VERIFICATION OF THE ZINC OXIDE ASSAY METHOD IN SIMANOVSKY OINTMENT FOR ITS CHEMICAL STABILITY ESTIMATION

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Abstract. Stability is the main quality parameter of any medicine. The literature contains a large number of methods for the known active ingredients analysis, but often there is no information about their use in the separate dosage forms analysis. The Simanovsky ointment often is being prepared in pharmacies of Ukraine. One of its main active ingredient is zinc oxide. Verification of the zinc oxide assay method in the medicine composition was done for proving the possibility of its use for the ointment chemical stability analysis.

Optimal approach to ointment sample preparation was chosen. The complexometry method was used for the zinc oxide assay in the ointment. The obtained values of all method validation parameters don't exceed the permissible criteria. The requirements for accuracy

(*δ,%=0.51≤1.02*) and precision (Δ_Z*=1.13≤3.20*) are fulfilled. The method was tested on the ointment samples. Obtained RSD value (*RSD=0.37 %*) proves the good reproducibility of the results.

Key words: compounding ointment, zinc oxide, complexometric titration, verification.

1. Introduction. Stability is the main indicator of the quality and safety of any medicine [1-4]. The problem of stability applies to all medicines without exception. The stability of the dosage form is influenced by the environment factors, the properties of the dosage form components, storage conditions, the manufacturing process, packaging, etc. [1, 2]. To date, the literature has enough information on the stability of many known active ingredients with the methods of their quantitative analysis. However, when considering the question of chemical stability of a particular dosage form the study should be conducted in view of its composition, manufacturing technology and packaging of the medicine [2]. Therefore, during the chemical stability study of the dosage form necessary to assess the possibility of existing methods using for analyzing its active ingredients or developing new ones. According to the modern requirements in the preparation of medicines in pharmacies, the definition of the term of their shelf life can based on literature data or on proven scientific results of the stability study [4, 5]. In this case, only validated methods of quantitative analysis should be used [5]. Especially important stability estimation of extemporaneous medicines which are made for stock. It will determine the possibility of extending their shelf life.

Simanovsky ointment often preparing in the compounding pharmacies of Ukraine. Its composition: phenylephrine hydrochloride 0,02; menthol 0,04; zinc oxide 0,24; wool fat 4,0; white soft paraffin 6,0. Due to the optimal combination of components, it is quite effective in

treating many diseases of the upper respiratory tract. To study the ointment chemical stability necessary to carry out quantitative determination of its active ingredients.

The aim of our work was verification of the zinc oxide assay method in the Simanovsky ointment for its subsequent use for the analysis of the dosage form chemical stability.

2. Materials and methods. Volumetric glassware of class A, reagents which meet the requirements of the SPhU, analytical balance AXIS ANG 200 (Poland), zinc oxide substance (series L08131014 produced by Zinsa, Peru) were used for the analysis.

Test solution. To 2.000 g of ointment was added 10 ml of dilute acetic acid R. The mixture was boiled for 5 minutes in the water bath. After cooling, the mixture was filtered through a paper filter to the 500 ml conical flask, preventing the fat falling on the filter. Extraction with 5 ml of dilute acetic acid R was repeated 2 more times, filtering through the same filter in the same conical flask. The volume of the solution was adjusted to the mark 200 ml with water R, and added about 50 mg of xylenol orange triturate R and hexamethylenetetramine R until the solution become violet-pink. To obtained solution, 2 g of hexamethylenetetramine R in excess was added and titrated with 0.1 M sodium edetate until the violet-pink colour changes to yellow.

1 ml of sodium edetate is equivalent to 8.14 mg of zinc oxide.

3. Results and discussion. SPhU [6], as well as European Pharmacopoeia [7], recommend to use the complexometric method for quantitative analysis of zinc oxide substance. However, in the literature there is no description of this method using for the ointment quantitative analysis. Therefore, it was verified in accordance with modern requirements [5, 7]. Total assay method uncertainty was calculated before its verification (Table 1). Taking into

account tolerances ± 10 % in the quantitative content of the extemporal ointments active ingredients, the maximum permissible uncertainty is 3.20%.

The obtained value of the method total uncertainty is much less than the total permissible uncertainty (0.48 <3.20), which indicates the possibility of obtaining the correct results using this method.

Table 1: Calculation of total uncertainty of zinc oxide assay method

Operation of analysis	*The parameter of calculation formula*	*Uncertainty, %*
Determination of 0.1 M sodium edetate molarity		
Weighting of zinc on the analytical balance, g	m_c	0.0002/0.120×100=0.17
Dilution in the volumetric flask, ml	200	0.10
Titration with 0.1 M sodium edetate	V_T (25 ml burette)	0.05/18.35×100=0.27
Uncertainty of the results of the molarity definition	$\Delta_{Cprac.} = \sqrt{0.17^2 + 0.27^2 + 0.10^2} = 0.33$	
Determination of zinc oxide content		
Weighing of ointments on the analytical balance, g	m_{st}	0.0002/2.00 × 100=0.01
Dilution in the volumetric flask, ml	200	0.10
Titration with 0.1 M sodium edetate	V_T (10 ml burette)	(0.02/5.90)×100=0.34
Total uncertainty of assay method	$\Delta_{tot.} = \sqrt{0.33^2 + 0.01^2 + 0.10^2 + 0.34^2} = 0.48$	

In the process of further verification of the methodology, all necessary validation characteristics were defined: linearity, accuracy, precision and reproducibility. Determination of linearity was performed in the range from 80 to 120 % from the nominal concentration of the zinc oxide in the ointment taking into account the tolerances of the quantitative content deviation. In the chosen range, five concentrations with three parallel analysis were analyzed. The slope of calibration curve, intercept, the residual standard deviation and the correlation coefficient were determined (table 2).

Table 2: Results of linear dependence parameters study of zinc oxide assay method

Validation characteristic	*Value*	*Permissible criteria*	*Conclusion on compliance*
b	0.98	-	-
S_b	0.094	-	-
(b-1)	0.050	-	correspond
a	2.20	statistical insignificance $a \le t(95\%, n-2) \times S_a$ *(a≤1.68)* practical insignificance *a≤5,12*	fulfilled by the second criterion
S_a	0.95	-	-
S_0	0.52	*max S_0=1.81*	correspond
S_Y	14.75	-	-
r	0.9994	*min r=0.9925*	correspond

All linearity parameters meet the criteria. A calibration curve was constructed by results of the linearity study (Figure 1).

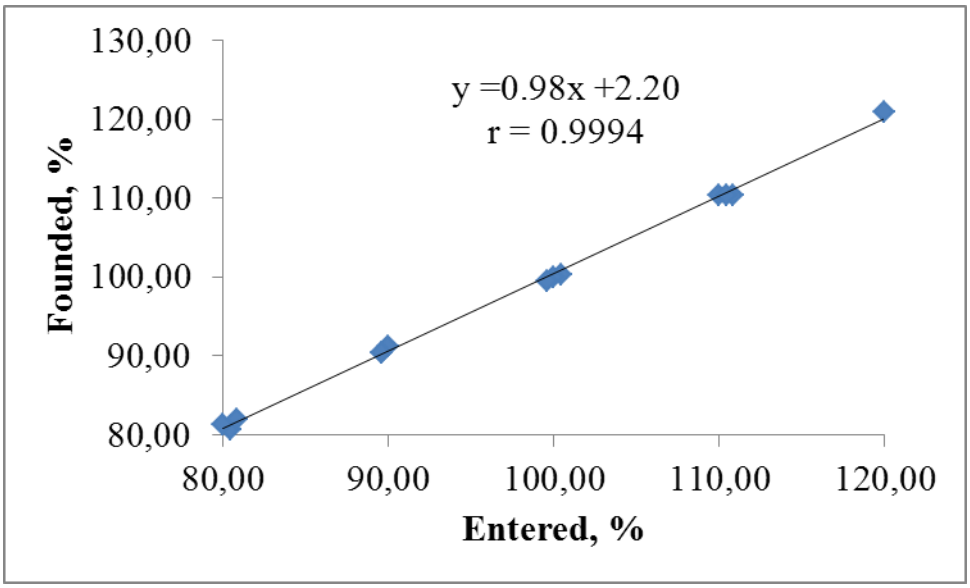

Figure 1: Graph of the linear dependence of sodium edetate volume from zinc oxide concentration in normalized coordinates

The study of the accuracy and precision parameters was performed with using the results of linearity study. Systematic error (δ, %) and Δ_Z values with their criteria were determined during the study (Table 3).

Table 3: Results of precision and accuracy study of zinc oxide assay method

Validation characteristics	*The obtained value*
$\overline{Z}$	100.51
S_Z	0.64
Δ_Z	1.13
Criterion of one-sided confidence interval $\Delta_Z \leq \Delta_{As}$ (1.13≤3.20)	
δ	0.51
Criterion of statistical insignificance δ, %≤0.29	
Criterion of practical insignificance δ, %≤0.32×Δ_{As} =1.02	

The obtained results testify the compliance of the accuracy and precision parameters with the requirements of the SPhU. Results of these parameters studying testify that the method can be used for analysis of the Symanovsky ointment chemical stability.

An ointment analysis using this method was conducted. Zinc oxide practically insoluble in in water and in ethanol (96 per cent). It dissolves in dilute mineral acids [6, 7]. Since the base of the ointment is insoluble in dilute mineral acids, the extraction of zinc oxide is best to carry out with their use. According to the recommended method of the zinc oxide substance assay [6, 7], dilute acetic acid are using for its dissolution. Therefore, it was used for its extraction from the ointment base. Since parts of the ointment base can pass into solution, it was recommended to filter the solution during sample preparation.

Calculation of the quantitative content of zinc oxide was carried out by equation:

$$X = \frac{V \cdot K \cdot T \cdot m_{by\_prescr.}}{m_{oint\_sample}}$$

where: V – volume of 0,1 M sodium edetate, ml;

T – titer of zinc oxide by 0,1 M sodium edetate, g/ml;

$m_{by\_prescr.}$ – total ointment weight by prescription, g;

m_{oint_sample} – weight of the ointment sample for quantitative determination, g.

The study of the method reproducibility on the ointment sample was conducted with six parallel analysis. The low value of the relative standard deviation (*RSD=0.37 %*) indicates the proper reproducibility of the method.

Table 4: Results of the reproducibility study of zinc oxide assay method in ointment

Sample №	$m_{oint\ sample}$, *g*	*Found, g*	*Content of zinc oxide, %*

1.	1.9976	0.2374	98.92
2.	2.0634	0.2389	99.54
3.	2.1140	0.2380	99.17
4.	1.9930	0.2393	99.71
5.	2.0618	0.2395	99.79
6.	2.0714	0.2384	99.33
Mean		0.2386	99.41
RSD, %		0.37	0.37

The average value of the zinc oxide content in the ointment samples is 0.2386 g (99.41 % from the prescribed amount). The obtained data testify the compliance of the ointment quality with the SPhU requirements and possibility of using the method for determining the quantitative content of zinc oxide in the Symanovsky ointment.

4. Conclusions. The method of complexometric titration was verified for zinc oxide assay in the composition of the Simanovsky compounding ointment.

Validation characteristics of the chosen method were studied. The obtained parameters of linearity, accuracy and precision testify its correctness and the possibility of use in other laboratories to determine the quantitative content of zinc oxide in the studied ointment and to analyze the ointment stability.

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