VERIFICATION OF THE QUANTITATIVE DETERMINATION METHOD FOR PHENYLEPHRINE HYDROCHLORIDE IN SOLUTION FOR INJECTIONS

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ABSTRACT

The spectrophotometric method for quantitative determination of phenylephrine hydrochloride solution for injections has been verified. The method was tested on model mixtures and a batch sample of the drug. The method proposed was verified by the following parameters: specificity, linearity, precision (convergence), accuracy, robustness, the range of application. These metrological characteristics do not exceed the acceptance criteria according to the SPhU. Based on these results the method can be correctly reproduced and is suitable for use in pharmaceutical analysis of injectable medicines containing phenylephrine hydrochloride.

Key words: Verification, Phenylephrine hydrochloride, Solution for injections, Spectrophotometric method, Quantitative determination.

INTRODUCTION

Validation of analytical methods starting from the stage of development and continuing at the stage of mass production and storage of medicines helps to produce quality and competitive products and is an invariable constituent for development of analytical documentation[1-8].

The procedure of analytical methods validation is presented in detail in the State Pharmacopoeia of Ukraine[9] and is part of the principles and requirements of Good Manufacturing Practice of medicines[10-12]. At the same time, when including the existing pharmacopoeial methods to monographs of the SPhU, it is sufficient to carry out verification for them.

Sympathomimetic phenylephrine hydrochloride, $C_9H_{14}ClNO_2$ [(R)-1-(3'-hydroxyphenyl)-2-(methylamino)ethanol hydrochloride] is a synthetic analogue of adrenal hormones (adrenaline and noradrenaline), has a direct stimulatory effect predominantly on postsynaptic α-adrenergic receptors. The medicine exhibits vasoconstrictor effects similar to norepinephrine, but the vasopressor effect of phenylephrine is less pronounced, but longer. It is the world's only non-prescription agonists since in the mean therapeutic doses it almost does not show a significant stimulatory effect on the central nervous system and is part of many drugs used in emergency treatment, otorhinolaryngology, ophthalmology and the treatment of infectious diseases[13-17].

Phenylephrine hydrochloride and its medicines are presented in monographs of the SPhU[7] and EP 7[18] (substance), in the USP[19] and BP[20] (substance, solution for injections, eye and nasal drops, etc.).

Phenylephrine hydrochloride in ampoules with the concentration of 1% and the volume of 1 mL is produced by such enterprise of Ukraine as GNCLS (SE "State Research Center of Drugs" Kharkiv, Ukraine).

The aim of our study was verification of the spectrophotometric method for quantitative assessment of phenylephrine hydrochloride in solution for injections according to the requirements of the project for the SPhU monograph.

EXPERIMENTAL

Materials and methods

The object of the study was "Mesatone, a solution for injections, 1%", 1 mL, in ampoules No.10, batches 30110, 60911, 81111 (GNCLS), which is a sterile solution of phenylephrine hydrochloride in water for injections, a standard sample is the substance of phenylephrine hydrochloride (Unichem laboratories Ltd., India), batch RRRRN/1104 dated 01/10/10.

Analytical research was performed by absorption spectrophotometry in the UV- and visible region using an Evolution 60S v4.003 spectrophotometer. During the study AXIS model ANG 200 laboratory electronic balance, measuring glassware class A (first class), reagents and solvents complying with the SPhU requirements were used.

Model solutions and reference solution were prepared according to the rules of Good Laboratory Practice.

Preparation of solutions and the range for determining the method

Test solution: Dilute the volume of the medicine accurately measured, which is equivalent to 10 mg of phenylephrine hydrochloride (1 mL of 1% solution of Mesatonum for injections) with 0.1 M solution of hydrochloric acid to the volume of 200 mL.

Reference solution: Prepare a reference solution of the standard sample of phenylephrine hydrochloride in 0.1 M solution of hydrochloric acid with the concentration of 50 mg/mL of phenylephrine hydrochloride.

Measure the optical density at 273 nm relative to the compensation solution (0.1 M solution of hydrochloric acid) three times with removing the cuvette. In parallel determine the optical density of the standard solution. Measure the content of phenylephrine hydrochloride in mg per 1 mL of solution for injections.

RESULTS AND DISCUSSION

The quantitative content of phenylephrine hydrochloride in solution for injections has been proposed to be carried out using the method of absorption spectrophotometry in the ultraviolet region by the standard method chosen by us for verification of the method.

The research conducted has confirmed that the UV absorption spectrum of phenylephrine hydrochloride in the range from 230 nm to 300 nm is characterized by the absorption maximum at the wavelength of 273 nm. Data in Fig. 1 show that the absorption spectra of the test solutions and standard solution practically coincide.
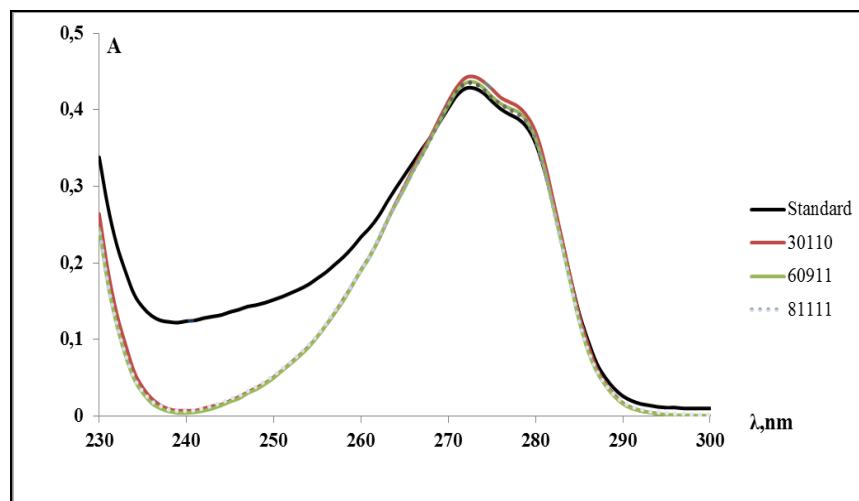
Fig. 1: The absorption spectrum of phenylephrine hydrochloride

To check robustness of the method for quantitative determination the stability of solutions was studied in time[21]. The check of solutions stability was performed for 60 min for the standard solution. It has been found that the analytical solution is stable for an hour, and it is sufficient in order to determine the optical density (Table 1).

Table 1: The study of stability of the analytical solution

No. of solution	Term of stability studies, t, min.					Average
	0	**15**	**30**	**45**	**60**	
A_{st}	0.428	0.428	0.428	0.428	0.428	0.428

Linearity of the method was investigated in the concentration range of 80-120%. Nine model solutions of phenylephrine hydrochloride accurately weighed with the concentrations of 80%, 85%, 90%, 95%, 100%, 105%, 110%, 115%, 120% of the nominal concentration were prepared. The linear characteristics b, Sb, a, Sa, Sr, r correspond to the acceptance criteria, linearity of the method is observed within the whole range of application (80-120%) (Table 2, Fig. 2).

Table 2: Metrological characteristics of the linear dependence

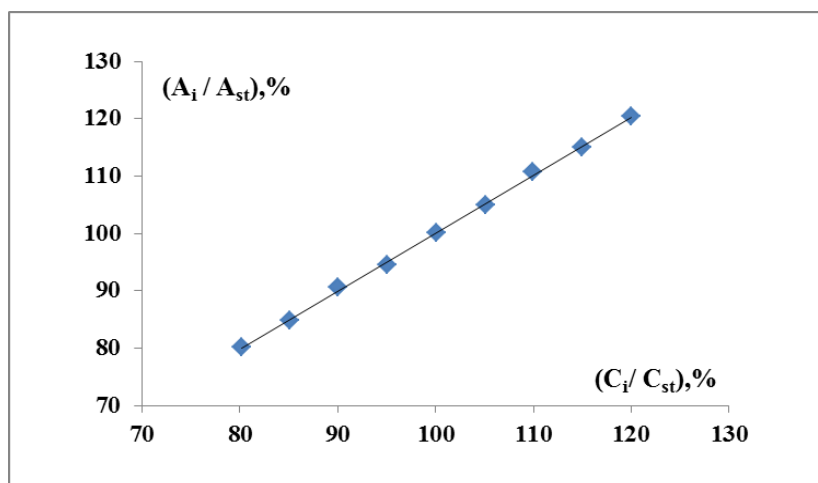| Parameter	Value	Requirements		Estimation of results (satisfied or not satisfied)
b	1.0107	-		-
s_b	0.0101382	-		-
a	| -0.97 |	≤ 1.94	≤ 2.6	satisfied by the first criterion
s_a	1.02263	-		-
s_0	0.39147	-		-
s_0/b	| 0.39 |	≤ 0.84		satisfied
s_Y	13.65			
r	0.999590	≥ 0.99808		satisfied

Fig. 2: The ratio of the optical density in relation to the ratio of the concentration of phenylephrine hydrochloride solution

Precision and accuracy was assessed from the linear data. The results of precision and accuracy determination and the correspondent criteria for the allowed content + 5% are presented in Table 3.

Table 3: The results of analysis for model mixtures and their statistical processing

No. of the model solution	Concentrations of the components		
	Introduced in % to the concentration of the reference solution $(C_i/C_{st}) \cdot 100\%$	Found in % to the concentration of the reference solution $(A_i/A_{st}) \cdot 100\%$	Found in % to the introduced $Z_i = (A_i/A_{st}) \cdot 100/(C_i/C_{st})$
1	80.12	80.10	99.97
2	85.11	84.81	99.64
3	90.01	90.65	100.71
4	95.00	94.56	99.54
5	100.10	100.16	100.06
6	105.09	105.04	99.94
7	109.99	110.63	100.58
8	114.99	114.95	99.97
9	119.98	120.46	100.40

Mean, $\bar{Z}$ %	100.09
Relative standard deviation, s_z%	0.40
Relative confidence interval $\Delta\% = t (95\%.8) \cdot s_z = 1.860 \cdot s_z =$	0.74
Critical value for convergence of results $\Delta_{As}, \% \leq$	0.25
Systematic error $\delta = \vert X - 100 \vert$	0.09
1. Criterion of the systematic error insignificance	Satisfied by the first criterion
2. $\delta \leq \tilde{\Delta} / 3 = 0.7439/3 = 0.2479$	
3. If not satisfied, then $\delta \leq 0.512$	
The overall conclusion of the method	Correct

As shown in Table 3, it is the precision method because the relative value of the relative confidence interval found is less than the critical value for convergence results: $\Delta\% = 0.24 \le 1.6$ (the systematic error of the method is significantly less than the regulated allowed content). The criterion of the systematic error insignificance $\delta \le \Delta/3 = 0.744/3 = 0.2479$ is performed; therefore, the method is correct.

The total uncertainty of analysis was calculated using the formula:

$$\Delta_{As} = \sqrt{\Delta^2_{Sp} + \Delta^2_{FAO}}$$

where Δ_{Sp} – is the uncertainty of the sample preparation;

Δ_{FAO} - is the expected measurement uncertainty (the final analytic operation).

$$\Delta_{As} = \sqrt{\Delta^2_{SP} + \Delta^2_{FAO}} = \sqrt{0.43^2 + 1.014^2} = 1.10$$

Thus, the expected results of the total uncertainty of the analysis results of the method for quantitative determination does not exceed the critical value (1.6%).

To confirm specificity of the analytical method the systematic error introduced to the solvent and excipients was calculated.

Requirements for the total uncertainty of the analysis results (Δ_{As}) are expressed as one-sided confidence interval for the probability of 95%:

$$\Delta_{As}(\%) \le \max \Delta_{As} = B \cdot 0.32 \, ; \; \Delta_{As} \le 1.6\% \; .$$

Requirements for the systematic error are:

$$\delta(\%) \le \max \delta = 0.32 \cdot \max \Delta_{As} \, ; \delta \le 0.512\%$$

Measurement of the optical density of placebo solution (A_{blank}) was conducted three times with removing the cuvette. In parallel the optical density for the reference solution (A_{st}) was measured. As a result, $A_{blank} = 0.001$; $A_{st} = 0.428$. It has been found that the placebo effect is 0.234%, and it does not exceed the determination error, which is 0.512%.

CONCLUSION

The spectrophotometric method for quantitative determination of phenylephrine hydrochloride solution for injections has been verified by such validation characteristics as specificity, linearity, accuracy, convergence and robustness. These metrological characteristics of the method do not exceed the acceptance criteria according to the SPhU.

The experimental data obtained show that the method can be correctly reproduced and can be recommended for use in laboratories for drug quality analysis when analyzing phenylephrine hydrochloride solution for injections.

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