Volgograd
State Medical University of
the Ministry of Health of the Russian Federation

GENERAL PHARMACEUTICAL CHEMISTRY

GEISMAN A.N. CHEMICAL METHODS PHARMACOPOEIA ANALYSIS – AUTHENTICITY OF MEDICINES OF ORGANIC NATURE IDENTIFICATION OF ORGANOELEMENT DRUGS

Lesson 7
V semester

Volgograd, 2021

Discipline

GENERAL PHARMACEUTICAL CHEMISTRY

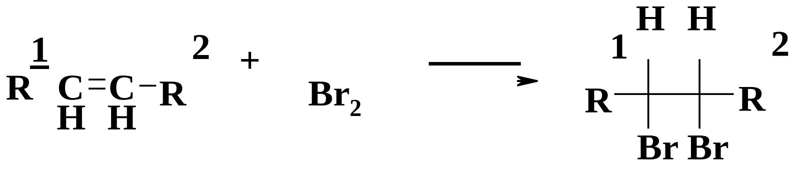
CHEMICAL METHODS OF PHARMACOPOEIAL ANALYSIS – AUTHENTICITY OF MEDICINES OF ORGANIC NATURE (IDENTIFICATION OF FUNCTIONAL GROUPS)

IDENTIFICATION OF ORGANOELEMENT DRUGS

Lesson № 7

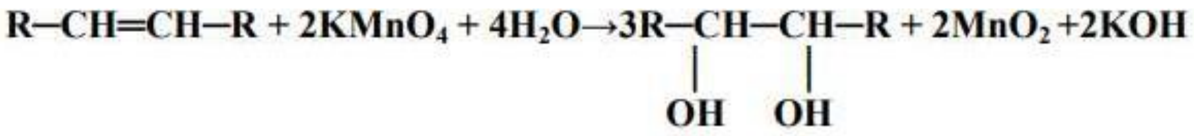
1.1 DETECTION OF MULTIPLE CONNECTIONS

❖ **The addition of bromine**. 1 ml of a solution of the test substance is placed in a dry test tube and a bromine solution is added drop by drop, with light shaking. The disappearance of the yellow color is observed:



$$R^{1}-\underset{H}{C}=\underset{H}{C}-R^{2} + Br_2 \longrightarrow R^{1}-\underset{Br}{\overset{H}{C}}-\underset{Br}{\overset{H}{C}}-R^{2}$$

❖ **Sample with potassium permanganate (Wagner reaction).** In a slightly alkaline environment, under the action of potassium permanganate, the substance is oxidized with the rupture of a multiple bond.



$$R{-}CH{=}CH{-}R + 2KMnO_4 + 4H_2O \rightarrow 3R{-}\underset{OH}{CH}{-}\underset{OH}{CH}{-}R + 2MnO_2 + 2KOH$$

2. IDENTIFICATION OF ORGANOELEMENT COMPOUNDS

Since the atoms of most organoelement compounds are covalently bound, preliminary mineralization is a necessary condition for testing their authenticity. In this case, the organic part of the molecule is partially or completely destroyed to carbon monoxide (IV) and water. Other elements form the corresponding ions. One of the ways to detect nitrogen, sulfur and halogens that are part of organic compounds is the **Lassen** sample.

Method: *20-50 mg of the substance is placed in a refractory glass tube and ground together with a piece of sodium the size of a pea. The liquid is previously removed from the surface of the sodium piece, and the edges are cut off so that the surface is shiny.*

The tube is heated initially with a weak flame, moving it from the top to the bottom of the tube (wearing protective glasses!). Sodium gradually melts and interacts with the substance. After the reaction, the sample is strongly calcined for several more minutes. The red-hot test tube is immersed in another test tube, into which 6 ml of water is pre-poured (be careful with protective glasses!). The outer tube should be fixed in a glass, while the test tube with the sample bursts, and there is an outbreak of hydrogen released as a result of the interaction of excess sodium with water. To completely separate the coal, the mixture is ground in a mortar and filtered. The filtrate is divided into two parts and used to detect sulfur and halogens.

2.1 SULFUR DETECTION

1. Detection in a solution obtained after decomposition by the Lassen method under the action of sodium nitroprusside.

A few drops of freshly prepared **sodium nitroprusside** solution are added to a part of the filtrate. A purple coloration appears, usually turning into a blood red:

$$Na_2S + Na_2[Fe(CN)_5NO] = Na_4[Fe(CN)_5NOS]$$

2. Detection in a solution obtained after decomposition by the Lassen method using lead acetate.

When heating a part of the filtrate with 2 ml of dilute hydrochloric acid, hydrogen sulfide is released, detectable by the blackening of paper impregnated with a solution of lead acetate.

$$\mathbf{Na_2S + 2\ HCl = 2\ NaCl + H_2S}$$

$$\mathbf{H_2S + Pb(CH_3COO)_2 = PbS + 2\ CH_3COOH}$$

3. Detection in the form of sulfate (oxidative mineralization). When treating organic substances with nitric acid, hydrogen peroxide or potassium permanganate, sulfur is oxidized to sulfate, which is most easily detected when barium chloride is added.

$$\mathbf{SO_4^{2-} + Ba^{2+} = BaSO_4}$$

In addition to the “wet” mineralization method, the so-called “dry” mineralization is used, which is carried out by heating a dry substance by fusing with a sintering mixture (**$Na_2CO_3 + KNO_3$**).

4. Detection of sulfur by direct interaction with sodium hydroxide.

It is most typical for compounds containing a thioester or thioketone group. The medicinal sulfur-containing organic substance is fused with sodium hydroxide or heated with a 10% solution of it. Covalently bound sulfur forms sulfide, which is proven by smell; by reaction with sodium nitroprusside or after acidification by darkening a filter paper soaked in lead acetate (see method 1).

2.2 DETECTION OF ORGANICALLY BOUND HALOGENS

Since halogen atoms are not ionogenically bound to the organic part of the molecule, in order to detect them, the substance must be mineralized, that is, to break the bond of the halogen with carbon and transfer the covalently bound halogen to an ionic state. The choice of the mineralization method is determined by the strength of the carbon-halogen bond (it falls from fluorine to iodine), as well as the structure of the halogen-containing LV (aliphatic, aromatic, heterocyclic series) and can be carried out in several ways:

1) Direct interaction with the reagent (iodoform with silver nitrate solution in nitric acid medium);

2) Hydrolytic cleavage with an aqueous (bromized) or alcoholic (chloroethyl) alkali solution (the method is used for halogen-containing aliphatic series LV);

3) Reducing mineralization with hydrogen at the time of separation (Zn + NaOH, Zn + CH_3COOH) or **metallic sodium** (for fluorine derivatives):

4) Oxidative mineralization:

a. combustion in a flask with oxygen (for all halogen-containing substances);
b. calcination with a sintering mixture (for all except fluorinated ones);
c. “dry” (heating without reagents);
d. ”wet” (heating with concentrated sulfuric acid);
e. Beilstein test;

❖ **Hydrolytic decomposition for LV containing halogen in the aliphatic chain (except fluorine)** is carried out by heating with an aqueous (bromized) or alcoholic (chloroethyl) alkali solution, the resulting halide ion is proved by conventional analytical reactions.

❖ **Recovery methods.** Reactions of reduction of halogen derivatives are usually reduced to the effect on the drug:

➢ atomic hydrogen, which is produced by the interaction of metallic sodium with anhydrous alcohol,

- powdered zinc with a solution of acetic or mineral acid or with a solution of caustic soda.

As a result of the reaction, sodium halide or hydrochloric acid is formed, which are further determined by the methods adopted for inorganic halides:

$$R{-}Hal + C_2H_5OH + 2\,Na \longrightarrow R{-}H + NaHal + C_2H_5ONa$$

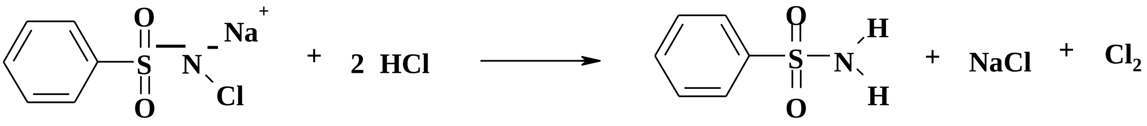
$$R{-}Hal + Zn + 2\,NaOH \longrightarrow R{-}H + NaHal + Na_2ZnO_2 + H_2O$$

$$R{-}Hal + Zn + 2\,CH_3COOH \longrightarrow R{-}H + HHal + Zn(CH_3COO)_2$$

In the analysis of chlorine derivatives, sodium chloride is formed, which is identified by the release of a white precipitate after acidification with nitric acid and the addition of a silver nitrate solution.

$$NaCl + AgNO_3 = AgCl + NaNO_3$$

During the reduction of bromine derivatives, they are detected by the reaction of halogen release (if sodium bromide is the release of bromine).



$$Cl_2 + 2\ NaBr = 2\ NaCl + Br_2$$

❖ **The sintering method** can be used in the presence of chlorine and sulfur in one compound, followed by the detection of formed chloride and sulfate ions. In this case, 0.1 g of the drug is mixed in a porcelain crucible with 0.3—0.5 g of the sintering mixture and calcined. After cooling, the contents of the crucible are dissolved in 5 ml of hot water and filtered. The filtrate is acidified with nitric acid and 0.5 ml of silver nitrate solution is added; a white precipitate, soluble in ammonia solution, falls out.

❖ **Iodine detection**. Iodine is detected either by heating the iodine derivative in a test tube on a burner flame, or by acting with concentrated sulfuric acid:

$$R-CH_2-I \xrightarrow[H_2SO_4\ \text{конц.}]{t} I_2$$

The release of purple iodine vapors or the purple coloration of chloroform extraction (after addition of chloroform to the mineralization) is observed.

Sintering with a mixture of potassium nitrate and sodium carbonate can also be applied:

$$R-CH_2-I \xrightarrow{KNO_3,\ Na_2CO_3,\ t} NaI$$

Then iodide ions are detected in one of the following ways:

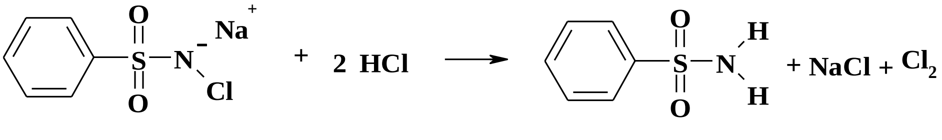
➢ Reaction with silver nitrate: a yellow curd precipitate of silver iodide, insoluble in ammonia and nitric acid, precipitates:

$$I^- + AgNO_3 \longrightarrow AgI\downarrow + NO_3^-$$

➢ Reaction with 0.1 M sodium nitrite solution. A brown precipitate with a reddish tinge (iodine) falls out.

$$NaI + NaNO_2 + HCl\ \ =\ \ NaCl + I_2 + NO\ + H_2O$$

➢ Reaction with chloramine B solution in an acidic environment:



$$Cl_2 + 2\,KI = 2\,KCl + I_2$$

The resulting iodine turns the chloroform layer purple.

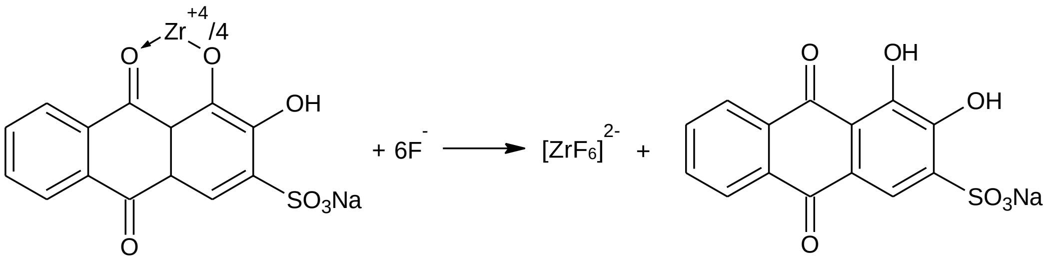
❖ **The Beilstein test.**

A small amount of the test substance is placed on a piece of copper oxide reinforced in a platinum wire, or on a rolled-up copper mesh and heated in a non-luminous flame zone of a gas burner. The staining of the flame in green indicates the presence of halogen. Pure green color is characteristic of iodine, bluish-green — chlorine or bromine. The copper oxide or copper mesh must be pre-calcined until the flame stops turning green. You can also use a copper wire bent into a loop at the end for the Bailyshtayia sample.

The Beilstein test does not always give the correct result. Some compounds containing nitrogen and sulfur at the same time, but not containing halogen, can also cause a similar flame color. Substances that form carbon monoxide when heated, such as formic acid, can also behave. In addition, a positive Beilstein test is given by urea.

❖ **Fluoride detection.**

Due to the high strength of the fluorine-carbon bond, mineralization is carried out by the action of a strong reducing agent, metallic sodium, when heated. The resulting fluoride ion is proved indirectly by the destruction of zirconium - alizarin dye due to the binding of Zr+4 into a more durable complex with fluoride, while the color of the solution changes from red-purple to yellow. The method is used to detect fluorine in halothane (fluorotane), fluorouracil, tegafur (fluorofur), fluoroquinolones, etc.



2.3 DETECTION OF ORGANICALLY BOUND PHOSPHORUS

❖ Phosphorus-containing compounds are mineralized with a mixture of concentrated sulfuric and nitric acids or a mixture for sintering to phosphate ions, which are detected by the reaction of formation of ammonium phospholybdate (yellow precipitate):

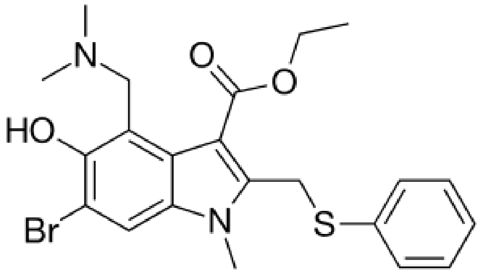
$$H_3PO_4 + 12(NH_4)_2MoO_4 + 21HNO_3 \rightarrow (NH_4)_3PO_4 \cdot 12MoO_3\downarrow + 21NH_4NO_3 + 12H_2O$$

CONTROL QUESTIONS ON THE TOPIC OF THE LESSON

1. Which functional groups are hydrolysis reactions used to identify?
2. Which functional groups can be detected by acylation reactions? What reagents are used in these methods?
3. What reactions prove the reducing properties of the aldehyde group?
4. What functional groups can be detected using a hydroxamine sample? How is this test performed?
5. Which compounds can be identified using the diazotization and nitrogen combination reaction? What reagents are used to carry out this reaction? Under what conditions is the reaction carried out?
6. Which functional groups can be detected by complexation reactions? Give examples of medicinal substances containing these PHG.
7. What methods of mineralization of organic compounds are known to you?
8. What explains the choice of mineralization method? What mineralization methods are used to determine phosphorus and sulfur?
9. What are the features of mineralization and detection of organically bound fluorine? Give examples of fluorinated organic medicinal substances.

SITUATIONAL TASK 1

The drug “Umifenovir” is used as an antiviral agent and has the following formula:



1. Name the functional groups included in the structure of the active substance.
2. Which heterocycle is the basis of the structure of umifenovir?
3. Suggest qualitative reactions that allow us to detect functional groups in the structure of umifenovir. Specify the reagents that will be required for this. Give the expected chemistry of the reactions.
4. How can a sulfur atom be detected in an aryl sulfide fragment?
5. How is the mineralization of organic compounds carried out to detect organically bound bromine? What rapid tests can be used to detect organically bound halogens?

TEST TASKS

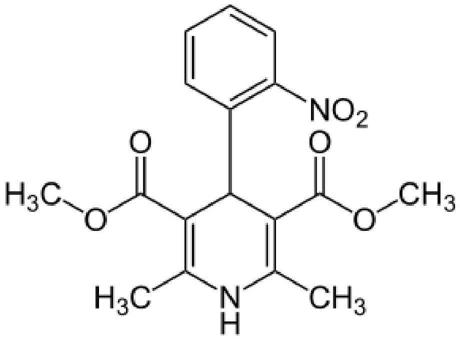
1. The Beilstein sample is not used for the analysis of compounds containing an atom

A. of A. Chlorine
B. B. Iodine
C. C. Fluorine
D. D. Bromine

2. The reaction of the formation of an azo dye (azo combination) refers to the reactions

A. Nucleophilic addition
B. Electrophilic substitution
C. Nucleophilic substitution
D. Radical substitution

3. Connection with the structural formula



It contains the following functional groups:

A. Aromatic amino group
B. Nitro group
C. Carbonyl group
D. Ester group

4. Oxidative mineralization includes:

A. Fusion with alkali
B. Combustion in a flask with oxygen
C. Fusion with metallic sodium
D. Heating with a sintering mixture

5. The following reagents are consistently used to conduct the hydroxamine test:

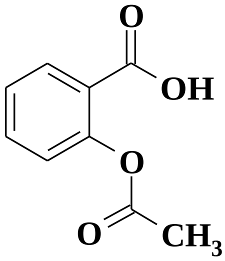
A. Hydroxylamine hydrochloric acid solution, hydrochloric acid solution, iron (III) chloride solution
B. Hydroxylamine alkaline solution, hydrochloric acid solution, iron (III) chloride solution

C. Hydroxylamine alkaline solution, iron (III) chloride
solution
D. Iron(III) chloride solution, hydroxylamine alkaline solution, hydrochloric acid solution

6. Reactions of formation of colored complexes with metal ions are characteristic of compounds containing:

A. The aldehyde group
B. The sulfamide group
C. The primary aromatic amino group
D. The hydrazide group

7. Products of acid hydrolysis of a compound having a structural formula



It can be detected by reactions:

A. “Silver mirror”
B. Complexation with iron ions
C. Formation of ethyl acetate
D. Hydroxamine sample

8. The reaction of formation of a complex compound of dark blue color with copper ions is characteristic for:

A. Glycerin
B. Ethanol
C. Aniline
D. Ethyl Acetate

9. Glucose is characterized by reactions with:

A. 2,4-Dinitrochlorobenzene
B. Phenylhydrazine
C. Fehling reagent
D. Picric acid

10. The benzo isonitrile sample is typical for compounds containing

A. secondary aliphatic amino
group
B. A primary aromatic amino
group
C. An aldehyde group
D. A tertiary aromatic amino group

11. After mineralization of iodine-containing compounds to iodide ions and addition of silver nitrate solution to the mineralization, the following precipitates:

A. A white precipitate, easily soluble in ammonia solution
B. Yellow precipitate, insoluble in nitric acid and ammonia solution
C. Yellowish precipitate, insoluble in nitric acid and slightly soluble in ammonia solution
B. Black precipitate, insoluble in mineral acids