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DEVELOPMENT OF THE QUANTITATIVE DETERMINATION METHOD OF THE ACTIVE INGREDIENTS IN ALTABOR SUBSTANCE

T.V.Krutskykh, A.S.Shalamay*

National University of Pharmacy
53, Pushkinska str., Kharkiv, 61002, Ukraine. E-mail: tvkrut@ukr.net
* JSC SIC "Borshchahivka CPP", Kyiv

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The method of quantitative determination of active ingredients in Altabor substance based on absorption of the ellagitannin complex by ions of iron (II) has been developed. The linear characteristics of the method developed have been identified. The linear dependence of absorbance of the ellagitannin complex of Altabor with ions of iron (II) on the concentration of Altabor has been proven. The accuracy of ellagitannin determination in Altabor substance has been studied. It has been found that the method proposed is characterized by acceptable accuracy and can be used to quantify the active ingredients in Altabor substance.

РОЗРОБКА МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ ДІЮЧИХ РЕЧОВИН У СУБСТАНЦІЇ АЛЬТАБОР
Т.В.Крутських, А.С.Шаламай
Ключові слова: *альтабор; елаготаніни; субстанція; кількісне визначення*
Розроблена методика кількісного визначення діючих речовин у субстанції Альтабор, яка ґрунтується на поглинанні комплексу елаготанінів іонами заліза (II). Визначено лінійні характеристики розробленої методики. Доведена лінійність залежності оптичної густини комплексу елаготанінів Альтабору з іонами заліза (II) від концентрації альтабору. Досліджено точність визначення елаготанінів у субстанції Альтабор. Встановлено, що запропонована методика характеризується прийнятною точністю і може бути застосована для кількісного визначення діючих речовин у субстанції Альтабор.

РАЗРАБОТКА МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ДЕЙСТВУЮЩИХ ВЕЩЕСТВ В СУБСТАНЦИИ АЛЬТАБОР
Т.В.Крутских, А.С.Шаламай
Ключевые слова: *альтабор; эллаготанины; субстанция; количественное определение*
Разработана методика количественного определения действующих веществ в субстанции Альтабор, которая основывается на поглощении комплекса эллаготанинов ионами железа (II). Определены линейные характеристики разработанной методики. Доказана линейность зависимости оптической плотности комплекса эллаготанинов Альтабора с ионами железа (II) от концентрации альтабора. Исследована точность определения эллаготанинов в субстанции Альтабор. Установлено, что предложенная методика характеризуется приемлемой точностью и может быть использована для количественного определения действующих веществ в субстанции Альтабор.

At JSC SIC "Borshchahivka CPP" Altabor substance was obtained from the collective fruit of black alder (*Alnus glutinosa*) and grey alder (*Alnus incana*) by the original technology. It is a complex of ellagic tannins, which is characterized by the amount of the monomer, of dimeric and oligomeric substances based on hexaoxydiphenic and valoniac acid [1, 2, 3, 4]. Ellagic dimers of the substance are presented by substances of two types: dimers based on glucose and hexaoxydiphenic acids, including alnusaponin, and dimers based on one residue of hexaoxydiphenic acid and two monosaccharides, aldopentose – alnitannins. In addition to the tannin complex, Altabor contains phenolic acids, carbohydrates, water-soluble polysaccharides, xanthones, and free amino acids presented by alanine, arginine, aminobutyric and glutamic acid.

Altabor substance is not described in the SPhU and world Pharmacopoeias. The quality control of the raw material – the collective fruit of alder – is described by the State Pharmacopoeia of the USSR, XI-th ed. The previous literature review has shown that the methods for quantitative determination of tannins (ellagitannins, gallotannins) can be divided into several groups: gravimetric – based on the adsorption with hide powder or precipitation of tannins with gelatin, salts of heavy metals; titrimetric – based on redox reactions, primarily using potassium permanganate; photometric – based on the reactions with salts of iron (III) and heteropoly acids; combined – both approaches of two methods mentioned above are used [5, 6]. Quantitative determination of tannins is also proposed to perform by HPLC.

Experimental Part

Many studies contain the comparative analysis of methods for determining tannins in various objects of analysis that are used most commonly in standardization of the medicinal plant raw material and herbal medicines. After having analyzed the methods

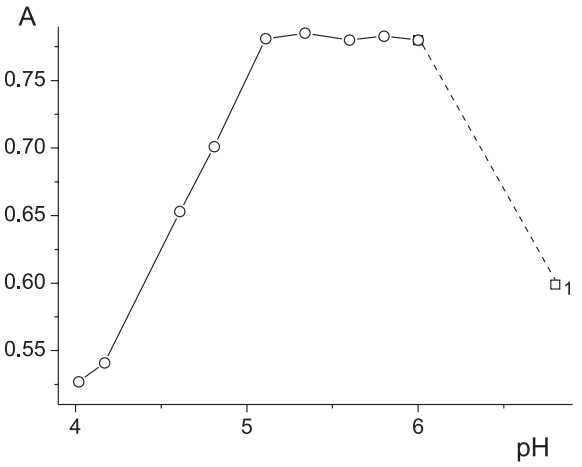

Fig. 1. The dependence of absorption of the ellagitannin complex of Altabor with ions of iron (II) on pH. The retention time is 120 min. λ = 550 nm. l = 10 mm. The reference solution is water.

of quantitative determination of tannins we make the following conclusions:

1. Titrimetric methods of analysis, including Leventhal method, do not provide selectivity and the correct assessment of the content of ellagitannins in the substance since it has been found that Altabor contains reducing sugars.

2. Photometric (based on redox reactions) and gravimetric methods do not provide acceptable selectivity.

3. A combined spectrophotometric method for determination of tannins prevails the methods mentioned above by selectivity. However, when working with a dosage form systematically underestimated results were obtained.

4. We had to refuse of using the known complexometric method for determining tannins. It has been found that in interaction of the substance with the precipitant a viscous resinous dark brown precipitate forms. The aggregate state of the precipitate does not allow to remove effectively the excess of zinc and provide the acceptable reproducibility of the results of determination.

During the research it was found that the ellagitannin complex of Altabor substance formed a precipitate in aqueous solutions not only with d-metal cations, and in the presence of cations of alkaline earth elements. However, it has been determined that in the presence of iron salts (II) in a weakly acidic and neutral medium the colour changes from yellow to blue with a slight reddish tint as a result of formation of the water soluble complex compound. The colour is associated with the change of the π-electron system of aromatic rings of derivatives of hexaoxydiphenic, valoniac and gallic acids due to the inductive effect caused by ionization of hydroxyl and carboxyl groups of the tannins hydrolyzed with iron ions (II) [7].

It was previously found that the colloidal chemical state of the complex formed depends not only on the pH of the solution, but on the composition of the buffer mixture as well. Thus, the use of acetate buffer prepared with ammonium acetate and acetic acid causes coagulation of the complex. At the same time in the presence of acetate buffer with sodium acetate and acetic acid absorption of the complex studied increases with increasing the pH of the solution, and at pH about 5.0 it reaches the plateau (Fig. 1). At pH over 6, a standard phosphate buffer with pH 6.8, which appeared be of little use, was used since colouring of the complex developed less compared to acetate buffer (point "1").

The intensity of the colour develops over time; therefore, to reduce the time of analysis, the reaction mixture is heated on a water bath or incubated at the temperature ranging from 77°C to 82°C for 40 min. The temperature control is very important since at 89°C coagulation of the complex of iron (II) with tannic acid occurs. At lower temperatures, for example at 70°C, the retention time should be increased because, otherwise, the overestimated results will be obtained.

When increasing the reaction temperature the colour intensity of the solution of the complex of iron (II) with tannin is slower compared to that for the substance under the same conditions. However, the colour intensity of the reference solution and test solution becomes even for 35 min, and then it is practically unchanged over time (Fig. 2).

It is well known that the molar and specific absorption rates depend on the composition of the complex compound, and when there is increase in the number of ligands, these values tend to increase too. Therefore, the effect of the amount of iron in determining the content of ellagitannins in the substance was studied. It has been found that the change in the volume of the reagent solution in the interval (50-150)% given in the project of Specifications does not affect accuracy and correctness of determining ellagitannins in the substance.

In the conditions proposed linearity of dependence of absorption of the ellagitannin complex with ions of iron (II) was studied with the change in the concentration of Altabor. Altabor substance of batches 30905 (Fig. 3-a) and 51005 (Fig. 3-b) obtained from the raw material of various suppliers was used in the experiment. Tab. 1 shows the linear characteristics of the method developed [8, 9, 10].

The acceptance criteria: The correlation coefficient (r)≥0.990. The value of the constant term (a) is insignificant at $a \leq \Delta_a$. When $a \geq \Delta_a$, it is significant. The systematic error is ≤1.0%.

High correlation coefficients (r) were obtained. Free member (a) in the case of Altabor substance of batch 30905 is significant, but the systematic error is 0.24%. Free Member (a) in the case of Altabor substance of batch 51005 is insignificant. Thus, the

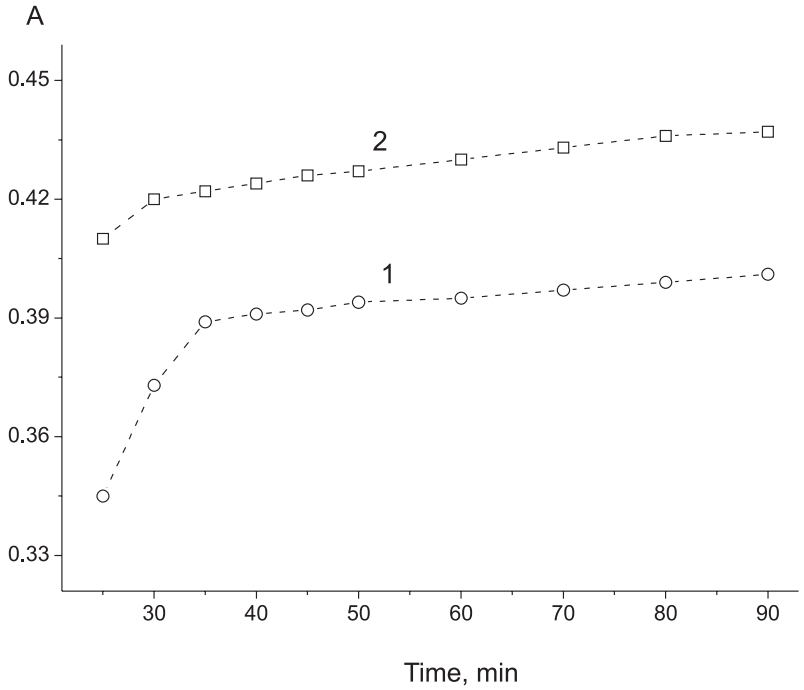

Fig. 2. The intensity of absorption of complexes of tannic acid (1) and ellagitannins of Altabor (2) with iron (II) with increasing the retention time at the temperature of 80°C.

Table 1

Characteristics of linearity of the method developed

	r	*a*	*b* (*a*≠0)	Δ*a*	Db	δ_a, %
c. 30905	0.999	0.0176	7.1970	0.0075	0.1362	0.24
c. 51005	0.999	-0.0020	7.2803	0.0033	0.0645	-

The limit of detection – 0.0028 mg/ml. The quantification limit – 0.0051 mg/ml.

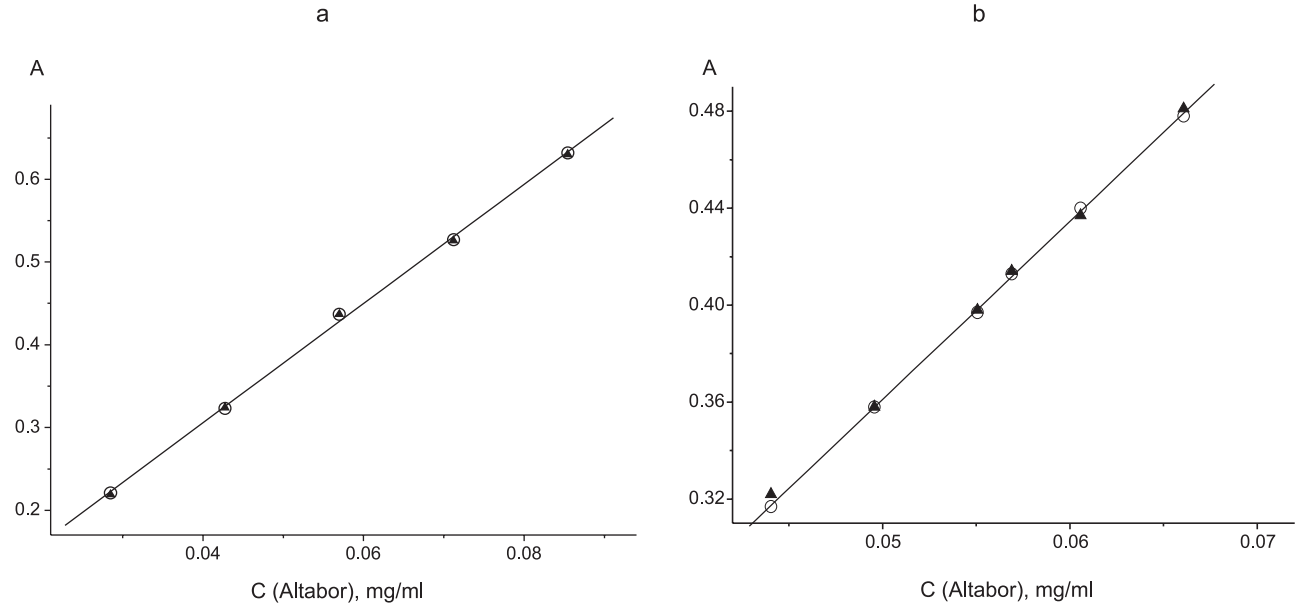

Fig. 3. Absorption of the complex of iron (II) with ellagitannins of Altabor of batches 30905 (a) and 51005 (b) in the medium of acetate buffer with pH 5.30 at 550 nm. Compensation solution – water.

linear dependence of absorbance of the complex of ellagitannins of Altabor with ions of iron (II) on the concentration of Altabor has been proven. The results of the study of accuracy of ellagitannin determination in Altabor substance calculated with reference to tannic acid are presented in Tab. 2.

As can be seen from the data, the method proposed is characterized by acceptable accuracy.

Thus, quantitative determination of the active ingredients in Altabor substance was performed by absorption spectrophotometry in the following way [11, 12]. The reagent solution and the buffer solution, the test solution and the reference solution were prepared; then their optical density was measured.

Reagent solution. Place 0.50 g of *iron (II) ammonium sulphate R* in a 50 ml volumetric flask, add 25 ml of 10 g/l of *potassium sodium tartrate R* and mix until complete dissolution of the sample. Dilute the volume with the same solvent to the mark and mix.

Buffer solution. Place 90 ml of 16.4 g/l *sodium acetate anhydrous R* in a 100 ml volumetric flask, dilute the solution to the volume with the solution of 12 g/l of *glacial acetic acid R* and mix. If necessary, adjust the pH of the buffer solution to 5.30±0.05 with 1 M *sodium hydroxide solution* and 12 g/l of *glacial acetic acid R.*

Test solution (a). Place approximately 50 mg (accurate weight) of the substance in a 100 ml volumetric flask and dissolve in 50 ml of *water R.* Dilute the volume with the same solvent to the mark and mix. Centrifuge the solution at speed of 8500 rpm for 15 min. For further research use the supernatant solution.

Test solution (b). Place 3.0 ml of the test solution (a) in a 25 ml volumetric flask, add 5.0 ml of the buffer solution and 1.0 ml of the reagent solution, dilute the solution to the volume with *water R* and mix. Close the flask with a stopper and heat on a water bath at a temperature of 77°C to 82°C for 40 min, cool under running cold water to the room temperature and mix.

Reference solution. Place approximately 50 mg of *tannic acid SS* dried to the constant weight at a temperature of 100°C to 105°C in a 200 ml volumetric flask, dissolve in 50 ml of *water R*. Dilute the volume with the same solvent to the mark and mix.

Place 3.0 ml of the solution obtained in a 25 ml volumetric flask, add 5.0 ml of the buffer solution and 1.0 ml of the reagent solution, dilute the solution to the volume with *water R* and mix. Close the flask with a stopper and heat on a water bath at a temperature of 77°C to 82°C for 40 min, cool under running cold water to the room temperature and mix.

Table 2

Statistical processing of the results of accuracy of determining ellagitannins

$\bar{X}$	n	P, %	t (95%)	RSD	$x_i \pm \Delta x_i$, %
59.87	6	95	2.571	0.57	1.46
59.94	6	95	2.571	0.76	1.95
The critical value				1.24	3.2

Measuring the optical density of the test solution (b) and the reference solution at 550 nm water *R* was used as a compensation solution.

The content of the total ellagitannins (X) in the substance was calculated with reference to tannic acid and a dried substance, in percent, by the formula:

$$X = \frac{A \cdot m_0 \cdot 3 \cdot P \cdot 100 \cdot 25 \cdot 100 \cdot 100}{A_0 \cdot 200 \cdot 25 \cdot 100 \cdot m \cdot 3 \cdot (100 - W)} = \frac{A \cdot m_0 \cdot P \cdot 50}{A_0 \cdot m \cdot (100 - W)},$$

where: A – is the optical density of the test solution (b); A_0 – is the optical density of the reference solution; m_0 – is the sample weight of tannic acid *SS*, mg; m – is the sample weight of the substance, mg; P – is the content of tannic acid specified in the certificate of tannic acid *SS*, %; W – is the weight loss on drying, %.

The content of ellagitannins calculated with reference to tannic acid and a dried substance must not be less than 45.0%.

Conclusions

1. The method of quantitative determination of active ingredients in Altabor substance based on absorption of the ellagitannin complex by ions of iron (II) has been developed.

2. The linear characteristics of the method developed have been identified. The linear dependence of absorbance of the ellagitannin complex of Altabor with ions of iron (II) on the concentration of Altabor has been proven.

3. The accuracy of ellagitannin determination in Altabor substance has been studied. It has been found that the method proposed is characterized by acceptable accuracy of the data.

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