Development of a spectrophotometric technique for the quantitative determination of ademol

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

According to statistics, craniocerebral injuries are often the cause of disability among the population. The choice of medicines for traumatic brain injuries is one of the most difficult problems in the complex treatment of such patients. Ademol (1-adamantylethyloxy-3-morpholino-2-propanol hydrochloride) is a promising medicinal substance that has a huge positive therapeutic effect of a protective act on the damaged brain and can be presented in medicinal preparations in different dosages. Therefore, the urgent task is the development of highly accurate, reliable, affordable, and fast methods of quantitative determination of ademol.

The aim of the work is to study the optimal conditions for the "ademol – bromocresol green" reaction and to develop an express, sensitive and easy-to-implement method for the quantitative determination of ademol.

Materials and methods. Bromocresol green (BCG) grade "chemically pure" and acetone grade "pure for analysis" were used as reagents and solvents. Analytical equipment: spectrophotometer Specord 200, electronic scales AVT-120-5DM, ultrasonic bath Elmasonic E 60H, class A measuring vessels.

Results. A new method of quantitative determination of ademol by the spectrophotometric method was developed. The optimal conditions of the flow between the substance to be determined and the reagent have been studied, the concentration limits have been established, in which subordination to the basic law of light absorption is observed. A forecast of the complete uncertainty of the results of the specified method of quantitative determination was made to assess the correctness of the reproduction of the developed method in other laboratories. The proposed method is relevant according to the requirements of the State Pharmacopoeia of Ukraine.

Conclusions. According to the experimental data, the method of quantitative determination of ademol can be correctly reproduced and suitable for use.

Key words: spectrophotometry, analysis, ademol, bromocresol green, State Pharmacopoeia of Ukraine, validation.

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Розробка спектрофотометричної методики кількісного визначення адемолу

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За статистикою, черепно-мозкові травми часто стають причиною інвалідності серед населення. Вибір лікарських засобів при травматичних ушкодженнях головного мозку – одна з найскладніших проблем у комплексі лікування таких хворих. Адемол (1-адамантилетилокси-3-морфоліно-2-пропанол гідрохлорид) – перспективна лікарська речовина, що має позитивний терапевтичний ефект захисної дії на мозок при його ураженні, може міститися в лікарських препаратах різного дозування. Тому актуальним завданням є розроблення точної, достовірної, доступної та швидкої методики кількісного визначення адемолу.

Мета роботи – вивчення оптимальних умов перебігу реакції «адемол – бромкрезоловий зелений» і розроблення експресної, чутливої та простої для виконання методики кількісного визначення адемолу.

Матеріали та методи. Як реактив і розчинник використовували бромкрезоловий зелений (БКЗ) кваліфікації «х. ч.», ацетон кваліфікації «ч. д. а.». Аналітичне обладнання: спектрофотометр Specord 200, ваги електронні АВТ-120-5DM, ультразвукова баня Elmasonic E 60H, мірний посуд класу А.

Результати. Розробили нову методику кількісного визначення адемолу спектрофотометричним методом. Вивчили оптимальні умови перебігу реакції між речовиною, що визначали, та реагентом, а також встановили межі концентрацій, у яких спостерігали підпорядкованість основному закону світлопоглинання. Здійснили прогноз повної невизначеності результатів цього методу кількісного визначення для оцінювання коректності відтворення розробленої методики в інших лабораторіях. Запропонована методика є актуальною за вимогами Державної фармакопеї України.

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Висновки. Згідно з експериментальними даними, методика кількісного визначення адемолу може бути коректно відтворена та придатна для використання.

Ключові слова: спектрофотометрія, аналіз, адемол, бромкрезоловий зелений, Державна фармакопея України, валідація.

Актуальні питання фармацевтичної і медичної науки та практики. 2023. Т. 16, № 1(41). С. 28–32

Traumatic brain injury (TBI) in practical health care today is called a "silent epidemic" due to the growing scale of this problem, low awareness of its significance, and incomplete epidemiological data [1]. Today, the main component of intensive therapy for TBI is a therapy that includes a number of measures aimed at preventing the development and correcting the manifestations of homeostasis disturbances that accompany traumatic brain damage. According to modern ideas, appropriate protection of the brain against the background of traumatic damage can be provided by cerebroprotectors [2,3]. The search for new substances, which could become the basis for the creation of a new domestic cerebroprotective agent, is an urgent task of pharmacology and does not raise doubts about its expediency [4].

The positive therapeutic effect that was obtained against the background of experimental TBI from the therapy with ademol – 1-adamantylethyloxy-3-morpholino-2-propanol hydrochloride (adamantane derivative), an in-depth study of additional influencing factors [5] and the prospects of using ademol as a neuroprotective agent [6], necessitates the development of quality control methods for this medicinal substance.

The use of modern physical-chemical methods of analysis allows to ensure of the appropriate level of quality control of medicinal products at all stages of production – from the substance to the finished product. Therefore, for modern pharmaceutical analysis, an urgent goal is the development of highly accurate, accessible, and express methods of quantitative determination of medicinal substances, both in the substance and in the composition of medicinal preparations.

At the moment, no information has been found regarding the availability of an express, sensitive and selective method for the quantitative determination of ademol. The described HPLC method for the quantitative determination of memantadine hydrochloride (another adamantane derivative) in tablets is characterized by high sensitivity, but at the same time, it is difficult to perform and requires expensive equipment [7]. Existing spectrophotometric methods for the quantitative determination of amantadine hydrochloride (adamantane derivative) [8–10] have inherent availability and selectivity, but some of them are time-consuming (for example, extractive techniques) and require additional sample preparation steps, while others use unavailable reagents.

Therefore, the expediency of developing new, simple, express methods for the quantitative determination of ademol as a promising neuroprotective agent is beyond doubt.

Aim

The aim of the work is to develop a convenient, economical, sensitive method of quantitative determination of ademol by reaction with bromocresol green by the spectrophotometric method.

Materials and methods

Research objects, solvents, and equipment. The object of the study is a working standard sample of ademol (PubChem CID – 2769721; UA/4845/01/01). As reagents and solvents, we used bromocresol green of the "chemically pure" qualification and acetone of the "pure for analysis" qualification. Analytical equipment: spectrophotometer Specord 200, electronic scales ABT-120-5DM, class A measuring vessels.

Determination was developed on the basis of the Department of Analytical Chemistry, Zaporizhzhia State Medical University.

The general method of quantitative determination of ademol. A precise measure of ademol (0.03300 g) is placed in a measuring flask with a capacity of 100.0 ml, dissolved in 4.00 ml of purified water, and brought up to the mark with acetone, mixed. 1.00 ml of the resulting solution is placed in a volumetric flask with a capacity of 10.00 ml, treated with 1.50 ml of a 1.00 % acetone solution of bromocresol green, and brought up to the mark with acetone. The absorbance of the test solution is measured against the background of the compensating solution, which does not contain the test substance, at 410 nm.

Results

Many factors influence the results of analytical reactions. Therefore, it is possible to objectively choose the optimal conditions for quantitative spectrophotometric analysis only after conducting preliminary studies. To develop a methodology for the quantitative determination of ademol based on the reaction with sulfonphthalein dye, the following factors that can affect the speed and completeness of the reaction were studied, namely, the composition of the solvent, the number of added reagents, the stability of the studied solutions over time.

When choosing a solvent, the solubility of the investigated substance and reagent, as well as the maximum value of the optical density of the obtained solutions, were taken into account. Analyzing data from the literature [11], acetone was chosen as the optimal solvent, in the presence of a small amount of purified water (≤4 %), necessary for dissolving the amount of ademol.

In order to choose the optimal reagent for the development of a method for the quantitative determination of the studied medicinal substance, the spectra of interaction products with the most common sulfonphthalein dyes were compared. The main criterion for choosing a reagent was the maximum value of the optical density of the reaction product. Therefore, a 0.030 % water-acetone solution of ademol and 1.00 % solutions of sulfonphthalein dyes (bromocresol purple (BCP), bromothymol blue (BTB) and bromocresol green) in acetone were prepared. Next, 1.00 ml of the investigated solutions

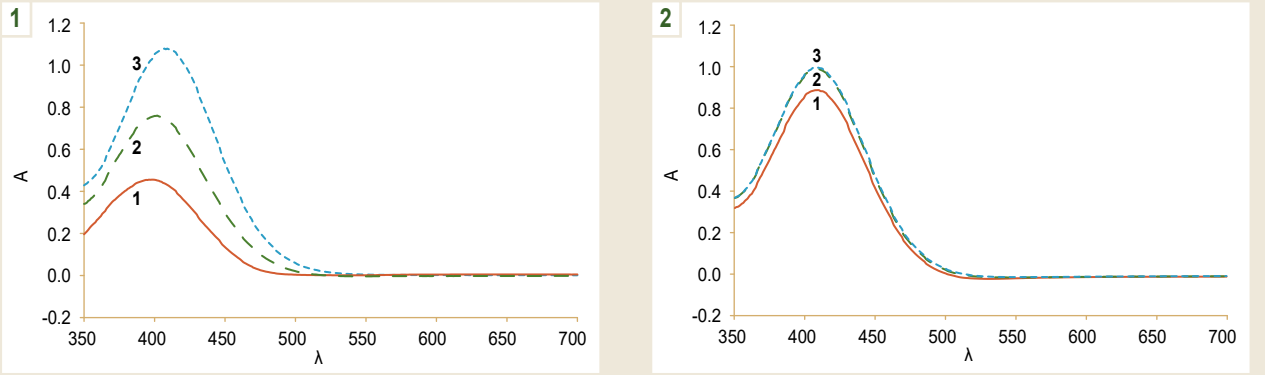

Fig. 1. Absorption spectra of the reaction products of ademol with BTB (1), BCP (2), BCG (3).

Fig. 2. The graph of the dependence of the absorption of the products of the ademol reaction on the amount of added 1.0 % BCG solution (1 – 1.00 ml; 2 – 1.50 ml; 3 – 2.00 ml).

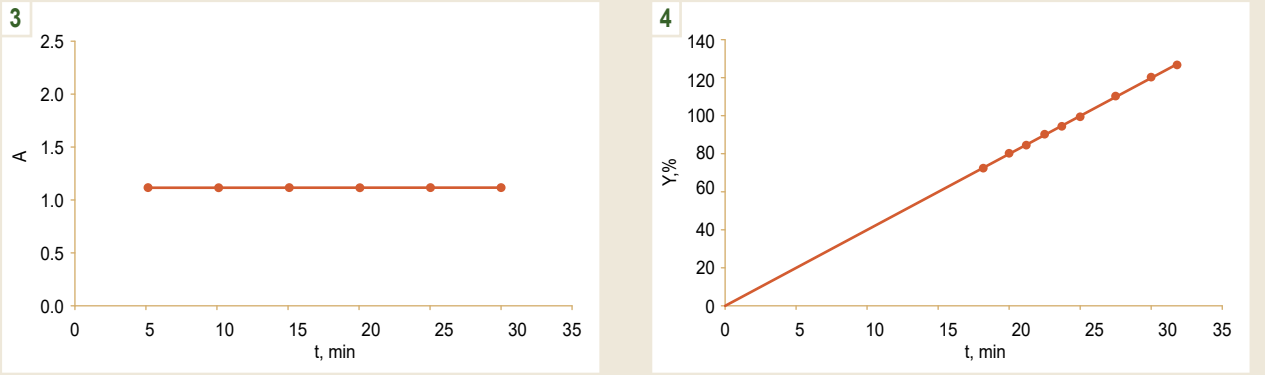

Fig. 3. Graph of dependence of the absorption of reaction products of ademol with BCG on time.

Fig. 4. Graph of the dependence of the optical density on the concentration of ademol.

were placed in 10.00 ml volumetric flasks, 1.00 ml of reagent was added, and acetone was brought up to the mark. The absorption of the obtained solutions was measured against the background of compensation solutions that did not contain the substance under study. According to *Fig. 1*, BCG showed the greatest activity in the reaction with ademol, therefore, for the further development of quantitative determination methods, we focused on this reagent.

The next stage of the study was to determine the amount of reagent required for the complete course of the reaction. It was established experimentally, based on the maximum yield of the reaction product, that is, on the basis of the maximum value of the optical density. For this, 1.00 ml of 0.030 % water-acetone solution of ademol and 1.00 ml were added to 10.00 ml volumetric flasks. 1.50 and 2.00 ml of 1.00 % acetone solution of BCG. The absorbance of the studied solutions was measured against the background of compensating solutions at 410 nm. Based on the obtained data, a graph of the dependence of the absorption value on the amount of the added reagent was plotted (*Fig. 2*).

As can be seen from the graphical dependence, the optical density of the investigated solutions increases from 1.00 to 1.50 ml, but when the volume of the added reagent increases from 1.50 to 2.00 ml, the optical density of the obtained solutions does not increase at all. Therefore, a reagent volume of 1.50 ml was chosen for the further development of the method of quantitative determination of ademol.

At the next stage of the research, the stability of the analyzed solutions over time was investigated. To do this, the optical density of the obtained solutions was measured under optimal conditions for 30 min with an interval of 5 min. It was established that the studied solutions are stable for at least 30 minutes (*Fig. 3*).

Linearity and range of application of the technique. Linearity was determined within the limits of concentrations in which obedience to Beer's law is observed, namely 0.0024–0.0042 g/100 ml. Solutions with a known concentration were obtained by diluting a standard 0.033 % ademol solution to 0.70 ml, 0.80 ml, 0.85 ml, 0.90 ml, 0.95 ml, 1.00 ml, 1.10 ml, 1.20 ml, 1.30 ml were placed in flasks with a capacity of 10.00 ml, treated with 1.50 ml of a 1.00 % acetone solution of bromocresol green, brought up to the mark with acetone and determined according to the above general method [12]. Based on the obtained data, a graph of the dependence of absorption on the concentration of the substance under study was constructed (*Fig. 4*).

Parameters of linear dependence were calculated using least squares regression analysis [13].

Table 1. Parameters of linear dependence

Parameters	Value
Regression equation	$A = b \cdot C + a$
Angular coefficient *b*	1.0013
Free member *a*	0.2218
s_b	0.7711
s_a	0.0078
Correlation coefficient *r* (*n* = 9)	0.9998
$s_{x,0}$ (%)	0.4032

Table 2. Prediction of the uncertainty of the method sample preparation

Sample preparation operation	Calculation formula parameter	Uncertainty, %
The investigated solution		
1) Taking the weight	m_0	0.20 mg/33 mg·100 % = 0.60 %
2) Bring to volume in a 100 ml volumetric flask	100	0.12 %
3) Taking a 1 ml aliquot with a pipette	1	0.74 %
4) Bring to volume in a 10 ml volumetric flask	10	0.50 %
$\Delta_{SP} = \sqrt{(0.60^2+0.12^2+0.74^2+0.50^2)} = 1.08$ %		

Found: $y_i = 1.0013\ x_i + 0.2218$. Obtained values: coefficients b, a, standard deviations for b and a– s_b, s_a, the residual standard deviation $s_{x,0}$(%) and the correlation coefficient *r* are given in the *Table 1*.

The closer the absolute value |r| to unity, the less random the observed linear relationship between *x* and *y* values. According to literature data and previously performed calculations, the correlation coefficient for this linear dependence must meet the criterion and be ≥0.9784.

The statistical quality of the obtained model is characterized by the residual standard deviation s_y, which has the dimension of the signal, or the residual standard deviation along the abscissa $s_{x,0}$, which has the same dimension as the substance content. According to the SPU, $s_{x,0}$(%) should not exceed 0.7917 ($\Delta As/t(95;n\text{-}2)$), which also meets the proposed criteria.

Thus, based on the obtained data, the linearity of the method is confirmed in the entire specified concentration interval, and the range of application of the method is 70–130 % of the nominal concentration of ademol.

Complete uncertainty of the analytical technique. Usually, the development of the technique is carried out in one laboratory, the level of equipment of which may be significantly higher than the permissible SPU [13], therefore, when reproducing this technique in another laboratory, the technique will give incorrect results. To exclude this, a forecast of the full uncertainty of the analysis results must be made, which should not exceed the maximum permissible uncertainty of the analysis results maxΔAs [14].

The formula for calculating the total uncertainty forecast is given below.

$$\Delta_{As} = \sqrt{(\Delta_{SP}^2 + \Delta_{FAO}^2)},$$

where Δ_{SP} – the uncertainty of the sample preparation method;

Δ_{FAO} – predicted uncertainty of the final analytical operation (0.70 % for spectrophotometry in the visible region of the spectrum).

The prediction of the uncertainty of sample preparation (Δ_{SP}) of the determination of the ademol content is given in the *Table 2*.

Having analyzed the *Table 2*, it can be seen that the most important uncertainty in sample preparation is introduced by operations 1 – weighing the sample to prepare the analyzed solution, 3 – taking an aliquot with pipettes of 1.00 ml, as well as 4 – bringing it to volume in volumetric flasks at 10.00 ml.

This distribution of the uncertainty of sample preparation is quite typical and acceptable for the quantitative determination of medicinal substances.

$$\Delta_{As} = \sqrt{(\Delta_{SP}^2 + \Delta_{FAO}^2)} = \sqrt{(1.08^2 + 0.70^2)} = 1.28\ \%.$$

Therefore, the predicted total uncertainty of the results of the analysis of the quantitative determination of ademol in the substance (1.28 %) does not exceed the maximum permissible uncertainty of the methodology for substances (1.50 %) and meets the requirements of the SPU.

Discussion

Summarizing the literature data reviews, no information has been found regarding the availability of any methods for the quantitative determination of ademol, and the presented spectrophotometric methods for determination another adamantane derivatives in UV and visible spectrum usually require increasing selectivity [8] or based on reactions which require special conditions and additional procedures such as extraction [9], pH control, etc.

The accuracy and selectivity of chromatographic methods [7] are not in doubt, but the sustained sample preparation stage, high cost of equipment, and consumables reduce their availability.

Therefore, the development of direct, non-extractive, selective, and sensitive spectrophotometric methods for the quantitative determination of ademol is certainly a promising area for improving the quality control of pharmaceuticals.

The actuality of developed new, simple, express method for the quantitative determination of ademol as a promising neuroprotective agent is beyond doubt.

The use of aqueous, aqueous-acetone solutions, in terms of "green chemistry", is one more advantage of the developed method, more rational than using only organic solvents, taking into account the solubility of the interacting products.

And of course, it is appropriate to implement the research results in practical pharmacy, analysis of future dosage forms with ademol.

Conclusions

1. A sensitive, economical, and rapid spectrophotometric method for the quantitative determination of ademol by reaction with bromocresol green was developed, the optimal conditions for the course of the interaction were investigated, the limits of concentrations in which obedience to Beer's law observed were determined, the parameters of linear dependence were calculated according to the requirements of the SPU.

2. A forecast of the complete uncertainty of the results of the specified method of quantitative determination was made to assess the correctness of the reproduction of the developed method in other laboratories.

Conflicts of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

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