**Pharmacognostic analysis of medicinal plant raw material**

Standardization and quality control of medicinal plant materials and its preparations are carried out in accordance with the requirements of common and particular articles of various pharmacopoeias Medicinal material and its products is full if they conform with the normative document. This conformity is determined by the pharmacognostic analysis. Pharmacognostic analysis is a complex of methods for analysis of plant and animal raw material, which allows to determine its identity and purity.

**General requirements**

Pharmacognostic analysis of medicinal plants is to determine: 1) authentication, 2) cleanliness, 3) good quality. Determining the authenticity of raw materials is reduced to determine whether such sample name under which he acted. Clean medicinal plants is determined by the absence of harmful impurities and to mix. Permitted impurities must not exceed certain standards. The high quality of raw materials depends on a number of reasons. It is determined by the accuracy and timeliness of the collection of raw materials, drying, lack of mold and pests, normal humidity, ash content and the content of active substances.

The investigated material can be: 1) solid (totum), 2) cutting (concisum), crushed (contusum), 3) powder (pulveratum). When his trial had to resort to various methods of analysis: 1) macroscopic, 2) microscopic, 3) merchandising, 4) phytochemical and 5) biological.

Guidelines for the researcher of raw materials are GFH or other scientific and technical documentation. Until recently, the use of the State Standards (GOST), temporal specifications (WTU). GOST remain only on drug-technical material that has applications in other industries (food, ether-oil, etc.).

Each pharmacopoeia article provides as follows:

1. Name of medicinal plants in Latin and own country languages, indicating the applicable parts of the plant.

2. Introductory part, which specifies the term collection of raw materials, the shape of workpiece material (dried or fresh), the scientific botanical name of the generating plants and the family in Latin language, the most important synonyms, and ways to use raw materials.

3.Exact macroscopic description of the solid raw material, its color, smell, taste and medium-size to determine the authenticity of raw materials for cutting raw materials specified size pieces.

4.Mikroskopic description of whole raw powder and to clarify the definition of authenticity.

5.Numeral indicators, giving the rules required for high quality food: in the moisture, ash and permissible impurity.

6.Quantitative definition of extractive and active substances, methods of analysis and rules of their content, if required.

7.Packing and marking GOST.

8.Storage and transportation according to the instructions of the Ministry of Medical Industry.

9. Storage life.

10.Highest single and daily doses for tough materials.

Compliance with the requirements of Pharmacopeia to the quality of raw materials, it is compulsory for all raw materials, let off from the warehouse pharmacies and processing of herbal and chemical-pharmaceutical plant.

**Pharmacognostic analysis**

Consumers use only approved certified raw material, which meets the requirements of analytical normative documents. Identity, purity and quality of each medicinal raw material are indicated in analytical normative documentation. These indicators are determined by usage of pharmacognostic analysis.

 Authenticity is a conformity of the  investigated MRM with its name under which it was received for analysis.

Diagnostic features – is a set of morphological, anatomical and chemical features, which are characteristic for investigated object and permitted to identify it.

Purity is the absence of [impurity](https://en.wikipedia.org/wiki/Impurity) or [contaminants](https://en.wikipedia.org/wiki/Contaminants) in a raw material.  A high quality is a conformity of the investigated medicinal material with all the requirements of normative document.

Pharmacognostic analysis comprises the batch quality (commodity), macroscopic, microscopic and phytochemical analyses. In some cases the biological activity of material is established ( for example. the material containing the cardiac glycosides).

Medicinal plant material can be standardized in entire, crushed, powdered, filter bags, briquette, granules and medical species. It is necessary to use certain methods of batch quality analysis in every case.

Batch quality analysisrefers to MPM examination and sampling of material in bulk, the further determiation for the presence of admixtures, degree of fragmentation and contamination of the medicinal plant material by drug-eating insects, quantities of moisture and ash, active or extractive matters.

Macroscopic analysis is used to establish the identity of medicinal plant material and some parameters of its quality.

Microscopic identification is a main method for the identification of crushed medicinal plant material (chopped, powdered, cut-pressed, briquettes, granules, collection and oth.), and also the whole material in the presence of morphological similar medicinal plant material.

Phytochemical analysis is used for detection of active substances and concomitant substances that are present in medicinal plant materials, as well as determination of the amount of biologically active substances by chemical, physicochemical and chromatographic methods. Chemical reactions used for identification of medicinal material according to technique and character of results are divided into:

- qualitative reactions. These reactions are carried out by adding the appropriate reagent to the extraction of the medicinal plant material; it can be carried out with sublimate – product of sublimation.

- microchemical reactions. Chemical reactions are carried out with microscopic analysis, the results of reactions are observed under microscope.

Chromatographic analysis is a component of phytochemical analysis for determination, separation and identification of mixture of natural compounds.

Luminescent analysis - a set of methods of analysis based on the observation of luminescence of substance stimulated by UV-light (photoluminescence). It is used for microscopic and chromatographic analysis to determine the identity of medicinal material.

**The batch quality analysis**

The purity, quality and authenticity are determined in batch quality analysis. For this purpose, samples are tested for fragmentation, the presence of particles lost their natural color, parts of plants prepared as raw materials, organic and mineral impurities, humidity, and the degree of contamination (granary insects). During the packaging and transportation the part of raw material turns into a powder, it has a negative effect on quality and impair the appearance of of raw material. There is the permissible content of the crushed particles for each kind of raw material. However, their average content should not exceed 2-5% (20% for a chamomile flower).

Each material has unique colour. However the improper drying changes the natural colour of raw material. For example, the leaves are blackened, flowers are got brown and faded. The number of particles of raw materials lost their natural color, on average, is no more than 5%.

Other parts of plant can get into material during the collection. For example, flower, fruit, branches and oth. can get into leaf material. However, their content should not exceed 2-5% in raw materials.

During the procurement of raw materials, foreign organic impurities can get into - parts of foreign plants. The organic impurities max. for herb and leaves – 1-5%.

It is possible the presence of mineral impurities in raw materials (sand, stone, soil, etc.). However, the permissible content of such impurities in the raw material is from 0.5 to 2% (for rhizomes with valerian roots up to 3%).

 After determination of the finely divided particles in the raw materials, the remaining impurities are determined in the sieve. For this, the raw material is placed on a wooden board or paper, the impurities are separated and weighed with an error of 0.1 g and the percentage in raw materials is calculated.

The number of pests in the sample is determined and counted for 1 kg of raw material for determination of the degree of contamination of raw materials with pest control. There are 3 degrees of contamination of medicinal plant material. I degree-for small insects, not more than 20 insects; II degree - more than 20 ticks; III degree - insects form solid masses. The contaminaiton by the largest insects in 1 kg of plant material: I degree- 1-5, II degree- 6-10, III degree – more 19 insects. Along with the degree of contamination of the raw material during the analysis, the amount of contaminated particles of the material is determined in percentage

Raw materials contaminated with pests are disinfected and sieved. At I degree, raw materials can be permitted for medical use, at II degree the raw material can be used for the production of medicinal products. At III degree, the raw material is destructed or used for processing to obtain the active substances.

The results are recorded in the protocols after the completion of the batch quality analysis.

**Macroscopic method of analysis**

The identification of medicinal plant material and some parameters of quality are determined according to morphological characteristics by macroscopic analysis.

*Macroscopic identity* consists of the visual examination or by loupe the external (morphological) characteristics of raw material, the determination of size, color, odour and carrying out some microchemical reactions.

Macroscopic analysis is carried out on fresh, dried, wetted or softened entire or grinded plant objects.

*The preparation of sample of plant material for analysis*

Fresh raw material is studied without preliminary treatment. Dried raw material (small and leather leaves, fruits, semens, bark and underground organs) is laid on rubber sheet or a dark paper for visual observation or use a magnifying lens (×6—10) or stereomicroscope.

Juicy fruits changed in shape during the drying, thin leaves, flowers, wrinkled and contracted parts of plant (fragments of stems with leaves and flowers) preliminary soften in quantity of 2-5 in humidified box or by immersion in water for 5-10 min.

Softened raw materials are placed on a glass, oilcloth or smooth dark paper and carefully streched. The flowers are first examined entirety, and then prepared for examination of the internal structure. In the fruits, the pericarp and seeds are studied.

*Appearance.* Appearance of plant raw materials is determined by visual comparison with reference sample or by description in the analytical normative document. The sequence of organoleptic characteristics of medicinal plant material is given in the schemes.

*Size.* For large objects (from 3 cm or more), 10-15 measurements by graduated ruler in millimeters are carried out. Small objects (up to 3 cm in size) are aligned on a sheet of calibrated paper, 20-30 measurements are carried out and calculate the average value. The size of spherical seeds is determined by sieving through a sieve with the appropriate size.

*Colour*. Examine the plant material under the diffuse daylight. Mark the color of raw materials on the surface of the organ (for leaves - both surfaces), as well as on a break or cut of raw materials (roots, rhizomes, bark).

*Odour.* Place a small portion of the sample in the palm of the hand or in a beaker of suitable size, and slowly and repeatedly inhale the air over the material..Sometimes pour a small quantity of boiling water onto the crushed sample in a beaker to enhance the strength of the odour.

*Taste*. The taste of fresh and dried plant materials is determined by direct taste (without swallowing) or taste 10% decoction. *Note!* The taste of the poisonous plant material is not determined!

In addition to the external examination, very simple, qualitative chemical reactions are carried out on dry raw materials (the presence of starch, inulin, lignin, mucus, glycosides, etc.), which contribute for identification and quality of medicinal plant material.

The qualitative reactions are carried out on the dried material, with powder or scraping or extraction.

A conclusion is made about the conformity of medicinal plant raw material to its name under which it was received for analysis after macroscopic examination and qualitative reactions.

**1. Leaf – *Folia*** (SP XI, edit. I, p. 252). Leaves as medicinal plant material is dried or fresh developed leaves or small leaves of compound leaf with petiole or without it.

**2. Flowers – Flores** (SP XI, edit. I, p.257). Flowers as a medicinal plant material are dried flowers, inflorescences or its parts collected at the beginning of bloom or in bud-formation period. In world practice, inflorescences are included in a separate morphological group of raw materials - "Inflorescencia".

**3. Furits – *Fructus* (SP** XI, edit. I, p. 258-261). Fruits as medicinal materials are ripe, dried or fresh fruits, collective fruit and its parts. The fruit consists of pericarp (pericarp) and seeds.

**4. Seeds – *Semina*** (SP XI, edit. 1, p. 258-261). Seeds as medicinal materials are ripe whole seeds and individual cotyledons.

**5. Herbs – *Herbae*** (SP XI, edit. I, p. 256). Herb as a medicinal material is a dried or fresh aboveground parts of plants collected during flowering, budding or fruiting. Herb consists of stems with leaves and flowers, partly with buds and unripe fruits. In some plants, only the apexes of a certain length are collected, while in others, the entire aboveground part is collected. In rare cases, the aerial part together with the roots.

**6. Bark – *Cortex*** (SP XI, edit. I, p. 261). A bark as medicinal material is an outer part of the trunks, branches and roots of trees and shrubs located to the periphery of the cambium. The bark is usually prepared in the spring during the sap movement and dried.

 **7. Roots, rhizomes, tubers, bulbs, bulbotubers– *Radices, Rhizomata, Tubera, Bulbi, Bulbitubera*** (SP XI, edit. I, p. 263). Roots, rhizomes, tubers, bulbs, bulbotubers as medicinal material are dried, rarely fresh, underground organs of perennial herbaceous plant, purveyed in autumn or in early spring, purified or cleaned from the soil, freed from dead parts, stems and leaves. Large underground organs are cut into parts before drying (length or crosswise).

**MACROSCOPIC ANALYSIS OF RAW MATERIALS**

- Aims to determine the authenticity of medicinal plants on the outside and morphological characteristics (shape, size, color, taste, smell, etc.)

**Analysis of leaves**

*a) small and leatherback (cranberries, bearberry)*

During drying, these leaves do not change the shape and size, so the dry leaves in the quantity of 5-10 pieces laid out on a sheet of blank paper and check:

- The size of the ruler (in mm)

- The edge of the sheet

- Venation

- Shape of a leaf

- Color sheet

- The nature of the upper and lower sides of the paper (shiny, matte, the presence of glands, etc.)

- The smell - when there is a grinding of raw materials between your fingers or pouring hot water

- The taste of water extract

*b) Leaves not leathery, thin (plantain, coltsfoot)*

These leaves, after drying, often lose their shape, so in the dry state is determined only:

- Smell

- Color

- The taste of water extract

All other external signs define mixing with warm water after the leaves in hot water. Leaves are removed from the water, laid out on a blank sheet of paper, straightened and conduct further analysis.

**Analysis flowers**

In the dry form is determined by:

- Color

- Smell

- Type of inflorescence

- Type of perianth

To determine the structure of the flower of his soaked in hot water and then analyzed:

- The structure of the cup, whisk

- The number of stamens and pistils, and their character

**Analysis of fruits, seeds**

*a) seeds, dried fruits* after drying, its shape and size do not change, so in the dry state determined absolutely everything, laying on a blank sheet of paper:

- Dimensions

- Form

- Availability of spare ribs

- Color

- Smell

- Taste

*b) Juicy fruit*

In the dry form is determined by:

- Color

- Pubescence

- Type of fruit

- Smell (if wash with hot water)

After mixing with warm water is spread on a clean sheet of paper and determine:

- Form

- Dimensions (length and width)

- Availability of special items (hulled outgrowth, the remainder of the cup)

Then cut and learn:

- Availability of seeds or seed

- Their shape and number

***Analysis of grass***

Adding the definition of various morphological traits groups:

Characteristics of stem:

- Length

- Diameter

- Form

- Character (ribbing, etc.)

Next, determine the parameters of leaves, flowers and fruits (see above).

**Analysis of the cortex**

We define the following indicators:

- Length and width

- The form of pieces of bark (grooved or tubular form) or flat pieces

- Thickness of the cortex (not more than 6 mm, optimum - 3.1 mm)

- The nature of the outside (the color, their shape, size, location)

- Color and character of the interior (brown, yellowish-green, with dots or ridges or smooth)

- Smell when pouring hot water when grinding

- Taste only in broth

**Analysis of underground organs**

In the dry form is defined:

- Shape (cylindrical, flattened, curved)

- The color of outside and in the fresh tissue

- The nature of the cut or break (even, smooth, splintery, hairy)

- The smell is often determined during the grinding or the pouring of raw hot water

- Taste only in broth

**Microscopic method of analysis**

The most important place is the microscopic analysis for determination of plant material. Microscopic method of analysis is used for identification of medicinal plant material. This method of analysis is particularly important for crushed, cut-pressed and briquitte material. Microscopic analysis is based on the detection of anatomical diagnostic features of the object under a microscope. The methodology and technique of application of this method and also the main approaches to the analysis of various morphological groups of plant materials are described in general notices. Also the most important anatomical and diagnostic characteristics of leaves, flowers, roots and other organs are summarized. The certain medicinal plant material is considered in specific monographs, where the main anatomo-diagnostic features are listed separately for whole, cutted and powdered material.

The microscopic analysis makes an objective assessment of the identity of medicinal plant material. However, there are difficulties in the analysis of grinded medicinal plant materials and collections. The main diagnostic features - trichomes are broken off, crystals of calcium oxalate are fallen out or join to other particles (in collections to particles of other plants) during the grinding, which creates additional difficulties for determination the identity of medicinal plant material. In addition, it is known that some close plants have similar anatomo-diagnostic features, different in size and frequency of occurrence. Therefore, in modern foreign pharmacopoeias, where microscopic analysis is also commonly used to determine the identity of medicinal plant materials, in addition to a mere listing of anatomo-diagnostic features, their sizes are additionally regulated (in particular, in the Pharmacopoeia of Germany). Usually the identity of medicinal plant materials is determined by qualitative reactions and the method of microscopic analysis. The value of examination can take place in analysis of whole and crushed plant material. However, the determination of identity on the morphological features of briquettes, filter bags, powders is not possible. It is possible to determine only color, taste (not always), odour, which carry insignificant informativeness about the identity of medicinal plant material for these dosage forms.

The use of qualitative reactions for determination of the identity of medicinal plant material is acceptable for all the listed dosage forms. However the medicinal plant material contains a complex of the biologically active substances, which requires the development of thorough methods of biologically active substances purification and it is a reason of the decrease in the level of confidence of the identity results.

Microscopic analysis gives the most reliable results for determination the identity. In recent years, the use of medicinal plant materials as powder has caused some changes of microscopic analysis. Some authors have determined the influence of the grinding ratio on the determination of the authenticity of the medicinal plant material of various pharmacological groups, the effect of the grinding ratio on the diagnostic features on the powder of plant raw materials, the effect of the grinding ratio and excipients in the microscopic study of tablets of plant powder on the diagnostic features of plant material, the determination of criteria of identity of plant tablets, studied the possibility of identification plant powders in complex criteria for the authenticity of plant powders to improve the analysis of briquettes, and also studied the exact microscopic diagnosis of medical species. The regulatory documents for plant powder and medications on the were drafted on the basis of results. In recent years, medicinal plant powdered materials are commonly used in medical practice, the determination of anatomo-diagnostic features of leaves and stems is not enough to determine the diagnostic characteristics of herbs.. It is also important to take into account on the anatamical-diagnostic characteristics of flowers and fruits. An attention should be paid to the petiole structure by leaf examination. Pollen of flowers and herbs has very important diagnostic characteristics. Despite the fact that the pollen was not studied microscopically previously. Anatomical -diagnostic features are a set of signs of the anatomical structure of material that distinguish this medicinal plant material from other species in the diagnosis of its identity.

Diagnostically significant characteristics are anatomical and diagnostic features that clearly distinguish this medicinal plant material from the other species. These characteristics are presented in sufficient quantity in the analyzed object and they are preserved by grinding the medicinal plant material to a powder of particle size - 0.5 mm.

Diagnostically significant particles are fragments of a powder carrying one or more diagnostically significant characteristics.

Microscopic analysis can not be the final criterion for the identification of medicinal plant material. Only in conjunction with other methods of analysis (macroscopic, phytochemical, etc.) the identity of the object of study can be reliably established. Optical equipments and auxiliary tools are required for carrying out of microscopic analysis. They include microscope, magnifying glass, polaroid, objective and ocular micrometers. Set of botanical instruments are used for the preparation of cross-section. Most often it is a razor and in special cases, if it is required to obtain a very thin sections - a microtome.

Various reagents are used for microscopic analysis. These reagents are divided into two groups: 1) indifferent and enlightening; 2) reagents for microchemical reactions. Indifferent and enlightening liquids include water, mixture of glycerol – water (1:2), 5% solution of chloralhydrate, aqueous solution of alkalines, solution of hydrogen peroxide and oth. The composition of the reagents for microchemical reactions is presented in the appropriate articles. Solutions for microchemical reactions are directly reagents for determination the identity of various biologically active substances. The technique of micropreparations is diverse and depends on its morphology, as well as on the state of the material – whole, cut or powdered. Micropreparations with various techniques are placed on a slide with applied inclusive liquid and covered with a cover glass.

*Histochemical reactions.* Carrying out the histochemical reactions is an integral part of the microscopic analysis. On the one hand, they enables to establish the presence of active substances in the medicinal plant material (fixed and essential oils, resins, contents of malleus, mucilagines, inulin, alkaloids, tannins, etc.) and their localization in plant tissues, on the other hand, the various parts of the cell are determined by histochemical reactions, the nature of the membrane, its lignification, the content of the cell sap. The necessary histochemical reactions are carried out on the transverse cut of the softened material or with a dry powder (scraping) of the plant organs.

*Microsublimation.* Microsublimation of active substances has a diagnostic significance for powders of some kinds of medicinal plant material (bark and underground organs). The powder of the crude material is placed on the bottom of test tube at a height of about 3 mm for carrying out sublimation. The tube is held horizontal and heated at the location of the powder in the burner flame. The sublimation of substances is deposited along the cool surface of test tube. The chemical reaction metioned in private article is carried out with the resulting sublimate.

Herbal medicine are produced in different forms. Medicinal plant material can be whole, crushed and powdered, the particle size of the powder varies widely. The assortment of dosage forms of plant material includes materials of various degree of crushing for preparation of infusions and decoctions, as well as briquettes, filter bags, collections, tablets.

**Luminescent microscopic method analysis**

Contained in plants of active substances in the anatomical specimens give a fluorescent microscope, a bright, colorful fluorescence, and various chemicals have different characteristic color. For example, tropan alkaloids give yellow fluorescence; anthganglycosides - various shades of orange, depending on the substance. The fluorescent microscopy was used primarily to identify the localization of active substances in plant tissues. The intensity of fluorescence is tentatively points to a greater or lesser concentration of these substances.

**The main methods of phytochemical analysis of medicinal plant material.** Phytochemical analysis enables to carry out qualitative and quantitative determination of active substances in plant materials by chemical and physicochemical methods.

Modern normative documents on medicinal plant materials include the standardization of biologically active substances as one of the most important numerical characteristics. Qualitative and quantitative determination are carried out by the chemical and physicochemical methods. Solven extraction or steam distillation is often used for isolation of organic compounds from natural objects. In both cases, a mixture of components is obtained, then it is purified from markers and ballast substances, divided into separate fractions or individual substances by operations: sequential treatment of the mixture with different solvents, distribution of substances between two immiscible solvents, chromatography methods.



**Chromatographic methods of pharmacognostic analysis**

The chromatographic method is the most frequently applied in phytochemical analysis. One of the most common method of phytochemical analysis. This method is used for separation of multi-component mixture for detection or identification. According to the mechanism of spearation there are three main types of chromatography: adsorption, partition and ion exchange chromatography. This method is based on the the differential adsoption of the components on the adsorbent (stationary phase) (adsorption or ion exchange chromatography) or the differences between two immiscible liquid phases, one of which is stationary (partition achromatography). According to purposes and tasks of analysis the various sorbents and types of chromatography are used: column, paper and thin layer chromatography. Paper and thin layer chromatography enable to work with a small amount of organic compounds and don’t require any expensive equipment. Chromatographic methods are widely applied in phytochemistry due to the simplicity, selectivity, rapidity, automation and combination of other physical-chemical methods. The chromatographic methods are characterised by the universality, i.e. possibility to use them for separation and identification of solid, luquid and gas. The particular value lies in the possibility to separate the compounds with close properties effectively, carry out both qualitative and quantitative anaylyses of resarched objects. The nature of mobile and stationary phases, mechanism of interaction between phase and separated substances, the technique of experiment are taken into account in the classificaiton of chromatorgraphic methods. Chromatographic methods are classified into gas, liquid, liquid-liquid, ion exchange chromatography and oth.

 Paper Chromatography (PC). In paper partition chromatography the separation of substances occurs due to the difference in partition between two phases. One of the phase is mobile and consists of the mixture of organic solvents. Other phase is stationary and it is the water trapped between the fibres of the paper. The solutions of analysing substances are applied on paper with micropipette, microsyringe or calibrated glass capillary. Then chromatography is carried out in appropriate system of solvents and conditions.

 Thin layer chromatography (TLC)*.* In this chromatographic method the adsorbent is a thin, uniform layer (usually about 0,24 mm) of a dry, finely powdered material applied to a suitable support, such as a glass plate or an aluminium or plastic foil. The mobile phase moves up the plate by capillary aciton. Chromatographic process depends on adsorbent, its treatment and nature of solvents employed. Confinement of a TLC plate in a chamber which has its headspace (the air in the chamber) saturated with solvent vapor. The chromatographic plate is often composed of glass for possibility to observe the movement of the mobile phase along the plate. Solid sorbents are silica gel, aluminium oxide, cellulose, sephadex, kieselguhr or ion-exchange resin. A thin layer can be impregnated with buffer solutions to produce an acid, neutral or base layer. Before the applicaiton, the plates can be activated by heating in a thermostat at a temperature of 100 to 105 ° C for 1 hour. TLC is carried out in a horizontal or vertical position.

The most reliable and effective methods are gas-liquid (GLC) and high-pperformance liquid chromatography (HPLC). GLC is based on the partition of substances between two phases, one of them is mobile. The mobile phase is an inert gas (helium, argon, nitrohen and oth.), the stationary phase is a liquid, applied on inert solid.  The sorbent is placed in U-shaped or spiral chromatographic column. The automatic device records the separated substances at the exit by their physical and chemical properties. The device records the qualitative and quantitative composition of mixture. The gas-liquid chromatography allows to analyse the mixture of volatile substances anf their derivatives.

In recent years HPLC has been succesfully developed. It is a variant of column chrromatography. The eluent (mobile phase) moves at high speed through the column due to the high pressure. This type of chromatography is a convenient for the separation, preparative isolation and qualitative and quantaticw analyses of nonvolatile thermolabile compounds. The common and specific reagents are used for the qualitative analysis of active substances or individual components. The most convient method for the deteciton is paper and thin layer chromatography. On chromatograms the active substances are displayed after vision in UV-light (flavonoids, coumarins and oth.) or or after treating with specific reagents (alkaloids, saponins, amino acids, etc.). There are opportunities for the identification of dominating substances by the characteristic fluorescence or the colour with reagents, R value, and by comparison with standard samples.

The methods based on the chemical and physical properties of analysed compounds are used for quantitative analysis. The main requirements of these methods of analysis are accuracy and sensitivity.

 Classical qunatative analysis is divided into gravimetric and titrimetric methods. The optical methods are often used, but the electrochemical methods are rarely used.

*Gravimetric analysis*is the quantitative isolation of a substance by precipitation from various solvents and weighing of the precipitate using analytical scales.

*Titrimetric (volumetric) methods* are various and depend on the chemical properties of analysed compounds. The direct and back titration are used for this purpose.  The titrimetric methods are based acid-base, oxidation-reduction reactions, precipitation reaction and formation of complex compounds. There are titrimetric methods by oxidants – permanganatometry and iodometry.

Optical methods include photometry, fluorometry, densitometry with use of paper and thin layer chromatography and polarymetry.

Photometric analysis is based on the measurement of optical density of analyzed solution in ultraviolet, visible and infrared spectra. Photocolorimetry and spectrophtometry are often used for the quantitative determiantion of some natural compounds in material and medication.

Spectrophotometric analysis is the absorption of monochromatic radiation with a particular wavelength by a substance. It is necessary to follow the Bouguer-Lambert-Beer law in quantitative determination of solutions by this method. Photocolorimetric methods of analysis are based on measuring the absorbance of non-monochromatic light of compounds in the visible part of the spectrum.

The concentration of substances in solution by the photometry is carried out by 3 methods - calculation by the molar absorbance coefficient; determination of the concentration of the analyzed compound by comparing the values of the optical densities of its and the standard solution and the calibration curve.

Fluorescence analysis is  to measure the integrated intensity of the light emitted by a luminescent probe. This is the most sensitive method of analysis of coumarins, flavonoids, anthraquinones.

Polarimetry is based on the ability of substances to rotate the plane of polarization. It is possible to determine only optically active compounds (for example, alkaloids, terpenoids, glycosides, etc.) by this method. Commonly used electrochemical methods for raw material analuysis are potentiometric titration and polagraphy. The methods of analysis based on physical properties include the method of distillation or steam distillation of volatile substances.

 **Luminescence analysis**

Luminescence is determined in solutions with a concentration of 10̄̄̄҅⁵-10̄҅6 mol / l. This method is a set of methods of analysis based on the observation of luminescence. The method is widely used in pharmacy, since fluorescent substances are often found in medicinal plant materials and among medicinal preparations. It is found that most alkaloids fluoresce in the solid state. For example, tropane alkaloids: hyoscyamine - fluoresce red-violet light, scopolamine - blue. The alkaloid strychnine gives blue-green fluorescence, berberine - golden yellow. Bright fluorescence is characteristic for anthracene derivatives contained in the bark of buckthorn, rhubarb, horse sorrel, madder, for flavonoids, coumarins and some other organic and inorganic compounds. Vitamin B2 (riboflavin) has a bright fluorescence. Its neutral solutions in water and alcohol give yellow-green light fluorescence. When the substances of medicinal plants do not luminesce, they are treated with detection reagents.

Fluorometry and spectrofluorometry are used for carrying out the analysis.

**BIOLOGICAL METHOD OF ANALYSIS**

Biological analysis is used in cases when the quality of medicinal raw materials can not be determined by chemical or physical methods. This method, in particular, is determinant for the analysis of medicinal plant material containing cardiotonic glycosides.

This method allows to determine the effect of the test materials in experimental animals: frogs, guinea pigs, cats, pigeons. The result is expressed in units of action (usually the lowest dose that causes a specific physiological effect). The activity of raw materials is expressed in units of 1 g of raw materials. This method is used in pharmaceutical laboratories and further described in the course of pharmacology.

**MERCHANDISING ANALYSIS**

Merchandising analysis is to determine the purity and the purity of medicinal raw materials. Raw examine the content of impurities and the degree of damage to pests. Impurity called foreign objects falling into a raw material in the process of harvesting. Among them may be unnecessary parts of the same plant, for example in sheet raw material is part of the stems, flowers and fruit in the grass - stalks with roots, thick lignified part of the raw materials. In the fruits and seeds are mixed unripe, broken seeds, rotten fruit, bound together in clumps, part of the peduncles, stems. In the roots and rhizomes may be part of the stems, too thin or woody roots in the cores - pieces of twigs, wood, etc.

When careless collecting, drying, storage for raw materials will be exposed to foreign plants, captured by chance, various organic litter - hay, straw and mineral admixtures - sand, stones, earth.

If you are too slow and irregular drying raw materials may change color, part of his dark, brown, together with the changes their chemical composition.

From entering the laboratory average sample Weigh average sample for merchandising analysis. Size medium-sized samples for each object set GFH and depends on the morphology of raw materials. Thus, on average taking 100 g of berries, flowers and seeds - 200 g, herbs, roots, rhizomes - 400 g, bark - 500

The analysis begins with the definition of minced raw materials, for which sample was sifted through a sieve with the big holes. Too much grind spoils the appearance of raw materials and reduces its quality. The balance hinges after determining chopped examine the content of permissible impurities. This sample was poured on analyzable board, large sheet of glossy paper, oilcloth, or other plate, and dismantle it piece by piece in a row by hand or using special wooden spatulas. Each kind of impurity allocate separately, weighed and calculated the percentage.

To determine the degree of destruction of raw materials pests in the dropout rate obtained in the determination of shredded them by hand or sift through a sieve, and then add up the number.

Important indicators for assessing the quality of raw materials are moisture and ash content. Ash content is called fireproof residue obtained after combustion and calcination of raw materials. This balance includes all elements of the plant ash and extraneous mineral admixtures (ground, stones, sand), falling in the raw material in the collection. Also determine the ash insoluble in 10% hydrochloric acid, which is called the "sand", as her form mineral impurities, while natural ash is always soluble.

Non-standard raw materials, depending on the defect sent for recycling, removal of impurities, with excessive chopped object can be sent to galen plant. Raw materials, does not meet the standard requirements, destroyed. The results will be recorded and conclude about the purity of raw materials.

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