УДК 615.074

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VALIDATION OF HPLC METHOD FOR DETERMINING THIOCTIC ACID IN THE COMPOUND DRUG FOR EXTERNAL USE TO TREAT DIABETIC ULCERS

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The pharmacopoeia definition procedure of thioctic acid by a high performance liquid chromatography in the new complex medicine was adopted and validated. It was shown that the validation criterion of the procedure is conformed to the requirements of the State pharmacopoeia of Ukraine for assay with limits of active ingredients ±5 %. There is no influence of matrix components on validation criteria.

Today, one of the most important issues around the world is diabetes mellitus (DM) due to the increasing number of patients, the severity of the disease, early disability and high mortality, as the consequence of a variety of complications which accompany the disease [1,2,3,4].

The most widespread DM complication is polyneuropathy and the consequent emerging diabetic ulcers (DU), which in turn may lead to the need for amputation of a diabetic patient's leg [2,5,6]. Diabetes is often asymptomatic, but leads to micro trauma (due to the sensory loss), followed by the formation of leg ulcers. It has been proved that 80 % of patients with diabetes who underwent amputation of the lower limbs, had a trauma or foot ulcers in their case history [2,6,7,8].

For the successful healing of lower limbs diabetic ulcers, the following conditions are necessary: metabolic compensation, control of the wound and

inflammation process (antibiotic therapy), unloading of the affected limb, and preservation of the blood flow in the lower limbs [2, 5, 6, 8]. The creation of new effective compound medicines for diabetes and its complications treatment, with no side effects is an urgent problem of modern pharmacy [2,3,4,7].

Now, the most common treatment for diabetes and its complications are the drugs with thioctic acid (Espa-lipon (Espharma, Germany), Thiogamma (Worwag, Germany), Alpha-lipon (Werhle, Germany), Dialipon ("Farmak" Ukraine), but there is no topical medication based on the given substance [2, 3,4,9,10].

In the development of modern compound drugs several active substances are usually used, which may combine several of joined pharmacological effects and therefore increase and enhance the pharmacological actions of monopreparations.

We have developed a combined topical medication in the form of a gel [11,12] for the treatment of diabetic ulcers based on thioctic acid and allantoin and the methods of its quality control.

Using the previously conducted complex research (technological, physico-chemical, microbiological, biological) we developed a rational composition of the gel. As active substances were selected thioctic acid (antioxidant, hepatoprotective, hypoglycemic activity) and allantoin (restorative, anti-inflammatory, wound-healing effect). As a basis, we chose gel: carbomer gelling agent of «Ultrez 10" brand, as a neutralizer - trometamol. Propylene glycol was taken to dissolve the thioctic acid, and sodium benzoate as a preservative [13,14,15].

For the new topical drug, which is in the process of pharmaceutical development at the department of biology, physiology and anatomy of the National University of Pharmacy under the supervision of prof. Maloshtan L.N. the anti-inflammatory, anti-oxidant effects and the ability to accelerate the processes of neogenesis and tissue granulation were experimentally established [16].

It is known that the components of the dosage form, especially those which significantly exceed the content of the active substance, have an impact on the reproducibility of determination methods and validation parameters. Therefore, the study of the influence of validation parameters on the compliance with the

established acceptability criteria is relevant in the development of new medicines.

We carried out the research on determination of the quantitative content of the developed gel active components: thioctic acid, allantoin, and sodium benzoate as a preservative.

In accordance with the requirements of the State Pharmacopoeia of Ukraine (SPU), in the development of analytical methods of quantity determination of active ingredients in dosage forms it is necessary to calculate the acceptability criteria of validation features, to develop a standardized procedure for the analytical methods validation and to conduct the experimental study on the metrological characteristics of the method.

The acceptable method of determining the thioctic acid is a pharmacopoeia method of high performance liquid chromatography [17].

The purpose of the study was to adapt the pharmacopoeia technique and its validation for the quantitative determination of thioctic acid in the new combined drug.

Material and method

The object of the study was a combined drug, 100 ml of which contain 1.0 grams of thioctic acid, 1.0 grams of trometamolum, 0.1 grams of allantoin and excipients: carbopol Ultrez 10NF, sodium benzoate, and the necessary amount of water.

The reagents and solvents used: thioctic acid thioctic acid RS, potassium dihydrogen phosphate, phosphoric acid, methanol, acetonitrile, purified water.

Analytical equipment: liquid chromatograph Prostar-210, by «Varian Chromatography System», U.S., electronic scales Precisa XT 220A, measuring vessels of A class.

Chromatography is carried out by a liquid chromatograph under the following conditions:

- apHera ™ C18 Polymer of 150 x 4.6 mm column, which is filled with a sorbent with particle size of 5 microns;
- mobile phase: a mixture of 8 volumes of acetonitrile, 41 volumes of 0.7

g / l solution of potassium dihydrogen phosphate (pH 3.0) and 51 volumes of methanol;

- detection at 215 nm wave length;
- column temperature 35° C;
- eluent speed: 0.7 ml/min;
- dosing volume: 50 ml.

Validation criteria [18]. Uncertainty of sample preparation, specificity, linearity, convergence, correctness, stability, intralaboratory accuracy. The validation procedure is described in detail in [19,20].

Spare solution of thioctic acid in a solvent (a mixture of equal volumes of acetonitrile and 0.7 g / l solution of potassium dihydrogen phosphate (pH 3.0) of 0.5 mg/ml comcentration. *Model solutions* are made from the spare ones by means of solvent dilution to the concentrations of 0.03 mg/ml - 0.07 mg/ml.

"Placebo". Based on the composition of the drug there were trometanol, allantoin, carbopol Ultrez 10NF, sodium benzoate and water in the "placebo".

Acceptability criteria. Due to the ICH requirement to normalize the API content in the finished medicinal product within ± 5%, the maximum value was $\Delta_{As} = 1{,}6$ *u* $\delta = 0{,}512$.

Results and discussion

Uncertainty of sample preparation. Based on the method of solutions preparation and the use of high performance liquid chromatography technique the complete predicted uncertainty of the method is $1{,}45 < \Delta_{As} = 1{,}6$. The data obtained showed no effect of sample preparation on the results of the determination.

Specificity, correctness, convergence. Typical chromatograms of the "placebo" solution (a) and the test solution (b) are shown in Fig. 1.

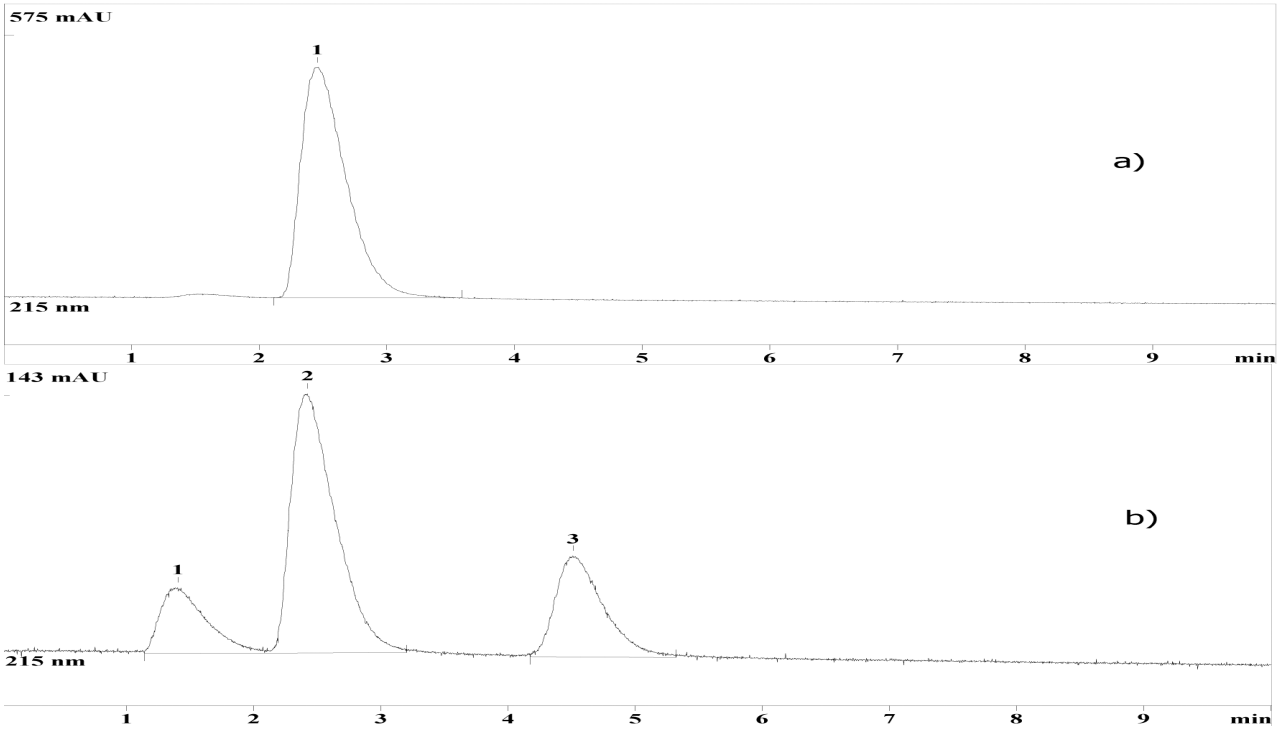

Fig.1. Typical chromatograms of solutions: standard solution (a) and the test solution (b).

Table 1 shows the determination of validation criteria of "correctness" and "convergence."

Table 1

The output data and results of the validation criteria "correctness " and "convergence" determination

№ model solution	Model solution concentration mg/ml	Concentration in normalized coordinates Xi %	Average peak areas	Average peak areas in normalized coordinates, Yi,%	$Z_i = \frac{Y_i}{X_i} \times 100\%$
Standart solution	0,053		1495,0482		
1	0,032	60,00	898,9235	60,13	100,21
2	0,037	70,00	1041,0162	69,63	99,47
3	0,042	80,00	1202,5533	80,44	100,54

4	0,048	90,00	1353,6203	90,54	100,60
5	0,053	100,00	1495,7130	100,04	100,04
6	0,058	110,00	1630,3272	109,05	99,13
7	0,064	120,00	1794,8556	120,05	100,04
8	0,069	130,00	1951,9055	130,56	100,43
9	0,074	140,00	2086,5196	139,56	99,69

Mean Z =	100,02	
Standard deviation SDZ =	0,50	
Confidence interval $\Delta\% = t(95\%; f) \times SD_Z =$	0,93	$< \max \Delta_{As}$ =1,6
Insignificant systematic inaccuracy test		
Systematic inaccuracy δ[Z-100]=	0,02	<0,31
		<0,51

From Table 1 one can see the fulfillment of the "correctness" and "convergence" criteria due to SPU requirements.

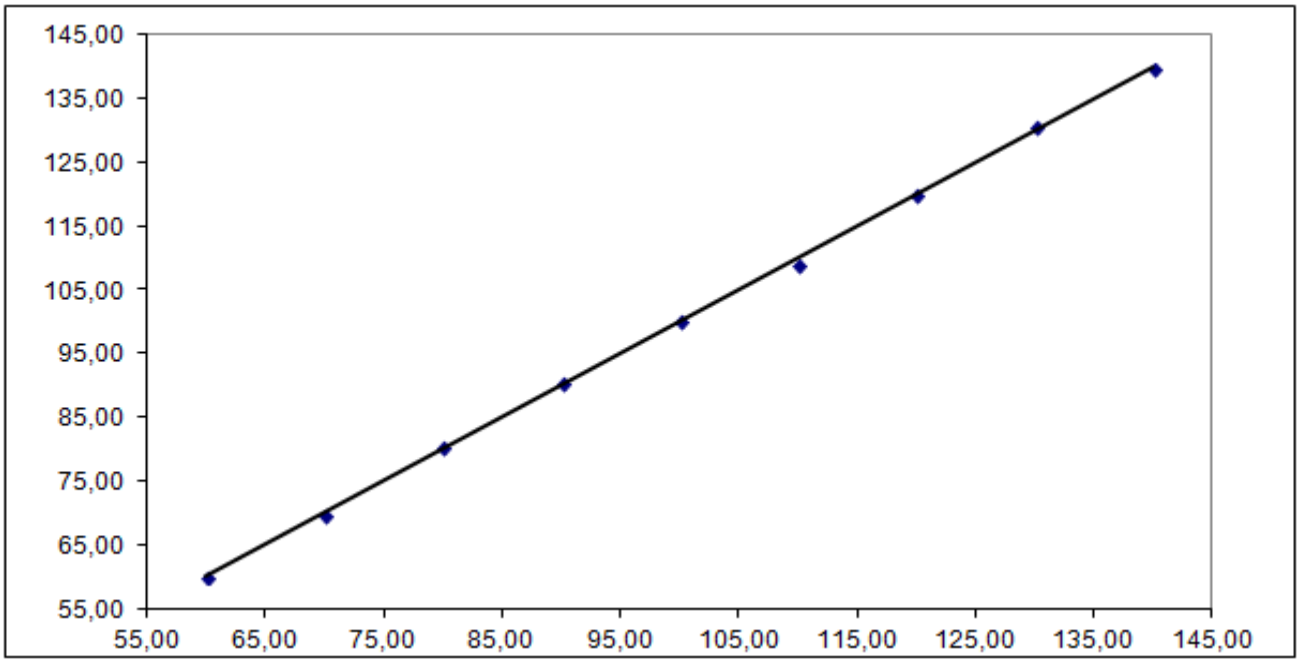

Fig. 2. The calibration line of the peak areas dependence of thioctic acid in model solutions on its concentration in them.

Linearity. According to the information received the calibration line was built, which is shown in Fig. 2.

Table 2 shows the determinaition of validation criteria "linearity".

Table 2

"Linearity" validation criteria determination

Critical parameter	Obtained result	Maximal value
The number of freedom degrees (f)	7	
Student's coefficient ($t_{(0,95,f)}$)	2,36	
Slope angle (b±Sb)	0,99±0,01	
Free term (a±Sa)	0,3±0,7	<1,28
Residual standard deviation (RSD0)	0,50	<0,85
Correlation coefficient (r(x))	0,99983	>0,99945
Determination limit	2,36	
Quantification limit	7,14	

Having examined the data from Table 2 one can see the correspondence of the"linearity" criterion with the SPU requirements.

Intralaboratory accuracy. Based on the determination of thioctic acid in the samples in 3 different days by different chemists the absence of influence of random factors in the reconstruction technique in the laboratory was found: the acceptance criteria $\Delta Z_{\text{int}\,ra} = 1{,}4 < \max \Delta_{As} = 1{,}6$.

Stability of solutions was determined during 6 hours. At a particular period the stability of solutions and their suitability for HPLC analysis was established: the acceptance criterion is 0,49 < max δ = 0,512/

Conclusion

1. The pharmacopoeia method for the quantitative determination of thioctic acid in the new compound drug was adapted.

2. The validation of the developed technique was carried out. It was shown that the validation criteria meet the requirements of SPU to the methods of quantification with the tolerance of the active substance of ± 5%.

3. It was found that there are no effects of other components of the dosage form on the validation criteria of the method.

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