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-adamantylethoxy-3-morpholinol-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.
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-adamantylethoxy-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 memantine 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.
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 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].
Usually, the analysis results max should not exceed the maximum permissible uncertainty of the analysis results, which may give incorrect results. To exclude this, a forecast of the full uncertainty of the analytical technique is carried out in one laboratory, the technique will be reproducing this technique in another laboratory, the technique must meet the criterion and be ≥0.9784.

The statistical quality of the obtained model is characterized by the residual standard deviation $s_x$, 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 ($\Delta As(\tau(95;m-2))$, 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 max$\Delta As$ [14].

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

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

where $\Delta SP$ – the uncertainty of the sample preparation method;

$\Delta FAO$ – 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 ($\Delta_{SP}$) 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.

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**Table 1. Parameters of linear dependence**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation</td>
<td>$A = b \cdot C + a$</td>
</tr>
<tr>
<td>Angular coefficient $b$</td>
<td>1.0013</td>
</tr>
<tr>
<td>Free member $a$</td>
<td>0.2218</td>
</tr>
<tr>
<td>$s_a$</td>
<td>0.7711</td>
</tr>
<tr>
<td>$s_b$</td>
<td>0.0078</td>
</tr>
<tr>
<td>Correlation coefficient $r$ (n = 9)</td>
<td>0.9998</td>
</tr>
<tr>
<td>$s_r(%)$</td>
<td>0.4032</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample preparation operation</th>
<th>Calculation formula parameter</th>
<th>Uncertainty, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>The investigated solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1) Taking the weight</td>
<td>$m_0$</td>
<td>0.20 mg/33 mg; 100 % = 0.60 %</td>
</tr>
<tr>
<td>2) Bring to volume in a 100 ml volumetric flask</td>
<td>100</td>
<td>0.12 %</td>
</tr>
<tr>
<td>3) Taking a 1 ml aliquot with a pipette</td>
<td>1</td>
<td>0.74 %</td>
</tr>
<tr>
<td>4) Bring to volume in a 10 ml volumetric flask</td>
<td>10</td>
<td>0.50 %</td>
</tr>
</tbody>
</table>

$\Delta_{SP} = \sqrt{(0.60^2+0.12^2+0.74^2+0.50^2)} = 1.08 \%$
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.

References


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