**Lesson 1**

**Methods for the quantitative determination of medicinal substances.**

**Quantitative analysis of drugs.**

The final step in the pharmaceutical analysis of a drug substance is quantitation. It is performed after the drug substance has been identified and the presence of an acceptable amount of impurities has been established. The choice of the optimal method of quantitative determination is determined primarily by its ability to evaluate the medicinal substance by the physiologically active part of the molecule. In practice, this is difficult to do. Usually, the quantitative content of the drug is determined by some of its chemical properties associated with the presence of a particular functional group.

Four groups of methods are used for the quantitative analysis of medicinal substances: chemical, physical, physicochemical and biological.

General requirements for methods of quantitative determination:

1. High sensitivity and specificity of the main reaction.

2. Simplicity and accessibility of the technique.

3. Availability in practice of used reagents.

4. Speed of execution.

5. The interaction of the analyte with the titrant should proceed stoichiometrically, to the end.

6. Possibility of fixing the equivalence point.

7. Minimum consumption of reagents and sample.

8. Accuracy of the method.

9. No influence of impurities, fillers, solvents in the analysis.

Quantitative determination is based on the physicochemical and biological properties of drugs.

Analysis methods:

— physical

– chemical

— physical and chemical

— biological

The choice of the optimal method of chemical determination is determined primarily by its ability to evaluate the medicinal substance by the physiologically active part of the molecule. Almost all ready-made forms now have high-performance liquid chromatography. On the one hand, this is an undoubted progress, since the method reliably allows one to identify an individual substance, on the other hand, the method is relative, expensive, and unsuitable for on-line analysis. Sufficiently reliable in-line analysis is possible when using one chromatograph for one type of analysis, and even then, subject to a number of requirements.

The method of subdivision into physical and physico-chemical methods of analysis is rather conditional. Purely physical methods include methods based on the properties associated with the state of aggregation of a substance:

1. The melting point of a substance. The melting temperature, as we have already said, determines the nature of the crystal lattice and other properties of the substance. The method is of little use for quantitative determination, although it is possible in some cases to estimate the quantitative content of a substance from the melting point. More often it is used for polymeric substances in order to determine the limit of polymerization.

2. Boiling point. The use of such an indicator for determining the quantitative content of a substance is of little use, more often it is used for qualitative analysis and authenticity testing.

3. The density of matter. The density of a substance, in principle, can be used for quantitative determination, however, the accuracy of this method is low and at present it is not used in the pharmacopoeia for the quantitative determination of medicinal substances.

Among the physical methods, a certain niche is occupied by optical methods of non-destructive testing. This group includes methods based on determining the refractive index of a light beam in a solution of the test substance (refractometry), measuring the interference of light (interferometry), the ability of a substance solution to rotate the plane of a polarized beam (polarimetry).

Refractometry. The method is mainly used to determine the authenticity of liquid medicinal substances (nicotinic acid diethylamide, tocopherol acetate, methyl salicylate), in intra-pharmacy control of dosage forms. Including the analysis of double and ternary mixtures. It is very rarely used for quantitative determination: volumetric refractometric analysis and refractometric analysis by the method of complete and incomplete extraction. For example, it is used when other methods fail to obtain a stable result.

Interferometry is an exotic method and is rarely used.

Polarimetry. Used to test the authenticity of medicinal substances in the molecules of which there is an asymmetric carbon atom. In pharmaceutical chemistry, there are few examples of the use of polarimetry in quantitative analysis, and then only when the ratio of optical isomers is regulated.

X-ray spectral methods of analysis. They can be used quite successfully in the presence of heavy elements in the molecule (cobalt, gold, platinum, etc.). In practice, such methods are not found in pharmaceutical analysis, however, so far. Probably in the future it is possible to create compact and cheap devices suitable for pharmaceutical analysis.

Physical and chemical methods of analysis.

1. Methods based on the absorption of radiation (absorption methods).

Absorption methods are based on the properties of molecules or atoms to absorb radiation of a certain frequency.

Atomic absorption spectrophotometry is based on the use of ultraviolet or visible radiation of resonant frequency. Absorption is associated with the transition of outer shell electrons to an excited level, which is characterized for each specific atom or molecule by a certain frequency. Objects that absorb radiation are atoms in the gaseous state (less often molecules). The essence of the method is the transmission of light through a cloud containing atoms or ions of the test substance. This cloud is created either by introducing a solution of a substance into the flame of a burner with a high-temperature flame (acetylene with oxygen, nitrous oxide with hydrogen, etc.), or through a special device that converts the solution into a vapor state.

Ultraviolet and visible spectrophotometry. The simplest and most widely used method of analysis in pharmacy. It is used at all stages of pharmaceutical analysis (testing for purity, authenticity, quantification), but the most reliable data is obtained when using the method for the quantitative determination of medicinal substances. The absorption process is due to the electronic transitions of the electrons involved in the valence bonds of the molecules and ultimately reflects the properties of the entire molecule as a whole. Good objects for spectrophotometry are aromatic and heteroaromatic compounds.

Differential methods make it possible to expand the scope of spectrophotometry in pharmaceutical analysis. They allow to increase its objectivity and accuracy, as well as to analyze high concentrations of substances. In addition, these methods can be used to analyze multicomponent systems without their preliminary separation.

New possibilities in the field of identification and quantification are opened up by the use of derivative UV spectrophotometry. The method is based on the selection by mathematical methods of individual bands from the UV spectrum, which is the sum of overlapping absorption bands or bands that do not have a clearly defined maximum. The accuracy of this method, however, is significantly lower, but it allows one to analyze mixtures without high costs and complex extractions.

A variation of spectrophotometry is photocolorimetry - a method for the quantitative determination of substances in the visible region. The method is based on the study of either colored compounds or the analysis of derivatives, for example, complexes into which the test compound is transferred. The method is still used for quantitative determination (furatsilin, furadonin), it is also used for the determination of substances in dosage forms.

Infrared (IR) spectroscopy. The essence of the method lies in the absorption of radiation in the IR region (from 200 to 4000 cm-1) by a substance molecule. IR spectra arise as a result of transitions between the vibrational levels of the ground electronic state of the system under study. Depending on this, there are deformation, stretching vibrations. The region of deformation vibrations is called the region of fingerprints, it is by it that substances are identified. IR spectroscopy is used almost exclusively for qualitative analysis and identification of the authenticity of substances. This is due to the fact that, as a rule, IR spectra are taken for substances that are in a crystalline state or in the form of a suspension in perfluoro-vaseline oil or in a tablet with KBr. Solution IR spectroscopy is limited to a few solvents and is rarely used. At the present stage, Fourier transform IR spectroscopy has begun to be quite successfully used, which makes it possible to determine a number of parameters, including the concentration of water, the content of the main substance, and, in the presence of a standard sample, to determine the concentration of impurities practically without sample preparation. This method is one of the most promising non-destructive testing methods.

Methods based on the emission of radiation.

This group of methods includes flame photometry, fluorescence, and radiochemical methods. Fluorescent methods are based on the ability of certain substances to fluoresce when exposed to UV radiation. This ability is due to the structure of either the organic compounds themselves or the products of their dissociation, solvolysis and other transformations caused by the action of various reagents. The intensity of fluorescence depends on many factors, including the concentration of the substance, though only at sufficiently low concentrations.

Fluorimetry can be used for both quantitative and qualitative analysis. Quantitative analysis is performed on spectrofluorimeters.

A variation of fluorescence is chemiluminescence - a method that uses the energy that occurs during a chemical reaction. This energy serves as a source of excitation. It is emitted during oxidation by some barbiturates (especially phenobarbital), aromatic acid hydrazides, and some other compounds. The method is little used in pharmaceutical chemistry, but it allows the determination of very low concentrations of substances in biological material.

Electrochemical methods.

Potentiometry is a method based on measuring the equilibrium potentials that arise at the boundary between the test solution and an electrode immersed in it. The method of potentiometric titration is used, which consists in establishing the equivalent volume of the titrant by measuring the EMF of the indicator electrode and the reference electrode immersed in the analyzed solution.

A variation of this titration is amperometric titration with two indicator electrodes, which are under a small voltage. The method is often used for nitrite and iodometric titrations.

Among electrochemical methods, polarographic methods stand apart - the method measures the current strength. Occurring on the microelectrode during electroreduction or electrooxidation of the analyte in solution. For quantitative determination, polarography was used in the analysis of cardiac glycosides, some vitamins.

Separation methods.

From this group of methods in pharmaceutical analysis, chromatography, electrophoresis and extraction are used.

Chromatographic methods for the separation of substances are based on their distribution between two phases: mobile and stationary. According to the mechanism of the separation process, chromatographic methods are classified into ion-exchange, adsorption, sedimentary, partition, redox chromatography.

Adsorption chromatography is based on the selective adsorption of individual components from a solution of a mixture of substances. Alumina, silica gel, microcrystalline cellulose, etc. serve as the stationary phase.

Ion exchange chromatography uses ion exchange processes occurring between the adsorbent and electrolyte ions. The stationary phase is ion-exchange resins.

For quantitative determination in pharmaceutical analysis, adsorption - TLC or paper chromatography is used.

Electrophoresis. This method is included as a qualitative and quantitative analysis method. It is used most often for the analysis of complex protein molecules. In practice, combined methods of immunoelectrophoresis and the method of peptide maps are used.

Gas-liquid chromatography. Without dwelling especially on this method, there will be a separate lecture, it should be noted that this is an accessible method for the analysis of volatile substances or their derivatives. The advantage of the method is the combination of identification of the medicinal substance, assessment of its purity and impurities, and quantitative determination.

HPLC. High performance liquid chromatography is a typical example of partition chromatography. This is one of the most widely used methods for quantitative determination both in substances and in finished forms. It is also used for the identification of a substance and the determination and identification of impurities. UV, fluorimetric, electrochemical, mass spectrometric detectors are used as detectors.

Thermal analysis methods, which include thermogravimetry, derivatography, differential scanning calorimetry, are most often used to analyze moisture content, study the equilibrium state of liquid-crystals, especially when the crystallography index of a substance is important. For quantitative analysis, the methods are of little use and are not practically used.

Biological methods of analysis. Methods for the quantitative determination of the active substance by biological methods are based either on their physiological effect on the animal organism, or on the effect on test microorganisms. Biological methods are used when it is not possible to draw a conclusion about the purity or amount of the active substance or the amount of active substances using physicochemical or chemical methods. An example is some antibiotics, erythromycin, kanamycin, total extracts of cardiac glycosides, toxic substances such as snake or bee venom. So the toxicity of bee venom is carried out on pigeons and is indicated in pigeon units. Biological evaluation of the effectiveness of digitalis, adonis, strophanthus preparations is carried out on frogs, cats or pigeons and expressed, respectively, (ICE) frog action units, (CED) cat action units or pigeon action units (GED). Antibiotics that cannot be characterized by physicochemical methods, or antibiotics containing several active substances (tobramycin, kanamycin) are analyzed by the zone of growth inhibition of test microorganisms on a solid nutrient medium. Biological methods are also used to evaluate the pyrogenicity of drug solutions.

Chemical methods of quantitative determination.

Gravimetric (weight) method. The method is used mainly for inorganic compounds, rarely for the quantitative determination of some alkaloids in the form of picrates or silicotungstates and vitamins (for example, thiamine bromide and rutin).

titrimetric methods.

Titrimetric (volumetric) methods of analysis are based on an accurate measurement of the amount of a reagent (titrant) consumed in a reaction with a certain substance. During titration, titrant is added in small portions to a solution containing a precisely known mass (weight) of the analyte. After adding each new portion of the titrant in the system described by the chemical reaction equation, an equilibrium is established:

Reactions used in titrimetry must meet the following basic requirements:

- the reaction must proceed quantitatively, that is, the equilibrium constant of the reaction must be sufficiently high;

— the reaction must proceed at a high rate;

— the reaction should not be complicated by side processes;

— There must be a way to determine the end point of the titration.

If a reaction does not satisfy at least one of these requirements, it cannot be used in titrimetric analysis.

In titrimetry, there are three methods of titration: direct, reverse and indirect (substitutive).

In direct titration, analyte A reacts directly with titrant B:

In Back titration, an excess of titrant B is added to the analyte, the unreacted residue of which is titrated with titrant D:

A + B = C

Excess

B + D = E

In INDIRECT (substitutive) titration with titrant B, the product of the intermediate reaction G of the analyte A reacts with the auxiliary reagent F:

A + F = G

G + B = K

For titration, titrimetric methods use solutions of exactly known concentration, called TITRANTS or TITRATING SOLUTIONS. The concentration of a titrated solution is denoted by the terms MOLAR, NORMAL, TITER or TITTER FOR THE SUBSTANCE TO BE DETECTED.

Titration methods used in pharmaceutical chemistry are usually divided into:

1. Acid-base titration (in aqueous and non-aqueous media);

Alkalimetry, acidimetry in aqueous and non-aqueous media

2. Oxidation-reduction methods (redoxmetry);

Permanganatometry, bichromatometry, cerimetry, iodometry, iodine chlorometry.

3. Methods of precipitation titration;

Argentometry, mercurymetry, mercurometry, thiocyanatometry.

4. Complexometric titration;

5. Nitritometry.

6. Method of titration in a non-aqueous medium.

7. Complexometry.