**Lesson 2**

**Physical methods of analysis.**

A relatively large number of dosage forms used in modern pharmacy indicates the need for their preliminary systematization and the creation of a rational classification of dosage forms.

There are different classification systems for dosage forms based on different principles:

1) Classification according to the state of aggregation. All dosage forms are divided into 4 groups: solid, liquid, soft, gaseous.

Solid dosage forms: collections, powders, tablets, mustard plasters, capsules, etc.

Liquid dosage forms: solutions, suspensions, emulsions, drops, infusions, decoctions, potions, lotions, etc.

Soft dosage forms: ointments, patches, suppositories, gelatin capsules, pastes.

Gaseous LF: gases, vapors, aerosols.

The classification according to the state of aggregation is the oldest, it is convenient for the primary separation of LF. The state of aggregation partially determines the rate of action of the drug and, to a certain extent, is associated with certain technological processes.

As is known, pharmacopoeial analysis aims to establish the authenticity, determine the purity and quantify the active substance or ingredients of a complex dosage form. The determination of some constants - melting point, density, specific absorption rate, allows you to simultaneously draw a conclusion about the authenticity and purity of a given substance. Since the methods for determining certain constants for various preparations are identical, we study them in the general methods of analysis. Knowledge of the theoretical foundations and the ability to carry out the definition will be required in the subsequent analysis of various groups of drugs.

Pharmacopoeial analysis is an integral part of pharmaceutical analysis and is a set of methods for studying medicines and dosage forms set forth in the State Pharmacopoeia and other normative documents (FS, FSP, GOST) and used to determine authenticity, purity and quantitative analysis.

In the quality control of medicines, physical, physico-chemical, chemical and biological methods of analysis are used.

The physical methods of analysis include.

• description;

• solubility;

• physical constants (melting, boiling or distillation point, refractive index, specific rotation, density, spectral characteristics);

• transparency and color of solutions;

• acidity or alkalinity, solution pH;

• weight loss on drying;

• sulfate ash;

A private monograph for any drug begins with the "Description" section, which mainly describes the physical properties of the substance:

• state of aggregation (solid, liquid, gas), if it is a solid, then the degree of its dispersion (fine-crystalline, coarse-crystalline), the shape of crystals (acicular, cylindrical) is determined

• the color of a substance is an important indicator of authenticity and purity. Most drugs are colorless, that is, they are white. Coloring visually when determining the state of aggregation. A small amount of the substance is placed in a thin layer on a Petri dish or watch glass and viewed against a white background. In SP X1 there is an article "Determination of the degree of whiteness of powdered drugs." The determination is carried out by an instrumental method on special photometers "Specol-10". It is based on the spectral characteristic of the light reflected from the drug sample. The so-called reflection coefficient is measured - the ratio of the magnitude of the reflected light flux to the magnitude of the incident. The measured reflectances make it possible to determine the presence or absence of a color or grayish tint in substances by calculating the degree of whiteness (α) and the degree of brightness (β). Since the appearance of shades or a change in color is, as a rule, a consequence of chemical processes - oxidation, reduction, then already this initial stage of the study of substances allows us to draw conclusions.

The smell is rarely determined immediately after opening the package at a distance of 4-6 cm. No smell after opening the package immediately according to the method: 1-2 g of the substance is evenly distributed on a watch glass with a diameter of 6-8 cm and after 2 minutes the smell is determined at a distance of 4-6 cm .

 In the "Description" section, there may be indications of the possibility of changing substances during storage. For example, in the preparation of calcium chloride, it is indicated that it is very hygroscopic and blurs in air, and sodium iodide - in the air it becomes damp and decomposes with the release of iodine, crystalline hydrates, in case of weathering or non-compliance with the conditions of crystallization in production, will no longer have the desired appearance. in the form of crystals, nor in color.

Thus, the study of the appearance of a substance is the first, but very important step in the analysis of substances, and it is necessary to be able to relate changes in appearance with possible chemical changes and draw the right conclusion.

Solubility.

Solubility is an important indicator of the quality of a drug substance. As a rule, a certain list of solvents is given in the ND, which most fully characterizes this physical property, so that in the future it can be used to assess the quality at one stage or another of the study of this medicinal substance. Thus, solubility in acids and alkalis is characteristic of amphoteric compounds (zinc oxide, sulfonamides), organic acids and bases (glutamic acid, acetylsalicylic acid, codeine). The change in solubility indicates the presence or appearance during storage of less soluble impurities, which characterizes the change in its quality.

By solubility is meant not a physical constant, but a property expressed by approximate data and serving for an approximate characterization of preparations.

Along with the melting point, the solubility of a substance at constant temperature and pressure is one of the parameters by which the authenticity and purity (good quality) of almost all drugs are established.

It is recommended to use solvents of different polarity (usually three); the use of low-boiling and flammable (diethyl ether) or very toxic (benzene, methylene chloride) solvents is not recommended.

There are two ways to express solubility:

1. In parts (ratio of substance and solvent). For example, for sodium chloride according to FS, the solubility in water is expressed in a ratio of 1:3, which means that no more than 3 ml of water is needed to dissolve 1 g of a medicinal substance.

2. In conventional terms. For example, for sodium salicylate in PS, solubility is given in conditional terms - “we will very easily dissolve in water”. This means that up to 1 ml of water is needed to dissolve 1 g of a substance.

Table 1

Conditional terms of solubility

|  |  |  |
| --- | --- | --- |
| Условные термины | Сокращения | Количество растворителя (мл),необходимое для растворения 1гвещества |
| Очень легко растворим | оч. л. р. | до1 |
| Легко растворим | л. р. | Более 1 до 10 |
| Растворим | р. | » 10 до 30 |
| Умеренно растворим | ум. р. | » 30 до 100 |
| Мало растворим | м. р. | » 100 до 1000 |
| Очень мало растворим | оч. м. р. | » 1000 до 10000 |
| Практически не растворим | пр. н. р. | » 10000 |

The conditional term corresponds to a certain interval of solvent volumes (ml), within which one gram of the medicinal substance should be completely dissolved.

The dissolution process is carried out in solvents at a temperature of 20°C. In order to save the medicinal substance and the solvent, the mass of the drug is weighed in such a way (with an accuracy of 0.01 g) that no more than 100 ml is spent on establishing the solubility of water, and no more than 10-20 ml of organic solvents.

A medicinal substance (substance) is considered soluble if no particles of the substance are detected in the solution when observed in transmitted light.

Methodology. (1 way). The weighed mass of the drug, previously ground into a fine powder, is added to the measured volume of the solvent corresponding to its minimum volume, shaken. Then, in accordance with Table. 1, the solvent is gradually added to its maximum volume and continuously shaken for 10 minutes. After this time, particles of the substance should not be detected in the solution with the naked eye. For example, 1 g of sodium benzoate is weighed, placed in a test tube with 1 ml of water, shaken and 9 ml of water are gradually added, because. sodium benzoate is easily soluble in water (from 1 to 10 ml).

For slowly soluble drugs requiring more than 10 minutes for complete dissolution, heating in a water bath to 30 ° C is allowed. Observation is carried out after cooling the solution to 20°C and vigorous shaking for 1-2 minutes. For example, caffeine is slowly soluble in water (1:60), codeine is slowly and slightly soluble in water (100-1000), calcium gluconate is slowly soluble in 50 hours of water, calcium lactate is slowly soluble in water, boric acid is slowly soluble in 7 hours glycerin.

2 way. Solubility, expressed in parts, indicates the volume of solvent in ml required to dissolve 1 g of a substance.

Methodology. (Method 2) The mass of the medicinal product weighed on a manual scale is dissolved in the volume of the solvent indicated by the RD. Particles of undissolved substance should not be detected in the solution.

Melting point (T°pl)

The melting point is a constant that characterizes the purity of a substance and at the same time its authenticity. It is known from physics that the melting point is the temperature at which the solid phase of a substance is in equilibrium with the melt. A pure substance has a clear melting point. Since drugs can have a small amount of impurities, we will no longer see such a clear picture. In this case, the interval at which the substance melts is determined. Typically, this interval lies within 2°C. A longer interval indicates the presence of impurities within unacceptable limits.

According to the formulation of GF X1, the melting point of a substance is understood as the temperature interval between the beginning of melting (the appearance of the first drop of liquid) and the end of melting (complete transition of the substance into a liquid state).

If the substance has an indistinct beginning or end of melting, only the temperature of the beginning or end of melting is determined. Sometimes a substance melts with decomposition, in which case the decomposition temperature is determined, that is, the temperature at which a sharp change in the substance occurs (for example, foaming).

Methods for determining the melting point

The choice of method is dictated by two points:

 stability of the substance when heated and

 ability to be ground into powder.

Methods involve the use of 2 devices:

• PTP (device for determining Tm): familiar to you from the course of organic chemistry, allows you to determine the Tm of substances in the range from 20◦С to 360◦С

• A device consisting of a round-bottom flask with a test tube sealed into it, into which a thermometer is inserted with a capillary attached to it containing the starting substance. The outer flask is filled with ¾ of the volume of the coolant liquid:

 water (allows you to determine Tmelt up to 80◦С),

 vaseline oil or liquid silicones, concentrated sulfuric acid (allows you to determine Tm up to 260 ° C),

 a mixture of sulfuric acid and potassium sulfate in a ratio of 7:3 (allows you to determine Tm above 260°C)

The technique is general, regardless of the device.

Finely ground dry matter is placed in a medium-sized capillary (6-8 cm) and introduced into the device at a temperature 10 degrees lower than expected. By adjusting the rate of temperature rise, the temperature range of changes in the substance in the capillary is fixed. At the same time, at least 2 determinations are made and the arithmetic mean is taken.

Tm is determined not only for pure substances, but also for their derivatives - oximes, hydrazones, bases and acids isolated from their salts.

Distillation Temperature Limits (Boil Temperature)

The GF value is defined as the interval between the initial and final boiling points at normal pressure (101.3 kPa - 760 mm Hg). The interval is usually 2°.

Under the initial T ° boil. understand the temperature at which the first five drops of liquid were distilled into the receiver.

Under the final - the temperature at which 95% of the liquid passed into the receiver.

A longer interval than indicated in the corresponding API indicates the presence of impurities.

The device for determining the CCI consists of

• a heat-resistant flask with a thermometer into which liquid is placed,

• refrigerator and

• receiving flask (graduated cylinder).

The TPP observed in the experiment lead to normal pressure according to the formula:

Tisp \u003d Tnabl + K (p - p1)

Where: p - normal barometric pressure (760 mm Hg)

 p1 - barometric pressure during the experiment

 K - increase in Tbp per 1 mm of pressure

Thus, by determining the temperature limits of distillation, the authenticity and purity of ether, ethanol, chloroethyl, and halothane are determined.

Density

Density is the mass per unit volume of a substance. Expressed in g/cm3.

ρ = m/V

If the mass is measured in g, and the volume is in cm3, then the density is the mass of 1 cm3 of a substance.

Density is determined using a pycnometer (up to 0.001). or hydrometer (measurement accuracy up to 0.01)

See the device of devices in the GF X1 edition.

Density determination with a pycnometer

Pycnometers are cones with a long narrow neck, on which a ring mark is applied. On the flask, its volume is indicated, as a rule it is 5, 10 ml. They are made of thin glass and are designed to determine the density of solutions.

Method 1. The determination is carried out using a pycnometer with an accuracy of 0.001. A clean dry pycnometer is weighed to the nearest 0.0002 g, filled with purified water slightly above the mark with a small funnel, closed with a stopper and kept for 20 minutes. in a thermostat, which maintains a constant water temperature of 20°C with an accuracy of 0.1°C. At this temperature, the water level in the pycnometer is brought to the mark, quickly removing excess water with a pipette or a strip of filter paper folded into a tube. The pycnometer is again closed with a cork and kept in a thermostat for another 10 minutes, checking the position of the meniscus in relation to the mark. Then the pycnometer is removed from the thermostat, the inner surface of the neck of the pycnometer is wiped with filter paper, as well as the entire pycnometer outside. Leave under glass of analytical balance for 10 min. and weighed with the same precision.

The pycnometer is freed from water, dried, rinsing successively with alcohol and ether. (Drying the pycnometer by heating is not allowed). The remaining ether is removed by blowing air, the pycnometer is filled with the test solution, and then the same operations are performed as with purified water. Density – ρ20 (g/cm3)

Density when determined by a pycnometer is calculated by the formula:

|  |  |  |
| --- | --- | --- |
| ρ20 = | (m2 – m)\*0.99703 | + 0.0012 |
| m1 - m |
|  |  |  |

m is the mass of the empty pycnometer in grams

m1 - its mass with purified water in grams

m2 - its mass with the investigated liquid in grams

0.99703 – density of water at 20◦С

0.0012 – air density at 20◦С

Determination of density using a hydrometer

The hydrometer is a glass thin-walled, cylindrical vessel, expanding at the bottom and having a glass tank at the end, filled with shot, less often with mercury. At the top of the hydrometer there is a scale with divisions corresponding to the density of the liquid and an indication of the temperature at which the determination should be made. There are hydrometers for liquids lighter and heavier than water, for sulfuric acid, caustic alkalis, as well as a number of special hydrometers for measuring the density of alcohol (spiritometer), milk (lactomer).

Method 2. Density is determined with a hydrometer with an accuracy of 0.01. The liquid to be tested is placed in a cylinder and, at a liquid temperature of 20°C, a clean, dry hydrometer is carefully lowered into it, on the scale of which the expected density value is provided. The hydrometer is not released from the hands until it becomes obvious that it is floating, while it is necessary to ensure that the hydrometer does not touch the walls and bottom of the cylinder. The countdown is made after 3-4 minutes. after immersion according to the division on the hydrometer scale corresponding to the lower meniscus of the liquid. The reading of the density values of dark-colored liquids is carried out along the upper meniscus (when reading the eye should be at the level of the meniscus).

Method Limitations

• it is impossible to determine the density of highly volatile substances;

• low accuracy;

• the need to use a relatively large amount of analyzed liquid.

 Certain intervals of density values serve to confirm the authenticity and purity of ethanol, glycerin, vaseline, vaseline oil, chloroethyl, halothane, etc.

Viscosity (internal friction)

Viscosity is the property of fluid bodies to resist the movement of one part of them relative to another.

Viscosity types:

• Dynamic

• Kinematic

• Relative - the ratio of the viscosity of the investigated fluid to the viscosity of another fluid:

ηrel = ηх / ηо = tx ρx/ toо ρо

ηх , ηо are the viscosities of the studied and standard liquids

tx , tо – outflow time of the test and standard liquids

ρx , ρо are the densities of the corresponding liquids

• Specific

• Reduced

• Characteristic

The determination of viscosity is carried out on various types of viscometers - capillary, rotational and falling ball viscometers. To assess the quality of liquid preparations having a viscous consistency (vaseline oil, glycerin), the relative viscosity is usually determined.

Acidity, alkalinity, pH.

Certain information about the degree of purity of drugs is given by the pH value of solutions. According to this indicator, one can judge the presence of impurities of an acidic or basic nature.

The definition is carried out in several ways:

• By changing the color of acid-base indicators (approximate pH value).

• Tirimetrically by acid-base titration (quantitatively)

Indicators are known to be electrolytes that exist in two tautomeric forms. Depending on the concentration of hydrogen ions (the pH value of the medium), one of these forms predominates, which determines a certain color of the solution. However, the color of the indicator only indicates that the pH of the solution is in the range where one of the forms of the indicator dominates, but does not indicate the true value.

For example, an admixture of mineral acids in boric acid is determined using methyl red, which does not change color under the action of weak boric acid, but turns pink in the presence of mineral acid impurities.

In some cases, the content of impurities of a basic or acidic nature is quantified.

For example, to establish the permissible impurity of formic acid formed during storage of a formaldehyde solution, its alkalimetric determination is carried out. This impurity should not exceed 0.2% in the preparation.

In most cases, to characterize the acidity or basicity of solutions in the ND for medicinal substances, a section is introduced -

• Determination of pH.

Prepare solutions of a certain concentration according to the FS, in which the range of pH values is indicated.

For example, for a 5% solution of sodium sulfacyl - pH 8.5-9.5.

To determine the pH according to the Global Fund XI, vol. 1, p. 113 two methods are applied:

• potentiometric (accuracy 0.1)

• colorimetric (approximate value).

pH (hydrogen index)

is the negative decimal logarithm of the activity of hydrogen ions.

pH = - lg aH+

In practice, not activity values are used more often, but the values of hydrogen ion concentrations and pH are found by the formula

pH = - lg [H+]

For example, [H+] = 10-2, pH = 2.

Potentiometric method - determination of pH consists in measuring the EMF of a galvanic cell, consisting of two electrodes: an indicator and a reference electrode.

The difference in EMF between these two electrodes (the potential of the indicator electrode) is determined by the activity of hydrogen ions in the test solution, and the potential of the reference electrode (or standard electrode) is known.

SP XI recommends the use of glass (more often than others) and quinhydrone electrodes as indicator electrodes; the use of a hydrogen electrode is rarely allowed.

In practice, calculations are not made when determining pH, because the scale of laboratory pH meters is graduated in pH values.

Potentiometers, pH meters, ionomers are used to measure pH.

The colorimetric method for measuring pH is based on the use of indicators that change their color depending on the activity of hydrogen ions in a certain pH range (turn into a tautameric form).

For determination, a series (scale) of standard buffer solutions (5-6) is prepared with an interval of 0.2 pH or 0.1 pH. To an equal volume of the test and all buffer solutions of the scale, add the same volume of the indicator solution and compare the color. The pH value of a buffer solution equal in color corresponds to the pH of the test solution.

Precise determination is preceded by an approximate determination of pH using indicator paper or an indicator with a wide transition area. The indicator is selected so that the expected pH value falls into the central part of the indicator color transition interval and a series of standard buffer solutions.

Advantage of the method:

• gives less accuracy, but does not require the use of a device.

Disadvantages of the method:

• the determination is carried out visually and an error may be present,

associated with the subjective nature of the choice;

• it is impossible to use it in colored and turbid solutions, in

the presence of oxidizing or reducing agents, other substances,

capable of interacting with the indicator.

OFS GF XII "Ionometry" describes the determination of the concentration of ions in a solution using ion-selective electrodes, while the potentiometric determination of pH is a special case of determining the concentration of ions.

Applications in pharmaceutical analysis:

1. identification of substances

2. definition of purity

Determination of volatile substances and water

Volatile substances can get into the LP either due to insufficient purification from solvents and intermediate products during the production process, or as a result of the accumulation of decomposition products.

Water in medicinal substances can be contained in the form of capillary, absorption-bound, chemically bound (hydrated and crystalline) or free.

 "Water Determination", describes three methods for the determination of volatiles and water.

1. Drying method (applicable both to determine water and volatile substances): by the difference in the weight of the substance before and after drying. First weighing after 2 hours, then every hour. Drying is carried out to constant weight in an open bottle. Conditions are specified in private FS.

2. distillation (distillation) method - (applicable only to determine water): the substance is placed in a special device, toluene or xylene is added to it (liquids that do not mix with water, and, at the same time, are lighter than it), distillation is carried out. Water is collected in a graduated receiver, on top of which there is an organic solvent (this prevents the water from evaporating). When the volume of water ceases to increase, distillation is stopped and the volume of distilled water is noted.

3. method of titration with Fisher's reagent: this is a chemical aquametry method that makes it possible to quickly and accurately determine any amount of water in organic and inorganic substances (both hygroscopic and crystallization), in volatile substances - the advantages of the method. The device for the determination is a closed system isolated from the external environment - the disadvantage of the method: a buret, a vessel for supplying a reagent, a flask for titration. Fisher's reagent is a solution of sulfur dioxide, iodine and pyridine in methanol:

SO2 + I2 + + CH3OH

The method is based on the property of iodine to interact with sulfur dioxide only in the presence of water in two stages. The reaction products (H2SO4 and HJ) are bound by pyridine, which quantitatively shifts the equilibrium to the right.

H2O + SO2 + I2 + 3C5H5N →2C5H5N · HI + C5H5NSO3

C5H5NSO3 + CH3OH → C5H5N ⋅ HSO4CH3





The end of the titration is determined

• either visually by the transition of the color of the solution (from yellow to red-brown),

• or electrometrically “until the current stops completely”.

Restriction: the method cannot be used to determine water in substances that react with the components of the Fisher reagent (ascorbic acid, aldehydes, ketones, etc.).