Development and Validation of UV-Spectrophotometric Method for Quantitative Assessment of Metronidazole in the «Metromed® Neo» Solution for Infusion

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Abstract

Introduction: Metronidazole is widely used in medicine and issues of quality control of its dosage forms are relevant. Objective - development and validation of a UV spectrophotometric method for monitoring the quality of metronidazole in the «Metromed® Neo» solution for infusion.

Materials and Method: The object of the study was a solution for infusion containing metronidazole. The method used is UV spectrophotometry.

Results: The relative error of the developed UV spectrophotometric method for the quantitative assessment of metronidazole in the solution for infusion was 1.9%. Validation characteristics of the method are within acceptable limits.

Conclusion: A UV spectrophotometric technique was developed and validated to assess the quality of the solution for infusion containing metronidazole. The results of the studies make it possible to use the developed and validated methodology in assessing the quality of metronidazole.

Keywords: *Metronidazole, UV spectrophotometry, solution for infusion, quality assurance, validation.*

Introduction

Antibacterial therapy is one of the most important components of intensive therapy of purulent-septic diseases, and its effectiveness has a significant impact on the course and outcome of the disease. Of particular interest is metronidazole. The mechanism of action of metronidazole is the biochemical reduction of the 5-nitro group of metronidazole by intracellular transport proteins of anaerobic microorganisms and protozoa [1].

In the US Pharmacopoeia, for the assessment of the authenticity and quantitative content of metronidazole in both substance and injectable dosage form[2], a method of high-performance liquid chromatography was proposed[3]. Existing method for assessing the quality of metronidazole do not always meet modern requirements. They have several disadvantages: the complexity, the duration of the implementation, the use of toxic organic solvents, expensive reagents and devices. This suggests that the problem of improving existing and developing new method for the analysis of metronidazole is relevant[4].

Spectrophotometry as a physicochemical method is characterized by high sensitivity and expressiveness. The development of simple, affordable, and highly accurate method for controlling the quality of drugs is essential for pharmaceutical manufacturers[5]. However, it should be noted that in order to confirm and ensure the quality of pharmaceutical products, the control method used must be validated. Currently, on the validation of analytical works, there are a number of regulatory documents, as well as scientific works, which describe in detail the conditions for the validation of analytical method [7].

Aim of the study was to develop and validate a UV spectrophotometric method for monitoring the quality of metronidazole in the Metromed® Neo infusion solution.

Materials and Method

Experimental Part

The infusion solution containing metronidazole has the following composition:

Composition:

Metronidazole 500 mg

(Brit. F., Heb. F,)

Sodium Chloride 800 mg

(Brit. F., Heb. F., FS 42 Uz-0129-2011)

Water for injection up to 100 ml

(FS 42 Uz-0512-2012)

Research on the development of metronidazole quality control method began with a study of the physicochemical properties of the substance.

Metronidazole or 2- (2-methyl-5-nitroimidazol-1-yl) ethanol is a white to white powder with a greenish-yellow tint, slightly soluble in water, acetone and alcohol 96%.

Results

Preparation of a working standard sample of metronidazole: 40 mg of metronidazole is placed in a 100 ml volumetric flask, a 0.1 M solution of hydrochloric acid is added, and the volume of the solution is adjusted with the same solvent. 5 ml of the resulting solution is transferred into a volumetric flask with a capacity of 100 ml, the volume of the solution is adjusted with the same solvent. The UV spectrum of the resulting solution was determined in the wavelength range from 230 nm to 350 nm in a cell with a layer thickness of 1 cm (Fig. 1).

The above spectrum indicates that metronidazole has a maximum absorption at a wavelength of 277 nm.

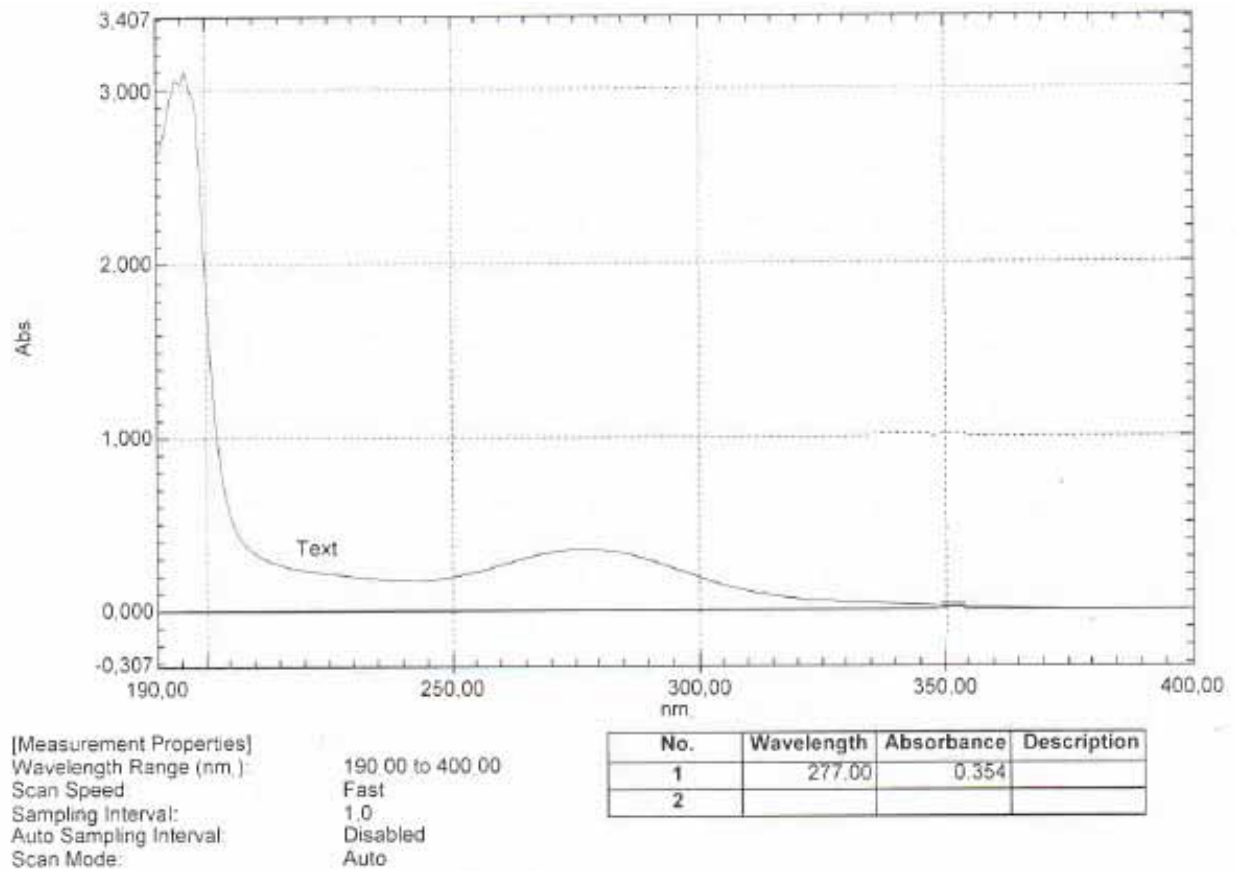

Fig. 1. UV spectrum of OCR metronidazole

To quantify metronidazole in the infusion solution, a test solution of metronidazole was previously prepared.

10 ml of the test solution of metronidazole was transferred into a volumetric flask with a capacity of 100 ml, the volume of the solution was brought to the mark with a 0.1 M hydrochloric acid solution, and stirred. 5 ml of the resulting solution was transferred into a volumetric flask with a capacity of 250 ml and the volume of the solution was adjusted to the mark with a 0.1 M hydrochloric acid solution and stirred. The optical density of the resulting solution was measured at a wavelength of 277 nm, using a 0.1 M hydrochloric acid solution as a reference solution.

The content of metronidazole in grams was calculated by the formula:

$$X = \frac{D \cdot 1 \cdot 100 \cdot 250}{375 \cdot 100 \cdot 0 \cdot 5} = \frac{D \cdot 5}{375},$$

where D is the optical density of the test solution.

375 - specific absorption rate of 1% solution of metronidazole.

The content of metronidazode (C6H9N3O3) in 1 ml of the drug should be from 0.0045 g to 0.0055 g.

Table 1: Metrological characteristics of the results of the quantitative assessment of metronidazole in the drug «Metromed® Neo», solution for infusion 500mg/100ml

The content of active substance, mg/100 ml	The limit of active substances in mg/ml	The average value of the quantity found, Xav	Sample Deviation, S	Relative Standard Deviation, RSD	Relative error, ε cf
Metronidazole					
500 mg	from 4.5 mg to 5.5 mg in 1 ml of the drug	4,720	0,1257	1,0%	1,9688
		4,759			
		4,773			

The results presented in table 1, indicate the reliability of the data.

Discussion

The next stage of the research was the validation of the developed UV spectrophotometry technique. Validation parameters were - correctness; precision (convergence, reproducibility), linearity, specificity.

1. The correctness of the analytical method characterizes the proximity of the test results obtained by this method to the true value.

Correctness was evaluated on the basis of at least 9 determination results at a minimum of 3 concentration levels in the limit of the analytical region (for example, 3 replicates of the determination for 3 analytical concentrations). Prepared solutions containing metronidazole in the amount of 80%, 100%, 120% of the declared value. The optical density of metronidazole in all mixtures was determined in three parallel determinations, and the quantitative determination of the active substance in each solution was calculated.

Acceptance criterion: the percentage of recovery when using concentrations of 80, 100 and 120%, adjusted to 100%, should be in the range from 98.0% to 102.0%.

Recovery percentage (R):

$$R = \frac{A}{B} * 100\%$$

where R (Recovery) - output, A – measured content, B-specified (actual) level.

The results of the analysis are presented in the table:

Table 2: Correctness of the UV spectrophotometric method for the quantitative determination of metronidazole in the drug «Metromed® Neo», 500mg/100ml solution for infusion

The claimed amount of metronidazole in test solutions, mg/ml	Found amount of metronidazole in test solutions, mg	Regeneration, %	Metrological characteristic
3,3	3,31	100,3	X_{av} = 99,0; S = 0,96; S^2 = 0,92; Δx = 2,22; Δx av = 0,74; E_{av} = 0,74
3,3	3,29	99,9	
3,3	3,28	99,3	
5,0	4,95	99,0	
5,0	4,88	97,6	
5,0	4,99	99,8	
6,0	5,97	99,5	
6,0	5,88	98,0	
6,0	5,88	98,0	
The average percentage of regeneration		99,0%	

From the results of the table it is seen that the average percentage of metronidazole regeneration is 99.0%, which indicates satisfactory correctness of the method.

2. The precision of the analytical method characterizes the degree of closeness of the independent results of individual tests obtained in specific specified conditions. This characteristic depends only on random factors and is not related to the true value of the measured quantity. It is expressed by the standard deviation - the coefficient of variation. Extreme precision indicators - convergence and reproducibility.

2.1. Convergence characterizes the degree of consistency of the results of measurements (tests) obtained by the same method at identical test objects, in the same laboratory, by the same operator, using the same equipment, within a short period of time.

Acceptance criterion: the coefficient of variation of parallel determinations for 6 measurements should be no more than 2%.

Table 3: The convergence of the UV spectrophotometric method for the quantitative determination of metronidazole in the drug "Metromed® Neo", solution for infusion 500mg/100ml

Solution No.	Found amount of metronidazole in test solutions, mg/ml	Arithmetic mean of all definitions, $\bar{X}$	Standard deviation, S	Coefficient of variation, CV
1	4,77	4,96	0,093	1,8
2	4,99			
3	5,00			
4	5,00			
5	5,01			
6	4,98			

The results presented in table 3 indicate a high convergence of the obtained data.

2.2. Reproducibility characterizes the measure of coincidence of the measurement results obtained by the same method on identical samples, in different laboratories, by different operators, using different equipment, a relatively long period of time between measurements, separate, presumably identical samples taken from the same batch of material.

Acceptance criterion: the coefficient of variation in each of the levels of accuracy is determined. The criterion of instrumental reproducibility is 1%, intralaboratory reproducibility is 2% for the quantitative determination of the main component.

The method for the quantitative determination of metronidazole in the analyzed medicinal product was tested in two laboratories:

1. Laboratory of expertise and standardization of medicines JV LLC Remedy Group.
2. Scientific laboratory LLC Medstandart.

The results of the analysis are presented in the table:

Table 4: Reproducibility of the UV spectrophotometric method for the quantitative determination of metronidazole in the drug «Metromed® Neo», 500mg/100ml solution for infusion

Lab Name	Solution No.	Found amount of metronidazole in test solutions, mg	Arithmetic mean of all definitions, $\bar{X}$	Standard deviation, S	Coefficient of variation, CV
Laboratory of Expertise and Standardization of Medicines JV Remedy Group LLC.	1	4,77	4,96	0,0928	1,82
	2	5,00			
	3	4,99			
	4	5,00			
	5	4,98			
	6	5,01			
Scientific laboratory LLC Medstandart.	1	4,86	4,92	0,075	1,6
	2	4,88			
	3	4,85			
	4	4,9			
	5	5,02			
	6	5,01			

The calculated values of the coefficient of variation are within acceptable limits and indicate the reproducibility of the developed technique.

3. Linearity is established on the basis of test results that are proportional to the concentration of the analyte in the sample within the analytical method. The linearity of the results can be represented graphically in the form of a dependence of analytical signals on the concentration of a substance (at least 5). The following parameters are used to confirm the linearity of the analytical technique: the regression coefficient and the slope of the regression line.

The determination is usually carried out on solutions with concentrations of 80, 90, 100, 110 and 120%.

Acceptance criterion: the technique is considered linear if the correlation coefficient between a series of obtained values is not lower than 0.995.

The results of the study of linearity must be indicated in the graphic image.

Table 5: Linearity of the UV spectrophotometric method for the quantitative determination of metronidazole in the drug «Metromed® Neo», 500mg/100ml solution for infusion

№	The concentration of the solution,%	The concentration of the solution, mg/ml	Optical density value	Correlation coefficient, R
1	80	0,008	0,283	0,9993
2	90	0,009	0,315	
3	100	0,010	0,354	
4	110	0,011	0,389	
5	120	0,012	0,420	

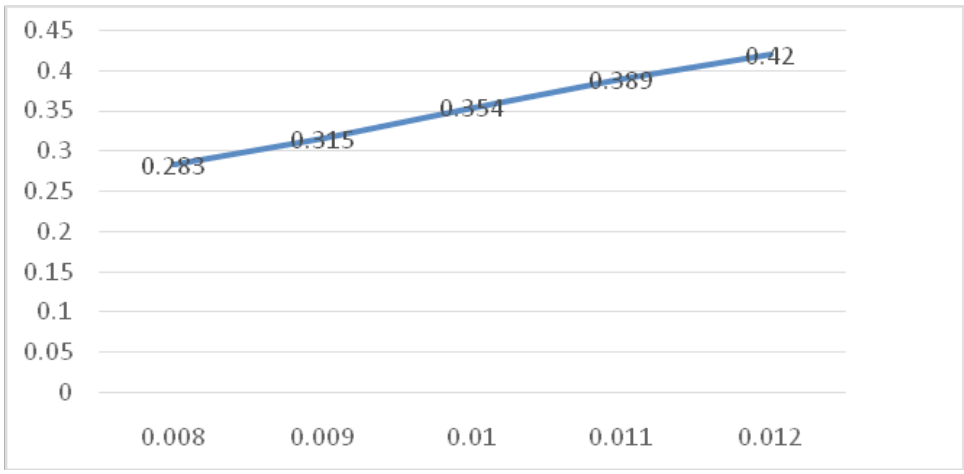

Fig. 2. The linear dependence of the optical density of metronidazole on its concentration in the test solution

From the data presented in table 5 and the graphic image, we can conclude that there is a linear dependence in a given concentration range. The value of corelation is within the acceptable range.

3. The specificity of the analytical method is determined by its ability to reliably determine the drug substance in the presence of impurity compounds, degradation products and excipients.

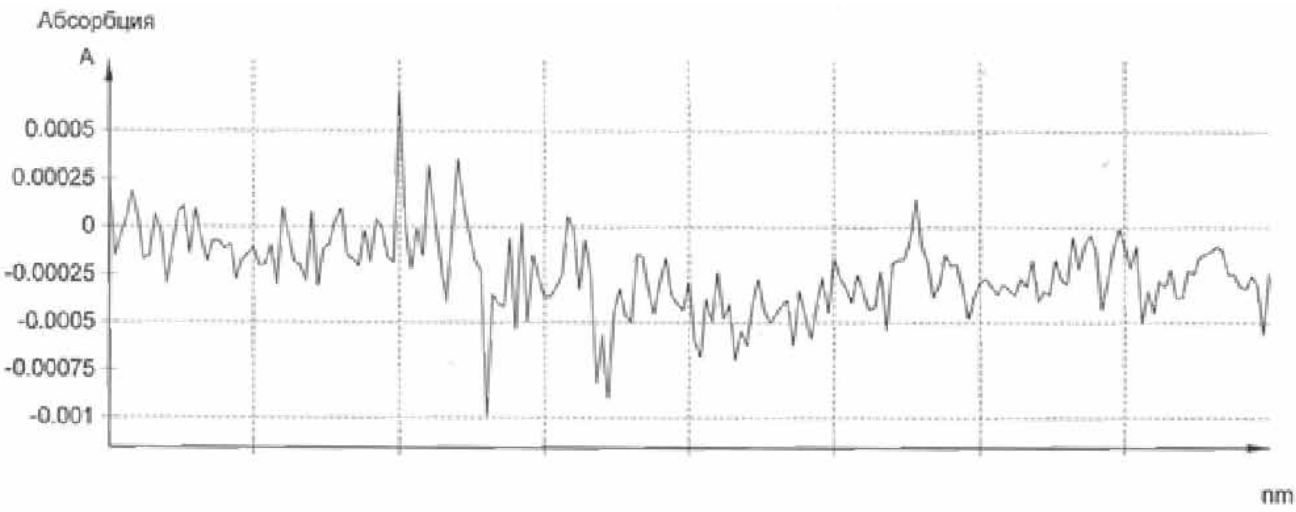

Fig. 2. The placebo absorption spectrum in the range from 250 nm to 320 nm

Preparation of a placebo solution: 800 mg of sodium chloride salt is dissolved in water for injection up to 100 ml. 10 ml of the resulting solution was transferred into a volumetric flask with a capacity of 100 ml, the volume of the solution was adjusted to the mark with a 0.1 M hydrochloric acid solution, and stirred. 5 ml of the resulting solution was transferred into a volumetric flask with a capacity of 250 ml and the volume of the solution was adjusted to the mark with a 0.1 M hydrochloric acid solution and stirred. The optical density of the resulting solution is measured at a wavelength of 277 nm, using a 0.1 M hydrochloric acid solution as a reference solution.

Acceptance criterion: impurities of the active substance, excipients and solvent for the test sample should not interfere with the quantitative determination of the basic substance.

From this spectrum it is seen that in the wavelength range from 250 nm to 320 nm, the placebo solution has no absorption in the UV light region. The maximum optical density obtained in a given wavelength range was 0.0005, which does not affect the quantification of the active substance.

Conclusion

Methodology was developed and validated for the quantitative assessment of metronidazole in the Metromed® Neo infusion solution. The obtained values of the validation characteristics are within acceptable limits, which allows using the developed technique in assessing the quality of the infusion solution.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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