Ministry of Health of the Russian Federation
Volgograd State Medical University

Department of Pharmaceutical and Toxicological Chemistry

GENERAL PHARMACEUTICAL CHEMISTRY

CHEMICAL METHODS FOR THE ANALYSIS OF PHARMACEUTICAL SUBSTANCES

Lesson 1
V term

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Discipline

GENERAL PHARMACEUTICAL CHEMISTRY

LESSON №1 Chemical methods for the analysis of pharmaceutical substances.	Chemical methods for the analysis of pharmaceutical substances. Classification of methods. Analysis criteria.

QUESTIONS FOR THE LESSON

1. What is pharmaceutical analysis and what is it for?
2. What are the objects of pharmaceutical analysis?
3. What does a qualitative analysis consist of?
4. What is quantitative analysis for?
5. Chemical methods for the qualitative analysis of drugs
6. Where can you conduct qualitative reactions? What does the choice of technique depend on?
7. The identification of inorganic medicinal substances
8. Identification of organic medicinal substances.
9. Identification of organoelement medicinal substances.
10. Chemical methods of quantitative analysis of drugs. Classification
11. Criteria for chemical analysis of medicinal substances. List and define.
12. Errors in pharmaceutical drug analysis
    a. Determinate Errors
    b. Instrumental Errors
    c. Personal Errors

PHARMACEUTICAL ANALYSIS

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds.

Pharmaceutical analysis is intended to either **identify** or **quantify** one or more substances in a given sample of pharmaceutical interest.

The **objects** of pharmaceutical analysis are normally active pharmaceutical ingredients (APIs), excipients, contaminants, degradation products, impurities, drug substances, and drug metabolites.

A substance to be identified or quantified is termed **analyte**.

The **samples** in pharmaceutical analysis are typically pharmaceutical ingredients, pharmaceutical preparations, or biological fluids like human blood and urine. The samples consist of one or several analytes, and a sample matrix which is defined as the rest of the sample.

Qualitative analysis includes *identification* of the active pharmaceutical ingredient and *determination of impurities* (goodness).

Identification is intended to confirm the identity of the analytes.

Quantitative analysis (also termed quantitation or quantification) is intended to measure the exact concentration or the exact amount of the analyte in a given sample.

As an example, paracetamol tablets containing 250 mg of paracetamol per tablet as the active ingredient have to be controlled prior to release from production. This is accomplished by pharmaceutical analysis. Paracetamol is the analyte, whereas the rest of the tablet, consisting of different inactive substances, is the sample matrix. Identification of paracetamol in the tablets is performed to make sure that the tablets contain the correct active ingredient, whereas quantitation is performed to measure the content of paracetamol and to check that this result is within the specification of the pharmaceutical manufacturer.

CHEMICAL METHODS FOR THE QUALITATIVE ANALYSIS OF DRUGS

The methods are based on chemical reactions. An obligatory condition for an objective test is the identification of those ions or functional groups included in the structure of molecules that determine pharmacological activity.

Since many medicinal substances contain the same ion (functional group), this makes it possible to conduct a single identification using chemical reactions and combine them into a pharmacopoeia article "General identity reactions".

Qualitative reactions can be carried out:

- ✓ on a slide;
- ✓ on filter paper;
- ✓ in a microprobe;
- ✓ in a test tube;
- ✓ on a slide under a microscope.

The choice of the technique depends on the amount of the substance, the lower limit of detection and the maximum dilution. Obviously, at a low concentration, the reaction can be negative. Therefore, it is better to use a small volume of solution, but with a high concentration of the substance.

To carry out a drip reaction on a slide, 0.03 ml of solution is enough, 0.01 ml of solution is enough to carry out the same reaction under a microscope, and up to 5 ml of solution is usually used in a test tube. It should be said that the sensitivity of the reaction is relevant in cases when a substance with high pharmacological activity and with a low dosage is analyzed.

The identification of inorganic medicinal substances

The identification of inorganic medicinal substances is an identification based on the detection by chemical reactions of cations and anions that make up their molecules.

To identify inorganic medicinal substances , use:

1. **Precipitation reactions** of anions and cations with the formation of substances insoluble in water, which can be characterized by color, solubility (in acids, alkalis, organic solvents), the ability to form complex compounds soluble in excess of reagents, etc.
2. **Redox reactions.**
3. **Reactions of neutralization and decomposition of anions** (by smell, release of oxides and dioxides).
4. **Changing the color of a colorless flame**. This test is used to detect metal cations (Li^+, Na^+, K^+, Ca^{2+},Ba^{2+}, Cu^{2+}), as well as organically bound halogens (Beilstein test).
5. **Changes occurring during heating and calcination of drugs** (pyrolysis reactions). Such changes include:
   - ✓ decomposition with color change;
   - ✓ release of gas (carbon dioxide, ammonia, hydrogen sulfide, dioxide, oxygen, nitrogen, etc.);
   - ✓ loss of crystallization water with a change in the color
   - ✓ of the calcined substance;
   - ✓ decomposition with the release of chemically bound water and by changing the composition of the substance (for example, during calcination of boric acid).

Identification of organic medicinal substances

Chemical reactions used to establish the authenticity of organic medicinal substances can be divided into three groups:

- **general reactions of organic compounds** (substitution, transformation of substituents, oxidation-reduction);
- **reactions of formation of salts of complex compounds;**

- **reactions used to identify organic bases and their salts.**

Tests are carried out using reactions to a particular functional group. In this case, the formation of a soluble or insoluble reaction product in water, changes in the color of the solution, the appearance of a characteristic odor. As reagents, both inorganic ions and complex compounds and organic substances of various chemical structures are used.

Identification of organoelement medicinal substances.

Elemental analysis is used to test substances containing atoms of sulfur, phosphorus, halogens, arsenic, bismuth, mercury in a molecule. Since the atoms of these elements in these medicinal substances are not ionized, preliminary mineralization is a necessary condition for testing their identity. As a result, the organic part of the molecule is destroyed (the conversion of carbon, hydrogen and oxygen into CO_2 and H_2O), and the above atoms form the corresponding ions, which are identified by sedimentary reactions to inorganic ions.

CHEMICAL METHODS OF QUANTITATIVE ANALYSIS OF DRUGS

Quantitative determination of medicinal substances is carried out using

- ✓ chemical,
- ✓ physical,
- ✓ physico-chemical
- ✓ biological methods.

Chemical methods have advantages, since these methods are absolute, they do not require the use of standard samples. Chemical methods are economically sound, do not require expensive equipment.

Chemical methods of quantitation of medicinal products are divided into

- ✓ gravimetric (weight)
- ✓ titrometric (volumetric).

Volumetric analysis essentially comprises of the most precise and accurate measurement of interacting solutions or reagents. It makes use of a number of graduated apparatus, such as: graduated (volumetric) flasks, burettes, pipettes and measuring cylinder of different capacities (volumes).

However, it is pertinent to mention here that quite a few techniques related to measurement of pharmaceutical substances and reagents involved is more or less common to both gravimetric and volumetric analysis.

Besides, in *gravimetric analysis*, some more additional techniques play a vital role, namely : precipitation, filtration, washing of the precipitate and ignition of the precipitate.

CRITERIA FOR CHEMICAL ANALYSIS OF MEDICINAL SUBSTANCES

1. ***Selectivity*** (specificity, selectivity) – the ability to unambiguously evaluate the determined component by the selected method independently of other substances present (impurities, decomposition products, etc.) in the test sample within a given range of application.

   For identity testing methods

   The validated technique (or a set of techniques) must provide reliable information about the presence of this active substance in a substance or medicinal form if it contains components provided for by the formulation, which is subject to experimental confirmation.

   Identity of the active substance in a pharmaceutical substance or drug is established in comparison with a standard sample or by physico-chemical or chemical properties uncharacteristic of other components.

   For methods of quantification and testing for impurities.

   The same approaches are used for the validated method of quantitative determination and testing for impurities - it must be experimentally confirmed that the presence of accompanying components does not affect the result of the analysis.

2. ***Detection limit*** is the smallest amount (concentration) of a detectable substance in a sample that can be detected using a validated technique.

   The limit of quantitative determination is the smallest amount (concentration) of a substance in a sample that can be quantified using a validated technique with the required accuracy and intra–laboratory (intermediate) precision.

   The sensitivity of qualitative reactions is influenced by such factors as:

   - volumes of solutions of reacting components,
   - concentrations of reagents,
   - pH of the medium,
   - temperature,
   - duration of the experience.

3. ***The analytical area of the technique*** is the interval between the upper and lower values of the analytical characteristics of the component being determined in the object of analysis (its quantity, concentration, activity, etc.).

4. ***The linearity of the technique*** is the presence of a linear dependence of the analytical signal on the concentration or amount of the substance being determined in the analyzed sample within the analytical area of the technique.
5. ***Correctness*** is a reflection of the difference between the true content of the determined component and the experimental results of the analysis.
6. ***Reproducibility (precision)*** is a characteristic of the "dispersion" of results near the average value of the determined value.
7. ***Stability (robustness)*** is the ability to preserve the characteristics found for it under optimal (nominal) conditions, given in the table, with probable small deviations from these conditions of analysis.

ERRORS

Errors in pharmaceutical drug analysis are normally of three types, namely :

1. Determinate Errors
2. Instrumental Errors
3. Personal Errors

Determinate Errors

Errors caused due to either incorrect adoption of an assay method or an incorrect graduation read out by an analyst are termed as determinate errors. Such errors, in principle may be determined and corrected. In usual practice the determinate errors are subtle in nature and hence, not easily detected.

A typical example of determinate errors:

Gravimetric Analysis: Where a compound is precipitated from a solution and the analyst believes that the analyte has been removed from the solution completely. Actually a small portion of the substance under investigation shall remain in solution. This sort of error is normally so insignificant that it is often neglected.

Instrumental Errors

Nowadays, both microprocessor based and computer-aided analytical instruments have more or less replaced the manually operated ones in any reasonably good analytical laboratory. One of the most prevalent determinate errors is caused by analytical intruments which are found to be ‘out of calibration’.

Hence, it is very essential that such instruments need to be calibrated periodically, for instance, a pH meter; a single-pan electric balance; an UVspectrophotometer.

In a similar manner, the calibration of glassware, such as: volumetric flasks, pipettes, burettes, measuring cylinders are duly carried out by specific methods recommended by regulatory documentation at specified temperatures.

Personal Errors

In addition to errors caused due to improper assay methods or faulty instruments, it may also be due to the analyst. A few typical examples are cited below:

- ✓ Physical Impairment : A person suffering from colour blindness may not be in a position to assess colour-changes precisely ; or if he uses bifocals he may not take the burette readings accurately.
- ✓ Learning-Curve Syndrome : An analyst must practise a new assay method employing ‘known’ samples before making an attempt to tackle an unknown sample, thereby minimising the scope of personal errors.