Validation of spectrophotometric methods of assaying metronidazole in capsules

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Introduction

The international community imposes stringent requirements for the quality and safety of products. Nevertheless, counterfeiting of medicines is a special social danger and an urgent problem. Therefore, the suggested methods of quality control must comply with regulatory requirements and fully confirm the quality of goods.

Validation of quality control methods is recommended by the State Pharmacopoeia of Ukraine (SPhU) and the world's leading Pharmacopoeias during the registration of medicines. This procedure aims to experimentally verify the correctness and accuracy of the above methods[1–3, 6, 7, 10–13].

The guarantee of the safety and effectiveness of drugs is also their stability and absence of side effects of interaction that can take place in the organism, with different active ingredients. This applies to simultaneously appointed drugs and components of food, drinks, mineral water, food additives that people use independently in their daily diet. Patients rarely adhere to the recommended diet, without changing their eating habits. Therefore, the study of influence of the most common cases of interaction on bioavailability and pharmacological activity of prescribed drugs is relevant.

In order to experimentally prove the expediency or inadmissibility of the combined use of metronidazole as a helicobacter drug with others drugs, food, mineral water and other beverages rich in metal cations, we carried out validation of analytical method of assaying metronidazole in capsules by the standard method within the UV-spectrophotometric method according to the SPhU requirements, which is planned for use in studying the bioavailability of products of metronidazole interaction with metal salts.

Experimental methods

The object of study is metronidazole capsules "TRIKACIDE" with the content of the active substance constituting 500 mg (manufactured by PHARMASCIENCE INC., Quebec, Canada, Series: 6452653).

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The standard sample substance of metronidazole (manufactured by LUOTIAN HONGYUAN BIOCHEMICAL CO., LTD, China Series: 08111803) was used to prepare the metronidazole reference solution.

An "Evolution 60S" Spectrophotometer (USA), a "Specord 200" Spectrophotometer (Germany), a AB 204 S/A METTLER TOLEDO analytical balance as well as a class A measuring vessel and reagents that conform to the SPhU were used in the study.

Methods of assaying metronidazole in capsules

The exact weight amount of the contents of 20 capsules, equivalent to 0.1000 g of metronidazole (approximately 0.1209 g) is placed in a 100.0 ml volumetric flask, 50 ml of 0.1 M hydrochloric acid solution is added, shaken for 15 minutes, brought to the mark with the same solvent. The obtained solution is then filtered, the first and the last portions of the filtrate are rejected. The aliquot of 1.0 ml of the resulting solution is taken and placed in a 100.0 ml volumetric flask and adjusted to the mark with the same solvent. The measurement of absorbance is carried out on a spectrophotometer at 277 nm wavelength in a ditch with a layer thickness of 10 mm. The measurement of the metronidazole standard sample solution absorbance was carried out at the same time[10–13].

Compensation solution is 0.1 M hydrochloric acid solution.

The content of the active ingredient, according to the SPhU should be between 90–110% of the nominal content per average weight of the capsules contents (0.450–0.550)[13].

Linearity, accuracy, precision and reproducibility were studied in model mixtures of a series of samples containing the known amounts of active substances and excipients. The study was conducted in the application range of 80–120% method with 5% increments. Absorbance was measured three times with the cell removed. The average value was used for the results calculation [1–6, 8–9, 14].

Results and discussion

The graph (Figure 1) confirms the linear dependence of absorbance on the concentration of the metronidazole solution[1, 4, 5, 12].

The calculation of the linear dependence for metronidazole was performed by the least squares method (Table 1).

According to Table 1, the parameters of linear dependence correspond to the requirements, confirming the linearity of the proposed methods.

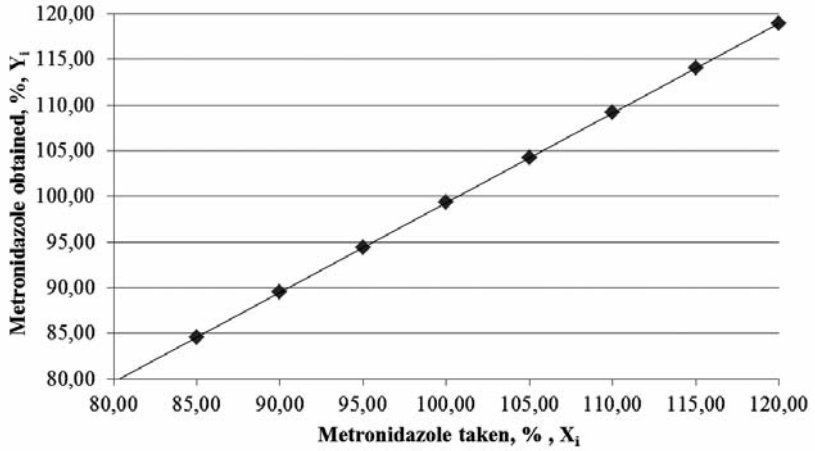

Fig. 1. The graph of absorbance dependence on metronidazole solution concentration

The criterion of practical uncertainty of methods for systematic error was 0.66%, executed at γ, % = 0.66 ≤ 1.024, characterizing the accuracy of the proposed spectrophotometric method of assaying in the range of applying the 80–120% method.

This method is characterized by convergence, as the relative confidence interval, Δ_Z, % = *t(95%,8)* · S_z = *1.0957* is below the critical value for convergence of results, which is in this case 3.20% (Table 2).

The research of reproducibility is a necessary condition if the method is planned to be included to the pharmacopeia and to be transferred to another laboratory.

Table 1. The Metrological characteristics of linear dependence for metronidazole

Parameter	Value	Criterion (for tolerances 90–110%), n = 9	Conclusion
b	0.9820	–	–
S_b	0.0163	–	–
a	1.1217	1. ≤ 1.8946 · S_a = 3.1174	
		2. if not executed 1), then ≤ 2.56	corresponds
S_a	1.6454	–	–
S_o	0.6320	S_o/b = 0.6436 ≤ 1.6890	corresponds
r	0.9990	≥ 0.9924	corresponds

Table 2. The results of metronidazole model mixtures analysis

No	Aliquot, ml	Introduced to nominal amount (X_i, %)	Absorbance, A_1 (A_{st} = 0.383)	Obtained for the nominal volume (Y_i, %) to introduced	Obtained in % $Z_i = 100\ Y_i / X_i$
1	0.80	80.00	0.305	79.72	99.65
2	0.85	85.00	0.324	84.68	99.63
3	0.90	90.00	0.344	89.82	99.80
4	0.95	95.00	0.359	93.82	98.76
5	1.00	100.00	0.384	100.17	100.17
6	1.05	105.00	0.397	103.66	98.72
7	1.10	110.00	0.416	108.62	98.74
8	1.15	115.00	0.435	113.58	98.76
9	1.20	120.00	0.459	119.84	99.87
Average value, Z, %					99.34
Relative standard deviation, S_Z, %					0.5893
Relative confidence interval, Δ_Z, % = t(95%, 8) · S_z					1.0957
Critical values for convergence of results, Δ_{AS}, %					3.20
Systematic error, δ, %					0.66
Criterion of systematic error statistical uncertainty, δ, %					0.37
Criterion of systematic error practical uncertainty, δ, %					1.02
Overall conclusion about the method					correct

Table 3. Results of the test solution temporal stability

	t, min					Average	RSD_t, %	$\Delta_t = 2.1318 \cdot RSD_t$
	0	15	30	45	60			
A	0.368	0.368	0.368	0.367	0.367	0.368	0.14	0.31
A_0	0.363	0.362	0.361	0.362	0.363	0.362	0.21	0.45

This parameter characterizes the accuracy of interlaboratory study methods on a series of test solutions[1–2, 4, 5, 12, 15]. The reproducibility was studied by measuring the optical absorption of the series of analytical solutions in different laboratories, with different equipment, by different analysts.

The data show that the proposed method can be correctly reproduced in other laboratories and characterized by the relative confidence interval of 100 ± 0.58% with a probability of 95%. Value $\Delta_{x,r}$, % = 0.58 ≤ 3,2. The interlaboratory systematic error constituted 0.54%.

The projected total uncertainty of results of assaying methods Δ_{AS} is 1.16% and not greater than the critical value (3.20%), which is insignificant.

The data confirm that the solutions are stable during the research time. The stability of solutions confirms an insignificant impact of reagents, equipment, and the subjective factor if the method is transferred.

To assess the "robustness", the stability of the test solutions was studied by measuring their absorbance every 15 minutes during an hour [1, 4–6] (Table 3).

Conclusions

Validation characteristics for the proposed assaying method were defined. Eligibility criteria for content tolerances of ± 10% were used.

The researched validation characteristics confirm linearity, precision (convergence, reproducibility), as well as the accuracy of the proposed method.

The prediction of total uncertainty of the proposed methods, determined with the use of the methods transferred, meets the eligibility criteria, which confirms the possibility of using the method of assaying the metronidazole capsules in other laboratories.

Conflicts of interest: none.

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