1. SAFETY RULE AT WORKING ON LABORATORY OF ORGANIC CHEMISTRY

1.1 General Provisions

Chemistry wet laboratories contain certain inherent dangers and hazards. You must learn how to work safely with these hazards in order to prevent injury to yourself and others around you. Experiments should not be performed without an instructor in attendance and must not be left unattended while in progress. The following guidelines are to help you:

1. Make sure you are familiar with all the safety information given to you about each experiment before starting the experiment. This includes your manual, these safety guidelines, any posted information or any other information provided to by your TA.
2. Do not work alone. Always have someone present in the laboratory that knows what to do in the event of an emergency.
3. Avoid cluttering the lab bench or hood. Clutter can lead to spills, so return items promptly to their proper locations. Do not place personal items, suchas bags and backpacks, on the floor as they may cause others to trip. Promptly notify the laboratory instructor of any spills and clean them up as instructed. Spills must be cleaned up as soon as possible to prevent others from exposure to the spilled chemical. Inform the laboratory instructor of any conditions that seem unsafe.
4. Tie long hair back. Wear safety goggles and a lab coat. Even if you aren't clumsy, someone else in the lab probably is. If you take even a few chemistry courses you will probably see people set themselves on fire, spill acid on themselves, others, or notes, splash themselves in the eye, etc. Don't be the bad example to others, remembered for all time for something stupid!
5. Do not bring food, drinks, or cosmetics into the laboratory. Eating, drinking, or applying cosmetics in the laboratory can introduce toxic or corrosive chemicals into your system. You particularly want to avoid any contamination to your mouth or eyes. Not only should you not bring in food or drinks, but you shouldn't taste or smell chemicals or biological cultures already in the lab.
6. Know the location and use of all safety equipment. All laboratories will have an eyewash station, safety shower, fume hood, a fire extinguisher, and a carrier for the safe transport of chemicals. Also know the location of the nearest emergency alarm.
7. Be familiar with the experiment before beginning the lab. Pay particular attention to cautions given in the procedure and by the laboratory instructor. Learn about hazards associated with the chemicals you will be using by looking up and reading their Material Safety Data Sheet (MSDS).

1.2 Safety regulations during the work with acids and alkalis

1. It is necessary to store the concentrated acids and alkalis in an exhaust case in strong ware on the pallet.

2. All works with acids and alkalis need to be carried out in goggles.

3. Concentrated hydrochloric and nitric acids it is possible to pour only in an exhaust case. Dilution of acids should be carried out in heat-resistant ware, at the same time acid needs to be flowed to water in the small portions, when hashing (it is impossible to flow water to the concentrated acid as in this case a large amount of warmth, waters as less dense substance is distinguished, boils on the surface of acid, and liquid can be thrown out from a vessel).

4. At dissolution of hydroxides of sodium and potassium pieces of alkali can be taken only tweezers or the pallet, but not hands; dissolution of these substances should be carried out by small portions.

1.3 Safety measures during the work with flammable liquids (FL)

Classification of flammable liquids (FL) according to the degree of danger. Depending on the FL flashpoint is conventionally assigned to one of three digits which are represented in Table 1:

Table 1

Discharge	Characteristics of liquid	Flash point, ° C:	
		in an open crucible	in a closed crucible
I	Particularly dangerous	Up to -18	Up to -13
II	Permanently dangerous	from -18 to 23	from -13 to 27
III	Hazardous at elevated temperature	from 23 to 61	from 27 to 66

The third category includes: amyl acetate, anisole, acetylacetone, benzyl chloride, bromobenzene, butanol, hexyl chloride, decane, diamyl ether, diketene, N, N-dimethylaminoethanol, dimethyl sulfate, N, N-diethylaminoethanol, diethylcarbonate isoamyl acetate, kerosines, xylene, methyl acrylate, morpholine, formic acid, octylamine, pentanol, propylbenzene, propanol, turpentine, styrene, acetic acid, acetic anhydride, chlorobenzene, cyclohexanone and others.

Flammable liquids (FL) are liquids that are capable of burning themselves after removing the ignition source and having a flash point not exceeding 61 ° C.

Combustible liquids (CL) are liquid substances having a flashpoint above 61 ° C in a closed crucible or above 66 ° C in an open crucible and capable of burning after removing the ignition source.

Work with flammable liquids. When working with flammable liquids should be followed so basic principles:

1. Work only in a fume hood.

2. Do not allow flammable vapors to enter the atmosphere (prevent the formation of fire and explosive mixtures).

3. Works with flammable liquids (FL) should be carried out far away from fire. It is forbidden to heat flying and flammable liquids (acetone, air, alcohols, petroleum air, gasoline, benzene, carbon sulfur) on an open flame. For heating of FL it is possible to use a water bath or an electric tile with the closed spiral, at the same time the flask has to be supplied with the water refrigerator.

4. It is impossible to heat combustible substances in open vessels. It should be done in flasks with the return refrigerator.

5. It is necessary to overtake FL in the device with the water refrigerator or on the rotor evaporator. It is impossible to overtake liquids dry – it can lead to explosion or the fire. Devices which contain FL should be disassembled after removal of all sources of a flame (the lit gas burners, spirit-lamps, electric tiles with an open spiral, etc.) and full cooling of a flask.

6. It is strictly forbidden to pour out FL in the sewerage, buckets and boxes for garbage as accidentally thrown match can cause the fire.

7. FL have to be stored in metal cases in the quantities which aren't exceeding daily requirements.

1.4 Security measures at leak of gas and suppression of the local fire and the burning clothes

1. At emergence of the fire it is necessary to remove quickly all combustible substances far away from the place of ignition, to disconnect the gas highway, all electric devices and to stop active access of air to laboratory.

2. The suppression of a flame by water can lead to expansion of the seat of fire if the substance insoluble in water (for example, ether, benzene, gasoline, turpentine metal sodium, etc.) is burning. In this case, the flame should be extinguished by sand or a fire-prevention blanket. In case of more extensive fire area it is necessary to use the fire extinguisher.

3. Soluble in water flammable substances, such as alcohol, acetone and others, can be extinguished with water.

4. If on someone the clothes light up, it is necessary densely on to cover the lit-up fabric with a fire-prevention blanket. At ignition of clothes it is impossible to run as it promotes distribution of a flame.

1.5 First aid at burns and poisonings with chemicals

1. At thermal burns of the first degree (redness and a swelling) the burned place should be processed spirit solution of tannin, 96% ethyl alcohol or solution of permanganate of potassium. At burns of the second and third degree (bubbles and ulcers) only the disinfecting lotions from potassium permanganate solution then it is necessary to see a doctor are admissible.

2. At burns acids it is necessary to wash out the struck place a large amount of flowing water, and then 3% solution of a hydrocarbonate of sodium then – again water.

3. At burns alkalis it is necessary to wash out the defeat center flowing water, and then the diluted solution of boric or acetic acid.

4. At hit of alkali or acid in eyes it is necessary to wash out their flowing water (3 – 5 min.), and then solution of boric acid (in case of alkali hit) or a sodium hydrocarbonate (in case of acid hit) then to see a doctor.

5. At burns phenol the center of defeat should be processed 70% ethyl alcohol, and then glycerin before disappearance of white spots on skin. At poisoning with vapors of phenol it is strictly forbidden to drink milk.

6. At burns bromine he needs to be washed away 96% alcohol or the diluted alkali solution then to grease the place of defeat with burns ointment and to see a doctor. At poisoning with vapors of bromine it is deeply necessary to inhale several times fumes of ethyl alcohol, and then to drink milk.

7. At hit on skin of caustic organic substances, not soluble in water, they need to be washed away a large amount of suitable solvent. After first-aid treatment the victim has to be sent to a first-aid post**.**

1.6 Chemical dishes for laboratory work of organic chemistry

Flasks, glasses, test tubes, cups, funnels, refrigerators, dephlegmators and other vessels of various designs belong to the main laboratory chemical glassware. Most often chemical ware is made of glass of various brands. Such ware differs in resistance to influence of the majority of chemical reagents, is transparent, easily washes.

a b c

Adapters: Wurz's (b) and Claisen (b), to a round bottom flask for distillation. Vacuum and simple (c) receiving adapters connect the outlet of a condenser to a receiving flask.

a b

c

Flasks: The Kleisen flask (a) with a refrigerator is used for distilling organic compounds under reduced pressure; Wurz flask (b) is used for distillation; the flasks (c) are used for distillation and synthesis of chemicals.

a b

Three-(a) and Two-neck (b) distilling flasks with angled necks allow to connect three components for complex distillations.

a b c

d

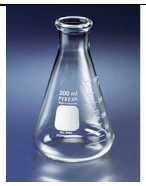
Complex for vacuum filtration (a): A Buchner funnel (b), Filtering flask (c), Schott's laboratory funnel (d)

a b

c

Condensers: Liebig (a) and a Graham (or reflux) (b) condenser are used to cool and condense hot vapor as part of a distillation and synthesis apparatus. Dезphlegmators (c) - reflux condensers serve for more careful separation of fractions of the mixture during its fractional distillation.

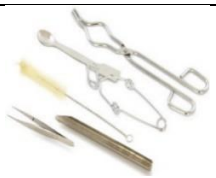
Separatory funnels are used in a variety of inorganic and organic chemistry processes



Conical or flat bottom flask

Glass laboratory

Desiccators allow to cool and store objects in a low-humidity atmosphere.

	beakers	
 Laboratory funnel	 The commonly used accessory tools	 Assorted solid, 1-hole and 2-hole stoppers
 Bunsen Laboratory Stand. An extension clamps and rings are very versatile in setting up distillation or other laboratory equipment.		

1.7Rules for assembling installations for the performance of organic syntheses

The choice of the device (equipment's) for synthesis is determined, first of all, by the task facing the experimenter, the reaction conditions, and also by the properties of the starting materials and final products.

Assembly of the installation should be carried out with great care and accuracy, as this is an indispensable condition for successful and safe operation. The assembled units must be not only literate in design, but also have an attractive appearance.

General rules for assembling instruments. Separate parts of the installation must be connected with each other carefully, picking up plugs, tubes and other parts even before the device is fixed to a tripod. If the device is assembled on thin sections, they should be pre-lubricated. The dishes are selected in such a size that the reacting substances occupy not more than half the volume (or no more than 2/3 of the volume). If the reaction mixture is heated, a round-bottomed flask of the appropriate size is necessarily used. After assembling the individual parts of the installation, they are fixed in the legs of the tripod. The installation is always assembled, beginning with its supposed "top" or from the main unit. For example, when assembling a plant for simple distillation, first fix the Wurz flask on the tripod, then attach a descending refrigerator to it, then an allonge, and finally bring the receiver under it. The entire installation should be assembled in one plane or along one line (except in some cases), without distortions or stress of the glass parts of the device. This is especially important when working with standard

sections, when they should be connected to each other without much effort on the part of the experimenter. At the same time, it must be ensured that the sealing conditions are met when connecting the individual parts of the device. If the glass parts of the installation are heavy enough (for example, a flask with a reflux condenser, a stirrer, a dropping funnel, a thermometer, etc.), then a few paws should be attached to the tripod. At the same time, reflux condensers, agitators, reflux coolers are fixed strictly vertically, and descending refrigerators are sloped so that the liquid flows into the receiver without getting into the plugs. If the unit is designed for operation at atmospheric pressure, it is necessary that it is freely communicated with the atmosphere in order to avoid increasing the pressure in the system. To protect the reactants from the action of air moisture (if necessary), use a calcium chloride tube.

Getting started, you should once again carefully inspect the device and make sure of the correctness of its assembly.

LABORATORY WORK №1-3

Phenols

All phenols contained in medicinal plants are formed from carbohydrates and their conversion products. The atomicity of phenols is determined by the number of OH groups. Plants are dominated by 2 and 3 atomic phenols.

All phenols are acids, soluble in water and water-soluble organic mixtures, alcohols, acetone.

Quantification of phenols in plant raw materials

About 1 g of finely ground raw material (accurately weighed) is placed in a 50 ml flask and 30 ml of ethyl alcohol is added. The flask is connected to a reflux condenser and heated to a moderate boil in a boiling water bath. After cooling, the extract is poured through a folded filter into a 100 ml volumetric flask. The extraction is repeated under the same conditions. The extract in the flask is adjusted to the mark with the same solvent.

An aliquot of the obtained extract (0.1-05 ml) is made up to 7 ml with water in a graduated tube with a ground stopper. The mixture is mixed well and 0.5 ml of Folin-Denis reagent is added. After stirring for 3 minutes, 1 ml of saturated sodium carbonate solution is added and the mixture is stirred well and left for 40 minutes.

Then measure the optical density in a 1 cm cuvette at 725 nm.

Water with reagent (9.5 ml + 0.5 ml of reagent) is used as a control. The calculation is carried out according to the calibration curve built for the known phenol (pyrocatechol), M 110; concentration 1 * 10-4 mol / l or 1.10 mg in 100 ml of water (0.5; 1.0; 1.5; 2.0; 2.5; 3.0 ml).

Folin-Denis reagent: 100 g of NaWO * 2H2O, 20 g of phosphoromolybdic acid and 750 ml of water, boiled for 2 hours in a flask with a reflux condenser and the volume is brought to 1000 ml.

Quantification of polyphenols

About 1 g (accurately weighed) of the contents of capsules (dragees, tablets) or 2 g of crushed vegetable raw materials are placed in a conical flask with a capacity of 100 ml, add 50 ml of purified water and boil in a steam bath for 2 hours.

The mixture is cooled and filtered into a volumetric flask with a capacity of 100 ml, the volume is brought up to the mark with purified water, stirred. [5 ml of the resulting solution is placed in a volumetric flask with a capacity of 25 ml, the volume is brought up to the mark with purified water, stirred] *

5 ml of the resulting solution is placed in a volumetric flask with a capacity of 50 ml, 1 ml of a solution of tungstate-phosphoric acid is added, the volume is brought up to the mark with 15% sodium carbonate solution, and mixed.

Measure the optical density of the resulting solution 2-3 minutes after adding the last reagent at a wavelength of 715 nm in a cuvette with a layer thickness of 10 mm, using purified water as a reference solution.

In parallel, the optical density of the pyrogallol CO is measured, exactly 2 minutes after the addition of the last reagent and within 15 minutes after the pyrogallol dissolution.

The content of the sum of polyphenols, in terms of pyrogallol, in percentage (X) is calculated by the formula:

$$\frac{D_1 \bullet m_0 \bullet 100 \bullet [25] \bullet 50 \bullet 5 \bullet 5 \bullet 100 \bullet [100]}{D_0 \bullet m_1 \bullet [5] \bullet 5 \bullet 100 \bullet 100 \bullet 50 \bullet [(100 - W)]}$$

where: D1 is the optical density of the analyzed solution at a wavelength of 715 nm;

D1 is the optical density of the pyrogallol CO solution at a wavelength of 715 nm;

m1 is the mass of the crushed medicinal plant material or medicinal product, g;

m0 is the mass of a sample of pyrogallol CRM - 0.0500 g;

W- loss in mass upon drying,%

* dilute the test solution if necessary

Preparation of a pyrogallol CRM solution: 0.05 g (accurately weighed) pyrogallol of the highest purity is placed in a volumetric flask with a capacity of 100 ml, dissolved in purified water, the volume is brought up to the mark with purified water and stirred.

5 ml of the resulting solution is placed in a volumetric flask with a capacity of 100 ml, the volume is brought up to the mark with purified water and mixed.

5 ml of the resulting solution is placed in a volumetric flask with a capacity of 50 ml, add 1 ml of a solution of tungstate phosphoric acid, mix, bring the volume to the mark with 15% sodium carbonate solution, mix.

The solution is prepared in a dark place!

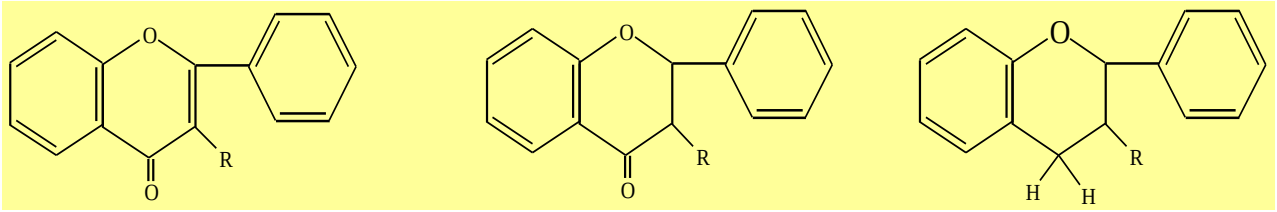
Preparation of a solution of tungstate-phosphoric acid: To 10 g of sodium tungstate add 8 ml of phosphoric acid 85%, 75 ml of purified water and heat for 3 hours with an air cooler. Then it is cooled and the volume is brought up to 100 ml with purified water, stirred.

Any phenol or phenolic acid similar to those contained in the raw material can be used as CO.

Flavonoids

Flavonoids belong to one of the most common groups of natural compounds. Most of them are found in plants in the form of glycosides, the variety of which is due not only to the position and a large set of sugars, but also to the difference in the magnitude of oxide cycles, as well as the configuration of glycosidic bonds.

The process of extracting flavonoids can be combined with the hydrolysis of glycosidic forms with hydrochloric or sulfuric acids when heated. The selective extraction method consists in the extraction of flavonoids from plant materials with various solvents in a specific sequence. With the help of low-boiling petroleum ether and carbon tetrachloride, first, the raw materials are degreased, and waxy and resinous substances are removed. Then, to isolate flavonoids, the plant raw materials are extracted with alcohols, acetone and their different percentages of aqueous solutions. The resulting extract is evaporated, hot water is added to the residue, and non-polar compounds (chlorophyll, fatty oils, essential oils) are removed with chloroform or carbon tetrachloride. Flavonoids from the aqueous phase are extracted sequentially with ether (aglycones), ethyl acetate (monosides) and butanol (biosides, triosides, etc.).



R= H флавон	R= H флаванон	R= H флаван
R=OH флавонол	R=OH флаванонол	R=OH флаванол

Quantitative determination of flavonoids (in terms of quercetin)

An analytical sample of raw materials is ground to a particle size passing through a sieve with holes 1 mm in diameter.

About 1 g (accurately weighed) of the raw material is placed in a flask with a thin section with a capacity of 150 ml, 30 ml of 90% alcohol containing 1% concentrated hydrochloric acid is added, the flask is connected to a reflux

condenser and heated in a boiling water bath for 30 minutes. The flask is then cooled to room temperature and filtered through a filter paper into a 100 ml volumetric flask. The extraction is repeated 2 more times as described above. The extracts are combined, filtered through the same filter into the same volumetric flask, the filter is washed with 90% alcohol and the volume of the filtrate is adjusted to the mark with 90% alcohol (solution A).

In a volumetric flask with a capacity of 25 ml, place 2 ml of solution A, add 1 ml of a 1% solution of aluminum chloride in 95% alcohol and bring the volume of the solution to the mark with 95% alcohol. After 20 min, the optical density of the solution is measured on a spectrophotometer at a wavelength of 430 nm in a cuvette with a layer thickness of 10 mm.

The reference solution is a solution consisting of 2 ml of solution A, brought to the mark with 95% alcohol in a 25 ml volumetric flask.

The content of the sum of flavonoids in terms of quercetin and absolutely dry raw materials in percent (X) is calculated by the formula:

$$X=\frac{D \bullet 25 \bullet 100 \bullet 100 \bullet 100}{764.6 \bullet m \bullet 2 \bullet (100-W)}$$

Where,

D is the optical density of the test solution at a wavelength 430 nm;

764.6 - specific absorption rate of a complex of quercetin with 1% a solution of aluminum chloride at a wavelength of 430 nm;

m is the mass of raw materials in grams;

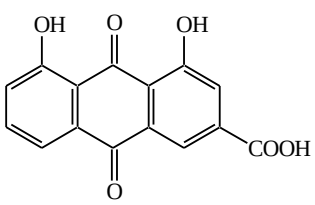
W - loss in mass during drying of raw materials in percent

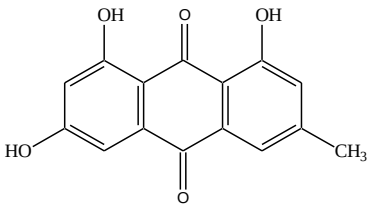
LABORATORY WORK

Anthraquinones

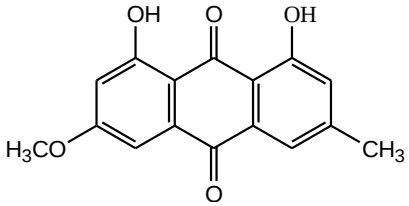
Anthracene is a group of natural substances containing three condensed rings, of the general formula C6-C2-C6.

There are oxidized, reduced and condensed derivatives of anthraquinones, some examples of structures are presented below:

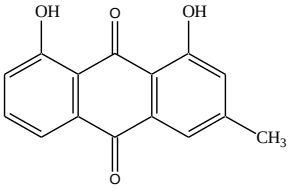


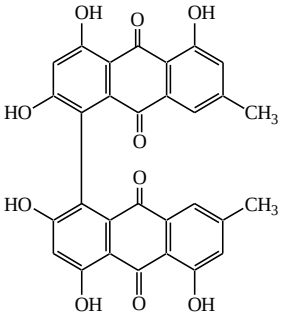


emodin



fiscionrhein





chrysophanic acid **5,5'-diemodin**

All of them can contain OH, OCH3, C (O) H, COOH, C1 to C5 radicals, carbohydrate fragments as substituents.

Depending on the nature of the substituents, they dissolve in non-polar (aglycones) and polar solvents when heated.

The reduced forms are oxidized, especially when heated, to the corresponding oxidized forms. Due to the formation of phenolates, they are highly soluble in alkalis.

Quantification of anthraquinones

An analytical sample of raw materials is ground to a particle size passing through a sieve with holes 1 mm in diameter.

About 1 g (accurately weighed) of the raw material is placed in a flask with a thin section with a capacity of 100 ml, 15 ml of 10% sulfuric acid (7.5 ml of glacial acetic acid) are added and heated under reflux in a boiling water bath for 1 hour. It is cooled, 50 ml of chloroform (ethyl acetate) are added through the refrigerator and the mixture is refluxed for another 1 hour.

The mixture is cooled, the extraction is filtered into a separatory funnel, 20 ml of alkaline-ammonia solution are added, and the mixture is shaken for 5-7 minutes. After complete delamination, the transparent red bottom layer is decanted. The treatment is repeated until the color of the alkaline-ammonia layer ceases, mix.

Measure the optical of the obtained alkaline-ammonia extracts at a wavelength of 525 ± 5 nm in a cuvette with a layer thickness of 10 mm, using an alkaline-ammonia solution as a reference solution.

The content of anthraquinone derivatives in terms of n chrysophanic acid is calculated by the formula:

$$X=\frac{C \bullet 100 \bullet 100 \bullet 100}{m \bullet (100-W)}$$

Where,

C is the content of anthraquinone derivatives in 1 ml of the test solution, found from the calibration graph, in grams;

m is the weight of the sample of raw materials, in grams;

W - loss in weight during drying of raw materials, in percent

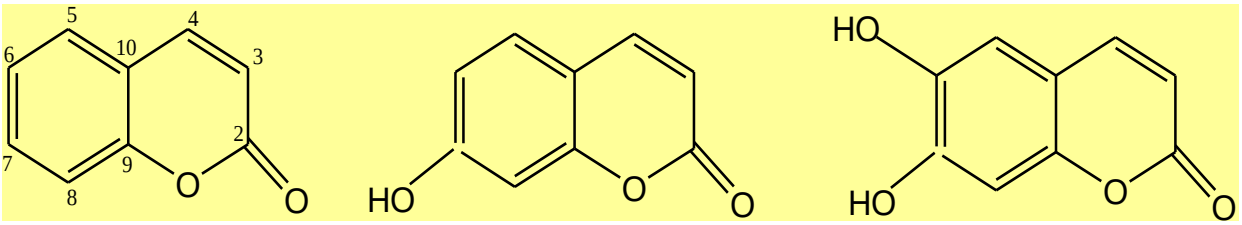
LABORATORY WORK

Coumarins

The companion of coumarins in plants are oxycinnamic acids, from which they are formed in the process of biogenesis.

The variety of coumarins is based on the benzene and pyran rings, which make up the skeleton of any coumarin.

Plants most often contain coumarin, its hydroxy derivatives, furo-, pyranocoumarins and glycosidated forms.



Coumarin **Umbelliferon** **Esculetin**

Quantification of coumarins

About 2 g of crushed raw materials (exact navska) are placed in a flask with a capacity of 100 ml, 50 ml of chloroform are added and heated with stirring for 2 hours in a boiling water bath with a reflux condenser, filtered through a paper filter.

Place 20 ml of the filtrate in a separatory funnel, add 1 g of sodium chloride, shake for 5 minutes, filter.

The chloroform extract is evaporated to dryness in a boiling water bath.

The dry residue is dissolved in 10 ml of ethyl alcohol 96%, quantitatively transferred with 10 ml of ethyl alcohol 96% into a volumetric flask with a capacity of 25 ml, the volume of the solution is brought to the mark with ethyl alcohol 96%.

[5 ml of the analyzed solution is placed in a volumetric flask with a capacity of 50 ml, the volume of the solution is brought to the mark with 96% alcohol, stirred] (if necessary).

The optical density of the solution is measured at a wavelength of 272 nm in a cuvette with a layer thickness of 10 mm, using 96% ethyl alcohol as a reference solution.

The content of coumarin derivatives in absolutely dry raw materials in terms of CO as a percentage is calculated by the formula:

$$X = \frac{D \bullet 25 \bullet [50] \bullet 100 \bullet 100}{734 \bullet 20 \bullet m \bullet [5] \bullet (100 - W)}$$

where: D is the optical density of the test solution at a wavelength of 272 nm;

734 - specific absorption index of CO of coumarin at a wavelength of 272 nm;
m is the weight of the sample of raw materials, g;

W is the loss in oil during drying of raw materials,%.

Determination of biologically active substances

Determination of flavonoids in quercetin

Put 1 g of raw material of exactly weight (d = 1 mm) in a 150 ml sanded flask, add 1 ml of concentrated HCl 30 ml of 90% alcohol, connect it to the return cooler and heat in boiling water for 30 minutes. Then cool the flask to room temperature and strain it through a filter paper into a 100 ml flask. Repeat the above experiment once more, then soak in 95% alcohol for 30 minutes. Filter the resulting solution (mixture) over the above 100 ml flask, rinse the filter with 90% alcohol, add 90% alcohol (solution A) to the mark of the flask (100 ml).
Transfer 2 ml of solution A and 1 ml of 1% solution of aluminum chloride in 90% alcohol to a 25 ml volumetric flask and add 90% alcohol to the mark. After 20 minutes, measure the optical density of the solution on a spectrophotometer AP-101 with a cuvette of wavelength λ = 430 nm and a thickness of 10 mm. As a replacement solution, take solution A with a volume of up to 25 ml of 90% alcohol.
When calculating quercetin, the amount of flavonoids is calculated using the following formula (in%):

$$X = \frac{D \cdot 25 \cdot 100 \cdot 100}{764{,}6 \cdot 2 \cdot M \cdot (100 - W)} \qquad (5)$$

Where: D is the optical density of the solution;

Formed aluminum chloride and quercetin at 764.6 - 430 nm
absorption index of the complex;

m - raw material (in grams);

W is the amount of raw material lost during drying (in%).

Quantities identification of coumarins

Place 2 g of accurately weighed, ground raw material in a 100 ml flask. Pour 50 ml of chloroform on it, connect to the refrigerator and heat in a water bath for 2 hours, stirring. Filter the juice through a paper filter. Pour 20 ml of the filtrate into the separation funnel, add 1 g of NaCl, mix for 5 minutes, then filter. The chloroform solution is dried in a water bath. Dissolve the dry residue in 10 ml of

96% ethyl alcohol, pour into a 25 ml volumetric flask and fill with 96% ethyl alcohol. Pour into a cuvette 10 mm thick and measure the optical density at a wavelength of 272 nm.

96% ethyl alcohol is used as a substitute solution. Calculates the percentage of coumarin derivatives as absolute dry CO of the raw material:

$$X = \frac{D \cdot 50 \cdot 100 \cdot 100}{734 \cdot 20 \cdot M \cdot (100 - W)} \quad (8)$$

Where: 734 is the absorption index of coumarin at a wavelength of 272 nm;

M - weight of raw materials, g;

W - loss of maca during drying,%.

Determination of the amount of organic acids

Put 5 g of raw material in a flask and add 40 ml of water. Boil the mixture in the back refrigerator for 2 hours. The mixture is filtered into a 25 ml volumetric flask and made up to volume. Remove the aliquot (10 ml) from the solution and pour into a 500 ml flask, add 200-300 ml of water, 1 ml of an alcoholic solution of 1% phenolphthalein, 2 ml of a solution of 0.1% methylene blue powder, a solution of blue ash-1 solution. vibrates to a red color.

The absolute content of all organic acids (X) in malic acid is determined by the following formula:

$$X = \frac{0.0067 \cdot 25 \cdot 100 \cdot 100}{m \cdot 10 \cdot (100 - W)} \quad (9)$$

Where: 0.0067 g - malic acid corresponds to 1 ml of NaOH;

V - the amount of NaOH used for titration, ml;

M - mass of raw materials, g;

W - moisture content of raw materials,% .

Paper chromatography

System of solvents used for paper chromatography:

1) Butanol: acetic acid: water (BCC) (40: 12.5: 29)
2) 6% - vertical acetic acid
3) Butanol: acetic acid: water (6: 7: 3) + 0.01 g of ninhydrin
4) EA: HAc: water (5: 3: 2)
5) Benzene: acetic acid: water (6: 7: 3)
6) Butanol: acetic acid: water (6: 7: 3)

Determinants used for paper chromatography

Determinants used for paper chromatography:
1) Aluminum chloride
1% solution of aluminum chloride in ethanol is used for the determination of flavonoids.
2) Diazotized p - nitroaniline (DZPNA)
Prepare 0.3% p-nitroaniline solution in 8% hydrochloric acid, add a few drops of 5% sodium nitrite and mix before use, prepare the mixture only when used. The solution prepared for the chromatogram is coated, dried at room temperature and then treated with 20% soda solution.
3) Dissolve 0.4 g of salicylic acid and 0.5 ml of toluidine in 10 ml of 97% ethanol. The chromatogram is treated with a detector, dried and heated at 1050C for 5 minutes.
4) Ninghydrin reagent
A 1% solution of ninhydrin in acetone detects amino acids.
5) Vanillin reagent
Determines 1% vanillin solution in hydrochloric acid, catechins, condensed tannins.
6) Ammonia vapor
Defines flavonols, flavonols.