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RESEARCH ARTICLE

Validation of the Quantitative Determination Method of the active substance in Angiolin eye drops by spectrophotometry

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ABSTRACT:

Today, cataracts are the main cause of vision loss. Therefore, the creation of new ophthalmic medicines (drugs), namely eye drops, based on the active substance angiolin ((S)-2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate) is an urgent task. Previously, we developed a method of standardization of the active substance – by the spectrophotometry. According to the requirements of the SPhU, the next step was the validation of the developed methods. That became the purpose of our work. Validation of the method of quantitative determination of active substances was carried out according to the following indicators: specificity, linearity, range of application, accuracy, correctness and robustness. In the course of the conducted studies, it was established that the method is characterized by sufficient convergence and correctness, as the criterion of insignificance of the systematic error of the method is fulfilled. The systematic error of the method satisfies the requirements of statistical and practical insignificance. The high value of the correlation coefficient r = 1.0000 and 0.99994 satisfies the requirements of the acceptance criterion (r = 0.9998) and confirms the linearity of the dependence between the amount of Angiolin taken and found in the range from 80% to 120%, according to its nominal content in the drug. The requirements for the linear dependence parameters (a, SD_0/b, r) of the Angiolin determination method are met in the entire concentration range from 80% to 120% of the nominal value. Thus, the developed method of standardization of active substances in Angiolin eye drops is valid and can be introduced into the project of QCM (quality control methods).

KEYWORDS: Angiolin, Eye Drops, Quantitative Determination, Spectrophotometry, Validation.

INTRODUCTION:

According to the WHO, the number of people blind due to cataracts in the world may increase to 40 million in 2025. On average, according to statistics, more than 70% of the population over the age of 72 and about 20% of the population aged 40-60 years suffer from cataracts. Cataract in Ukraine ranks first in prevalence among diseases of the eyes and auxiliary apparatus. Today, cataract is considered not only as one of the urgent ophthalmological problems, but also as an important medical and social problem.[1]

The following drugs are used for the treatment of cataracts: Oftan Catachrom (Santen JSC, Finland), Taufon (Farmak, PJSC, Kyiv, Ukraine), Quinax (Alcon - Couvreur, Belgium), Vita-iodurol (Excelvision AG for Novartis Pharma AG, France/Switzerland), Potassium iodide (Unimed Farma LLC, Slovak Republic). Thus, it was established that the assortment of drugs for the treatment of cataracts is limited and mainly consists of manufacturers of foreign origin. Despite the scientific achievements of recent years, an extremely important task is the creation of new ophthalmic medicines, namely: eye drops, which continue to remain the most common and widely used medicinal form due to the traditionality of production and ease of use.

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Therefore, the creation of new ophthalmic drug of domestic production based on the original substance is an urgent and promising task. At the Department of Pharmaceutical Chemistry, the employees of the Zaporizhzhia State Medical University (ZSMU) together with the specialists of the "Farmatron" SPA under the leadership of Professor I.A. Mazur synthesized a new compound, which was named Angiolin.

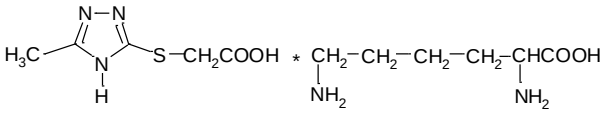

Fig. 1.1. 2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate

In previous studies, the pharmacological effectiveness of Angiolin eye drops was studied in concentrations: 0.5%, 1%, 1.5%, 2% and 2.5%. As a result of studies, it was established that 1% Angiolin eye drops, which exhibit anti-inflammatory, wound-healing, and reparative effects, were the most effective.[2-5]

Previously, we developed a method of standardization of the active substance – by the spectrophotometry method. Therefore, the next task, according to the requirements of the State Pharmacopoeia of Ukraine (SPhU), is to validate the developed methodology according to the following indicators: specificity, linearity, range of application, accuracy, correctness and robustness.

The purpose of our work is to validate the method of quantitative determination of the active substance in Angiolin eye drops by the spectrophotometry method.

MATERIALS AND METHODS:

For this, we used the substance angiolin (manufacturer: State Enterprise "Chemical Reagent Plant" of the Scientific and Technological Complex "Institute of Monocrystals" of the National Academy of Sciences of Ukraine, series 2451117) and the pharmacopoeial standard sample ((S)-2,6-diaminohexanoic acid of 3-methyl-1,2,4-triazolyl-5-thioacetate. 1% "Angiolin" eye drops were made in laboratory conditions, which included such excipients as: sodium chloride, methylcellulose and purified water.[6-7]

Based on the materials of previous studies, it was proposed to carry out quantitative determination of angiolin content by UV spectrophotometry in the range of 200-300nm, cuvette thickness 10mm. The content of angiolin in eye drops should be from 0.0095g/ml to 0.0105g/ml.[8-10]

Criteria for suitability of validation characteristics of the technique. The preparation of the test solution and the comparison solution of angiolin was carried out according to the following method: in 9 measuring flasks with a capacity of 100ml, put the amount of angiolin substance indicated in the Table 1.1, add 10ml of purified water to each flask, mix for 20 minutes, bring the volume of the mixture up to the mark with the same solvent and mix for another 5 minutes. Transfer 5ml of the obtained solution to a 50ml volumetric flask and make up to the mark with water. 10ml of the resulting solution is transferred to a 25ml volumetric flask and brought up to the mark with water. Model mixtures are presented in Table 1.

Table 1: Model mixtures

No. model number	Angiolin	
	Weight of the sample (mg)	Content, in % of the nominal value
1	160,00	80
2	170,00	85
3	180,00	90
4	190,00	95
5	200,00	100
6	210,00	105
7	220,00	110
8	230,00	115
9	240,00	120

RESULTS:

Specificity. After preparing the solutions according to the method presented above, we analyze them using a modified spectrophotometry method. Acceptability criteria are: 1) Spectra with an absorption maximum coinciding with the spectrum of angiolin on the diagram of the tested solution must be absent from the diagram of the "placebo" solution of the drug (sample 0); 2) The spectrum of angiolin on the diagram of the tested solution must coincide with the spectrum of angiolin on the diagram of the comparison solution.

The specificity of the method of identification and quantitative determination of angiolin is demonstrated in Fig. 1.2.

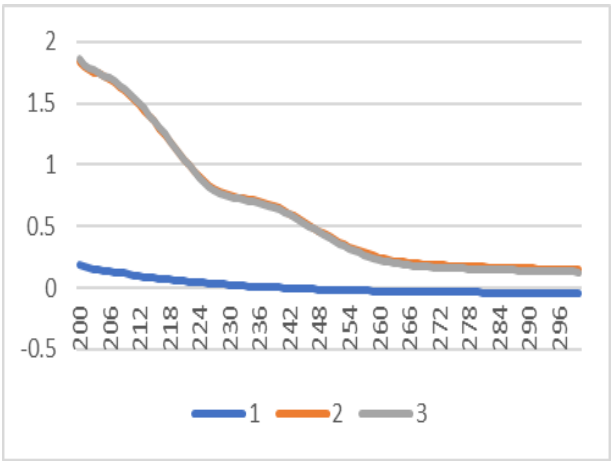

Fig. 1.2 UV spectra of solutions: 1 - "placebo" solution of the drug, 2 - tested solution of the drug, 3 - comparison solution of Angiolin eye drops

As can be seen from Fig. 1.1, the light absorption spectrum of the placebo solution does not have a light absorption maximum at a wavelength of λ = 238 nm, the influence of the optical density of the placebo solution on the analysis result is insignificant compared to the maximum permissible uncertainty of the analysis results.

Therefore, the proposed method, which allows determining Angiolin in the presence of all other components in eye drops by spectrophotometry, is specific.

The next task was the preparation of model tested solutions. The characteristics of correctness and precision were studied on model solutions of the drug with concentrations of Angiolin corresponding to 80%, 85%, 90%, 95%, 100%, 105%, 110%, 115% and 120%.

The range of application of the analytical method is the interval between the minimum and maximum concentrations (quantity) of the analyzed substance in the sample (including these concentrations), for which it is shown that the analytical method has the required correctness, convergence and linearity.[11-17]

Linearity is the ability of the technique (within the range of application) to give values directly proportional to the concentration (amount) of the analyzed substance in the sample. The linearity characteristic was studied in the range of angiolin concentrations from 80% to 120% in relation to the nominal value.

The graph of linear dependence is presented in Fig. 1.3, and the results of calculations of parameters of linear dependence in Table 1.2. and 1.3.

As can be seen from the presented data, the requirements for parameters of linear dependence are met, i.e., the linearity of the method of quantitative determination of angiolin is confirmed in the concentration range from 80% to 120% of the nominal value for the content limits of ± 5%.

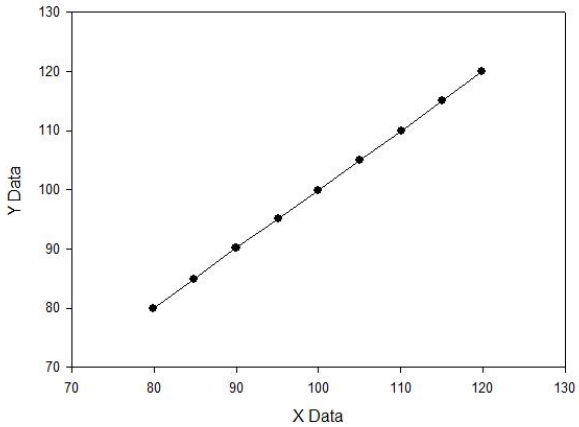

Fig. 1.3 Linear dependence of the found concentration of Angiolin on its input concentration in normalized coordinates by the spectrophotometry

Correctness characterizes the degree of correspondence between the known content of the substance to be determined in the solution and its content in the solution, which is determined by this method.

Convergence characterizes the *precision* of the technique when it is carried out under the same conditions over a short period of time. At this stage, the convergence is investigated on 9 model mixtures, which cover the range of application of the technique.

The correctness and convergence of the method/technique was checked by the "entered-found" method. The results of quantitative determination of angiolin in model solutions in the range of analytical concentrations and the results of calculations of metrological characteristics are presented in the Table 1.2 and 1.3.

Table 1.2 Metrological characteristics of the linear dependence of the found concentration of angiolin on its input concentration

Parameters	Value	Requirements 1	Requirements 2	Conclusion
B	0,9974			
S_b	0,0034			
A	0,2480	≤ |0,24|	≤ |2,6|	Sustained by 1 criterion
S_a	0,3381			
SD_0	0,1303			
SD_0/b	0,01306	≤ |0,84|		Executed
R	1,0000	> |0,99810|		Executed

Table 1.3 Results of the analysis of model solutions containing from 80% to 120% of angiolin in relation to the nominal concentration, and their statistical processing

No. Solution	Amount of angiolin, g (mst = 0.2000)	Entered in % from nominal concentration (Xi, actual, %)	Average optical density (A_i^st= 0.643)	Found in % from nominal concentration (Yi, %)	Found in % to entered Zi = 100 • (Yi/Xi)
1	0,1598	79,89	0,517	79,99	100,13
2	0,1698	84,85	0,549	84,91	100,07
3	0,1799	89,98	0,582	90,08	100,12
4	0,1905	95,18	0,615	95,04	99,86
5	0,1999	99,94	0,646	99,92	99,98
6	0,2101	105,03	0,678	104,93	99,90
7	0,2204	110,16	0,705	109,96	99,82
8	0,2302	115,10	0,743	115,01	99,92
9	0,2397	119,86	0,776	120,03	100,14
Average, Zavg,% =				99,99	
Relative standard deviation,RSDz, % =				0,12	
Relative confidence interval Δz % = t (95 %, 9 – 1) x RSDz = 1.86 x 0.12 =				0,22	
Critical value for convergence of results ΔAs, % =				1,6	
A systematic error δ % = | Zcp – 100 | =				0,01	
Criterion of insignificance of systematic error: 1) statistical insignificance: δ<Δz : √9 = 0.22/3 = 0.07 % > 0.01 % If not performed 1), το δ ≤ maxδ: 2) practical insignificance: δ% ≤ 0.32 × 1.6 = 0.51 % > 0.01				Executed Executed	
General conclusion about the methodology				CORRECT	

From the data given in the Table 1.3, it turns out that the method of quantitative determination of angiolin in eye drops by UV spectrophotometry is characterized by sufficient correctness and convergence (precision) over the entire range of concentrations (from 80% to 120%) and is correct.

As evidenced by the data given in the Table 1.3, in the range of angiolin concentrations from 80% to 120% in relation to the nominal concentration, the method of its quantitative determination does not have a significant systematic error.

DISCUSSION:

Summarizing all of the above, we can say that the technique/method is characterized by sufficient convergence, since the found value of the relative confidence interval of the value Δ_Z for angiolin does not exceed the critical value for the convergence of results (1.6%) (Table 1.2, 1.3). Also, the method is characterized by sufficient correctness, since the criterion of insignificance of the systematic error of the method is fulfilled. The systematic error of the method satisfies the requirements of statistical and practical insignificance. The high value of the correlation coefficient r = 1.0000 and 0.99994 satisfies the requirements of the acceptance criterion (r = 0.9998) and confirms the linearity of the dependence between the amount of Angiolin taken and found in the range from 80% to 120%, according to its nominal content in the drug. The requirements for parameters of linear dependence (a, SD_0/b, r) of the Angiolin determination method are met in the entire concentration range from 80% to 120% of the nominal value. [18-21]

FINDINGS:

1. As a result of the conducted studies, it was established that the developed method of standardization of the active substance in 1% Angiolin eye drops is valid according to the following indicators: specificity, correctness, precision (convergence) and linearity.

2. The total predicted uncertainty of the analysis results does not exceed the critical value regulated by the State Pharmacopeia of Ukraine.

DIRECTIONS FOR FURTHER STUDIES:

The developed and validated method of determining the active substance in Angiolin eye drops can be introduced into the project of the QCM.

GRATITUDE:

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CONFLICT OF INTEREST:

None.

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