Development of the Spectrophotometric Method for the Determination of Metoprolol in Tablets by using Bromothymol Blue

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Recieved: March 07, 2023; Accepted: August 11, 2023
DOI: 10.17721/moca.2023.63-69

A simple, eco-friendly, rapid, and economic spectrophotometric method for the determination of metoprolol tartrate in tablets has been developed. The procedure for metoprolol determination is based on a reaction with bromothymol blue (BTB) in acetonitrile medium. The maximum formation of the colored product was observed in acetonitrile at the absorption maximum at a wavelength of 402 nm. The stoichiometry of molecular complexes was determined by Job's method and it was found to be 1 to 1. The optimal concentration of BTB was $1.6 \cdot 10^{-4}$ M. The linear relationship between absorbance at λ_{max} and concentration of analyte ranging 16.00-24.00 μg mL^{-1} was found. Regression analysis of Beer's law plot at 402 nm yielded the regression equation, y = 0.0199x + 0.0721. High values of correlations coefficient R^2 = 0.9990 and small values of intercept validated the linearity of calibration curve and obedience to Beer's law. The calculated LOD and LOQ values were 1.90 and 5.78 μg mL^{-1}, respectively. The proposed green spectrophotometric method is simple, rapid, and suitable for routine pharmaceutical analysis.

Keywords: metoprolol, bromothymol blue, spectrophotometry, validation, quantitative determination, pharmaceutical analysis, tablets

Diseases of the cardiovascular system occupy a significant part in the general list of human illness. Among them: arterial hypertension, myocardial infarction, chronic heart failure, various types of arrhythmias, and others. For the treatment of the above-mentioned conditions are used the means of neurohumoral modulation, hemodynamic support and drugs used to improve the prognosis regarding the duration and quality of life of patients. Beta adrenoblockers cover a large share among these medicines. Metoprolol tartrate is cardioselective drug with hight lipophilic effect. Chemically is bis [(2RS)-1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl) amino]propan-2-ol] (2R, 3R)-2,3-dihydroxybutanedioate, - water-soluble molecule (0.402 mg/mL), log P = 1.8, pKa (strongest acidic) = 14.09, pKa (strongest basic) = 9.67 [1]. European Pharmacopoeia (EP) has a monograph on the substance metoprolol tartrate [2], but there is no monograph on tablets or pharmaceutical formulation. Summarizing the review of the scientific literature about the existing methods of determining metoprolol was established that scientists have been described a number of spectrophotometric [3-10] and chromatographic [11-20] methods of analysis. However, many of them are limited in their applications, difficult to perform and time consuming, whereas simple spectrophotometric methods are very few and generally include extraction or dilution of the drug followed by measurement of absorbance in the UV-region which leads to lack of specificity and may be affected by other UV-absorbing drugs, dyes and flavors, or degradation products. Spectrophotometric methods with various reagents for the determination of metoprolol in drug demonstrated in Table 1. The spectrophotometric method of the determination of metoprolol developed by scientists Mustafa Cesme et al. based on interaction with Cu (II) salts in Britton-Robinson buffer solution [3]. Scientists Nabil et al. developed a spectrophotometric method for the determination of metoprolol by the reaction with bromothymol blue (BTB) in buffer pH 3.4 and chloroform medium and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in a acetonitrilic solution at maximum wavelengths of 413 nm and 457 nm respectively [4]. The first method described in the article, which was based on the interaction of metoprolol and BTB, is interesting but during a careful analysis of this method, we drew attention to the large amount of metoprolol taken for analysis (equivalent to 100 mg), large waste, and the use of chloroform (40 mL). All of the above does not correspond to the principles of «green chemistry». Donchenko A. and Vasyuk S. developed the spectrophotometric method for the determination of metoprolol in pharmaceutical forms by using 2,3-dichloro-1,4-naphthoquinone in the medium of dimethylformamide (DMF) [5].

Table 1. Overview of the spectrophotometric methods for the determination of metoprolol in drug.

№, Ref.	Drug	Reagent	Medium	λ_{max}, nm	Conc. range	LOD/LOQ, μg mL^{-1}	AGREE assessment	Comments
1. [3]	Pharmaceutical dosage forms	Cu(II) salt	Britton-Robinson buffer solution	675	8.5-70 μg mL^{-1}	LOD 5.56		direct determination
2. [4]	In bulk, tablets and capsules	Bromothymol blue (BTB) or 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)	buffer pH 3.4, chloroform(BTB), acetonitrile (DDQ)	413 (BPB); 457 (DDQ)	2.0-40.0 μg mL^{-1} in BPB method; 5.0-25.0 μg mL^{-1} in DDQ method	BTB LOD 0.363 LOQ 1.10; DDQ LOD 0.746 LOQ 2.286		extraction-photometric determination
3. [5]	In pure and dosage forms	2,3-dichloro-1,4-naphthoquinone	Dimethylformamide (DMF)	493	18.00-28.00 mg/100 mL	LOD 1.38, LOQ 4.49		direct determination
4. [6]	Pharmaceutical preparations	zero-, first-, second- and thirdorder derivative	Methanol	240-310 - zero order; the first derivative at 265, 278, 285; the second derivative at 276, 279, 287, 282; the third derivative at 275, 278, 281	-	-		direct determination
5. [7]	In bulk and tablet formulation	-	Phosphate buffer 6.8	223 and 226	5-25 μg mL^{-1}	LOD 0.0498 and 0.765, LOQ 0.232 and 0.280		direct determination
6. [8]	Pharmaceutical formulations	Alkaline potassium permanganate at 25+/-1 °C	0.60 M NaOH	610	1.46·10^{-6}-8.76·10^{-6} M	LOQ 0.04 and 0.10 microg ml-1		direct determination
7. [9]	Tablets	Bromo-phenol blue (BPB)	Methanol	595	9.56 -15.02 μg mL^{-1}	LOD 0.81 LOQ 2.67		direct determination
8. [10]	Tablets	Bromocresol green (BCG)	Methanol	624	5.47-38.30 μg mL^{-1}	LOD 0.41 LOQ 1.24		direct determination

All the described spectrophotometric methods for the determination of metoprolol were carried out using direct determination, except for the methods described in the article [4], where extraction-photometric determination was required. Described spectrophotometric methods have a large number of disadvantages such as toxic solvents were used, big amount of waste, long sample preparation. Nowadays, the requirements for pharmaceutical analysis have been changed. The environmental friendliness of analytical methods is no longer a luxury, but a necessity.

Previously our scientific group had been developed ecofriendly spectrophotometric methods for the determination of metoprolol based on the reaction with sulfophthalein dyes, namely bromphenol blue (BPB), bromcresol green (BCG) in methanolic solution at maximum wavelengths of 595 nm and 624 nm, respectively [9, 10]. Therefore, we continued the development of a spectrophotometric method for the determination of metoprolol in tablets with other sulfophthalein dyes and settled on bromothymol blue (BTB). The aim of our work was to develop a simple, ecofriendly and rapid spectrophotometric method for the determination of metoprolol tartrate in tablets, suitable for routine pharmaceutical analysis.

Materials and Methods

Objects of study, solvents and equipment. A double–beam Shimadzu UV-Visible spectrophotometer, with spectral bandwidth of 1 nm wavelength accuracy ± 0.5 nm, Model–UV 1800 (Japan), Software UV-Probe 2.62, and a pair of 1 cm matched quartz cells, was used to measure absorbance of the resulting solution. Other used instruments: Analytical Balance RAD WAG AS 200/C, pH–meter I-160MI.

All the chemicals used were of analytical reagent grade. Pharmacopeial standard sample (CRS) of metoprolol tartrate and BTB were provided by Sigma-Aldrich (≥ 98%, HPLC). The used dosage forms of metoprolol tartrate were 50, 100 mg (tablets).

Procedure for the determination of metoprolol tartrate with BTB. 5.48 mg of CRS metoprolol tartrate was transferred into a 50.00 mL volumetric flask with 35 mL acetonitrile. The mixture was shaken and diluted to volume with acetonitrile. Aliquot 1 mL was added to 1 mL of $1.6 \cdot 10^{-4}$ M acetonitrile solution of BTB. The volume 10.00 mL was made up to the mark by adding acetonitrile. The absorbance of the resulting solution was measured measured against the blank solution (a solution containing all components except the analyte) at a wavelength of 402 nm.

Procedure for tablets for the determination of metoprolol tartrate with BTB. Twenty tablets were accurately weighed and grounded. A quantity of powder containing 5.48 mg of metoprolol tartrate was transferred into a 50.00 mL volumetric flask with 35 mL acetonitrile. The mixture was shaken for 15 min, diluted to volume with acetonitrile and then filtered using 0.2 μm Nylon filter membrane. Aliquot 1 mL was added to 1 mL of $1.6 \cdot 10^{-4}$ M acetonitrile solution of BTB. The volume 10.00 mL was made up to the mark by adding acetonitrile. The absorbance of the resulting solution was measured against the blank solution (a solution containing all components except the analyte) at a wavelength of 402 nm.

Results and Discussion

Our scientific group has conducted a number of studies based on the interaction of metoprolol tartrate with sulfophthalein dyes, which can exist in solutions in the form of two protonated forms. The formation of the first form involves the splitting of a proton and the formation of a quinoid colored structure [9,10].

Choice of reaction conditions

Before starting the development of the analytical method, it is necessary to study the conditions of the reaction between metoprolol and BTB. Metoprolol forms complexes with BTB with an absorbance maximum at a wavelength of 402 nm (Fig. 1). To select the optimal solvent, series of experiments were conducted using methanol, ethanol, chloroform, ethyl acetate, and acetonitrile. Methanol and ethanol solutions were unsuitable for further use, as no absorbance was detected. The maximum absorbance of metoprolol was monitored in a solution of ethylacetate, chloroform and acetonitrile. (Fig. 2). For further studies we chose acetonitrile, because in this medium the maximum analytical signal was observed. Acetonitrile is also a satisfactory solvent according to the principles of «green chemistry». Optimal concentrations of BTB was $1.6 \cdot 10^{-4}$ M.

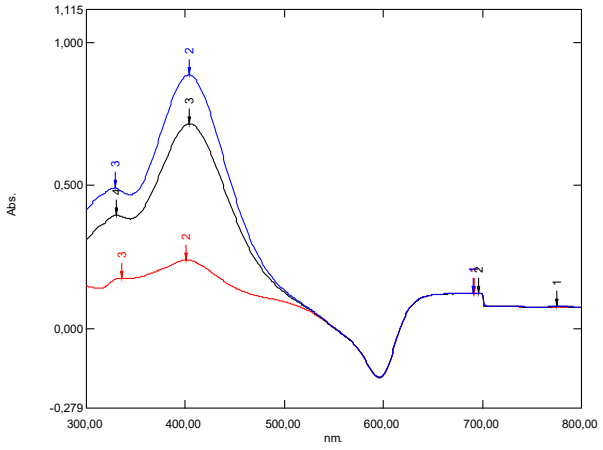

Fig. 1. Absorbance spectra of the reaction product of metoprolol tartrate ($C_M = 1.60 \cdot 10^{-4}$ M) from BTB ($C_M = 1.60 \cdot 10^{-4}$ M) against acetonitrile (blue), against BTB (black) and BTB against acetonitrile (red).

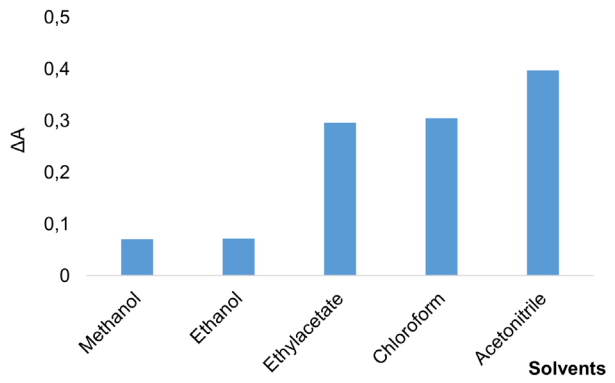

Fig. 2. Effect of solvents on the formation of metoprolol-BTB complex ($1.6 \cdot 10^{-4}$ M solutions at 402 nm).

The next stage of research was the determination of the stability of the solution over time (45 min). This is an important stage of research, because if there is no stability of the formed reaction product, then the further development of the analytical method is impossible. The results of the conducted studies showed that the change in absorbance during 45 min is insignificant (-0.005), which indicates that the reaction product is stable over time. However, we recommend determining the absorbance immediately.

Determination of stoichiometric coefficients was carried out by the Job's method.The graph of the dependence of the amount absorbance on the ratio of the volumes of the components of the isomolar series is presented on Fig. 3.

According to the data represented in Fig. 3, the stoichiometric ratios of the reactive components of «metoprolol tartrate - BTB» is 1 : 1.

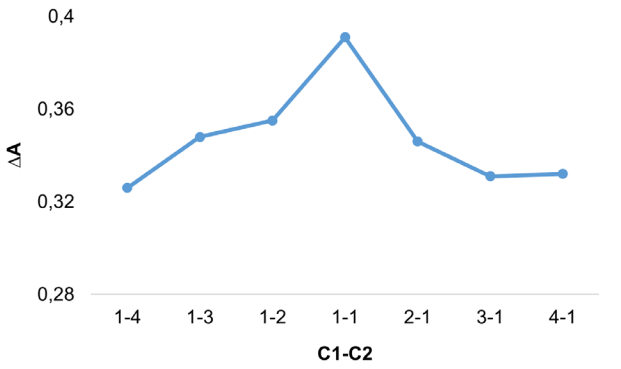

Fig. 3. Graph of the dependence of the absorbance on the composition of the isomolar solution : C_1 – $1.6 \cdot 10^{-4}$ M metoprolol tartrate; C_2 – $1.6 \cdot 10^{-4}$ M solution BTB) at 402 nm.

Validation of analytical method

The spectrophotometric method has been validated in accordance with the requirements of SPhU (State Pharmacopoeia of Ukraine) [21] (specificity, linearity, range of application, accuracy, precision and robustness).

Specificity

The study of the specificity of the analytical method was carried out using the absorbance of excipients of tablets («placebo»). The absorbance of excipients is insignificant (the found value of δnoise is 0.26 %) and does not exceed the acceptance criterion.

Linearity and range of application

Determination of linearity was performed over the calibration range of the method using model solutions. The results were statistically processed by the method of least squares in accordance with the requirements of the SPhU. It was found that there is a linear relationship between absorbance and concentration of metoprolol tartrate (Table 2). The analytical method was linear in the concentration range of 16-24 μg mL^{-1}. LOD and LOQ were 1.90 μg mL^{-1} and 5.78 μg mL^{-1}.

Table 2. Analytical parameters.

Parameter	Value	Acceptability criteria
Beer's law limits (μg mL^{-1})	16.00-24.00	-
Molar absorptivity	$1.60 \cdot 10^4$	-
Sandell sensitivity (μg cm^{-2})	0.0428	-
Limit of detection (μg mL^{-1})	1.90	-
Limit of quantification (μg mL^{-1})	5.78	-
Intercept (a)	0.0721	$\|a\| \le 2.6$
Slope (b)	0.0199	-
Correlation coefficient (r)	0.9990	> 0.9968
Standard deviation of intercept (Sa)	0.0115	-
Standard deviation of slope (Sb)	0.0016	-

Accuracy and precision

The deviation of the average value "found/put" from 100% was calculated according to the equation:

$$\delta = |\bar{z} - 100|,$$

The maximum permissible deviation value of "found/put" from 100% was calculated according to the equation:

$$\delta_{max} = 0.32 \cdot \Delta_{As},$$

The confidence interval of the spread of "found/entered" values was calculated according to the equation:

$$\Delta_z = S_z \cdot t(95\%, m - 1),$$

where: S_z - standard deviation of «found/put» ratios for all solutions; $t(95\%, m - 1)$ - the one-sided Student's coefficient for a probability of 95% (for and degrees of freedom 7—1=6 is 1.9432.

The calculation results are given in Table 3, 4.

Table 3. Accuracy and precision parameters.

Parameter	Metoprolol
Z_{cp}	100.01 %
δ	0.01 %
δ_{max}	0.51 %
Δ_{lin}	0.82 %
Δ_{max}	1.60 %

Table 4. The results of the study of the precision of the method.

Drug	$\overline{X}$, g	S	RSD	Δx
Metoprolol 0.05 g (Farmak, № 10822)	0.05	$1.71 \cdot 10^{-4}$	0.68	0.07
Metoprolol 0.1 g (Farmak, № 30421)	0.10	$2.36 \cdot 10^{-4}$	0.58	0.28

Robustness

During the study of robustness it was found that the analyzed solutions were stable for 45 min, and fluctuations in the amount of added reagent (BTB solution) within ± 16.67 % did not significantly affect the absorbance (Table 5).

Table 5. Determination of the robustness of the analytical method.

Amount of BTB, mL	% BTB	ΔA
0.90	90.00	0.388
0.95	95.00	0.389
1.00	100.00	0.391
1.05	105.00	0.392
1.10	110.00	0.395

Assessment of the impact of analytical methods on the environment

In connection with the increase in environmental pollution, there is a need to create green analytical methods, which will have a positive effect not only on the environment, but also on the researchers/chemists who conduct the analysis. One of the main tasks in the development of the methodology is the determination of greenness using the analytical eco-scale and the AGREE (Analytical GREEnness) program [22, 23]. The number of points according of the analytical eco-scale was 88, which indicates an excellent green result (Table 6). The score according to the pictogram of analytical method using AGREE tool was 0.76 (Fig. 4).

Fig. 4. Pictogram of analytical method using AGREE tool.

Table 6. Analytical eco-scale for assessing the «greenness» of the developed method.

Parameters	Penalty points
Reagents BTB	1
Acetonitrile	3
Energy	0
Waste	8
Total number of penalty points	12
Ball of analytical eco-scale	88
Conclusion	Exellent «green» analysis

Presented results in Table 6 and Fig. 4 demonstrated that proposed spectrophotometric method developed by our scientific group was ecofriendly.

Conclusions

A simple, ecofrendly, rapid and economic spectrophotometric method for the determination of metoprolol tartrate in tablets based in reaction with BTB has been developed. The maximum formation of the colored product was observed in acetinitrile at the absorption maximum at a wavelength of 402 nm. The stoichiometry of molecular complexes was determined by Job's method and it was found to be 1 to 1. It was determined that the optimal concentration of BTB is $1.6 \cdot 10^{-4}$ M. The absorbance of the analytical form is stable at least for 45 min. The linear relationship was established between absorbance at 402 nm and concentration of drug in the range 16-24 μg mL^{-1}. Proposed green spectrophotometric method is simple, rapid and suitable for routine pharmaceutical analysis.

Funding

This research was funded by the Ministry of Health of Ukraine, under the project number 0120U104201.

Acknowledgments

The authors would like to thank all the brave defenders of Ukraine who made the finalization of this article possible.

Conflicts of Interest

The authors declare no conflict of interest.

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