# СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

*Recommended by Doctor of Pharmacy, professor V.S.Bondar*

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## DETERMINATION OF THE FOOD AZO DYE CARMOISINE VS CHLORPHENIRAMINE MALEATE ION ASSOCIATE STRUCTURE

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*Key words: carmoisine; chlorpheniramine maleate; ion associates; HPLC*

*Recently different excipients, including colouring agents, are often used to give a marketable appearance and improve the consumer characteristics of drugs. Usually they are applied in the composition of medicines for internal use (tablets, capsules, syrups, troches, lozenges, etc). Synthetic azo dyes – a group of compounds obtained by the coupling reaction of sulfonaftilamines and diazotised sulfoanilines with aromatic or heterocyclic phenols are the most widely used in the pharmaceutical practice. Compounds of this group give bright, easily reproducible colours, they are resistant to the light, oxidants, reducing agents, pH changes and less sensitive to different types of technological impact. Synthetic azo dyes are poorly absorbed from the intestines after intake, but they can form ion associates with salts of organic bases, including with drugs, and it may alter their bioavailability. The aim of our work was to determine the partition coefficient of the ion associate of a synthetic food azo dye carmoisine with the medicinal substance chlorpheniramine maleate in the water-chloroform system and to study the stoichiometric ratio of the components in this ion associate. The partition coefficient in the water-chloroform system has been determined spectrophotometrically. It is 3.44, indicating that the ion associate is more soluble in water than in chloroform. The stoichiometric ratio of carmoisine and chlorpheniramine maleate in the resulting ion associate has been determined by HPLC, and it is 1:2.*

In today's competition producers face the challenge not only to provide consumers with qualitative, effective and safe drugs, but also to defend the competition in its segment of the pharmaceutical market. Various excipients, including food dyes, are widely used to improve the consumer characteristics. In the pharmaceutical industry synthetic azo dyes, in particular carmoisine (E 122), are the most commonly used [6-9]. Carmoisine, a red coloured synthetic food azo dye, is a derivative of diazosulfonaphthalenes, a crystalline substance that is soluble in water [5, 10].

Earlier when developing the method for quantitative determination of carmoisine in the composition of drugs we have found that it is capable to form ion associates with organic amines, particularly with such medicinal substance as chlorpheniramine maleate and to extract with an aqueous solution of chloroform in this form [3, 4].

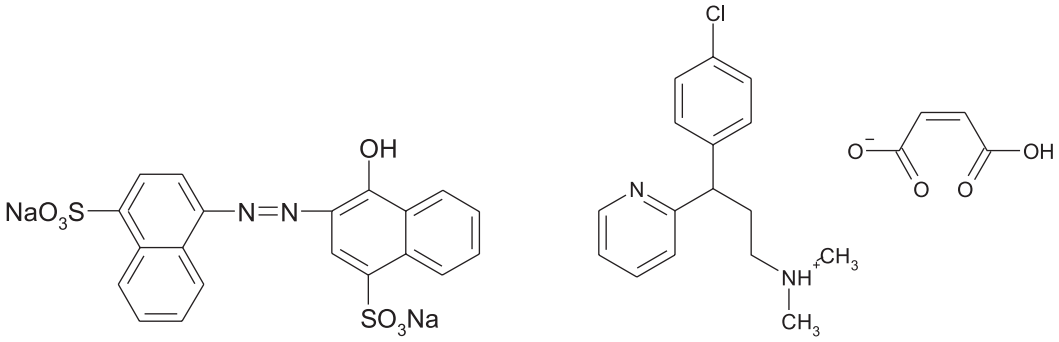
Chlorpheniramine maleate (3-(4-chlorophenyl)-N,N-dimethyl-3-pyridin-2-yl-propan-1-amine) is the active pharmaceutical ingredient of the synthetic origin. It is a white crystalline powder, easily soluble in water, soluble in ethanol and poorly soluble in diethyl ether. It exhibits the cholinolytic and antihistaminic action [2].

The aim of our research is to calculate the partition coefficient in the water-chloroform system and to study further the ratio of the components in the ion associate formed.

**Materials and Methods**

To calculate the partition coefficient in the water-chloroform system the extraction was performed according to the following procedure: to a separating funnel place 4.0 ml of 0.001 M carmoisine solution and 6.0 ml of 0.001 M solution of chlorpheniramine maleate, add 30.0 ml of phosphate buffer solution with pH 4.2 and extract with 20.0 ml of chloroform saturated with water. Measure the absorbance of the extract at the absorption maximum of 523 nm. The blank solution is chloroform. Then to the chloroform extract add 25.0 ml of the buffer solution and perform the extraction once more. Measure the absorbance of the resulting aqueous layer on a spectrophotometer at the wavelength of 517 nm. The blank solution is phosphate buffer solution.

*The method for obtaining the associate.* Prepare aqueous solutions of chlorpheniramine maleate and carmoisine in equal molar concentrations. In a separation funnel place 15.0 ml of phosphate buffer solution, add 1.5 ml of chlorpheniramine maleate, 3.5 ml of carmoisine and mix. Then carry out a single extraction using 10.0 ml




Carmoisine Chlorpheniramine maleate

Sheme

of chloroform. Transfer the chloroform extract of the ion associate into an evaporating dish, evaporate chloroform, dissolve a dry residue in 96% ethanol, transfer quantitatively into a 10.0 ml volumetric flask and dilute to the volume with the same solvent.

The extract obtained was determined chromatographically. In parallel, under the same conditions, aqueous standard solutions used to obtain the associate were chromatographed.

In our work the following reagents were used: a standard sample (SS) of chlorpheniramine maleate and carmoisine, phosphate buffer solution with pH 4.2, chloroform, purified water.

The analytical equipment was Agilent 1100 liquid chromatograph, Axis electronic analytical balances and measuring glassware of class A.

The chromatographic conditions were as follows:

- the column – 150×3.9 Xterra RP18; grain size is 5 micron;
- the mobile phase – acetonitrile/water, 50/50;
- detection at wavelength – 215 nm;
- the eluent rate – 1 ml / min;
- the dosing volume – 5 microlitres.

**Results and Discussion**

With the help of the absorbances obtained and the specific absorption rate calculated previously the concentrations of carmoisine in chloroform and buffer solution were calculated. The partition coefficient was calculated by the formula:

$$K = C_2 / C_1,$$

where: $C_2$ − is the carmoisine concentration in water after the second extraction; $C_1$ – is the concentration of carmoisine in chloroform.

The data obtained has shown that the partition coefficient in the water-chloroform system is 3.44, i.e. the ion associate formed is more soluble in water than in chloroform.

Carmoisine is a disodium salt of dibasic sulfoacid, which dissociates in the aqueous solution to form an anion with a charge of 2-, and chlorpheniramine maleate containing the dimethylamino group and the pyridine ring in its composition is the salt of a weak dibasic conjugated organic acid. Theoretically, it can provide two series of salts − with the protonated dimethylamino group and a monobasic cation of maleic acid, wherein the negative charge is delocalized over the entire molecule, or the protonated dimethylamino group and the pyridine nitrogen, as well as a dibasic cation of maleic acid. The latter version is less likely because of the reduced basicity of the pyridine nitrogen and the low

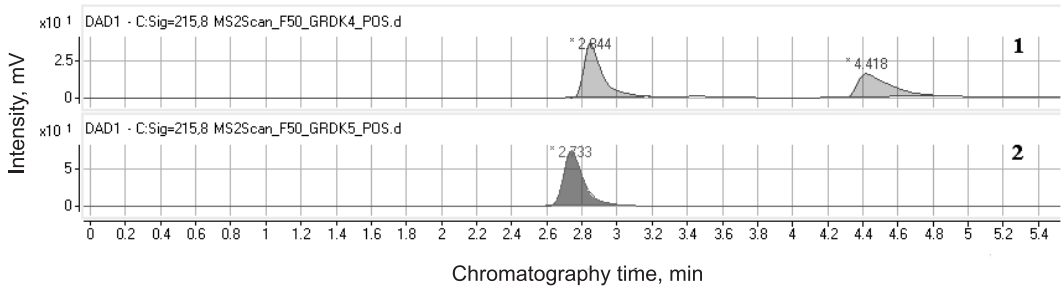



Fig. 1. The scheme of chromatogram of standard solutions of chlorpheniramine maleate (1) and carmoisine (2).

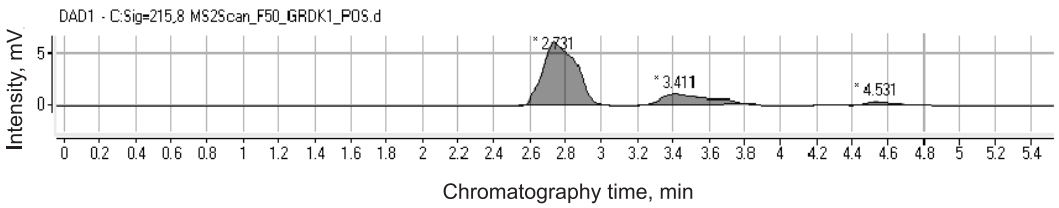



Fig. 2. The scheme of the chromatogram of the ion associate chloroform extract.

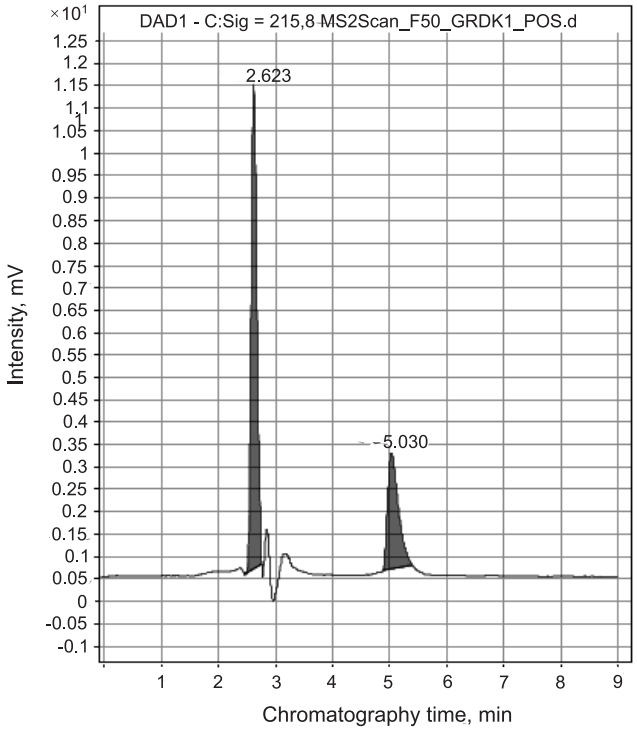



Fig. 3. The scheme of the chromatogram of the ion associate chloroform extract in 0.01 M NaOH solution.

acidity of maleic acid according to the second stage of dissociation due to delocalization of the first negative charge. It can be assumed that one molecule of carmoisine and two molecules of chlorpheniramine will enter the reaction of the ion associate or, less likely, the ratio of the components will be 1:1.

This assumption was verified by the isomolar series method, the result of which showed that the optimal ratio was 3:7 [1].

During investigation by the HPLC method there are two clearly separated peaks corresponding to chlorpheniramine and maleate on the chromatogram of the standard solution of chlorpheniramine maleate (Fig. 1). In the chromatogram of the standard solution of the dye a clear peak, which corresponds to carmoisine, is observed (Fig. 1).

In the chromatogram obtained with the chloroform extract of the ion associate (Fig. 2), there is an unseparated peak, indicating that in these chromatographic conditions the ion associate is quite stable and not subjected to chromatographic separation.

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, quantitatively transferred to a 10.0 ml volumetric flask and diluted to the volume with the same solvent.

In the chromatogram of the extract obtained (Fig. 3) the characteristic peak of carmoisine and the characteristic peak of chlorpheniramine are clearly visible. The relation of peak areas of carmoisine and chlorpheniramine shows that the ratio of the components in the associate is 1:2.

CONCLUSIONS

1. The partition coefficient calculated in the water-chloroform system is 3.44; the ion associate formed is more soluble in water than in chloroform.

2. It has been determined by HPLC that the stoichiometric ratio of chlorpheniramine maleate and carmoisine is 1:2.

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**ВСТАНОВЛЕННЯ СТРУКТУРИ ІОННОГО АСОЦІАТУ ХАРЧОВОГО АЗОБАРВНИКА КАРМОЇЗИНУ З ХЛОРОФЕНІРАМІНУ МАЛЕАТОМ**

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***Ключові слова:*** *кармоїзин; хлорофеніраміну малеат; іонні асоціати; ВЕРХ*

*Останнім часом для надання товарного вигляду і поліпшення споживчих характеристик лікарських засобів часто застосовують різні допоміжні речовини, в тому числі і барвники. За-*

*звичай підфарбовують ліки для внутрішнього застосування (таблетки, капсули, сиропи, драже, пастилки та ін.). Найбільш широко у фармацевтичній практиці використовуються синтетичні азобарвники – група речовин, отриманих за реакцією азосполучення діазотованих сульфоанілінів і сульфонафтиламінів з ароматичними чи гетероциклічними фенолами. Сполуки даної групи дають яскраві, легко відтворювані кольори, стійкі до дії світла, окисників, відновників, змін pH і менш чутливі до різних видів технологічного впливу. Синтетичні азобарвники після прийому всередину погано всмоктуються з кишечника, проте вони здатні утворювати іонні асоціати з солями органічних основ, в тому числі і з лікарськими засобами, що може змінювати їх біодоступність. Метою нашої роботи було визначення коефіцієнта розподілу іонного асоціату харчового синтетичного азобарвника кармоїзину з лікарською речовиною хлорофеніраміну малеатом у системі вода-хлороформ та дослідження стехіометричного співвідношення компонентів у цьому іонному асоціаті. Коефіцієнт розподілу в системі вода-хлороформ визначали спектрофотометрично. Він становить 3,44, тобто іонний асоціат краще розчиняється у воді, ніж у хлороформі. Методом ВЕРХ встановлено стехіометричне співвідношення кармоїзину та хлорофеніраміну малеату в утвореному іонному асоціаті, яке становить 1:2.*

**ОПРЕДЕЛЕНИЕ СТРУКТУРЫ ИОННОГО АССОЦИАТА ПИЩЕВОГО АЗОКРАСИТЕЛЯ КАРМОИЗИНА С ХЛОРФЕНИРАМИНА МАЛЕАТОМ**

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***Ключевые слова:*** *кармоизин; хлорфенирамина малеат; ионные ассоциаты; ВЭЖХ*

*В последнее время для придания товарного вида и улучшения потребительских характеристик лекарственных средств часто применяют различные вспомогательные вещества, в том числе и красители. Обычно подкрашивают лекарства для внутреннего применения (таблетки, капсулы, сиропы, драже, пастилки и др.). Наиболее широко в фармацевтической практике используются синтетические азокрасители − группа веществ, полученных по реакции азосочетания диазотированных сульфоанилинов и сульфонафтиламинов с ароматическими или гетероциклическими фенолами. Красители данной группы дают яркие, легко воспроизводимые цвета, устойчивые к свету, окислителям, восстановителям, изменениям pH и менее чувствительные к различным видам технологического воздействия. Синтетические азокрасители после приема внутрь плохо всасываются из кишечника, однако они способны образовывать ионные ассоциаты с солями органических оснований, в том числе и с лекарственными средствами, что может привести к изменению их биодоступности. Целью нашей работы было определение коэффициента распределения ионного ассоциата пищевого синтетического азокрасителя кармоизина с лекарственным веществом хлорфенирамина малеатом в системе вода-хлороформ и исследования стехиометрического соотношения компонентов в этом ионном ассоциате. Коэффициент распределения в системе вода – хлороформ определяли спектрофотометрически. Он составляет 3,44, что говорит о лучшей растворимости ассоциата в воде. Методом ВЭЖХ установлено стехиометрическое соотношение кармоизина и хлорфенирамина малеата в образованном ионном ассоциате, которое составляет 1:2.*