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# CHROMATOGRAPHIC RESEARCH METHODS

## WORKBOOK

## FOR PRACTICAL CLASSES AND

## SELF-STUDY

**for students of the educational program 'Pharmacy'**

**MINISTRY OF HEALTH OF UKRAINE**
**NATIONAL UNIVERSITY OF PHARMACY**
**Pharmaceutical Chemistry Department**

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## WORKBOOK
## FOR PRACTICAL CLASSES AND
## SELF-STUDY

**for students of the educational program 'Pharmacy'**

**student _____ year _____ group**

_____
(first name, last name)

_____
(academic year)

**Kharkiv**
**NUPh**
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Б 88

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Б 88 **Chromatographic research methods :** workbook for practical classes and self-study for students of the educational program 'Pharmacy' / V. A. Georgiyants, O. A. Yevtifieieva, N. M. Smielova, H. V. Hryhoriv, V. A. Chornyi, K. A. Taran, I. V. Bezruk, V. A. Mishchenko – Kh. : NUPh, 2023. – 63 p.

The workbook as a teaching and methodical guide was created according to the approved program of the educational component 'Chromatographic research methods'. It contains research on qualitative and quantitative analysis using chromatographic methods of analysis; educational tasks; 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 care.

**UDC 543.544:001.891.5 (072)**

# INTRODUCTION

The educational component ‘Chromatographic research methods’ provides students with theoretical knowledge about the basics of the chromatographic separation process and practical skills in paper, thin-layer, liquid, ion-exchange, ion, exclusion and gas chromatography.

One of the forms of the educational process planned for this educational component is practical classes, during which students under the guidance of a teacher conduct experimental research with the aim of practical consolidation of theoretical knowledge, acquire the skills to work with laboratory equipment and devices, master the methods of chromatographic research and their results processing.

To conduct practical classes, specially equipped educational laboratories with equipment, research objects, reagents, as well as high-quality educational and methodological support are necessary.

This workbook was created for the preparation of students for practical classes of the educational component ‘Chromatographic research methods’ with the aim of further meaningful performance of experimental tasks during practical classes. Each topic contains a list of basic concepts, schemes and tables, the filling of which is aimed at thorough mastering and structuring of the theoretical material, as well as a detailed description of experimental work on the corresponding topic, which is planned to be performed during the practical class.

According to the given order of conducting the research, the student should make notes of observations, write down the appropriate reaction equations, make the necessary calculations and draw conclusions based on the results of the conducted experiment.

## *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!*

Familiar with the rules and safety measures during work in the chemical laboratory of the Pharmaceutical Chemistry Department

| signature | First name, last name |
|---|---|

(Date)

**The theme of the lesson**:
*Physico-chemical foundations of the chromatographic separation process. Terminology in chromatography. Classification of the chromatographic methods of research. Solubility, pH and lipophilicity of substances. Influence of factors on chromatographic separation. Application, advantages and disadvantages of chromatographic research methods.*

**Give the definition of the terms:**

*Chromatography is a science*

*Chromatography is a process*

*Chromatography is a method*

**Give the definition of the basic constituent components of a chromatographic process:**

| *Mobile phase* | *Analyzed probe* | *Stationary phase* |
|---|---|---|
| ↓ | ↓ | ↓ |
| | | |

**Define the main mechanisms of substance separation in chromatography:**

**Indicate in which of the definitions the process of "sorption" and in which of "desorption" is described?**

| | |
|---|---|
| Physico-chemical process of the substance (sorbate) adsorption in the liquid or gas phase by another substance – a solid or liquid absorber (sorbent) | Physico-chemical process of releasing a substance (sorbate) from a solid or liquid absorber (sorbent) |
| ↓ | ↓ |
| | |

**What is the difference between the processes of "adsorption" and "absorption"?**


***Adsorption*** –

The substance, on the surface of which concentration of another substance occurs, is called _____, and the one, which is released from the gas or liquid form and remains in the surface, is _____.

***Absorption*** *(separation)* –

The substance, which soaks (absorbs) the components, is called _____, and the one, which is adsorbed from the gas or liquid phase – _____.

**Give the definition of the terms and supply the examples among the medicinal products:**

| | |
|---|---|
| *The polar substance* – | Examples: |
| *The non-polar substance* – | Examples: |
| *The hydrophilic substance* – | Examples: |
| *The hydrophobic (lipophilic) substance* – | Examples: |

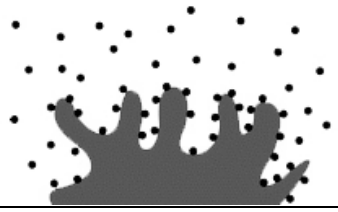
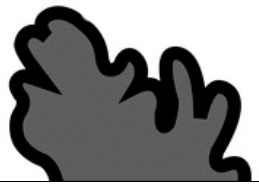
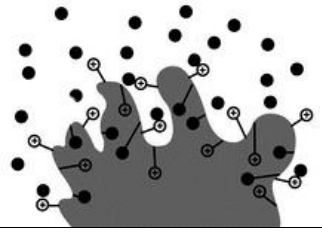
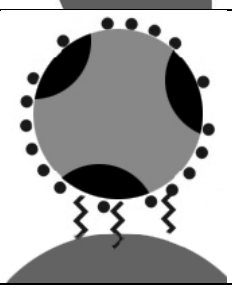
| | |
|---|---|
| *The amphiphilic substance* | Examples: |

**Give the classification of chromatographic methods of analysis according to the aggregate state of the mobile phase:**

| | |
|---|---|
| **1.** | **1.** |
| **2.** | **2.** |
| | **3.** |

**Give the classification of chromatographic methods of analysis according to the technique (method) of carrying out the process of separation phase:**

**Give the classification of chromatographic methods of analysis according to the nature of the processes, which occur during the separation of components (according to the mechanism of interaction of the sorbent and sorbate):**

| | |
|---|---|
|  | *Adsorptive chromatography* |
|  | *Separation chromatography* |
|  | *Ion exchange chromatography* |
|  | *Exclusion chromatography* |
|  | *Affinity chromatography* |
|  | *Sediment chromatography* |

**Give the classification of chromatographic methods of analysis according to the purpose of conducting the chromatographic processes:**

**Indicate the possibilities of applying chromatographic research methods:**

**Indicate advantages and disadvantages of the chromatographic research methods:**

| *Advantages* | *Disadvantages* |
| --- | --- |
| | |

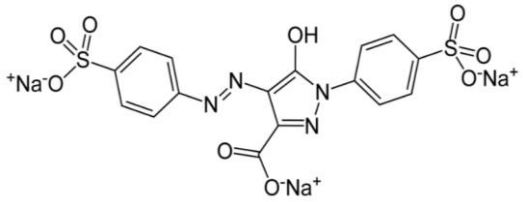
## LABORATORY WORK No. 1

**Separation of the mixture of hydrophobic and hydrophilic substances (The experiment "The Banner of Ukraine")**

An emerald-green carbonated drink called "Tarhun" was taken for the analysis. The classic recipe for making the drink includes the use of such components as water, citric acid, sugar and tarragon extract (a plant also known as tarragon, which gave the drink its name). The real "Tarhun" from the tarragon extract is often produced of the yellow color nowadays but in green bottles to match the consumer habits.

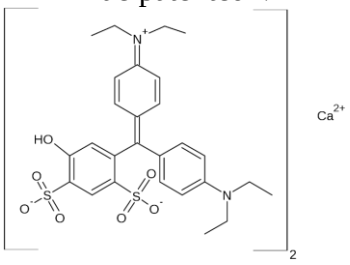
On the shelves in the shops we can come across the modern analogue of "Tarhun", for getting the green color of which orange-yellow (E102) and blue (E131) dyes are used.

Light-yellow dye (E 102) –
Tartrazine



$C_{16}H_{10}N_4Na_2O_9S_2$
Molecule mass: 512,38
***Well soluble in water, not soluble in organic solvents***

Red-blue dye (E 131) –
Blue patented V

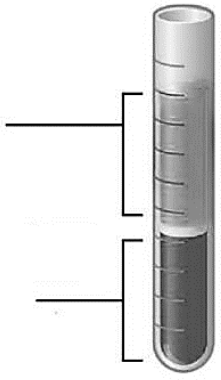


$C_{54}H_{62}CaN_4O_{14}S_4$
Molecule mass: 579,72
***Well soluble in water***

THE ORDER OF CONDUCTING THE EXPERIMENT:

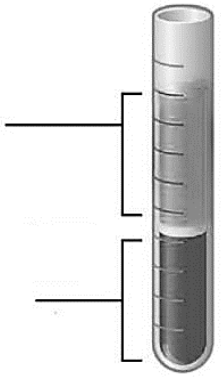
1. Take a clean dry 50 ml test tube and place 5 ml of "Tarhun" drink.
2. Add 5 ml of chloroform to the test tube, shake and mark the separation of the mixture into the layers, consider their coloring.

*Observations*



3. Place into a 2 ml test tube 5 mg/ml of novocaine solution and mix intensively, consider the coloring of the layers.

*Observations*



4. Draw conclusions.

(Date)

**The theme of the lesson**:
*The theoretical foundations and practical application of the paper chromatography method in the pharmacopoeial analysis of medicinal products.*

**Give the definition of the terms:**

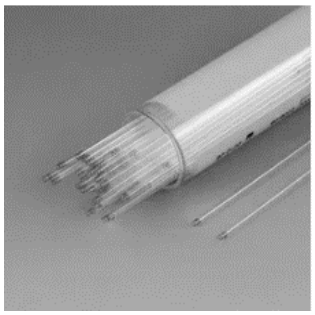
*Paper chromatography –*

*Chromatographic paper –*

**State the mobile and stationary phases in the paper chromatography method:**

| *Mobile phase* | *Stationary phase* |
|---|---|
| ↓ | ↓ |
| | |

**Specify the equipment and materials used in the paper chromatography method:**

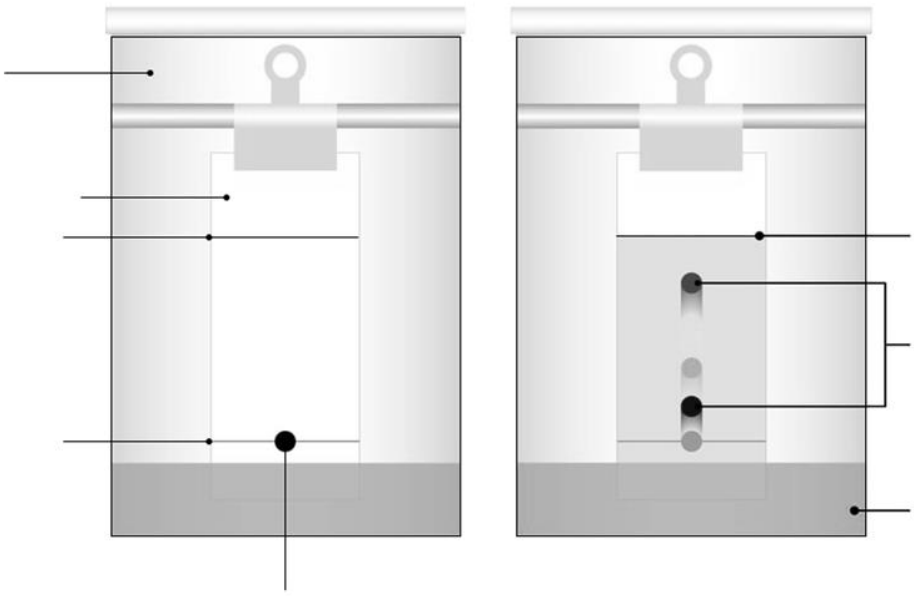
**State the requirements for chromatographic paper:**

**Specify the peculiarities and purpose of chromatographic paper types:**

| *Hydrophilic chromatographic paper* | *Hydrophobic chromatographic paper* |
|---|---|
| ↓ | ↓ |
| | |

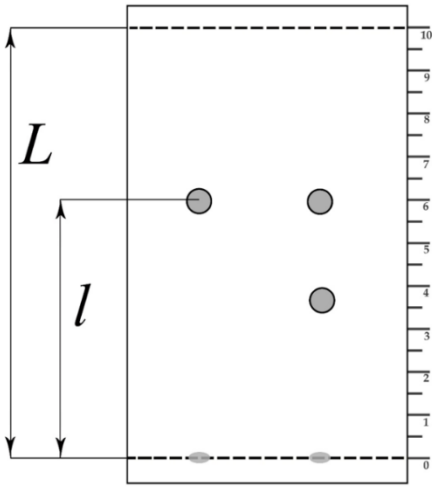
**Specify the requirements for the analytical solvents used for conducting the mobile phase:**

**Mark *chromatographic chamber, chromatographic paper, the place for applying the test solution, the "start" and "finish" lines, mobile phase, the received chromatographic zones in the picture*:**



**Give the definition of retardation factor ($R_f$ ), specify the formula for its calculation:**

*Retardation factor, $R_f$ –*




$R_f$ =

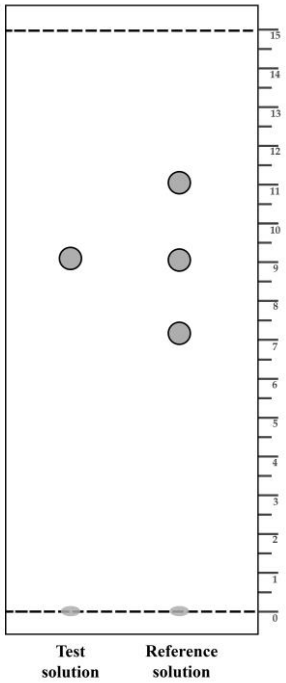
*L* - _____

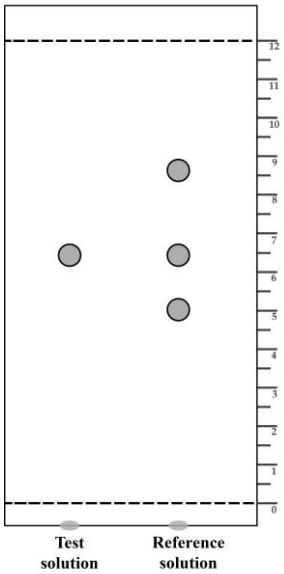
_____

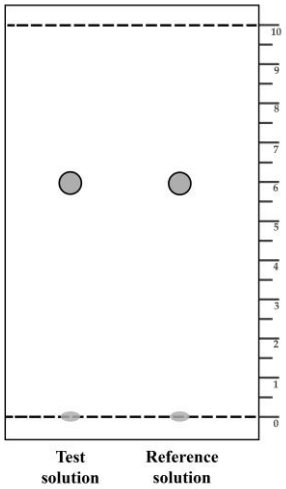
*l* - _____

_____

**Calculate the retardation factor ($R_f$) for the chromatographic zones of the test and reference solutions, draw conclusions:**

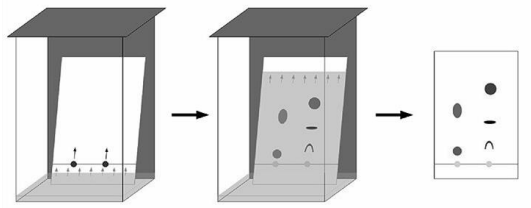
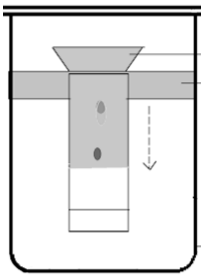
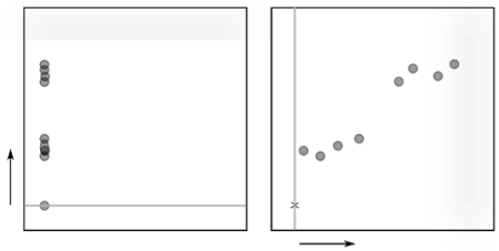
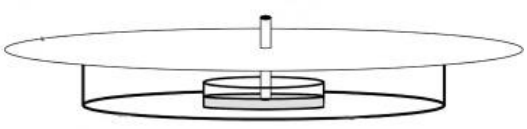



| *Calculations:* | *Calculations:* | *Calculations:* |
|---|---|---|
| | | |

**Give a name to the ways of conducting paper chromatography, characterize them in short:**

| | |
|---|---|
|  | |
|  | |
|  | |
|  | |

**Specify the advantages and disadvantages of the paper chromatography method:**

| *Advantages* | *Disadvantages* |
| --- | --- |
| ↓ | ↓ |
| | |

## LABORATORY WORK No. 2

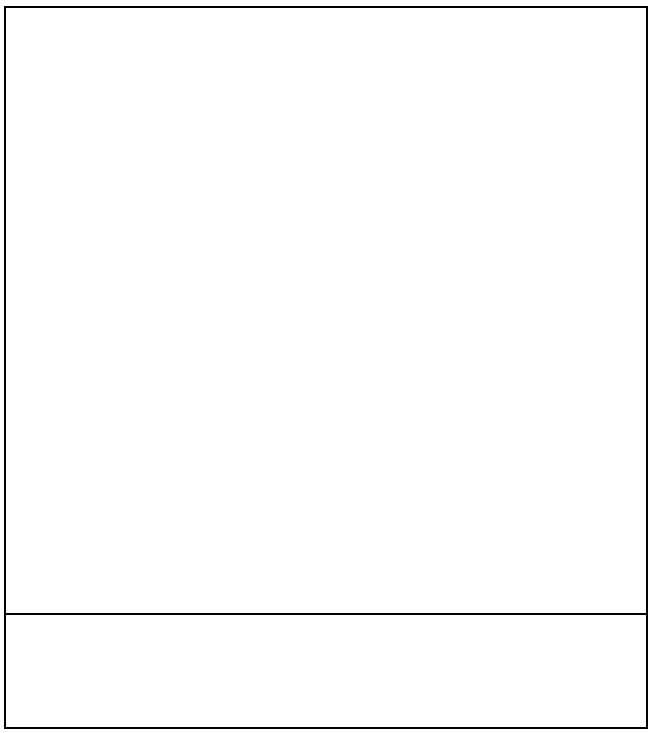
**Determination of ink composition by the paper chromatography method**

*The aim of the work:* to master the technique of conducting ascending paper chromatography, determine the composition of ink pens, markers, felt-tip pens, etc.

*Equipment and reagents:* paper for chromatography; samples of ink of different chemical composition (pens, markers, felt-tip pens); alcohol (96 %); bromothymol blue solution; phenolphthalein solution; chemical beaker with a volume of 200 ml; glass; spray; measuring cylinder; tweezers; pencil; ruler.

THE ORDER OF CONDUCTING THE EXPERIMENT:

1. Prepare and cut out paper for chromatography of the necessary size. With the help of the pencil and ruler mark a line on it, where the ink samples will be applied (the "start" line). Move 10 cm from the "start" line and draw the "finish" line.
2. Apply the ink samples onto the "start" line.
3. Place 20 ml of alcohol (96 %) into the chemical beaker and cover it with glass.
4. With the help of laboratory tweezers place paper for chromatography with the applied samples into the beaker with the mobile phase (alcohol 96 %) for the place of sample application to be higher than the level of the solvent.
5. Wait until the solvent reaches the "finish" line and with the help of tweezers take out paper for chromatography, let it air dry.
6. Place the substance detection solution (bromothymol blue or phenolphthalein solution) into a plate spraying device and spray onto the surface of paper for chromatography.
7. Analyze the results and compare the ink composition in different samples. Draw the obtained results.



8. Calculate the retardation factor ($R_f$) for the obtained chromatographic zones:

9. Draw conclusions regarding the ink composition.

## LABORATORY WORK No. 3

**Separation of a mixture of iron (III) ions and copper (II) by circular (radial) paper chromatography method**

*The aim of the work:* to master the technique of conducting the experiment by circular (radial) paper chromatography method and separate the mixture of iron (III) ions and copper (II).

*Equipment and reagents:* circular paper for chromatography 10 cm in diameter*;* 0.1 % solution containing the mixture of iron (III) ions and copper (II); alcohol (96 %); potassium ferrocyanide solution; desiccator with a lid; calibrated capillaries or microsyringe; spray; measuring cylinder; tweezers; pencil; ruler.

THE ANALYSIS TECHNIQUE:

***The test solution.*** 0.1 % solution containing the mixture of iron (III) ions and copper (II).

***Paper for chromatography:*** circular paper for chromatography 10 cm in diameter, the width of the "wick" is 5 mm.

***The volume of probe application.*** 5 μl of the test solution.

***The mobile phase.*** *Alcohol (96 %),* 10 ml.

***Chromatography.*** Paper for chromatography with the applied probes is placed into the chromatographic chamber. When the application zone is washed out and the mobile phase passes a certain distance, the paper is removed, the "finish" line is marked with a pencil.

***Eluent Removal.*** Chromatographic paper is dried in air or in a stream of air.

***Detection of chromatographic zones.*** It is carried out by spraying the chromatographic paper with a solution of potassium ferrocyanide ($K_4[Fe(CN)_6]$).

***The result.*** Retardation factor ($R_f$) values are calculated. The conclusion is made.

THE ORDER OF CONDUCTING THE EXPERIMENT:

1. Prepare 0.1 % solution containing the mixture of salts of iron(III) chloride and copper(II) sulfate.
2. *Chromatographic chamber preparation.* A desiccator can be used as chromatographic chamber with the idea of obtaining circular chromatogram. Place 10 ml of mobile phase (alcohol 96 %) into the chromatographic chamber, close with the lid for saturation.
3. *Chromatography paper preparation.* Mark the places of probe application on the paper for chromatography and draw the outline of the "wick" 5 mm in width.
4. *Probe application.* Apply 5 μl of the test solution onto the center of the chromatographic paper, dry the application area.
5. *Chromatography*. Place the paper into the chromatographic chamber so that the "wick" touches the mobile phase and close the chamber lid.
6. When the mobile phase covers 2/3 of the plate, remove the paper for chromatography, mark the distance the mobile phase has covered, and air dry.
7. *Searching for chromatographic zones (detection).* Apply a solution for detecting chromatographic zones – a solution of potassium ferrocyanide ($K_4[Fe(CN)_6]$) onto the paper. Specify the coloring of the obtained chromatographic zones. State the reaction equation underlying the detection of the compounds and indicate the analytical effect.

$Fe^{3+} + K_4[Fe(CN)_6] \rightarrow$

coloring –

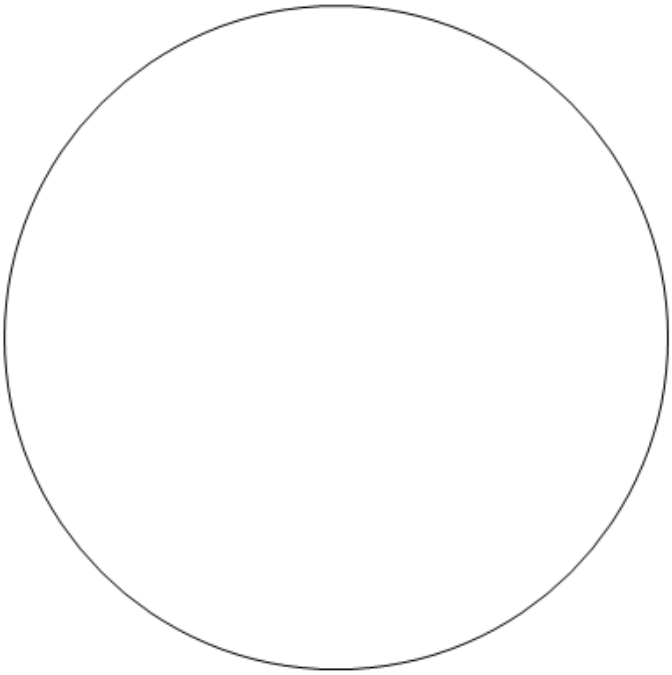
| |
|---|
| $Cu^{2+} + K_4[Fe(CN)_6] \rightarrow$ |

coloring –

8. Calculate the retardation factor ($R_f$) for the obtained chromatographic zones.

| |
|---|
| |

9. Draw the obtained chromatographic zones.



10. Draw conclusions.

(Date)

**The theme of the lesson**:
*The theoretical foundations and practical application of thin-layer and high-performance thin-layer chromatography methods in the pharmacopoeial analysis of medicinal products.*

**Give the definition of the terms:**

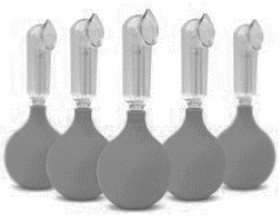
*Thin-layer chromatography (TLC) is*

*High-performance thin-layer chromatography (HPTLC) is*

**State the mobile and the stationary phases in TLC/HPTLC methods:**

| *Mobile phase* | *Stationary phase* |
|---|---|
| ↓ | ↓ |
| | |

**Specify the equipment and materials used in TLC/HPTLC methods:**



**Compare TLC/HPTLC methods:**

| *Parameter* | *Thin-layer chromatography (TLC)* | *High-performance thin-layer chromatography (HPTLC)* |
|---|---|---|
| Thickness of the sorbent layer | | |
| Size of the sorbent particles | | |
| Volume of the application probe | | |
| Plate size | | |
| Sensitivity | | |
| Effectiveness of separation | | |
| Analysis duration | | |

**Calculate the weight of the substance that needs to be weighed on the analytical scales to prepare solutions of a given concentration:**
*example:*

| | |
|---|---|
| **1.0 %** amlodipine solution, **10 ml** | *calculations:*<br>1.0 % solution means that 1.0 g is needed for preparation of the solution with the volume of 100 ml. Accordingly, for preparing 10 ml of the solution it is necessary to take the substance:<br>1.0 g – 100 ml<br>X g – 10 ml<br>X = 0.1 g |

| | |
|---|---|
| **2.5 %** bisoprolol solution, **20 ml** | *calculations:* |
| **10.0 %** piracetam solution, **100 ml** | *calculations:* |
| **0.5 %** analgin solution, **20 ml** | *calculations:* |

| | |
|---|---|
| **4.0 %** paracetamol solution, **25** ml | *calculations:* |
| **1.5 %** nifuroxazide solution, **50 ml** | *calculations:* |

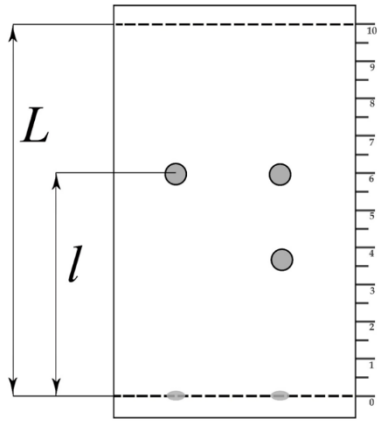
**Calculate the volumes of the mobile phase components that need to be mixed for obtaining the given amount of the mobile phase (eluent):**
*example:*

| | |
|---|---|
| *Alcohol (96 %) – water (70:30),* **50 ml** | *Alcohol (96 %)* – **35 ml**<br>*Water* – **15 ml** |

| | |
|---|---|
| *Glacial acetic acid – chloroform – water (70:60:10),* **28 ml** | *Glacial acetic acid* – _____ **ml**<br>*Chloroform* –_____ **ml**<br>*Water* – _____ **ml** |
| *Glacial acetic acid – water – dioxane (10:25:65),* **20 ml** | *Glacial acetic acid* – _____ **ml**<br>*Water* – _____ **ml**<br>*Dioxane* – _____ **ml** |
| *Water – methanol – glacial acetic acid – ethylene chloride (10:15:25:50),* **40 ml** | *Water* – _____ **ml**<br>*Methanol* – _____ **ml**<br>*Glacial acetic acid* – _____ **ml**<br>*Ethylene chloride* – _____ **ml** |
| *Concentrated ammonia solution – methanol – methylene chloride (2:20:80),* **51 ml** | *Concentrated ammonia solution* – _____ **ml**<br>*Methanol* – _____ **ml**<br>*Methylene chloride* – _____ **ml** |
| *Concentrated ammonia solution – cyclohexane – alcohol (96 %) (6:30:72),* **36 ml** | *Concentrated ammonia solution* – _____ **ml**<br>*Cyclohexane* –_____ **ml**<br>*Alcohol (96 %)* –_____ **ml** |

**Give the definition of retardation factor ($R_f$), specify the formula for its calculation:**

*Retardation factor ($R_f$) –*

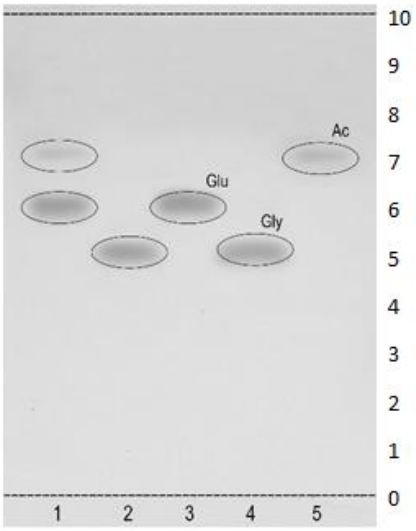



$R_f$ =

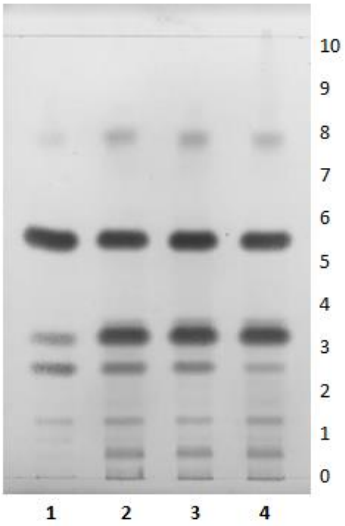
*L* - _____

*l* - _____

**Calculate the retention factor *($R_f$)* for the test substances and reference solutions in the chromatograms shown below:**




**1** – test solution of medical product № 1;
**2** – test solution of medical product № 2;
**3** – reference solution of glutamic acid ;
**4** – reference solution of glycine;
**5** – reference solution of ascorbic acid




**1** – reference solution: the upper zone – alantolactone, the next zone – isoalantolactone, the next zone – dihydroisoalantolactone;
**2-4** – test solution: rhizomes and roots of Inula helenium

| *Calculations:* | *Calculations:* |
|---|---|
| | |

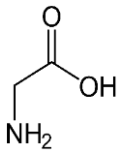
**State the advantages and disadvantages of TLC/HPTLC methods:**

| *Advantages* | *Disadvantages* |
|---|---|
| ↓ | ↓ |
| | |

## LABORATORY WORK No. 4

**Identification and determination of glycine substance purity by the method of thin-layer chromatography**

The substance of glycine, which is used for manufacturing medicinal products "Glycine", "Glycised", etc., was sent to the control and analytical laboratory for analysis. Analyze the glycine substance and draw a conclusion regarding its quality.




***Glycine*** ($C_2H_5NO_2$, aminoacetic acid, aminoethanoic acid) is the simplest aliphatic amino acid. It is used in medicine as a nootropic medicinal product that stimulates mental activity, improves memory and learning ability.

*The aim of the work:* to master the technique of conducting the experiment by thin-layer chromatography method; identify and define the glycine substance purity.

*Equipment and reagents:* a plate for thin-layer chromatography "Silica gel 60"; glycine substance; standard sample of glycine and glutamic acid; *alcohol* (96 %); *water R*; *ninhydrin R1 solution*; chromatographic chamber; calibrated capillaries or microsyringe; spray; thermal cabinet; a flask with a lid; measuring cylinder; tweezers; pencil; ruler.

THE ANALYSIS TECHNIQUE:

***The test solution (a).*** Dissolve 0.01 g of *glycine substance* in *water R* and dilute to 25.0 mL with the same solvent.

***The test solution (b).*** Dilute 2.5 mL of *test solution (a)* to 25.0 mL with *water R*.

***Reference solution (a).*** Dissolve 10 mg of *glycine CRS* in *water R* and dilute to 25.0 mL with the same solvent.

***Reference solution (b).*** Dilute 1.0 ml *of the test solution (a)* to 200.0 mL with *water R*.

***Reference solution (c).*** Dissolve 10 mg of *glycine CRS* and *glutamic acid CRS* in *water R* and dilute to 25.0 mL with the same solvent.

***Plate***. A plate for thin-layer chromatographers "Silica gel 60".

***Development***: 10 cm.

***Application***: 5 μL.

***Mobile phase.*** *alcohol (96 %) – water* (70:30).

***Chromatography.*** The plate is placed into the pre-saturated chromatographic chamber. Elution is carried out until the mobile phase reaches the "finish" line.

***Removal of eluent.*** The plate is dried in the air**.**

***Detection:*** It is carried out by spraying the plate with a solution of *ninhydrin R1* and subsequent heating at temperatures from 100 °C to 105 °C for 5 min.

***Suitability of the chromatographic system.*** The chromatogram of the reference solution (c) should show two clearly separated chromatographic zones.

***Identification.*** On the chromatogram of *the test solution* (a), the zone of glycine should be detected at the level of the zone of glycine on the chromatogram of the reference solution (a) and the reference solution (c).

***Purity definition.*** On the chromatogram of the test solution (a) any zone, except for the basic one, should not be more intensive than the zone on the chromatogram of the test solution *(b)* (0.5 %).

THE ORDER OF CONDUCTING THE EXPERIMENT:

**I. Preparation of the mobile phase (eluent)**

The mixture is prepared as the mobile phase:

| |
|---|
| *alcohol (96 %) – water R* (70:30) |

Calculate the volumes of the mobile phase components needed to mix in order to get the set amount of eluent:

| |
|---|
| *Calculations:* |

Measure all the components of the mobile phase into the flask with a lid and mix.

**II. Preparation of the chromatographic chamber**

Use a clean, dry chromatographic chamber for the analysis. The technique states that a saturated chromatographic chamber is used for the analysis, so prepare a saturated chromatographic chamber based on the technique given below. To do this, cut out a rectangular piece of filter paper and place it on one side of the chromatographic chamber. Pour the mobile phase onto the bottom of the chamber, tightly close the lid, leave for 1 hour for saturation.

**III. Preparation of the test and reference solutions.**

Prepare test and reference solutions following the technique.

**IV. Preparation of the TCL plate**

Cut out a chromatographic plate of the necessary size. With the help of a ruler and a graphite pencil draw the "start" and "finish" lines, also mark the areas for applying the test and reference solutions.

**Note!** The substance solution is applied in the form of rounded spots.

- The distance from the bottom edge of the plate to the "start" line – 1.5 cm
- The distance to be covered by the mobile phase – _____ cm (the distance from the "start" line to the "finish" line).
- The distance from the left and the right edge of the plate to the probe application zones – 1.5 cm.
- The distance between the probe application zones – 1.0 cm.

**V. Probe application**

Specify the probe application volume:

*Test solution (a)* – _____ µl

*Test solution (b)* – _____ µl

*Reference solution (a)* – _____ µl

*Reference solution (b)* – _____ µl

*Reference solution (c)* – _____ µl

Apply the test and reference solutions onto the TLC plate, drying the chromatographic zone thoroughly between each application.

**Note!** Before the application, first rinse the syringe three times with alcohol (96 %) and then three times with the test or reference solution.

**V. Chromatography**

With the help of laboratory tweezers place the TLC plate into the chromatographic chamber, carefully dipping the ends of the plate into the mobile phase. As soon as the mobile phase passes from the "start" to the "finish" line, remove the plate and leave it in the air to remove excess mobile phase.

**VIII. Searching for the chromatographic zones (detecting)**

For detecting chromatographic zones according to the technique *ninhydrin R1* solution is used, which after heating in a thermal cabinet with α-amino acids gives characteristic coloring. State the reaction underlying the detection and specify the analytical effect:

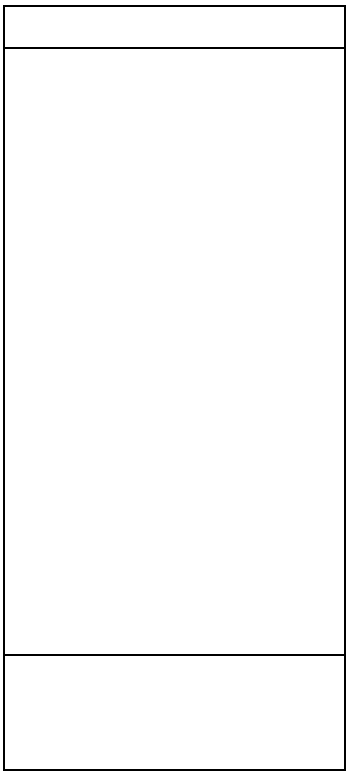
| |
|---|
| |

Place *ninhydrin R1* solution into the atomizer and process the TLC plate. Then place the plate in a thermal cabinet at a temperature of 100–105 °C for 5 minutes.

**IX. Parameter calculation, drawing the conclusions**

To obtain the chromatographic zones of the test solutions and reference solutions, calculate the retardation factor ($R_f$):

Draw the obtained chromatogram:



Make a conclusion regarding the substance quality.

## LABORATORY WORK No. 5

**Identification of biologically active substances of medicinal herbal raw material by the high-performance thin-layer chromatography method on the hardware complex Camag®**

*The aim of the work:* to master the technique of conducting the experiment by the high-performance thin-layer chromatography method on the hardware complex Camag®, identify biologically active substances of medicinal herbal raw material.

*Equipment and reagents:*

THE ANALYSIS OF THE TECHNIQUE, ORDER OF CONDUCTING THE EXPERIMENT:

To obtain the chromatographic zones of the test samples and reference solutions, calculate the retardation factor ($R_f$):

Draw the obtained chromatogram:

Make a conclusion regarding the quality of the medicinal herbal raw material.

(Date)

**The theme of the lesson**:
*The theoretical foundations and practical application of the liquid chromatography method and its use in the pharmacopoeial analysis of medicinal products.*

**Give the definition of the terms:**

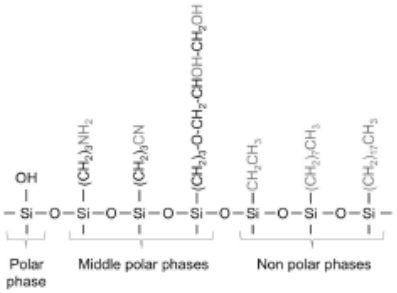
*Liquid chromatography is*

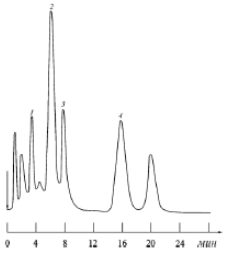
*Liquid chromatographer is*

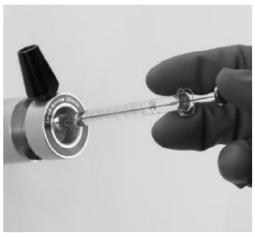
**Specify the mobile and stationary phases in the liquid chromatography method:**

| *Mobile phase* | *Stationary phase* |
|---|---|
| ↓ | ↓ |
| | |

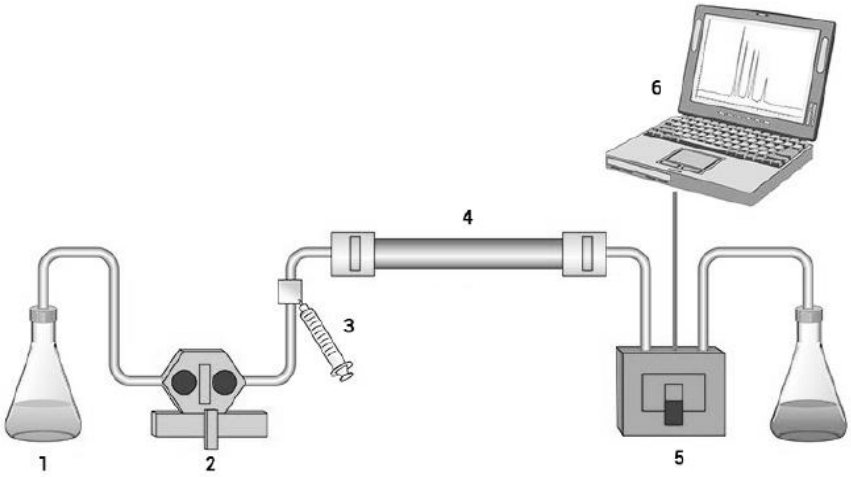
**Indicate the equipment and materials used in liquid chromatography method:**





**Name the main blocks of a liquid chromatographer and their purpose:**




| | *The name of the block* | *Its purpose* |
|---|---|---|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |

| | *The name of the block* | *Its purpose* |
|---|---|---|
| 6 | | |

**Name the main advantages and disadvantages of using silica gel as a stationary phase in liquid chromatography. Give examples of modified silica gel.**

| |
|---|
| |

**Characterize the ways of presenting a mobile phase in the liquid chromatography method, specify advantages and disadvantages**

| *Isocratic elution* ↓ | *Gradient elution* ↓ |
|---|---|
| | |
| Advantages: | Advantages: |
| Disadvantages: | Disadvantages: |

**Describe the variants of normal-phase and reversed-phase liquid chromatography:**

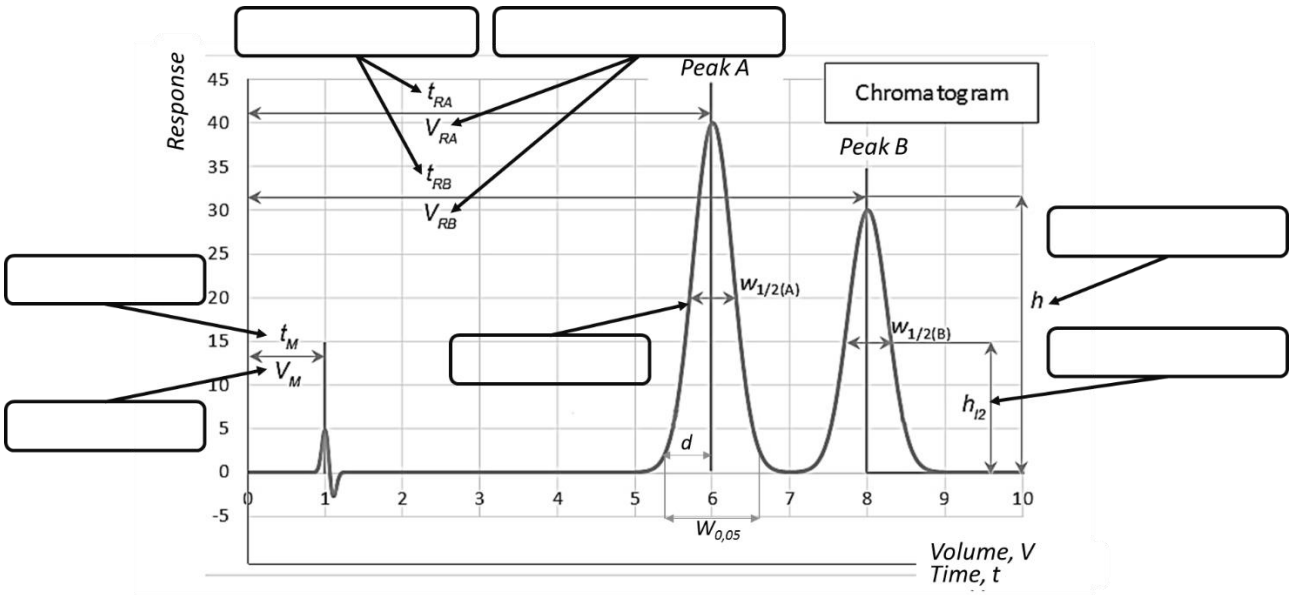
| *Normal-phase chromatography* ↓ | *Reversed-phase chromatography* ↓ |
|---|---|
| | |

| | |
|---|---|
| | |

**Describe the types of detectors in the liquid chromatography method:**

| *The detector type* | *Substances it detects* | *Sensitivity, selectivity* | *Disadvantages* |
|---|---|---|---|
| Spectrophotometric | | | |
| Diode-matrix | | | |
| Refractometric | | | |
| Conductometric | | | |
| Fluorimetric | | | |
| Mass detector | | | |

**Specify the chromatogram parameters and name the terms used to characterize it:**




*Chromatogram is*

*Chromatographic peak is*

*The basic line –*

*Retention time ($t_R$) –*

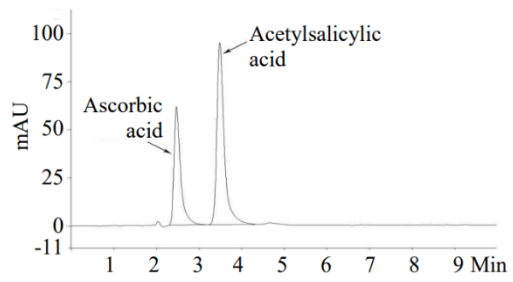
*Retention volume ($V_R$) –*

*Hold-up time ($t_M$) –*

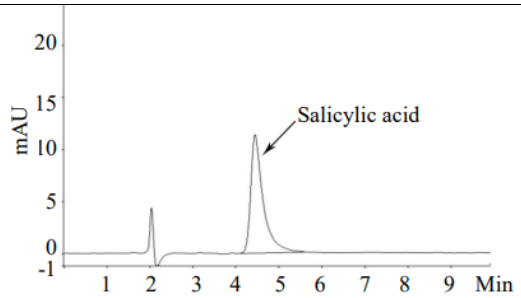
*Hold-up volume ($V_M$) –*

*Relative (corrective) holding time -*

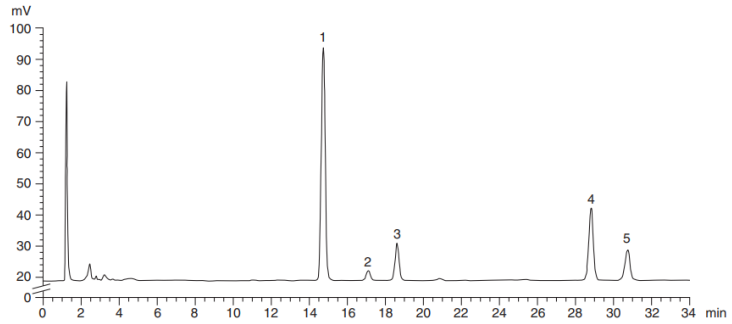
**Indicate the retention time ($t_R$) for the substances under analysis using the chromatograms below:**




*Ascorbic acid – _____ min*
*Acetylsalicylic acid – _____ min*




*Salicylic acid – _____ min*




*Peak № 1 (Bilobalid) – ____ min*
*Peak № 2 (Ginkgolid J) – ___ min*
*Peak № 3 (Ginkgolid C) – ___ min*
*Peak № 4 (Ginkgolid A) – ___min*
*Peak № 5 (Ginkgolid B) – ___ min*

**Supply the formulae for calculating the general characteristics of the chromatogram and make calculations:**

| *Relative (corrective) holding time:* | *Resolution ($R_s$):* | *Symmetry factor ($A_s$):* |
|---|---|---|
| | | |

| | |
|---|---|
| Calculate the corrective retention time of valerian and caproic acid if the retention time of substances is: for the solvent – 2.5 min, for valerian acid – 10.5 min, for caproic acid – 11.8 min. | *Calculations:* |
| Calculate the corrective retention time of isopropanol if the retention time is: for the solvent – 1.2 min, for isopropanol – 6.3 min. | *Calculations:* |
| Calculate the corrective retention time of camphor when the retention time equals: for the solvent – 0.5 min, camphor – 5.6 min. | *Calculations:* |
| Calculate the corrective retention time of acetaldehyde if the retention time equals: for the solvent – 1.5 min, acetaldehyde – 9.3 min. | *Calculations:* |
| Calculate the corrective retention time of methanol when the retention time equals: for the solvent – 1.5 min, methanol – 3.8 min. | *Calculations:* |
| Calculate the resolution *($R_s$)* if it is known that the duration of the first peak output is | *Calculations:* |

| | |
|---|---|
| 2,52 min, of the second one – 7,21 min. The peak width at half of the height for the first one is 0,2 min, for the second one – 0,55 min. | |
| Calculate the resolution *($R_s$)* if it is known that the duration of the first peak output is 4,68 min, of the second one – 10,44 min. The peak width at half of the height for the first one is 0,35 min, for the second one – 0,62 min. | *Calculations:* |
| Calculate the resolution *($R_s$)* if it is known that the duration of the first peak output is 3,18 min, of the second one – 4,24 min. The peak width at half of the height for the first one is 0,18 min, for the second one – 0,42 min. | *Calculations:* |
| Calculate the resolution *($R_s$)*, if it is known that the duration of the first peak output is 5,48 min, of the second one – 1,80 min. The peak width at half of the height for the first one is 0,54 min, for the second one – 0,62 min. | *Calculations:* |
| Calculate the resolution *($R_s$)* if it is known that the duration of the first peak output is 8,25 min, of the second one – 6,30 min. The peak width at half of the height for the first one is 0,66 min, for the second one – 0,25 min. | *Calculations:* |
| Calculate the symmetry factor *($A_s$)* if it is known that the peak width at 1/20 of the height is 10 mm, the width of the front edge of the peak is 6 mm. Make a conclusion about the conformity of the symmetry ratio value to the requirements of the SPU. | *Calculations:* |
| Calculate the symmetry factor *($A_s$)* if it is known that the peak width at 1/20 of the height is 24 mm, the width of the front edge of the peak is 10 mm. Make a conclusion about the conformity of the symmetry ratio value to the requirements of the SPU. | *Calculations:* |
| Calculate the symmetry factor *($A_s$)* if it is known that the peak width at 1/20 of the height is 36 mm, the width of the front edge of the peak is 24 mm. Make a conclusion about the conformity of the symmetry ratio value to the requirements of the SPU. | *Calculations:* |
| Calculate the symmetry factor *($A_s$)* if it is known that the peak width at 1/20 of the height is 18 mm, the width of the front edge of the peak is 8 mm. Make a conclusion about the conformity of the symmetry ratio value to the requirements of the SPU. | *Calculations:* |

| | |
|---|---|
| Calculate the symmetry factor *($A_s$)* if it is known that the peak width at 1/20 of the height is 25 mm, the width of the front edge of the peak is 13 mm. Make a conclusion about the conformity of the symmetry ratio value to the requirements of the SPU. | *Calculations:* |

**State the advantages and disadvantages of the liquid chromatography method:**

| *Advantages* | *Disadvantages* |
|---|---|
| ↓ | ↓ |
| | |

## LABORATORY WORK No. 6

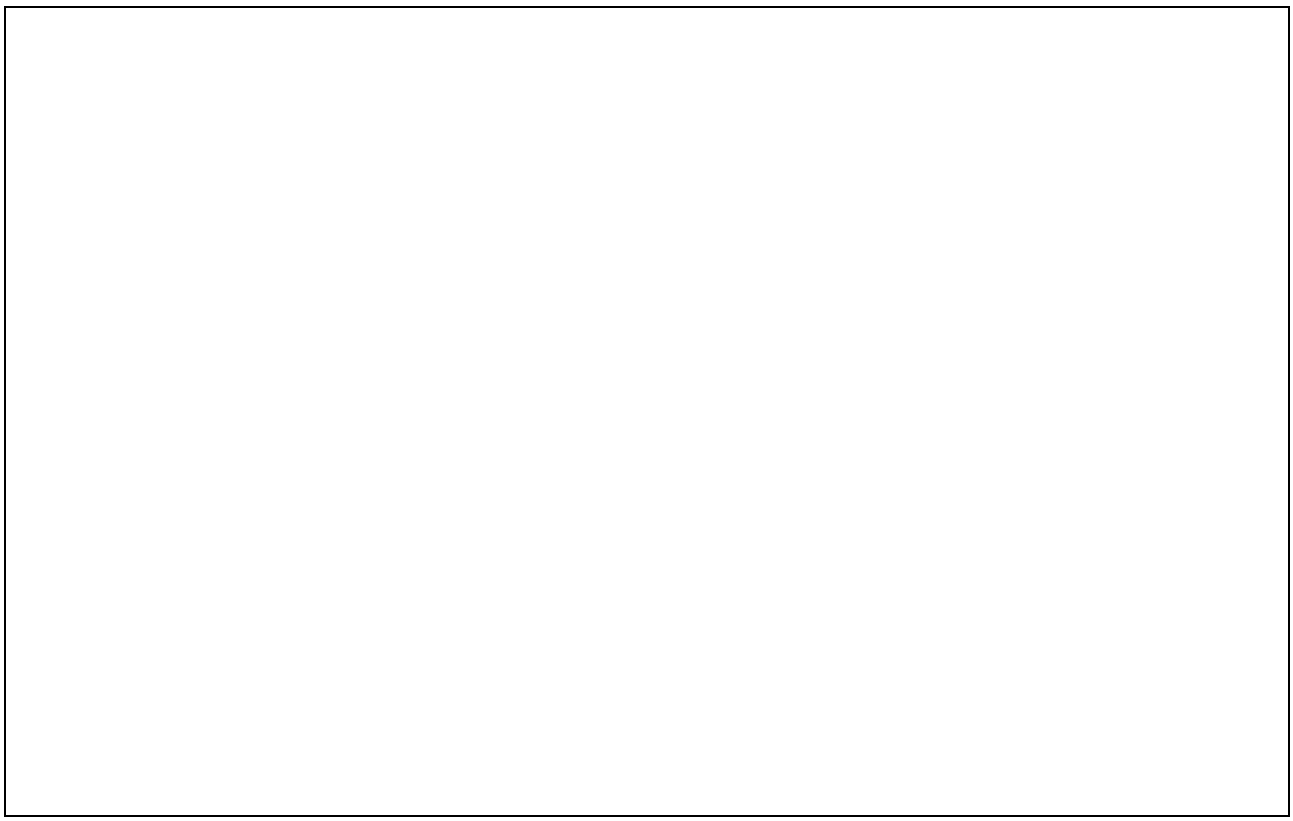
**The analysis of the substance, medicinal products or medicinal herbal raw material by the liquid chromatography method**

*The aim of the work:* to master the technique of conducting an experiment by the liquid chromatography method, carry out the analysis of the substance, medicinal products or medicinal herbal raw material.

*Equipment and reagents:*

THE ANALYSIS TECHNIQUE, THE ORDER OF CONDUCTING THE EXPERIMENT:

The place for the chromatogram:



Draw a conclusion regarding the quality of the substance, medicinal product or medicinal herbal raw material:

(Date)

**The theme of the lesson**:
*Theoretical foundations of ion, ion exchange and exclusion chromatography methods, their application in the pharmacopoeial analysis of medicinal products.*

**Give the definition of the terms:**

*Ion-exchange chromatography is the method*

*Ion-exchange is the process*

*A counterion is*

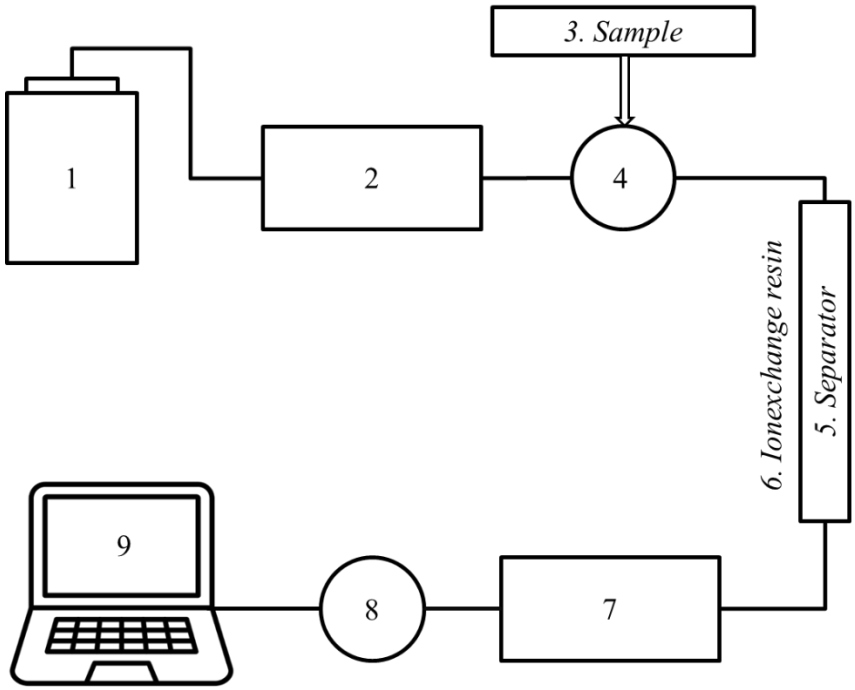
**Specify the mobile and stationary phases in ion, ion exchange and exclusion chromatography methods:**

| *The method title* | *Mobile phase* ↓ | *Stationary phase* ↓ |
|---|---|---|
| Ion chromatography | | |
| Ion-exchange chromatography | | |
| Exclusion chromatography | | |

**Indicate which of the definitions describes the "cation exchange process" and which one the "anion exchange process"?**

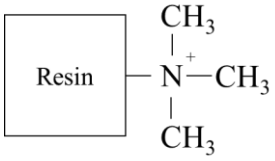
| | |
|---|---|
| In the chromatographic process the positively charged analytes bind to the negatively charged resin and exchange ions. ↓ | In the chromatographic process the negatively charged analytes bind to the positively charged resin and exchange ions. ↓ |
| | |

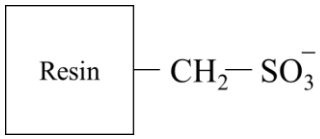
**Name the main blocks of the device for the ion exchange chromatography and their purpose:**

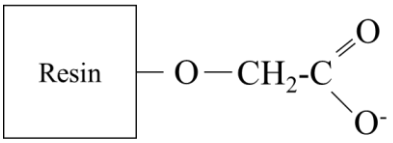


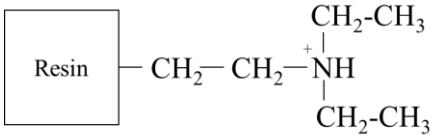

| | *The block name* | *Its purpose* |
|---|---|---|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |
| 6 | | |
| 7 | | |
| 8 | | |
| 9 | | |

**Indicate the types of ion exchange resin:**




**Give the characteristic of the ion exchange resin, supply examples:**

| | |
|---|---|
| *Strong acid cation exchangers* – | Examples: |
| Weak acid cation exchangers – | Examples: |
| Strong alkaline anion exchangers – | Examples: |
| Weak alkaline anion exchangers – | Examples: |

**State the main kinds of detectors in the ion chromatography method and give the examples of the substances that can be defined with the help of these detectors:**

**Indicate advantages and disadvantages of ion-exchange chromatography:**

| *Advantages* | *Disadvantages* |
|---|---|
| ↓ | ↓ |
| | |

## LABORATORY WORK No. 7

**Determination of salt content (potassium or sodium chloride) in the test sample by the method of ion exchange chromatography**

*The aim of the work:* to master the technique of conducting the experiment by ion exchange chromatography, determine the salt content (potassium or sodium chloride) in the test sample.

*Equipment and reagents:* potassium (sodium) chloride substance; ionite; *water R*; universal indicator; a solution of 0.1 M sodium hydroxide or 0.1 M hydrochloric acid; a solution of 2 M solution of hydrochloric acid or 2 M solution of sodium hydroxide; methyl orange; phenolphthalein.

THE ANALYSIS TECHNIQUE:

Potassium (sodium) chloride substance is used as the test solution. At the first stage of the analysis it is necessary to prepare the chromatographic column. Ionite is usually saturated with *water R* followed by regeneration of the column. The chemical exchange of ions, which is the basis of ionite regeneration, can be characterized by the reactions:

H-form cation exchanger: $R\text{-}Me^+ + H^+ \leftrightarrow R\text{-}H + Me^+$

OH-form anion exchanger: $R\text{-}An^- + OH^- \leftrightarrow R\text{-}OH + An^-$

pH of the chromatographic column liquid is checked before the use to prevent errors during the analysis. The suitability of the chromatographic column is checked using a universal indicator.

Next, the aqueous solution of the tested product is passed through the chromatographic column for the purpose of ion exchange.

The chemical process underlying the stoichiometric ion exchange during the determination can be characterized by the reactions:

H-form cation exchanger: $H\text{-}R + Na^+ \leftrightarrow Na\text{-}R + H^+$ (HCl)

OH-form anion exchanger: $R\text{-}OH + Cl^- \leftrightarrow R\text{-}Cl + OH^-$ (NaOH)

The solution of the substance, formed after the ion exchange, is collected in a flask for titration, and titrimetric determination and calculation of the substance content are carried out.

THE ORDER OF CONDUCTING THE EXPERIMENT:

1. Take a chromatogtaphic column, filled with cationite (anionite), and pass 20-30 ml of *water R* through it.
2. Next, pass through the column 25-30 ml of 2 M solution of hydrochloric acid (if it is a KU-1 cationic column) or 2 M solution of sodium hydroxide (if it is an AB-17 anionic column) at a speed of 1 ml/min.

3. Wash the ionite from excess acid or alkali by passing 20-30 ml of *water R* through the column.

4. Place a drop of the solution flowing from the column on a strip of universal indicator paper. The color of the indicator should match the color given by *water R*.

5. 50.0 mg of the tested medicinal product is weighed with the help of analytical scales and dissolved in 15-20 ml of *water R*. The obtained solution is entered 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.

6. The solution of acid or alkali that has been released is titrated according to standard solutions of 0.1 M sodium hydroxide or 0.1 M hydrochloric acid. Based on the obtained data, the substance composition is calculated.

1 ml *0.1 M hydrochloric acid solution* corresponds to 5,844 mg NaCl.

1 ml *0.1 M sodium hydroxide solution* corresponds to 5,844 mg NaCl.

The reaction equation:

Calculations:

Conclusions:

(Date)

**The theme of the lesson**:
*The theoretical foundations of the gas chromatography method and its practical application in the pharmacopoeial analysis of medicinal products.*

**Give the definition of the terms:**

*Gas chromatography is*

*Gas chromatographer is*

**Indicate the mobile and stationary phases in the gas chromatography method:**

| *Mobile phase* | *Stationary phase* |
|---|---|
| ↓ | ↓ |
| | |

**State the requirements needed for the analyzed substances in the gas chromatography method:**

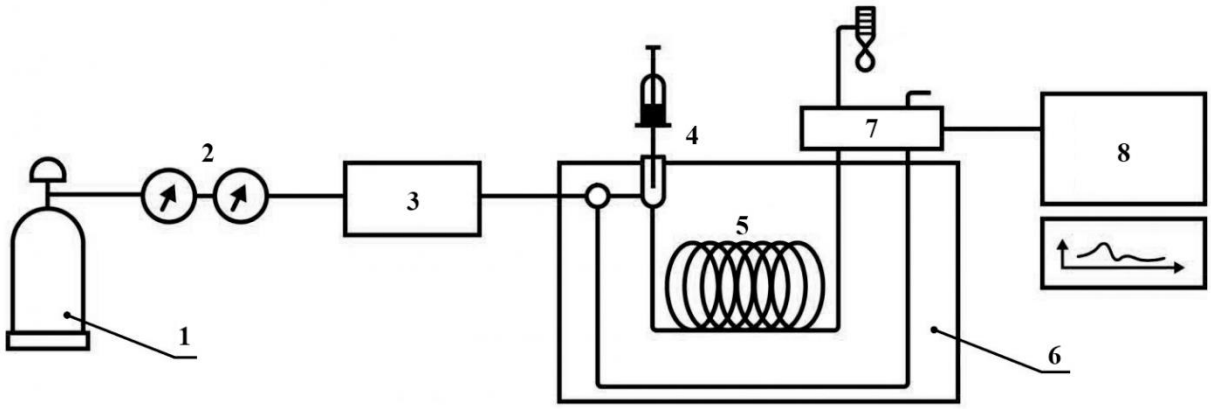
**Give the definition of the terms:**

*A volatile compound is –*

*Thermal lability is –*

*Derivatization is –*

**Name the main blocks of the gas chromatographer and their purpose:**




| | *The block name* | *Its purpose* |
|---|---|---|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |
| 6 | | |
| 7 | | |
| 8 | | |

**Specify the gas kinds used in gas chromatography and their purpose:**

| | | |
|---|---|---|
| | | |
| ↓ | ↓ | ↓ |
| Purpose: | Purpose: | Purpose: |
| Examples: | Examples: | Examples: |

**Characterize the column types in gas chromatography, indicate their advantages and disadvantages:**

| *Inserts (embossed)* | *Capillary* |
|---|---|
| ↓ | ↓ |
| | |
| Advantages: | Advantages: |
| Disadvantages: | Disadvantages: |

**Characterize the temperature mode types in the gas chromatography method:**

| *Isothermal* | *Programmed* |
|---|---|
| ↓ | ↓ |
| | |

**Characterize the detector types in the gas chromatography method:**

| *The detector name* | *The substances it defines* | *Sensitivity, selectivity* | *Disadvantages* |
|---|---|---|---|
| Beam ionization detector | | | |
| Katarometer | | | |
| Electron capture detector | | | |
| Thermoion detector | | | |
| Beam photometric detector | | | |

**Indicate the stages of the chromatographic process while conducting an experiment with the help of the gas chromatography method:**

**State the advantages and disadvantages of the gas chromatography method:**

| *Advantages* | *Disadvantages* |
|---|---|
| ↓ | ↓ |
| | |

**Compare the gas and the liquid chromatography methods:**

| *Parameter* | *Gas chromatography* | *Liquid chromatography* |
|---|---|---|
| Mobile phase | | |
| Stationary phase | | |
| Column kinds | | |
| Column length | | |
| Column diameter | | |
| Substances that can be defined | | |

## PRACTICAL WORK No. 1

### Determination of residual quantities of organic solvents

Study the SPU 2.4.24 "Identification of residual solvents and control of their quantities" article. According to this article, the control over the presence of the residual quantities of organic solvents can be conducted with the help of any validation method. 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 quantities of organic solvents determined?

## PRACTICAL WORK No. 2

### The analysis of essential oils by the gas chromatography method

Select the SPU monographs 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 the reasons for using the GC method in such a case.

## LABORATORY WORK No. 8

**The analysis of the substance, medicinal products or medicinal herbal raw material with the help of the gas chromatography method**

*The aim of the work:* to master the technique of conducting an experiment with the help of the gas chromatography method, carry out the analysis of the substance, medicinal products or medicinal herbal raw material.

*Equipment and reagents:*

THE ANALYSIS TECHNIQUE, ORDER OF CONDUCTING THE EXPERIMENT:

The place for the chromatogram:

Draw a conclusion regarding the quality of the substance, medicinal product or medicinal herbal raw material:

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