The study of properties an ion associate of food azo dye carmoisine with myramistin

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ABSTRACT

Carmoisine is the synthetic food dye, which is used in the manufacturing of some medicals and food products. Previously, we had already been established, that it is able to form the ion associates with some organic amines and their salts, including medicinal substances, and it was capable in this form to be extracted from aqueous solution. The aim of our research was studying the properties of formed ion associate between carmoisine and myramistin - quaternary ammonium compound. As the result of the work performed, that absorption spectra of mixture of carmoisine and myramistin was characteristic by bathochromic shift of the aromatic and long wavelength peak maxima in visible area of the initial dye and by hypochromic effect, reflected particularly in the area of the maximum of the dye. The partition coefficients for various solvents ror the associate were spectrophotometrically established and it is shown, that the highest ratio was observed when extraction associate with chloroform from the aqueous solution without addition of buffer. The dependence of the absorbance on the pH value of the aqueous medium was studied and shown, that change in the pH range from 2.0 to 8.0 had no significant effect on degree of the extraction with chloroform the associate. The dependence of the absorbance of myramistin with carmoisine associate on extraction time was studied and it was shown, that the optimal time was 2 minutes The stoichiometric ratio of the components in the resulting ion associate was established with isomolar series method and was confirmed with HPLC and it was 1:1.

Keywords: carmoisine, myramistin, ion associates, extraction, spectrophotometry, HPLC.
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INTRODUCTION

Every manufacturer wants to attract the largest number of consumers to his product in evolving competitive environment. A lot of attention is paid to improvement of consumption properties and visual appeal of goods along with safe, effective, and high quality drugs. It is possible to achieve similar purpose for pharmaceutical market by applying of various excipients, including dyes. Due to increasing number of synthetic molecules enter body; more research is done to study the effects of these molecules on the human body. Food dyes are the ones of widely used groups of excipients in food and pharmaceutical industries, that include a carmoisine [1, 2].

We have found that this dye was capable to form ion associates with some organic amines and their salts, including medicinal substances, and it was capable in this form to extract from aqueous solution with organic solvents [3, 4, 5]. We have analyzed the chemical structures of the carmoisine and myramistin and we have suggested that these substances were also able to form an ion associate.

Myramistin is a drug belonging to the group of cationic antiseptics and surfactants (quaternary ammonium compounds), and it has anti-microbial, anti-inflammatory and local immunoadjuvant action. The drug is active

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against variety of pathogenic microorganisms including viruses, fungi, bacteria and protozoa. Molecules of substance act on outer shell of microbial cell that leads to its destruction and death. Local immunostimulatory effect of the drug is provided through the activation function of phagocytic cells (phagocytes and macrophages). [6, 7, 8].

Carmoisine   **Myramistin**
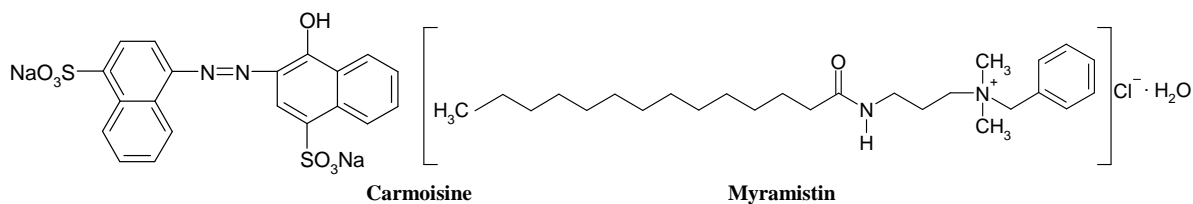
The aim of our research was studying properties of ion associate between carmoisine and myramistin.

MATERIALS AND METHODS

During carrying out of experimental researches the following materials and methods were used: synthetic food dye carmoisine (series 657334(1-20)-657383(1-20)) and medicinal substance myramistin. Analytical researches were performed on a spectrophotometer Evolution 60S and on liquid chromatograph Agilent 1100, pH values were measured with potentiometer pH-150 MI. For operation we used AXIS ANG200 analytical balances, Class A measuring glassware and excipients, that meet requirements of the State Pharmacopoeia of Ukraine (SPhU) [9] which are harmonized with the European ones [10].

For preparation of test solutions of dye and myramistin water R was used. During researches the following organic solvents were used: chloroform, butanol and ethyl acetate marks "chemically pure".

RESULTS AND DISCUSSION

The absorption spectra of aqueous solutions of carmoisine and myramistin were measured. 1.0 ml of 1×10^{-3} M carmoisine solution were placed into a 25.0 ml volumetric flask and then it was taken to the mark with water R. In parallel, 5.0 ml of 1×10^{-3} M myramistin solution were placed into another 25.0 volumetric flask and then it was taken to the mark with water R. The spectra of obtained solutions were measured against water R (Figure 1).
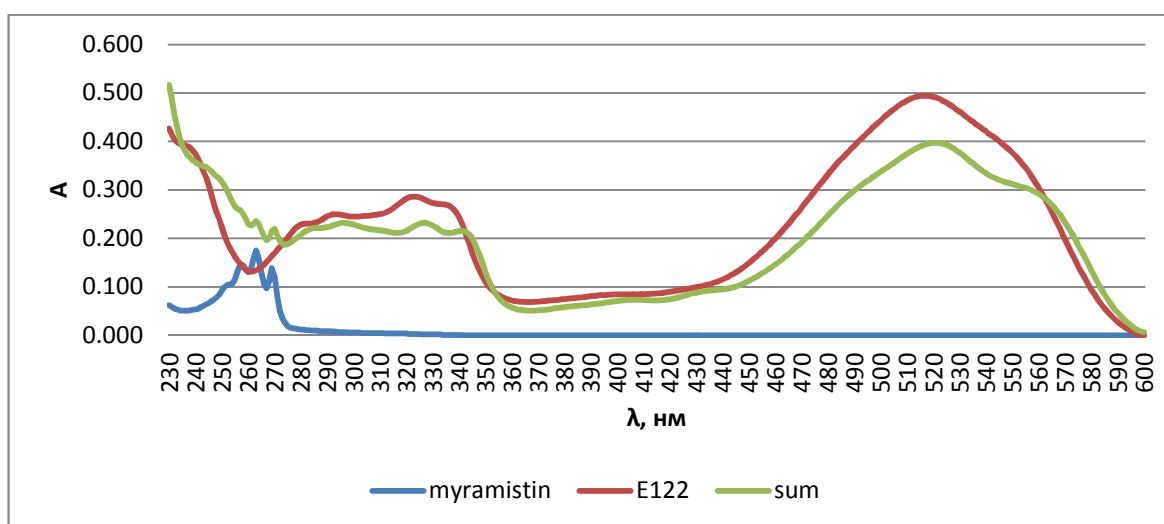

Fig. 1. The absorption spectrum of aqueous solutions myramistin, carmoisine and their mixture

Analysis of the myramistin aqueous solution spectrum showed the presence of typical "benzene" absorption bands with distinct maximums at 257 nm, 263 nm and 269 nm. These absorption bands were due to the presence of the aromatic ring in the myramistin structure. Carmoisine molecule consisted of two residues of naftensulfonate sodium conjugated via diazo group, one of which contained a hydroxyl group. The absorption spectrum of the carmoisine aqueous solution in the UV light was represented by a broad band in the region of 280-350nm. The band could be distinguished in 4 absorption bands corresponding $\pi \rightarrow \pi^*$ transitions of aromatic system. There was the presence of a wide plateau observed at the 360-430nm in visible area; the plateau became an intense long-wavelength absorption

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band with maximum at the 516-519nm. There was a slight inflection of the absorption band in the area of 535-540 nm, which indicated that it had a total character. Simultaneously, in the same conditions total absorption spectrum of mixture of two solutions was measured. 1.0 ml of 1×10^{-3} M carmoisine solution were placed into a 25.0 ml volumetric flask, 5.0 ml of 1×10^{-3} M myramistin solution were added and then they were taken to the mark with water R. As a blank solution water R was used (Figure 1). Analysis of total spectrum of the mixture of two solutions was characterized by superposition of two acquisitions of substances at 250-270 nm with a somewhat smoother. But well distinct peaks corresponded to the maximum in the absorption spectrum of the molecule of myramistin. For all spectrum (except for the area at 250-270 nm) hypochromic effect was typical, especially in the area of maximum dye - at 516-519 nm. Also, obtained spectrum was characterized with 3-4 nm bathochromic shifts of carmoisine aromatic peaks and 4 nm bathochromic shifts of a long-wavelength peak in visible region with more contoured inflection of the absorption band at 535-540 nm, that could indicate of the mutual influence of the molecules associated with the ion associate formation Based on these results it could be assumed that physicochemical interaction between myramistin and dye occurred. And it was reflected in the character of the obtained solution mixture spectrum.

Establishment of partition coefficients in various solvents at different pH values
To calculate partition coefficients: 1×10^{-3} M aqueous solutions of carmoisine and myramistin were used. Required pH value was created by using of phosphate buffer solutions.

Procedure of implementation: 0.5 ml of myramistin solution, 0.5 ml of carmoisine solution and 49.0 ml of phosphate buffer solution were placed into a separating funnel and they were shaken for 2 minutes. Stock solution was measured spectrophotometrically at the wavelength of 450-550 nm against a blank solution. Formed ion associate was extracted once with organic solvent – butanol, chloroform or ethyl acetate, in a portion of 25.0 ml. The spectrum of aqueous layer after extraction was also measured over the wavelength range 450-550 nm against a blank solution. During extraction with chloroform 0.25 ml of 1×10^{-3} M myramistin solution were used. Necessity of measurement spectrum in this range was explained by the possibility of maximum displacement due to the effect of an organic solvent on properties of the formed associate. There the values of the absorption at the maximum were found and concentration of carmoisine in the initial solution and in the aqueous solution after extraction by the standard method were calculated. Concentration of carmoisine in the extraction was found by difference of the concentrations of the initial solution and of the aqueous solution after extraction. The partition coefficient was calculated with formula:

$$K = \frac{C_2}{C_1}, \text{ where:}$$

C_1 was the carmoisine concentration in the initial water solution;
C_2 was the carmoisine concentration in the obtained extract solution.

The results are given in Table 1.
As a result of the research it is possible to assert, that the ion associate formed was practically insoluble in ethyl acetate at all investigated pH values. It should also be noted, that initial carmoisine was soluble in butanol and obtained coefficient didn't indicate the formation of the associate.

Table.1. The partition coefficients of the associate for some solvents

solvent	pH	C_2/C_1
butanol	4,2	2,41
	6,2	2,34
	-	2,71
chloroform	4,2	1,39
	6,2	1,49
	-	1,82
ethyl acetate	4,2	0,59
	6,2	0,37
	-	0,48

These results allowed to suggest that when developing control methods of equipment cleaning after using it in food dyes manufacturing (in particular, carmoisine manufacture) its reaction with myramistin and the subsequent extraction of the associate with chloroform from the wash water could be used.

It was known that the formation of ion associates was affected by the acidity of the medium, so we studied the dependence of the absorbance of the associates extracts from the pH value of the aqueous medium. For this purpose

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0.5 ml of 1×10^{-3} M myramistin solution, 0.1 ml of 14×10^{-3} M carmoisine solution and 20.0 ml of corresponding phosphate buffer solution with various pH (2.0 to 8.0) were placed into a separating funnel and they were mixed for 2 minutes. Ion associates obtained were extracted once with 10.0 ml of chloroform. Measurements were made against the reference solution, which was used as water-saturated chloroform. The measurements data are shown in Table 2 and Fig.2.

Table 2 The dependence on extraction an ion associate of pH value

pH	absorbance	pH	absorbance	pH	absorbance
2,0	0,637	4,5	0,652	6,5	0,674
2,5	0,654	5,0	0,664	7,0	0,664
3,0	0,636	5,5	0,676	7,5	0,617
4,0	0,635	6,0	0,651	8,0	0,649

Data in the Table follows that change in the pH range from 2.0 to 8.0 had no significant effect on the degree of extraction associate with chloroform.
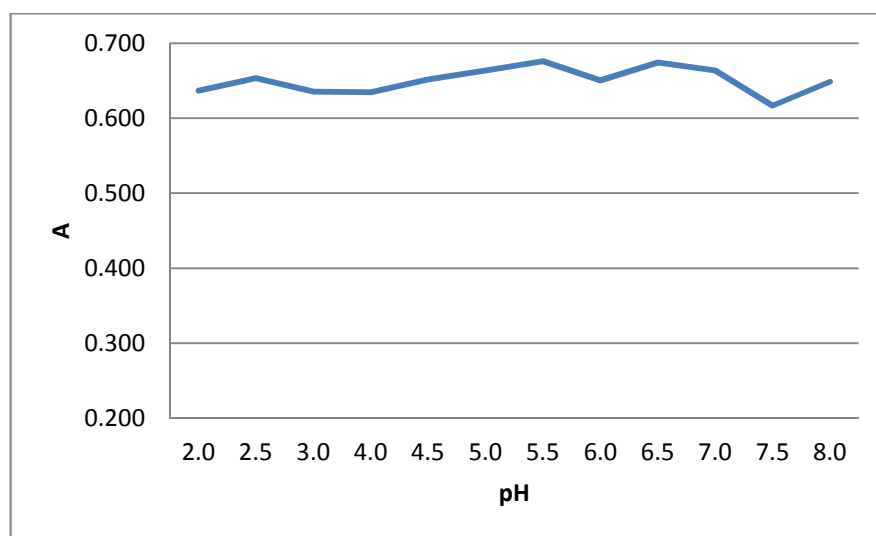

Fig.2. The dependence of absorbance (A) on pH value for the ion associate between carmoisine and myramistin

Completeness of transfer of the colored associate from aqueous phase to the organic one and, consequently, the intensity of the color of the extract depended on the contact time of the two phases. Therefore, we also studied the dependence of the absorbance of carmoisine associate with myramistin from the time of extraction. For research of this dependence, 0.5 ml of 1×10^{-3} M myramistin solution, 0.1 ml of 14×10^{-3} M carmoisine solution and 20.0 ml of water R were placed into a separating funnel. 10.0 ml of chloroform were added to the obtained solution and the mixture was shaken vigorously. The time of extraction was increased in each subsequent experience. The extracts were measured at the wavelength of 521 nm. The obtained data are presented in Table 3.

Table 3 Absorbance of ion associate extracts depending on the extraction time

The extraction time	0,25 min	0,5 min	1 min	2 min	3 min	5 min
Absorbance	0,430	0,582	0,608	0,622	0,620	0,619

Data in the Table 3 follows that for the complete extraction of the ion associate it was necessary to spend 2 minutes in case of using chloroform as the extracting agent.

Carmoisine is a disodium salt of dibasic sulfonic acid, which dissociates in aqueous solution to form an anion with a charge $^{-2}$. And myramistin is a salt with a quaternary ammonium cation with charge $^{+1}$. It can be assumed that one molecule of carmoisine and one or two molecules of myramistin will come into reaction of the formation of ion associate.

With the aim of studying the stoichiometric ratios of the reactants in the reactions, spectrophotometric analysis for solutions of colored products formed by reactions was performed. The basis of this law was a combination of using the law of mass action and the basic law of light absorption. For this the most common method of

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spectrophotometric study of the reactants stoichiometric ratios named "The Method of Isomolar Series" was used [9].

Dye and drug solutions of the same molar concentrations (1×10^{-3} M) were prepared for the experiment. They were mixed in antisymbatic ratios (1:9 to 9:1) wherein the total number of moles of both components in a total volume remained unchanged. For research of this dependence a measured amount of the carmoisine and myramistin solutions was placed in a separatory funnel, 20.0 ml of water R were added and it was extracted with 10.0 ml of chloroform. The extracts were measured at the wavelength of 521 nm against a blank solution. Plotted versus the optical density of the volume ratio of component isomolar series based on the data obtained. Results are presented in Table 4 and Fig. 3.

Table 4 The studying results of the stoichiometric ratio of the components

Ratio	Volume of myramistin	Volume of carmoisine	Absorbance
9:1	4,50	0,50	0,181
4:1	4,00	1,00	0,322
2,33:1	3,50	1,50	0,827
1,5:1	3,00	2,00	1,222
1:1	2,50	2,50	2,822
1:1,5	2,00	3,00	1,238
1:2,33	1,50	3,50	0,824
1:4	1,00	4,00	0,505
1:9	0,50	4,50	0,344

Analysis of the data suggested that the stoichiometric ratio of the components in the formed ion associate was 1:1.
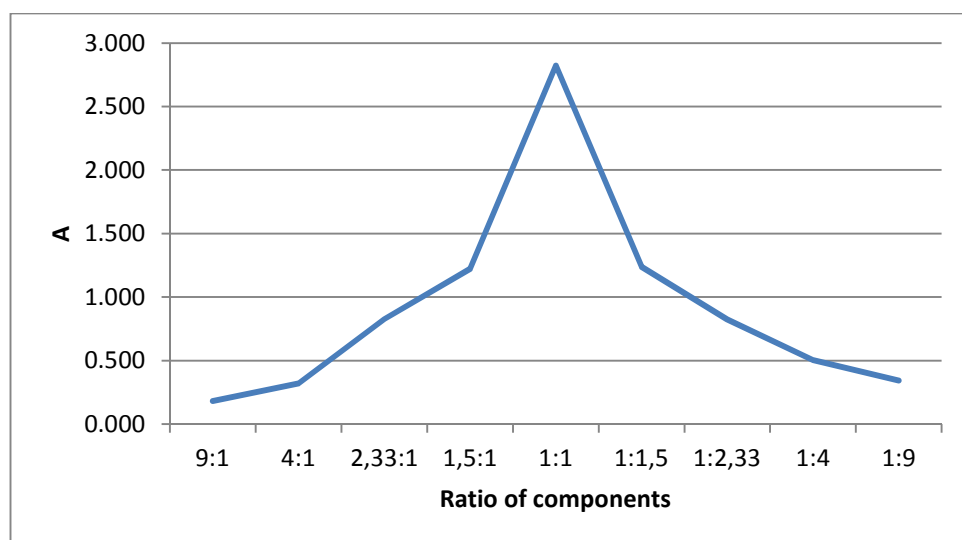

Fig. 3. The dependence of absorbance (A) of the molar ratio of carmoisine and myramistin

In order to confirm obtained results about stoichiometric ratios of reacting substances in the studied reactions with isomolar series, analysis with HPLC was performed.

The procedure for obtaining the associate: Aqueous solutions of myramistin and carmoisine were prepared in equal molar concentrations (1×10^{-3} M). 15.0 ml of water R were placed in a separation funnel, 2.5 ml of myramistin solution and 2.5 ml of carmoisine solution were added and they were mixed. Then a single extraction was carried out using 10.0 ml of chloroform. The chloroform extract of the ion associate was transferred into an evaporating dish, then it was evaporated with chloroform, a dry residue was dissolved in 96% ethanol, then it was transferred quantitatively into a 10.0 ml volumetric flask and it was diluted to the volume with the same solvent. Obtained extract was determined chromatographically. In parallel, under the same conditions, aqueous standard solutions used to obtain the associate were chromatographed.

The chromatographic conditions were as follows:
the column – 150×3.9 Xterra RP18; grain size was 5 micron;
the mobile phase – acetonitrile/water, 50/50;
detection at wavelength – 215 nm;

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the eluent rate – 1 ml / min;
the dosing volume – 5 microlitres.

The results are shown in Figure 4, 5 and 6.

During investigation with HPLC method, a clear peak with retention time was 2,289 min, corresponding to myramistin on the chromatogram of the standard solution of myramistin was observed (Fig. 4). In chromatogram of the standard solution of the dye a clear peak with retention time was 6,647 min, corresponding to carmoisine, was observed (Fig. 4).
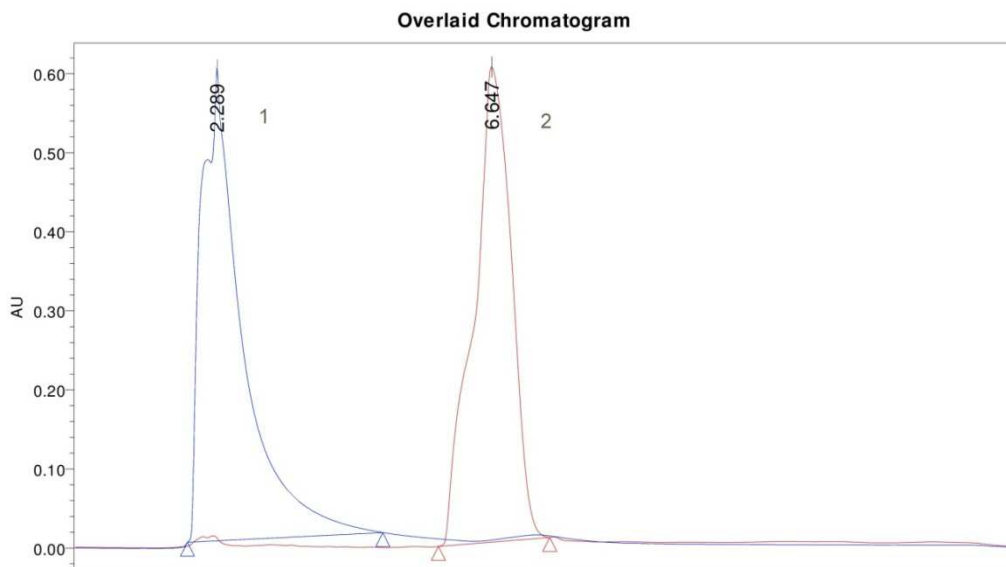

Fig. 4. The scheme of chromatogram of carmoisine (1) and myramistin (2) standard solutions

In the chromatogram obtained with the chloroform extract of the ion associate (Fig. 5), there was a clear peak, which corresponded to carmoisine and a not completely separated peak, indicating that by dissolving the dry residue in 96% alcohol the associate was partially dissociated.
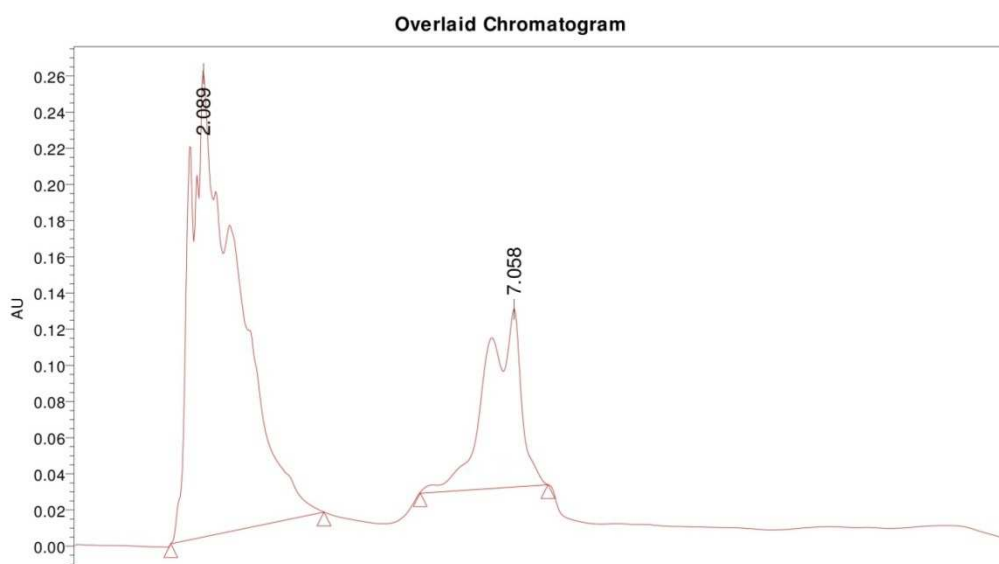

Fig. 5. The scheme of chromatogram of the ion associate chloroform extract

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We decided to try to destroy the resulting ion associate using 0.01 M sodium hydroxide. To do this, the resulting dry residue was dissolved in 0.01 M solution of NaOH and 96% alcohol, quantitatively transferred to a 10.0 ml volumetric flask and diluted to volume with the same solvent.

In the chromatogram of the extract obtained (Fig. 6) the characteristic peak of carmoisine and the characteristic peak of myramistin were clearly visible. Relation of peak areas of carmoisine and myramistin showed that ratio of the components in the associate was 1:1.
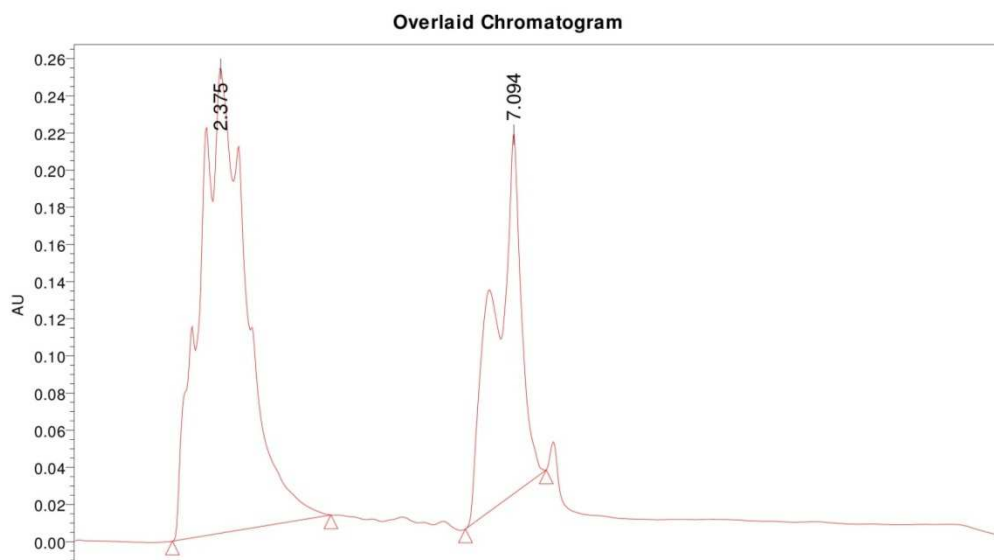

Fig. 6. The scheme of the chromatogram of the ion associate chloroform extract in 0.01 M NaOH and 96% alcohol solutions

CONCLUSION

1. Absorption spectra of aqueous solutions of myramistin, carmoisine solutions and their mixture were investigated. Bathochromic shift of the aromatic and long wavelength peak maxima in visible area of the initial dye was shown. Hypochromic effect was evident, reflected particularly in the area of the maximum of the dye. It could be explained by the mutual influence of the molecules related with the formation of ion associate.
2. Partition coefficients for different solvents for associate were established. The highest ratio was observed to extraction associate with chloroform from the aqueous solution without addition of buffer.
3. The dependence of the absorbance on the pH value of the aqueous medium was studied. And it was established that change in the pH range from 2.0 to 8.0 had no significant effect on degree of the extraction with chloroform the associate of carmoisine with myramistin.
4. The dependence of the absorbance of associate of myramistin with carmoisine on extraction time was studied. The optimal time was 2 minutes.
5. The stoichiometric ratio of the components in the resulting ion associate was established with isomolar series method, which consisted 1:1.
6. The stoichiometric ratio of the components in the ion associate was confirmed with HPLC and it was 1:1.

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