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DEVELOPMENT AND VALIDATION OF A METHOD FOR SIMULTANEOUS QUANTITATIVE DETERMINATION OF ALBENDAZOLE AND PRAZIQUANTEL IN COATED TABLETS "AP-HELMIN"

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Adapting modern methods of quantitative analysis of active substances in their joint content in the dosage form and validating them is an integral process of pharmaceutical development. We have developed a drug in the form of coated tablets for the treatment of helminthiases of the digestive system in adults. A feature of this drug is the composition of the API of albendazole and praziquantel in a ratio of 1:4.

The aim *of this research is to develop methodology for quantitative analysis of both substances by the method of liquid chromatography, determination of their possible mutual influence on the process, as well as validation of the proposed methods.*

Materials and methods. *To meet the research's set purpose, the following tasks were identified: choosing the most rational method for the quantitative determination of albendazole and praziquantel; confirming the absence of the mutual influence of APIs on the results obtained; and validating the selected methods of albendazole and praziquantel analysis.*

Object of the research conducted included coated tablets "AP-helmin", series 1-5.2021; pharmacopoeial standard sample (PSS) of albendazole, and PSS praziquantel. Quantitative determination of albendazole and praziquantel was conducted according to SPU, method 2.2.29.

Results. *The article describes the conditions and stages of the quantitative determination of albendazole and praziquantel and the main indicators of method validation.*

Conclusions. *It was proven that quantification with the liquid chromatography method of both substances is validated, and the substances do not affect each other's analysis in the coated tablets "AP-helmin" following the project of QCM for this drug. All calculated parameters meet the required validation criteria*

Keywords: *validation, liquid chromatography, quantitative analysis, albendazole, praziquantel, coated tablets*

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1. Introduction

Parasitic diseases are one of the most common among the population. Recently, there has been a clear trend towards an increase in the incidence of invasive etiology, in particular, helminthiasis. According to the latest data from the World Health Organization, helminthiasis affects about 4.5 billion people. In Europe, one in three people is affected. 85–95 % of the adult population of the United States have pathogens of parasitic infections (according to Dr. R. Andersen).

At the Department of Pharmaceutical Technology of Drugs of the National University of Pharmacy there was created the medicine in the form of coated tablets "AP-helmin", the API of which are albendazole and praziquantel in a ratio 1:4. This medicine is intended for the treatment of helminthiasis of the digestive system in adults. The effect of the therapeutic dose on the body was confirmed in models of ascariasis in piglets, spontaneous toxocariasis and dipilidiosis in dogs and spontaneous paraspidoderosis and hymenolepidosis in white rats. The absolute efficacy of coated tablets "AP-helmin" in all studied cases of intestinal helminthiasis was established, which indicates their high level of specific pharmacological activity. Indicators of haematological studies in piglets free from intestinal helminths before and 24 and 72 hours after drug administration were within the physiological norm. Clinical examination of experimental animals confirmed the low degree of toxicity of the drug [1, 2]. The novelty of the developed drug is protected by a patent of Ukraine for invention No. 124898, "Anthelmintic medicine based on albendazole and praziquantel" (December 8, 2021) and utility model patent of Ukraine No. 142220", Anthelmintic medicine based on albendazole and praziquantel" (May 25, 2020) [3].

Developing qualitative and quantitative analysis methods in the composition of medicine is a mandatory point of quality control. Thus, the purpose of this research is to develop methods for the qualitative and quantitative analysis of albendazole and praziquantel in coated tablets "AP-helmin".

Following the recommendations of the European Pharmacopoeia, the identification of albendazole, as well

as praziquantel, should be performed by infrared absorption spectrophotometry or gas-liquid chromatography [4]. The resulting spectrum should be identical to the standard sample of the substance albendazole or the standard sample of the substance praziquantel, respectively.

Validation is performed to confirm the method of quantitative determination of albendazole and praziquantel by liquid chromatography's compliance with the criteria of acceptability in tablets "AP-Helmin."

Validation was performed according to the requirements of the State Pharmacopoeia of Ukraine (SPhU) [5].

The research was performed in the State Research Laboratory for Quality Control of Medicines (National University of Pharmacy, Kharkiv, Ukraine) in the period June 2021–February 2022.

2. Planning (methodology) of research

In order to meet the set aim of the research, the following tasks of the study were identified (Fig. 1).

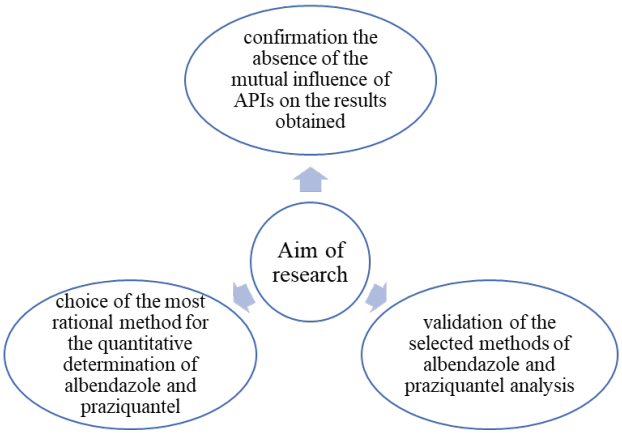

Fig. 1. General methodology of the research

The equipment used for validation has been fully certified (Table 1).

Table 1

Equipment used in the research

Control and measuring devices and auxiliary equipment	Certification data*
Liquid chromatograph ProStar, by "Varian"	Certificate of verification of the measuring instrument No. 84343/2, valid until September 13, 2022
Analytical scales ABT 120-5DNM, by "Kern&Sohn Gmbh"	Certificate of verification of the measuring instrument No. 87881/1, valid until August 15, 2022
Measuring vessels of 1 class of accuracy (accuracy class A): measuring flasks, pipettes	

*Note: * – certificate of verification of measuring equipment is issued by an authorized organization SE "Kharkiv Regional Research and Production Centre for Standardization, Metrology and Certification"*

3. Materials and methods

Reagents. As the reagent of the research, water for chromatography and acetonitrile for chromatography (p. UN1648, valid until 08.2022) were used.

Objects of research. Objest of the research conducted included coated tablets "AP-helmin", series 1-5.2021; pharmacopoeial standard sample (PSS) of albendazole, and PSS praziquantel.

Method of quantitative determination of albendazole and praziquantel. Quantitative determination of albendazole and praziquantel was conducted according to SPU, method 2.2.29.

Test solution. Place 8.0 mg of powdered tablet mass in a volumetric flask (50.0 ml), add 40 ml of methanol R, sonicate for 10 min. Then, bring the volume of the solution with the same solvent to the mark, mix and filter through the paper filter "blue tape". Place 5.0 ml of the obtained solution in a volumetric flask (100.0 ml) and bring to the mark with methanol R.

Reference solution a. Place 250.0 mg of PSS albendazole in a volumetric flask (50.0 ml), dissolve in 30 ml of methanol R, sonicate for 10 minutes Then bring the volume of the solution with the same solvent to the mark, mix and filter through paper filter "blue tape".

Reference solution b. Place 400.0 mg of PSS praziquantel is placed in a volumetric flask (50.0 ml), dissolve in 30 ml of methanol R, and sonicate for 10 minutes. Then bring the volume of the solution with the same solvent to the mark, mix and filter through paper filter "blue tape".

Reference solution c. Place 2.0 ml of reference solution a and 5.0 ml of reference solution b in a volumetric flask (100.0 ml), bring to the mark with methanol R and mix.

The solutions are used freshly prepared.

Before chromatography, the solutions are filtered through a membrane filter with a pore size of not more than 0.45 μm.

Chromatography is performed on a liquid chromatograph with a spectrophotometric detector under the following conditions:

– column size 150×4.6 mm, filled with *octadecylsilyl silica gel for chromatography R* (for example, Symmetry C18, size 150x4,6 mm, by "Waters") with pre-column, 5 μm, for which the conditions of the suitability of the chromatographic system;

– mobile phase flow rate:1.5 ml/min;

– column temperature: 45 °C;

– wavelength detection at 225 nm;

– injection volume: 20 μl.

– mobile phase: *water R – acetonitrile R* (60:40), degassed in any convenient way;

– elution mode – isocratic.

Chromatograph solvent (blank chromatogram), reference solution, and test solution during 15 min.

The chromatographic system is considered suitable using the following conditions for the reference solution:

– peak symmetry coefficient of albendazole and praziquantel ranges from 0.8 to 1.5;

– separation coefficient of the peaks of albendazole and praziquantel is not less than 1.5.

Formula 1 calculates the content of albendazole (X_1) or praziquantel (X_2) in one tablet in mg, based on the tablet's average weight:

$$X_{1/2} = \frac{S_1 \times m_0 \times 2 \times 50 \times 100 \times P \times b}{S_0 \times 50 \times 100 \times m \times 5 \times 100} = \frac{S_1 \times m_0 \times P \times b}{S_0 \times m \times 250}, \quad (1)$$

where S_1 – average area of the peaks of albendazole (praziquantel), calculated from the chromatograms of the test solution;

S_0 – average area of the peaks of albendazole (praziquantel), calculated from the chromatograms of the reference solution;

m – weight of the tablet portion taken to prepare the test solution, mg;

m_0 – weight of the PSS albendazole (PSS praziquantel) portion taken to prepare the reference solution, mg;

P – content of the main substance in PSS albendazole (PSS praziquantel) taken to prepare a reference solution, %.

b – average weight of the tablet, mg.

The content of albendazole and praziquantel in one tablet in terms of the average weight of the tablet should be:

– at the time of preparation: albendazole: 95.0–105.0 mg (±5 %), praziquantel: 380.0–430.0 mg (±5 %);

– during storage: albendazole: 92.5–107.5 mg (±7.5 %), praziquantel: 370.0–430.0 mg (±7.5 %).

Forecast of uncertainty of analysis results.

The maximum allowable total relative uncertainty of the method of analysis of finished drugs Δ_{AS} % is associated with the tolerance of the content of the analyzed substance in accordance with the specification (B).

In the case of coated tablets "AP-helmin", the quantitative content of albendazole and praziquantel should be within ±5 % (according to the requirements of the quality control methods (QCM) project for the drug-coated tablets "AP-helmin", therefore max Δ_{AS} is ≤1.6 %.

The forecast of the uncertainty of sample preparation was calculated based on the general requirements of the SPU for laboratory equipment (Tables 2, 3).

Determination of the uncertainty of the final analytical operation Δ_{FAO} is carried out for the test solution and the comparison solution. When calculating confidence intervals, one-sided Student's coefficient for a probability of 95 % and the corresponding number of degrees of freedom are used. Confidence intervals for the comparison solution and the test solution are calculated for the average of 5 results (the maximum number of measurements according to the MQQ of the medicine, methods of SPhU 5.3 N.2, 2.4 and 2.6 were used).

$$\Delta_{FAO}^{cm} = \frac{1}{\sqrt{5}} \times t(95\,\%, n-1) \times RSD;$$

$$\Delta_{FAO}^{smp} = \frac{1}{\sqrt{5}} \times t(95\,\%, n-1) \times RSD.$$

According to the requirements of the suitability of the chromatographic system of the quantitative determination method, the relative standard deviation of five parallel determinations should be no more than 1.0 %. When n=5, t(95 %, n–1)=2.1318:

$$\Delta_{FAO}^{cm} = \frac{1}{\sqrt{5}} \times 2.1318 \times 1.0\,\% = 0.9534;$$

$$\Delta_{FAO}^{smp} = \frac{1}{\sqrt{5}} \times 2.1318 \times 1.0\,\% = 0.9534.$$

The total uncertainty of the final analytical operation:

$$\Delta_{FAO} = \sqrt{\left(\Delta_{FAO}^{smp}\right)^2 + \left(\Delta_{FAO}^{smp}\right)^2} = 1.35.$$

Table 2

Calculation of uncertainty of sample preparation for analysis of albendazole (Δ_{sp})

Sample preparation operation	Value	Uncertainty, (Δ) %
Test solution		
Portion (m)	800 mg	0.025
Bringing to volume	50 ml	0.17
Aliquot	5 ml	0.37
Bringing to volume	100 ml	0.12
Reference solution		
Portion (m)	250 mg	0.080
Bringing to volume	50 ml	0.17
Aliquot	2 ml	0.61
Bringing to volume	100 ml	0.12
Complete uncertainty of sample preparation Δ_{sp} %		0.78
Uncertainty of the final analytical operation Δ_{FAO} (liquid chromatography) *		1.35
Complete uncertainty of the analysis methodology Δ_{AS} % $\Delta_{AS}\% = \sqrt{(\Delta_{sp}\%)^2 + (\Delta_{FAO}\%)^2}$		1.56

Table 3

Calculation of uncertainty of sample preparation for analysis of praziquintel (Δ_{sp})

Sample preparation operation	Value	Uncertainty, (Δ) %
Test solution		
Portion (m)	800 mg	0.025
Bringing to volume	50 ml	0.17
Aliquot	5 ml	0.37
Bringing to volume	100 ml	0.12
Reference solution		
Portion (m)	400 mg	0.050
Bringing to volume	50 ml	0.17
Aliquot	5 ml	0.37
Bringing to volume	100 ml	0.12
Complete uncertainty of sample preparation Δ_{sp} %		0.60
Uncertainty of the final analytical operation		1.35
Δ_{FAO} (liquid chromatography) *		
Complete uncertainty of the analysis methodology Δ_{AS} %		1.48
$\Delta_{AS}\% = \sqrt{(\Delta_{sp}\%)^2 + (\Delta_{FAO}\%)^2}$		

Complete uncertainty of the method of analysis of albendazole Δ_{AS} %:

$$\Delta_{AS} = \sqrt{(\Delta_{sp})^2 + (\Delta_{FAO})^2} = 1.56.$$

The complete uncertainty of the praziquantel analysis methodology Δ_{AS} %:

$$\Delta_{AS} = \sqrt{(\Delta_{sp})^2 + (\Delta_{FAO})^2} = 1.48.$$

Thus, the calculated total uncertainty of the analysis method Δ_{AS} % is less than maxΔ_{AS} (1.56 %<maxΔ_{AS}=1.6 % and 1.48 %<maxΔ_{AS}=1.6 %), which meets the requirements for this parameter.

Therefore, the uncertainty of sample preparation and analysis as a whole should ensure sufficient measurement accuracy.

4. Result

Validation studies.

Specificity.

The specificity of the method was confirmed by comparing the chromatograms of the reference solution, test solution, blank solution, and placebo solution.

The retention time of the albendazole and praziquantel peaks on the chromatograms of the test solution corresponds to the retention time of the albendazole and praziquantel peaks on the chromatograms of the reference solution (approximately 6.0 min and 7.4 min, respectively).

The blank chromatogram revealed no peaks whose retention time coincided with the retention time of the albendazole and praziquantel peaks. There are no peaks on the chromatograms of placebo solutions that would coincide with the peaks of albendazole and praziquantel. Chromatograms of the blank solution, reference solutions, placebo solution and test solution are shown in Fig. 2–7.

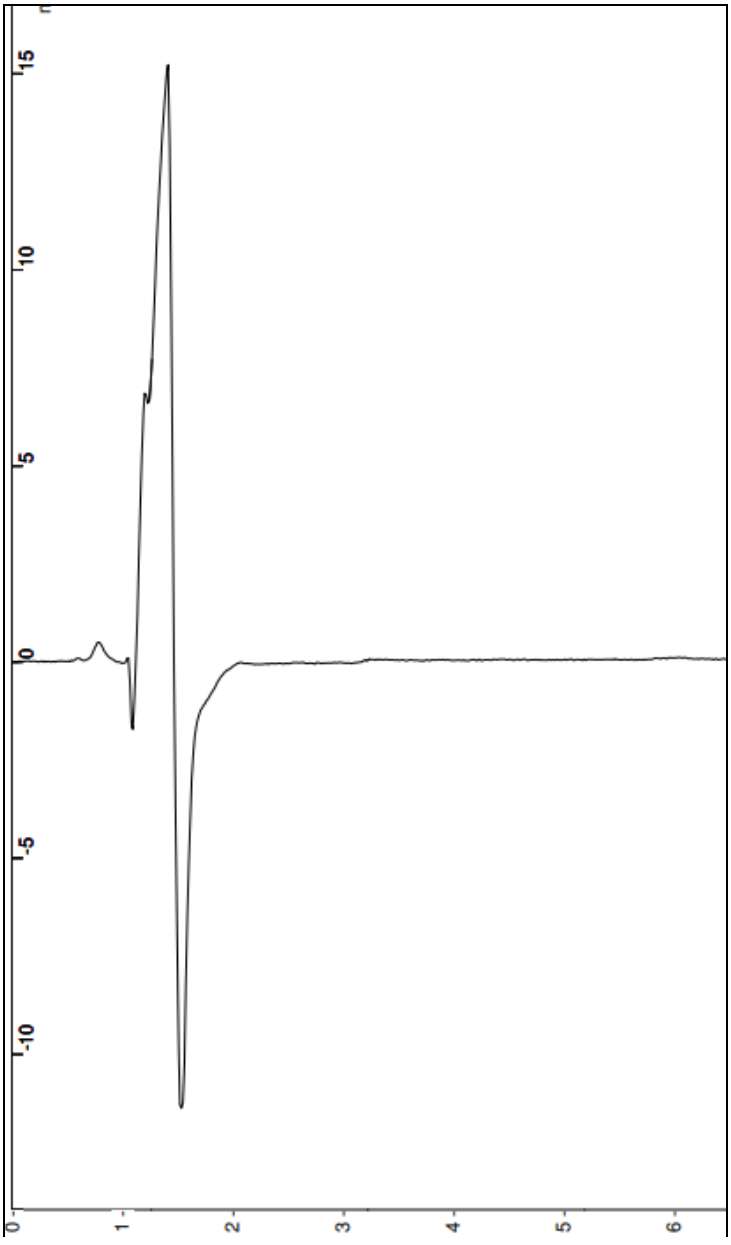

Fig. 2. Chromatogram of the blank solution

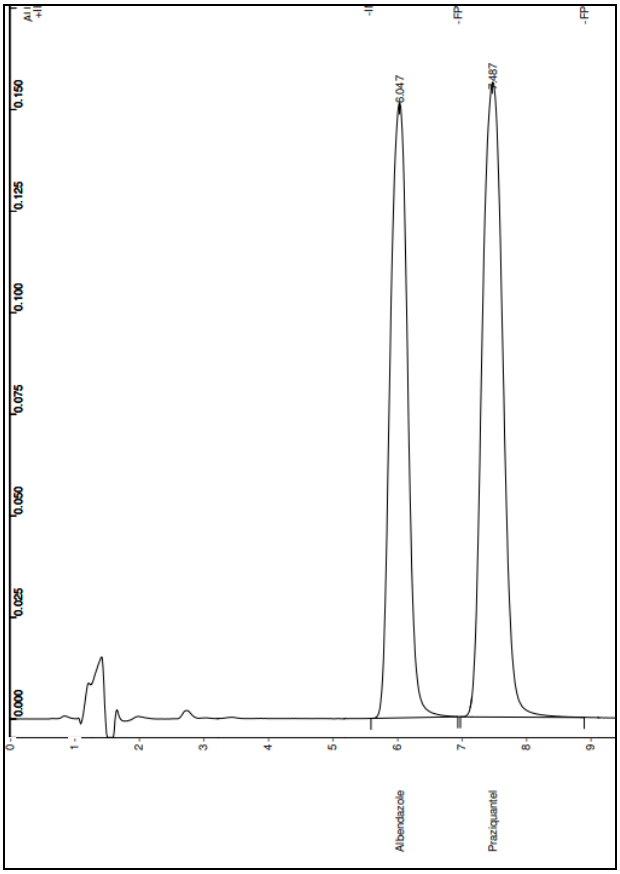

Fig. 3. Chromatogram of the test solution

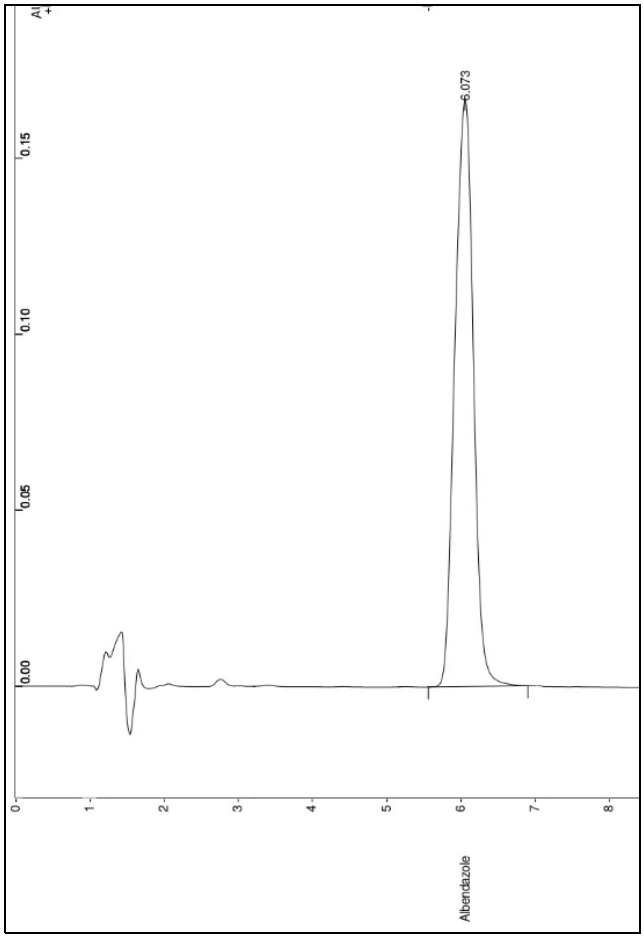

Fig. 4. Chromatogram of *reference solution a*

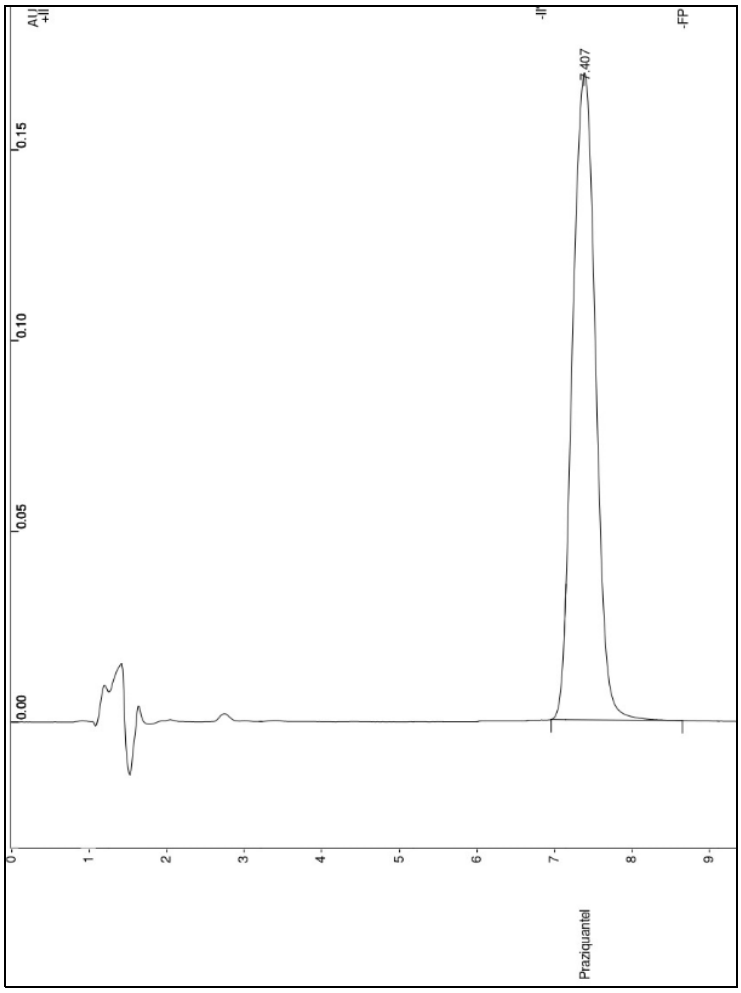

Fig. 5. Chromatogram of *reference solution b*

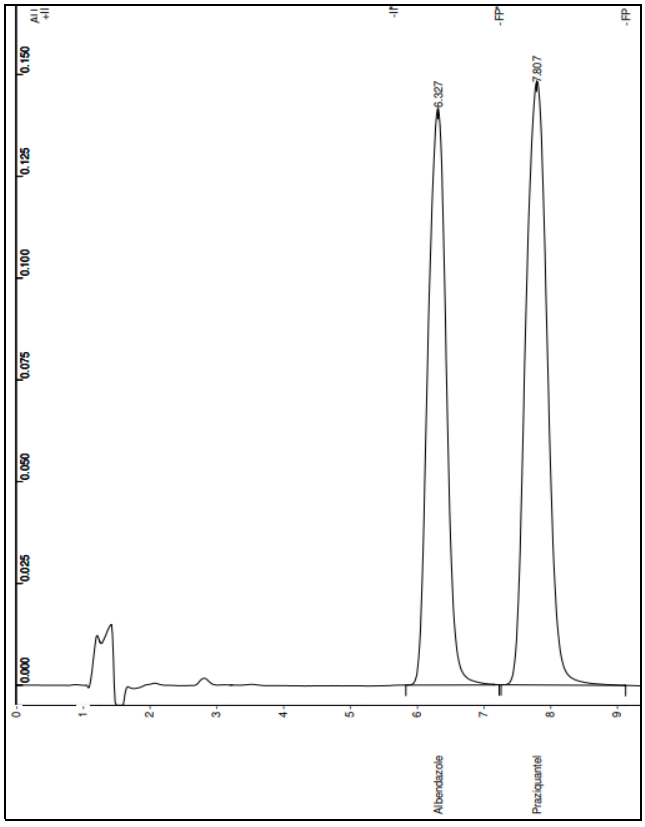

Fig. 6. Chromatogram of *reference solution c*

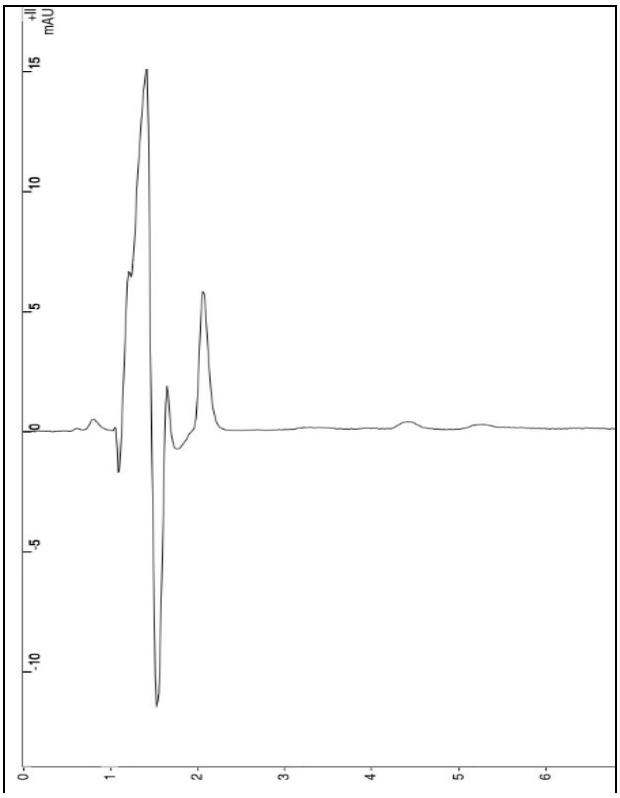

Fig. 7. Chromatogram of placebo solution

Linearity.

The quantification method should be linear within the range of application, which should overlap the possible values of the concentrations of the active substance. SPU sets the range of application of quantitative methods as 80–120 %.

To confirm the method's linearity, 9 model solutions were prepared, the concentration of which varies evenly within the range of application (step – 5 %).

Calculations and criteria are given for normalized values of the standard solution (Tables 4, 5) by the formulas (3), (4):

$$X_i = C_i / C_{st} \cdot 100; \quad (3)$$

$$Y_i = S_i / S_{st} \cdot 100. \quad (4)$$

In Fig. 8, a graph of the linear dependence of the analytical signal on the actual concentration of the solution, constructed in normalized coordinates, based on the data in the Table 4 is presented.

Table 4

Calculation of linearity parameters of the method of quantitative determination of the standard solution of albendazole according to the project of QCM

No.	C, %	C_i	$C_{i/st}$	The average value of the peak area	$S_{i/st}$
1	80	0.080	80.0	11249956	80.12
2	85	0.085	85.0	11883547	84.63
3	90	0.090	90.0	12610971	89.81
4	95	0.095	95.0	13436396	95.69
5	100	0.100	100.0	14089179	100.34
6	105	0.105	105.0	14714210	104.79
7	110	0.110	110.0	15399158	109.67
8	115	0.115	115.0	16191913	115.31
9	120	0.120	120.0	16833544	119.88
Standard	–	0.100	–	14041821	–

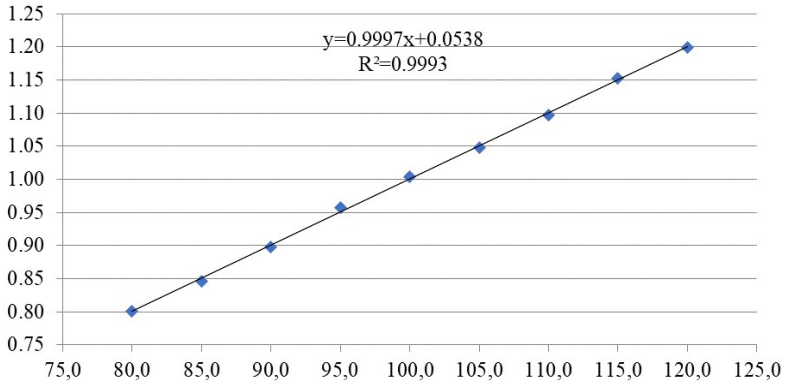

Fig. 8. Graph of linear dependence ($Y_i = b*X_i + a$) for standard albendazole solution

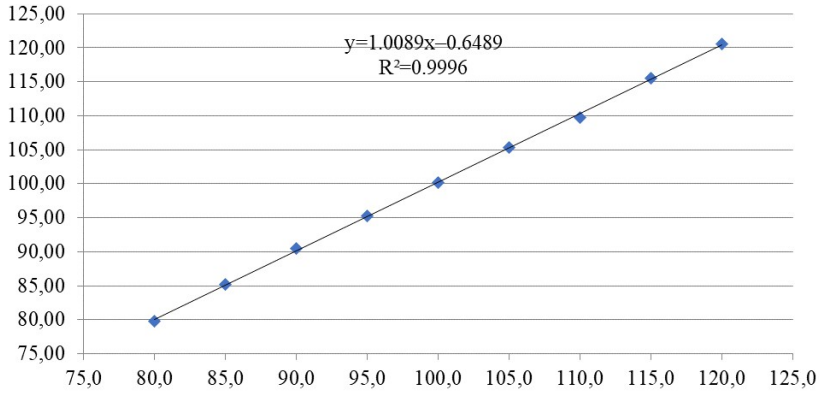

Fig. 9. Graph of linear dependence (Y_i=b*X_i+a) for standard praziquantel solution

Table 5

Calculation of linearity parameters of the method of quantitative determination of the standard solution of praziquantel according to the project of QCM

No.	C, %	C_i	$C_{i/st}$	The average value of the peak area	$S_{i/st}$
1	80	0.320	80.0	13831505	79.81
2	85	0.340	85.0	14764724	85.19
3	90	0.360	90.0	15677943	90.46
4	95	0.380	95.0	16511162	95.27
5	100	0.400	100.0	17368305	100.21
6	105	0.420	105.0	18254615	105.33
7	110	0.440	110.0	19023512	109.77
8	115	0.460	115.0	20030009	115.57
9	120	0.480	120.0	20887332	120.52
Standard	–	0.400	–	17331048	–

In Fig. 9, a graph of the linear dependence of the analytical signal on the actual concentration of the solution, constructed in normalized coordinates based on the data in Tab. 5 is presented.

Eligibility criteria are given in Tables 6, 7.

Table 6

Data of verification of linearity of the method of quantitative determination of albendazole

No.	Parameter	Requirements	Value obtained	Fulfillment of the criterion
1	$\|a\|$	≤2.6	0.054	Carried out
2	S_0	≤0.84	0.425	Carried out
3	r	>0.9981	0.9997	Carried out

Table 7

Data of verification of linearity of the method of quantitative determination of praziquantel

No.	Parameter	Requirements	Value obtained	Fulfillment of the criterion
1	$\|a\|$	≤2.6	0.649	Carried out
2	S_0	≤0.84	0.499	Carried out
3	r	> 0.9981	0.9998	Carried out

Correctness.

To determine the correctness within the range of use of the analytical method, 9 test solutions were prepared, observing all stages of the analytical method. The concentration of albendazole and praziquantel in solutions ranged from 80 % to 120 %. The determination of the correctness parameters and eligibility criteria are presented in Tables 8, 9.

Table 8

Calculation of the correctness parameters of the standard solution of albendazole

Introduced in % to the concentration of the reference solution (C_i/C_{st})*100 %	Found in % to the concentration of the reference solution (A_i/A_{st})*100 %	Found in % of introduced (A_i/A_{st})*100/(C_i/C_{st})
80	80.12	100.15
85	84.63	99.56
90	89.81	99.79
95	95.69	100.72
100	100.34	100.34
105	104.79	99.80
110	109.67	99.70
115	115.31	100.27
120	119.88	99.90
Z_{avr}		100.03
Relative standard deviation, s_z %		0.37
Relative confidence interval ΔZ=t(95 %, 8)*s_z=1.8595*s_z		0.69
The critical value for the convergence of results δ % ≤		1.6
Systematic error δ=$\|Z_{avr}$–100|		0.03
Criterion of statistical insignificance: δ≤ΔZ/3=0.69/3=0.23 (0.03≤0.23);		Carried out
If not carried out, the criterion of practical insignificance: δ≤0.512 (0.03≤0.512)		Carried out
General conclusion about the method		Correct

Fulfilment of the criteria of correctness for the determination of albendazole and praziquantel in coated tablets “AP-helmin” by liquid chromatography are given in Table 10.

Table 9

Calculation of the correctness parameters of the standard solution of praziquantel

Introduced in % to the concentration of the reference solution $(C_i/C_{st})*100$ %	Found in % to the concentration of the reference solution $(A_i/A_{st})*100$ %	Found in % of introduced $(A_i/A_{st})*100/(C_i/C_{st})$
80	79.81	99.76
85	85.19	100.23
90	90.46	100.51
95	95.27	100.28
100	100.21	100.21
105	105.33	100.31
110	109.77	99.79
115	115.57	100.50
120	120.52	100.43
Z_{avr}		100.23
Relative standard deviation, s_z %		0.28
Relative confidence interval $\Delta Z=t(95\ \%,8)*s_z=1.8595*s_z$		0.52
The critical value for the convergence of results δ % ≤		1.6
Systematic error $\delta=\lvert Z_{avr}-100\rvert$		0.23
Criterion of statistical insignificance: $\delta\leq\Delta Z/3=0.69/3=0.23$ (0.03≤0.23);		Carried out
If not carried out, the criterion of practical insignificance: δ≤0.512 (0.03≤0.512)		Carried out
General conclusion about the method		Correct

Table 10

Results of the correctness assessment by two criteria

Parameter	Value	Requirements for statistical insignificance	Requirements for practical insignificance	Fulfillment of the criterion
$\lvert\overline{Z}-100\rvert$	0.03	≤0.23	≤0.512	Carried out by two criteria
$\lvert\overline{Z}-100\rvert$	0.23	≤0.17	≤0.512	Carried out by one criterion

Precision.

Precision was investigated on the tested solutions prepared for the definition of criterion "Correctness". The precision criteria of a quantitative determination of albendazole and praziquantel of standard solution are fulfilled (Table 11).

Table 11

Results of the evaluation of albendazole and praziquantel standard solution precision

Parameter	Value	Criterion	Fulfillment of the criterion
ΔZ	0.69	≤1.6 %	Carried out
ΔZ	0.52	≤1.6 %	Carried out

Intra-laboratory precision.

Results of the study of 6 trials of one sample by two analysts on different days during one working week using different measuring vessels are used. Determination of intra-laboratory precision parameters and calculation of its criteria are presented in Tables 12, 13.

Table 12

Determination of intra-laboratory precision parameters of albendazole

No.	Analyst No. 1	Analyst No. 2
1	101.43	103.15
2	102.03	102.54
3	102.35	103.12
Average	101.93	102.94
General average		102.44
Relative standard deviation, RSD %		0.60
Confidence interval, $(\Delta_{intra}=t(95\ \%, m*n-1)*RSD, \%=0.26*RSD, \%$		1.39

Table 13

Determination of intra-laboratory precision parameters of praziquantel

No.	Analyst No. 1	Analyst No. 2
1	101.60	101.93
2	101.78	101.08
3	101.89	100.52
Average	101.76	101.18
General average		101.47
Relative standard deviation, *RSD* %		0.51
Confidence interval, $(\Delta_{intra}=t(95\ \%, m*n-1)*RSD, \%=0.26*RSD, \%$		1.18

Fulfilment of the criteria of intra-laboratory precision for determining albendazole and praziquantel in coated tablets "AP-helmin" by liquid chromatography are given in Table 14.

Table 14

Results of the assessment of intra-laboratory precision

Parameter	Criterion requirements	Value obtained	Fulfillment of the criterion
Δ_{intra}	≤1.6	1.39	Carried out
Δ_{intra}	≤1.6	1.18	Carried out

Robustness.

Study of the stability of the test solution and reference solution was performed after 24 hours. The results are presented in Tables 15, 16.

Table 15

Determination of the stability of the standard solution of albendazole and praziquantel

The average value of S peaks of freshly prepared solution	The average value of S peaks of the solution after 24 hours	Parameter changes as a percentage
14217199	14275727	0.412
17215122	17283733	0.399

Thus, the differences between the obtained values of the peak areas should not exceed the criterion of insignificance compared to the maximum allowable uncer-

tainty of the analysis results ($\Delta_{AS,\ insig}$), i.e. 0.512 %. According to the determination results, the test solution was stable for 24 hours.

Table 16

Determination of the stability of albendazole and praziquantel in the test solution

The average value of S peaks of freshly prepared solution	The average value of S peaks of the solution after 24 hours	Parameter changes as a percentage
14917733	14910614	0.048
17750469	17683504	0.377

Therefore, the findings found that the chromatographic system was compliant with the requirements of the test to verify the suitability of the chromatographic system for albendazole and praziquantel quantitative determination in coated tablets "AP-helmin" (Table 17).

Table 17

Compliance with the requirements of the test to verify the suitability of the chromatographic system for mutual albendazole and praziquantel quantitative determination

Parameter	Requirements	Found
Peak separation ratio of albendazole and praziquantel	Not less than 1.5	2.6
Symmetry coefficient of the albendazole peak for the reference solution c	from 0.8 to 1.5	1.00
Symmetry coefficient of the praziquantel peak for the reference solution c	from 0.8 to 1.5	0.99

5. Discussion

The obtained results confirm that the method of quantitative determination of albendazole and praziquantel in coated tablets "AP-helmin" is specific.

The findings confirm that the method of quantitative determination of albendazole and praziquantel in coated tablets "AP-helmin" in the concentration range from 80 % to 120 % is linear; it satisfies the criteria of acceptability of the validation indicator "Correctness". The method is characterized by sufficient convergence, as the value of the relative confidence interval of the value is 0.69 and 0.52 %, which is less than the critical value for the convergence of results (1.6 %) and satisfies the criteria of acceptability of the validation indicator "Precision". The chosen method of quantitative determination of albendazole and praziquantel in coated tablets "AP-helmin" meets the eligibility criteria of the test "Intra-laboratory precision" as well.

The obtained results coincide with similar studies by other authors. In particular, spectrophotometric methods for determining pure albendazole and in the composition of solid and liquid dosage forms are sensitive, fast, economically available and subject to validation [6–12]. In the simultaneous determination of albendazole and other anthelmintic agents, for example, ivermectin, other researchers often turn to LC, GLC, and HPLC [13–17]. However, no validation data is provided. The number of publications on the study on the determination of praziquantel is lower compared to the number of publications on albendazole. The gas-liquid chromatography method is considered the most effective for this substance [18, 19]. Materials on the simultaneous determination of albendazole and praziquantel are extremely scarce. Available publications on the use of spectrophotometric methods [20]. There are few reliable studies on the validation of these methods because the combination of these substances in the proposed ratio (1:4) is new, which confirms the relevance and timeliness of the described validation studies.

Practical relevance. The practical relevance of the results lies in validating the method for the joint qualitative determination of albendazole and praziquantel by the method of liquid chromatography in the coated tablets "AP-helmin".

Research limitations. The presented validation results of the method for joint quantitative determination of albendazole and praziquantel are limited to the dosage form of coated tablets, the technology of their preparation, and the ratio of the APIs.

Prospects for further research. Prospects of further research include 2 main directions: 1 – investigation of the validating parameters of joint quantitative determination of albendazole and praziquantel in other dosage forms as they are developed; 2 – investigation of the validating parameters of quantitative determination of albendazole in the dosage form of chewable pastilles under the conditional name "Albenpast".

6. Conclusions

According to the findings it can be concluded that the method of liquid chromatography is considered validated and can be used for quantitative analysis of albendazole and praziquantel in the coated tablets "AP-helmin" in accordance with the project of QCM for this drug. All calculated parameters meet the required validation criteria.

Conflict of interest

The authors declare that they have no conflict of interest related to this research, whether financial, personal, authorship, or otherwise, that could affect the research and its results presented in this paper.

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Data availability

Data will be made available at a reasonable request.

Use of artificial intelligence

The authors confirm they did not use artificial intelligence technologies when creating the current work.

References

1. Semchenko, K., Vyshnevska, L., Polovko, N. (2018). Study of anthelmintic activity and acute toxicity of medicine of combined composition. ScienceRise: Pharmaceutical Science, 6 (16), 8–12. https://doi.org/10.15587/2519-4852.2018.151428

2. Semchenko, K., Vyshnevska, L. (2020). Study of the specific activity of anthelmintic drug "Ap-helmin." Ukrainian Biopharmaceutical Journal, 2 (63), 54–57. https://doi.org/10.24959/ubphj.20.271

3. Semchenko, K. V., Vyshnevska, L. I. (2019). Pat. No. 124898 UA. Antyhelmintnyi zasib na osnovi albendazolu ta prazykvantelu. MPK: A61K 31/4184, A61K 31/47, A61P 33/00. No. a201910236; declareted: 07.10.2019, published: 08.12.2021, Bul. No. 49.

4. European Pharmacopoeia (2021). Strasbourg: European Directorate for the Quality of Medicines & Health Care. Available at: https://pheur.edqm.eu/subhome/10-8

5. State Pharmacopoeia of Ukraine. Vol. 1 (2015). Kharkiv: State Enterprise Ukrainian scientific pharmacopoeial centre of quality of drugs, 1128.

6. Anil, W., Subhash, G., Roshan, I., Badri, P. N. (2008). Validated liquid chromatographic method for simultaneous estimation of albendazole and ivermectin in tablet dosage form. Indian Journal of Chemical Technology, 15, 617–620.

7. Khanji, M., Kawas, G., Haroun, M., Rasheed, M. A., Sakur, A. A. (2020). Quantitative determination of albendazole forced degradation percentages by densitometric thin layer chromatographic method. Research Journal of Pharmacy and Technology, 13 (5), 2207–2213. https://doi.org/10.5958/0974-360x.2020.00396.0

8. Kumari, N., Singh, B. (2019). Quality By Design: A systematic approach for the development of analytical method validation. Journal of Drug Delivery and Therapeutics, 9, 1006–1012.

9. Fregonezi-Nery, M. M., Baracat, M. M., Kedor-Hackmann, É. R. M., Pinheiro, R. M. (2001). Determination of albendazole in oral suspension. Analytical Letters, 34 (8), 1255–1263. https://doi.org/10.1081/al-100104151

10. Swamy, N., Basavaiah, K. (2014). Simple and rapid spectrophotometric assay of albendazole in pharmaceuticals using iodine and picric acid as CT complexing agents. Brazilian Journal of Pharmaceutical Sciences, 50 (4), 839–850. https://doi.org/10.1590/s1984-82502014000400019

11. Refat, M. S., Mohamed, G. G., Fathi, A. (2011). Spectrophotometric Determination of Albendazole Drug in Tablets: Spectroscopic Characterization of the Charge-transfer Solid Complexes. Chinese Journal of Chemistry, 29 (2), 324–332. https://doi.org/10.1002/cjoc.201190086

12. Tella, A. C., Olabemiwo, O. M., Malwi, M. O., Obiyenwal, G. K. (2010). Developing a spectrophotometric method for the estimation of albendazole in solid and suspension forms. International Journal of Physical Sciences, 5, 379–382.

13. Ahmed, D. A., Abdel-Aziz, O., Abdel-Ghany, M., Weshahy, S. A. (2018). Stability indicating determination of Albendazole in bulk drug and pharmaceutical dosage form by chromatographic and spectrophotometric methods. Future Journal of Pharmaceutical Sciences, 4 (2), 161–165. https://doi.org/10.1016/j.fjps.2018.02.001

14. Ferencz, E., Kelemen, É.-K., Obreja, M., Sipos, E., Vida, S., Urkon, M., Szabó, Z.-I. (2021). Computer-assisted UHPLC method development and optimization for the determination of albendazole and its related substances. Journal of Pharmaceutical and Biomedical Analysis, 203, 114203. https://doi.org/10.1016/j.jpba.2021.114203

15. Saini, G., Singh, B., Vyas, M., Durgapal, S., Rangra, N., Suttee, A. (2024). RP-HPLC method development and validation of Albendazole and its impurity. BIO Web of Conferences, 86, 01046. https://doi.org/10.1051/bioconf/20248601046

16. Rashed, N. S., Zayed, S., Abdelazeem, A., Fouad, F. (2020). Development and validation of a green HPLC method for the analysis of clorsulon, albendazole, triclabendazole and ivermectin using monolithic column: Assessment of the greenness of the proposed method. Microchemical Journal, 157, 105069. https://doi.org/10.1016/j.microc.2020.105069

17. Varghese, S., Vasanthi, P., Ravi, T. (2011). Simultaneous densitometric determination of ivermectin and albendazole by high-performance thin-layer chromatography. Journal of Planar Chromatography – Modern TLC, 24 (4), 344–347. https://doi.org/10.1556/jpc.24.2011.4.13

18. Saleh, H., Schnekenburger, J. (1992). Determination of praziquantel and of praziquantel in tablets by gas–liquid chromatography. The Analyst, 117 (9), 1457–1460. https://doi.org/10.1039/an9921701457

19. Mainardes, R. M., Cinto, P. O., Gremião, M. P. D. (2006). High-performance Liquid Chromatography Determination of Praziquantel in Tablets and Raw Materials. Acta Farmacutica Bonaerense, 25 (4), 567–570.

20. Soto, C., Contreras, D., Orellana, S., Yañez, J., Toral, M. I. (2010). Simultaneous Determination of Albendazole and Praziquantel by Second Derivative Spectrophotometry and Multivariated Calibration Methods in Veterinary Pharmaceutical Formulation. Analytical Sciences, 26 (8), 891–896. https://doi.org/10.2116/analsci.26.891

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