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Аналітична хімія

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VALIDATION OF METHOD OF METRONIDAZOLE POLAROGRAPHIC DETERMINATION IN INFUSIONS SOLUTIONS

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The validation of the method of metronidazole polarographic determination in infusion solutions has been performed. This method is based on the reduction of the nitro group of metronidazole on the mercury dropping electrode. The main validation parameters such as the specificity, the robustness, the linearity, the accuracy as well as the precision have been determined within the used method. The technique conforms the current requirements for the methods of quantification of substances in medical products.

Keywords: polarography, metronidazole, nitro group, antibiotic, validation.

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1. Introduction

The safe and effective use of medicines (drugs) requires multilevel quality control at all stages of the manufacture, from substance synthesis to finished dosage forms. Quality control of medicines requires the use of sensitive and selective methods for determining the content of active substances.

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol, CAS number 443-48-1, fig. 1) belongs to the group of nitroimidazole medicines, which is used mainly for the treatment of infections caused by susceptible organisms, in particular, anaerobic bacteria (Bacteroides, Fusobacterium, Campyobacterium, Clostriubacterium, Treponema, Histomonas). Metronidazole is suspected of being genotoxic, cancerogenic, and mutagenic, as are their hydroxyl metabolites having retained the original nitroimidazole ring [1, 2]. For this reason, Metronidazole has already been banned in Europe by Council Regulation 613/98/EEC [3]. Therefore, the quantitative determination of metronidazole substance content in drugs is very important.

The HPLC with a complex mobile phase and/or mass spectroscopic detection is often used for the metronidazole determination [4–7]. These methods are reliable, but expensive, and often require complicated and time consuming sample preparation. Simple spectrophotometric methods are used to determine NIM, but these methods are less sensitivity and selectivity than voltammetry [8–10]. A cyclic voltammery determination of metronidazole on a paste carbon electrode showed an irreversible peak of metronidazole recovery at a potential of approximately –0.4 V, but the sensitivity of this method is not sufficient [11]. The State Pharmacopoeia of Ukraine [12] regulates the determination of metronidazole in solutions for infusions by the potentiometric titration.

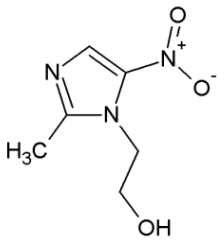


Fig. 1. Structural formulas of metronidazole

The development of new methods remains important considering the growing quantity of counterfeit medicines. Recently electrochemical methods have begun a new stage of rapid development caused the qualitative and quantitative determination of organic substances. Voltammetry is one of the most versatile electrochemical methods. Nitroimidazoles are electrochemically active substances.

Current requirements for quality control of medicinal products include the mandatory validation of methods of identification and quantification. According to the requirements of the European, American Pharmacopoeia and the State Pharmacopoeia of Ukraine [12–14] for analytical methods of quantification, the following analytical characteristics should be determined: the specificity, the robustness, the linearity, the accuracy in-laboratory precision and reproducibility in interlaboratory experiment. Therefore, we validated the method of polarographic determination of metronidazole in solution for infusion.

The developed technique is characterized by a low limit of detection ($LOQ = 4.5 \cdot 10^{-7}$ M), simplicity and economy.

2. Materials and experimental procedures

The object of the study is solution for infusions of metronidazole 5 mg/ml, 2 ml in a vial (Pharmaceutical Firm Darnitsa).

Preparation of the test sample solution (TSS): 1.0 ml of test solution for infusions was added to a 100.0 ml volumetric flask and distilled water was added to the mark. The concentration of metronidazole such TSS is $3.0 \cdot 10^{-4}$ M.

Metronidazole standard produced by Sigma Aldrich (St. Louis, MO, USA) with a quantitative content of 98 % active ingredient was used. The working solution of standard samples (SSS) of metronidazole was prepared by dissolving the exact amount of standard in 15 mL of 2 M hydrochloric in 100.0 mL volumetric flask and distilled water was added to the mark. In this case, the concentration of analyte was $1.0 \cdot 10^{-3}$ M in final volume.

The Britton-Robinson (BR) buffer was used to provide the required pH value. The procedure of BR buffer preparation: 20.2 g of Na2B4O7·10H2O, 28.7 mL of glacial acetic acid and 17.6 mL of orthophosphoric acid were dissolved in 1.0 L volumetric flask. The necessary pH value was obtained by adding 2.5 M sodium hydroxide solution (monitored at pH meter), then distilled water was added up to flask mark. All these reagents were “pure for analysis”.

Preparation of the placebo solution: an aliquot of sodium chloride, sodium hydrophosphate dodecahydrate, citric acid monohydrate were added into a 25 mL volumetric flask to obtain a solution with the necessary concentration, then 2 mL of BR buffer with necessary pH (9.6) was added to the flask, and distilled water was added to the mark.

Working solutions were obtained as follows: an aliquot of SSS was added into a 25 mL volumetric flask to obtain a solution with the necessary concentration, then 2 mL of BR buffer with necessary pH (9.6) was added to the flask, and distilled water was added to the mark. The obtained working solutions were introduced into the cell and deoxygenated with argon for 10 min. Voltammograms were recorded in the range of potentials from 0.0 to –1.5 V.

The study of the electrochemical process was performed using cyclic voltammetry, and the linear sweep VA method was used for the development of an analytical technique.

Voltammetric measurements were performed on digital device MTech OVA-410 [15] and temperature-controlled three-electrode cell. A static mercury drop indicator electrode (SDME), a saturated calomel reference electrode and platinum wire auxiliary electrode were used. The accuracy of the potential measurement is 1 mV. The uncertainty of the current measurement is 0.1 %. The employed SDME had the following characteristics: m = $5.94 \cdot 10^{-4}$ g/s; τ = 10 min in 0.2 M NH4Cl with an open circuit. Potentials sweep rate v = 0.5 V/s.

The pH of the solutions was measured potentiometrically using MV 870 DIGITAL-pH-MESSERÄT pH-meter.

3. Results and discussion

The maximum permissible uncertainty (Δ_{As}) for the metronidazole determination in infusion solution with a tolerance of B = 5 % calculated according to [14, 16, 17] is

$$\Delta_{AS} \leq B \cdot 0.32 = 7.5 \cdot 0.32 = 2.4\ \%.$$

The complete uncertainty of the analysis results consists of the uncertainty of the sample preparation and the uncertainty of the final analytical operation. The uncertainty of sample preparation Δ_{SP} for metronidazole solution was calculated using the method of sample preparation of the standard sample and the test solution, as well as performing the analytical reaction of determination by the formula:

$$\Delta_{SP} = \sqrt{\sum_i \Delta_i^2},$$

where Δ_i are the individual components of uncertainty specified in [17].

The results of the calculations are given in table. 1.

So, $\Delta_{SP} = \sqrt[2]{0.12^2 + 1.18^2 + 0.23^2 + 0.5^2} = 1.31\ \%$.

The uncertainty of the final analytical operation was calculated by the formula:

$$\Delta_{FAO} = 1.65 \cdot \sqrt{\frac{2 \cdot s_i^2}{3}},$$

where S_i = 0,1 % – uncertainty of current measurement (on the passport of the device):

$$\Delta_{FAO} = 1.65 \cdot \sqrt{\frac{2 \cdot 0.1^2}{3}} = 0.08\ \%.$$

The uncertainty of the sample preparation and the uncertainty of the final analytical operation make up the total uncertainty of the analysis results Δ_{As}:

$$\Delta_{As} = \sqrt[2]{(\Delta_{SP})^2 + (\Delta_{FAO})^2} = \sqrt[2]{1.31^2 + 0.08^2} = 1.31\% \leq 2.4\ \%.$$

The calculated value of complete uncertainty of the results of analysis does not exceed the maximum permissible uncertainty of analysis. Moreover, it is indicated that the sample preparation and analytical measurements of the signal do not cause a significant error in the results of the analysis.

Table 1

Calculation uncertainty of sample preparation for the determination of metronidazole in infusion solutions

Sample preparation	The parameter of the calculation formula	The uncertainty over [17]	
		SSS	TSS
Selection of sample standard metronidazole sample	m_0	1.18 %	–
Pipette aliquot selection 2 ml	2	0.50 %	
Bring to volume in a volumetric flask 100 ml	100	0.12 %	
Pipette aliquot selection 1 ml	1	0.60 %	
Bring to volume in a volumetric flask 25 ml	25	0.23 %	

The validation characteristic of robustness was investigated during the development of the method of polarographic determination of metronidazole: stability of solutions over time and influence of pH. The results of the study of the robustness of the method of metronidazole polarographic determination are listed in table. 2.

The obtained results show that the change in the values of the metronidazole recovery current with the change of the investigated factors is insignificant compared to the maximum permissible uncertainty of the analysis results. Therefore, the method is stable and the results of the analysis are reliable with slight changes in the conditions of analysis.

Table 2

The results of the study of the robustness of the method of polarographic determination of metronidazole, $C_{TSS} = 5.0 \cdot 10^{-5}$ M

Factor	Limits of change of factor	Current I, μA	Calculation due [17] δ, %
pH of the polarographic solution	9.4	1.451	0.31
	9.5	1.448	
	9.6	1.454	
	9.7	1.453	
	9.8	1.452	
Stability of the polarographic solution over time, min	5	1.450	0.23
	10	1.449	
	20	1.453	
	30	1.452	
	60	1.452	

The specificity of the method was investigated comparing polarograms of TSS and placebo (fig. 2, *a*). The influence of the placebo should not exceed 0.77 % from the maximum permissible uncertainty (Δ_{As}):

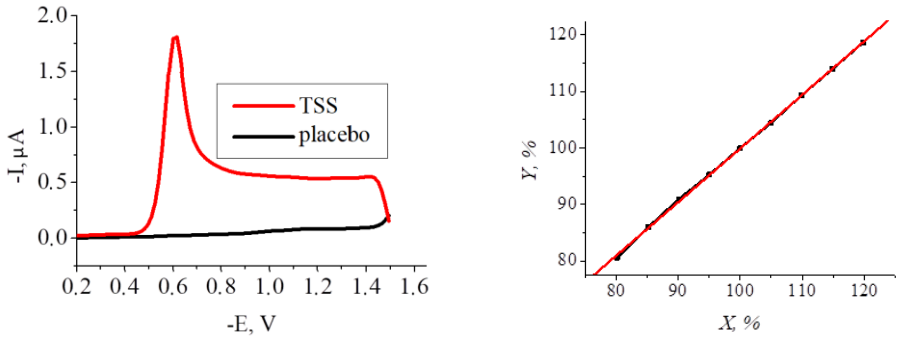
$$max\delta \leq 0.32 \cdot \Delta_{As} = 0.32 \cdot 2.4 = 0.77\ \%.$$

The influence of the current of the placebo solution on the result analysis is insignificant in comparison to the maximum permissible uncertainty:

$$\delta = \frac{I_{placebo}}{I_{TSS}} \cdot 100\ \% = \frac{0.013}{1.775} \cdot 100\ \% = 0.73\ \% < 0.77\ \%.$$

According to the validation data the method allows to determine metronidazole, while all the components are present.

For the linearity study, nine model solutions were prepared within the range of application of the method (from 80 % to 120 % relative to the nominal content of metronidazole in the infusion solution) (table. 3, fig. 2, *b*).



a *b*

Fig. 2. Polarograms of TSS and placebo solution (*a*); graph of current versus concentration of metronidazole in normalized coordinates (*b*)

Table 3

The results of the analysis of model solutions

No. Model solution	Introduced SSS V, ml	Concentration model solution, C·10^{-5}, M	Introduced, $X_i = \frac{C_i}{C_{cm}} \cdot 100$ %	Current value I, μA	Found, $Y_i = \frac{I_i}{I_{cm}} \cdot 100$ %	Found in % to introduced $Z_i = \frac{Y_i}{X_i} \cdot 100$
1	0.60	0.75	75.00	0.63	75.84	101.12
2	0.65	0.81	81.00	0.68	81.13	100.16
3	0.70	0.87	87.00	0.73	87.38	100.43
4	0.75	0.94	94.00	0.78	93.99	99.99
5	0.80	1.00	100.00	0.83	100.00	100.00
6	0.85	1.06	106.00	0.88	106.13	100.12
7	0.90	1.12	112.00	0.94	113.10	100.98
8	0.95	1.19	119.00	1.00	120.31	101.03
9	1.00	1.25	125.00	1.05	126.68	101.34

The parameters of linearity, accuracy and precision calculated by [12] in the table. 4, for the determination of metronidazole in infusion solutions are presented.

The calculated one-sided confidence interval in this preparation is less than the maximum permissible according to the State Pharmacopoeia of Ukraine. This indicates that the technique meets all the pharmacopoeial requirements of the criteria of accuracy and precision.

Using the linearity parameters S_a and b, it is possible to estimate the detection limit (LOD) and the limit of quantification (LOQ) by the

$$\mathrm{LOD} = 3.3 \cdot \frac{S_a}{\mathrm{b}}; \qquad LOQ = 10 \cdot \frac{S_a}{\mathrm{b}}.$$

For linearity in normalized coordinates, LOD and LOQ values are calculated as a percentage of the concentration of the comparison solution, which makes it possible to estimate a certain “margin of safety” of the procedure. Therefore,

$$\mathrm{LOD} = 3.3 \cdot \frac{1.12}{1.023} = 3.61\ \%;$$

$$\mathrm{LOQ} = 10 \cdot \frac{1.12}{1.023} = 10.95\ \%,$$

that significantly less than the lower concentration range (80 %), therefore, does not affect the accuracy of the analysis.

Table 4

Results of the linearity, accuracy and precision validation of metronidazole in infusion solutions

Parameter	Value	Critical values	Conclusion
Linearity			
Slope *b*	1.023	0.975–1.025	Maintained
S_b	0.011		
Intercept *a*	1.6	2.6	Maintained
S_a	1.12		
Residual standard deviation S_0	0.52	0.84	Maintained
Criterion for the linear correlation coefficient Rc	0.99951	0.99810	Maintained
Correctness and precision			
Average value Z, %	100.6	97.5–102.5	Maintained
Relative standard deviation S_z, %	0.57		
Relative reliable interval Δ_{As}.% = t(95 %, 8)·S_z	1.1	1.6	Maintained
Systematic error, δ	0.44	0.51	Maintained

For the in-laboratory precision study, five samples were analyzed for one batch of the drug on two different voltammetric units (table 5, experiments 1 and 2) on different days in one laboratory, as well as by the 2 different analysts (experiments 2 and 3) that used different laboratory glassware. For all results, a single mean value of metronidazole content (Z), relative standard deviation (SD_z), and relative reliable interval (Δ_{intra}) were calculated according to [12, 16, 17].

Table 5

The results of the in-laboratory precision testing of the metronidazole quantification in infusion solution

Number of solution	Value Z_i, %		
	Experiment 1	Experiment 2	Experiment 3
1	100.45	99.25	100.65
2	99.93	99.65	98.93
3	100.25	100.62	99.28
4	99.29	99.06	100.36
5	98.96	98.98	99.25
Average	99.76	99.51	99.69
Consolidated average Z_{intra}		99.65	
S_z, %	0.63	0.67	0.76
SD_z, %		0.69	
Δ_{intra}		1.21	

The Δ_{intra} value is 1.2 %, which complies with the requirements

$$\Delta_{intra} \leq max\delta = 1.21\% \leq 1.60\,\%$$

and indicates that the developed method satisfies the requirement of validation criteria and is suitable for the quantitative determination of metronidazole in solution for infusion.

Therefore, the results of the validation evaluation confirmed the validity of the method of quantification of metronidazole in infusion solutions. The proposed method can be used to develop analytical regulatory documentation for a medicinal product, namely to quantify metronidazole in solution for infusion, in the practice of laboratory of pharmaceutical analysis. The proposed method for sensitivity and selectivity outperforms spectrophotometric methods for the determination of nitroimidazoles and is more expressive and economically more advantageous than chromatographic techniques.

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ВАЛІДАЦІЯ МЕТОДИКИ ПОЛЯРОГРАФІЧНОГО ВИЗНАЧЕННЯ МЕТРОНІДАЗОЛУ В РОЗЧИНІ ДЛЯ ІНФУЗІЙ

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Проведено валідацію методики полярографічного визначення метронідазолу в розчині для інфузій "Метронідазол-Дарниця" – виробництва ПрАТ "Фармацевтична фірма «Дарниця»". Методика ґрунтується на відновленні нітрогрупи метронідазолу на р.к.е. Визначено головні валідаційні параметри: робасність, лінійність, правильність, прецизійність у всьому діапазоні застосування методики. З'ясовано, що методика відповідає сучасним вимогам до методик кількісного визначення речовин у лікарських засобах. Техніка відповідає сучасним вимогам щодо методів кількісної оцінки речовин у лікарських засобах: вона характеризується низьким значенням залишкового стандартного відхилення S_0 = 0,52, низькою межею виявлення (C_{min} = $4{,}5 \cdot 10^{-7}$ М). Обчислене значення повної невизначеності результатів аналізу дорівнює Δ_{As} = 1,31 % і не перевищує гранично допустиму невизначеність результатів аналізу 1,6 %, а також свідчить про те, що стадії пробопідготовки та вимірювання аналітичного сигналу не викликають суттєвої похибки результатів аналізу.

Опрацьовану методику можна використати для розроблення аналітичної нормативної документації щодо лікарських засобів, у практиці державних лабораторій контролю якості лікарських засобів, а також заводських лабораторій фармацевтичних компаній. Запропонована методика чутливості та селективності переважає над існуючими спектрофотометричними методами визначення нітроімідазолів, вона є більш експресною та економічно сприятливішою, ніж хроматографічні методи.

Ключові слова: метронідазол, валідація, нітрогрупа, антибіотик, полярографія.

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