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Application of test-kits with heavy metals salts for analysis of salts of benzoic and salicylic acids in extemporal medicines

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ABSTRACT

Test systems are simple, effective, affordable and express analysis tools used in medical practice, to diagnose the state of the human organism, and for the quality control of medicines. The aim of the work wasto check the possibility of using test-kits based on filter paper for high-quality rapid analysis of salts of benzoic and salicylic acids while conducting the pharmaceutical analysis of extemporal medicinal preparations (EMP). Test-kits were prepared by the way of physical immobilization of reagents solutions (iron (III) chloride and copper (II) sulfate) on the matrix surface with further drying. The objects of the experiment were 1%, 2% and 5% aqueous solutions of sodium salts of benzoic and salicylic acids. The results of the investigation show that using test-kits based on filter paper with heavy metal salts makes it possible to identify sodium benzoate and sodium salicylate in extemporal medicines: sodium benzoate with the help of test-kits with $FeCl_3$starting from 7 mg/ml (1% aqueous solution) and with the help of test-kits with $CuSO_4$starting from 14 mg/ml (2% solution); sodium salicylate with the help of test-kits with $FeCl_3$ and $CuSO_4$starting from 14mg/ml (2% solution).For simultaneous identification of sodium benzoate and sodium salicylate in a polycomponent EMP test-system with$CuSO_4$ can be used.

Keywords: extemporal medicines, chemical test-kits, sodium benzoate, sodium salicylate.

INTRODUCTION

At present in Ukraine the traditions of extemporal production of medicinal preparations remain and are being improved[1]. The State Pharmacopoeia ofUkraine (SPU) includes articles and requirements regulating the quality of medicinal preparations produced in pharmacies [2], taking this into consideration there is the necessity of developing and standardization of the techniques of analysis of mono- and polycomponent extemporal medicinal preparations (EMP). Taking into consideration the level of material and technical basis of the most of pharmacies which produce EMP in Ukraine, one of the priority directions of solving the given problem is the retrieval and development of new techniques based on the principle of express analysis and testing.

The techniques of analysis with the use of test-systems and test-means on the basis of filter paper (FP)are used wider and wider in medical practice as well as for the analysis of chemical compounds, food products, water and ground [3-6]. These analytical means are produced through physical and chemical immobilization, application of nanoparticles, etc. [3,7,8]. However, the use of available in the market test-systems for identification of the components of EMP has a number of problems [9], which can be solved at the expense of developing and application to practice of the test-systems of direct pharmacy production.

In the preceding publications the possibility of using test-systems based on filter paper modified with heavy metals salts for identification of preparations of the derivatives of pyrazol, sulphanilamideand vitamins [10,11] in pharmacy produced monocomponent medicinal preparations was investigated. Taking into consideration the obtained results it was decided to extend the spectrum of use of the test-systems under investigation. As the following objects of the research sodium salts of benzoic and salicylic acids having broad application in production of EMP and which when

combined with the saults of trivalent iron are able to create stable coloured complexes were chosen[2,12], the techniques of identification of these substances with the help of cation Cu^{2+} are also known [13]. The mechanisms of chemical processes of creation of coloured complexes resemble, that allows to use the data of the reaction for developing specific techniques of identification with the help of test-systems. Taking into consideration positive experience of the former research [10] of using test-system with 2% alcoholic solution $FeCl_3$(iron (III) chloride solution S_3), it was decided to produce a test-system modified with 1,3% aqueous solution [2] of this reagent. The use of this solution will allow to use the reagent more effectively while making test-means.

The aim of this work is to prove the possibility of identification of sodium salts of benzoic and salicylic acids with the help of test-systems based on filter paper modified with heavy metals salts ($FeCl_3$, $CuSO_4$) as parts of mono- and polycomponent medicinal preparations of extemporal production.

MATERIALS AND METHODS

To carry out the research the following preparations were used: monocomponent medicinal preparations of 1%, 2% and 5% aqueous solutions of sodium benzoate and sodium salicylate, polycomponent EMP – a powder for internal administration [14]:

Rp.: Natrii benzoas
Natrii salycilas ana 3,0
Natrii sulfas 4,0
Natrii hydrogencarbonas 6,0
Misce, fiat pulvis
Da tales doses numero 5
Signa:internal

For test-systems production the following preparations were used: *the matrix* – sheet filter paper of the brand "Ф"; *reagents:* $FeCl_3*6H_2O$ (p.) № of consignment 187-226 Ukraine; $CuSO_4*5H_2O$ (p.) № of consignment 42 Ukraine. *Substances* – sodium benzoate № of consignment 20120612, produced in China, sodium salicylate № of consignment 0503036, produced in China. For preparation of aqueous solutions purified water *S* was used.

Original and model solutions of sodium benzoate and sodium salicylate were made by dissolving exact weights of substances in *purified water S* to the adjusted concentration. Solutions of copper (II) sulphate and iron (III) chloride were made according to the requirements of SPU [2].

Test-systems were made through physical immobilization, according to the techniques [10,11]. The test-system modified with *iron (III) chloride solution* S_2, which was called FP $FeCl_3S_2$,was made according to the scheme described in [10].

The express analysis of monocomponent dosage forms was conducted according to the technique mentioned in [10,11], the analytical effect was compared with the colouring of the blank experiment (purified water S) and parallel researches-comparisons [2,12,13], which were modified through the equivalent reduction of the number of reagents in the compound, the techniques conducted drop wisely on the object-plate were selected.

The analysis of the combination of the components under investigation – sodium benzoate and sodium salicylate, was conducted according to the following technique: 1 drop of the compound solution was deposited on the surface of test-system, the analytical effect formed on the surface of the test-system was compared with the similar experiment on the object-plate, whereupon the data was compared with the results received at the stage of identification of sodium benzoate and separately on the surface of each test-means.

RESULTS AND DISCUSSION

The research involved three stages. At the first stage the analysis of monocomponent aqueous solutions of sodium benzoate and sodium salicylate in the given concentrations was conducted with the help of the test-system with heavy metals salts (FP $FeCl_3S_1$,FP $FeCl_3S_2$, FP $CuSO_4$). The second stage was devoted to the study of the possibility of simultaneous identification of sodium salicylate and sodium benzoate in an aqueous solution with the help of test-systems. At the third, closing, stage orienting at the data received at the preceding stages, the analysis of polycomponent prescription was conducted.

The results received at the first stage of the research are presented in table 1.

Table 1 The analytical effects of test-systems with heavy metals salts for the analysis of 5% aqueous solutions of sodium benzoate and sodium salicylate

substance	FP $FeCl_3S_1$	r.c.	b.e.	FP $FeCl_3S_2$	r.c.	b.e.	$FPCuSO_4$	r.c.	b.e.
sodium benzoate	pinkish yellow fur spot	+	yellow	pinkish yellow fur spot	+	yellow	blue fur spot	+	light blue
sodium salicylate	bluish purple spot	+		purple spot	+		green spot	+	

r.c. – research-comparison
b.e. – blank experiment

On the surface of test-systems, it is characteristic of sodium benzoate to make up a spot with prominent borders, which is distinguished by higher intensity of the colouring in the centre of the deposited drop of solution; for sodium salicylate it is characteristic to have the colouring changing with smooth intensity over the entire surface of the deposited drop without distinct borders. Analytical effects on the surface of test-systems correspond to those received in the researches-comparisons, the comparison of analytical effects of principal and blank experiments exclude the possibility of receiving false-positive results, all this attests that the use of test-systems with the salts of Fe^{3+} and $Cu^{2+}$for identification of sodium benzoate and sodium salicylate is possible and promising. The colouring on the surface of test-systems $FPFeCl_3S_1$ and $FPFeCl_3S_2$ differs as in the case of conducted researches-comparisons, this is due to the difference in the concentration of the solutions which the test-systems were modified with, that in its turn causes different proportion of salicylate ion and iron cation contained in the complex. These differences are irrelevant and allow to use both test-systems for identification of sodium salicylate in monocomponent liquid dosage forms. One more difference between the stated test-means is that due to the lighter basic colouring of the test-system $FPFeCl_3S_2$ the analytical effect of sodium benzoate detection is more contrastive, which simplifies the process of visual fixation of the result of the research.

The studies of the soundness of the results reflection in the range of application (70-130%) for each test-system were conducted according to the standardized procedure [15]. The experiments were carried out separately for each concentration of the solutions of sodium benzoate and sodium salicylate (1%, 2%, 5%). The results of the soundness studies are presented in tables 2, 3.

Table 2 The study data of the soundness of results reflection of the reaction of identification of sodium benzoate in monocomponent liquid dosage forms

		FP $FeCl_3S_1$	FP $FeCl_3S_2$	FP $CuSO_4$	FP $FeCl_3S_1$	FP $FeCl_3S_2$	FP $CuSO_4$	FP $FeCl_3S_1$	FP $FeCl_3S_2$	FP $CuSO_4$	FP $FeCl_3S_1$	FP $FeCl_3S_2$	FP $CuSO_4$	FP $FeCl_3S_1$	FP $FeCl_3S_2$	FP $CuSO_4$
range, %		70			85			100			115			130		
1%	*m, g*	0,1761			0,2115			0,2503			0,2872			0,3274		
	C, mg/ml	7,04			8,46			10,01			11,49			13,10		
	R, %	100	100	0	100	100	10	100	100	45	100	100	80	100	100	98
	Δ C	6,05														
2%	*m, g*	0,3517			0,4235			0,5002			0,5742			0,6506		
	C, mg/ml	14,07			16,94			20,01			22,97			26,02		
	R, %	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	Δ C	11,96														
5%	*m, g*	0,8746			1,0702			1,2497			1,4315			1,6243		
	C, mg/ml	34,98			42,81			49,99			57,26			64,97		
	R, %	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	Δ C	29,99														

The conducted experiment showed that sodium benzoate can be identified with the help of test-systems of modified $FeCl_3$ with the acceptable soundness of results starting with the concentration of 1% (the range of application 7,06 - 13,11 mg/ml). Both test-systems despite the concentration of the reagent can fully respond the requirements as for identification of sodium benzoate in the given concentrations. The test-system $FPCuSO_4$ gives the possibility to identify this preparation starting with the concentration of 2% (14,01 – 26,02 mg/ml), the use of the given test-means for identification of sodium benzoate in the concentrations of 1% and lower is impossible because of weak contrast of the spot of the formed fur against the whole surface of the test-system.

The graphical reflection of the data from table 2 – the "effectiveness curve" of identification of 1% aqueous solution of sodium benzoate with the help of test-systems (FP $FeCl_3S_1$, FP $FeCl_3S_2$, FP $CuSO_4$) is depicted in figure 1.

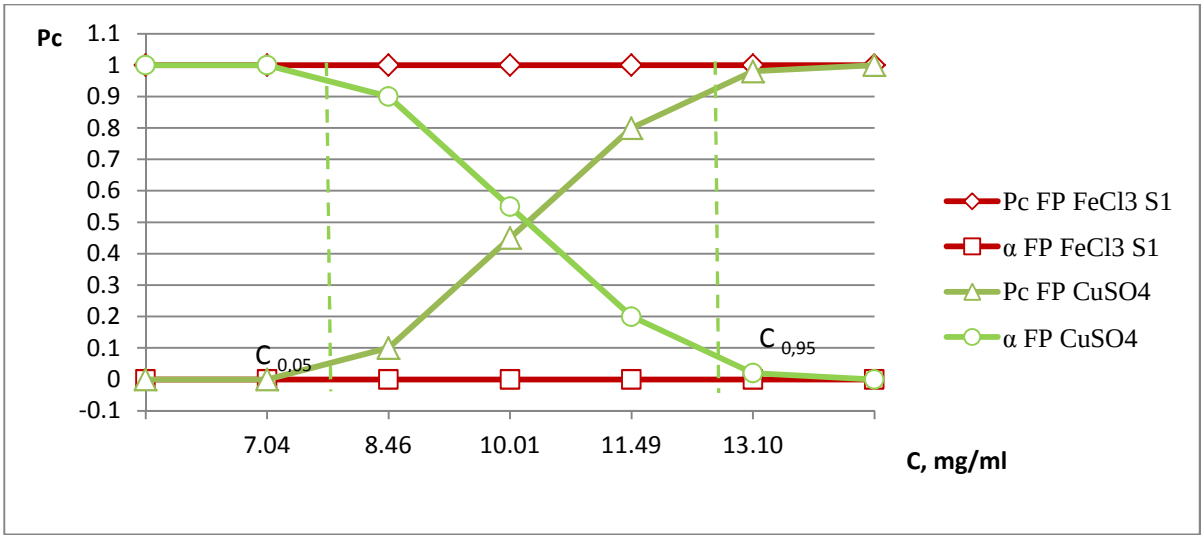

Figure 1.The "effectiveness curve" of identification of sodium benzoate in 1% aqueous solution with the help of test-systems with $FeCl_3$ and $CuSO_4$

Table 3 The study data of the soundness of results reflection of the reaction of identification of sodium salicylate in monocomponent liquid dosage forms

		FP $FeCl_3S_1$	FP $FeCl_3S_2$	FP $CuSO_4$	FP $FeCl_3S_1$	FP $FeCl_3S_2$	FP $CuSO_4$	FP $FeCl_3S_1$	FP $FeCl_3S_2$	FP $CuSO_4$	FP $FeCl_3S_1$	FP $FeCl_3S_2$	FP $CuSO_4$	FP $FeCl_3S_1$	FP $FeCl_3S_2$	FP $CuSO_4$
range, %		70			85			100			115			130		
1%	*m, g*	0,1752			0,2145			0,2505			0,2865			0,3254		
	C, mg/ml	7,01			8,58			10,02			11,46			13,02		
	R, %	5	0	0	25	10	0	40	35	15	75	70	60	100	95	95
	Δ C	6,01														
2%	*m, g*	0,3497			0,4362			0,5010			0,5741			0,6514		
	C, mg/ml	13,99			17,45			20,04			22,96			26,06		
	R, %	100	95	95	100	100	100	100	100	100	100	100	100	100	100	100
	Δ C	12,07														
5%	*m, g*	0,8850			1,0632			1,2523			1,4402			1,6392		
	C, mg/ml	35,40			42,53			50,09			57,61			65,57		
	R, %	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	Δ C	30,17														

According to the results of the research it was defined that the test-systems under investigation, with the accepted level of soundness, allow to identify sodium salicylate in 2% aqueous solution. As for 1% aqueous solution, though at the upper point of the range of application (130%) the indices of soundness reached the magnitude accepted to the requirements it is not enough to recommend these test-systems for identification of sodium salicylate in the concentrations of 1% and lower (figure 2).

To conduct the research at the second stage it was decided to use the mixture of 2% aqueous solution of sodium salicylate and sodium benzoate because starting with this very concentration test-systems start identifying sodium salicylate effectively.

The conclusions as for the convenience of test-systems for completing of the given task were made on the basis of the character of the formed analytical effect: simultaneous visible demonstration of properties, characteristic for both components of the mixture, or development of a new specific analytical effect were considered to be a positive result; and the negative result was the development of an analytical effect which is characteristic only of one of the components under investigation.

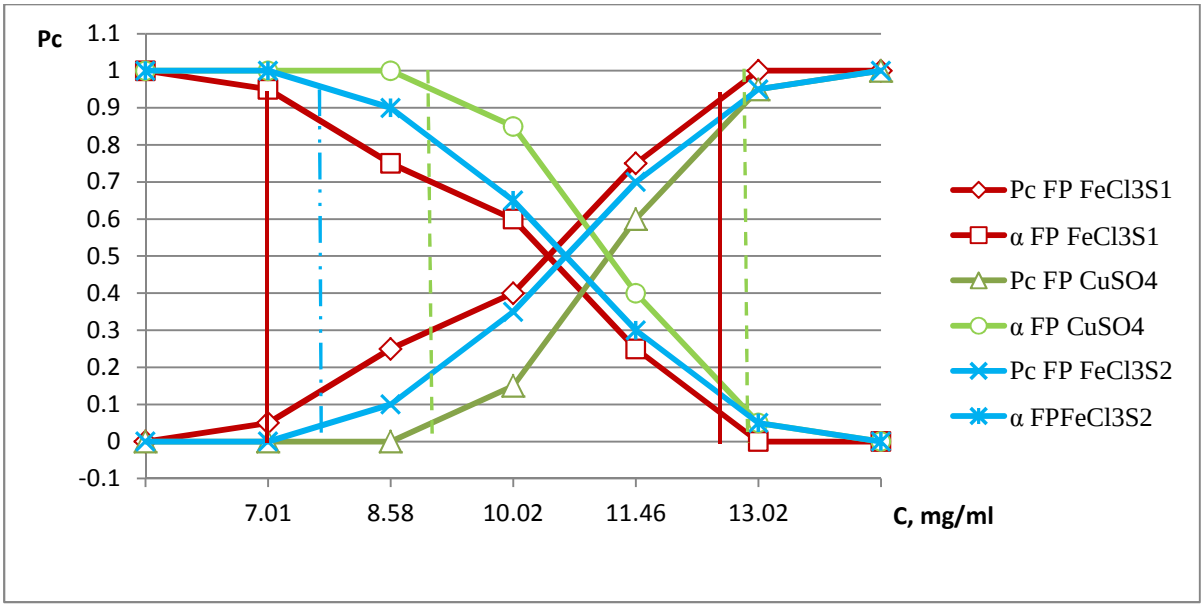

Figure 2.The "effectiveness curve" of identification of sodium salicylate in 1% aqueous solution with the help of test-systems with $FeCl_3$ and $CuSO_4$

A number of samples of the compound, which differed in a balanced change of the components proportion, were exposed to the analysis, in order to define the correlation between the change of concentration of sodium benzoate or sodium salicylate with the change of the analytical effect. The results, received while conducting the research are depicted in table 4. In table 5, the results of researches-comparisons for each component under investigation are represented.

Table 4 The change of the analytical effect depending on the change of the proportion of sodium benzoate and sodium salicylate in the compound

b./s.*	FP $FeCl_3$ S_1	FP $FeCl_3$ S_2	FP $CuSO_4$
10:1	Reddish brown colouring with a purple edging, a brown spot of fur in the centre	red colouring with a purple edging, a reddish brown spot of fur in the centre	Blue colouring, stronger than in the research-comparison
5:1	Reddish brown colouring with a purple edging, a brown spot of furinthecentre	Reddishbrown colouring with a purple edging, a brown spot of fur in the centre	turquoisecolouring, in time changes into blue
3:1	purple colouring, there is a dark erspotoffur	Reddishpurple colouring, there is a dark erspotoffur	Emerald colouring with a dark spot offur, in time blue spots are developed
2:1	purple colouring	Reddish purple colouring	Emerald colouring with a dark spot offur, in time blue spots are developed
1:1	bluish purple colouring	purple colouring	Intensive grassy green colouring with a dark spot offur
1:2	bluish purple colouring	purple colouring	Intensive grassy green colouring with a dark spot offur
1:3	bluish purple colouring	purple colouring	Intensive grassy green colouring
1:5	bluish purple colouring	purple colouring	Intensive grassy green colouring
1:10	bluish purple colouring	purple colouring	Grassy green colouring

** - proportion of the components of sodium benzoate and sodium salicylate*

Table 5 The analytical effects received on the surface of test-systems in researches-comparisons with 2% aqueous solutions of sodium benzoate and sodium salicylate

component	FP $FeCl_3S_1$	FP $FeCl_3S_2$	FP $CuSO_4$
sodium benzoate	pinkish yellow spot of fur	pinkish yellow spot of fur	blue spot of fur
sodium salicylate	bluish purple colouring	purple colouring	Grassy green colouring

The basic point of analysis is the proportion 1:1 as in this very proportion sodium benzoate and sodium salicylate are presented in a polycomponent prescription mentioned at the beginning of this work. As it can be seen from the results presented in tables 4 and 5, on the surface of test-systems FP $FeCl_3$ $S_1$and FP $FeCl_3$ S_2 bluish purple and purple colouring is developed correspondingly. These analytical effects correspond to those received as a result of the interaction of the mentioned test-systems with 2% aqueous solution of sodium salicylate. As the proportion of the components is changed, the analytical effect changes correspondingly: when the portion of sodium benzoate is enlarged in the compound, the colouring of the analytical effect gets red tones and a reddish brown spot of fur

appears; while enlarging the portion of sodium salicylate, the analytical effect remains similar to that of the research-comparison with sodium salicylate.

For test-system FP $CuSO_4$ it is characteristic to have a change of the analytical effect comparing with the researches-comparisons – in the proportion 1:1 the colouring becomes more intense, comparing with the research with sodium salicylate, and a dark spot of fur develops. This analytical effect indicates the possibility that this test-system gives an opportunity to identify the components under investigation while their simultaneous presence in a solution.This thesis can be proved while studying the analytical effects for other components proportions, in particular, in the range of proportions 2:1 – 5:1 on the surface of test-system two colourings develop gradually – at first it is emerald, which fully or partially changes into blue. During the enlargement of the portion of sodium benzoate in the solution (10:1)the identification of sodium salicylate becomes impossible, in its turn the enlargement of the presence of sodium salicylate (1:3 and more) minimizes the chances of identification of sodium benzoate. For the proportion 1:10 the intensity of grassy green colouring on the surface of the test-system is similar to that of the research-comparison for sodium salicylate.

Having analysed the received data the following conclusion can be made. The test-systems based on $FeCl_3$ can be relevant for identification of both components of the compound under investigation starting with the proportion 5:1 towards sodium benzoate. For completing the set task, that is the analysis of the stated polycomponent prescription, the use of these test-systems is not possible. Due to the development of a specific analytical effect (intensification of the colouring and development of a spot of fur), the test-system FP $CuSO_4$ can identify simultaneous presence of sodium benzoate and sodium salicylate in a compound at the proportion of the components 1:1, and also 2:1 – 5:1 towards sodium benzoate and 1:2 towards sodium salicylate.

For the third stage of the research the test-system FP $CuSO_4$ was chosen. For identification of sodium benzoate and sodium salicylate consisting in a polycomponent EMP with the help of the mentioned test-system the following technique was used: 0,1 g of the powder under investigation was dissolved in 1ml of *purified water S*, 1 drop of the received solution was deposited on the surface of the test-system – the development of intensive grassy green colouring with a dark spot of fur in the centre was recorded.

For test-system FP $CuSO_4$ the study of the specifity of soundness of the reproduction of the reaction results in the range 70-130% separately for each of the two components under investigation of the prescription mentioned at the beginning of this work (the mass of the other component remained stable). The results of the conducted experiment are represented in table 6.

Table 6 The resultsofthe study of the specifity of soundness of the reproduction of the reaction results in the range 70-130%

range,%	**70**	**85**	**100**	**115**	**130**
sodium benzoate					
m, g	0,0133	0,0157	0,0192	0,0218	0,0242
C, mg/ml	13,30	15,70	19,20	21,80	24,20
analytical effect	intensive grassy green colouring	intensive grassy green colouring, a spot of fur develops	intensive grassy green colouring, a spot of fur	Intensive grassy green colouring, a spot of fur, in some time blue colouring is developed	Emerald colouring, a spot of fur, in some time blue colouring is developed
R, %	100	100	100	100	100
sodium salicylate					
m, g	0,0130	0,0156	0,0189	0,0215	0,0245
C, mg/ml	13,00	15,60	18,90	21,50	24,50
analytical effect	intensive grassy green colouring, a spot of fur develops	intensive grassy green colouring, a spot of fur	intensive grassy green colouring, a spot of fur	intensive grassy green colouring	intensive grassy green colouring
R, %	100	100	100	100	100

As it can be seen from the results represented in table 6, the test-system FP $CuSO_4$ can be used for simultaneous identification of sodium benzoate and sodium salicylate in a polycomponent medicinal preparation of pharmacy production. The presence of other components of this EMP (sodium sulphate and sodium hydrocarbonate) does not affect the results of identification of the components under investigation because of their resistance for the reagent test-means.

CONCLUSION

1. Test-systems based on filter paper modified with the salts of heavy metals Fe^{3+}andCu^{2+} give a possibility to identify sodium benzoate and sodium salicylate in aqueous monocomponent solutions of EMP.

2. Minimal ranges of application of the test-systems modified with $FeCl_3$ ($FPFeCl_3S_1$ and $FPFeCl_3S_2$) made up: for sodium benzoate –7,06-13,11 mg/ml (1% aqueous solution); for sodium salicylate –14,08-26,11 mg/ml (2% aqueous solution); test-system FP $CuSO_4$– 14,01-26,02 and 14,08-26,11mg/ml for sodium benzoate and sodium salicylate correspondingly.
3. The difference in the concentrations of the solutions of iron (III) chloride, which the test-systems $FPFeCl_3S_1$ and $FPFeCl_3S_2$ were modified with, does not significantly affect the results of the research, that is why in order to use the reagent more effectively and to enhance the convenience of experiment results fixation, the test-system made on the basis of iron (III) chloride solution S_2 (concentration 13 g/l or 1,3%) can be recommended to use.
4. For simultaneous identification of sodium benzoate and sodium salicylate in a polycomponent EMP the test-system FP $CuSO_4$ can be used since on its surface the analytical effect corresponding to both components under investigation – intensive grassy green colouring with a dark spot of fur, is developed; with a high soundness of the results reproduction this technique can be used starting with the concentration 13,3 – 24,2 mg/ml for sodium benzoate and 13,00 – 24,5 mg/ml for sodium salicylate.

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