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CHROMATOGRAPHIC RESEARCH METHODS

GUIDELINES

MINISTRY OF HEALTH OF UKRAINE
NATIONAL UNIVERSITY OF PHARMACY

CHROMATOGRAPHIC RESEARCH METHODS

GUIDELINES

for students of the educational program ‘Pharmacy’

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Chromatographic research methods: guidelines for students of
Б 88 educational program ‘Pharmacy’ / V. A. Georgiyants, N. M. Smielova, H. V. Hryhoriv, O. A. Yevtifieieva, V. A. Chornyi, H. I. Severina, V. O. Grudko – Kharkiv: National University of Pharmacy, 2023. – 34 p.

Guidelines are compiled for students of the educational program ‘Pharmacy’.

Guidelines contain theoretical information on the subject and practical tasks on chromatographic research methods; information about the rules of work in the laboratory; a list of safety measures while working in the laboratory and methods of providing primary medical aid; topic, purpose, theoretical questions and questions for self-study, tasks for practical work, methodological instructions for completing assigned tasks, tasks for independent work, demonstration experiments, questions for self-control and test tasks.

Guidelines will help students in preparation and during classes in the educational component ‘Chromatographic research methods’.

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The purpose and tasks of the educational component ‘Chromatographic research methods’, its place in the educational process

The training of specialists in the field of chemistry and pharmacy at the modern level requires knowledge related to the theoretical foundations of certain instrumental methods, as well as practical skills in using applied and empirical data to solve routine tasks. A topical direction is the study of the features of pharmacopoeial analysis and quality control of active pharmaceutical ingredients, medicinal products, medicinal plant raw materials and herbal medicinal products using modern chromatographic research methods.

According to the program and curriculum, the educational component ‘Chromatographic research methods’ consists of one module.

General theoretical questions from the educational component ‘Chromatographic research methods’ are presented in lectures, which also consider the general principles of modern chromatographic methods of analysis and how to use them to solve theoretical and practical problems in the pharmacopoeial quality control of medicinal products.

Classes are provided with appropriate tools, equipment, reagents, tables, and slides. Workbook is provided for each class.

At the end of studying the educational component ‘Chromatographic research methods’, students have a semester control of the module.

These guidelines are compiled according to a single methodological system to help students in preparing for practical classes and conducting semester control of the module.

The educational component ‘Chromatographic research methods’ should ensure the acquisition of the following competencies by those obtaining a higher education:

- ✓ the ability to apply knowledge in practical situations;
- ✓ the to abstract thinking and analysis;
- ✓ the ability to learn and be diligently educated;
- ✓ the ability to evaluate and ensure the quality of the work performed;
- ✓ the ability to conduct research at the appropriate level;
- ✓ the ability to work with laboratory equipment and devices, methods of experimental research and process the results of conducted research.

As a result of the study of the educational component ‘Chromatographic research methods’, the student should know:

- ✓ theoretical foundations and principles of chromatographic methods of analysis;
- ✓ approaches to the selection of the most effective chromatographic methods for the separation and determination of the components of the analyzed samples based on their physical and chemical properties.

During the performance of laboratory practice, a student should be able to:

- ✓ analyze the information obtained as a result of the application of chromatographic methods, generalize, systematize and use it in professional activities;
- ✓ use the data of chromatographic research methods to monitor the effectiveness and safety of the use of medicinal products;

- ✓ evaluate and ensure the quality of the application of chromatographic methods for identification, determination of purity and quantitative content of biologically active substances in medicinal products;
- ✓ choose the most effective chromatographic methods for the analysis of substances based on their physical and chemical properties;
- ✓ to understand the rules of operation and safety techniques when working with equipment.

After studying the educational component ‘Chromatographic research methods’, the student should have:

- ✓ skills in planning the analysis of an unknown organic substance using chromatographic methods of analysis;
- ✓ skills of sample preparation and analysis of medicinal products during research using chromatographic methods;
- ✓ reasonably understand the choice of chromatographic methods of analysis in the pharmacopoeial analysis of organic substances;
- ✓ understand the rules of operation and safety techniques when working with devices used in chromatographic analysis;
- ✓ analysis technique using modern equipment.

Rules of work in the laboratory of the Pharmaceutical Chemistry Department

1. Before starting to perform laboratory tasks, students should familiarize themselves with the rules of work in the laboratory, instructions on safety, and the plan of fire prevention measures.
2. Before the start of the class, the students on duty receive in the laboratory the equipment and reagents necessary for the class, which they are obliged to return after the end of the laboratory work.
3. Students must maintain cleanliness and order in the laboratory. It is allowed to work only in a medical gown and a special headgear. Only objects necessary for conducting research should be on the desktop.
4. Performance of laboratory tasks is allowed after preliminary preparation. The teacher monitors the readiness of students to perform laboratory work.
5. Used solutions containing concentrated acids, alkalis, organic solvents, reagents with poisonous substances and precious metals are poured into specially marked glasses.
6. It is not allowed to take any reagents out of the laboratory and conduct additional experiments without the consent of the teacher.
7. After finishing the work, it is necessary to thoroughly wash the used dishes, put the workplace in order, turn off the water and electrical appliances.

It is necessary to remember that cleanliness, accuracy and painstaking work are the keys to the successful completion of a laboratory task.

Safety measures during work in the laboratory and methods of providing first aid

1. When working in the laboratory, it is necessary to strictly observe all safety measures in accordance with the rules and instructions.
2. Work with concentrated acids and other substances that emit caustic or poisonous vapors, as well as with substances and solutions that have a strong unpleasant smell, is carried out under hood.
3. All operations with diethyl ether and other flammable substances must be performed with extreme caution away from open flames, hot surfaces, electric and electrostatic sparks.
4. When working with harmful and poisonous substances (salts of barium, mercury, lead, arsenic, copper, metallic mercury, cyanide compounds, hydrogen sulfide, etc.), it is necessary to work carefully so that they do not enter the human body. It is forbidden to eat food in the chemical laboratory.
5. In case of cuts, make sure that there are no fragments of glass left in the wound, treat it with iodine solution and bandage it.
6. In case of burns, it is necessary to make a compress with a solution of tannin.
7. If concentrated sulfuric acid comes into contact with the skin, it is necessary to first wipe the affected area with a dry cotton swab or napkin. Then rinse with plenty of water. In case of burns of the skin, mucous membranes or eyes with acids, first wash

the affected area thoroughly with water, and then with a 2% solution of sodium bicarbonate.

8. In case of caustic alkali burns, wash the affected area well with water, and then with a 1% solution of acetic or citric acid.

9. In case of skin burns with phenol, bromine and similar substances, wash the affected area with a large amount of alcohol and apply burn ointment.

10. In case of poisoning with chlorine, bromine, nitrogen oxides and other similar substances, the victim should be given to sniff a tampon soaked in ammonia solution, and then take him out into the fresh air, give him a drink of milk.

11. In case of severe burns, injuries and poisoning, after providing first aid, the victim should be immediately sent to the hospital.

12. The first-aid kit should always have dressings, solutions and medicines necessary for first aid.

13. In the event of a fire hazard, it is necessary to immediately turn off the exhaust ventilation and the power circuit breaker, warn the teacher or laboratory assistant, and take measures to eliminate the fire.

14. Insignificant centers of fire are covered with sand, covered with a fire blanket or extinguished with a fire extinguisher.

15. In the event of a fire, it is necessary to leave the laboratory quickly and in an organized manner, remove the victims and provide them with first aid.

In case of an accident, contact the teacher immediately!

Workbook

The results of the completed laboratory tasks and the theoretical processing of the material students put in the workbook.

The title page of the workbook must contain: the name of the educational component; name and surname of student; group number, course, specialty.

In the workbook, the date and topic of the lesson, the purpose of the study, calculation tasks, materials in the form of diagrams and tables for independent filling of the student should also be given.

For each topic, laboratory work is offered for acquiring practical skills, which includes:

- ✓ the name of the analysis (description of the research object);
- ✓ type of control performed (qualitative, quantitative, etc.);
- ✓ regulatory documentation according to which the research is conducted;
- ✓ research methods, reactions, graphical representation of the obtained chromatogram, observations;
- ✓ conclusions obtained during the research, their compliance with the specified requirements.

TOPIC 1. Physico-chemical bases of the process of chromatographic separation. Terminology in chromatography. Classification of chromatographic research methods. Solubility, pH and lipophilicity of substances. Influence of factors on chromatographic separation. Application, advantages and disadvantages of chromatographic research methods.

Aim: To study the physico-chemical bases of the chromatographic separation process, the constituent components of the chromatographic process. **Get acquainted** with the history of the invention and development of chromatography; characteristic features of chromatographic research methods; factors affecting the process of separation of substances. **Know** the basic mechanisms of separation of substances; classification of chromatographic research methods; practical application, advantages and disadvantages of chromatographic methods.

Theoretical questions

1. Familiarize yourself with general concepts and terminology in chromatography, the phenomena on which chromatographic methods of separation of substances are based.
2. Describe the mandatory components of the chromatographic process and their relationship: mobile phase, stationary phase, analyzed substance (mixture of substances).
3. Specify the physico-chemical basis of the chromatographic separation process and the characteristic features of chromatographic research methods.
4. What are the main mechanisms of separation of substances that prevail in chromatographic methods of analysis?
5. Specify the factors affecting the separation of substances in chromatographic methods.
6. Give the classification of chromatographic research methods according to the technique (method) of the separation process; by the type of contacting phases (by the aggregate state of the moving and stationary phases); by the nature of the processes occurring during the separation of components (by the mechanism of interaction of the sorbent and sorbate); depending on the purpose of the chromatographic process.
7. What are the main parameters of the chromatographic process? What theories of chromatographic separation exist? What is the essence of the theory of theoretical plates in chromatography? What is the essence of the kinetic theory of chromatography? What are the main factors causing blurring of chromatographic zones?
8. What is a chromatogram?
9. Separation parameters. Retention factor. Resolution. Efficiency of the chromatographic column. Plate number. The height equivalent to a theoretical plate. Selectivity.

Theoretical questions for self-study

1. Get acquainted with the history of the formation and development of chromatographic research methods, name the leading scientists in this field.
2. What are polar and nonpolar compounds? How does the solubility, pH, lipophilicity of substances affect their separation in chromatographic methods?
3. Describe the current state of chromatographic methods of analysis and their areas of application, value and place among other analytical methods.
4. State the advantages and disadvantages of chromatographic methods.
5. What is the physical meaning of the Van Deemter equation?

Practical tasks

Learn about different chromatographic research methods and the equipment used. Separate the mixture of substances according to the given methods of analysis.

Methodical instructions for performing assigned tasks

The following objects are offered for mastering the techniques and assimilation of the material: fizzy drink ‘Tarhun’.

Tasks for independent work

Complete the topic ‘Physico-chemical basics of the chromatographic separation process’ in the workbook ‘Chromatographic research methods’, complete the test tasks.

Experimental study

For a better understanding of the material, it is recommended to perform an experimental study: Separation of a mixture of hydrophobic and hydrophilic substances in the drink ‘Tarhun’ (experiment ‘Flag of Ukraine’).

Place 5 ml of ‘Tarhun’ in a clean, dry 50 ml test tube, add 5 ml of chloroform, shake and fix the separation of the mixture into layers, note the color of the layers. After that, add 2 ml of 5 mg/ml novocaine solution to the test tube and intensively mix, note the color of the layers, draw a general conclusion.

Questions for self-control

1. What are the mandatory components of the chromatographic process?
2. By what criteria is it possible to classify chromatographic methods?
3. What factors affect the separation of substances in chromatographic methods?
4. In which areas of human life are chromatographic methods of analysis used? Indicate what are the areas of their use in the quality control of medicinal products?
5. Describe the advantages and disadvantages of chromatography methods.

Tests

1. Mandatory components of the chromatographic system are:
A. stationary, mobile phase, analyzed compound
B. stationary phase only
C. mobile phase only
D. only the analyzed compound

2. The solvent or mixture of solvents moving along the stationary phase during the chromatographic process is:
A. eluent or mobile phase
B. stationary phase
C. sorbent
D. detection reagent

3. Specify the mandatory component of the chromatographic process, the main function of which is the sorption and retention of the substance and which is a solid adsorbent with a developed surface or a liquid film fixed on a solid support:
A. Stationary phase
B. Mobile phase
C. Test compound
D. All options are correct

4. Chromatography is a set of methods for detecting, purifying and separating the components of a mixture based on the difference in:
A. affinity for mobile and stationary phases
B. melting points
C. boiling points
D. optical density

5. The physicochemical process of absorption of a substance (sorbate) in the liquid or gas phase by another substance – a solid or liquid absorber called a sorbent is:
A. Sorption
B. Desorption
C. Migration
D. Ion exchange

TOPIC 2. Theoretical foundations and practical application of the paper chromatography method in the pharmacopoeial analysis of medicinal products.

Aim: To study the physico-chemical basics of the paper chromatography method and the mandatory components of the chromatographic process. **Get acquainted** with the history of the method; research procedure, the equipment and materials used in the paper chromatography method; ways of conducting research depending on the technique of execution; calculation and use of the retardation factor; practical application, advantages and disadvantages of the method.

Theoretical questions

1. What are the features of performing chromatography on paper?
2. What factors affect the process of separation of substances in the paper chromatography method?
3. What is the difference between partition and adsorption paper chromatography?
4. Describe the components of the chromatographic process in the paper chromatography method: stationary and mobile phase, analyzed sample (mixture).
5. What are the types and requirements for normal-phase and reversed-phase chromatographic paper? What is the function of chromatography paper?
6. What types of chromatographic chambers are used in paper chromatography? What is their purpose?
7. Indicate what are the requirements for analytical solvents used for as the mobile phase (eluent)?
8. What devices are used to apply the test sample to chromatographic paper?
9. Describe the methods of identifying chromatographic zones in paper chromatography? What equipment is used?
10. List the main stages of conducting research in the analysis of substances by the method of chromatography on paper.
11. Methods of performing paper chromatography: one-dimensional ascending and descending chromatography; two-dimensional chromatography; circular (radial) chromatography.
12. Calculation of the retardation factor. What is its connection with the physical and chemical properties of the substance? What factors affect it?

Theoretical questions for self-study

1. Give the classification of chromatographic methods according to the technique of conducting the research, indicate what kind of chromatography on paper belongs to it?
2. Give a general description and note the features of planar (plane) chromatography methods as liquid chromatography methods.
3. Get acquainted with the history of the discovery of the paper chromatography method.

4. Describe electrophoretic chromatography on paper.

5. State the advantages and disadvantages of the paper chromatography method, its field of application.

Practical tasks

Familiarize yourself with the paper chromatography analysis technique and the equipment used in the research. Separate the mixture of substances according to the given methods of analysis.

Methodical instructions for performing assigned tasks

The following objects are offered for mastering the techniques and the material: various inks (pens, markers); a mixture of iron (III) and copper (II) salts.

Tasks for independent work

Complete the topic ‘Paper chromatography’ in the workbook ‘Chromatographic research methods’, complete the test tasks.

Experimental study

For a better understanding of the material, it is recommended to conduct an experimental study:

1. Determination of ink composition by the method of ascending paper chromatography.

Chromatographic paper of the required size is prepared. With the help of a pencil and a ruler, mark a line on it where the ink samples will be applied (the start line) and draw the finish line 10 cm from the start line. Ink samples are applied to the start line.

Place 20 ml of alcohol (96 %) in a chemical beaker and cover it with glass. With the help of laboratory tweezers, the chromatographic paper with the applied samples is placed in a beaker with a mobile phase (alcohol) in such a way that the place of application of the samples is above the level of the solvent. When the solvent reaches the finish line, the chromatographic paper is removed with tweezers and dried in air.

A solution for detecting substances (bromothyme blue or phenolphthalein solution) is placed in a device for spraying the plates and sprayed onto the surface of the chromatography paper.

The obtained results are analyzed and the composition of the ink in different samples is compared.

2. Separation of iron (III) and copper (II) salts by circular (radial) paper chromatography.

A 0.1 % solution containing iron (III) and copper (II) ions is used as the tested solution. A desiccator is used as a chromatographic chamber for obtaining a circular chromatogram. Using a measuring cylinder, measure 10 ml of the mobile phase (alcohol 96 %) and place it in the chromatographic chamber, cover it with a lid and

leave it for saturation. On round chromatographic paper with a diameter of 10 cm, mark the place of sample application and draw the contours of the wick with a width of 5 mm. 5 μl of the tested sample is applied to the center of the chromatographic paper. Place the chromatographic paper in the chromatographic chamber so that the wick touches the solvent. Close the camera cover. When the mobile phase passes 2/3 of the plate, the paper is taken out and the distance traveled by the mobile phase is noted, the paper is dried in air. To detect chromatographic zones, chromatographic paper is treated with a solution of potassium ferrocyanide, the color is noted, and the retardation factor (R_f) are calculated for the chromatographic zones obtained, and appropriate conclusions are drawn.

Questions for self-control

1. Describe what the mobile and stationary phase are in the paper chromatography method?
2. Describe the procedure for conducting research using the paper chromatography method.
3. What types of paper chromatography do you know?
4. How is the retardation factor calculated? What is its use in practice?
5. In which industries is the method of paper chromatography used, its advantages and disadvantages?

Tests

1. A saturated chromatographic chamber is:

A. chamber in which the filter paper was placed, the required amount of mobile phase was added, closed with a lid and left for some time to saturate

B. a chamber to which the required amount of mobile phase was added and closed with a lid

C. chamber without moving phase

D. empty chamber

E. there is no correct answer

2. A solvent or a mixture of solvents that move the tested sample along the stationary phase in the paper chromatography method:

A. Mobile phase

B. Stationary phase

C. Sorbent

D. Fixing agent

3. What is the name of the method of paper chromatography analysis, where a circle of the required diameter is cut out of chromatographic paper, a solution of the test substance is applied to the center of the circle, a small strip (wick) is cut on the circle, the strip is folded back, and placed in a chromatographic chamber with a mobile phase:

A. Circular (radial) chromatography
B. One-dimensional ascending chromatography
C. One-dimensional descending chromatography
D. Two-dimensional chromatography

TOPIC 3. Theoretical foundations and practical application of thin-layer and high-performance thin-layer chromatography methods in the pharmacopoeial analysis of medicinal products.

Aim: To study the physico-chemical basics of thin-layer and high-performance thin-layer chromatography methods and mandatory components of the chromatographic process. **Get acquainted** with the history of methods; research procedure. **Get familiarized with** the equipment and materials used in the thin layer chromatography method; calculation and use of the retention factor; practical application, advantages and disadvantages of methods.

Theoretical questions

1. What are the features of performing chromatography in a thin layer of sorbent?
2. Describe the components of the chromatographic process in thin-layer and high-performance thin-layer chromatography methods: stationary and mobile phase, analyzed sample.
3. What are the main separation mechanisms in thin-layer chromatography?
4. What are the types and requirements for chromatographic plates? Describe the components of the plate: substrate, sorbent, binding component. Name the differences between thin layer chromatography plates and high performance thin layer chromatography plates.
5. What types of chromatographic chambers are used? What are the features of their preparation for analysis?
6. Indicate what are the requirements for analytical solvents used for the mobile phase (eluent)?
7. What devices are used to apply the sample? What are their advantages and disadvantages?
8. Describe the methods of detecting chromatographic zones in thin-layer and high-performance thin-layer chromatography methods? What equipment is used?
9. State the main stages of conducting research in the analysis of substances by the method of chromatography in a thin layer.
10. How are chromatographic zones detected after elution? What equipment is used?
11. Peculiarities of conducting research on the Camag® hardware complex.
12. Identification, determination of purity and quantitative determination of substances: peculiarities of conduct, used equipment, evaluation of parameters.

Theoretical questions for self-study

1. Give the classification of chromatographic methods according to the technique of performing the research, indicate what type belongs to a thin layer chromatography?
2. Give a general description of the methods related to planar (plane) chromatography.

3. Familiarize yourself with the history of the discovery of the thin-layer chromatography method.

4. How is the retardation factor calculated in thin-layer and high-performance thin-layer chromatography methods?

5. How do you check the suitability of plates for thin-layer and high-performance thin-layer chromatography?

6. State the advantages and disadvantages of the thin-layer and highly efficient thin-layer methods, the fields of their application.

Practical tasks

Familiarize yourself with examples of calculating the concentration of a substance in a solution, the volumes of the mobile phase components, the retardation factor, and complete the calculation tasks in the workbook (topic: 'Thin layer chromatography').

Familiarize yourself with thin-layer and high-performance thin-layer chromatography techniques and the equipment used in the research. According to the given methods of analysis, investigate the quality of substances according to the given methods.

Methodical instructions for performing assigned tasks

The following objects are offered for mastering the techniques and assimilation of the material: glycine substance, standard samples of glycine and alanine, marigold tincture.

Tasks for independent work

Complete the topic 'Thin-layer chromatography' in the workbook 'Chromatographic research methods', complete the test tasks.

Experimental study

For better assimilation of the material, it is recommended to perform the following experimental studies:

1. Identification of the glycine substance by the method of thin-layer chromatography.

A 0.01% solution of the glycine substance is used as the test solution, and a 0.01% aqueous solution of a glycine standard sample is used as a comparison solution. Weigh 0.01 g of the glycine substance on an analytical balance and place it in a 100.0 ml volumetric flask, dissolve it in a small amount of water R and then bring it up to the mark with water R, mix. A solution of a standard substance is prepared according to a similar method.

A clean, dry chromatographic chamber is lined with filter paper. The mobile phase is a mixture of *alcohol (96%) R - water R* (70:30), for the preparation of which

all the components of the mobile phase are measured into a separate flask with a lid and mixed. The mobile phase is placed on the bottom of the chromatographic chamber, wetting the filter paper, tightly closed with a lid and left for 1 hour for saturation.

For the study, a chromatographic plate 'Silica gel 60' with a size of at least 4x12 cm is used. Using a ruler and a graphite pencil, receding 1.5 cm from the lower edge of the plate, draw a start line. Mark the distance that the mobile phase must travel according to the method (10 cm), mark the finish line, and also, leaving at least 1 cm from the right and left edges, mark and sign two areas for applying the tested solution and the comparison solution.

Calibrated capillaries or a microsyringe are used to apply the analyzed solutions. Before application, the device is washed first three times with ethanol, and then three times with the test solution or the reference solution.

With the help of laboratory tweezers, the plate is placed in the chromatographic chamber, carefully immersing its ends in the mobile phase. After the mobile phase has passed from the start line to the finish line, the plate is removed and left in air to remove excess mobile phase.

To detect chromatographic zones, a solution of *ninhydrin R1* is used, which is placed in a sprayer and applied to the plate. Then the plate is placed in an oven at a temperature of 100-105 °C for 5 minutes.

For the obtained chromatographic zones of the tested sample and the comparison solution of glycine, the retention coefficients are calculated, and a conclusion is drawn regarding the quality of the substance.

2. Determining the purity of the glycine substance (substances detected by ninhydrin) by the method of thin-layer chromatography.

To conduct research, use:

- ✓ tested solutions (a) – 1% aqueous solution of glycine substance;
- ✓ tested solutions (b) – 0.1% aqueous solution of glycine substance;
- ✓ comparison solution (a) – 0.1% aqueous solution of the glycine standard sample;
- ✓ comparison solution (b) – 0.0005% aqueous solution of glycine standard sample;
- ✓ comparison solution (c) – 0.1% aqueous solution of a standard sample of glycine and alanine.

A clean, dry chromatographic chamber is lined with filter paper. The mobile phase is a mixture of *alcohol (96%) R - water R* (70:30), for the preparation of which all the components of the mobile phase are measured into a separate flask with a lid and mixed. The mobile phase is placed at the bottom of the chamber, wetting the filter paper, tightly closed with a lid and left for 1 hour for saturation.

For research, a chromatographic plate 'Silica gel 60' is used. With the help of a ruler and a graphite pencil, receding 1.5 cm from the lower edge of the plate, draw the start line. Mark the distance that the mobile phase must pass according to the method (10 cm), mark the finish line, and, leaving at least 1 cm from the right and left edges, mark and sign the areas for applying the tested solutions and comparison solutions.

Calibrated capillaries or a microsyringe are used to apply the analyzed solutions. Before application, the device is washed first three times with ethanol, and then three times with the test solution or the reference solution.

With the help of laboratory tweezers, the plate is placed in the chromatographic chamber, carefully immersing the ends of the plate in the mobile phase. After the mobile phase has passed from the start line to the finish line, the plate is removed and left in air to remove excess mobile phase.

To detect chromatographic zones, a solution of *ninhydrin R1* is used, which is placed in a sprayer and applied to the plate. Then the plate is placed in an oven at a temperature of 100-105 °C for 5 minutes.

The suitability of the chromatographic system is assessed by reference solution (c): the chromatogram must have two clearly separated chromatographic zones of glycine and alanine.

Make a general conclusion about the purity of the glycine substance according to the test solution (a): any zone, except for the main one, should not be more intense than the zone on the chromatogram of the comparison solution (b) (0.5%).

3. Identification of biologically active substances of marigold tincture by the method of high-performance thin-layer chromatography on the Camag® hardware complex.

Before the start of the study, each unit of the Camag® hardware complex is connected to the electrical network and preparatory work is carried out to set up the system, including installing the appropriate microsyringe needle, filling the container for its washing, turning on the nitrogen supply, etc.

In the software of the visionCATS device, a new method is created and the parameters necessary for operation are adjusted in accordance with the research methodology. During the analysis, the name of the procedure and a brief description of the operator's necessary actions will be displayed on the on-duty computer monitor.

The TLC plate is placed in a visualizer (CAMAG TLC Visualizer 2) and scanned in several modes: in daylight and at a wavelength of 365 nm. After that, the plate is removed and installed in the plate holder of the autosampler (CAMAG ATS4).

As a test solution, a marigold tincture is used, as a comparison solution – a methanol solution of the standard substances of caffeic acid (0.01%), chlorogenic acid (0.01%) and rutin (0.025%). Test solutions and reference solutions are placed in vials and installed in the cuvette compartment of the autosampler. The appropriate command is run on the computer to start applying the test and control solutions to the plate.

The plate with the applied substances is fixed in the plate holder of the automatic elution chamber ADC 2. Prepare the mobile phase *formic acid anhydrous R – water R – ethyl acetate R* (10:10:80), place 25 ml of the resulting mixture in the compartment for saturation of the chromatographic chamber and 10 ml to the chromatography department. Run the appropriate command on the computer to start elution. After the procedure, the plate is dried at a temperature of 100-105 °C in a drying cabinet.

The warm plate is placed in a device for spraying plates (CAMAG Spray) and with the help of a sprayer, a detection solution is applied – 10 g/l of *diphenylboric acid*

aminoethyl ether R in *methanol R*, then a solution of 50 g/l *macrogol 400 R* in *methanol R*. Dry in air within 30 min.

Rescan the plate in a visualizer (CAMAG TLC Visualizer 2) under daylight and at a wavelength of 365 nm.

In the visionCATS software, set the start line and the finish line, sign the obtained chromatographic zones and calculate the retardation factor. Then make a conclusion about the position of the chromatographic zones.

Questions for self-control

1. Describe the mobile and stationary phase for thin-layer and high-performance thin-layer chromatography methods.
2. Describe the procedure for conducting research using thin-layer and high-performance thin-layer chromatography methods.
3. What is the fundamental difference between thin-layer and high-performance thin-layer chromatography methods?
4. How are the methods of thin-layer and high-performance thin-layer chromatography used in the quality control of medicinal products? What are their advantages and disadvantages?

Tests

1. In the method of thin-layer chromatography to detect chromatographic zones of substances that fluoresce or phosphoresce, or when a corresponding fluorescent indicator is applied to the plate, use:

A. UV cabinet (UV lamp) or visualizer
B. detector
C. sprayer
D. chromatographic column
E. there is no correct answer

2. Thin-layer chromatography (TLC) is a method of separation and analysis of a mixture of substances, which is based on the fact that:

A. the stationary phase is applied in a thin layer on a glass, plastic or aluminum substrate; the mobile phase is liquid and is a soluble or a mixture of solvents.
B. the stationary phase is used, which is a special type of paper (chromatographic paper), and the mobile phase is liquid and is a mixture of solvents.
C. the stationary phase is placed in a chromatographic column, and the mobile phase is a liquid that is fed under pressure.
D. the stationary phase is placed in a chromatographic column and the mobile phase is a pressurized gas.

3. The stationary phase in the thin-layer chromatography method is:

A. A sorbent layer applied to a glass, aluminum or plastic substrate
B. Solvent or sum of solvents

C. Chromatographic column
D. Special types of paper

4. Which of the sorbents is most often used as a stationary phase in the thin-layer chromatography method:
A. Silica gel
B. Plastic
C. Glass
D. Polyethylene oxide

5. What is the name of the glass container with a lid, in which the mobile phase is placed and chromatography is performed in the thin-layer chromatography method:
A. Chromatographic chamber
B. Chromatographic column
C. UV cabinet
D. Measuring flask

TOPIC 4. Theoretical foundations of the liquid chromatography method and its application in the pharmacopoeial analysis of medicinal products.

Aim: To study the physico-chemical basics of the liquid chromatography method and the mandatory components of the chromatographic process. **Get acquainted** with the history of the method; research procedure. **Know** the equipment and materials used in the liquid chromatography method; calculation and use of chromatographic parameters (retention time, peak area etc.); practical application, advantages and disadvantages of the method.

Theoretical questions

1. Describe the components of the chromatographic process in the liquid chromatography method: stationary and mobile phase, analyzed sample (mixture).
2. Describe the types of liquid chromatography, what is their fundamental difference: adsorption chromatography, partition chromatography, high-performance liquid chromatography, ion exchange chromatography, chiral chromatography?
3. What types of physical and chemical interactions occur in the method of liquid chromatography?
4. What is the structure and principle of operation of a liquid chromatograph?
5. Indicate what are the requirements for analytical solvents used for the manufacture of the mobile phase (eluent)? Eluting power of the solvent, classification of solvents by polarity.
6. List the main stages of research in the analysis of substances by the method of liquid chromatography.
7. Sorbents for liquid chromatography. Polar and non-polar sorbents. Modification of sorbents.
8. What types of chromatographic columns are used in the method? What is their purpose?
9. What types of pumps are used to supply the mobile phase?
10. What is an injector? What types of injectors are there?
11. Indicate what types of detectors are used in liquid chromatography? What are their functions? Sensitivity and selectivity of detectors. Direct and indirect detection.
12. Combination of two independent methods – chromatography and mass spectrometry. What are the requirements for a mass spectrometer? Mention the advantages of the method and the field of application.
13. Normal-phase, reversed-phase: differences, analytical possibilities.
14. Adsorption and distribution liquid chromatography. Advantages and limitations.
15. What is a chromatogram? Chromatographic peak, retention time (volume), dead time (volume).
16. What is peak area? What is it used for?
17. Retention factor. Distribution constant. Symmetry factor. Resolution. Relative retention. Plate number.

Theoretical questions for self-study

1. Basics of the concept of theoretical plates. The number of theoretical plates and the efficiency of the column. The concept of a height equivalent to a theoretical plate (VETT). Disadvantages of the concept of theoretical plates.

2. Kinetic theories of chromatography. Factors influencing the erosion of zones (eddy diffusion, molecular diffusion, mass transfer resistance in mobile and stationary phases). Dependence of VETT on the flow rate. Van Deemter equation.

3. Chromatography of hydrophilic interactions.

4. Fields of application of the liquid chromatography method, its advantages and disadvantages.

Practical tasks

Familiarize yourself with examples of calculating chromatographic parameters and complete the calculation tasks in the workbook (topic: 'Liquid chromatography').

Methodical instructions for performing assigned tasks

The following objects are offered for mastering the methods and assimilation of the material: chromatograms of medicines analysis.

Tasks for independent work

Complete the topic 'Liquid chromatography' in the workbook 'Chromatographic research methods', complete the test tasks.

Experimental study

For a better assimilation of the material, it is recommended to familiarize yourself with the equipment and procedure of the liquid chromatograph, using for research a medicine or medicinal plant material that is currently being analyzed in the laboratory.

Questions for self-control

1. Describe what the mobile and stationary phase are in the method of liquid chromatography?

2. What types of liquid chromatography methods do you know?

3. Describe the main components of a liquid chromatograph, their functions.

4. What is the procedure for liquid chromatography research?

5. What is a chromatogram? What chromatographic parameters are used to describe the chromatogram?

6. How is the method of liquid chromatography used in the quality control of medicinal products, advantages and disadvantages of the method?

Tests

1. A component of a liquid chromatograph that analyzes substances after their separation on a column and transmits information to the data collection and processing system:

A. Detector.
B. Pump.
C. Mobile phase (eluent).
D. Chromatographic column.
E. Injector.

2. To identify the medicinal product by the method of liquid chromatography, the parameter is used:

A. retention time *(t_R)*
B. retardation factor *(R_f)*
C. peak area (S)
D. refractive index

3. To identify the medicinal substance, the analyst uses the method of liquid chromatography, the stationary phase of which is:

A. A sorbent placed in a column
B. Chromatographic paper
C. The sorbent layer applied to the substrate
D. Chloroform
E. Activated charcoal.

4. A graphic representation of the detector operation, which is a sequence of detector signals produced when separate components of the mixture exit the column, is called:

A. Chromatogram.
B. Spectrum.
C. Scheme.
D. Diagram.
E. Timeline.

TOPIC 5. Theoretical foundations of ion, ion exchange and exclusion chromatography methods, their application in the pharmacopoeial analysis of medicinal products.

Aim: To learn and master the theoretical foundations of ion, ion exchange, exclusion, and affinity chromatography methods, their possibilities, advantages and disadvantages. **Learn** the types of equipment and their purpose. **Be able** to choose equipment depending on the nature of the analyte. Understand the similarities and differences between liquid chromatography methods.

Theoretical questions

1. What are the features and principle of the ion exchange chromatography method? What are the advantages and disadvantages of the method?
2. Describe the types of ion exchange chromatography and their analytical capabilities: cation-exchange and anion-exchange chromatography.
3. Types of buffer solutions and types of ion exchange polymers in ion exchange chromatography.
4. What are the features of ion chromatography? What are the advantages and disadvantages of single-column and double-column ion chromatography?
5. What are the features and principle of the exclusion chromatography method? What are the retention patterns and types of sorbents used? What is gel chromatography and what are the requirements for gels? Indicate the most common sorbents in the method and describe their properties.
6. Indicate the areas of application of different types of chromatography.

Theoretical questions for self-study

1. Theoretical foundations of chromatographic separation. The concept of ion exchange, cationites, anionites, ampholytes. The principle of operation of the ion exchanger.
2. Counterion selectivity and ion exchange efficiency, quality of chromatographic separation. Ion exchange resins. Buffer solutions.
3. Application of ion exchange chromatography for separation and determination of cations (sodium, potassium, calcium and magnesium) and anions (chlorides, sulfates, carboxylic acids) and other biologically active substances (antibiotics, glycans).
4. Application of ion exchange chromatography for purification and separation of proteins, enzymes, sugars/carbohydrates.

Practical tasks

1. State the main stages of determining the quantitative content of the analyte (tested sample) by the method of ion exchange chromatography. Explain the basis for using this method.

2. To determine the content of the tested sample of the medicinal substance by the method of ion exchange chromatography.

Methodical instructions for performing assigned tasks

The following objects are offered for mastering the techniques and assimilation of the material: sodium chloride, potassium chloride.

Experimental study

For a better understanding of the material, it is recommended to perform an experimental determination of the content of potassium (sodium) chloride in the tested sample by the method of ion exchange chromatography.

The substance potassium (sodium) chloride is used as the tested sample.

To prepare (regenerate) cationite (KU-1) and anionite (AB-17), 25-30 ml of a 2M solution of hydrochloric acid or sodium hydroxide, respectively, are passed through the column at a speed of 1 cm^3/min. Then the ionite is washed from excess acid or alkali with purified water. The completeness of washing is checked by a universal indicator. For this, a drop of the solution flowing from the column is placed on a strip of indicator paper. The color of the indicator should match the color of purified water.

A portion of the drug under study is dissolved in 15-20 ml of purified water. The studied solution is introduced into the prepared column and passed at a rate of 1 ml/min. The solution flowing from the column is collected in a conical flask for titration. The resulting acid or base solution is titrated with standard base or acid solutions, respectively. Based on the obtained data, the content of the substance in the substance is calculated.

Tasks for independent work

Complete the topic ‘Ion exchange chromatography’ in the workbook ‘Chromatographic research methods’, complete the test tasks.

Questions for self-control

1. Describe the procedure for carrying out research using the ion exchange chromatography method.

2. Give the scheme of cation and anion exchange. Explain the principle of its operation.

3. Describe the processes underlying ionite regeneration.

Tests

1. Chromatographic methods of analysis are distinguished by the mechanism of interaction of sorbent and sorbate. Select the appropriate partitioning mechanism for ion exchange chromatography

A. On the different solubility of compounds that are distributed on the stationary phase.

B. On different capacity for adsorption by a solid sorbent.

C. On the different ability of substances to ion exchange.

D. On the formation of precipitates of different solubility of the studied compounds with a sorbent.

E. On the formation of coordination compounds of different stability in the phase or on the surface of the sorbent.

2. A component of an ion exchange chromatograph that reduces the background conductivity of chemicals and improves the conductivity measurement of the studied ions

A. Detector.

B. Pump.

C. Suppressor.

D. Chromatographic column.

E. Injector.

3. The chemical laboratory received a preparation that is a mixture of glucose and mannose. To determine these substances in the mixture, the analyst uses the method:

A. Spectrophotometry

B. Polarimetry

C. Liquid chromatography

D. Ion exchange chromatography

E. Gas chromatography.

TOPIC 6. Theoretical foundations of the gas chromatography method and its application in the pharmacopoeial analysis of medicinal products.

Aim: To learn and master the theoretical foundations of the gas chromatography method, its possibilities, advantages and disadvantages. **Learn** the components of a gas chromatography device, their purpose, and be able to compare it with a liquid chromatography device. **Understand** the similarities and differences between liquid and gas chromatography methods.

Theoretical questions

1. Give a general description of gas chromatography as a method of separation and determination of volatile compounds.
2. Indicate the main nodes and describe the principle scheme of gas chromatograph
3. Types of gas chromatography: gas-adsorption and gas-liquid chromatography, their analytical capabilities.
4. What types of gases are used in gas chromatography? What is a carrier gas? What function does it perform?
5. What are the requirements for substances for their determination by gas chromatography?
6. What is the difference between packed and capillary columns for gas chromatography?
7. What types of injectors are there? What are their differences and areas of application?
8. Classification of detectors and their most important characteristics. The sensitivity of detectors, the principle of their operation.
9. Gas chromatography-mass spectrometry. Specify the advantages of the method and the field of its application.
10. Programming the temperature regime in gas chromatography.
11. Identification and quantitative calculations. Analytical capabilities of gas chromatography.

Theoretical questions for self-study

1. Theoretical foundations of chromatographic separation. Retention factor. Distribution constant. Symmetry factor. Resolution. Relative retention. Plate number.
2. Specify the scope of the gas chromatography method, its advantages and disadvantages.

Practical tasks

1. Read article 2.4.24 of the State Pharmacopoeia of Ukraine ‘Identification of residual solvents and control of their quantities’. According to the article, control of the presence of residual amounts of organic solvents can be carried out by any validated methods. However, the most widely used is the gas chromatography method.

Explain the rationale for using the gas chromatography method for this purpose, and how exactly are residual amounts of organic solvents determined?

2. Select the monographs of the State Pharmacopoeia of Ukraine related to the analysis of essential oils (for example, ‘Peppermint oil’). Compare the methods proposed for the identification and quantification of this class of compounds. Clarify of the reason for using the gas chromatography method in this case.

Methodical instructions for performing assigned tasks

For mastering the methods and assimilation of the material, relevant articles of the SFU are offered.

Tasks for independent work

Complete the topic ‘Gas chromatography’ in the workbook ‘Chromatographic research methods’, complete the test tasks.

Questions for self-control

1. Describe the procedure for conducting research using the gas chromatography method.
2. Draw a diagram of a gas chromatograph. Explain the principle of its operation.
3. Describe the main parameters of the chromatogram.
4. What are the main differences between the gas chromatography method and the liquid chromatography method.

Tests

1. The separation of substances by the gas chromatography method occurs due to the different speed of movement of substances in the column. What is the mobile phase in this method of analysis?

A. Carrier gas
B. Solid substance
C. Solvent
D. Water

2. Substances can be analyzed by the gas chromatography method:

A. Volatile
B. Thermolabile

C. With a molecular weight greater than 500

3. The following cannot be used as a carrier gas:
A. Chlorine
B. Nitrogen
C. Helium
D. Hydrogen

4. To identify the compound by the gas chromatography method, a detector is used:
A. Mass spectrometric
B. Thermoconductometric
C. Flame ionization detector
D. Thermoionic

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