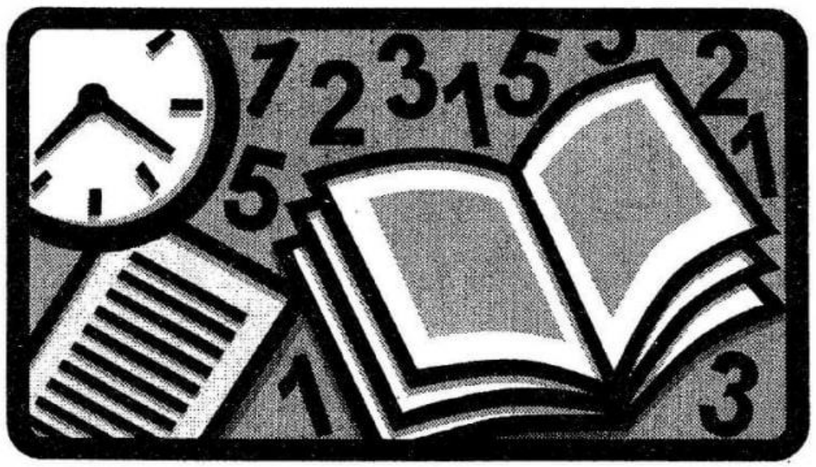
WORKING JOURNAL 2

IN TOXICOLOGICAL AND FORENSIC CHEMISTRY

STUDENT(S) OF THE COURSE GROUP



Class no.

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Date.

TOPIC: GROUP OF SUBSTANCES ISOLATED FROM BIOLOGICAL MATERIAL BY POLAR SOLVENTS ("MEDICINAL POISONS")

GENERAL AND SPECIFIC ISOLATION METHODS:

1. Isolation with water acidified with oxalic acid (method of A. A. Vasilieva)

The crushed object (organs of the corpse) is placed in a flask or beaker and poured with double the volume (relative to weight) of distilled water, then the mixture is acidified with a 10% oxalic acid solution to pH 2-3 according to universal indicator paper. After two hours of infusion with constant stirring of the mixture, an aqueous extract is obtained, which is filtered through a double layer of gauze. The infusion operation is repeated for one hour with a single volume of acidified water. The filtered acidic aqueous extracts are combined, placed in a separating funnel and shaken three times with separate portions of chloroform (15, 10, 10 ml). The chloroform extracts are combined and filtered through a small paper filter, previously moistened with chloroform, into a dry flask labelled "Acidic chloroform extracts". The aqueous layer in the separating funnel is alkalised with 25% ammonia solution to pH 8-9 using universal indicator paper and shaken again three times with separate portions of chloroform (15, 10, 10 ml). Combine the chloroform extractions and filter them as described above into a flask labelled "Alkaline chloroform extractions". When extracting poisons with chloroform from alkaline aqueous extractions, stable emulsions are formed, which can be destroyed by centrifuging the contents of the funnel or adding sodium sulfate to the mixture.

2. Isolation with ethanol acidified with oxalic acid (Stas-Otto method)

The crushed object (organs of the corpse) is placed in a glass or jar, poured with 96% ethanol until a mirror-like surface is formed, the mixture is acidified with a 10% alcohol solution of oxalic acid to pH 2-3 according to universal indicator paper and left for a day with periodic stirring. After this period, the acidic alcohol extracts are separated by filtering through an ethanol-soaked paper filter. The extraction operation is repeated 2-3 times. The acidic alcoholic extracts are combined and evaporated in a water bath at 40-50°C to a syrupy consistency. The syrupy liquid is treated with 96° ethanol, adding it dropwise until the ethanol no longer causes cloudiness of the liquid. The precipitate is allowed to settle and then filtered through a small filter (5-6 cm in diameter) previously moistened with alcohol. The filter is washed with a small amount of alcohol. The filtrate is condensed in a porcelain cup in a water bath to a syrupy consistency and the precipitation operation is repeated again. This is done until the ethanol no longer precipitates the proteins. Then the extract is evaporated again in a water bath to a syrupy consistency, the residue is dissolved in 25-30 ml of warm distilled water and the cloudy solution is filtered through a small smooth filter moistened with water into a separating funnel. The water-alcohol solution is extracted three times with chloroform in portions of 15, 10 and 10 ml. The chloroform extracts are filtered through a chloroform-soaked filter into a dry flask labelled "Acidic chloroform extracts". The aqueous residue in the separating funnel is alkalised with 25% ammonia solution to pH 8-9 using universal indicator paper and the chloroform extraction is carried out again as described above. The combined chloroform extractions are placed in a dry flask labelled "Alkaline chloroform extractions".

3. Isolation with water acidified with sulfuric acid (method by V. F. Kramarenko)

100 g of crushed biological material is placed in a flask or beaker, poured with 0.01 M sulfuric acid solution until a mirror-like surface is formed. If the pH of the solution after stirring the contents of the flask is higher than 2.5, add 20 % sulfuric acid solution dropwise: up to the specified pH value. The mixture is left for 2 hours, shaking occasionally. After this time, the aqueous extracts are separated and filtered through cheesecloth. The extraction operation is performed three times. The acidic extracts are collected together and centrifuged. The supernatant is discarded, and the precipitate is re-poured with 20-30 ml of 0.01 M sulfuric acid solution (pH 2.5), mixed, infused for 2 hours, and then the extracts are centrifuged. The centrifugates are combined, saturated with ammonium sulphate and left for 1-2 hours. If a precipitate forms, it is separated by centrifugation. The protein-free acidic liquid is extracted twice with ether in 40 ml portions. The ether layer is separated, and a 20% sodium hydroxide solution to pH 8.5-9 is added to the aqueous extraction and the basic substances are extracted three times with separate portions of chloroform equal to one third of the volume of the aqueous phase. The chloroform extractions are combined, filtered through a dry filter, and then the solvent is distilled off in a water bath at 40-50°C to dryness. Depending on the task at hand, the dry residue is dissolved in 5-6 ml of chloroform or 10 ml of 0.1 M hydrochloric acid solution and tested for alkaloids. If necessary, the ether extract obtained from the acidic aqueous extraction is also tested for substances extracted from the acidic aqueous medium with an organic solvent.

4. Isolation with acetone (Kartashov's method)

5 g of homogenised internal organ tissue is placed in a 20 ml penihydrin vial, 5 ml of acetone is added; the mixture is stirred, covered with a plastic cap and shaken on an automatic shaker for 10 min. Then the contents of the vial are centrifuged for 5 min at 2500 rpm and the supernatant is poured off through a small cotton swab into a 30 ml vial. The extraction operation is repeated 3 more times. To the combined acetone extracts, add 20 mL of 0.5 M hydrochloric acid solution and extract 2 times with 10 mL of n-hexane. The organic phase is separated and discarded. From the suitable phase, the substances are extracted with ether 2 times in 10 mL. The ether extracts are combined, filtered through a paper filter and evaporated under warm air to dryness. The dry residue is tested for acidic substances. The aqueous phase is alkalised to pH 11, 5 g of sodium chloride is added and extracted 2 times with 10 ml of ether. The ether extracts are combined, filtered, the solvent is evaporated, and the dry residue is tested for basic substances.

5. Rapid method for the isolation of phenothiazine derivatives with acidified acetonitrile (method of E. M. Salomatin)

50 g of crushed biological material is placed in a flask, acidified with 10% hydrochloric acid solution to pH 2-3, and the poisons are extracted with 100, 50 and 50 ml of acetonitrile for 30, 15 and 15 min using a mechanical shaker. The acetonitrile extracts are filtered through a paper filter moistened with distilled water into a separating funnel containing 500 ml of 2.5% aqueous sodium sulfate solution. The contents of the separating funnel are stirred until a homogeneous solution is formed, acidified with 6 M hydrochloric acid solution to pH 2.0-3.0 (according to the universal indicator) and extracted three times for 10 min with 100 mL portions of ether. The acidic aqueous-acetonitrile solution remaining after the ether extraction is alkalised with saturated aqueous sodium hydroxide solution to pH 13 using universal indicator paper and extracted three times for 10 min with 100 mL portions of ether. The ethereal extracts are evaporated under vacuum on a rotary evaporator at 40°C to a volume of 35-40 ml and filtered into a 50 ml volumetric flask through a 5-6 cm diameter paper filter containing 1.5-2 g of anhydrous sodium sulfate. The evaporating flask and filter are washed with 10-15 ml of ether, which is added to the filtrate in the measuring flask. The contents of the flask are brought to the mark and examined**.**

6. Isolation of barbiturates with alkaline water (Valov's method)

Water and 10% sodium hydroxide solution (180 and 20 ml, respectively) are added to 100 g of crushed biological material. The mixture is stirred and left for 30 min with occasional stirring, then filtered and centrifuged for 30 min at 3000 rpm. Add 120 ml of 10% sodium tungstate solution and 0.5 M sulfuric acid solution to the centrifuge to pH 2. After that, the mixture is heated for 20 min in a boiling water bath and then centrifuged for 30 min. The centrifuge is filtered through a cotton swab, discarding the precipitate. The swab is washed with 10 mL of water, adding it to the strained centrifuge. An equal volume of ether is added to the acidic extraction and shaken for 15 min. Separate the organic phase and shake with 50 mL of 10% sodium hydroxide solution, then separate the aqueous layer, acidify it with 25% sulfuric acid solution to pH 2 and shake with an equal volume of ether. Separate the ether layer and analyse the extract for barbiturates.

7. Isolation of barbiturates with water acidified with sulfuric acid (method of V. I. Popova)

Pour 100 g of crushed biological material (liver, kidneys, brain) with 0.01 M sulfuric acid solution (80 ml), bring the liquid to pH 2.0-3.0 with 30% sulfuric acid solution and leave for 2 hours with periodic stirring. Then the extract is drained off, the infusion operation with acidified water is repeated 2 more times for an hour, pouring new portions of 0.01 M sulfuric acid (80 ml each). The extracts are combined, filtered through 3 layers of gauze, the volume is measured, and then centrifuged (3000-5000 rpm) for 20-30 min. 25 or 50 ml of the centrifugate is injected into a column (40 x 2.5 cm) filled with Sephadex G-25 gel (dry particle size 100-300 µm). The clamp on the polyethylene tube at the bottom of the column is opened: the extract is absorbed by the gel (a small layer of liquid should remain above the gel). After that, 2 mL of 0.01 M sulfuric acid solution is added to the column twice, each time opening the clamp.

For elution of barbiturates, the column is connected to the vessel above filled with 0.01 M sulfuric acid solution and the clamp at the bottom of the column is opened. The first 150 mL of eluate is discarded, and the next 200 mL is transferred to a separating funnel with 50 mL of chloroform; the contents of the funnel are shaken for 10 min. The extraction with new portions of chloroform is carried out 2 more times. The chloroform extractions are combined, evaporated at 40°C to dryness and examined.

8. Isolation of metabolites of 1,4-benzodiazepine derivatives (method of B. M. Izotov et al.)

Add 6 M hydrochloric acid solution in a 1:2 ratio to 25 g of organ homogenate and hydrolyse the sample in a reflux flask in a glycerol bath at 140-145°C for 60 min. The hydrolysate is centrifuged at 5000 rpm for 15 min, filtered and extracted three times in 50, 25, 25 ml portions with a chloroform-pentanol mixture (9:1). The organic phase is separated into a 100-mp volumetric flask, filtered through a layer of anhydrous sodium sulfate, and brought to the mark, after which it is tested for benzophenones (metabolic products of 1,4-benzodiazepine derivatives).

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Date.

TOPIC: DIRECTED AND INDIRECT INVESTIGATION OF "ACIDIC" CHLOROFORM EXTRACTIONS (BARBITURIC AND SALICYLIC ACID DERIVATIVES, PYRAZOLONE, PURINE)

TLC "screening" (one of the options) of substances that are included in "acidic" chloroform extractions

1 ethane (in general solvent systems)

On a chromatographic plate with a fixed layer of silica gel, 0.1-0.2 ml of the extract, equivalent to the extraction from 1-2 g of organ, is applied at three points, which are 2 cm apart. The plate with the samples is placed in a chamber containing an acetone-chloroform solvent system (1:9) at the bottom. After the chromatogram develops and the plate is dried, separate chromatographic bands (samples after separation) are developed. After closing the 2nd and 3rd bands, the first band is treated with a 5% mercuric sulphate solution and then a 0.1% solution of diphenylcarbazone in chloroform. In the presence of barbiturates, spots coloured blue-violet or red-violet appear. Then the 1st and 3rd lanes are closed, and the second is treated with a 10% solution of ferric (III) chloride. In the presence of pyrazolone derivatives, coloured spots appear: blue, blue, blue-violet, red-violet; salicylic acid - blue-violet. After that, lanes 1 and 2 are closed, and the third lane is treated with Dragendorff's reagent and then with a 10% sulfuric acid solution. In the presence of substances of a weakly basic nature (caffeine, amidopyrine, antipyrine, diazepam, nitrazepam), orange, orange-brown, yellow-orange spots are formed.

Evaluation of the test results. In the absence of the above coloured spots, the test of the "acidic" chloroform extract is terminated; if the result is positive, the analysis is continued.

Depending on the suspected compounds in the extract sample, a confirmatory stage of TLC "screening" is performed in private solvent systems with a witness.

Stage 2 (in private solvent systems)

Conditions of TLC screening of barbiturates:

System: chloroform-n-butanol-25% ammonium hydroxide solution (70:40:5); sorbent: KSC silica gel buffered with 0.033 M boric acid solution; witness: cyclobarbital, number of samples applied - two (one for development, the other for elution).

Conditions for TLC screening of caffeine and pyrazolone derivatives:

System: acetone-cyclohexane (5:1); sorbent: basic aluminium oxide; witness: depending on the colour on chromatograms with ferric (III) chloride solution - if the red colour is stable, antipyrine, if the purple colour disappears, amidopyrine, if the pink colour disappears, 4-monomethylaminoantipyrine (a product of analgin metabolism). When forming coloured spots with only one modified Dragendorff reagent, caffeine is used as a witness.

Evaluation of the test results. If the confirmatory stage of the TLC screening is positive, chemical reactions are performed and the absorption spectrum of the detected substance in the UV region is recorded.

№	Research methodology	Observations.	Reaction chemistry	Chemical and toxicological assessment reactions

1.	**<u>Chemical methods of</u>** research *Salicylic acid* **<u>Reaction of tribromophenol formation</u>** Add a few drops of distilled water to the residue after removing the chloroform in the porcelain cup, stir and add 2-3 drops of saturated bromine water solution.			
2.	**<u>Colour reaction with ferric oxide chloride solution</u>** Add 1 drop of freshly prepared ferric chloride solution to the residue after removing the chloroform in a porcelain beaker. Place 1 drop of freshly prepared ferric oxide chloride solution on the filter paper and dry it. Then, 1-2 drops of the chloroform extraction test are applied to the same spot.			
3.	*Independent work* **<u>Methyl acetylate formation reaction</u>** Put a few drops of the chloroform extract under test into a test tube, evaporate under low heat in a water bath, and add 2-3 drops of concentrated sulfuric acid, 2-3 drops of methyl alcohol to the residue and heat in a water bath. The characteristic odour of salicylic acid methyl ester appears.			
1.	***Derivatives of barbituric acid (barbital, barbamyl, phenobarbital)*** **General reactions of barbiturates** **<u>Reaction with ammonia solution of cobalt nitrate or cobalt acetate</u>** A drop of chloroform extraction is applied to a piece of paper treated with a 1% alcohol solution of cobalt nitrate and dried. Then fumigate with ammonia vapour (by holding a glass containing concentrated			

	ammonia to the throat).			
2.	**<u>Reaction of isolation of the acidic form of barbiturates A</u>** few drops of a chloroform solution of the substance under study are layered on a slide, removing the chloroform at room temperature. The next drop is layered after the previous one has evaporated. The dry residue is dissolved in a drop of concentrated sulphuric acid. After 3-5 minutes, one drop of distilled water is placed next to this drop, and then they are carefully combined using a capillary. After 10-20 minutes, and in case of small amounts of barbiturate after 1-2 hours, the appearance of a crystalline precipitate characteristic of each individual barbiturate is observed. Describe the crystal shapes and draw them for each barbiturate.			
3.	**Private reactions of barbiturates** **<u>Reaction with chloroacetic acid</u>** A drop of chloroform solution is added to the dry residue on the slide (after removing the chloroform). After 10-15 minutes, the formation of crystalline precipitates is observed under the microscope. If the precipitate does not form for a long time, 1-2 crystals of fire iodine are added to the drops on the slides and the preparations are examined again after 10-15 minutes under the microscope. Sketch the crystal shape for each of the chloroquinic acid barbiturates.			

4.	**<u>Reaction with iron iodide complex</u>** One drop of iron iodide complex is added to the dry residue on the slide; after 10-15 minutes, the formation of characteristic crystal growths is observed. If the crystalline precipitate is too abundant, the reaction mixture is carefully evaporated on a slide over a flame of an alcohol flask, and then a drop of distilled water is added to the dry residue. After 10-15 minutes, the preparation is again examined under a microscope. Draw the shape of the crystals obtained for each barbiturate.			
5.	**<u>Reaction with a copper-nickel complex</u>** Add one drop of copper nickel complex to the dry residue of the test substance on the slide. After 10-15 minutes, the formation of crystalline sediments. Draw the shape of the crystals.			
1.	*Purine derivatives (caffeine)* **General reaction** **<u>Reaction of murexide formation</u>** Place 5-6 drops of the chloroform solution of the substance to be tested in a porcelain cup and evaporate the solvent without heating. To the dry residue is added 0.5-1 ml of saturated bromine water solution and evaporated to dryness in a water bath. One drop of 25% ammonia solution is added to the brownish residue on a glass rod.			
2.	**<u>Reaction with Nessler's reagent</u>** The caffeine solution with Nessler's reagent is heated for 1-2 min in a boiling water bath.			

1.	*Pyrazolone derivatives (antipyrine, analgin)* **Fire retardant** **<u>Reaction with ferric (III) chloride</u>** A few drops of the chloroform extraction are added to a porcelain cup and evaporated to dryness. A drop of 5% ferric chloride solution is added to the dry residue.			
2.	**<u>Reaction for the formation of nitrosoantipyrine</u>** Pour 3-5 ml of the chloroform extraction into a porcelain cup and evaporate to dryness in a water bath. Dissolve the dry precipitate in 3-5 drops of water, add 2-4 drops of 10% sulfuric acid solution and 2-3 drops of saturated sodium nitrite solution.			
3.	*Independent work* **<u>Reaction of nitrogen dye formation</u>** Pour 2-5 drops of chloroform extraction from an acidic medium into a test tube and evaporate to dryness in a water bath. Add 1-2 drops of water to the dry residue. A drop of ice-cold acetic acid and a drop of 5% potassium nitrite solution are added to the resulting solution. The mixture is left for 5 minutes with occasional shaking. Then a small amount of sodium azide is added to the test tube. After the gas bubbles have ceased to form, add 3-4 crystals of α-naphthylamine and heat the test tube in a water bath for 1-2 minutes.			

1. 2.	**Analgin** **<u>Reaction with ferric (III) chloride</u>** A few drops of the chloroform extraction are added to a porcelain cup and evaporated to dryness. A drop of 5% ferric chloride solution is added to the dry residue. **<u>Sample with lignin</u>** Apply 2-3 drops of the chloroform solution to a single point on a piece of newsprint. A characteristic colouration is observed, which is sharply enhanced when the stain is treated with dilute hydrochloric acid.			

Class no.

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Date.

TOPIC: TARGETED AND UNDIRECTED CHLOROFORM EXTRACTION FOR ALKALOIDS (TROPANE, PYRIDINE AND PIPERIDINE DERIVATIVES)

TLC "screening" (one of the variants) of substances to be included in "alkaline" chloroform extraction

Conditions for TLC screening: extract samples are applied as described for "acidic" chloroform extraction; system for the preliminary stage of TLC screening: chloroform-dioxane-acetone-25% ammonium hydroxide solution (45:47.5:5:2.5). Developers for individual samples (bands): 10% solution of sulfuric acid in ethanol for phenothiazine derivatives (red, blue spots); 10% solution of ferric chloride (III) for pyrazolone and phenothiazine derivatives (red, blue, blue colour of spots); Dragendorff reagent for all compounds containing tertiary nitrogen (orange-brown colour of spots). Evaluation of the analysis results. In the absence of characteristic spots on the chromatograms, the study is completed. If coloured spots with the same Rf value are formed after development with all of the above reagents, a confirmatory stage of TLC "screening" for phenothiazine derivatives is performed; with the Dragendorff reaction and a solution of ferric (III) chloride for pyridine and piperidine derivatives, quinoline, isoquinoline, tropane, indole, as well as for 1,4-benzodiazepine and p-aminobenzoic acid derivatives.

Conditions for the confirmatory stage of TLC "screening"

For alkaloids: chloroform-diethylamine system (9:1); silica gel sorbent KSC; Dragendorff developer.

№	Research methodology	Observations.	Reaction chemistry	Chemical and toxicological assessment reactions
1.	Confirmatory studies on substances that enter the "alkaline" chloroform extraction **Pyridine and piperidine derivatives (anabasin, nahicarpine)** **Anabasin** **Reaction with Dragendorff reagent** Apply 2-3 drops of a solution of the test substance in chloroform to a glass slide and evaporate to dryness. To the dry residue add a drop of 0.1 M hydrochloric acid and a drop of Dragendorff's reagent. After 10-15 min, the characteristic crystals are viewed under a microscope.			

2.	**<u>Reaction with picric acid</u>** Apply 2-3 drops of a solution of the test substance in chloroform to a glass slide and evaporate to dryness. To the dry residue add a drop of 0.1 M hydrochloric acid and a drop of 0.5% picric acid solution. After 10-15 min, the characteristic crystals are examined under a microscope.			
3.	**<u>Reaction with 1% Reinecke's salt solution</u>** Apply 2-3 drops of a solution of the test substance in chloroform to a glass slide and evaporate to dryness. To the dry precipitate add a drop of 0.1 M hydrochloric acid and a drop of a freshly prepared solution of 1% Reinecke's salt. After 10-15 min, the characteristic crystals are examined under a microscope.			
1.	**Pachycarpine** **<u>Reaction with iodine solution in potassium iodide (Bouchard's reagent)</u>** Apply 2-3 drops of a solution of the test substance in chloroform to a glass slide and evaporate to dryness. To the dry residue add a drop of 0.1 M hydrochloric acid and 1-2 drops of iodine solution in potassium iodide. After 10-15 min, the characteristic crystals are examined under a microscope. Draw the crystals.			
2.	**<u>Reaction with picric acid</u>** Apply 2-3 drops of a solution of the test substance in chloroform to a glass slide and evaporate to dryness. To the dry residue add a drop of 0.1 M hydrochloric acid solution and 1-2 drops of picric acid solution. After 10-15 min, the characteristic crystals are examined under a microscope. Draw the crystals.			

3.	**<u>Reaction with cobalt rhodanide complex</u>** Apply 2-3 drops of a solution of the test substance in chloroform to a glass slide and evaporate to dryness. To the dry residue add a drop of 0.1 M hydrochloric acid solution and 1-2 drops of cobalt rhodanide complex solution. After 5-10 min, the characteristic crystals are examined under a microscope. Draw the crystals.			
1.	**Tropane derivatives (atropine, scopolamine, cocaine)** *General reaction (except for cocaine)* **<u>Vitaly-Maurin's reaction</u>** Add a few drops of concentrated nitric acid to the dry chloroform residue in a porcelain beaker and carefully evaporate to dryness in a water bath. This operation is repeated at least 3 times and cooled. Then a freshly prepared alcohol solution of potassium hydroxide is added to the residue. Observe the characteristic colouration.			
2.	*Confirmatory reactions* **Atropine** **<u>Reaction with Reinecke's salt</u>** Apply 2-3 drops of a solution of the test substance in chloroform to a glass slide and evaporate to dryness. To the dry precipitate add a drop of 0.1 M hydrochloric acid and a drop of a freshly prepared solution of 1% Reinecke's salt. After 10-15 min, the characteristic crystals are examined under a microscope. Draw the crystals.			

3.	**<u>Reaction with picric acid</u>** Apply 2-3 drops of a solution of the test substance in chloroform to a glass slide and evaporate to dryness. To the dry residue add a drop of 0.1 M hydrochloric acid solution and 1-2 drops of picric acid solution. After 10-15 min, the characteristic crystals are examined under a microscope.			
4.	**Scopolamine** **<u>Reaction with Reinecke's salt</u>** Apply 2-3 drops of a solution of the test substance in chloroform to a glass slide and evaporate to dryness. To the dry precipitate add a drop of 0.1 M hydrochloric acid and a drop of a freshly prepared solution of 1% Reinecke's salt. After 10-15 min, the characteristic crystals are examined under a microscope. Draw the crystals.			
5.	**<u>Reaction of</u>** cocaine **<u>permanganate formation</u>** A few drops of the chloroform solution under investigation are gradually layered on a glass slide; the organic solvent is evaporated without heating, and 1 drop of a 10 % solution of hydrochloric acid is added to the residue. The liquid is evaporated at room temperature. The operation is repeated 2-3 times. Then 1 drop of 1% potassium permanganate solution is added to the dry residue. After 10-15 min, the characteristic crystals are examined under a microscope. Draw the crystals.			

Class no.

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Date.

OBJECTIVES: TARGETED STUDY OF "ALKALINE" CHLOROFORM EXTRACTIONS FOR ALKALOIDS (QUINOLINE, ISOQUINOLINE, INDOLE DERIVATIVES, ACYCLIC ALKALOID EPHEDRINE)

№	Research methodology	Observations.	Reaction chemistry	Chemical and toxicological assessment reactions
1.	**Isoquinoline derivatives (morphine, codeine)** *General reactions* **Reaction with formaldehyde in concentrated sulphuric acid (Markey's reagent)** A few drops of the chloroform solution under test are placed in a porcelain cup, and the solvent is evaporated without heating. To the dry residue add 1 drop of freshly prepared Markey's reagent (1 drop of formalin in 1 ml of sulfuric acid).			
2.	**Reaction with a solution of ammonium molybdate in concentrated sulphuric acid (Frede's reagent)** Place a few drops of the chloroform solution to be tested in a porcelain cup and evaporate the solvent without heating. Add 1 drop of Frede's reagent to the dry residue.			
3.	**Reaction with sodium vanadate in concentrated sulphuric acid (Mandelin's reagent)** A few drops of the chloroform solution to be tested are placed in a porcelain cup and the solvent is evaporated without heating. Add 1 drop of Mandelin's reagent to the			

	dry residue.			
4.	**Morphine** **<u>Reaction with ferric oxide chloride</u>** Place a few drops of the chloroform solution to be tested in a cup and evaporate the solvent without heating. Add 1 drop of freshly prepared ferric oxide chloride solution to the dry solution.			
1.	**Quinoline derivatives (quinine)** **<u>Fluorescence reaction</u>** A portion of the chloroform test solution is placed in a test tube, and the chloroform is evaporated by heating in a warm water bath. To the dry residue add 1 ml of distilled water and 1 ml of 10% sulfuric acid. The characteristic fluorescence is observed under UV light.			
2.	**<u>Reaction of thalloylquinone formation (in drop modification by L.V. Peysakhovich)</u>** Apply 5-10 drops of chloroform extraction to the filter paper, moisten the stain with a drop of water and subject it to successive treatment with bromine water vapour (until the stain turns yellow) and 25% ammonia solution.			
1.	**Acyclic alkaloid ephedrine** **<u>Reaction with a solution of ninhydrin in n-butanol</u>** A few drops of the test chloroform solution are applied to the Silufol plate, the organic solvent is evaporated without heating. A solution of ninhydrin in n-butanol is then added. The silica plate is			

	heated in a sand bath until the characteristic colour develops.			
1.	**Indole derivatives (strychnine)** **<u>Oxidation reaction with potassium bichromate in concentrated sulfuric acid</u>** 4-5 drops of the chloroform solution of the substance to be tested are placed in a porcelain cup and, after evaporation of the chloroform, 1 drop of concentrated sulfuric acid and a small crystal of potassium bichromate are added to the dry residue.			
2.	**<u>Reaction with sodium vanadate in concentrated sulphuric acid (Mandelin's reagent)</u>** A few drops of the chloroform solution to be tested are placed in a porcelain cup and the solvent is evaporated without heating. Add 1 drop of Mandelin's reagent to the dry residue.			

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Date.

TOPIC: Directed and indirect research of "LUCH" chloroform extraction for synthetic "medicinal poisons" (derivatives of 1,4-BENZDIAZEPINE, Phenothiazine, p-AMINOBENZOIC ACID)

№	Research methodology	Observations.	Reaction chemistry	Conclusion.
1.	**Derivatives of 1,4-benzodiazepines** **<u>Methodology for acid hydrolysis</u>** 2 mL of the chloroform extract is evaporated to dryness in a flask in a boiling water bath. To the dry residue is added 5 mL of 6N hydrochloric acid and the contents of the flask are heated with reflux condenser in a boiling water bath for 60 min. The hydrolysate is cooled and neutralised with saturated sodium hydroxide solution to pH 7-9. The resulting solution is transferred to a separating funnel and the hydrolysate products of 1,4-benzodiazepines-aminobenzophenones are extracted with an equal volume of chloroform. The organic phase is separated and filtered through anhydrous sodium sulfate. The extract is evaporated to a volume of 0.2 ml and examined. After transferring to a chromatography plate, view under UV light. **<u>Reaction of nitrogen dye formation</u>** The chromatography plate is treated with reagents in sequence: 1 % sodium nitrite solution, followed by 2 M hydrochloric acid solution and			

	alkaline β-naphthol solution.			
1.	**Phenothiazine derivatives (aminazine, levomepromazine)** **Reaction with concentrated sulfuric acid** 4-5 drops of chloroform solution of the test substance are placed in a porcelain cup and after evaporation of the chloroform, 1 drop of concentrated sulfuric acid is added to the dry residue.			
2.	**Reaction with concentrated nitric acid** Place 4-5 drops of the chloroform solution of the substance to be tested in a porcelain cup and, after evaporation of the chloroform, add 1 drop of concentrated nitric acid to the dry residue.			
3.	**Reaction with concentrated hydrochloric acid** Place 4-5 drops of the chloroform solution of the substance to be tested in a porcelain cup and, after evaporation of the chloroform, add 1 drop of concentrated hydrochloric acid to the dry residue.			
1.	**Derivatives of p-aminobenzoic acid (novocaine)** The chloroform extract is transferred to a chromatographic plate or paper in a single spot, which, after drying, is treated with a drop of 2 M hydrochloric acid solution, then a drop of 1% sodium nitrite solution, and after 2-3 min - a drop of alkaline β-naphthol solution.			

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Date.

TOPIC: QUANTIFICATION OF "MEDICINAL POISONS" IN EXTRACTS FROM CADAVERIC ORGANS

Quantitative determination of aminazine with ferric (III) chloride

Reagents:

1. 5 % solution of ferric (III) chloride.
2. Distilled water.
3. Extract from biological material containing aminazine (task).

Method of determination

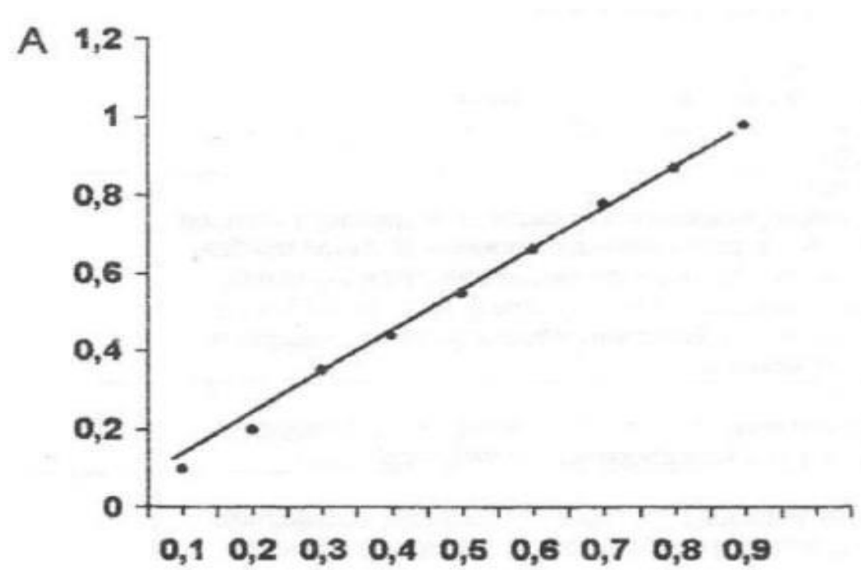
Pour 5 ml of the aqueous extract containing aminazine into a test tube, add 1 ml of 5 % ferric (III) chloride solution (immediately before the measurement). Stir. The coloured solution is transferred to a cuvette with a layer thickness of 10 mm and the optical density is measured using a KFC-2 photoelectrocolourimeter at 540 nm. The reference solution is a mixture of 5 ml of distilled water and 1 ml of ferric (III) chloride solution.

The concentration of aminazine in the extract is determined from the calibration chart. After that, the formula is used to determine the content of aminazine in 100 g of biological material.

X=C*20,

where X is the aminazin content in 100 g of biological material; C is the concentration of aminazin in the sample (sample volume 5 ml).

Graduation chart for the quantitative determination of aminazine



With mg in the sample

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TOPIC: EXAMINATION OF BIOLOGICAL FLUIDS FOR "MEDICINAL" POISONS IN ACUTE INTOXICATION

Objects of study: biological fluids that are expected to contain salicylic acid, paracetamol, aminazine, quinine, barbiturates, codeine, 1,4-benzodiazepine derivatives.

№	Research methodology	Observations.	Reaction chemistry	Conclusion.
I. 1.	Without isolation **Salicylic acid and salicylates** Add 3 drops of 5% ferric chloride solution to 1 ml of urine.			
2.	**Paracetamol** Add 2-3 drops of 10% hydrochloric acid solution to 1 ml of urine and cool. Then 2-3 drops of 1% sodium nitrite solution and 2-3 drops of freshly prepared 1% β-naphthol solution in 10% caustic soda solution are added to the cooled mixture. A large excess of β-naphthol solution interferes with this reaction.			
3.	**Aminazin** To 1 ml of urine add 1 ml of FPN reagent (ferric chloride (III), hydrochloric acid, nitric acid).			
4.	**Quinine** Add 3 ml of 10% sulfuric acid solution to 2 ml of urine. A characteristic fluorescence is observed under UV light.			

II. 1.	<u>After isolation</u> **Barbiturates** 5 ml of urine is added to the dividing funnel, to which			

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TOPIC: DETERMINATION OF SENSITIVITY AND SPECIFICITY OF CHEMICAL REACTIONS SUITABLE FOR RAPID ANALYSIS OF ACUTE POISONING

<u>Objects of analysis:</u> model chloroform solutions of atropine, aminazine, amitriptyline (1000 µg/ml), 1% aqueous solution of pachycarpine.
<u>Reagents:</u> 1% aqueous solution of Reinecke's salt (ammonium tetrarhodanodiaminochromium chromate) NH_4 [$Cr(SCN)_4$ (NH)$_{32}$]; 0.5% aqueous solution of picric acid, cobalt rhodanide complex.

<u>A method of analysis to determine the sensitivity of chemical reactions (using pachycarpine as an example).</u>

Apply 1 drop of a 1% pachycarpine solution to a glass slide and add 1 drop of cobalt rhodanide complex, and then combine them with a glass rod. If no characteristic crystals form immediately, place the slide in a humid chamber for 5-10 minutes. At the same time, apply a drop of the corresponding reagent to the slide as a control experiment. To determine the sensitivity, a number of successive dilutions are performed and the limit of detection of pachycarpine is established, and then the limit dilution is calculated using the formula.

<u>Results and discussion</u>
The results of the research are presented in Table 1.
The limiting dilution is calculated by the formula:

where - V is the volume of the droplet, ml (0.05 ml) m is the detection limit, m C is the limiting dilution.

Table 1

Indicators of sensitivity of microcrystalloscopic reaction of pachycarpine with cobalt rhodanide complex

Substance (chemical formula)	Re.	The shape of the crystals	Sensitivity	
			Detection limit, μg	Limit dilution

A method of analysis to determine the specificity of chemical reactions.

Chloroform solutions of atropine, aminazine, and amitriptyline are used as test objects. Apply 2-3 drops of the test chloroform solution to a glass slide, evaporate the chloroform to dryness at room temperature. To the dry residue add 1 drop of 0.1 M hydrochloric acid and 1 drop of the appropriate reagent (1% Reinecke's salt solution, picric acid). If no characteristic crystals are observed immediately, the slide is placed in a humid chamber for 5-10 minutes. At the same time, a drop of the corresponding reagent is applied to the slide as a control experiment. The results of the study are recorded in Table 2.

Table 2

Results of the specificity of microcrystalloscopic reactions of nitrogen-containing drugs

№	The drug (chemical formula of the drug)	Re.	Sediment characteristics (amorphous, crystalline)	The shape of the crystals
1.	Aminazin	Salt Reinecke		
2.	Atropine	Salt Reinecke		
3.	Amitriptyline	Salt Reinecke		
4.	Aminazin	Pikrinova acid		
5.	Atropine	Pikrinova acid		
6.	Amitriptyline	Pikrinova acid		

According to the table, identify the most specific reactions for drugs suitable for rapid analysis of acute poisoning.

Conclusion:

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TOPIC: APPLICATION OF THE TC METHOD IN THE SCREENING OF MEDICINAL SUBSTANCES IN BIOLOGICAL FLUIDS

Objects of study: chloroform extracts from urine, which are supposed to contain quinine, novocaine, aminazine, salicylic acid, antipyrine; standard chloroform solutions of quinine, novocaine, aminazine, salicylic acid, antipyrine.

Reagents and equipment: HPLC or Sorbfil chromatography plates; solvent systems: chloroform-acetone (9:1), chloroform-acetone-isopropanol-25% ammonia solution (7:7:7:2); developers: Dragendorff's reagent, 5% ferric chloride (III) solution. Chromatographic chambers, capillaries.

Method of analysis for acidic, neutral and slightly basic substances. The analysed extracts (samples) and standard solutions of aminazine, antipyrine, and salicylic acid are applied to the chromatographic plate. The sample plate is placed in a chamber with a chloroform-acetone (9:1) solvent system at the bottom. After the chromatogram develops and the plate is dried, it is treated with a 5% solution of ferric chloride (III) (in the presence of substances of acidic, neutral, weakly basic nature, spots of a characteristic colour appear). Calculate the value of Rf.

Method of analysis for basic substances. The analysed extracts (samples) and standard solutions of aminazine, quinine and novocaine are applied to the chromatographic plate. The plate with the samples is placed in a chamber containing a solvent system of chloroform-isopropanol-acetone-25% ammonia solution (7:7:7:2). After the chromatogram develops and the plate is dried, it is treated with Dragendorff's reagent (orange-brown spots appear in the presence of basic substances).Calculate the value of Rf.

Draw the results of the chromatographic studies (Figs. 1, 2) and enter them in Tables 1 and 2.

Table 1

Results of TLC studies of extracts containing some acidic, neutral and slightly basic substances

Object of research	Solvent system	Developing agent	Colour of the stain after developer treatment	The value of Rf

Conclusion:

Table 2

Results of the TLC study of extracts containing some basic substances

Object of research	Solvent system	Developer reagent	Colour of the stain after developer treatment	The value of Rf

Conclusion:

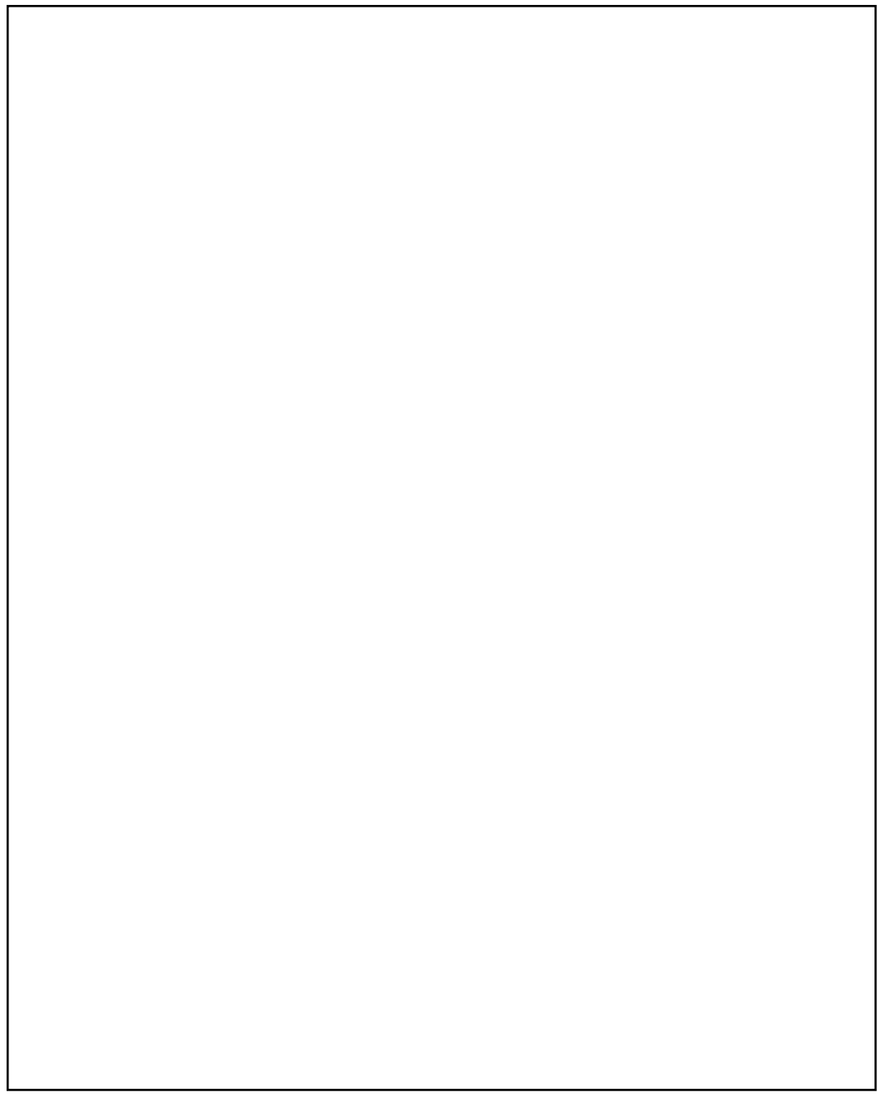

Fig. 1. Scheme of application of samples of analysed extracts and standard solutions of acidic, neutral and slightly basic substances.

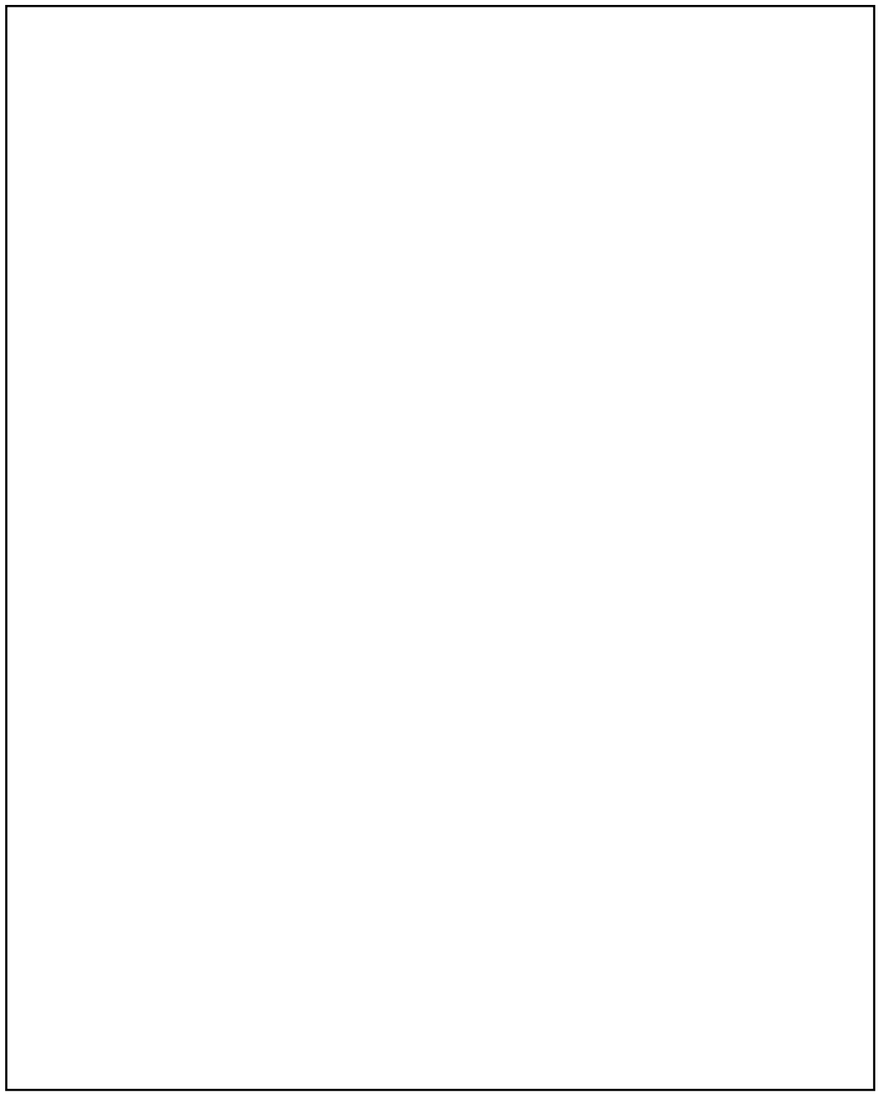

Fig. 2. Scheme of application of samples of analysed extracts and standard solutions of basic substances.

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TOPIC: QUANTITATIVE EXTRACTION-PHOTOMETRIC DETERMINATION OF NARCOTIC AND INTOXICATING SUBSTANCES WITH BROMOTHYMOL BLUE AND SELECTION OF CONDITIONS FOR ANALYSIS. APPLICATION OF THE DEVELOPED METHOD IN THE RAPID ANALYSIS OF BIOLOGICAL FLUIDS

The extraction-photometric method of analysis is based on the formation of ionic associations of positively charged ions of nitrogen-containing organic substances and negatively charged ions of acidic dyes. The ionic associations are extracted with organic solvents and stain the organic phase, which is photometrically measured using photoelectrocolourimeters or spectrophotometers.

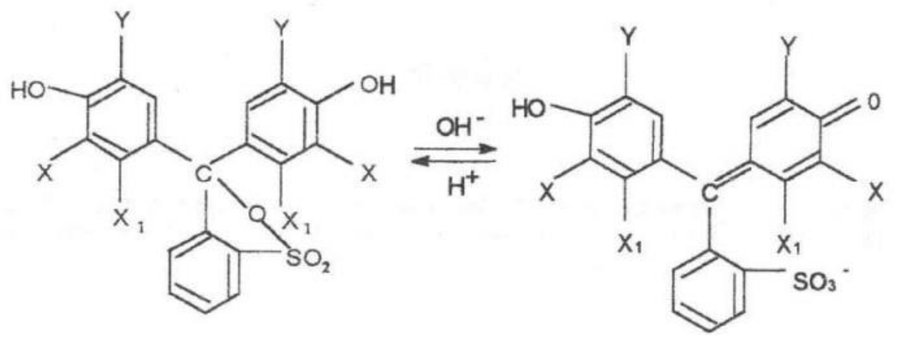
To increase the sensitivity of the method, ionic associates are destroyed by alkaline or acid solutions with subsequent colour enhancement.

Extraction photometry is characterised by high sensitivity, reliability, speed, the ability to concentrate micro quantities of drugs, and to perform analysis without additional thorough purification, as the reference solution is a "blank" sample.

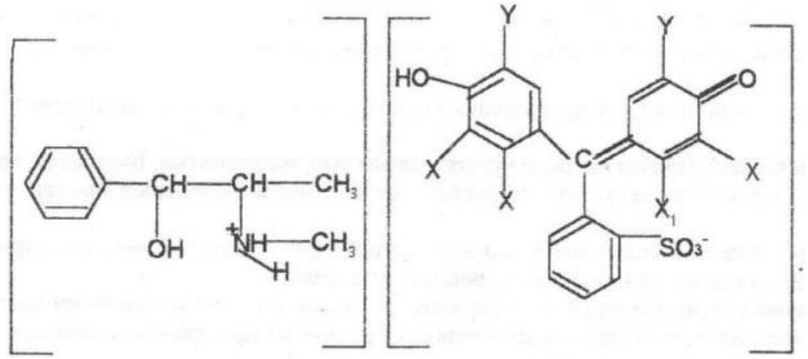
Among the acid indicators in the analysis of narcotic and intoxicating drugs of a basic nature, the most commonly used are: methyl orange, tropheoline OO, sulfophthalein dyes.

The ability of sulphophthalein indicators to form ionic associations with drugs depends on the pH of the medium, the pH of the drug and dye, the presence and location of substituents in the drug and dye molecules, and the nature of the organic solvent.

Ionisation of sulphophthalein dyes:



Possible composition of the ionic association of ephedrine and sulfophthalein indicator:



In extraction-photometric analysis, sulfophthalein dyes are widely used: bromothymol blue (ETC); bromophenol blue (BPS); thymol blue (TS).

Substitutes	BTS	BFS	CU
X	-Br	-Br	-
X_1	$-CH_3$	-	$-CH_3$
Y	$-C H_{37}$	-Br	$-C H_{37}$

EXPERIMENTAL PART

Objects of analysis: standard aqueous solution of ephedrine hydrochloride (200 µg/ml).
Reagents: 0.04% aqueous solutions of BTS, BFS, TS; phosphate buffer solution (pH 7.6); 0.02 M aqueous solution of hydroxide.
Equipment: photoelectrocolourimeter KFC-2, distribution funnels.

Methodology of the analysis

I. The most sensitive indicator is selected in dividing funnels, into which 5 ml of buffer solution with pH 7.6, 1 ml of 0.04% indicator solution, 1 ml of standard solution of the drug and 5 ml of chloroform are added. Shake for 2-3 minutes, separate the chloroform layer and measure the optical density of the yellow-coloured solution using a CFC-2.2 max photoelectrocolourimeter (440 ± 10 nm, cuvette 10 mm); reference solution - chloroform. The results are listed in Table 1.

Determining the sensitivity of the indicator

Table 1

Indicators	Optical density (A)
BTS	
BFS	
TC	

Based on the data in Table 1, select the most sensitive indicator that forms stable ionic associations that can be easily extracted with chloroform at pH 7.6.

II Increasing the sensitivity of the analysis method. The yellow colour of the extract is due to the absorption of the uncharged form of the dye, which dissociates by the sulpho group. To increase the sensitivity of the method, 5 ml of 0.02 M sodium hydroxyl solution is used, under the influence of which the dye passes into an aqueous solution and turns it blue, the optical density (A) of these solutions is high (Table 2).

Table 2

Optical density values of coloured aqueous and chloroform solutions

The content of the drug in the in the stock solution, µg/ml	**Optical density of coloured solutions**	
	Chloroform (440 ± 10 nm)	**Aqueous (590 ± 10 nm)**
50		
100		

Note: the reference solution is a mixture of reagents.

III The choice of a light filter is made by determining the highest value of the optical density of solutions of different concentrations. The results are shown in Table 3.

IV Selection of a cuvette. A calibration graph is used to calculate the content of ephedrine hydrochloride in solutions. The working length of the cuvette affects the angle of inclination of the calibration graph to the abscissa axis. Cuvettes are selected so that the measured optical densities fall within the range of values from 0.1 to 1.0, which ensures sufficient accuracy of the optical density measurement. Cuvettes with a liquid layer thickness of 5.10 and 20 mm were used for the measurement. The results of the study are presented in Table 4.

Table 3

Dependence of the optical density of dyed solutions on the used light filter

$\lambda_{эф.}$ of the light filter, nm	**Optical density of coloured solutions obtained at the drug content, μg**	
	50	100
400 ± 10		
440 ± 10		
490 ± 10		
540 ± 10		
590 ± 10		
670 ± 10		

Table 4

Dependence of the optical density of coloured solutions on the thickness of the liquid layer

Drug taken, μg	**Optical density of solutions with layer thickness, mm**		
	5	10	20
20			
50			

Conclusion:

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TOPIC: DETERMINATION OF "MEDICINAL" POISONS IN BIOLOGICAL FLUIDS BY ENZYME-LINKED IMMUNOSORBENT ASSAY

Enzyme-linked immunosorbent assay for opiates

№	Stages of enzyme-linked immunosorbent assay	Scheme of transformations
1.	Adsorption of modified MB (protein-bound) on a plate	MB +M+ ATP -
2.	Rinse your tablet	MBATP + MATP
3.	Introduction of urine specimens containing morphine (M)	conjugate laundering
4.	Addition of horseradish peroxidase (HRP)-labelled antibodies and incubation	MATII
5.	Rinse your tablet	IBATP + N_2 O_2 +
6.	Substrate application (H_2 O_2 + o-phenylenediamine)	+ o-phenylenediamine
7.	Determination of the optical density of a coloured solution	Colouration, the intensity of which decreases with an increase in the content of morphine in the urine

Accounting for analysis results

The results of the analysis are evaluated visually by comparing the colour of the morphine standard wells with the colour of the wells in the test samples, or spectrophotometrically at a wavelength of 492 nm (IFCO-2 playtest).

The amount of morphine in the test samples is determined by a calibration graph of the dependence of the average optical density of two or three parallel determinations on the concentration of morphine standard in semi-logarithmic coordinates: C is plotted on the abscissa axis (C is the concentration of morphine in ng/ml), and the ratio of optical density A/A_0 is plotted on the ordinate axis (A is the optical density of the standard solution, and A_0 is the optical density of the "blind" experiment in the absence of morphine).

The data for the construction of the graduation graph are entered in the table

Table.

Data recording form for building a calibration graph in ELISA

№	C, ng/ml	Lg C	A	A_0	A/A_0
1.	500	2, 7	0, 40	0, 88	0, 47
2.	250				
3.	125				
4.	62				
5.	31				
6.	15				
7.	7, 5	0, 9	0, 68	0, 88	0, 80

Perform the determination of opiate content by ELISA in model urine samples using a calibration chart.

Building a calibration graph

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TOPIC: SPECTRAL AND CHEMICAL METHODS FOR THE DETERMINATION OF CARBON MONOXIDE (II) IN BLOOD
CREDIT LESSON

№	Research methodology	Observations.	Chemistry of reactions	Conclusions.
1.	Spectral analysis of blood The blood to be tested is diluted with water until a light pink solution is obtained. When this solution is examined spectroscopically, the corresponding spectral bands are clearly visible. The spectrum of blood oxyhaemoglobin has two absorption bands between the Fraunhofer D and E lines at wavelengths of 577-589 and 536-556 km. The spectrum of carboxyhaemoglobin has two absorption bands at wavelengths of 564-579 and 523-536 nm. After adding one volume of a freshly prepared solution of ammonium sulfide or other reducing agents (hydrazine hydrate, sodium dithionite, etc.) to 4 volumes of aqueous blood solution, oxyhaemoglobin is converted to deoxyhaemoglobin, which has a single broad absorption band at 543-596 nm. Carboxyhaemoglobin is not reduced by ammonium sulphide and other reducing agents. Therefore, after the addition of reducing agents, the absorption bands of carboxyhaemoglobin do not disappear.			

2.	<u>Chemical research methods</u> Blood containing carboxyhaemoglobin does not change or slightly changes its colour after the addition of certain reagents, and normal blood that does not contain carboxyhaemoglobin changes its colour significantly under the influence of these reagents. 2.1 Reaction with caustic soda solution (Hoppe-Seiner test). 2.2 Reaction with ammonium sulphide (Salkowski-Katayama test). 2.3 Reaction with quinine and ammonium sulphide (Khoroshkovich-Marx test). 2.4 Reaction with potassium hexacyano(III) ferrate and potassium dichromate (Sidorov's test). 2.5 Reaction with potassium hexacyano(III) ferrate (Bürker test). 2.6 Reaction with potassium hexacyano(III) ferrate and acetic acid (Wetzel's test).			

	2.7 Reaction with tannin (Kunkel-Wetzel test).			