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β-alanine spectrophotometric determination in reaction with sodium salt of 1,2-naphthoquinone-4-sulfonic acid

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Key words: *Spectrophotometry, Analysis, Beta-Alanine,* 1,*2-naphthoquinone-4-sulfonate, Validation Studies.*

Aim. The new spectrophotometric method for the quantitative determination of β-alanine in pharmaceutical formulations has been developed.

Methods and results. This method is based on the measurement of aqueous β-alanine solutions absorption at 470 nm. The proposed method is actual according to the validation requirements of Ukrainian Pharmacopeia. The analytical method was optimized and validated by establishing the linearity (the correlation coefficient r = 1,000), precision (*RSD%* = 0,806, n = 9) and the accuracy ($\bar{z}$ = 100,8 *%*).

Conclusion. According to the experimental data, the technique can be correctly reproduced and it is suitable for using in laboratories of the State Inspection for Quality Control of Medicines and QCD of the chemical-pharmaceutical enterprises.

Спектрофотометричне визначення β-аланіну за реакцією з натрієвою сіллю 1,2-нафтохінон-4-сульфокислоти

К. П. Портна, С. О. Васюк

З метою вдосконалення контролю якості фармацевтичних препаратів, що містять β-аланін, розробили нову спектрофотометричну методику кількісного визначення β-аланіну в лікарській формі, що ґрунтується на вимірюванні абсорбції водних розчинів β-аланіну за довжини хвилі 470 нм. Доведено відповідність розробленої методики вимогам Державної фармакопеї України за основними валідаційними характеристиками: лінійністю (коефіцієнт кореляції r = 1,000), прецизійністю (*RSD%* =0,806, n = 9), правильністю ($\bar{z}$ = 100,8 *%*), діапазоном застосування (62–138%), робасністю. Статистичні показники підтверджують високу точність і коректність запропонованої методики, а також можливість її застосування в лабораторіях із контролю якості лікарських засобів і відділах технічного контролю хіміко-фармацевтичних підприємств.

Ключові слова: *спектрофотометрія, кількісне визначення, β-аланін, натрієва сіль 1,2-нафтохінон-4-сульфокислоти, валідація.*

Запорізький медичний журнал. – 2015. – №1 (88). – С. 95–98

Спектрофотометрическое определение β-аланина по реакции с натриевой солью 1,2-нафтохинон-4-сульфокислоты

Е. П. Портная, С. А. Васюк

С целью усовершенствования контроля качества фармацевтических препаратов, содержащих β-аланин, разработана новая спектрофотометрическая методика количественного определения β-аланина в лекарственной форме, которая основана на измерении абсорбции водных растворов β-аланина при длине волны 470 нм. Доказано соответствие разработанной методики требованиям Государственной фармакопеи Украины согласно таким основным валидационным характеристикам: линейность (коэффициент корреляции r = 1,000), точность (*RSD%* = 0,806, n = 9), правильность ($\bar{z}$ = 100,8 *%*), диапазон применения (62–138%), робастность. Полученные статистические показатели свидетельствуют о высокой точности и корректности предложенной методики, а также возможности ее применения в лабораториях контроля качества лекарственных средств и отделах технического контроля химико-фармацевтических предприятий.

***Ключевые слова*:** *спектрофотометрия, количественное определение, β-аланин, натриевая соль 1,2-нафтохинон-4-сульфокислоты, валидация.*

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At the current stage of the development of pharmaceutical science and practice, the pharmaceutical market is continually expanding. Available techniques and express analysis methods serve as the main tool in the system of ensuring an appropriate level of drugs quality control. Thus, the British Pharmacopeia (BP) and the United States Pharmacopeia (USP) offer a titrimetric method of quantitative determination of alanine content in a substance, namely, acidimetric titration in non-aqueous medium (titrant – perchlorate acid $HClO_4$, medium – a mixture of anhydrous formiate acid HCOOH and anhydrous or glacial acetate acid CH_3COOH in the ratio 1:10), with indicating (naphtholbenzein solution) or potentiometric fixation of the titration end point [1,2]. In spite of high accuracy of the titrimetric analytical methods for determination of drug substances in pharmaceutical compositions, the use of instrumental methods of analysis is more common due to their higher sensitivity. Herewith the chromatographic [3–5] and optical [6,7] methods of analysis of β-alanine are described.

Some of the offered methods require usage of expensive equipment, unobtainable reagents; some are hard in implementing, or are offered only for active pharmaceutical ingredients. Therefore, the expediency of development of new simple valid methods of quantitative determination of β-alanine specifically in pharmaceutical formulations is unquestionable.

The aim of the research

Development and validation of convenient, cost-efficient, sensitive method of quantitative determination of β-alanine in the pharmaceutical formulations through reaction with the sodium salt of 1,2-naphthoquinone-4-sulfonic acid and establishment of stoichiometric proportions of the components of the reaction mixture «β-alanine-reagent».

Materials and methodology

Objects of study, reconstitution solutions and equipment

Study subject – medication «Abufen» (Laboratories *Bouchara Recordati s.a.s, France*), series 13080.

As reagents and solution, sodium salt of 1,2-naphthoquinone-4-sulfonic acid with purity qualification CP (chemically pure), 0,01 M solution of NaOH and purified water were used. As a standard, a working reference sample of β-alanine was used.

Analytical equipment used: spectrophotometer Specord 200, electronic scales ABT-120-5DM, volumetric glass ware of class A.

General method of the quantitative determination of β-alanine content

The aliquot part (0,00065 g) of the aqueous solution of β-alanine is poured into a measuring flask of 25,00 ml volume, treated with 1,00 ml of 1,0% aqueous solution of sodium salt of 1,2-naphthoquinone-4-sulfonic acid, with 1,50 ml of 0,01 M solution NaOH, and mixed up. A received solution is heated up for 10 minutes on the water-bath under temperature of 60°C, then is cooled down and filled to the mark with the purified water. Absorbance of the test solution is measured at 470 nm against the background of the compensation solution that does not contain any test solution.

Determination of β-alanine in pharmaceutical composition

At determining of the β-alanine in pharmaceutical formulation «Abufen», the exact weighted amount of a tablet mass (about 0,0150 g) is put into a 25,00 ml volumetric flask and filled to the mark with water. The amount of 1,00 ml of the resulted solution is poured into a 25,00 ml volumetric flask, then treated with 1,00 ml of 1,0% aqueous solution of the sodium salt of 1,2-naphthoquinone-4-sulfonic acid, 1,50 ml of 0,01 M solution of NaOH, and mixed up. The received reaction mixture is heated up on the water bath at 60°C for 10 minutes, then cooled down and filled to the mark with the purified water. In parallel, the experiment with a working reference solution is carried out.

The calculation of the content of the active agent is performed according to the typical formula:

$$\chi = \frac{A \cdot \rho_{gen}}{A_0 \cdot \rho \cdot l \cdot 100} \cdot k \qquad (1.1)$$

where A – absorbance of the testing solution;

p_{gen} – tablet mass average, g;

A_0 – absorbance of the reference solution;

p – weighted amount of the pharmaceutical formulation, g;

l – layer thickness, cm;

k – nominal ratio based on dilutions and the reference solution concentration.

Results and discussion

For the development of a method of quantitative determination of β-alanine based upon its reaction with the sodium salt of 1,2-naphthoquinone-4-sulfonic acid, there have been studied the factors affecting the character of the absorbance spectrum and the value of absorbance, in particular – the nature of the solution medium, the amount of added reagents, pH of the reaction mixture, conduction period of the reaction and the reaction products resistance in time. When choosing a solution medium, a solubility of the testing substances, reagents and the maximum value of absorbance of the resulted solution were taken into account.

It was experimentally established that the reagent interacts with β-alanine in the aqueous medium to form a colored composition with maximum light absorbance at 470 nm (*fig. 1*). Given that, the optimum amount of 1,0% reagent needed to form the reaction resultant with maximum absorbance is 1,00 ml.

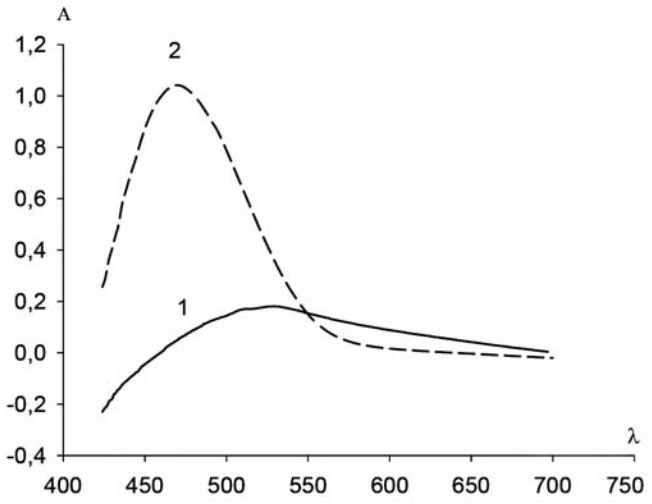

Fig. 1. Absorption spectra of: 1 – aqueous solution of the 1,2-naphthoquinone-4-sulfonate, 2 – reaction resultant of β-alanine with 1,2-naphthoquinone-4-sulfonate.

For more complete conduction period of reaction it is necessary to prepare an alkaline medium and heat it on the water bath. It was experimentally established that the maximum absorbance was reached with adding of 1,50 ml of 0,01 M NaOH solution and heating the reaction mixture for 10 min at 60°C.

Target values of the detection threshold and molar absorption coefficient are indicative of a high sensitivity of the reaction (*table 1*).

Table 1

Optical specifications and basic parameters of linear dependency of 1,2-naphthoquinone-4-sulfonate and β-alanine reaction

Molar absorbance rate ε	3330,3
Sendel factor W_S	0,0267
Detection threshold C_{min} (mcg/ml)	1,33
Equation of linear regression	Y = bX + a
Slope coefficient b±(s_b)	0,3377±(0,0018)
Intercept term of a linear regression a±(s_a)	0,0109±(0,0048)
Residual standard deviation $S_{x.0}$%	0,170
Correlation coefficient r	1,000

Establishment of the stoichiometric ratios of components of the reaction mixture «β-alanine – sodium salt of 1,2-naphthoquinone-4-sulfonic acid» was performed by means of the most common methods: by isomolar series and the molar-ratio method (saturation method) [8].

Isomolar series method is based on identifying of correlation between isomolar concentrations of the reacting agents that correspond to the maximum output of the compounds developed. Given that, the resultant output-solution composition curve is characterized by the turning point, which location is associated with stoichiometric factors *m* and *n* of the reaction resultant M_mR_n *(fig. 2)*.

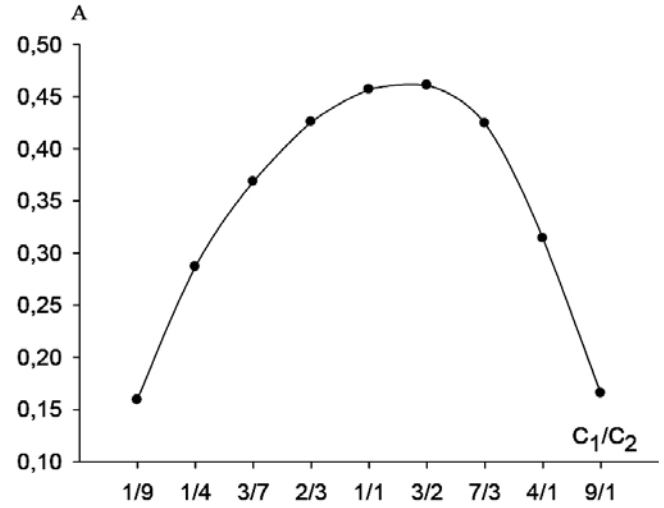

Fig. 2. Dependence diagram of the absorbance from the composition of the isomolar solution (C_1 – 0,007 M solution of β-alanine, C_2 – 0,007 M solution of 1,2-naphthoquinone-4-sulfonate).

The essence of the molar-ratio method lies in establishing of the dependency relation of the absorbance value and the concentration of one of the components with the constant concentration of the other component and vice versa. The correlation is established with the help of the transverse line lowered from the intersection of two curves *(fig. 3)*.

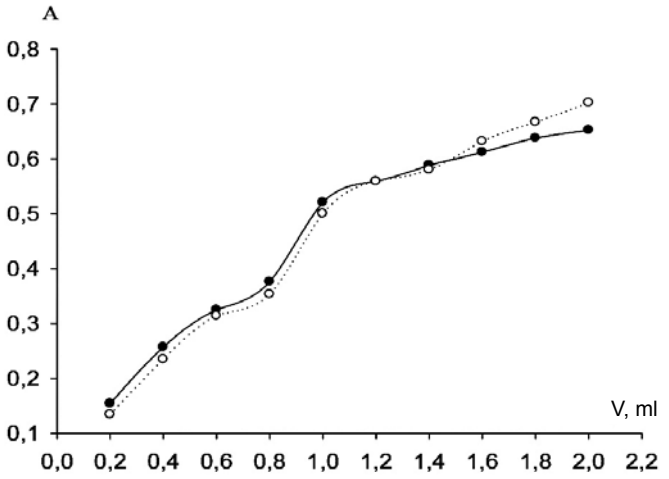

Fig. 3. Saturation curves: 1 – β-alanine at constant concentration of the reagent (1,00 ml 0,007 M solution); 2 – reagent at constant concentration of β-alanine solution (1,00 ml 0,007 M solution).

Stoichiometric ratios of the reactants of «1,2-naphthoquinone-4-sulfonate-β-alanine» obtained by the method of isomolar series and saturation method definitely agree with each other and make up 1:1, accordingly.

Determination of the main validation characteristics

Linearity was determined within the range of concentrations, in which a subjection to the Beer's law was observed. The solutions with the established concentration obtained by diluting of the reference solution of β-alanine were determined by the introduced general procedure. On the basis of the obtained data, a dependence diagram of absorbance and concentration of a testing substance was drawn (*fig. 4*), and the parameters of linear dependency were calculated (*table 1*).

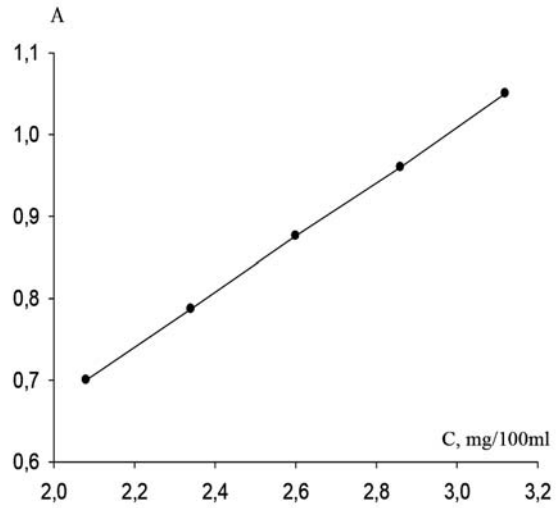

Fig. 4. Absorbance – β-alanine concentration dependency diagram.

The numeric indicators of linear dependency, obtained in line with regulations of SPU (State Pharmacopoeia of Ukraine), showed that all requirements as to the parameters to linear dependency have been met; thereby the technique linearity can be confirmed across the range of the selected concentrations [9].

According to SPU, the procedure used is *accurate on the level of convergence*, in case one-sided confidence interval (Δ_{xr}) does not exceed a maximum permissible uncertainty of analysis (Δ_{As}%). Basing on the data presented in the *table 2*, the technique is regarded accurate.

Table 2

Determination of the accuracy of the quantitation of β-alanine in tablets «Abufen»

Metrological characteristics					
$\bar{\chi}$	*S*	*RSD, %*	Δ_{xr}	$\Delta_{\bar{x}r}$	Δ_{As}%
0,402	$3{,}24 \cdot 10^{-3}$	0,806	1,49	0,499	3,20

Accuracy of the results was established by the addition method. In regard to the condition $|\bar{Z} - 100| \leq \Delta_{\bar{z}}$, the difference $|\bar{Z} - 100|$ is statistically significant, in this case SPU recommends to use a criterion of practical insignificance of the obtained systematic inaccuracy as regards to a maximum permissible uncertainty of analysis. The procedure is considered correct, as the condition $| \bar{Z} - 100 | \leq 0{,}32 \cdot \Delta_{As}$% is met [9]. The obtained data are given in *Table 3*.

Table 3

Determination of the validity of the quantitation of β-alanine in tablets «Abufen»

Metrological characteristics					
$\bar{Z}$ *(n=9), %*	*RSD, %*	$\lvert\bar{Z} - 100\rvert$	Δ_z	$\Delta_{\bar{z}}$	Δ_{As}%
100,8	0,605	0,800	1,12	0,375	3,20

The application range of the analytical procedure is the interval between the minimum and maximum concentrations of the testing substance, for which it was shown that the procedure exhibits a required linearity, validity and accuracy. Based on

the results of the undertaken study, the application range for the developed methodology is 62–138 % and ranges within the performance period for the procedures of quantitative determination according to SPU requirements (80–120%) [9].

Robustness assessment was carried out at the stage of technique development, as well as there were determined the stability of testing solutions in time and the influence of the quantity of the added reagents on the results of the determination. It was established that the test colored solutions are stable for no less than 60 min, and variations in the quantity of added reagent (a solution of the sodium salt of 1,2-naphthoquinone-4-sulfonic acid) within ± 10–20% do not affect the value of the absorbance.

Conclusions

1. There has been developed a highly sensitive, cost-effective and convenient spectrophotometric method of quantitative determination of β-alanine content in the pharmaceutical formulation.
2. By means of the method of isomolar series and saturation method, there have been established stoichiometric ratios of the reaction mixture components of «β-alanine-the sodium salt of 1,2-naphthoquinone-4-sulfonic acid», which made up 1 : 1.
3. It has been proved that the developed technique of the quantitative determination based on such characteristics as linearity, accuracy, validity, application range and robustness, is consistent and features easiness in implementation and availability; hence, it can be used in Quality Control procedures for medicines.

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