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Research Article

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Development of the extraction-photometric method for quantitative determination of Timolol Maleate

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ABSTRACT

The extraction-photometric method for quantitative determination of timolol maleate has been developed. This method is based on the ion associate formation with methyl orange dye; its chloroform solution has the absorption maximum at the wavelength of 426 nm. The method allows to determine the content of timolol maleate in the range of concentrations from $22.0 \cdot 10^{-4}$ to $2.2 \cdot 10^{-3}$ g/ml. During the work the reagents for forming the ion associate (0.1% solution of methyl orange, pH of phosphate buffer solution) have been selected. The stability of the chloroform solution of the ion associate has been studied. The following validation characteristics have been studied: robustness (the analytical solution is stable for an hour), linearity (a=-0.29% ≤ max a, 5.1%; b=1.0064; the correlation coefficient is 0.9992), accuracy (0.34% ≤ max δ, 1.02%), precision (1.13% ≤ max Δas 3.2%); they indicate the correctness of the given method for quantitative determination.

Key words: timolol maleate, quantitative determination, validation, spectrophotometry

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INTRODUCTION

Timolol maleate − (S)-1-(tert-butylamine)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)hydroxy] propan-2-ol – is a nonselective β-blocking agent, which unlike all other drugs of this group does not possess the intrinsic sympathomimetic activity. Since 1978 it has been used for treating chronic wide-angle glaucoma, and in some cases for treating secondary glaucoma [1]. Timolol maleate is manufactured by different domestic and foreign producers in the form of eye drops, being very seldom in tablet dosage forms.

For quantitative determination of timolol maleate in the substance such methods as acid-base potentiometric titration [7,8], voltammetry [2], HPLC [3,11,12], UV-spectrophotometry [4] are recommended. In literature the methods of chromatography [5] and spectrophotometry [7] are described for analysis of the substance in eye drops.

Often in finished dosage forms the excipients with the aromatic structure are used; they can interfere with determination of timolol maleate by the method of direct spectrophotometry. The extraction-photometric method with the use of synthetic organic dyes is of interest. This method of analysis is highly sensitive, rapidly developed and promising as it allows to determine the active substance in the presence of additional ingredients.

The aim of the work is to develop the extraction-photometric method for determination of β-blocking agent of timolol maleate based on the ion associate formation with methyl orange.

EXPERIMENTAL SECTION

The reference standard of timolol maleate (India), batch No.20121413P was used in the work. Reagents were methyl orange dye of analytical grade; purified water; 96% alcohol; ammonium acetate of analytical grade; phosphate

buffer solution with pH=3.5; chloroform. The reagents used were prepared according to the requirements of the SPhU [6].

Analytical research was performed on an Evolution 60S spectrophotometer of Thermo Fisher Scientific company, USA, a pH-150MI potentiometer using "AXIS" ANG 200 electronic laboratory balances (Poland), measuring glassware of class A.

Method:
Place 0.05 g of timolol maleate (accurately weighed) into a 50 ml volumetric flask, dilute to the volume with purified water. Transfer the accurately measured volume of 0.5 ml of the test solution into a separating funnel, add 3 ml of 0.1% solution of methyl orange, 0.75 ml of 5% potassium bichromate solution, 10 ml of buffer solution with the pH=3.5, 10 ml of chloroform. Shake for 3 min and leave for 5 min to separate the layers. Filter the chloroform extract through the paper filter into a 50 ml volumetric flask. Repeat the extraction with chloroform twice more using 10 ml of chloroform each time. Filter through the same filter into a volumetric flask, dilute to the volume with chloroform and mix. Measure the optical density of the solution obtained at the wavelength of 426 nm in the cell with the layer thickness of 10 mm. Use chloroform as a compensation solution.

RESULTS AND DISCUSSION

By its chemical properties timolol maleate is the salt of an organic base, therefore, we assumed that this compound can form ion associates with acid indicators. For this purpose we applied the most frequently used dyes such as bromothymol blue, bromophenol blue and methyl orange. The reaction of the ion associates formation occurs most often at pH=7.5 [9,10]. It has been found that under these conditions when using bromophenol blue the ion associate is formed, but the indicator partially passes into the chloroform extract. While carrying out the reaction with bromothymol blue the ion associate is not formed. The ion associate is formed with methyl orange, but colouring of the chloroform layer is unstable. While carrying out the reaction of the ion associate formation at pH=3.5 it has been found that the most stable compound is formed when using methyl orange as a dye.

When studying the absorption spectrum of the chloroform solution of the timolol ion associate with methyl orange in the range from 220 nm to 550 nm it has been found that absorption maxima are observed at the wavelengths of 289 and 426 nm (Fig.1).

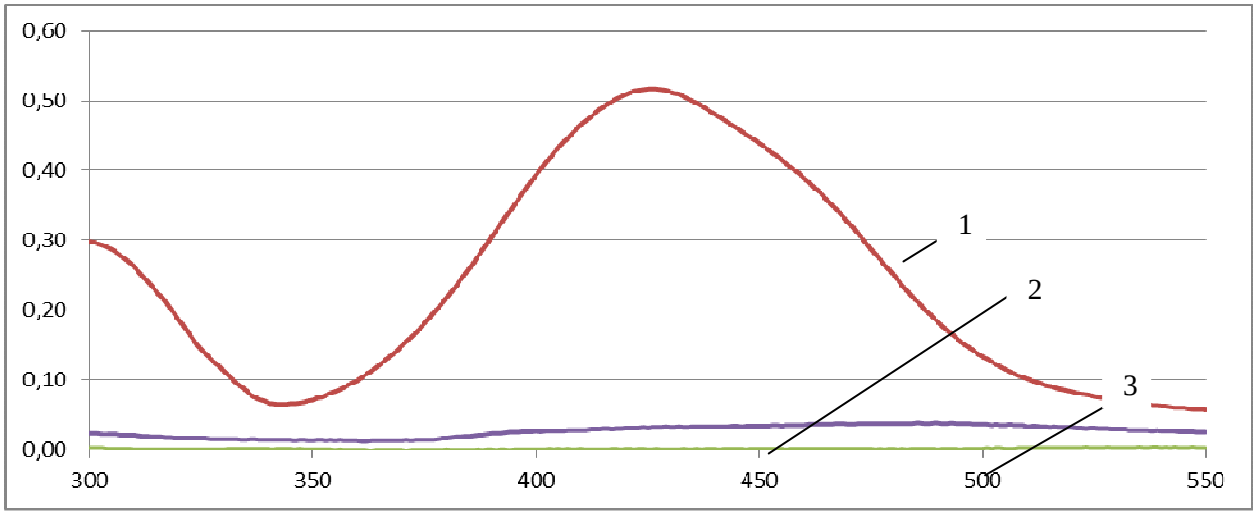

Fig. 1 – The absorption spectrum of chloroform extracts:
1 – the ion associate of timolol maleate substance with methyl orange; 2 – timolol maleate without methyl orange; 3 – methyl orange

Timolol maleate is poorly soluble in chloroform, and methyl orange is practically insoluble; they do not affect the character of the absorption spectrum of the chloroform solution of the ion associate (absorption spectra correspond to the absorption spectra of the solvent), therefore, the maximum at the wavelength of 426 nm was chosen as an analytical band. It has been experimentally proven that the mole ratio of timolol maleate to methyl orange is 1:9.

For further application of the method in pharmaceutical analysis such main validation characteristics as specificity, linearity, precision (convergence), accuracy were studied.

The stability test for the test solution and the reference solution is one of the elements for studying robustness of the method, and it should be performed before checking all other validation characteristics. To assess robustness the stability of the chloroform solutions (the test solution and the reference solution) was checked by measuring the optical density three times removing the cell at the wavelength of 426 nm within an hour in 15, 30, 45, 60 min. The results of statistical processing of the experimental data and their assessment comparing them with the critical values are given in Table 1.

Table 1. Stability of solutions in time

Optical density	stability studies *t*, min					Mean	RSDt	Δt	$\delta_{max,}$%
	0	15	30	45	60				
A_o	0.522	0.522	0.521	0.521	0.519	0.522	0.1161	0.2475	1.54
A	0.523	0.524	0.523	0.522	0.522	0.524	0.1453	0.3097	

The systematic error δ introduced by their instability does not exceed the limit value $\delta_{max,}$%. As we can see, $\Delta_t \leq max\ \delta$, i.e. the test solution and the reference solution are stable within 1 hour.

Linearity of the method was studied in the range of 80-120% by measuring the optical density of chloroform solutions of the ion associates [6].

Calculation of the parameters of the linear dependence Y=b·X+a was conducted by the least square method. The calculation data – b, s_b, a, s_a values (the residual standard deviation) and r (the correlation coefficient) – are presented in Table 2; the straight line obtained in the normalized coordinates is given in Fig. 2.

As can be seen from Table 2, the requirements of the State Pharmacopoeia of Ukraine [6] to the parameters of the linear dependence are met, i.e. linearity of the method is confirmed within the range of the concentrations studied.

Table 2. Metrological characteristics of the linear dependence Y = b·X + a of timolol maleate

Parameters	Values	Criteria (for tolerances 90-110%, g=9)	Conclusion (satisfied or not)
b	1.0064	-	-
s_b	0.0155	-	-
a	-0.29	1) ≤ 1.8946·s_a = 5.1; 2) if it is not satisfied 1), then ≤ 5.1;	satisfied
s_a	1.5634	-	-
r	0.9992	≥ 0.99236	satisfied

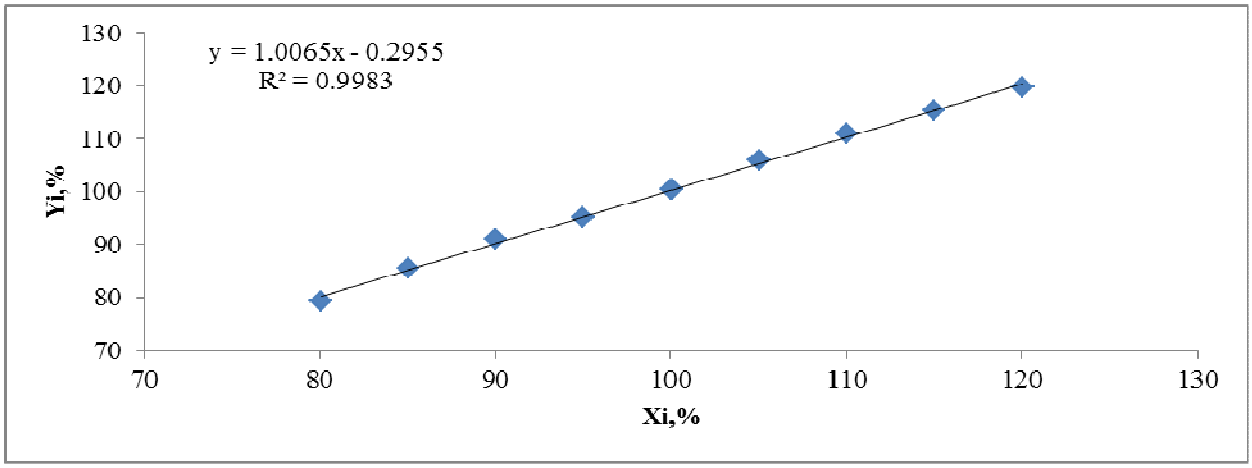

Fig. 2 – The linear dependence of the optical density from the concentration of timolol maleate in the normalized coordinates

The method of analysis is characterized by sufficient convergence and accuracy within the whole range of concentrations of 80-120% (Table 3).

As can be seen from the data of Table 3, the method is precise since the value of the relative confidence interval found is less than the critical value for convergence of results: Δ% = 1.13 ≤ 2.24 (the systematic error of the method is significantly less than specified tolerances). The criterion of the systematic error insignificance is performed – δ≤ 0.24, therefore, the method is correct.

As a result of the experimental studies it has been proven that the method allows to determine reliably the quantitative content of timolol maleate. The validation procedure gives the possibility to recommend the extraction-photometric method in the drug quality control laboratories.

Table 3. The results of analysis for test solutions and their statistical processing

No. of the test solution	Introduced in % to the concentration of the reference solution (Xi act%)	Optical densities Ai (Ast=0.522)	Found in % to the concentration of the reference solution (Yi%)	Found in % to the introduced Zi=100(Yi/Xi)
1	80.02	0.414	79.31	99.11
2	85.01	0.446	85.44	100.51
3	90.01	0.475	91.00	101.10
4	95.00	0.497	95.21	100.22
5	100.02	0.524	100.38	100.36
6	105.02	0.553	105.94	100.87
7	110.01	0.579	110.92	100.83
8	115.01	0.602	115.33	100.27
9	120.01	0.625	119.73	99.77
Mean, $\overline{Z}$ %				100.34
Relative standard deviation, s_z%				0.6096
Relative confidence interval, Δas%=t(95%,8)· Sz				1.1335
Critical value for convergence of results Δas				2.24%
Systematic error δ=$\lvert \overline{Z} - 100 \rvert$				0.34
Criterion of the systematic error insignificance 1) *δ≤Δas/(g)^0.5=0.72/√9* 2) if it is not satisfied 1), then δ≤0.72				0.24
The overall conclusion of the method:				correct

CONCLUSION

1. The extraction-photometric method for quantitative determination of timolol maleate based on the reaction of the ion associate formation with a dye has been developed.
2. The conditions of the extraction-photometric method such as pH and the amount of the dye have been selected, the ratio of timolol maleate to methyl orange has been determined, the analytical band has been selected.
3. The validation procedure of the method for quantitative determination of timolol maleate has been conducted; it confirms specificity, linearity, precision (convergence), accuracy and the range of application.

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