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DEVELOPMENT AND VALIDATION OF A PROCEDURE FOR QUANTITATIVE ANALYSIS OF MOXIFLOXACIN IN THE "MOXIFLOXAZOL" MEDICINE

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In this work, the absorption spectra of moxifloxacin in ethanol were studied and it was found that quantitative analysis of the medical product should be rationally carried out at λmax = 296 nm. Experimental data on the quantitative analysis of moxifloxacin in the "Moxifloxazol" medicine are presented. The relative error of the analysis is not higher than ±1.82%. The sensitivity of the determination of moxifloxacin is 0.213 μg /ml. The developed procedure was validated according to the following validation characteristics: specificity, linearity, correctness and precision. The moxifloxacin content in the ointment is calculated by the method of the calibration graph equation. The calculated content is within the range of 0.0482–0.0544 g and corresponds to the standards of permissible deviations.

Keywords: moxifloxacin, Tizol gel, quantitative analysis, UV spectrophotometry, validation

Moxifloxacin is a chemotherapeutic agent of the fluoroquinolone group with a wide spectrum of bactericidal action. Due to the ability to suppress microorganisms which are resistant to other antibiotics, the relevance of expanding the nomenclature of dosage forms that include it increases [8,9]. The substance of moxifloxacin hydrochloride is represented by crystalline powder from light yellow to yellow and characterized by moderate solubility in water, low solubility or very low solubility in 96% alcohol. Currently, the European Pharmacopoeia recommends using the high-performance liquid chromatography (HPLC) analysis for quantitative determination of the moxifloxacin hydrochloride substance and its dosage forms [10]. This method is also regulated by the draft pharmacopoeial monograph "PM Moxifloxacin hydrochloride", considered at the Council of the Ministry of Health of the Russian Federation on the State Pharmacopoeia. In addition, a number of studies describe the possibility of using the UV spectrophotometry method for quantitative analysis of moxifloxacin [2,6].

We offer an ointment under the conventional name "Moxifloxazol" containing 0.05 g of moxifloxacin and Tizol gel up to 10.0 g. The medicine may be in demand in the treatment of a number of dermatological, dental and ophthalmological

diseases caused by pathogenic microflora. The modern low-toxic base "Tizol" will contribute to the increased conductivity of the medicine to the lesion, as well as provide anti-inflammatory, antiseptic, antipruritic and analgesic effects [5]. When developing new medicines, the "Quantitative Determination" test is important, which allows assessing the quality of the finished medical product [3,4].

The purpose of this work is to develop and validate the procedures of quantitative analysis of moxifloxacin in the "Moxifloxazol" medicine.

MATERIALS AND METHODS

The moxifloxacin hydrochloride substance (Neuland Laboratories Limited, India, PM 000-715-081118, 2018), titanium-containing Tizol gel (Olympus LLC, Yekaterinburg, Russia, FSP 3157-06), solutions of moxifloxacin in 95% ethyl alcohol (RFK CJSC, Russia, FS.2.1.0105.18), hydrochloric acid 0.01 mol/l (Bashkir Soda Company, Russia), ointment under the conventional name "Moxifloxazol" containing 0.5% of the medicine in the Tizol gel were used in this study. The study was carried out using a spectrophotometer SF-2000 (OKB SPECTRUM CJSC, St. Petersburg, Russia).

Quantitative determination of moxifloxacin was carried out by an easy-to-perform UV spectrophotometric method, which is used in the analysis of the medicine in tablets and is not inferior to chromatography in accuracy. The weight percent and the weight of moxifloxacin in grams were calculated using a calibration graph.

When constructing the calibration graph, a 0.02% standard solution (reference standard) of the medicine in ethyl alcohol was prepared. Then a variable amount of milliliters (from 0.2 ml to 1.2 ml) of the test solution was transferred to a measuring flask with capacity of 25 ml and the volume of liquid in the flask was brought to the mark with ethanol. The optical densities of the obtained solutions were measured using a spectrophotometer at a wavelength of 296 nm. Based on experimental data, a calibration line was constructed in coordinates A – C, μg/ml (Fig. 2). To obtain objective results of the analysis, eight parallel experiments were conducted using 0.6 ml of the initial solution. The moxifloxacin content as a percentage was calculated using the formula (1):

$$W = \frac{C(mox) \cdot V(total) \cdot V_2 \cdot 100}{10^6 \cdot a(mox) \cdot V_1}, \quad (1)$$

where C(mox) – the concentration of moxifloxacin, calculated by the equation of the calibration graph, μg /ml; V(total) – the volume of ethyl alcohol containing the weight of moxifloxacin, 100 ml; V_1, V_2 – dilution factor, 0.6 ml and 25 ml; a(mox) – moxifloxacin weighed sample, 0.02 g.

The model mixture was prepared taking into account the solubility of moxifloxacin in ethyl alcohol (moxifloxacin 0.05 g, ethanol 200 ml). Research procedure: 4 ml of ethanol solution is introduced into a measuring flask and the volume of liquid in the flask is brought to 25 ml with ethanol. Next, ethanol is added to 3 ml of the resulting solution to a total volume of 25 ml. The optical density of the solution is measured with respect to ethanol at a wavelength of 296 nm. The weight of moxifloxacin in the simulated solution is found by the formula (2):

$$m(mox) = \frac{C(mox) \cdot V(total) \cdot V_2 \cdot V_3}{10^6 \cdot V \cdot V_1}, \quad (2)$$

where m(mox) – the weight of moxifloxacin, g; V(total) – the volume of the simulated solution, 200 ml; V – the volume of the simulated solution taken for analysis, 4 ml; V1, V2, V3 – reciprocal dilution, 3 ml, 25 ml, 25 ml, respectively.

Quantitative analysis of moxifloxacin in the ointment "Moxifloxazol" was carried out as follows: 4 ml of 0.01 mol/l hydrochloric acid

solution and ethanol were added to the ointment weighed sample (about 0.10 g) to obtain a total volume of 30 ml. The mixture was stirred and filtered through a folded filter with discarding the first portion of the filtrate. Then 7 ml of ethanol was added to 3 ml of the resulting solution and the mixture was scanned photometrically at a wavelength of 296 nm. The compensation solution was an ethanol extract from 0.10 g of Tizol gel, obtained similarly to the study of moxifloxacin. The concentration of the medicine in the sample (μg/ml) was found by the equation of the calibration graph, and the weight percent and the weight in ointment were calculated by the formulas (3, 4):

$$m(mox) = \frac{C(mox) \cdot V(total) \cdot V_2 \cdot P}{10^6 \cdot a(ointment) \cdot V_1}, \quad (3)$$

$$W = \frac{C(mox) \cdot V(total) \cdot 100 \cdot V_2}{10^6 \cdot a(ointment) \cdot V_1}, \quad (4)$$

where a(ointment) is the weighed sample of ointment taken for analysis, g; P is the weight of the dosage form, 10.0 g; V_1, V_2 is the dilution factor, 3 ml and 10 ml, respectively; V(total) is the volume of ethyl alcohol containing the weighed sample of ointment, 30 ml.

RESULTS AND DISCUSSION

The conducted spectral analysis showed that for quantitative spectrophotometric determination of moxifloxacin in the ointment "Moxifloxazol" it is rationally to use absorption bands within the wavelengths of 280–310 nm with maximum absorption λ = 296 nm (Fig. 1)

As experimental data have shown, the similar maxima and minima are observed on the ethanol absorption spectra of moxifloxacin together with the Tizol gel, as in the absence of a base (Fig. 1, curves 2, 3). Extreme points also coincide on the absorption spectrum of the ethanol extract of moxifloxacin from ointment (curve 4).

Validation of the procedure was carried out according to the State Pharmacopoeia of the Russian Federation of the XIV edition, OFS.1.1.0012.15 "Validation of analytical procedures".

Specificity

To determine the specificity using a spectrophotometer, the spectral data on placebo solutions (ethanol extract of Tizol gel and 95% ethanol) and a standard moxifloxacin solution were sequentially collected, according to the quantitative analysis procedure. The obtained spectra did not contain the peaks which were characteristic of the standard moxifloxacin solution (Fig. 1, curves 5 and 6).

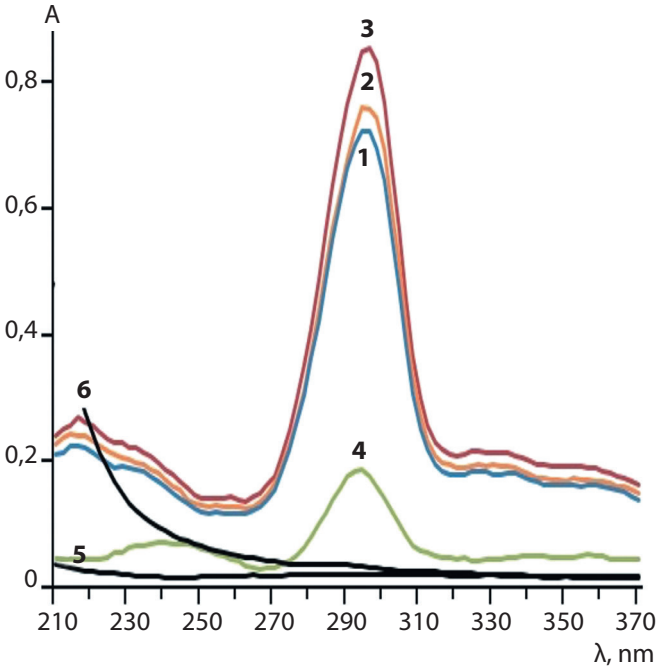

FIG. 1. *Dependence of light absorption of ethanol solutions of moxifloxacin and placebo solutions on wavelength: 1 – moxifloxacin concentration 2.0×10^{-5} mol/L; 2 – moxifloxacin concentration 2.0×10^{-5} mol/L; Tizol gel 5.0×10^{-5} mol/L; 3 – moxifloxacin concentration 2.0×10^{-5} mol/L; Tizol gel 1.0×10^{-4} mol/L; 4 – ethanol extract of moxifloxacin from ointment ($C = 4.3\times10^{-6}$ mol/L); 5 – ethanol extract of Tizol gel 4×10^{-5} mol/L; 6 – 95% ethanol*

Table 1

RESULTS OF THE REGRESSION ANALYSIS OF MOXIFLOXACIN

x_i, µg/ml	y_i	x_iy_i	x_i^2	y_i^2	b	C, µg/ml
1.6	0.15	0.24	2.56	0.023	0.0938	0.213
3.2	0.30	0.96	10.24	0.090		
4.8	0.45	2.16	23.04	0.203		
6.4	0.60	3.84	40.96	0.360		
8.0	0.75	6.00	64.00	0.563		
9.6	0.90	8.64	92.16	0.810		
33.6	3.15	21.84	232.96	2.049		

Linearity

In order to establish the linearity of the procedure, the optical densities of moxifloxacin solutions were experimentally measured in the range of 1.6 µg/ml – 9.6 µg/ml. At least five parallel experiments were carried out, on the basis of which the regression analysis indicators were calculated (Table 1). The statistical insignificance of the free term of the linear dependence was estimated. Linearity was considered optimal at values of the correlation coefficient $|r| \geq 0.99$ (Table 2).

It was found that the sensitivity of the moxifloxacin assay is 0.245 µg/ml, the value of the correlation coefficient satisfies the condition $|r| \geq 0.99$. The value of the free term of the linear dependence is less than its confidence interval, which gives reason to proceed to the equation of a straight line passing through the origin (Table 2).

According to the data obtained (Table. 1) we built a calibration graph. There is a direct relationship between the concentration of moxifloxacin and the optical density (Fig. 2). This indicates the possibility of analyzing moxifloxacin by UV spectrophotometry in ointment.

Accuracy and precision

The repeatability (convergence) of the validated procedure was evaluated using model mixtures of moxifloxacin under the same laboratory conditions during a short period of time based on the results of eight parallel experiments. Intra-laboratory precision was determined on different days with the participation of two researchers (analysts). The obtained data were statistically processed (Table 3).

The obtained values of the standard deviation (precision) and relative error (accuracy) do not exceed the limits of 100±2,0%.

Analytical range

The interval between the upper and lower values of the moxifloxacin concentration, within which the acceptable accuracy, precision

Table 2

MOXIFLOXACIN REGRESSION EQUATION IN UV SPECTROPHOTOMETRY

Regression equation	Correlation coefficient	$\|a\| \leq t(P; f) \cdot S_a$ at P = 95%	Equation of a straight line
y = 0.0938x – 0.00028	0.9981	0.00028 < 0.043	y = 0.0938x

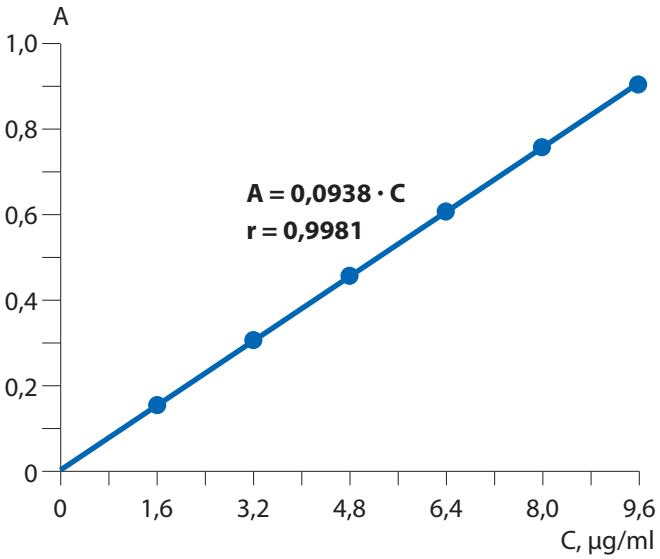

FIG. 2. *Calibration graph for the analysis of moxifloxacin*

and linearity of the procedure have been proven, ranges from 1.6 μg/ml to 9.6 μg/ml.

During the study, the moxifloxacin quantitative analysis procedure in a model dosage form was tested. Experimental data are specified in Table 4.

The weight of moxifloxacin in the simulated solution is from 0.0483 g to 0.0510 g with an acceptable range of 0.040–0.060 g [7].

Quantitative analysis of moxifloxacin in the ointment "Moxifloxazol" was carried out in an ethanol extract obtained from an exact weighted sample. Experiments have shown that about 22% of the medicine passes into the organic phase due to its low solubility in ethanol, while the weight fraction (%) increases in the presence of acid. To determine the optimal conditions for quantitative analysis, a model ointment with an exact content of moxifloxacin and a base was prepared. The studies were carried out in the presence of various volumes of 0.01 mol/l hydrochloric acid solution injected into ethanol. The results of the experiments are shown in Table 5.

Table 3

RESULTS OF THE EVALUATION OF THE ACCURACY AND PRECISION OF THE MOXIFLOXACIN SPECTROPHOTOMETRIC ANALYSIS PROCEDURE

First day			Second day			Metrological characteristics
A	Found		A	Found		
	C, μg/ml	x_i (W), %		C, μg/ml	x_i (W), %	
0.460	4.90	102.08	0.445	4.74	98.75	**First day** x = 100.05% S = 2.182 Sx = 0.772 ε_α = 1.82 A = ±1.82% Δ = 100.05 ± 1.82% **Second day** x = 100.08% S = 1.984 Sx = 0.702 ε_α = 1.66 A = ±1.66% Δ = 100.08 ± 1.66%
0.445	4.74	98.75	0.460	4.90	102.08	
0.450	4.80	100.0	0.440	4.69	97.71	
0.440	4.69	97.71	0.460	4.90	102.08	
0.445	4.74	98.75	0.440	4.69	97.71	
0.440	4.69	97.71	0.445	4.74	98.75	
0.465	4.96	103.33	0.460	4.90	102.08	
0.460	4.90	102.08	0.455	4.85	100.97	

Table 4

THE RESULTS OF THE ANALYSIS OF MOXIFLOXACIN IN A MODEL DOSAGE FORM BY THE METHOD OF THE EQUATION OF THE CALIBRATION GRAPH (A = 0,0938 · C)

№	Optical density	Weight, μg/ml	Found		Acceptance criteria	
			%	g	%	g
1	0.440	4.69	0.49	0.0489	±20.0	0.040–0.060
2	0.450	4.80	0.50	0.0500		
3	0.435	4.64	0.48	0.0483		
4	0.460	4.90	0.51	0.0510		
5	0.445	4.74	0.49	0.0494		
6	0.455	4.85	0.51	0.0505		

It has been found that when 4 ml of 0.01 mol /l hydrochloric acid solution is injected into the test solution, about 100% of moxifloxacin passes from the Tizol gel into an aqueous ethanol medium. We have accepted these analysis conditions as optimal.

According to the conducted research, a procedure of quantitative analysis of moxifloxacin in the ointment "Moxifloxazol" has been developed. The content of moxifloxacin in the ointment is in the range of 0.0482–0.0544 g (Table 6).

Table 5

DATA ON THE CHOICE OF OPTIMAL CONDITIONS FOR THE ANALYSIS OF MOXIFLOXACIN (A = 0,0938 · C)

№	Sampled				Optical density	Found W, %
	m (ointment), g	m (Tizol), g	Volume of 0,01 mol/L HCl, mL	Volume of ethanol, mL		
1	0.1033	0.1041	0.0	30.0	0.11 0.12	22.60 24.76
2	0.1023	0.1041	1.0	29.0	0.32 0.33	66.60 68.80
3	0.1047	0.1041	2.0	28.0	0.42 0.44	85.60 89.60
4	0.1056	0.1041	3.0	27.0	0.46 0.46	92.80 92.80
5	0.1030	0.1041	4.0	26.0	0.48 0.50	99.40 103.60
6	0.1017	0.1041	5.0	25.0	0.62 0.60	129.80 125.80

Table 6

RESULTS OF QUANTITATIVE ANALYSIS OF MOXIFLOXACIN IN OINTMENT BY THE METHOD OF EQUATION OF THE CALIBRATION GRAPH (A = 0,0938·C)

Sampled, g		Test results				Standard deviations	
ointment	Tizol	A	C, μg/ml	m, g	W, %	g	%
0.1038	0.1015	0.53	5.65	0.0544	0.54	0.040–0.060	±20.0
0.1038	0.1015	0.52	5.54	0.0534	0.53		
0.1038	0.1015	0.48	5.12	0.0493	0.49		
0.1038	0.1015	0.50	5.33	0.0513	0.51		
0.1038	0.1015	0.47	5.01	0.0482	0.48		
0.1038	0.1015	0.49	5.22	0.0503	0.50		

CONCLUSIONS

As a result of studying the ethanol absorption spectra of moxifloxacin, the optimal conditions for quantitative analysis of the semi-solid medicine have been established.

Based on experimental data, a procedure for quantitative analysis of moxifloxacin in a model mixture with a relative error of ± 1.82% is proposed.

The developed procedure for quantitative analysis of moxifloxacin in ointment based on the Tizol gel was validated according to the following validation characteristics: specificity, linearity, accuracy and precision. The studied validation characteristics meet the acceptance criteria.

The proposed procedure of quantitative analysis of moxifloxacin in the ointment "Moxifloxazol" shall be used rationally for inclusion in the regulatory documentation to assess the quality of the finished medicine.

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