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QUANTITATIVE SPECTROPHOTOMETRIC ANALYSIS OF THE MEDICINAL PRODUCT "METROKETOCONAZOLE"

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The results of experiments on the analysis of metronidazole and ketoconazole in a new medicinal product "Metroketoconazole" made on a titanium-containing basis ("Tizol" gel) are presented. Ultraviolet absorption spectra of ethanol solutions of ointment ingredients are presented. It is established that for quantitative determination it is necessary to use the wavelengths of 241 nm and 312 nm. Optimal conditions were selected and a method was developed for analyzing compounds in a model mixture with a relative error of ±1.47–1.73%. A procedure for quantitative spectrophotometric determination of metronidazole and ketoconazole in the studied soft dosage form with an error not exceeding the limits of permissible levels and deviations is proposed.

Keywords: ketoconazole, metronidazole, Tizol gel, quantitative analysis, spectrophotometry

Currently, the need for compounded ointments is increasing due to the growth of the nomenclature of finished pharmaceutical products and the development of factory pharmacy [1, 8]. In addition, new low-toxic ointment bases with pharmacological properties have appeared in practice. Such bases include the titanium-containing Tizol gel. We have proposed a soft dosage form consisting of 0.1 g of metronidazole, 0.1 g of ketoconazole and 9.8 g of Tizol gel, conventionally called Metroketoconazole. This medicine can be used as a promising chemotherapeutic and antifungal agent [6,7]. Due to the presence of the Tizol gel, the ointment will also have anti-inflammatory, local analgesic, antiseptic and antipruritic actions [3,5]. The gel, which has excellent intratissual conductivity, will bring metronidazole and ketoconazole to the affected area. Therefore, the Metroketoconazole ointment is of interest for physicians and patients. The introduction of new medicines into medical practice must be accompanied by the development of methods of analysis that allow us to determine the quality of their manufacture.

The purpose of the study is development of a method for the quantitative determination of metronidazole and ketoconazole in a soft dosage form on a titanium-containing base.

MATERIALS AND METHODS

Metronidazole substances (China, FS-000349, 2012), ketoconazole substances (India, FS-000507,

2013) were used in the work, the quality of which correspond to the regulatory documentation. As an ointment base, Tizol gel, produced by Olymp LLC (FSP 42-3157-06, Yekaterinburg), was used. The object of the study is Metroketoconazole in soft dosage form, containing 1.0% of metronidazole and ketoconazole in the Tizol gel. The experimental work was carried out by the method of spectrophotometry, which is a highly-demanded method in pharmaceutical analysis [2, 4], using a Russian-made spectrophotometer SF-2000 (OKB Spektr LLC, St. Petersburg) in quartz cells.

RESULTS AND DISCUSSION

As shown by experimental data, ethanol solutions of metronidazole and ketoconazole obey the basic absorption law, and their ultraviolet spectra (Fig. 1) are overlapped (λ=215–320 nm). It is difficult to quantify each medication, so we used the well-known method of K. Firordt, applied for the analysis of two-component mixtures.

According to this method, a system of equations is derived for the thickness of the working layer of 1 cm. The optical density in a mixture of the two compounds under study is written by the following equations:

$$A(\lambda_1) = \varepsilon_1(\lambda_1) \cdot C_1 + \varepsilon_2(\lambda_1) \cdot C_2,$$

$$A(\lambda_2) = \varepsilon_1(\lambda_2) \cdot C_1 + \varepsilon_2(\lambda_2) \cdot C_2,$$

where C_1 and C_2 – component concentrations, mol/l; $\varepsilon_1(\lambda_1)$, $\varepsilon_1(\lambda_2)$, $\varepsilon_2(\lambda_1)$, $\varepsilon_2(\lambda_2)$ – molar absorption coefficients at the wavelengths λ_1 and λ_2.

From the system of equations, the concentration of each component in the mixture was found according to the following formulas:

$$C_1 = \frac{\varepsilon_2(\lambda_2) \cdot A(\lambda_1) - \varepsilon_2(\lambda_1) \cdot A(\lambda_2)}{\varepsilon_1(\lambda_1) \cdot \varepsilon_2(\lambda_2) - \varepsilon_1(\lambda_2) \cdot \varepsilon_2(\lambda_1)},$$

$$C_2 = \frac{\varepsilon_1(\lambda_1) \cdot A(\lambda_2) - \varepsilon_1(\lambda_2) \cdot A(\lambda_1)}{\varepsilon_1(\lambda_1) \cdot \varepsilon_2(\lambda_2) - \varepsilon_1(\lambda_2) \cdot \varepsilon_2(\lambda_1)},$$

When developing a method for quantitative spectrophotometric analysis of metronidazole and ketoconazole in the ointment, the optimal wavelengths were selected and the molar absorption coefficients were calculated. To do this, a curve was constructed for the dependence of ε (ket) – ε (met) on the wavelength (Fig. 2). The extreme point on the curve is observed at a wavelength of 243 nm and located near the maximum absorption of ketoconazole (λ=241 nm) in an ethanol solution. In addition, the curve has a pronounced minimum at a wavelength of 312 nm, which is similar to the second maximum absorption of metronidazole. The results obtained give grounds to take the values of 241 nm and 312 nm as the optimal wavelengths.

To confirm the above data, a curve was constructed for the dependence of ε (ket)/ε (met) on the wavelength at which there is a maximum at λ=243 nm (Fig. 3). Therefore, λ=241 nm and λ=312 nm corresponding to the maxima in the absorption spectra of medicinal product were taken as the analytical wavelengths for

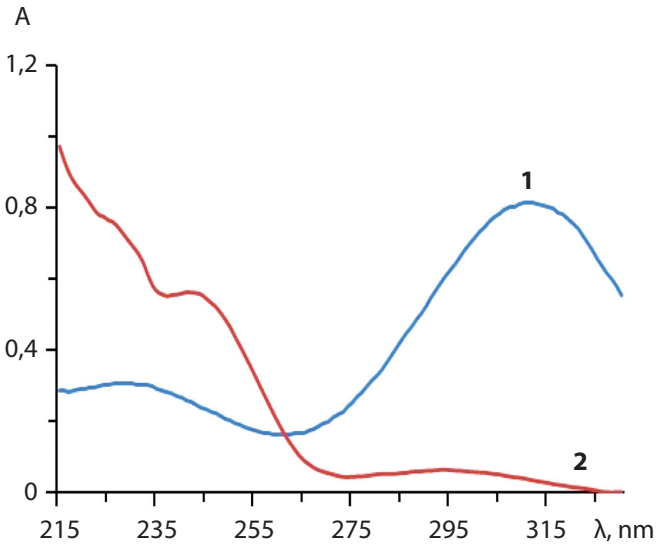

FIG. 1. *Absorption curves of metronidazole (1 – C=1,0 · 10⁻⁴ mol/L) and ketoconazole (2 – C=3,0 · 10⁻⁴ mol/L) in ethanol as a function of wavelength*

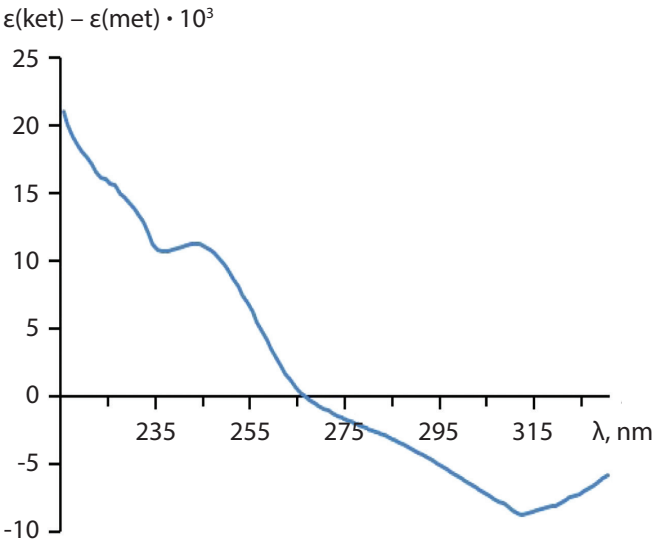

FIG. 2. *The curve of the dependence of ε(ket) – ε(met) on λ, nm*

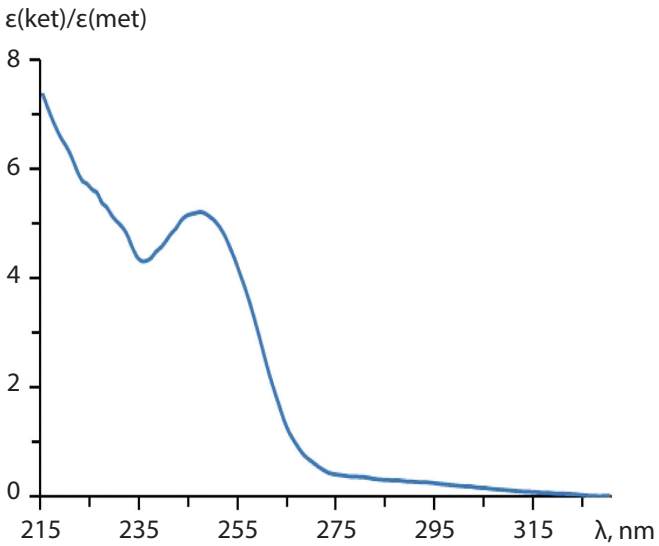

FIG. 3. *The curve of the dependence of ε(ket)/ε(met) on λ, nm*

the spectrophotometric analysis of metronidazole and ketoconazole in the mixture.

The quantitative determination of the ingredients of the soft dosage form by the spectrophotometric method was carried out at the selected wavelengths. For this purpose, the molar concentrations of ketoconazole were specified as C_1, the molar absorption coefficients – as $\varepsilon_1(241)$, $\varepsilon_1(312)$, and these parameters of metronidazole were specified as C_2, $\varepsilon_2(241)$, $\varepsilon_2(312)$. The system of K. Firordt's equations and the calculation of concentrations were expressed in the following form:

$$A(241) = \varepsilon_1(241) \cdot C_1 + \varepsilon_2(241) \cdot C_2,$$

$$A(312) = \varepsilon_1(312) \cdot C1 + \varepsilon_2(312) \cdot C_2,$$

$$C = \frac{\varepsilon_2(312) \cdot A(241) - \varepsilon_2(241) \cdot A(312)}{\varepsilon_1(241) \cdot \varepsilon_2(312) - \varepsilon_1(312) \cdot \varepsilon_2(241)},$$

$$C = \frac{\varepsilon_1(241) \cdot A(312) - \varepsilon_1(312) \cdot A(241)}{\varepsilon_1(241) \cdot \varepsilon_2(312) - \varepsilon_1(312) \cdot \varepsilon_2(241)}.$$

The calculated absorption coefficients of medicines at analytical wavelengths are shown in Table 1.

To develop a method for the spectrophotometric analysis of the components in the ointment, a model mixture containing 1.0% metronidazole and ketoconazole in ethyl alcohol was prepared. The analysis was carried out in the following way: 1 ml of the model mixture was introduced into a 50 ml volumetric flask and the volume of liquid in the flask was brought with ethanol up to the mark. Further, ethanol was added to 2 ml of the resulting mixture to obtain total volume of 25 ml and optical densities were measured at wavelengths of 241 nm and 312 nm using a spectrophotometer in a cell with a working layer thickness of 1 cm. The reference solution was ethanol.

Table 1

THE RESULTS OF CALCULATION OF MOLAR ABSORPTION COEFFICIENTS OF MEDICINES

Medical products	C, mol/L	A(241nm)	ε(241nm)	A(312nm)	ε(312nm)
Ketoconazole	$4.0 \cdot 10^{-5}$	0.563	14075	0.033	825
Metronidazole	$1.0 \cdot 10^{-4}$	0.259	2590	0.813	8130

Using the above formulas and the obtained values of optical densities, the concentration of ketoconazole (C_1) and metronidazole (C_2) was calculated and the mass fraction and mass of medicines in the model mixture were found:

$$m(med.) = \frac{C(med.) \cdot M(med.) \cdot V(total) \cdot V_2 \cdot V_3}{V \cdot V_1 \cdot 1000},$$

где W(med.) – mass fraction of a medicine, %; m(med.) – mass of a medicine, g; C(med.) – concentration of a medicine, mol/L; M(med.) – molar mass of metronidazole (171,16 g/mol) and ketoconazole (521,43 g/mol); V(total) – volume of a volumetric flask, 50 ml; V – volume of the mixture taken for analysis, 1 mL; V_1, V_2-dilution factor, 2 mL и 25 mL correspondingly; V_3 – total volume of the mixture, 10 mL; a(med.) – sample weight of medicinal product, g.

For the reliability of the experimental data, eight parallel determinations were performed, and the results were statistically processed (Table 2). The results of the studies showed that the relative error of the analysis of ketoconazole and metronidazole by the proposed spectrophotometric method is ±1.47% and 1.73%, respectively.

As experimental data have shown, ethanol solutions of ketoconazole practically do not absorb light at a wavelength of 312 nm at concentrations less than $4.0 \cdot 10^{-5}$ mol/L. This makes it possible to quantify metronidazole in the presence of ketoconazole, and the analysis of the two-component mixture is simplified. Therefore, the system of equations proposed above was expressed for $\varepsilon_1(312) = 0$ in the following form:

$$A(241) = \varepsilon_1(241) \cdot C_1 + \varepsilon_2(241) \cdot C_2,$$

$$A(312) = \varepsilon_2(312) \cdot C_2.$$

Molar concentrations of medical products were calculated using the following formulas:

$$C_1 = \frac{A(241) - \varepsilon_2(241) \cdot C_2}{\varepsilon_1(241)},$$

$$C_2 = \frac{A(312)}{\varepsilon_2(312)}.$$

Table 2

RESULTS OF STATISTICAL PROCESSING OF MEDICINE ANALYSIS DATA IN THE MODEL MIXTURE

No.	Obtained values				Metrological characteristics	
	Ketoconazole		Metronidazole			
	C, mol/L	W, %	C, mol/L	W, %		
1	$3.02 \cdot 10^{-5}$	98.42	$9.53 \cdot 10^{-5}$	101.95	Ketoconazole $\bar{\bar{w}} = 100.54\%$ $S = 1.766$ $S_{\bar{\bar{w}}} = 0.624$ $\varepsilon_\alpha = 1.48$ $A = \pm 1.47\%$ $\Delta = \bar{\bar{w}} \pm \varepsilon\alpha =$ $= 100.54 \pm 1.48\%$	Metronidazole $\bar{\bar{w}} = 100.26\%$ $S = 2.065$ $S_{\bar{\bar{w}}} = 0.730$ $\varepsilon_\alpha = 1.73$ $A = \pm 1.73\%$ $\Delta = \bar{\bar{w}} \pm \varepsilon\alpha =$ $= 100.26 \pm 1.73\%$
2	$3.13 \cdot 10^{-5}$	102.00	$9.19 \cdot 10^{-5}$	98.33		
3	$3.11 \cdot 10^{-5}$	101.35	$9.57 \cdot 10^{-5}$	102.41		
4	$3.02 \cdot 10^{-5}$	98.42	$9.19 \cdot 10^{-5}$	99.33		
5	$3.12 \cdot 10^{-5}$	101.68	$9.53 \cdot 10^{-5}$	101.95		
6	$3.13 \cdot 10^{-5}$	102.00	$9.19 \cdot 10^{-5}$	98.33		
7	$3.02 \cdot 10^{-5}$	98.42	$9.19 \cdot 10^{-5}$	98.33		
8	$3.13 \cdot 10^{-5}$	102.00	$9.57 \cdot 10^{-5}$	102.41		

Table 3

DATA FROM THE ANALYSIS OF MEDICINES IN THE MODEL MIXTURE

A(241)	A(312)	Concentration, mol/L		m_1(ket), r	m_2(met), r
		C_1(ket)	C_2(met)		
Firordt method					
0.68	0.80	$3.08 \cdot 10^{-5}$	$9.53 \cdot 10^{-5}$	0.1004	0.1019
0.70	0.80	$3.22 \cdot 10^{-5}$	$9.51 \cdot 10^{-5}$	0.1049	0.1017
0.70	0.85	$3.11 \cdot 10^{-5}$	$10.14 \cdot 10^{-5}$	0.1014	0.1085
0.69	0.80	$3.15 \cdot 10^{-5}$	$9.52 \cdot 10^{-5}$	0.1027	0.1018
0.70	0.84	$3.13 \cdot 10^{-5}$	$10.01 \cdot 10^{-5}$	0.1020	0.1071
Simplified Firordt method					
0.70	0.82	$3.12 \cdot 10^{-5}$	$10.09 \cdot 10^{-5}$	0.1017	0.1079
0.68	0.80	$3.02 \cdot 10^{-5}$	$9.84 \cdot 10^{-5}$	0.0984	0.1053
0.72	0.82	$3.26 \cdot 10^{-5}$	$10.09 \cdot 10^{-5}$	0.1062	0.1079
0.70	0.80	$3.16 \cdot 10^{-5}$	$9.84 \cdot 10^{-5}$	0.1030	0.1092
0.71	0.83	$3.17 \cdot 10^{-5}$	$10.21 \cdot 10^{-5}$	0.1033	0.1053

The masses of the study objects found in the model mixture are shown in Table 3. According to the results of parallel experiments, it was found that the content of ketoconazole (m_1), calculated by the Firordt method and a simplified system of equations, is in the range of 0.0984–0.1062 g, metronidazole (m_2) – 0.1017–0.1092 g, which corresponds to the permissible deviations in the mass of individual doses, presented in the Order of the Ministry of Health of the Russian Federation dated 26.10.2015 No. 751н "On approval of the rules for the manufacture and dispensing of medicines for medical use by pharmacy organizations, individual entrepreneurs licensed for pharmaceutical activities"

The method of analysis described above is proposed to be used for the quantitative determination of medicines in an ointment prepared on the basis of the Tizol gel. Procedure: in a glass chemical cup, place a sample of ointment of about 0.1 g (exact weight), add 25 ml of 95% ethanol solution, mix the mixture and filter it through a paper folded filter. After that, ethanol is added to 4 ml of the filtrate to obtain a total volume of 10 ml and the optical density of the solution is measured at wavelengths of 241 nm and 312 nm with respect to the reference solution (ethanol extract of the Tizol gel prepared in a similar way). According to the values of optical densities and molar absorption coefficients (Table. 1), calculate the molar concentrations of medicines using the above formulas. The content of metronidazole and ketoconazole in soft dosage form is calculated by the following formula:

$$m(med.) = \frac{C(med.) \cdot M(med.) \cdot V(total) \cdot V_2 \cdot P}{10^3 \cdot a(ointment) \cdot V_1},$$

where V(total) – the volume of ethanol in which the ointment weighed sample is dissolved, 25 ml; a (ointment) – the dosage form weighed sample, g; V_1, V_2 – the dilution factor, 4 ml and 10 ml, respectively; P – the mass of the dosage form, 10 g.

Error in the analysis of the studied medicines in the ointment (Table. 4) regardless of

Table 4

DATA FROM THE SPECTROPHOTOMETRIC ANALYSIS OF MEDICINES IN THE OINTMENT

Weighed sample		Obtained results				Permissible levels	
m(ointment), g	m(Tizol), g	C_1(ket), mol/L	C_2(met), mol/L	m_1(ket), g	m_2(met), g	g	%
Firordt method							
0.1039	0.1033	$3.11 \cdot 10^{-5}$	$10.14 \cdot 10^{-5}$	0.0975	0.1044	0.085–0.115	±15.0
0.1039	0.1033	$3.18 \cdot 10^{-5}$	$9.76 \cdot 10^{-5}$	0.997	0.1005		
0.1039	0.1033	$3.32 \cdot 10^{-5}$	$19.75 \cdot 10^{-5}$	0.1041	0.1004		
0.1039	0.1033	$3.23 \cdot 10^{-5}$	$9.88 \cdot 10^{-5}$	0.1013	0.1017		
Simplified Firordt method							
0.1042	0.1033	$3.12 \cdot 10^{-5}$	$10.09 \cdot 10^{-5}$	0.0976	0.1036	0.085–0.115	±15.0
0.1042	0.1033	$3.26 \cdot 10^{-5}$	$10.09 \cdot 10^{-5}$	0.1020	0.1036		
0.1042	0.1033	$3.09 \cdot 10^{-5}$	$10.21 \cdot 10^{-5}$	0.0966	0.1048		
0.1042	0.1033	$3.19 \cdot 10^{-5}$	$10.09 \cdot 10^{-5}$	0.0998	0.1036		

the method of calculation is within the permissible standard values in grams and deviations in percentages provided for in the regulatory documentation.

CONCLUSIONS

As a result of studying the optical properties of ethanol solutions of metronidazole and ketoconazole, it was found that their absorption spectra are overlapped.

The optimal conditions for the analysis were determined and the analytical wavelengths of 241 nm and 312 nm were selected for the quantitative spectrophotometric determination of medicines in the prescription using the K. Firordt method.

Studies on the analysis of the model mixture were carried out and a method was developed that allows quantifying the studied compounds with a relative error of ±1.47–1.73%.

A method of spectrophotometric analysis of metronidazole and ketoconazole in a soft dosage form on a titanium-containing basis with an error not exceeding the standard deviations is proposed

The developed method for the analysis of metronidazole and ketoconazole in a soft dosage form such as Metroketoconazole can be recommended for inclusion in the regulatory documentation for establishing the quality of ointment manufacturing.

REFERENCES

1. *G.V. Ayupova. Modern aspects of the development and improvement of the composition and technology of dosage forms // Medical Bulletin of Bashkortostan. 2016. – No. 11 (5). – pp. 87–90.*
2. *V.G. Belikov. Analysis of medicinal substances by photometric methods. Experience of work*

of domestic specialists // Russian Chemical Journal. 2002. – No. 46 (4). – pp. 52–56.

3. *L.V. Dyachkova. Study of the structural and mechanical properties of ointment bases // Bulletin of pharmacy. 2012. – No.3. – pp. 23–28.*
4. *T.G. Evstafieva, N.S. Bessonova, T.A. Kobeleva, A.I. Sichko. The use of spectrophotometry in the analysis of the new drug "Metatetrazol" // Health and education in the XXI century. 2018. – No. 20 (12). – pp. 55–59.*
5. *T.G. Evstafieva, N.N. Bacheva, N.S. Bessonova, T.A. Kobeleva, A.I. Sichko. Application of spectrophotometric analysis for establishing osmotic and transcutaneous activity of new dosage forms "Metamiosol" and "Phenylbutazol" // Medical science and education of the Ural. 2018. – No. 19 (3). – pp. 56–62.*
6. *M.V. Makhotina, A.Yu. Petrov. Comparative analysis of processes of compounded preparation of Tizol- based ointments // Fundamental science and technologies – perspective developments: materials of the XIV international scientific and practical conference. – North Charleston, USA, 2018. – 2. – pp. 96–98.*
7. *N.M. Nasybullina. Creation of dosage forms of anti-inflammatory and antimycotic agents based on liposomes for external use // Health as the basis of human potential: problems and ways to solve them. 2010. – No.5 (1). – p. 370.*
8. *R.R. Sabirzhan, S.N. Egorova. Pharmacy's manufacturing of dosage forms for medical and preventive institutions: the study of modern nomenclature // Scientific Bulletin of the Belgorod State University. 2012. – No. 18/2 (10). – pp. 31–35.*