + 3\text{NaOH} \rightarrow \text{\begin{align*}
\text{O} & \quad \text{Na} & \quad \text{Na} & \quad \text{O} \\
\text{O} & \quad \text{Na} & \quad \text{Na} & \quad \text{O} \\
\end{align*}} + \text{CH}_3\text{COONa} + 2\text{H}_2\text{O}
\]

By dilute sulfuric acid salicylic acid is formed, which is detected by

\[
\text{\begin{align*}
\text{O} & \quad \text{Na} & \quad \text{Na} & \quad \text{O} \\
\text{O} & \quad \text{Na} & \quad \text{Na} & \quad \text{O} \\
\end{align*}} + \text{H}_2\text{SO}_4 \rightarrow \text{\begin{align*}
\text{O} & \quad \text{Na} & \quad \text{Na} & \quad \text{O} \\
\text{O} & \quad \text{Na} & \quad \text{Na} & \quad \text{O} \\
\end{align*}} + \text{Na}_2\text{SO}_4
\]

- Iron (III) chloride solution (violet)
- Melting temperature (156-161°C)
- Formaldehyde in sulfuric acid media (Mark reagent).

Ethanol and concentric sulfuric acid is added at filtrate and acetic acid ethyl ester is formed with a special smell.

\[
\text{CH}_3\text{COOH} + \text{C}_2\text{H}_5\text{OH} \xrightarrow{\text{H}_2\text{SO}_4} \text{CH}_3\text{C}(\text{O})\text{OC}_2\text{H}_5 + \text{H}_2\text{O}
\]

- Aspirin also is determined by 2-4% amino-antipyrene and potassium hexa-cyanoferrate(III) in a chloroform media.
- After acidic hydrolysis of aspirin (concentric sulfuric acid and water) acetic acid and salicylic acid are formed. Acetic acid is identified by its specific smell, and salicylic acid is detected by formaldehyde (pink color is formed).

Quantitative analysis

79
Neutralization method (alkalimetry). It is titrated by 0.1 N NaOH. Indicator is phenolphthalein.

Neutralization (acidometry). After hydrolysis the addition quantity of NaOH is titrated by HCl.

UV- spectrometry (before doing hydrolysis with base).

Cerimetry (titrant is a cerium sulphate (IV). Drug can be oxidized up to glutaric acid, formic acid and in to other aliphatic acids.

**Pharmacological properties:** analgesic, anti-inflammatory, anti-fever, anti-aggregation. It is produces in the form of tablets (65, 81, 250, 325, 500 mg).

**Anthranilic acid derivatives (fenamates)**

The group of these derivatives represent as an isosteres of salicylic acid, in which molecule the hydroxyl group is substituted by the isostere amino group. Otherwise, the anthranilic acid derivatives are the isosteres of nitrogen containing salicylic acid. The individuality of these preparations are, that at first they have an anti-inflammatory property and after that an analgesic.

![Salicylic acid](COOH)

![Antranilic acid](NH)

In 1960 a number of N-substituted anthranilic acid derivatives were suggested by the research group of Park-Defis, which nowadays are known as phenama-acids: mefenamic acid, meclofenamic acid and flufenamic caid.

![Mefenamic acid](NH)

![Meclofenamate](Cl)

![Flufenamic acid](CF3)

**Amino phenols**

**Paracetamol**

Paracetamol is a white or weak yellow, odorless crystalline powder. 1 g is dissolved in 70 ml water and in 10 ml 96% alcohol.
**Identity**

- Phenolic hydroxyl group with iron (III) chloride solution forms a bluish-violet color.
- Heating with dilute hydrochloride acid the molecule is hydrolyzed. In the result p-aminophenol is formed which due to oxidation with potassium dichromate solution turns into quinonimine (quinoid-form), which than interacts with the p-aminophenol and forms an indophenol (violet color).

\[
\begin{align*}
\text{NH}_2\text{COCH}_3 & \xrightarrow{\text{H}_2\text{O}} \text{NH}_2\text{CH}_2\text{COOH} \\
\text{NH}_2 & \xrightarrow{\text{K}_2\text{Cr}_2\text{O}_7} \text{NH} - \text{O} \\
\text{NH}_2 & \xrightarrow{\text{H}_2\text{SO}_4} \text{N} - \text{N}
\end{align*}
\]

- Hydrolyzing by dilute sulfuric acid or hydrochloric acid an acetic acid smell is felt.
- Aromatic amino group which is formed after acidic hydrolysis is detected by azo-dye formation (dizotetion and azo-combination reactions).

\[
\begin{align*}
\text{NH}_2\text{COCH}_3 & \xrightarrow{\text{H}_2\text{O}} \text{NH}_2\text{CH}_2\text{COOH} \\
\text{NH}_2 & \xrightarrow{\text{NaNO}_2/\text{HCl}} \text{NH}_2\text{COCH}_3 \\
\text{NH}_2 & \xrightarrow{\text{NaOH}} \text{N} - \text{N}
\end{align*}
\]

- Paracetamol due to its phenolic hydroxyl group can form azo-dye with di-azonium salts.
- It forms dark-red color with the Mark reagent.
- Paracetamol has a reductive property (due to phenolic group) and reduce the silver from silver nitrate ammonia solution, mercury from Nessler reagent, Cu₂O from Fehling reagent.
- Paracetamol with nitric acid forms yellowish gray colored compound.

**Paracetamol quantitative analysis** is realized after acetyl group hydrolysis through these methods:
- Nitritometry, indicator is iodine-starch paper (bluing). Equivalent point also is detected by potentiometric method.

\[
\begin{align*}
\text{NaNO}_2 + \text{HCl} &\rightarrow \text{NaCl} + \text{HNO}_2 \\
\text{KJ} + \text{HCl} &\rightarrow \text{KCl} + \text{HJ} \\
2\text{HNO}_2 + 2\text{HJ} &\rightarrow \text{I}_2 + 2\text{NO} + 2\text{H}_2\text{O}
\end{align*}
\]

- Cerimetric method, titrant is a cerium (IV) sulphate, in the presence of potassium iodide and the formed iodine is titrated through the sodium thio-sulphate.
\[
2\text{Ce(SO}_4\text{)}_2 + 2\text{KJ} \rightarrow \text{J}_2 + \text{Ce}_2\text{(SO}_4\text{)}_3 + \text{K}_2\text{SO}_4
\]

\[
\text{I}_2 + 2\text{Na}_2\text{S}_2\text{O}_3 \rightarrow 2\text{NaI} + \text{Na}_2\text{S}_4\text{O}_6
\]

- New-phisico-chemical methods: Spectrophotometric, GLCh.

Drug forms are capsules, tablets, syrups, suppositories. It is also used in combination with analgin, dibazole, papaverine, phenobarbital, caffeine, codeine.

**Control tests**

1. Choose the reagents which are necessary for salicylic acid detection
   1. Mark
   2. FeCl₃ solution (PH 2-3)
   3. H₂SO₄ (conc. t° C)
   4. zincuranylacetate
   a/ 1.2.3 b/ 1.2 c/ 2.3.4 d/ 2.3

2. Choose the quantity detection methods for both salicylic acid and sodium salicylate
   1. acidometry
   2. bromatometry
   3. iodometry
   4. alkalimetry
   a/ all b/ 2.3.4 c/ 2.3 d/ 1.2.3

3. Which methods are used for acetyl salicylic acid quantity detection?
   1. alkalimetry
   2. acidometry
   3. cerimetry
   4. complexonometry
   a/ 1.2 b/ 1.2.3 c/ 3.4 d/ 1.3.4
4. Paracetamol due to phenolic hydroxyl group forms azo dye with
   a) diazonium salt
   b) NaNO₂, HCl, β-naphthol, OH⁻
   c) CH₃O H₂SO₄ /conc./
   d) AgNO₃, NH₄OH solution

5. Paracetamol quantity detection by nitritometry is implemented after
   a) oxdation
   b) acidic hydrolysis
   c) reduction
   d) pirolis

---

**Pyrazol derivatives**

Pyrazol represents two nitrogen containing five-member aromatic heterocyclic compounds which derivatives have a wide usage for colourants and in medicine. Pyrazol derivatives are: phenazone (antipyrine), metamizole (analgin), propiophenazone.

![Antipyrine (Phenazone)](image_url)
Phenazone synthesis is realized by Knor in 1884, due to condensation of aceto-acetic acid ether with phenyl-hydrazine. The same product is obtained from the condensation of diketen and phenylhydrazine, then after methylation in heating condition with benzosulfoacid methyl ester and basic hydrolysis phenazone is formed.

Phenazone is an odorless, white crystalline powder with a bitter taste. It very easily is dissolved in the water (1:1), and is easily soluble in the alcohol.

**Identity**

- Phenazone forms 4-nitrozophenazone (an enamel-green color) due to interaction with sodium nitrite in the acidic media. Due to this substituting reaction, phenazone can be differed from other pyrazole derivatives.

- In the result of interaction 4-nitrozophenazone and α-naphthyl amine azo-dye (red color) is formed
It can give reactions with general alkaloid precipitating reagents due to tertiary nitrogen.

Reactions with oxidizers: Phenazone forms a red color iron-phenazone or ferripirine (3C_{11}H_{12}ON_{2} \cdot 2FeCl_{3}), due to interaction with iron (III) chloride, which is discolored by mineral acids.

Phenazone forms 4-iodophenazon due to interaction with I_{2} (iodine solution is discolored).

Phenazone is oxidized by the mixture of potassium dichromate K_{2}Cr_{2}O_{7} and concentric sulfuric H_{2}SO_{4} acid and forms colored (green) products.

UV and IR spectroscopy are also used for phenazone.

**Quantitative analysis**

Spectroscopy

Photocolorimetry: It is based on nitrozophenazone formation reaction.

Iodometry: It forms 4-iodophenazon, due to the active hydrogen in the 4-th position, which is dissolved in the chloroform, and for prevention of the reverse reaction a sodium acetate must be added. The iodine addition is titrated by sodium thio-sulphate.

Phenazone is kept in the well closed orange vial and in the light protected place.
Metamizole sodium (Analgin)

![Chemical Structure of Metamizole Sodium](image)

1-phenyl-2,3-dimethyl-4-methylaminopyrazolon-5  
N-sodium methylsulfate

Analgin is a white or weak yellow colored crystalline powder, which is rapidly hydrolyzed by humidity.

**Identity**

- Metamizole identification is based on its expressed reductive property. It interacts with oxidizers: by iron (III) chloride it forms iron-metamizole (blue color).
- Metamizole is also oxidized by silver nitrate \( \text{AgNO}_3 \) and \( \text{NaNO}_2 \) in concentric sulfuric acid media, and by mixture of calcium hypochloride and calcium chloride \( \text{Ca(ClO)}_2, \text{CaCl}_2 \). In the presence of HCl, it is oxidized by KJ, at first strawberry color is formed which then is turned into a polyiodide dark precipitate.
- Sodium ion in the molecule of metamosol, is detected by the yellow color of flame or zinc-uranil acetate (white precipitate).
- Analgin, heating in the acidic media, is decomposed by forming a sulfuric gas and formaldehyde.

Sulfuric acid is detected by potassium iodide and starch solution by the help of blue track appearance in the wet filter paper.

\[
5\text{SO}_2 + 2\text{KIO}_3 \rightarrow 4\text{SO}_3 + \text{I}_2 + \text{K}_2\text{SO}_4
\]

Formaldehyde is detected by heating with the salicylic acid in the presence of sulfuric acid. Thus, aurinic dye is formed (a p-quinoidic product) red in a color.
Sulfur atom, containing in metamizole, is detected by melting it with the mixture of sodium and potassium carbonates Na₂CO₃ + K₂CO₃, after which the alloy must be cooled and dissolved in the HNO₃ nitric acid and should be filtrated. The formed sulfate ions are detected by barium chloride solution.

**Quantitative analysis**

- Spectrometry
- Iodometry (in the weak acidic aqua-alcoholic media).

\[
\begin{align*}
\text{NaO}_{3} & \quad \text{I}_2 \quad \text{H}_2\text{O} \\
\text{Na}^{+} & \quad \text{I}^{-} \quad \text{HI} \quad \text{CH}_2\text{O} \quad \text{NaHSO}_4
\end{align*}
\]

The end of titration is detected by iodine addition. Yellow color is formed.

**Phenylibutazone (butadione)**

It represents pyrazolidine-dion-3,5- derivative.

It is white or weak yellow crystalline powder. It poorly is dissolved in water, hardly in alcohol. Phenylibutazone synthesis is realized by reducing the nitrobenzole up to hydrazobenzole and than is condensed it with the malonic acid dichlorine anhydrate in the presence of sodium ethylate.
Identity

- It forms a cherry color with the heating by the H₂SO₄ sulfuric acid in the presence of sodium nitrite. Thus, occurs oxidation of hydrazobenzole by the formation of azobenzol.

- Phenylbutazone posses an acidic property due to the active hydrogen in the 4th position, in acetone solution.
  - Phenylbutazone interacts with the sodium hydroxide solution and forms soluble sodium salt, after which interacting with the salts of heavy metals complexes are formed.

A grey-bluish precipitate is formed with the copper (II) solution.
A white precipitate is formed with the silver nitrate solution.

Phenylbutazone interacts with general alkaloid reagents due to tertiary nitrogen.

**Quantitative analysis**

- Spectrometry
- Neutralization (it is titrated through the 0.1M sodium hydroxide solution in the presence of acetone), indicator phenolphthalein
- Iodine-chlorometry in the presence of potassium iodide. Titrant is a iodine-chlorine solution, indicator starch solution.

Usage: All mentioned drugs are used during pain in different origin. Analgine is included in composition of Caffetine, Anapirin, Tempalgin, Baralgine, Pentalgine drugs.

Control tests

1. Antipirine is detected by the following reagents
   a) silver nitrate solution
   b) sodium nitrite, $\text{H}^+$
c) diphenylamine
d) cobalt chloride solution

2. Analgin interacts with HCl and forms CH₂O, which is detected by
   a) FeCl₃ solution
   b) salicylic acid, conc. H₂SO₄
   c) diphenylamine
   d) cobalt chloride solution

3. Analgine quantity is detected by the following method
   a) acid base titration in non aqueose media (in glassial acetic acid)
   b) gravimetry
   c) iodometry
   d) nitritometry

4. Choose the products which is formed from hydrazobenzole oxidation
   a) benzoic acid
   b) benzaldehyde
   c) azobenzole
   d) hydrazine

5. Choose the product which is formed from the interaction of phenylbutazone and copper (II) sulphate in basic media
   a) precipitate /copper (II) complex salt /
   b) azobenzole
   c) hydrazobenzole
   d) Cu₂O sediment

**Opioid alkaloids**

**Phenantren isoquinoline (morphinan) derivatives**

Morphine, codeine and their derivatives are opioid alkaloids. Their semi-synthetic derivatives are: ethyl morphine, hydro morphine, oxy-morphine, hydrocodon, oxicodon, naltrexon and synthetic products: methadone, meperidin, phentanil, suphentalyl and others.

There are special opioid receptors in brain; μ, κ, δ and opioid receptors binded with μ-receptors.

**Enkephalines**: Are pento-peptides having 5 amino acid residues.

**Endorphines**: Contain 31 amino acids. These are endogen ligands which are able to bind with opioid receptors and posses an analgesic effect. Morphine connection with opioid receptors is
conditioned by structural and conformational similarity of its molecule with some parts of the enkephalines and endorphins. Morphine and its derivatives are exogenic analgesics and they stabilize neuropeptides, due to inactivating of their breaking enzymes known as enkephalinazes.

Morphine was obtained from its natural source in 1806 by Sertyurner from papaver somniferum L. milk, which is known as opium. When opium is dried it turns into a soft, yellowish-greenish-brown or with grey rough surface. It has a typical smell and is a heavy and hard mass (after remaining out in the air for some time). It is dissolved in water forming an acidic grey solution. Opium contains 25 different alkaloids, which contain 20-25% of opium common mass. It contains hydrocarbons, proteins, resins, fats and other substances.

The following alkaloids are important in the medicine: narcotine, codeine, papaverine and thebain. The quality of opium is detected by morphine’s content (5-20%).

Opium alkaloids, according to their chemical structure, are phenantrene (morphine, codeine), benzyl isoquinoline (papverine) and apomorphine derivatives. Alkaloids are in meconic acid and also in lactic acid and sulfuric acid salts form, except narcotine and papaverin, which basic properties are weakly expressed.

Alkaloids are obtained from opium and are differentiated from one another by using physicochemical methods, such as: chromatography and different methods of electrophoresis. Morphine structure was suggested by Robinson in 1923, but was finally justified by synthesis (1950-1951). Now there are different methods of morphine synthesis, but the most economically convenient source of morphine is its obtainment from natural source.

Morphine and its derivatives are N-methyl morphinan derivatives, in which A,B,C is partially hydrated phenantrene, C,D is hydrated isoquinoline, but D is piperidine. In their molecule structure there is an epoxy group, which is connected with carbon atoms.

Morphine has optical activity, which is conditioned by 5 asymmetric carbon atoms in its molecule (5, 6, 9, 13 14). There is a central carbon atom (C13), in morphine-like analgesics molecule, which substitutes are not hydrogen. One of the substitutes is benzene ring, but another is the tertiary nitrogen atom, which binds with the central carbon due to 2 carbon atoms. Electro acceptor groups are also necessary for molecule (keto, ester, double bonds).

**Morphine Hydrochloride**
Morphine molecule contains 2 hydroxyl groups: one phenolic (in aromatic ring), and the other is alcoholic.

Morphine is an amphoteric compound: its acidic property is conditioned by a phenolic hydroxyl group, but its basic property by tertiary nitrogen. Morphine reductive properties are conditioned by phenol, tertiary N, alcohol and partly by a hydrated phenantren group. Morphine solutions are easily oxidized due to both influences light and alkali solution. Morphine solution stability is noticed in pH 2.5-limits.

Morphine hydrochloride is a white needle-like crystal or crystalline powder. Over time it turns yellow in color. It slowly is dissolved in water and in organic solvents is dissolved very poorly (1:50). It also is dissolved in the both the acids and the base.

**Morphine's identity is realized by its acid-base and reductive properties.**

- With Mark reagent (\(\text{CH}_2\text{O} + \text{H}_2\text{SO}_4\)). A formed purple-red color turns into bluish-violet.
- With potassium hex cyan ferrate (III) \(\text{K}_3[\text{Fe(CN)}_6]\) (oxidizer) in acidic media pseudo morphine is formed. But adding an iron (III) chloride solution berlin azure (blue precipitate) is formed.

\[
3\text{K}_4[\text{Fe(CN)}_6] + 4\text{FeCl}_3 \rightarrow 2\text{K}_4[\text{Fe(CN)}_6] \cdot \text{Fe}_4[\text{Fe(CN)}_6] \cdot 12\text{KCl}
\]

Berlin azure

- Phenol hydroxide group is detected by a three-valence iron chloride.
- Morphine hydrochloride is dissolved in ammonia solution forming a white crystalline precipitate (morphine). It can be released and detected by its melting temperature. But if sodium hydroxide solution is added into that precipitate, the precipitate is dissolved turning into phenolat (due to phenol group).
- Chloride ion is detected by silver nitrate solution. A white precipitate is formed.
- Morphine becomes orange in color due to concentrated nitric acid, then color turns into yellow.
Morphine forms azodye with diazonium salts. The compounds containing a phenolic group, in which ortho and para positions are free can easily combined with diazonium salts forming azodye.

In the presence of concentrated sulfuric acid or hydrochloric acid in heating condition, morphine derivatives: morphine, codeine, ethylmorphine form apomorphine.

The presence of tertiary nitrogen is detected by alkaloid reagents. For morphine, the most sensitive reagent is the ammonium molybdate solution in concentrated sulfuric acid (Frede reagent). Concentrated sulfuric and nitric acid mixtures (Erdman reagent) oxidize morphine into apomorphine, which with the addition of nitric acid converts it into an intense red color.
By Mandeline reagent (ammonium vanadate solution in concentrated sulfuric acid) it forms a violet color.

Morphine’s reductive property is defined by a potassium iodate KIO₃ solution in the presence of sulfuric acid in a chloroform media. Chloroform layer turns pinkish-violet due to formed iodine.

Morphine in the presence of a peroxide solution, ammonia and copper sulfate forms a red color.

Morphine with Pillager reagent (conc. H₂SO₄, t°, I₂, NaHCO₃) forms a red color (o-quinon).

Morphine hydrochloride is detected by UV-spectrophotometry

**Quantity detection**

- Acid-base titration in glacial acetic acid in the presence of mercury (II) acetate solution. Morphine is titrated by perchloric acid until a formation of green color.
- Neutralization method in water-alcoholic media by chloroform addition: Titrant is 0,1M NaOH solution and indicator is an phenolphthalein.
- Argentometry-Volhard methpod (due to chloride ion), also potentiometric method.
- UV spectrophotometry.

**Pharmacological effect:** analgesic.

In high doses, morphine is a somnolent and can cause a pleasant feeling (euphoria), but in the case of double usage it causes an addiction (morphinism).

Pseudo morphine (2,2’-bismorphine) has been discovered in dogs’ tissues, which have addiction toward morphine. This is one of the reasons of causing an addiction. Pseudo morphine was also found in opium. Morphine is prescript in the list of “controlled drugs”, it must be kept in well-closed vials and safe from light place. Morphine hydrochloride is produced in 0,01 g tablets, 1ml 1% injected solution forms in ampoules.

**Codeinum**

Codeine contains 0,2-1% in opium: In1832 Robriquet discovered codeine for the first time from opium. But codeine’s natural sources can’t satisfy medicinal demands, due to which
it is obtained from morphine by a semi-synthetic method. Codeine is obtained by the Radion-Shapochnicov method from morphine and phenyl trimethyl ammonium hydroxide.

The letter is obtained from dimethyl phenyl amine and p-toluenesulfoacid methyl ester.

For getting codeine phosphate or sulfate in codeine's and phosphoric acid or sulfuric acid equivalent quantities add alcohol and let the preparation to crystallize from the solution.

Codeine is an odorless, white crystalline powder that has a bitter test. It dries in air. It is dissolved in boiling water, and easily is dissolved in 96% alcohol. In contrast to morphine, it doesn't soluble in bases. It is dissolved in acids and chloroform.

- **Identity**
  - With Mark reagent codeine becomes a bluish-violet then turns into yellow.
  - Codeine becomes an orange from addition of conc. HNO₃.
  - Codeine doesn’t dissolved in ammonium hydroxide solution (doesn’t have a phenol group).
  - Codeine interacts with iron (III) chloride solution after heated with concentrated sulfuric acid (hydrolysis). It then forms a blue color. Under influence of H₂SO₄ codeine turns into apomorphine and phenol groups are formed, which can interact with iron (III) chloride.
Tertiary nitrogen is detected by general alkaloid precipitated reagents.

Codeine becomes violet due to interaction with the Frede reagent.

It detects through the mixture of conc. H₂SO₄ + conc. HNO₃. The concentric sulfuric acid oxidizes codeine up to apomorphine, which because of nitric acid becomes an intense red color.

The phosphate ion, in the codeine phosphate, is detected through the silver nitrate. A yellow precipitate is formed.

\[ \text{PO}_4^{3-} + \text{Ag}^+ \rightarrow \text{Ag}_3\text{PO}_4 \downarrow \]

Codeine is detected by spectroscopy.

Codeine precipitate is formed from codeine phosphate by adding sodium hydroxide.

Melting temperature of sediment is detected (154-157°C):

Morphine residue in the codeine is defined by sodium nitrite solution in acidic media, after which ammonia solution is added and we get a colored ammonium salt.

Quantity detection

Codeine quantitatively detection analysis is realized by the neutralization method (has a strong basic properties) by titrating through the 0,1M hydrochloric acid in a water-alcoholic media. Indicator is a methyl red.

\[ \text{C}_{17}\text{H}_{17}\text{ON(OH)OCH}_3 + \text{HCl} = \text{C}_{17}\text{H}_{17}\text{ON(OH)OCH}_3 + \text{HCl} \]

Codeine phosphate quantity is detected by acid-base titration in non aqueous media (glacial acetic acid). Titrant is a 0,1M perchloric acid. Indicator is a crystalline violet.

UV spectrophotometry

Photocolorimetry

Pharmacological effects are: analgesic, sedative and anti-cough (relaxes cough formatting centers excites). It is produced in the form of tablet (0,01-0,02 g), powder (0,01-0,02 g).

Morphines semi-synthetic and synthetic derivatives according to pharmacological effect

Naltrexone Hydrochloride
It is an odorless crystalline powder. The specific rotation of solution is equal to (-187) - (-197°). It is dissolved in the water, alcohol, ether, and poorly dissolves in the chloroform. It is synthesized from thebaine by adding peroxide. Endiol is formed which is submitted to acidic hydrolysis by forming enketol, and the metoxy group is hydrolyzed. Oximorphone is formed by double bond hydration in the enketol. Afterwards it interacts with bromine-cyan and then it is N-alkilized by halogenalkil (bromine-methylcyclo-propyl) and Naltrexon is obtained.

Identity

Naltrexone hydrochloride easily is oxidized in neutral, acidic and basic environment.

- With Mark reagent yellow color is formed.
- With the mixture of concentric sulfuric acid and concentric nitric acid orange color is formed.
- Naltrexone base form is formed by ammonia hydroxide solution. The sediment is detected by melting temperature and in filtrate chloride ion is determined by silver nitrate.
- Keto group in naltrexone molecule is detected by 2,4-dinitro-phenyl-hydrazine solution. Yellow colored sediment of naltrexone 2,4-nitrophenyl hydrazone is formed.
Phenolic hydroxyl group is detected by iron (III) chloride solution.

Azo-dye is formed from the interaction of naltrexone hydrochloride and diazonium salt due to phenolic hydroxyl group.

Naltrexon is detected by IR specters (IR) (4000-400sm^-1).

**Naltrexone** quantitatively is detected by:
- Acid base titration in non-aqueose media (glacial acetic acid). Titrant is HClO₄, indicator crystalline violet.
- UV spectroscopy (maximum absorption 280 mn).
- HPLC (USA-pharmacopeia).

Naltrexon hydrochloride is differed from morphine by cyclo-propyl group, due to which it becomes an antagonist toward opium receptors. It is produced in the form of tablet and capsule forms (0,025-0,05 g).

**Apomorphine hydrochloride** is also semi-synthetic drug, and is classified to the apomorphine derivative.

**Apomorphine Hydrochloride**

It is a white or weak yellow or grey crystalline powder. It is hardly dissolved in water, alcohol, practically doesn't dissolved in ether and chloroform. Apo-morphine aqueous solutions under the light turns green in color and losses its activity. Its main reason is conditioned by the presence of 2 phenolic hydroxyl groups in the molecule, which are sensitive toward oxidizers.

**Synthesis**

Apo-morphine is synthesized by heating morphine with conc. HCl in an autoclave (140-150°C):
Identity

- Apo-morphine gives Vital-Morene reaction (like-tropan).
- Apo-morphine, in the presence of morphine is detected by ammonia solution in the chloroform media. Chloroform layer becomes violet in color.
- Chloride ion detection by silver nitrate solution.
- Apo-morphine hydrochloride crystals form a red color with nitric acid.
- Preparation interacts with 5% hydrocarbonate and 0,1M iodine solutions in ether presence. Ether layer is turned into reddish-violet, but water is turned green in color.

Preparation quantitative analysis is carried out in non-aqueous media by acid-base titration in glacial acetic acid and with mercury (II) acetate. It is titrated by 0,1M perchloric acid and crystalline violet is used as an indicator.

- UV spectroscopy (λ=272 nm)

Apomorphine possesses weak analgesic activity.

Trimeperidine Hydrochloride (Promedolum)

It has a little odor (sometime odorless) and is a white crystalline powder. It easily is dissolved in the water, alcohol and chloroform but in the ether it practically doesn't dissolve.

Promedol synthesis was carried out by Nazarov and his workers. Precursor is methyl-vinyl-allyl-keton, which interacts with methyl-amin.1,2,5-trimethyl 4-piperidone is formed which with phenyl litium lead to a formation of 1,2,5-trimethyl-4-phenylpiperidol 4-Li-
alcoholat. The letest interacts with propionic acid chlor anhydrid and promedol is formed.

Identity

- Tertiary nitrogen in Promedol is detected by general precipitating alkaloid reagents. Reacting with 1% picrinic acid, it forms a yellow promedole picrate.
- Chloride ion is detected by silver nitrate.
- The 0,5% chloroform solution slowly adding on the Mark reagent (CH₂O in concentrated sulfuric acid), between the two liquids surface a red ring will be noticable.
- Sodium cobalt nitrite in concentrated sulfuric acid (1% solution) with promedol forms a reddish-cherry color.
- With ammonium vanadate in concentrated sulfuric acid media forms a green color.

Quantitative analysis is realized by acid-basic titration in non-aqueous media (glacial acetic acid).

Control tests

1. Phenolic hydroxyl group in morphine molecule is detected by
   a) FeCls solution
   b) CuSO₄ solution
   c) CoCl₂ solution
   d) HCl
2. Morphine hydrochloride is not identified by
   a) CuSO₄ solution
   b) CH₂O + conc. H₂SO₄/Mark reagent/
   c) NH₄OH solution
   d) J₂, conc. H₂SO₄, NaHCO₃, t⁰/Pelagre reagent/

3. Morphine hydrochloride quantity is detected by
   a) iodometry
   b) acid base titration in non aqueose media /glassial acetic acid/
   c) nitritometry
   d) complexometry

4. Phosphate ion in codeine phosphate is detected by
   1. AgNO₃ solution
   2. magnesium mixture
   3. BaCl₂ solution
   4. CuSO₄ solution
   a/ 1.2 b/ 2.3 c/ 3.4 d/ 1.4

5. Morphine hydrochloride is determined by the following reagents
   1. K₃[Fe(CN)₆], FeCl₃ solution
   2. FeCl₃ solution
   3. alkaloid precipitating reagents
   4. CoCl₂ solution
   a/ 1.2.3 b/ 1.2 c/ 3.4 d/ 2.3

---

**ANTIDEPRESSANTS**

Medicinal products which are used for the treatment of depression are classified into different chemical groups. Such as:

- Izonicotinic hydrazide derivatives-nialamid
- Tricyclic antidepressants- imipramine, amitriptyline
- Other chemical classes-fluoxetine, etc.

Both the chemical and modern physicochemical methods are used for analysis of these products.

**Tricyclic antidepressants**
**Imipramin hydrochloride**

**N-(3-dimethylaminapropyl)-iminodibenzyl hydrochloride**

**Synthesis:** Precursor is orto-nitrotoluene, which interact with ethylformyl in the presence of sodium ethylate, 2,2-nitrodibenzyl is formed, which is turned into 2,2-diaminadibenzyl due to catalytic hydration. The latest in heating condition (207-280°C) forms and ammonia. The formed imidodibenzyl interacts with dimethylaminapropyl chloride, in the presence of sodium amide with toluene benzene mixture. As a result, imipramine hydrochloride is formed.
Tertiary nitrogen can be detected by general alkaloid precipitating reagents. With alkali solution the basic form is released which is detected by melting temperature. In filtrate chloride ion can be detected by silver nitrate.

\[
\begin{align*}
\text{Imipramine hydrochloride} & \quad \text{Amitriptyline hydrochloride} \\
\end{align*}
\]

IR and UV spectroscopy is also used for identification.

**QUANTITATIVE ANALYSIS**

Acid-base titration in non-aqueous media (media is glacial acetic acid, indicator is crystalline violet, in the presence of mercury acetate, titrant is 0.1M HClO₄.

Neutralization method also can be used: alkalimetry, titrant is sodium hydroxide, indicator is phenolphthalein.

Argentometry (Volhard method).

Imipramine hydrochloride is produced in the form of tablets.

5-(3-dimethylaminopropyldien)10,11-dihydroxybezacycloheptene hydrochloride

White crystalline powder, melting temperature is 195-199°C: It easily is dissolved in water, ethanol, chloroform, and practically doesn’t dissolved in ether.

It is produced in form of tablets, ind onjection solutions.

**Synthesis:** Raw substance are both phthalic anhydride and phenyl-acetic acid. Thus due to interaction 2-phenyl ethyl benzoic acid is formed, which can undergo cyclation in 170°C. The formed
compound is condensed with bromine magnesium-propyl-dimethylamine and at last hydrolysis can be released for the final formation of the preparation.

**IDENTITY**
- Tertiary nitrogen can be detected by general precipitating alkaloid reagents.
- With alkali solution the basic form is released which is detected by melting temperature. In filtrate chloride ion can be detected by silver nitrate.
- It has typical absorptions in the UV and IR specters.

**QUANTITATIVE ANALYSIS**
- Alkalimetry, neutralization according to HCl by 0.1M NaOH in ethanol, titration end is detected by potentiometric method.
- Acid-base titration in non-aqueous media (American pharmacopeia). Titrant is a 0.1M HClO4, indicator crystalline violet, media glacial acetic acid, in the presence of mercury acetate.
Fluoxetine hydrochloride

It is produced in the form of tablets and capsules 0.02g.

It represents aryl-oxy-propanol-amine derivative. It is used in medicine in form of hydrochloride. It is a white, odorless, crystalline powder. It is hardly dissolved in the water, but is soluble in both ethanol and methanol.

IDENTITY

- IR and UV spectrophotometry.
- HPLC.
- With alkali solution the basic form is released which is detected by melting temperature.

![Chemical structure diagram]

- In filtrate chloride ion can be detected by silver nitrate

\[
\text{NaCl} + \text{AgNO}_3 \rightarrow \text{AgCl} \downarrow + \text{NaNO}_3
\]

- After mineralization, fluorine ion detection is carried out: with calcium chloride, calcium fluoride is formed (white sediment).

\[
2\text{CaCl}_2 + \text{F}^- \rightarrow \text{CaF}_2 + 2\text{Cl}^-\]

- Reaction with iron rodanide, in result of which the noticeable red color is disappeared.

\[
[\text{Fe (CNS)\textsubscript{6}}]^{3-} + 6\text{F}^- \rightarrow 6\text{CNS}^- + [\text{Fe F\textsubscript{6}}]^{3-}
\]

- Reaction with zirconium alizarin complex, in the result free alizarin is released, which in contrast to violet complex has a yellow color.

![Chemical reaction diagram]

QUANTITATIVE ANALYSIS

106
- HPLC.
- Acid-base titration in non-aqueous media. Titrant is a 0.1M HClO₄, indicator crystalline violet, media glacial acetic acid, in the presence of mercury acetate.

\[
\text{N}^+\text{HCH}_3 + \text{HClO}_4 + \text{H}_2\text{Cl}_2\text{CO}_2\text{H} \rightarrow \text{N}^+\text{ClO}_4^- + 2\text{CH}_3\text{COOH} + \text{HgCl}_2
\]

- Spectrophotometry.

It is produced in form of tablets and capsules.

**Nialamidum /Nialamide/**

Izonicotinic acid 2-(2'-benzylcarbamoil)-ethylhydrazide

*It is produced* in tablets form. It is a white, odorless microcrystalline powder, with 151-153°C melting temperature.

Nialamide synthesis precursor is bromine propionic acid and thionyl chloride.

**Physico-chemical properties**
As an isoniazide derivative it undergoes tautomerization and depending on pH of media it can show both a basic and acidic properties and in pH=6,6-8,1 nialamide has non ionized form.

Identity chemical methods and quantitative analysis is based on; pyridine cycle determination, hydrazine residue existence, hydrolysis and oxide-reductive reactions, and absorption properties.

**IDENTITY**

- Pyridine cycle detection:
  - Nialamide is heated in aqueous bath with citric acid and acetic acid anhydrate, cherry color is formed.
  - The Zinke method: pyridine cycle splitting, by adding splitting agent 2,4-dinitrochlorbenzene or bromrodan/chlorrodan in a basic NaOH alcoholic solution, as a result glutacon aldehyde is formed.

- The formed glutacon aldehyde can be detected by interaction with primary amines, (mainly aniline is used). An aldehyde with amine forms condensed colored product (Schiff bases).
Reaction with bromine rodane or chlorine rodane as a splitting agent.

- Nialamide interacts with general alkaloid reagents as a compound containing tertiary nitrogen.
- Interaction with Fehling reagent is carried out due to hydrazine residue reductive property, at first gas bubbles are noticed, then Cu₂O brick-red precipitate is released.
- Nialamide identity can be proved in UV region by typical absorption.

**QUANTITATIVE ANALYSIS**

- Nitritometry: method is based on nitrozo compound formation (nialamide interacts with the 0.1N sodium nitrite in acidic media).
Internal indicator is a tropoloin 00 and methylene blue mixture. Iodine-starch paper is also possible to use as external indicator, thus from the addition of titrant will become blue.

\[
2KJ + 2NaNO_2 + 4HCl = J_2 + 2NO + 2NaCl + 2H_2O
\]

- Spectrophotometry.

NOOTROPICS

Aminalon

White hygroscopic crystalline powder, melting temperature is 200-205°C. It is easily dissolved in water, unsoluble in ethanol and other organic solvents.

Chemical properties

Aminalon, as all amino acids, has amphoteric properties. It is in zwiter ionic form in the both neutral media and solid state. It is melted at high temperature 200-205°C (one of the identification methods) and is dissolved in the both acids and basics.

Identity

- Aminalon gives ninhydrin reaction, as amino acid (despite the reaction with ninhydrin is mainly typical to \(\alpha\)-amino acids, GABA also gives this reaction), bluish-violet complex is formed.
- It can form dark blue complex compound with the copper sulfate in the basic media.

\[
2 \text{R-CH-COOH + CuSO}_4 + 2\text{NaOH} \xrightarrow{\text{O}} \text{CH}_2-\text{CH}_2-\text{CH}_2-\text{C}=\text{O} + \text{Na}_2\text{SO}_4 + 2\text{H}_2\text{O}
\]

- Aminalon is formed bright-raspberry color with alloxane on heating in aqueous bath.

- The carboxyl group in aminalone molecule show acidic properties. After neutralization by NaOH solution drug's basic properties is increased due to amine group and in phenolphthaleine media the solution is turned into pick color. When formaldehyde solution is added, the amine group is blocked (azometin group is formed), basic properties is disappeared and solution became colorless (Serense method). This reaction is also used in quantitative detection.

\[
\begin{align*}
\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{C}=\text{O} + \text{NaOH} & \xrightarrow{\text{H}_2\text{O}} \text{CH}_2-\text{CH}_2-\text{CH}_2-\text{C} = \text{ONa} \\
\text{H} - \text{C} - \text{H} & \xrightarrow{\text{N}=\text{C}} \text{CH}_2-\text{CH}_2-\text{CH}_2-\text{C} = \text{ONa}
\end{align*}
\]

- Interaction with potassium rodanide is also used as a specific reaction. Heating Aminalone and KSCN in equal quantities, and using filter paper which was before wetted by lead acetate, will turn black in color. Reaction occurs according to ammonium salts and KSCN interaction scheme.

\[
\text{KSCN} + \text{NH}_4\text{Cl} \xrightarrow{t^0} \text{NH}_4\text{SCN} + \text{KCl}
\]

Further heating of NH₄SCN will recombine the molecule to thionyl urea, which isomer form is decayed by forming H₂S. Interacting this with lead acetate black color is formed.

\[
2\text{NH}_4\text{SCN} \xleftrightarrow{\text{2NH}_4\text{SCN}} 2\text{S=CH} \xleftrightarrow{\text{HS}+\text{NH}_2} \text{H}_2\text{S} + \text{NH}_2\text{CN}
\]

\[
\text{H}_2\text{S} + \text{Pb(CH}_3\text{COO})_2 \xrightarrow{} \text{PbS}_4 + 2\text{CH}_3\text{COOH}
\]

Quantitative analysis
Keldal method is used for Aminalon.

Spectrophotometric method is based on colored product formation after interaction with alloxane.

Formaldehyde titration (phormol titration): based on blocking of amino group of aminalone, must be titrated by 0.1M sodium hydroxide solution.

Acid-base titration in non-aqueous media. Media is the glacial acetic acid, titrant 0.1N HClO₄, and indicator is a crystalline violet.

It is produced in the form of tablets.

Picamilon
Picamilonum

\[
\begin{align*}
\text{Picamilon} & \\
\text{Picamilonum} & \\
\end{align*}
\]

N-nicotinoil-γ-butyric acid sodium salt

White hygroscopic crystalline powder. It is easily dissolved in water, ethanol, and practically insoluble in ether and chloroform.

Identity

Identity of this preparation can be divided into:
I. Typical to pyridine cycle
II. Typical to amino acid part
III. Specific

I. Reactions typical to pyridine ring

- A colored reaction occurs with citric acid and acetic acid anhydride, violet color is formed.
- Zinke reaction is carried out by splitting regents 2,4-dinitrochlorine benzene and bromine rodane/chlorine rodane, in the NaOH alcoholic solution.
- Interaction with alkaloid reagents Lugol, Dragendorff, Mayer etc.

II. Reactions typical to amino acid moiety

The GABA is released from picamilon, in the basic media and in heating conditions, which can be identified by typical reactions, especially through ninhydrin.

III. Specific reactions

- Sodium ion detection, by yellow flame color, also by zinc-uranyl acetate.
- Picamilon has typical absorptions in the UV region.

Quantitative analysis
Quantitatively picamilon can be detected by acid-base titration in the non-aqueous media (media is glacial acetic acid and acetic acid anhydride, titrant is perchloric acid, indicator is crystalline violet).

It is produced in tablets form.

**PIRACETAM**

![Chemical structure of Piracetam]

White crystalline powder, melting temperature is 151-155°C, easily is dissolved in water, ethanol, unsuluble in ether.

The synthesis is α-pyrolidone, which is hardly alkylated according to nitrogen atom. Thus, piracetam synthesis is realized by its lactim form, which interacts with chloracetic acid ester.

**Chemical properties**

Piracetam represents itself both lactam and amid according to its structure.
Hydrolyzing in a basic media the cycle is broken dawn due to amide group and ammonia is released (smell or litmus paper)

It can give hydroxamate reaction as lactam and amide, due to interaction with hydroxyl amine solution in the presence of copper salt in the acidic media.

Piracetam can be detected by IR and UV spectroscopy.

In drug forms piracetam is identified by color reactions: by indophenol formation which is formed if piracetam interacts with sodium hypochloride and phenol. After which identification and quantity detection is realized by photocolorimetry method.

Quantitative analysis
Keldal method is used, after basic hydrolysis due to released ammonia.

It is produced: in tablets, capsules, granules (for syrup preparation) ampoules for injection.

Control tests

1. Choose the reagent for aminalone identity
   a) KMnO₄ solution
   b) alloxan, dimethylformamide, t⁰
   c) NaNO₂, HCl solution
   d) H₂O₂ solution

2. Aminalone quantity is detected by the following method
   a) nitritometry
   b) Keldal
   c) complexometry
   d) bromatometry

3. In picamilone structure pyridine ring is not identified by
   a) melting with citric acid and acetic acid anhydride
b) 2,4-dinitrochlorbenzen, OH⁻ alcoholic solution
c) chlorrodan
d) conc. H₂SO₄

4. Piracetam quantity is detected by
   a) acid base titration in non aqueous media (dimethylformamide)
   b) bromatometry
   c) Keldal
   d) gravimetry

5. Amino acid moiety in picamilone molecule is detected by the following reagent
   a) ninhydrine /after basic hydrolysis/
   b) alkaloid precipitating reagents
   c) 2,4-dinitrochlorobenzene, OH⁻
   d) H₂O₂ solution

**Antihystaminic drugs**

**Ethylene diamine and dimethylaamina ethanol derivatives**

Representatives of these group antihystaminic preparations are used in the medicine.
- Diphenhydramine hydrochloride (dimedrol)
- Chrolpyramine hydrochloride (suprastine)
- Ranitidine hydrochloride
- Famotidine

**Diphenhydramine hydrochloride (Dimedrol)**

**Diphenhydramine Hydrochloride**

It is a white odorless crystalline powder with appropriate m.t. 167-172°C. It very easily is dissolved in water, easily in ethanol and chloroform.

**Synthesis**

Precursors are benzhydrol and dimethyl-amino-ethylchloride.

**Identity**

Physicochemical and chemical methods are used for these preparations identity

**Physicochemical methods**

- IR spectroscopy.
- UV spectroscopy.
- Gas liquid chromatography. An individual method is for di-phenyl-hydramine identity and quantity detection.

**Chemical methods:**

- Containing tertiary nitrogen it interacts with general and specific alkaloid reagents. Dragendorf, Mayer, Frede (yellow color, which turns into red by heating), with picrinic acid, phosphor-wolfram and silicium-wolframic acids.
- Dimedrol does not formed a colored products with conc. H₂SO₄, but interacts with the mixtures of conc. nitric and sulfuric acids (1:9, Erdman reagent) and forms colored products.
• It interacts with Mark reagent.
• Dimedrol can form oxonium salt with conc. H$_2$SO$_4$. The formed bright yellow color is turned into reddish-brown.

\[
\begin{align*}
\text{C} & \quad \text{H$_2$SO$_4$} \\
\text{O} & \quad \text{C$_\text{BEC}$} \\
\text{N} & \quad \text{SO$_4^{2-}$} \\
\end{align*}
\]

As oxonium salts are instable compounds, thus they are easily hydrolyzed from water and a color disappears.

✓ Brown sediment is released by adding 0.1M HCl, CuSO$_4$ and ammonia rodanide into diphenyl hydramine water solution.
✓ Boiling with dilute nitric acid hydrolysis is realized (synthesis reverse process) and benzhydrol is formed.

\[
\begin{align*}
\text{C} & \quad \text{H$_2$O} \\
\text{O} & \quad \text{H} \\
\text{N} & \quad \text{O} \\
\end{align*}
\]

which can be detected after re-crystallization by m.t. /61-67 C\(^\circ\)/:
✓ Different fthaleinic, sulfo-fthaleinic dyes and azocompounds (methylen red, thymol blue, phenol red, tropeoline 0, 00, 000, etc.) can be used for di-phenyl hydramine identity, from these reagents the colored sediments are formed and different colored chloroform extracts are also formed.
✓ As a hydrochloride preparation a basic form can be precipitated by base, and chlorine ion can be detected as well.

\[
\begin{align*}
\text{Cl}^- & \quad \text{AgNO}_3 \rightarrow \text{AgCl}\downarrow + \text{NO}_3^- \\
\text{AgCl} + 2\text{NH}_3 \cdot \text{H}_2\text{O} \rightarrow [\text{Ag(NH}_3)_2]\text{Cl} + 2\text{H}_2\text{O}
\end{align*}
\]

Assay
As hydrochloride, acid base titration in non aqueous media is used with the presence of mercury acetate. Titrant is a 0.1 N HClO₄ indicator is a crystalline violet and media is a glacial acetic acid.

\[
\text{CH}_3\text{COOH} \quad 2 \quad \text{HCl} + 2\text{HClO}_4 + \text{Hg(CHO}_2\text{O)}_2 \quad \rightarrow \quad 2 \quad \text{CH}_3\text{COOH} \quad \text{N} \quad \text{CH}_3 \quad \text{ClO}_4^- + \text{HgCl}_2 + \text{HCl}
\]

According to PhA titration can be carried out also in acetic acid anhydride media without using mercury acetate, and there is not necessity for adding this reagent.

- Alkalimetry in a water media, is based on hydrochloric acid neutralization by sodium hydroxide. Titration is carried out in the ether presence, in which diphenhydramine basic form is dissolved (see reaction in identity).
- Iodine-chlorometry based on iodine monochloride reaction.

\[
\text{ICl} + \text{KI} \rightarrow \text{I}_2 + \text{KCl}
\]

\[
\text{I}_2 + 2\text{Na}_2\text{S}_2\text{O}_3 \rightarrow 2\text{NaI} + \text{Na}_2\text{S}_4\text{O}_6
\]

- Diphenylhydramine can be detected by argentometry due to chloride ion of the hydrochloride acid (Volhard method: reverse argentometric method).
Diphenylamine quantitatively can be detected by extraction-titrimetric method.

**Chloropiramin hydrochloride (Suprastin)**

It is a white crystalline powder.

**Identity**

**Physicochemical and chemical methods** are used for this preparation identification.

**Physicochemical methods**
- UV, IR scopy, TLC.

**Chemical methods**
- Having tertiary nitrogen it is detected by general and specific alkaloid reagents.
- Preparation chloroform solution with 1% picrinic acid solution, in water media is formed a yellow color (chloropiramin picrate).
- As hydrochloride preparation basic form can be precipitated by base, and a chloride ion is detected in the solution.

**Quantitative analysis**
- It can be detected by acid-base titration in non aqueous media as a hydrochloride. The glacial acetic acid and acetic acid mixture is used as a solvent /5:10/, indicator is a crystalline violet.

**Famotidine**
It is a white powder. It is sensitive toward light. It is easily dissolved in water, practically is not dissolve in ethanol, chloroform, ether, acetone. It is easily dissolved in dimethylformamide and glacial acetic acid.

Ranitidine hydrochloride

It is a white powder, with appropriate m.t. 140°C (by decaying). It is sensitive toward light humidity. It is dissolved in water, moderately in ethanol and is practically insoluble in the chloroform.

Synthesis
Precursors are furfuril alcohol, dimethylamine and paraform and the interaction is carried out in the mechanism given bellow.

Identity
Physico-chemical methods:
• IR and UV spectroscopy, Thin-layer-chromatography.

Chemical methods:
✓ Having tertiary nitrogen in its molecule it can be detected by general and specific precipitating alkaloid reagents.
As hydrochloride preparation basic form can be precipitated by base, and chlorine ion can be detected.

\[
\text{Ranitidine hydrochloride is detected by acid-base titration in glacial acetic acid media. The end of titration is detected by potentiometric method. Famotidine quantity is detected in the same conditions. 0.1M HClO₄ must be taken in both cases as a titrant.}
\]

- Ranitidin hydrochloride assay is detected through argentometry with potentiometry usage (Fayance method, direct argentometry) by 0.1 n silver nitrate solution.
- UV-spectrophotometric method. Optical density is also measured.
- HPLC is used.

**Control tests**

1. Choose the precursors for dimedrol synthesis
   1. benzhydrol
   2. dimethylaminaethylchloride
   3. dimethylamine
   4. furfuril alcohol
   a/ 1.2  b/ 1.3  c/ 3.4  d/ 2.4

2. Dimedrol is identified by
   1. CH₂O, H₂SO₄ /conc./ solution
   2. phosphor molibdenic acid
   3. picrinic acid
   4. H₂SO₄ conc., NH₄MoO₃ solutions
   a/ 1.2  b/ 1.2.3.4  c/ 3.4  d/ 1.2.3

3. Ranitidine hydrochloride quantity is not detected by
   a) HPLC
   b) argentometry
c) acid base titration in glacial acetic acid
d) acid base titration in dimethylformamide

4. Choose the precursors for ranitidine hydrochloride synthesis
1. furfuril alcohol
2. dimethylamine
3. paraform
4. benzhydrol
a/ 1.2.3  b/ 1.2  c/ 3.4  d/ 2.4

5. Choose the methods for ranitidine hydrochloride quantity detection
1. argentometry
2. UV spectrophotometry
3. HPLC
4. complexometry
a/ 1.2  b/ 1.2.3  c/ 1.2.4  d/ all

Drugs acting on blood system
4-oxicumarine derivatives
In medicine from 4-oxicumarine derivatives mainly are used Neodicumarin (ethylbiscumacetate), Syncumar (acenocumarol), Fepromaron and Varfarine in sodium or potassium salts.

General physical properties
- They are white, odorless crystalline powders.
- Badly soluble in water, soluble in acetone and basic solutions
They give typical absorptions in IR and UV-regions.

**General chemical properties**

**Acid-base properties**

4-oxicumarine derivatives possess acidic properties due to phenolic hydroxyl group in the reason of which they can form

- salts with NaOH
- colored complex compounds with FeCl₃ (grey-red).

![Chemical structure of neodicumarin](image)

**Neodicumarin (ethylibiscumacetate)**

**Synthesis:** The precursors of synthesis are 4-oxicumarine and glyoxalic acid ethyl ester. The letest is obtained from oxalic acid and ethanol.

![Synthesis reaction](image)

Glyoxalic acid ethyl ester interacts with 2mol 4-oxicumarin and forms ethylibiscumacetate

**Identity**

- The specific identification reaction for neodicumarene is the heating with concentric sulfuric acid. Yellow color is formed which is turned into orange. After dilution of solution bi-4-oxicumarinil-3-acetic acid (white sediment) is formed which is dissolved in bases.

- **Acetylation reactions**
Due to phenolic hydroxyl groups, neodicumarine, fepromarone, and acenocumarol can form esters with carbonic acids or their anhydrides. The formed esters are detected by typical melting temperatures.

**Hydrolytic splitting reactions**

4-oxicumarine derivatives contain lactone ring, thus undergo hydrolytic breaking in basic media. Thus ethylbiscumacetate and fepromaron melting with alkalis leads to a formation of salicylate ion, which after acidifying sediment form is released. Salicylic acid identity can also be detected by FeCl₃ (bluish-violet color).

Lacton ring splitting in neodicumarine molecule is realized due to hydrolysis in soft conditions. Reaction product being phenolic compound can be detected by both azo-dye formation with diazonium salts (cherry-red color) and indophenol dye formation with quinonimine (blue color).

**Fepromarone due to hydroxyl group interacts with diazonium salt. Orange azo-dye is formed.**
Acenocumarole after reduction of nitro group (primary aromatic amine is formed) gives diazonium salt formation and azoconjugation reactions:

After basic hydrolysis of neodicumarine the released ethanol is detected by iodoform formation reaction.

Hydroxamate reaction
Compounds of this group can give hydroxamate reaction, which is typical for all compounds containing lacton ring and complex ether group. Preparation is heated with hydroxylamine basic solution, afterwards interaction is carried out with Fe$^{3+}$ or Cu$^{2+}$ salts in acidic media.

Quantitative analysis
Neutralization in acetone media. Titrant is a NaOH, indicator is a methyl red and methylen blue mixture (mono-substituted salt is formed).

Acetylation method: after formation of neodicumarine diacetate by acetic acid anhydride in pyridin media, the surplus quantity of acetic acid anhydride undergoes hydrolysis. The formed acetic acid is titrated by NaOH in the presence of phenolphthalein.

$$ (\text{CH}_3\text{CO})_2\text{O} + \text{H}_2\text{O} \rightarrow 2 \text{CH}_3\text{COOH} $$

$$ \text{CH}_3\text{COOH} + \text{NaOH} \rightarrow \text{CH}_3\text{COONa} + \text{H}_2\text{O} $$

Quantitative analysis of fepromaron and acenocomarol is realized by neutralization, titrant is sodium hydroxide in the acetone media, as an indicator phenolphthalein is used.
Phenyline is a derivative of 1,3-indandion. White or yellow odorless crystals, melting temperature is 148-151°C. It is hardly dissolved in water, ethanol and ether, esially in chloroform, benzene. Water solution has orange color.

**Identity**
- Phenylin is formed red precipitate with NaOH due to tautomerism
- A bluish-violet color is formed with concentrated H₂SO₄
- Spectrophotometry method.

**Quantity analysis**

**Quantity detection** is realized by neutralization through the NaOH, indicator is a phenolphthalein.

- Phenyline quantity is detected by using bromation method in 10% alcohol solution.
After 5 minutes β-naphthol and KI solutions are added. The addition quantity of bromine is connected by β-naphthol.

Brom derivatives of phenindion interacts with KI.

The released iodine is titrated by 0.1M sodium tiosulphate solution, indicator is starch solution.

\[ \text{I}_2 + 2 \text{Na}_2\text{S}_2\text{O}_3 \rightarrow 2 \text{NaI} + \text{Na}_2\text{S}_4\text{O}_6 \]

Phenylin is indirect anticoagulant. It is used in thrombosis. It is produced in form of tablets.

**Control tests**

1. 4-oxicumarine derivatives are
   a) anticoagulants
   b) spasmolytics
   c) antiimmflamatory agents
   d) somnolents

2. Choose the reagent and condition of salicylate ion formation from neodicumarine
   a) KOH /melting/
   b) H$_2$SO$_4$ /hydrolysis/
   c) NaOH /solution/
   d) CuSO$_4$ solution

3. Choose the indicator for neodicumarine quantity detection by neutralization method in acetone media
a) NaOH solution  
b) acetone  
c) phenolphthalein  
d) mixture of methyl red and methylen blue

4. Choose the product which is formed after fepromaron melting with alkali /KOH/  
a) salicylate ion  
b) chroman  
c) 4-oxycumarine  
d) phenol

5. Choose the method for phenylin quantity detection  
a) acid-base titration in non aqueose media  
b) neutralization  
c) nitritometry  
d) iodochlorometry

Calcium antagonists  
Nifedipine

**Synthesis** is realized by condensation of nitro-benz-aldehyde with acetoacetic acid esters in the presence of ammonia.
It is a yellow crystalline powder, insoluble in water and easily decaying under the light. It can give typical absorptions in IR and UV regions.

**IDENTITY**

- It is detected by chromatography in tetrachlormethane: chloroform:propanol-1 (70:20:10) solvents system.
- Nifedipine solution forms a red color with KOH alcoholic solution in dimethylformamide media.

Nitro group gives diazotation reaction after reduction of it into amino group.

- It can give hydroxamate reaction as complex ether.
- By HPLC method

**Quantitative analysis** is realized by spectrophotometric and HPLC methods.

**Verapamil**
Identity

- Adding sulfuric acid and KMnO₄ into the preparation a reddish-violet precipitate is released.
- White sediment (preparation base form) is released adding NaOH on the preparation.
- Chloride ion detection

Quantitative analysis

1. Acid-base titration in non aqueous media in the presence of mercury acetate (media is glacial acetic acid, titrant is a HClO₄, indicator is a crystalline violet).
2. UV-spectroscopy

Spasmolytic drugs

Papaverin and Drotaverine (No-Spa)

Papaverin is an alkaloid, which is contained in the Papaver Somniferum raw fruits milk known as opium. It was obtained by Merk from opium in 1884, moreover alkaloid content in opium is 0,4-1,5%. Papaverine is differed from opium and other derivatives due to both chemical structure and pharmacological effects. According to chemical structure, it belongs to the isoquinoline derivatives, partly 1-benzyl isoquinoline derivatives.

Drotaverine hydrochloride /No-Spa/ represents Papaverine semisynthetic analogues.

Benzylisoquinoline derivative properties
Papaverine hydrochloride is a white, but drotaverine hydrochloride is a colored compound and both are moderate soluble in water. Papaverin and Drotaverine are used as spasmolytics and as brain and coronary vasodilators.

These drugs action mechanism is not completely studied yet, but is supposed that it is connected with phosphodiesterase enzyme inhibition, which increases cyclic AMP level.

**Papaverines synthesis** precursor is 3,4-dimethoxyphenyl-ethylamine, condensing it with 3,4-dimethoxyphenyl acetic acid (homoveratric acid) chlorine anhydride.
This method changed variant is used as well Pitet Gams synthesis. Precursor is 3,4-dimethoxyphenylmethylamino ketone, which is condensed with homoveratric acid chlorine anhydride.

**Identity**

Papaverine and Drotaverine hydrochlorides are detected by IR and UV spectroscopy and also by colored reactions, which are

- Benzylisoquinolines by general alkaloid and specific reactions, and by halogens (bromine, iodine) substituted reactions,
- Oxidation reactions are detected by color and fluorescent compounds formation,
- Reactions based on acid-base properties,
- Reactions conditioned by methoxy groups in the structure.

For example

- Papaverine hydrochloride gives a green color with ammonia molibdate in concentric sulfuric acid presence (Frede reagent).
- With iodine alcoholic solution diiod papaverine hydrochloride is formed (C$_{20}$H$_{19}$N$_{x}$I$_{2}$H$_{x}$I):
- With bromine water yellow brom papaverine hydrobromide sediment is formed (C$_{20}$H$_{20}$O$_{4}$N$_{x}$Br$_{x}$HBr).
- With concentric sulfuric acid in heating condition violet color is formed /oxidation reaction/.
- Papaverine hydrochloride with sodium acetate solution leads to a formation of its base form, which is identified by melting temperature (145-170°C).
- Drotaverine hydrochloride with sodium hydroxide solution forms drotaverine base form wich is detected by melting temperature (208-214°C).
- Papaverine hydrochloride with concentric nitric acid forms yellow color which after heating is turned into orange:
- With Mark reagent papaverine forms colored products, after adding bromine water and ammonia, violet sediment is formed which is dissolved in alcohol, forming reddish-violet solution. Reaction is conditioned by methylenebisappaverine sulfate formation.

- With acetic acid anhydride and sulfuric acid it forms yellow color with green fluorescence.

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Papaverine and Drotaverine hydrochlorides with KMnO₄ in acidic media can form a blue fluorescence. 

Drotaverine hydrochloride heating with FeCl₃ in concentrated sulfuric acid media green color is appeared, then after adding nitric acid it becomes reddish-brown.

Chlorine ion is detected in papaverine and drotaverine hydrochlorides by silver nitrate solution.

**Papaverine and Drotaverine hydrochlorides quantitatively are detected by:**

- Acid-base titration in a non-aqueous media in the glacial acetic acid media, in the presence of mercury acetate (titrant is HClO₄, indicator crystalline violet).
- Neutralization in alcohol media, titrant is NaOH, indicator is phenolphthaleine.
- Argentometry according to chlorine ion (Volhard method).
- Spectrophotometric, photocolorimetric and extraction-photometric methods are used for medicinal forms.

This drugs must be kept according to “B-list”, away from light to prevent oxidation. From the light and air they become yellow.

SAR studies have shown that benzene ring is responsible for activity as well. Due to this fact many drugs were synthesized, which are bendazole hydrochloride (dibazol), omeprazole, domperidone (motilium).

**Dibazolum**

**Dibazol /bendazol/**

2-benzyl benzimidazole hydrochloride

Bendazol-hydrochloride (dibazol) is benzimidazole derivative and papaverine synthetic analogue. Tertiary nitrogen atom is responsible for 2-benzimidazole basic properties.

Dibazol (bendazol) hydrochloride is quite soluble in water, it is easily dissolved in ethanol and is practically insoluble in ether.

Dibazole possess spasmolytic property on blood veins and on the internal organs smooths muscles. Dibazol can decrease arterial blood pressure by peripheral vein dilation.

Dibazole is used in combination with other antihypertensive drugs.

Dibazole base synthesis is realized from o-phenylenediamine and phenyl acetic acid by the following mechanism.
Identity

- IR and UV specters.

- An identity can be also realized by adding iodine solution into dibazole in weak acidic media. Reddish-silver sediment is formed, which is conditioned by dibazole polyiodide release.

- With ammonia hydroxide solution bendazole base form is realized which is swdiment and tdetected by melting temperature /182-186°C/, and in filtrate chlorine ione is detected by silver nitrate in the presence of nitric acid. White sediment is formed.

- Bendazole hydrochloride with cobalt nitrate alchoholic solution forms blue colored complex salt.

- With the mixture of ammonia vanadate and concentric sulfuric acid in the presence of chloroform red color is formed.

**Dibazole hydrochloride quantitatively is detected by:**

- Acid-base titration in a non-aqueous acetic acid media in mercury acetate presence or in formic acid and acetic acid anhydride mixture (titrant is perchloric acid, indicator is a crystalline violet).
Adding silver nitrate into dibazole hydrochloride alcoholic solution in the presence of addition quantity of concentric ammonia (the latest is taken for dissolve the formed silver chloride sediment), lead to release of dibazol silver salt (a white sediment), which quantitatively is detected by argentometry.

Then nitric acid is added on order to dissolve the sediment. The formed silver nitrate is titrated by ammonia radanite solution in the presence of iron ammonia alum (Volhard method).

In medicinal forms dibazole hydrochloride is detected by spectrophotometric method in 244 nm, 270 nm regions.

Complexometric extraction-photometric methods can be used as well.

High quality. 1,2-Phenylen diamine content is detected, that shouldn’t exceed 0.05%.

Dibazole must be kept in dry conditions and at room temperature.
Pharmacological classification of drugs providing cardio-vascular normal function is problematic, because of this their chemical classification is very suitable.

In organs and tissues drug that improve blood flow are used in angina treatment. These diseases reasons are injuries of myocardium blood flow and metabolism. There are different drugs, which can enhance coronary blood flow, lighten myocardium load, and improve metabolism processes. They are called antianginal (lat. angina pectoris) preparations, which include organic nitrates and nitrites.

Antianginal main drugs are organic nitrates: nitroglycerine, nitrosorbite, isosorbite mononitrate and erynite (first 3 are included in main drugs list).

It has been discovered that the esters of nitroglycerine and nitric acids are turned into nitration in the organism. They are reduced up to nitrogen monoxide due to both iron containing enzymes and blood hemoglobin.

\[
\text{NO}_3^- \cdot 3\text{Fe}^{2+} + 4\text{H}^+ \rightarrow \text{NO} + 3\text{Fe}^{3+} + 2\text{H}_2\text{O}
\]

The nitrogen monoxide weakens the smooth muscles of the vessels, decreases the blood pressure and takes off the hart ischemic pain. The scientists were endowed with the Nobel Prize for this discovering in 1998.

**Nitroglycerine**

*Nitroglycerol, Glyceryl trinitrate (CO1D AO2)*

In synthesis and storage should be careful, because from very light agitation (highly volatile) or high temperature (180°C) it can explode. Nitroglycerine is one of the powerful explosive substances. For this purpose in large quantities it was synthesized by Alfred Nobel (in1960). By exploiting temperature it exceeds 1,5 times of trotylline (1500 kkal/kg).

\[
4\text{C}_2\text{H}_5(\text{ONO}_2)_3 \xrightarrow{180^\circ\text{C}} 6\text{N}_2 + 12\text{CO}_2 + \text{O}_2 + 10\text{H}_2\text{O}
\]

Gaseous substances volume formed from exploitation is 713 1/kg. In medicine, very dilute solutions are used.

It is colorless or weak yellow, with not high density (0.829 g/sm³) oily compound. Less soluble in water, alcohol and well in organic solvents. Volatile, absorption from closing cotton, from one tablet into another migration are the reason of tablet’s instability. Poured nitroglycerine immediately must be overflow by alkali solution (hydrolysis). Nitroglycerine or its solutions even in few quantities by contacted with skin and mucous membrane can cause strong headache.
It is produced in 1% alcohol solution (Sol.Nitroglycerini 1%), tablet's (0,5 mg), 1% oily solution, long-term micro capsules- Sustac-mite (2,6 mg) and Sustac-forte (6,4 mg), Nitrong mite, forte (Nitrong):

It must be kept in well-closed vials, in cold, dark place, far from fire.

Nitroglycerine synthesis is carried out due to glycerin and nitric acid interaction in the presence of concentric sulfuric acid.

![Chemical Reaction](attachment:image)

Identity of compounds can be determined by reactions typical to nitrate ion and obtainment glycerin in result of hydrolysis. Nitrate ion is detected by diphenylamine sulfuric acid solution. Diphenylbenzidine imonium blue salt is formed.

For glycerin detection akrolein formation reaction is used. Heating mixture with potassium hydrosulfate, akrolein is formed with a distinct unpleasant smell.

For glycerin detection akrolein formation reaction is used. Heating mixture with potassium hydrosulfate, akrolein is formed with a distinct unpleasant smell.

Quality: Inorganic nitrate presence is controlled (see previous reaction). By chromatographic methods and IR-spectroscopy is confirmed that nitroglycerin tablet's contain also 10% diethyleneglycoldinitrate.

O$_2$N-O-CH$_2$CH$_2$-O-CH$_2$CH$_2$-O-NO$_2$

Quantity detection

- Nitroglycerine in the presence of oxygen (hydrogen peroxide) is subjected to hydrolysis. For 1 mol nitroglycerine neutralization, 5 mol of sodium hydroxide must be taken, 3 of which is used for hydrolysis, but 2 for formic acid formation and also for acetic acid neutralization.

  CoH$_5$(ONO$_2$)$_3$ + 5NaOH $\rightarrow$ NaNO$_3$ + 2NaNO$_2$ + CH$_3$COONa + HCOONa + 3H$_2$O

- By using HPLC. Preparation and standard main picks must be suit by both the control time and the surface size.

- In medicinal forms nitroglycerine quantitative detection is based on photometric method. After hydrolysis HNO$_3$ is realesed, which with phenol-2,4-disulfonic acid forms a 6-nitrophenyl-2,4-disulfonic acid. From ammonia addition molecule gets in dark yellow aci-form with a specific absorption (415 nm).
\[
\begin{align*}
\text{H}_2\text{C}-\text{ONO}_2 & \quad \text{CH}_3\text{COOH} & \quad 3\text{H}_2\text{O} & \quad \text{H}_2\text{C}-\text{OH} \\
\text{HC}-\text{ONO}_2 & \quad \text{CH}_3\text{COOH} & \quad \quad + & \quad 3\text{HNO}_3 \\
\text{C}-\text{ONO}_2 & \quad \text{CH}_3\text{COOH} & \\
\text{H}_2\text{O} & \\
\end{align*}
\]

\[
\text{OH} \quad \text{SO}_3\text{H} & \quad \text{HNO}_3 & \quad \text{CH}_3\text{COOH} & \quad \text{SO}_3\text{H} \\
\text{OH} \quad \text{SO}_3\text{H} & \quad \text{KNO}_3 & \quad \text{CH}_3\text{COOH} & \quad \text{SO}_3\text{H} \\
\text{OH} \quad \text{SO}_3\text{H} & \quad 3\text{NH}_4\text{OH} & \quad (\text{NH}_4)_3 \\
\text{OH} \quad \text{SO}_3\text{H} & \quad 3\text{NH}_4\text{OH} & \quad (\text{NH}_4)_3 \\
\text{OH} \quad \text{SO}_3\text{H} & \quad 3\text{NH}_4\text{OH} & \quad (\text{NH}_4)_3 \\
\end{align*}
\]

**Nitroglycerine content** is detected by calibrimetry curve based on the same colored product formation (6-nitrophenyl-2,4-disulfonic acid yellow ortho-quinoid form) which is released from the interaction of KNO₃ and 6-nitrophenyl-2,4-disulfonic acid in ammonia media.

Isosorbite monohydrate, nitrosorbite and erynite are also considered to the group of organic nitrates.

![Diagram of Nitroglycerine content](image-url)

The syntheses of these preparations are occurred by polyatomic alcohols (glycerine, sorbit, pentaeritrite) and nitric acid interaction in concentrated sulfuric acid presence.

Sorbet is obtained by glucose catalytic hydration (Ni). Interacting with nitric acid depending on mol ratio, isosorbit mononitrate or dinitrate can be obtained either.
These preparations are a white, insoluble in both water and alcohol crystalline powders. Nitrosorbite is produced in form of tablets (0.02g or 0.04g) and 1% solutions. Eflox long, Olokard retard are long acting forms of nitrosorbite.

For identification IR absorption spectrophotometry and chromatography is used.

Preparation must be kept in well-closed vials and safe from light.

Control tests

1. Choose the synthesis precursors for papaverine synthesis /by Piket-Gams method/
   1. 3,4-dimethoxyphenylmethilketon
   2. homoveratric acid chloranhydride
   3. benzoic acid
   4. methylaminoketon
   a/ 1.2   b/ 1.3   c/ 2.4   d/ 1.4

2. Choose the reagents for drotaverine hydrochloride identification
   1. KMnO₄, H⁺ solution
   2. H₂SO₄ /conc./, t⁰, FeCl₃ solution
   3. FeCl₃ solution
   4. alkaloid precipitating reagents
   a/ 2.3   b/ 1.2.4   c/ 1.4   d/ 3.4

3. Quantity detection methods for papaverine and drotaverine hydrochlorides are
   1. acid-base titration in non aqueose media
   2. neutralization
   3. argentometry
   4. nitritometry
   a/ 1.2.3   b/ 2.3   c/ 1.4   d/ 3.4

4. Bendazole hydrochloride quantity is detected by
   1. acid-base titration in glacial acetic acid media
   2. argentometry /Volhard/
   3. complexometry
   4. extraction-photocolorimetry
   a/ 1.2   b/ 1.3   c/ all   d/ 1.3.4
5. Nifedipine is identified by
1. NH₂OH, OH⁻, Fe³⁺, H⁺ solutions
2. KOH alcoholic solution, by dimethylformamide
3. AgNO₃ solution
4. Zn, HCl, NaNO₂, H⁺, β-naphthol, OH

a/ 1.4  b/ 1.2  c/ 3.4  d/ 1.2.4

Pyrimidine derivatives

Uracil derivatives general characteristic

Uracil is 1,2,3,4-tetrahydropyrimidine dion derivative and similar to barbituric acid derivatives it can have 2 tautomeric forms.

The general structure for uracil’s derivative is the following:

In case of hydrogen substitution in 1ˢᵗ, 5ᵗʰ, 6ᵗʰ -positions compounds can be obtained having certain pharmacological activity. 5-fluorine uracil derivatives (fторурацил, fторафур) possess anticancer activity. Methyluracil derivatives can stimulate the metabolism processes.

In medicine, fторурацил, methyluracil and nucleozides тегафур (fторафур), zidovudine, stavudine are used.
Uracil derivatives synthesis is realized by aliphatic compounds cyclation. S-methyl-iso-thio-urine and sodium formil-fluorine-acetic acid ester are condensed and form 2-methylthio-5-ftoruracyl, which is hydrolyzed in the presence of HCl and turned into 5-ftoruracil.

\[
\begin{align*}
\text{C}_2\text{H}_5\text{O}-\text{C}-\text{O} & \quad \text{H}_2\text{C}-\text{COOC}_2\text{H}_5 & \quad \text{C}_2\text{H}_5\text{ONa} & \quad \text{NaOCH}=\text{C}-\text{COOEt} \\
\text{NH}_2 & \quad \text{C}=\text{NH} & \quad \text{NaO}-\text{CH} & \quad \text{F} \\
\text{S}-\text{CH}_3 & \quad \text{H}_2\text{C}-\text{C} & \quad \text{F} & \quad \text{H}_2\text{C}-\text{S} \\
\end{align*}
\]

Ftorafur (tegafur) is obtained from ftoruracil.

Ftoruracil is a white or yellowish crystalline powder. It melts at 282-284°C. It is less soluble in water and alcohol. Ftorafur is a white crystalline powder. It is poorly soluble in water and alcohol. Ftorafur sodium salt is well soluble in water. It rapidly metabolized in blood and carbon atoms.

From the chemical point of view, ftoruracyl and tegafur have weak acidic properties due to lactam-lactim tautomism and give reactions typical to phenols: azo-conjugation, oxidation, substitution and condensation.

### Identity
- Ftoruracil and ftorafur in basic media with deiazonium salt form azo-dye.
- After mineralization fluorine atom can be determined (F⁻).
- The residue is dissolved in water (pH 4,0-5,0) and CaCl₂ solution is added. White opalescence is observed.
  \[2\text{F}^- + \text{CaCl}_2 \rightarrow \text{CaF}_2 + 2\text{Cl}^-\]
- For detecting fluorine-ion, samples can be burned in a test tube in the presence of oxygen and hydrogen peroxide. Adding iron rodanite into the filtrate a red color is formed.
  \[\text{Fe(NCS)}_3 + 6\text{F}^- \rightarrow [\text{FeF}_6]^{3-} + 3\text{NCS}^-\]
- With zirconium alizarine complex free alizarine is released
- Ftoruracil and ftorafur discolor bromine water.
  \[
  \begin{align*}
  \text{O} & \quad \text{O} \\
  \text{O} & \quad \text{H} \\
  \text{H} & \quad \text{H} \\
  \text{F} & \quad \text{Br}_2 & \quad \text{HBr} \\
  \end{align*}
  \]

- Ftoruracil with potassium permanganate solution in basic environment is oxidized and forms green colored compound.
Due to acidic properties uracil derivatives from salts:
- With AgNO₃ and HgCl₂ solutions ftoruracil and ftorafur can form white precipitate.
- With the addition of cobalt salts, violet sediment is formed.

Ftorafur is heated with NaOH 30% solution in Zn powder presence, in the result ammonia is released. The basic hydrolysis is carried out. Na-hypochlorite is added in a reaction mixture, which interacts with released NH₃ and forms a mono-chloramin. After phenol is added in a mixture (pH=11) and indophenol (blue) is formed.

\[
\text{NH}_3 + \text{NaOCl} \rightarrow \text{NH}_2\text{Cl} + \text{NaOH}
\]

Uracil derivatives can be detected by conc. H₂SO₄ and can give absorptions in UV region.

Ftoruracil and tegafur (ftorafur) can interact with hydroxylamine in pH-8 by forming a colored product.

**Quantitative detection**

- Photo-colorimetric method, spectrophotometric method.
- 0,1M AgNO₃ solution is added in ftoruracil water solution. The released nitric acid is titrated by 0,1M NaOH (indicator is phenol red) (indirect neutralization- alkalimetry).

\[
\text{HNO}_3 + \text{NaOH} = \text{NaNO}_3 + \text{H}_2\text{O}
\]

Acid-base titration in non aqueous media (dimethylformamide). Indicator is thymol blue. Titrant is 0,1M Na-methylate solution.

Bromatometry is used for Tegafur (ftorafur) detection. Bromine surplus is detected by iodometry.

\[
\text{KBrO}_3 + 5\text{KBr} + 6\text{HCl} \rightarrow 6\text{KCl} + 3\text{H}_2\text{O} + 3\text{Br}_2
\]

\[
\text{2KI} + \text{Br}_2 \rightarrow 2\text{KBr} + \text{I}_2
\]

\[
\text{I}_2 + 2\text{Na}_2\text{S}_2\text{O}_3 \rightarrow \text{NaI} + 2\text{Na}_2\text{S}_4\text{O}_6
\]
Ftoruracil and ftorafur should be kept in dry, safe places protected from light and in well-closed vials. Products must be kept in 15-25° C temperature. Ftoruracil is a toxic substance. Ftoruracil and ftorafur possesses anti cancer activity (are cytostatics). It can be used in stomach and gastrointestinal tract malignant tumors.

Dosage forms: Ftoruracil 5 ml 5% ampules, tegafur 10 ml 4% solution in Na-salt form. Ftorafur in comparison with ftoruracil is less toxic. These products are contraindicated in kidney and liver function disorder.

---

**Methyluracil**

Methyluracil 64-65% is obtained from diketen and urea in pyridine media.

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{C} \quad \text{NH}_2 \\
\text{O} & \quad + \quad \text{H}_2\text{C} & \quad \text{O} \\
& \quad \text{P} \quad \text{y} \quad \text{t}\degree \\
& \quad \text{H}_3\text{C} \quad \text{N} \quad \text{H} \quad \text{C} \quad \text{NH} \quad \text{O} \\
& \quad \text{O} \quad \text{O}
\end{align*}
\]

It is a white crystalline odorless powder. It is less soluble in water and alcohol. It is practically insoluble in ether and chloroform. Water solution pH =7. It undergoes sterilization in 100°c in 30 minutes.

**Identity**

- Methyluracil alcohol solution is heated and cooled. After cobalt nitrate alcohol solution and ammonia solution are added. A violet color is appeared.
- Methyluracil discolors bromine water.
- Methyluracil with p-nitrodiazobenzene forms reddish-orange sediment.

**Quantitative analysis**

Methyluracil quantitatively is detected through acid-base titration in non aqueous media (in dimethylformamide). Titrant is 0,1M sodium hydroxide solution in methanol and benzene mixture. Indicator is thymol blue in dimethylformamide media.
It is used in wounds, burnings and breakings. It is a leucopoiesis stimulator. It is produced in the form of tablets (0.5 g).

Zidovudine (Azidotimidine, Zidovudine)

This product belongs to the nucleozides. It was synthesized in 1964. Zidovudine is dissolved in ethanol and methanol. It is less soluble in chloroform, water and ether. It is a white or yellowish, odorless crystalline powder. T.m.=120-123°C. Specify rotation (+58)-(+61°) in 1% methanol solution. During synthesis, mixtures are obtained, which are similar by their structure.

Identity

- IR–spectroscopy and UV spectroscopy. NMR-spectroscopy, GLCh.

Quantitative analysis

- Chromatography

The product has been used in systemic viral infections treatment, but in 1985 for HIV. Action mechanism is conditioned by inhibition of transcriptase activity. The product completely does not treat. It is produced in the form of capsules (0.1 and 0.25 g).

Stavudine is zidovudine’s structural analogue. It is also used in HIV treatment. It is produced in the form of capsules (0.03-0.04 g).

Lamivudin (Lamivudinium)
By its structure, properties, analysis and pharmacological properties they are similar products. There is available a sulfur atom in the 4th position and instead of oxy group is amino. It is produced in combination with zidovudine 0.25 g. It is produced in the form of tablets.

**Purine synthetic derivatives**

Mercaptopurine, azathioprine and inozine (riboxine) are used in the medicine as anti-metabolites from purine derivatives.

---

**Mercaptopurine**

![Mercaptopurine Monohydrate](image)

6-mercaptopurine monohydrate

**Synthesis**

Mercaptopurine- synthesis precursors are hypoxantine and phosphorous pentasulfide in non aqueous pyridine media.

![Synthesis Reaction](image)

It is a yellow crystalline powder. Tm.=312-314°C. It is practically insoluble in water and other organic solvents. It is dissolved in warm water and easily is dissolved in alkali solutions.

**Identity**

- It forms a yellowish-green color with Na nitroprusside in the basic media; after acidification it is turned into dark green.
- Mercaptopurine forms yellowish-orange precipitate with copper chloride CuCl₂ and hydroxylamine hydrochloride NH₂OHxHCl in the basic media (hydroxamate formation reaction).
- With concentrated nitric acid HNO₃ a yellow color is formed.
In a concentrated sulfuric acid, ethanol and sucrose presence, a brawn color is formed.
With a lead acetate alcoholic solution a yellow sediment is formed.
Due to tertiary nitrogen interacts with general alkaloid precipitating reagents.
UV-spectroscopy.

**Quantitatively**
- Iodometric method. After mineralization with 30% NaOH solution sulfide ion is formed, which is titrated by iodine solution.
- Reverse argentometric method (Volhard method). 0,1M AgNO₃ solution is added by addition quantity, which is titrated by NH₄SCN, indicator is FeNH₄(SO₄)₂.

\[
\begin{align*}
\text{AgNO}_3 + \text{NH}_4\text{NCS} & \rightarrow \text{AgNCS} \downarrow + \text{NH}_4\text{NO}_3 \\
3\text{NH}_4\text{NCS} + \text{FeNH}_4(\text{SO}_4)_2 & \rightarrow \text{Fe(NCS)}_3 + 2(\text{NH}_4)_2\text{SO}_4
\end{align*}
\]

- Reverse mercurimetric method. The addition quantity of mercury nitrate is titrated by by NH₄SCN, indicator is FeNH₄(SO₄)₂.
- Acid-base titration in non aqueous media (in dimethylformamide). Titrant is 0,1M sodium hydroxide solution in methanol and benzene mixture. Indicator is thymol blue in dimethylformamide media.

It is produced in the form of tablets (0,05 g). Mercaptopurine is an anti-leukemic agent.

**Azathioprin (Azathioprine)**

It is a bright yellow, greenish crystalline powder. It is practically insoluble in water, alcohol and chloroform. It is easily soluble in alkali solutions.
Identity

- IR-spectroscopy, spectrophotometry
- Nitro group detection: hydration is carried out (Zn+HCl). After 5 minutes it becomes a yellow filtrate. Adding urea di-azotation reaction and azo-conjugate is realized. That is why NaNO₂+HCl is added, after which in basic media □-naphthol is added to form a pink sediment.

\[
\begin{align*}
\text{Zn} + \text{HCl} & \rightarrow \text{ZnCl}_2 + \text{H}_2 \\
\text{R-NO}_2 + 3\text{H}_2 & \rightarrow \text{R-NH}_2 + 2\text{H}_2\text{O} \\
\text{R-NH}_2 + \text{NaNO}_2 + 2\text{HCl} & \rightarrow [\text{R-N} \equiv \text{N}]\text{Cl} + \text{NaCl} + 2\text{H}_2\text{O}
\end{align*}
\]

Azathioprin quantitatively can be detected by

- Acid-base titration in non aqueous media. Media is a dimethyl formamide, in which azathioprine acidic properties is increased. Titrant is a tributylammonium hydroxide. Equivalency point is detected by potentiometry.
- Spectrophotometry.

It is kept in dry and protected place from light. Azathioprine is an immune-depressant. It is used in organs transplantation, for suppressing the organs incompatibility. It is produced in tablets (0,05 g):

Riboxin, inosin (Riboxinum, Inosine)

It is odorless, bitter in taste, white or yellowish crystalline powder. It slowly is dissolved in water, less in alcohol. Inosine is a natural hypoxanthene ribofuranosil derivative. It is a real metabolite for human organisms and as anabolic agent, it can increase myocardial energetic balance and is used in cardiology.

Synthesis:
- Inosine is obtained by biosynthesis (microbes Bacillus subtilis), after which through physicochemical methods, it is released from basic solution.

Identity
- Ribose residue is detected, in the inosine molecule, by FeCl₃ in the presence of conc. HCl and 10% orcin alcohol solutions. Mixture is heated for 20 minutes on an aqueous bath. A green color is formed.
- IR spectroscopy.
Quantitative analysis is carried out by spectrophotometric method ($\lambda=249$ nm).

Inozine is kept in room temperature, protected from light. Inosine is used for cardiovascular diseases (heart stroke, ischemia, arrhythmia and etc.). Produced in the form of tablets (0,2 g).

**Pyrazolopyrimidine derivative**

Pyrazolopyrimidine is a heterocyclic system, contains pyrimidine, pyrazol, due to their condensation 4H-pyrazolo-(3,4-d) pyrimidine is formed. Allopurinol is it’s derivative.

**Allopurinol**

![Allopurinol](image)

It is a white or grey crystalline powder. It is less soluble in water, alcohol, practically insoluble in both chloroform and ether. It easily is dissolved in an alkali solution.

**Identity**
- IR spectroscopy, spectrophotometry.
- It forms yellow sediment with Nessler reagent in alkali media ($t^\circ$).

**Quantity detection**
- Acid-base titration is carried out in the non aqueous media. Media is di-methylformamide solution. Titrant is a 0,1M sodium hydroxide solution with the methanol and benzol mixture. Indicator is a thimol-blue. The equivalency point is detected through potencometric method.

![NMR structure](image)

Dosage form is tablets (0,1g). It is used in hyper-uricemia treatment and prevention. It is contraindicated in kidney functional disorders and urea accumulation.

**Control tests**

1. Ftoruracil identity is detected by
1. diazonium salt solution
2. KMnO₄, OH⁻ solution
3. AgNO₃ solution
4. NH₂OH, OH⁻ solution

a/ 1.2.3  b/ 1.2.3.4  c/ 3.4  d/ 1.4

2. Choose the reagents by which are not used for mercaptopurine identity detection
   a) Na-³ nitropruside, OH⁻
   b) HNO₃/nitric acid/
   c) alkaloid precipitating reagents
   d) bromine water

3. Choose the quantity detection method for mercaptopurine
   1. Volhard
   2. reverse mercurimetry
   3. acid-base titration in dimethyl formamide
   4. acid-base titration in glacial acetic acid

   a/ 1.2  b/ 1.2.3  c/ 3.4  d/ 1.4

4. After mineralisation of mercaptopurine with 30% NaOH sulfur atom is converted into
   a) sulfide ion
   b) sulfite ion
   c) sulfate ion
   d) thiosulfite

5. Choose the methods and reagents for alopurinol identification
   1. IR-spectrophotometry
   2. photocolorimetry
   3. Nesler reagent
   4. alkaloid precipitating reagents

   a/ 1.2.4  b/ 1.2.3  c/ 3.4  d/ 1.2.3.4
Adrenal gland hormones and their semi-synthetic derivatives

Pregnan, androstan, estran derivatives are adrenal cortex layer hormones.

![Chemical structures of pregnan, androstan, and estran](image)

They are similar to each other according their chemical structure. Androstan differs from pregnan by ethyl substitute in its molecule and estran differs due to its aromatic ring, and hasn’t one methyl group. These hormones are derivatives of cyclopentanperhydrofenantrene.

![R-radical and hydrogen atoms](image)

R-radical and hydrogen atoms in 8, 9, 14 positions can be either cys or trans.

Hormones regulate the most important functions of the organism: metabolism, growth, gender development.

The **first class** of the hormones, containing 1 or 2 phenol hydroxyl and amino acidic or amino alcoholic groups. These are hormones or their semi-synthetic derivatives are produced from both the thyroid gland and the adrenal nuclear layer.

The steroid hormones of the **second class**, as cyclo-penthan-per-hydro-phenantren derivatives (hestagens, androgens, estrogens with their semi-synthetic derivatives) are produced from the adrenal cortex layer.

Insulin belongs to the **3rd class**, which is produced from the pancreatic gland of the humans and animals and due to their chemical structure it has a middle place between the peptides and proteins.

Corticosteroids are classified into mineralo-corticosteroids, which regulate saline homeostasis in the organism, practically not acting on the protein and carbohydrate metabolism and gluco-corticosteroids, which in contrary improve protein and carbohydrate metabolism.

Corticosteroids (deoxycorticosteron, cortisone, hydrocortisone, prednisone, prednizolon), hestagen hormones (yellow body hormones: progesterone, semi synthetic pregnin, nor-ethisteron) chemical structure is based on pregnan system.
For all corticosteroids it is typical
- Double bond in the 4th position
- Carbonyl in the 3rd position
- Hydroxyl or ketone group in the 11th position
- Hydroxyl groups presence in the 21 position
- Ketonic group in the 17th position

Raw substances for corticosteroids are animal (cattle) gland or steroidal natural substances (cholesterol). Cortisone was released from natural source in 1936, but complete synthesis was carried out by Woodwort in 1952, which has 30 stages process. Corticosteroids biological synthesis is carried out by enzymes in the organism. Corticosteroids are odorless crystalline powders that have white or weak yellow color. They are particularly insoluble in the water or hardly soluble in the alcohol. Prednizolone is dissolved in the alcohol. In 1956 cortisone was obtained from the solasodine in the industry. Solasodine is glucoalkaloid aglycon, which is obtained from plant source. During synthesis reaction pregnenolon is firstly obtained, and then progesterone.

Hormones molecule chemical structure consists of steroid system, hydroxyl, ketone groups, double bond, □-ketone group.

Identity

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The hormones containing \(-\)-ketol group have a strong reductive properties, due to which molecule is oxidized:

a) With Fehling reagent (weak oxidizer), \(-\)-ketolic group is oxidized up to carboxyl group and Cu\(_2\)O red sediment is formed.

\[
\begin{align*}
\text{CH}_2\text{OH} + 4\text{H}_2\text{O} + 4\text{COOK} &\rightarrow \text{CH}_\text{OH} + \text{COOK} + \text{H}_\text{COOH} + 2\text{Cu}_\text{2O} \\
\end{align*}
\]

b) Alcohol solution of prednizolon, hydrocortisone, cortisone acetate with silver nitrate ammonia solution (weak oxidizer) in heating condition black sediment is formed.

\[
\begin{align*}
\text{CH}_\text{OH} + 2\text{Ag(NH)}_\text{2}\text{NO}_3 + 2\text{H}_\text{2O} &\rightarrow 2\text{Ag} + \text{ONH}_4 + \text{HCOON}_4 + 2\text{NH}_\text{2NO}_3 \\
\end{align*}
\]

c) Interaction with strong oxidizers (KIO\(_4\), HClO\(_4\)) leads to a formation of formaldehyde, which is detected by chromo-tropic acid or salicylic acid in the presence of conc. sulfuric acid media.

d) With 0,5% tri-phenyl tetrazol chloride alcoholic solution in the presence of tetramethylammonium hydroxide a red colored farmazan is formed.

\[
\begin{align*}
\text{CH}_2\text{OH} + \left[\text{C}_\text{6H}_\text{5}\text{-N}\text{-N\text{-C}_\text{6H}_\text{5}}\right]^- &\rightarrow \text{COOH} + \text{C}_\text{6H}_\text{5}\text{-NH}\text{-N\text{-C}_\text{6H}_\text{5}} + \text{HCl} \\
\end{align*}
\]

For all steroid system containing hormones is typical the reaction with concentrated sulfuric acid and due to this interaction the colored compounds are formed, which can give fluorescence in UV-region. From corticosteroids dexametazon forms a red color, with greenish-brown fluorescence, hydrocortisone forms a yellow color, prednizolon forms a green color, which then turns into red, and cortisone acetate forms an orange color.

All hormones, except estrogens, in 3\(^{rd}\) position have a ketone group, due to which they can interact with hydroxylamine, hydrazine, phenylhydrazine, semicarbazide, and form several colored compounds. For example cortisone acetate interacting with phenyl hydrazine leads to a formation of phenyl hydrazon-cortizon acetate.
Hydroxamate formation reaction is used for esters detection. For example, cortisone acetate forms a grey cherry color and deoxycorticosterone acetate forms a reddish-violet color.

Acetates can be detected by basic hydrolysis (KOH) in the alcoholic solution. Afterwards, adding conc. sulfuric acid and ethanol in the mixture acetic acid ethyl ester smell is felt. This identification reaction is used for hydrocortisone acetate detection.

HPLC or thin-layer Ch also are used for corticosteroids (prednizolon, cortisone acetate, deoxycorteron acetate) detection; those results are compared with samples.

For quantity (also identity) detection are used

- Photocolorimetry.
- Spectrophotometry in the UV region, based on the colored and UV region fluorescence products in all below maintained reactions.

Deoxycorteron acetate oily solutions 0.005-0.01g is used for medicinal purpose.

Inserting fluorine atom in 9th or 6th and 9th positions corticosteroids semi-synthetic derivatives are obtained for increasing corticosteroids action. There are dexametazone, triamcinol, flumetazon pivalate, flucinolon acetonide.
Fluorine atom insertion into the molecule structure is carried out by fluorine-hydrogen and double bond formation in 1-2 positions by microbiological method. Spectrophotometry is used in UV region, photocolorimetry, HPLC are also used for the mentioned compounds identity and quantitative analysis.

Fluorine atom is detected by iron (III) thio-cyanate after preliminary mineralosation of the molecule through heating, in the fluorine derivatives.

\[ \text{Fe(SCN)}_3 + 6F^- \rightarrow [\text{FeF}_6]^{3-} + 3\text{SCN}^- \]

Flourine ion is detected also by calcium chloride and zirconium alizarine complex.

Fluorine derivatives possess more active pharmacological effect, than prednizolone. They also have anti-inflammatory and ant-allergic effect. Dexametazone 35 times is more active than cortisone. Dexametazone is prescribed in 0,002-0,003 g, and triamcinol in 0,01-0,02 g.

Flumetazone pivalate and fluocinolon acetonide are prescribed in 0,02-0,025 % ointment and emulsion form.

**Control tests**
1. Cortisone by industrial method is synthesised from
   a) cholesterine
   b) solasodine
   c) androstane
   d) progesterone

2. Due to ketol group, hormones /prednizolon, cortisone acetat/ are oxidized with
   1. Fehling reagent (t°)
   2. Ag[NH$_3$]$_2$NO$_3$ (t°) solution
   3. phenylhydrazine solution
   4. NH$_4$OH solution
   a/1.3 b/ 2.4 c/ 1.4 d/ 1.2

3. Cortison acetate is detected by
   1. phenylhydrazine solution
   2. NH$_2$OH, OH, FeCl$_3$ solutions
   3. conc. H$_2$SO$_4$
   4. salycilic acid solution
   a/ 1.2.3 b/ 1.2 c/2.3 d/ 1.4

4. After mineralization of corticosteroids fluorine derivetives fluorine aion is detected by
   a) BaCl$_2$ solution
   b) iron (III) thyocianate
   c) NaOH solution
   d) FeCl$_3$ solution

5. Choose the methods for corticosteroids fluorine derivetives identification
   1. spectrophotometry
   2. photocolorimetry
   3. HPLC
   4. iodometry
   a/ 1.2 b/ 2.3 c/ 3.4 d/ 1.2.3

Gestagen and their semi-synthetic derivatives
Gestagens are pregnane derivatives—yellow body hormones. Progesterone and its semi-synthetic derivatives Norethisterone and Medroxyprogesterone acetate are used in the medicine.

Gestagenes are used in ovary dysfunction.

Progesterone is extracted from pigs gland or synthesized from solasodine by semi-synthetic way (in cortisone synthesis). Progesterone is synthesized from cholesterol, diosgenin and 17-ketosteroid by industrial method. Pregnenolone is intermediate substance during synthesis, which is dehydrated by microbiological method and forms progesterone.

\[
\text{Cholesterol} \xrightarrow{\text{dehydration}} \text{pregnenolone} \xrightarrow{\text{dehydration}} \text{progesterone}
\]

The properties of gestagen hormones and their semi-synthetic derivatives

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Chemical structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td></td>
<td>White or weak yellow crystalline powder. M.t. 127-134 °C. Specify rotator 186°-196° (0,5% solution in ethanol)</td>
</tr>
<tr>
<td>Norethisterone</td>
<td></td>
<td>White or yellowish crystalline powder. M.T. 201-206 °C. Specify rotator ` 23°-27° (1% solution in chloroform)</td>
</tr>
<tr>
<td>Medroxyprogesterone Acetate</td>
<td></td>
<td>White, odorless crystalline powder. M.T. 204 °C. Specify rotator ` +45°- +51° (1% solution in dioxan)</td>
</tr>
</tbody>
</table>

Semi-synthetic preparation Norethysteron (norcolate) is a 19-nortestosterone derivative, in which molecule an ethylene radical is present. Medroxyprogesten is 6-methyl-17-hydroxyprogesterone acetate-acetylic derivative.
Physical properties: Progesterone, norethylsteron and medroxyprogesteron acetate are white or yellowish crystalline substances. They are practically insoluble in the water. Progesterone is dissolved in the ethanol, but norethysterion is very less soluble. They both are soluble in the chloroform.

Identity

- Progesterone is detected by colored reactions. Steroid cycle presence is detected by interacting with concentrated sulfuric acid. Yellow color is formed with green fluorescence. The two layers are discolored through the chloroform addition.
- Progesterone alcohol solution forms a red color interacting with m-dinitrobenzene in the basic media.
- Norethysterol is detected by oxime formation with hydroxylamine (due to steroid cycle ketone group in 3rd position).

\[
\text{Progesterone} + \text{NH}_2\text{OH} \rightarrow \text{Norethysterol} + \text{H}_2\text{O}
\]

In the result of interaction a crystalline substance is obtained, and melting temperature can be detected.
- Progesterone forms a-crystalline compound with the 2,4-dinitrophenylhydrazine, which is detected by melting temperature. At the same time, the quantity is detected by gravimetric method.

\[
\text{Progesterone} + \text{DNP} \rightarrow \text{2,4-dinitrophenylhydrazine}
\]

- The ester formation with acetic acid anhydride is conditioned by the presence of alcohol hydroxyl group in Norethysteron molecule (is determined by its melting temperature (158-163° C).

\[
\text{Progesterone} + (\text{CH}_3\text{CO})_2\text{O} \rightarrow \text{Acetylation}
\]

- In 3rd position carbonyl is combined with double bond in 4-5 positions and due to that the light absorption is appeared in UV-region (\(\lambda=241\text{nm}\)).

Quantitatively norethysterion is detected by spectrophotometry in UV region (263 nm), in the tablet forms.
- Preparations identity, quantity and foreign steroids presence are detected by HPLC.
- Progesterone quantitatively is detected by spectrophotometry;
Norethysteron quantitatively is also detected by indirect neutralization method. With silver nitrate hydrogen atom is substituted by silver cation in the ethylene radical and silver acetylenide and nitric acid are formed. The released nitric acid is titrated by NaOH.

The preparations must be kept in well-closed vials, safe from light and not high than 20-25°C. They are produced in injection 1% or 25% oily solution forms. Norethysteron is produced in “Norcolute” tablets form, which contains 0,005 g norethystirol. Medroxyprogesteron acetate possesses gestagenic and antitumor properties.

Medroxyprogesteron is produced in the form of tablettes (0,1- 0,4 g), injection solutions in the ampules (150 mg/ml), in the suspension form 3,3 ml (0,5 or 0,15 g/ml).

Estrogen hormones and their semi-synthetic derivatives

Estrogens are the natural follicle hormones, which are produced in the women ovaries in the period of gender development. They include estrane system in their structure.
It is known 3 natural estrogen hormones: Estron (3-oxy-17-oxoestran), estradiol (3,17-dioxyestran), estriol (3,16-,16-trioxyestran).

Estradiol is 2 times more active than estran, but it easily destructed and it has short action duration.

Estrogen hormones in the ether form present in the woman and female animal’s urine (10-25mg/l), which is the raw material for obtaining these hormones.

The urine is hydrolyzed by acid and free estrogens are extracted from the mixture by any organic solution.

The hormones become phenolates due to phenolic hydroxyl group by the help of alkali, the latter because of well solubility in the water can be cleaned from organic foreign mixtures by the help of organic solvents (extraction).

Estrone and Estriol except estradiol dipropionate and ethylene-estradiol are also used in the medicine.

Estrane is the precursor of the estradiol and estradiol dipropionate. It is hydrated (17-keto-group) up to estradiol and 3,17°-oxi-groups are acetylated.

<table>
<thead>
<tr>
<th>Estradiol derivative properties</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preparation</strong></td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Description</th>
<th>Melting Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethinylestradiol</td>
<td>17β-ethinylestratrien-1,3,5(10)-dien-3,17β</td>
<td>White crystalline. M.T.181-186°C. Specify rotator -27°—31° (0.4% solution in pyridine)</td>
<td></td>
</tr>
<tr>
<td>Mestranol</td>
<td>17β-ethinylestratrien-1,3,5(10)-dien-3,17β-3 methyl ether</td>
<td>White crystalline powder. M.T.149-154°C. Specify rotator +2°—8° (2% solution in chloroform)</td>
<td></td>
</tr>
<tr>
<td>Estradiol Dipropionate</td>
<td>estratrien -1,3,5(10)-dien-3,17β dipropionate</td>
<td>White, odorless crystalline powder. M.t.104-108°C. Specify rotator +37°—41° (1% solution in dioxane)</td>
<td></td>
</tr>
</tbody>
</table>

Ethynyl estradiol is obtained from estron by interacting with acetylene:

Estradiol derivatives are white, light yellowish crystalline compounds. They are practically insoluble in the water, easily soluble in both chloroform and ethanol. They are differed from each other by specify index; there are 4 asymmetric carbon atoms in their molecule.

**Identity**

- 0.005% alcohol solution of ethynyl estradiol can be detected by absorption specify index in 280 nm region, by spectrophotometric method.
- Ethynyl estradiol ethynyl group is detected by silver nitrate ammonia solution. Silver acetylenid is formed (the melting temperature is detected).
- Ethynyl estradiol can be detected through interaction with diazonium salts (sulfanylic acid, sodium nitrite and hydrochloride mixture) in the basic media: bis-diazo compound is formed (dark red).
This reaction is used in tablet forms for quantity detection by photocolorimetry.

- Ethinyl estradiol is detected by spectrocolorimetry in ethanol and sodium hydroxide mixture in \( \lambda = 220-330 \text{ nm} \) region.
- Ethinyl estradiol heating with benzoil chloride forms a benzoate (M.T. 199-202\(^\circ\)C) due to phenolic hydroxyl group.

There is phenol hydroxyl group in the estrogen structure in contrast to others, due to which they are detected by bromine water discoloring, nitration, iron (III) chloride solution, diazotation reaction.

- Estradiol diazopropionate basic hydrolysis product after acidification and clearing from the mixtures is melted in 173-179\(^\circ\)C (estradiol).
- Estradiol dipropionate is also detected by UV-spectroscopy in 0,01 % ethanol solution \( \lambda = 269, 276 \text{ nm} \).
- Mestranol specify index is detected in 0,005 % ethanol solution \( \lambda = 279 \text{ nm} \).
- Estrogens with concentrated sulfuric acid can form colored or fluorescenting compounds in. Ethinyl estradiol forms reddish-orange color with yellowish-green fluorescence (after adding water the color is changed into violet and violet precipitat is formed).
- Mestranol forms red color with red fluorescence.
- Estradiol dipropionate with concentrated sulfuric acid is hydrolyzed by forming estradiol and propionic acid. Afterwards heating it in the presence of ethanol propionic acid ethyl ester is formed, which has a specific smell’
Estradiol dipropionate, mestranol, ethinyl estradiol are identified by IR-spectrophotometry in 4000-200 sm⁻¹ region.

Estrogens can also be detected by HPLC comparing results with standard.

HPLC or thin-layer chromatographies are used for detection of foreign steroids by comparing results with the standard.

**Quantity detection**

Photocolorimetry, spectrophotometry in UV region are used for quantity detection, which are based on colored and product fluorescing in UV region.

Estradiol dipropionate is hydrolyzed by potassium hydroxide alcoholic solution (0.1N) and surplus quantity of KOH is titrated by hydrochloric acid: acidometry (neutralization method).

Ethinyl estradiol is kept in dry, well-closed orange glass vials.

Mestranol and estradiol dipropionate are kept in dry and safe from light conditions.

Ethinyl estradiol is produced in tablets (0.00001 and 0.00005 g). Mestranol is included in Infecundin active contraceptive tablets (mestranol 0.0001 g, noretinodrel 0.0025 g).

Ethinyl estradiol is contained in the following contraceptives; **marvelon, non-ovlon, ovidon**, which are used in the tablet form.

---

**Estrogen synthetic derivatives**

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Beside steroid system hydroxyl and keto group containing aromatic compounds also have estrogen activity, which are able to form hydrogen bonds with proteins in the organism, for example phenantrenen, diphenyl derivatives and others.

Synthetic estrogens advantages is their synthesis method convenient (have not complex chemical structure).

In medicine biphenyl ethane and stilbene derivatives are used.

![Diphenyl ethane](image1)

![stilbene](image2)

Hexestrol (sinestrol) is a diphenylethan derivative but stilben is a diethylstilbestrol derivative.

Synthetic estrogen molecules contain oxy-phenyl radicals, which are connected with the 6-membered carbon chain in the para position. Hexestrol is a hexenal derivative, and diethylstilbestrol is a hexenal-3 derivative.

These preparations are close to the natural hormones according their pharmacological effects. They are not destructed in the GIT, but are absorbed.

Sinestrol was synthesized by Magidsone at first time in 1937, and it is contained in the Anison oily ether. Anethol-bromide is formed by anethol and hydrobromide interaction. Then it interacts with the phenyl-magnesium-bromide and forms a hexestrole-dimethyl-ether, which is demethylized and we can get a mezo-isomer.
The properties of estrogen synthetic derivatives

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Chemical structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexestrol (sinestrol)</td>
<td>mezo-3,4-bis-(p-oxyphenyl)-hexan</td>
<td>White or, weak yellow, odorless crystalline powder.</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>trans-3,4-bis-(p-oxyphenyl)-hexen-3</td>
<td>White odorless crystalline powder.</td>
</tr>
</tbody>
</table>

The two preparations are white odorless crystalline powders (hexestrol can have yellow color). They are practically insoluble in the water, easily soluble in both ethanol and ether and less soluble in the chloroform.

Identity

- Formalin containing chloroform solution of Ssnestrol with concentrated sulfuric acid forms a cherry color (typical to phenols).
- Diethylstibestrol and its propionate in the same conditions turn into orange, which after dilution with the water is gradually discolored.
- Octestrol becomes bright yellow by interacting with concentrated sulfuric acid, but after diluting with the water it doesn’t discolored, thus the reason is not hydroxonium ion formation.
- Sinestrol, octestrol (phenol hydroxyl containing molecules) in glacial acetic acid discolor bromine water. Yellow sediment is released (tetrabromhexestrol).
- Hexestrol is identified by nitration reaction. With nitric acid in heating condition yellow color is formed.

- Diethylstilbestrol in the same conditions in the presence of liquid phenol forms an enamel-green color.
- Diethylstilbestrol is detected by iron (III) chloride solution (phenol groups).

The other drug solutions interacting with iron (III) chloride solution form a green color which is gradually turned into yellow color (the phenolic group is detected).
Estrogens are turned into appropriate ethers through the acetic acid anhydrate or benzoic acid chloride. The esters are crystalline powders and are differed from each other by their melting temperature.

- Diethylstilbestrol propionate after basic hydrolysis and working out by acid the obtained diethylstilbestrol is recognized by melting temperature (166-172°C).

- Esters are detected by hydroxamates formation reaction.

- Diethylstilbestrol after heating with acetic acid and vanillin, an hydrochloric acid is added and after cooling the chloramine solution must be also added. Afterwards a blue color is formed.

- Diethylstilbestrol solution becomes grey-yellow in color, due to interaction with glacial acetic acid and phosphoric acid on heating in aqueous bath. The color is disappeared in dilution by glacial acetic acid.

- Diethylstilbestrol is heated with acetic acid and vanillin, then HCl is added, after cooling the solution chloramine solution is added. Blue color is formed.

**Quantity detection**

- Strinestrol, octestrol and diethylstilbestrol in pyridine media by acetic acid anhydride surplus turn into the esters. Anhydride addition quantity is turned into acetic acid and is titrated by alkali solution.

- Diethylstilbestrol propionate is detected by the spent alkali solution quantity in hydrolysis reaction.

- Hexestrol quantity can be detected by bromatometry.

\[
\text{KBrO}_3 + 5\text{KBr} + 6\text{HCl} \rightarrow 3\text{Br}_2 + 6\text{KCl} + 3\text{H}_2\text{O}
\]

Released bromine interacts with hexestrol and forms a sediment in the form of tetrabromine derivative. Titrant surplus is detected by iodometry.
Photocolorimetric method is used based on colored product formation (bis-diazocompound) from the interaction of sulfanilic acid diazonium salt and hexestrol or diethylstilbestrol or octestrol.

The same reaction can be used in solutions by measuring optical density, for the same drugs quantity analysis (comparing with standard).

Preparations can be differed from each other due to different solubility in water. These preparations come near to the natural hormones due to their pharmacological effect. They don’t degrade in gastrointestinal system and rapidly are absorbed.

They are used in the treatment of some malignant tumors. They are kept in well-closed vials, from the light protected place. Sinestrol is produced in tablets form (1mg) and injected oily solutions form. 2% solution is used for the treatment of malignant diseases. 3% oily solution of diethylstilbestrol is used for the same purpose.

Control tests

1. Choose the synthesis precursor for both estradiol and estradiol propionate
   a) estran
   b) estriol
   c) estron
d) ropionic acid

2. Due to phenolic hydroxyl group ethynilestradiol is detected by
   1. diazonium salt solution
   2. conc. H₂SO₄
   3. FeCl₃ solution
   4. benzylchloride solution
   a/ 1.2   b/ 1.2.3.4   c/ 3.4   d/ 1.3.4

3. Phenolic hydroxyl group in ethynilestradiol is detected by
   1. FeCl₃ solution
   2. bromine water
   3. benzylchloride solution
   4. conc. H₂SO₄
   a/ 1.3   b/1.2.3   c/ 1.2.3.4   d/1.2

4. Choose the identity methods and reagents for estradiol dipropionate detection
   1. UV-spectrophotometry
   2. conc. H₂SO₄
   3. HPLC
   4. conc. H₂SO₄, ethanol, t⁰
   a/ 2.3   b/ 1.2.3.4   c/ 1.3   d/ 1.4

5. Estrogens quantity is detected by the following methods
   1. thin layer chromatography
   2. spectrocolorimetry
   3. mass-spectrophotometry
   4. HPLC
   a/ 1.2   b/ 1.2.3.4   c/ 2.3   d/ 2.3.4

Androgen hormones and their semi-synthetic derivatives

Androgens are produced in the male sexual glands (testiculs) during the period of sexual development. They are androstan derivatives
In 1931 androsteron was released from male urine by Butenant, then dehydroandrosteron. Afterwards, testosteron was released from the animal’s testiculs tissue, which 10 times excels androsteron by its physiological activity.

Testosterone synthesis can be realized from acylated dehydropregrenolon in industrial way, which is obtained from cholesterin (like cortizone), also from \( \square \)-sitosterine by microbial oxidation and side chain separation.

In 1936 has been discovered that testosterone action becomes more longer after its etherification. Due to this action, a testosterone propionate as a preparation was created, which is more active and more stable in keeping conditions in comparison with the all esters. It excels testosterone by its action duration.

Testosterone propionate is obtained from testosterone etherification with propionic anhydride in 110°-114°C conditions.

Methyltestosteron is a Testosteron semi-synthetic derivative, which can be synthesized from dehydroepiandrosteron and methyl-magnesium-iodide.
Testosterone and its semi-synthetic derivatives stimulate protein synthesis (anabolic), but show mostly androgen activity in the organism.

The methyl-androsterondiol, which is the intermediate product of methyltestosteron synthesis, excels androgen by anabolic properties, but methandrostenolon due to keeping of testosterone anabolic activity 100 times concedes the latter one by androgen properties.

Testosterone is used for the sexual immature, sexual system functional disorders, vessel, neuro disorders, and also for the malignant diseases.

Methandrostenolon and methylandrostendiol are prescribed in protein metabolic disorders, which is attend to serious traumas, coronary insufficient, ulcer diseases and myocardial stroke.

Androgen hormones mostly are odorless, white or weak yellow crystalline powders, which are used in medicine.

They are practically insoluble in the water, easily soluble in the ethanol, soluble in the chloroform and ether.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Chemical structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone Propionate</td>
<td><img src="image1" alt="Image" /></td>
<td>White crystalline powder.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M.T.118-123°C:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specify rotator +83° - +90° (1% ethanol solution):</td>
</tr>
<tr>
<td>Methyltestosterone</td>
<td><img src="image2" alt="Image" /></td>
<td>White or weak yellow crystalline powder, weak hydroscopic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M.T.162-168°C:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specify rotator +79° - +85° (1% ethanol solution):</td>
</tr>
<tr>
<td>Metandriole</td>
<td><img src="image3" alt="Image" /></td>
<td>White crystalline powder.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M.T.199-206°C:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specify rotator -70° - +77° (1% ethanol solution):</td>
</tr>
<tr>
<td>Metandienone (Methandrostenolon)</td>
<td><img src="image4" alt="Image" /></td>
<td>White crystalline powder.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M.T.13-170°C:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specify rotator 0 ±5° (1% ethanol solution) or -7 ±13°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2% ethanol solution):</td>
</tr>
</tbody>
</table>
Identity

➢ Preparations identity can be done by IR-spectroscopy.

➢ Androgens are detected by esters melting temperature, the latter were formed by acetic acid anhydride: methyl testosterone acetate (m.t.173-176°C), methandriol (m.t. 174 -180°C):

➢ Testosterone propionate and methyltestosteron have a keto group in the 3rd position and can form oxims with hydroxylamine, which melting temperature in the propionate case is equal to 166-170°C, but in methyltestosteron case it is equal to 210-216°C.

➢ Methandion is detected by methandienon 2,4-dinitrophenyl hydrazone formation (orange-red color) reaction by interacting with 2,4 dinitrophenylhydrazine.

➢ Testosterone propionate interacting with isoniaside forms a yellow isonicotinoil hydrazone. This reaction is used in drugs detection by spectrophotometry.

➢ Testosterone which is obtained in the result of testosterone propionate basic hydrolysis is melted in 150-156°C. Complex ether group is detected in this molecule by this method.

➢ The hydroxamate formation reaction with iron (III) chloride is used for testosterone propionate identity.
This reaction is distinctive for testosterone propionate from other drugs, which are not esters.

- Steroid compounds are detected by concentrated sulfuric acid. Methyltestosterone and metandriol can form a yellowish-orange color with green fluorescence but metandienone has a red color.
- UV-spectroscopy is used for androgen and anabolic drugs identity and quantitative analysis.
- NMR and mass-spectroscopy are used for these drugs detection.

**Quantitative detection method**

- Polarimetric method.
- HPLC method.

Androgen and anabolic steroid drugs must be kept in the well-closed vials and the place safe from light and humidity.

Testosterone propionate is used in 1% or 5% oily solutions form. Methyltestosterone is produced in (0.005 and 0.01g) tablets form. Methandienone is produced in 0.005 g tablets form. Methandriol is produced in 0.25 g tablets.

**Synthetic anabolic compounds**

**19-nortestosterone derivatives**

19-nortestosterone has anabolic properties. It is differed from testosterone by the absence of methyl radical in 19 position.

19-nortestosterone esters are Nandrolone phenylpropionate, which is called phenabolin and Nandrolone deconoate, which is called retabolyl.
### Nandrolone ethers properties

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Chemical structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nandrolone Phenylpropionate</strong></td>
<td>17α-áxy-19-nor-4-androsteron-3-on-17α-phenylpropionate (phenylpropionate 19-nortestosteron)</td>
<td>White crystalline powder:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M.T.95-99°C:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specify rotator</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+52° +58°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2%- chloroform solution):</td>
</tr>
<tr>
<td><strong>Nandrolone Decanoate (Retabolyl)</strong></td>
<td>19-nortestosteron17β-decanoate</td>
<td>White crystalline powder</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or colorless crystals.</td>
</tr>
</tbody>
</table>

Nandrolone Deconoate is synthesized from estradiol methyl ester by reducing the aromatic group, afterwards the hydrolysis must be done and the released product is oxidized up to keto group formation. The etherification is carried out by deconic acid chlorine anhydrate for preparation synthesis.

![Chemical structures](image)

Phenabolin and retabolil are the white crystalline powders that are practically insoluble in the water, poorly soluble in the alcohol and easily in the chloroform. Phenabolin is dissolved in the acetone, and retabolol in the ether.

**Identity and quantity detection**
5% Nandrolone phenyl-propionate is detected by IR-spectrophotometry, in the chloroform solution. Nandrolone phenyl-propionate quantitative analysis is realized by UV-spectrophotometry (\(\lambda = 240\ \text{nm}\)). Nandrolone decanate (retabolol) in the oily solution is detected by thin-layer chromatography. Retabolil quantitatively is detected by photocolorimetry. For this method retabolil with isonicotinic acid hydrazide forms yellow colored isonicotinoil hydrazone. It must be kept in the light protected place. In case of precipitate formation, the ampoules solution should be heated.

The two preparations have a strong and long anabolic effect. They are used in form of oils: methandienane in form of phenaboline (1% and 2.5% ampoules) and metandiole in form of retabolil (5% 1ml ampoules).

**Androstan synthetic acetoxi derivatives**

The structural changes in the androstan molecule, such as acetylation, the steroidal ring hydration, the chlorine atom insertion in the piperazine and into other substitutes brings pharmaceutical changes.

The pharmaceutical activity is lost or is decreased in its derivatives. For example androstane acetoxi derivatives can be transformed into anti-androgens and also can possess antitumor property, for example can also be muscular-relaxant.

These properties are explained, that in the pipecuronium molecule the distance between the quaternary nitro groups is the same, as it is in the natural d-tubocurarine molecule.

We will analyze two preparations synthetic ones; cyproterone acetate, which is called Androcur and bromide of pipecuronia, which is called Arduan.

These two preparations are white crystalline powders.
The properties of androstan acetoxy derivatives

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Chemical structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyproterone Acetate (Androcure)</td>
<td><img src="image" alt="Chemical structure of Cyproterone Acetate" /></td>
<td>White or almost white crystalline compound. M.T.~210°C; Specific rotator +152°-up to +157°:</td>
</tr>
<tr>
<td>Pipecuronium Bromide (Arduan)</td>
<td><img src="image" alt="Chemical structure of Pipecuronium Bromide" /></td>
<td>Crystalline powder white or almost white color.</td>
</tr>
</tbody>
</table>

Cyproterone acetate is practically insoluble in the water, less soluble in the ethanol, and is soluble in the methanol and acetone. Pipecuronium bromide is dissolved in the water and ethanol.

Identity and quantity detection

Cyproterone acetate identification:
- IR spectroscopy.
- The chlorine atom and acetyl substitute are detected in the cypreterone acetate after mineralization by potassium carbonate.
- It forms a red color by concentric sulfuric acid.
- It is detected by TLC in the tablets.

Cyproterone acetate quantitatively is detected by:
- UV spectroscopy (λ=282 mn)
- It is also detected by HPLC.

Pipecuronium bromide isidentity detection:
- With sodium tetraphenylborate solution in NaOH media white precipitate is formed.

The quantitative analysis is realized by photocoltimetry based on hydroxamate formation reaction.
These preparations are kept in the freezer compartment (+4°C). Cyproterone acetate is produced in tablet forms (0.05g).

This preparation has anti-androgen effect. Pimecromium bromide is used in the muscular weakness and in the surgery. It is produced in the form of lyophilized powder, ampoules (0.004g), and for infusion.

**Control tests**

1. Methyltestosterone is synthesised from
   1. dehydroepiandrosterone
   2. methylmagnesium iodide
   3. testosterone
   4. methyl iodide
   a/ 1.2  b/ 1.4  c/ 3.4  c/ 2.3  

2. Choose the reagents for testosterone propionate identity detection
   1. hydroxylamine
   2. isoniazine
   3. KOH solution
   4. NH₂OH, NaOH, FeCl₃, HCl solution
   a/ 1.2  b/ 2.3.4  c/ 1.3.4  d/ 1.2.3.4  

3. Choose the methods which are used for androgens detection
   1. UV-spectrophotometry
   2. nuclear magnetic resonance
   3. HPLC
   4. diazotation and azo-conjugation
   a/ 1.2  b/ 1.2.3  c/ 2.3.4  d/ 1.2.3.4  

4. Methyltestosterone is not identified by
   a) Acetic acid anhydride
   b) hydroxylamine
   c) conc. H₂SO₄
   d) NaNO₂, H⁺, β-naphthol, OH⁻

5. Ciproterone acetate /Androcure/ identity is detected by
   1. IR-spectrophotometry
   2. conc. H₂SO₄
3. TLC
4. AgNO₃ solution /after mineralisation/
   a/ 1.2    b/ 3.4    c/ 1.2.3.4    d/ 1.2.3