QUANTIFICATION OF ALBENDAZOLE IN TABLETS OF COMBINED COMPOSITION

K. Semchenko, L. Vyshnevska

The aim. *Treatment of helminthiases of the digestive system remains an actual task of the medicine and pharmacy worldwide. So, to meet the needs of the Ukrainian pharmaceutical market tablets based on albendazole and praziquantel were proposed. In order to establish the further production of the drug, studies were conducted on the development and validation of a method for the quantitative determination of one of the active substances – albendazole, in the presence of the second active substance praziquantel.*

Materials and methods. *The object of the study was samples of tablets under the conditional name "AP-helmin". Albendazole was extracted from tablets with ethyl alcohol when heated, 0.1 M sodium hydroxide solution was added and the obtained sample was evaluated spectrophotometrically in the ultraviolet light (308 nm). The method was conducted meeting the requirements of State Pharmacopoeia of Ukraine, harmonized with the relevant European Pharmacopoeia techniques.*

Results. *The samples comply with the Bouguer-Lambert-Behr law in the concentration range $1×10^{-3}$ – $1.2×10^{-2}$ mg/ml, the correlation coefficient is ≥ 0.9998. The studied method of quantitative determination of albendazole in the drug meets the criteria of acceptability for the range of determination ±5.0 % by validation characteristics: specificity, linearity, precision, accuracy.*

The criterion of insignificance of the systematic error of the method is fulfilled – the systematic error of the method (0.24) is practically insignificant, that is, the method of analysis is characterized by sufficient correctness in the whole concentration range from 80 to 120 %. All the validation characteristics respond to the acceptance criteria.

Conclusions. *A method for the quantitative determination of albendazole in tablets "AP-helmin" by spectrophotometry in ultraviolet light (308 nm) was worked out. It was experimentally confirmed that the second active pharmaceutical ingredient (praziquantel) had no affect the accuracy of the results obtained*

Keywords: *albendazole, tablets, quantitative analysis, praziquantel, spectrophotometry, UV, validation, anthelmintic drugs*

1. Introduction

The diversity of biological species on Earth ensures their constant interaction with each other: useful (symbiosis, reciprocity, commensalism), neutral or harmful (competition, amensalism, allelopathy, predation, parasitism). Among the above types of interaction, parasitism is of particular interest to medicine, including the negative impact on the human body of protozoa, helminths, arthropods, etc., which leads to a significant reduction in the quality of life of patients and the development of reversible or irreversible consequences [1].

Parasitic infections are one of the most acute and urgent problems of society. According to the World Health Organization, about 1.2 billion people are infected with parasitic infections each year.

Digestive helminthiasis are of particular interest, as the most common group of parasitic diseases in most countries with non-tropical climates including Ukraine (currently, there are known about 70 species of helminths in Ukraine out of more than 250) [2].

As the pharmaceutical market in Ukraine does not currently meet the needs of patients to the full, we offered a drug in the form of tablets of combined composition with a proven broad spectrum of anthelminthic activity against the helminthiasis of the digestive system [3–5].

The active pharmaceutical ingredients of the drug are the substances albendazole (methyl [5-(propylthio)-1H-benzoimidazol-2-yl]carbamate) and praziquantel ((RS)-2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one) as the most perspective for the treatment of intestinal helminthiasis [6].

As one of the stages of pharmaceutical development of a medicinal product for its further introduction into industrial production is the development of validated quality control techniques, the first step was a study to quantify active pharmaceutical ingredients.

In the literature for the quantitative determination of albendazole in mono-component tablets is conducted by the method of absorption spectrophotometry in the ultraviolet spectrum in an environment of methanol acidified with hydrochloric acid [7], in the visible region after reaction with 2,3-dichloro-5,6-dicyano-p-benzoquinone or 3,6-dichloro-2,5-dihydroxyp-benzoquinone [8] and complexation reactions with iodine or picric acid [9].

The purpose of our work is to develop and validate the quantitative determination of albendazole in tablets of combined composition by a spectrophotometric method, taking into account the presence of the second active pharmaceutical ingredient – praziquantel, and the influence of excipients. For the proposed method, the conditions of analysis, sample preparation and validation characteristics were determined.

2. Planning (methodology) of the research
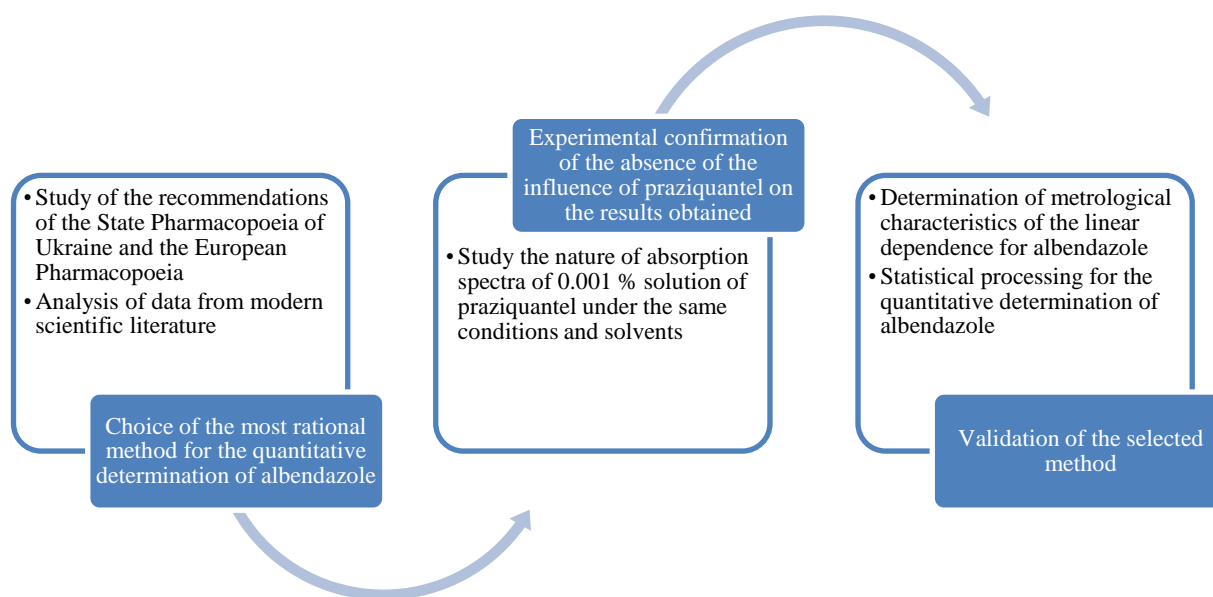The methodology of the research is shown in Fig. 1.

- Study of the recommendations of the State Pharmacopoeia of Ukraine and the European Pharmacopoeia
- Analysis of data from modern scientific literature

Choice of the most rational method for the quantitative determination of albendazole

Experimental confirmation of the absence of the influence of praziquantel on the results obtained

- Study the nature of absorption spectra of 0.001 % solution of praziquantel under the same conditions and solvents

- Determination of metrological characteristics of the linear dependence for albendazole
- Statistical processing for the quantitative determination of albendazole

Validation of the selected method

Fig. 1. Methodology of the research of albendazole quantification in tablets "AP-helmin"

3. Materials and methods
An experimental batch of tablets, substances of albendazole (series 170403, manufactured 04.2018) and praziquantel (series 20170702, manufactured 07.2019) were used in the research.

Class A measuring vessels, reagents meeting the requirements of State Pharmacopoeia of Ukraine [10], harmonized with the relevant European Pharmacopoeia [11] techniques, analytical balance AXIS ANG200 (Poland), spectrophotometer Evolution 60s (USA) were used in the experiment.

Albendazole was extracted from tablets with ethyl alcohol when heated, 0.1 M sodium hydroxide solution was added and the obtained sample was evaluated spectrophotometrically in the ultraviolet light at a wavelength of 308 nm. The samples comply with the Bouguer-Lambert-Behr law in the concentration range $1 \times 10^{-3} - 1.2 \times 10^{-2}$ mg/mk, correlation coefficient – ≥ 0.9998. The investigated method for quantifying albendazole in the drug meets the eligibility criteria for the range of determination ± 5.0 % for validation characteristics: specificity, linearity, precision, accuracy – within 80-120 % of the nominal content.

Method of spectrophotometric quantitative determination of albendazole

Test solution. The exact batch of tablet powder equivalent to 0.100 g of albendazole is shaken with 50 ml of ethyl alcohol 96 % when heated in a water bath, cooled, adjusted to 100.0 ml with the same solvent. The resulting solution is filtered through a "white tape" paper filter, discarding the first 15–20 ml of the filtrate. 1.0 ml

of the resulting solution is adjusted to 100.0 ml with 0.1 M sodium hydroxide solution.

Comparison solution. 0.100 g the substance of albendazole is dissolved in 50 ml of ethyl alcohol 96 % when heated in a water bath, adjusting the volume of the solution with the same solvent to 100.0 ml. 1.0 ml of the resulting solution is adjusted to 100.0 ml with 0.1 M sodium hydroxide solution.

Compensation solution. 0.1 M sodium hydroxide solution.

The optical density of the test solution and the comparison solution is measured at a wavelength of 308 nm relatively to the compensation solution.

The content of albendazole in grams, based on the average weight of the tablet and the nominal content of albendazole in the standard sample, is calculated according to the formula:

$$x, g = \frac{A \cdot m_{st} \cdot 100 \cdot 1 \cdot 100 \cdot m_{av.tabl.} \cdot \%st}{A_{st} \cdot m_b \cdot 100 \cdot 1 \cdot 100 \cdot 100}.$$

4. Results of the research
For the usage of the spectrophotometric method in the analysis of albendazole in the presence of praziquantel in the experimental dosage form, it was necessary to study the character of the UV absorption spectra of the test substance in different solvents. Tests were carried out for 0.001 % solutions of albendazole in ethyl alcohol 96 %, 0.1 M hydrochloric acid solution and 0.1 M sodium hydroxide solution (Fig. 2).
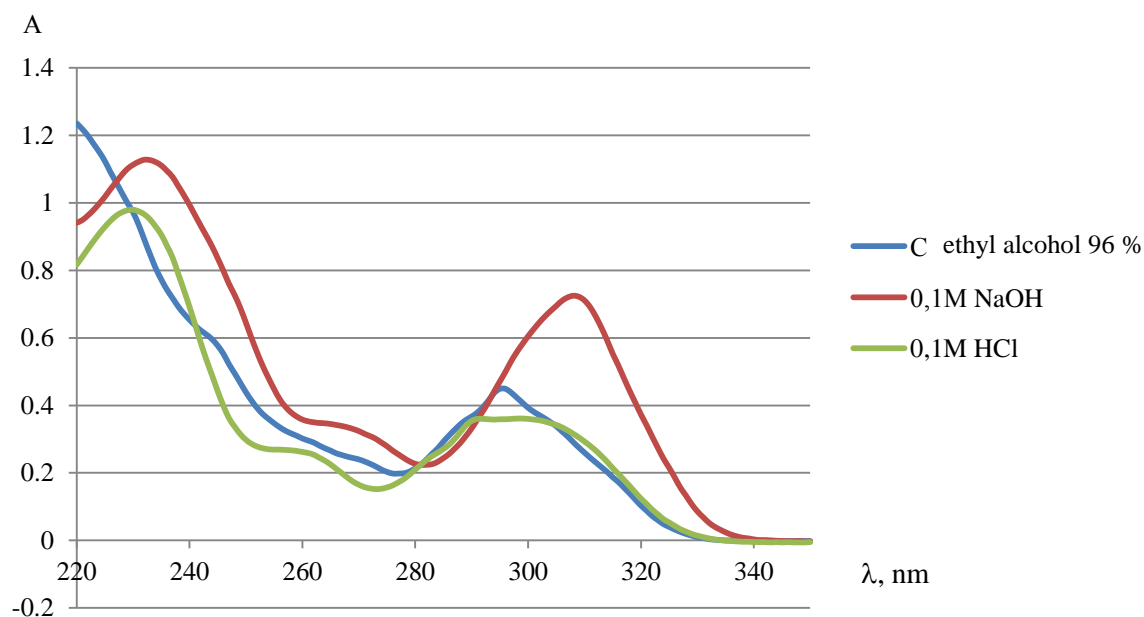
Fig. 2. UV absorption spectra of 0.001 % solutions of albendazole: 1 – in ethyl alcohol 96 %; 2 – in 0.1 M sodium hydroxide solution; 3 – in 0.1 M hydrochloric acid solution

It was established (Fig. 1) that in 0.001 % alcohol solution of albendazole, the absorption maximum is observed at a wavelength of 296 nm, at the transition to an acidic solvent, the maximum intensity decreases and it is observed at a wavelength of 292 nm, while at the same time the shoulder appears in the range 297–300 nm of the spectrum. In the UV spectrum of 0.001 % solution of a substance in 0.1 M sodium hydroxide solution, a hypochromic shift of the maximum position to 308 nm occurs with an increase in the absorption intensity.

When studying the nature of absorption spectra of 0.001 % solution of praziquantel under the same conditions and solvents, it was found that the UV spectra of praziquantel in alcohol, 0.1 M hydrochloric acid solution and 0.1 M sodium hydroxide solution are characterized by the presence of two weakly intense spots at the wavelengths of 264 nm and 272 nm (Fig. 3).

In the study of the change in optical density from the concentration of standard solutions of albendazole it was found that the subordination of the law of Bouguer-Lambert-Behr was observed in the range of concentrations from 1×10^{-3} to 1.2×10^{-2} mg/ml, the correlation coefficient is ≥ 0.9998 (Fig. 4).
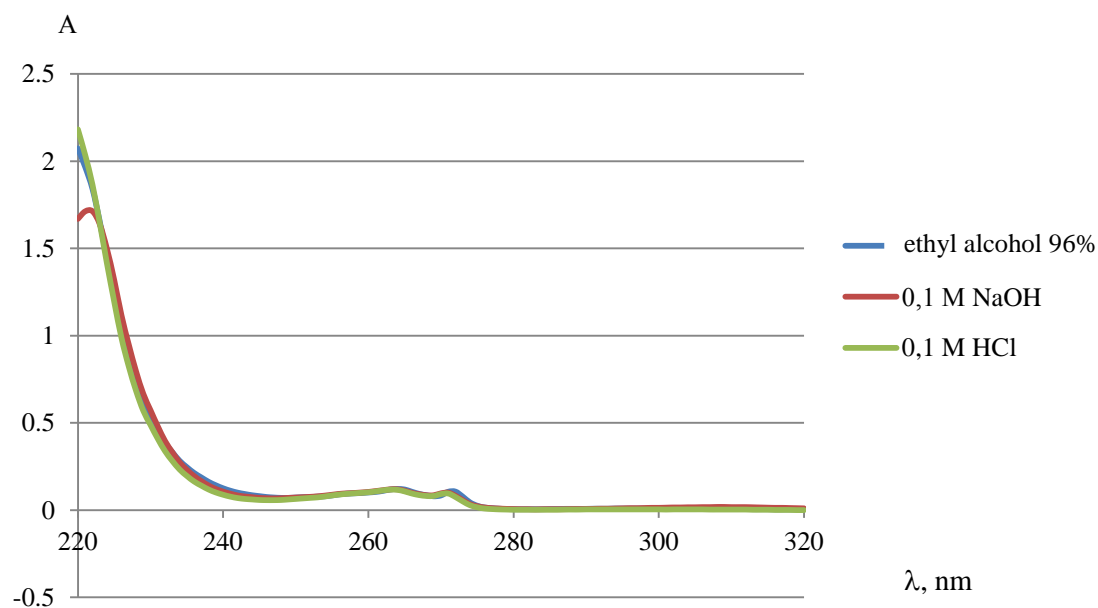

Fig. 3. UV absorption spectra of 0.01 % solutions of praziquantel:
1 – in ethyl alcohol 96 %; 2 – in 0.1 M sodium hydroxide solution; 3 – in 0.1 M hydrochloric acid solution
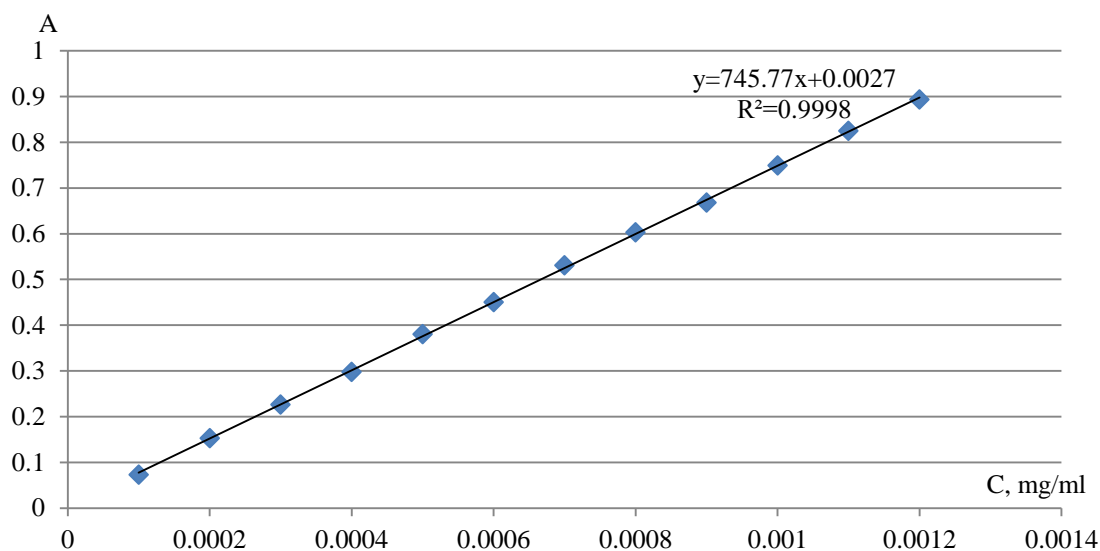
Fig. 4. Calibration graph of optical density versus concentration of standard albendazole solutions

The specificity of the technique was verified by measuring the optical density (A_{blank}) of the placebo solution and the optical density (A_{st}) of the comparison solution. It was found that: $A_{blank} = 0.002$; $A_{st} = 0.725$, that is, the placebo effect is $0.28\% \leq 0.51\%$ (the effect of background absorption on the measurement results is insignificant) (Fig. 5).

Linearity, convergence, correctness and the range of application of the method was determined on mixtures with a known content of albendazole in the range of 80 % to 120 % relatively to the maximum allowable value.

The comparison solution and the model solutions were prepared by one method, the actual values of X_i were found from the relation $X = A_i / A_{st} \cdot 100\%$ and were equal to the ratio of actual samples of albendazole, which was taken to prepare a model solution and a comparison solution. The linear dependence of the optical density on the concentration of albendazole in the normalized coordinates is shown in Fig. 6.

The least-squares method is used to calculate the linear dependence parameters $Y_i = b \times X_i + a$ for albendazole (Table 1).
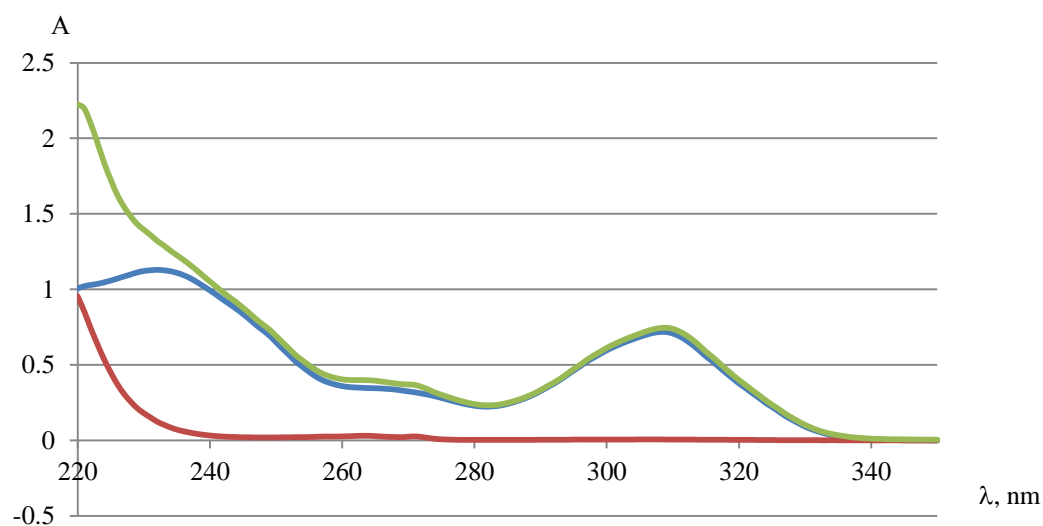

Fig. 5. UV absorption spectra of 1 – 0.001 % solution of albendazole (blue); 2 – model mixture (green); 3 – placebo in 0.1 M sodium hydroxide solution (red)
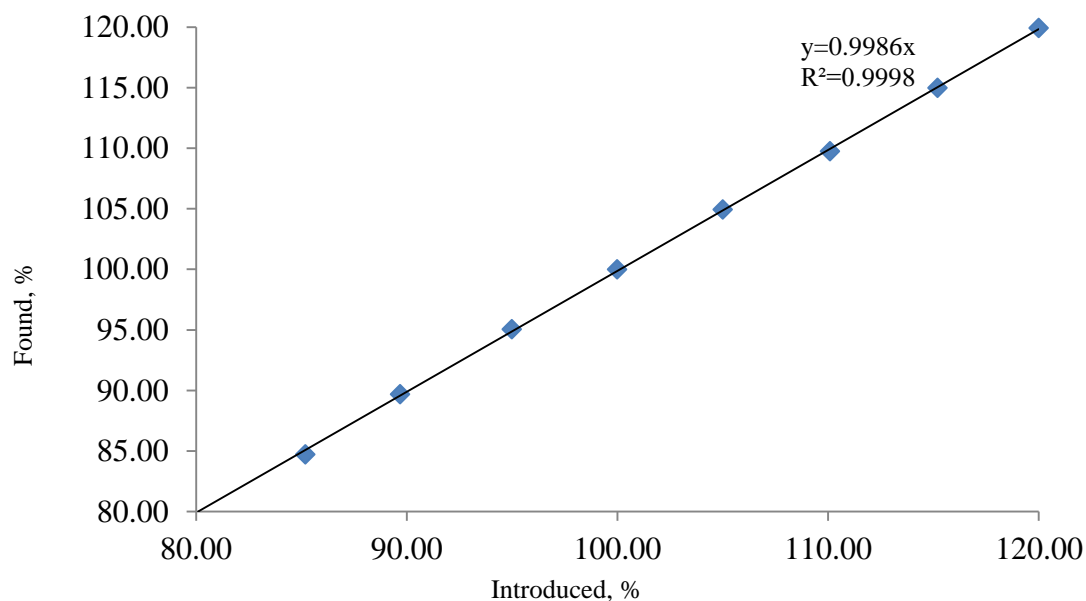
Fig. 6. Linear dependence of the optical density on the concentration of albendazole

Table 1

Metrological characteristics of the linear dependence for albendazole

Dimension	Value	Criterion (for tolerances 95.0 – 105.0 %), g=9)	Conclusion
b	0.9986	–	–
S_b	0.0049	–	–
a	-0.0132	1.≤1.8595·S_a=0.9195, 2. if not executed 1), then ≤1.6	Responds
S_a	0.4945	–	–
S_r	0.1901	≤0.84	–
r	0.9998	≥0.9981	Responds

As it is seen from Table 1, all the requirements for linear dependence parameters are fulfilled, i.e. the linearity of the method of quantitative determination of albendazole is confirmed throughout the concentration range (80-120 %). High value of the correlation coefficient for albendazole r = 0.9998 responds to the eligibility criteria (r = 0.9981) and confirms the linearity between the "introduced" and the "found" quantity of the test substance (Table 2).

For albendazole, the method of analysis is characterized by sufficient precision (convergence), since the value of the relative confidence interval of the value Z (0.15) is found smaller then critical value to convergence of results (1.60 %).

Table 2

The results of the analysis of model mixtures and their statistical processing for the quantitative determination of albendazole

No. of the model solution	Weight of albendazole, g m_{st}=0.1021 g	Introduced in % to the concentration of the comparison solution (X_i=C_i/C_{st}, %)	The average values of the optical density (A_i) (A_{st}=0.725)	Found in % to the concentration of the comparison solution (Y_i=A_i/A_{st}, %)	Found in % to introduced Z_i=100·(Y_i/X_i) %
1	2	3	4	5	6
1	0.0801	80.10	0.565	79.92	99.77
2	0.0852	85.20	0.599	84.72	99.44
3	0.0897	89.70	0.634	89.67	99.97
4	0.0950	95.00	0.672	95.05	100.05
5	0.1000	100.00	0.707	100.00	100.00
6	0.1050	105.00	0.742	104.95	99.95
7	0.1101	110.10	0.776	109.76	99.69
8	0.1152	115.20	0.813	114.99	99.82
9	0.1200	120.00	0.848	119.94	99.95

continuation of Table 2

average, $\bar{Z}$ %	99.85
Relative standard deviation, % $RSD_Z(\%) = \sqrt{\dfrac{\sum_i (Z_i - \bar{Z})^2}{n-1}} \times \dfrac{100}{\bar{Z}}$	0.1933
Relative confidence interval, $\Delta_Z(\%) = t(95, n-1) \times RSD_Z = 1{,}8595 \times RSD_Z$, %	0.3595
Critical value to the convergence of results ΔAs, % (marginal uncertainty)	1.60
Systematic error $\delta = \lvert \bar{Z} - 100 \rvert$	0.15
The criterion for the insignificance of a systematic error $\delta\% = \dfrac{\Delta_z}{\sqrt{n}} = \dfrac{\Delta_z}{3} = \dfrac{0.7296}{3} = 0.24$ (0.24≥0.67) if not executed 1), то $\delta \le \Delta_z$ (0.67≤0.7296)	Responds
General conclusion about the accuracy of the methodology	Correct

The criterion of insignificance of the systematic error of the method is fulfilled – the systematic error of the method (0.24) is practically insignificant, that is, the method of analysis is characterized by sufficient correctness in the whole concentration range from 80 to 120 % (Table 2).

Thus, for the quantitative determination of albendazole in tablets the method of spectrophotometry after extracting of the active substance from the tablet mass with ethyl alcohol was used. To further improving of the specificity of the technique and increasing the intensity of the absorption maximum 0.1 M sodium hydroxide solution was added and the study was performed at a wavelength of 308 nm.

5. Discussion of the results

The results obtained indicate the applicability of the pharmacopoeial method for the quantitative determination of albendazole in tablets with the concomitant content of praziquantel. The second substance does not affect the completeness and quality of the determination of albendazole, which allows the method to be validated and included in the appropriate section of the finished product quality control.

The findings correlate with the results of the other scientists' studies [12–14], which also experimentally confirm the absence of the effect of praziquantel on the results of the analysis of albendazole in the composition of the tablet mass. The proposed conditions for the spectrophotometric study are identical to those presented in other scientific papers on the determination of albendazole in tablet dosage forms [15].

The next step in the development of quality control methods for tablets based on albendazole and praziquantel will be the development and validation of a method for the quantitative determination of praziquantel.

Study limitations. The studies carried out are based on the development of the quantitative method on samples of tablets obtained in laboratory conditions, and do not fully reflect the possible risks of carrying out such a study on sample tablets of industrial production. Validation studies require supporting research.

The prospects for the further research. The article describes the main stages of the quantitative determination of albendazole in tablets "AP-helmin". The next stage of research is the development of a method for the quantitative determination of praziquantel and the subsequent adaptation of both proposed methods for use in quality control of this drug of industrial production.

6. Conclusions

Spectrophotometric method for quantitative determination of albendazole in tablets in the presence of other active substance praziquantel was developed.

The procedure of validation of the method of quantification of albendazole using the eligibility criteria for tolerances of ± 5.0 % was carried out, which confirms the specificity, linearity, precision (convergence), correctness and range of application of the proposed method.

Conflict of interests

Authors declare no conflict of interest.

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