DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE DETERMINATION OF CARVEDILOL IN TABLETS

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ABSTRACT

INTRODUCTION: Carvedilol is in a class of medications called beta blockers. It works by relaxing blood vessels and slowing heart rate to improve blood flow and decrease blood pressure.

AIM: The aim of this article is to develop a spectrophotometric method for the quantitative determination of carvedilol with diazonium salts, establish optimal conditions for the quantitative determination of carvedilol in drugs, and validate the developed methodology.

MATERIALS AND METHODS: All studies were conducted on the basis of the experimental pharmaceutical research department of the scientific medical laboratory center (NMLC) of the Zaporozhye State Medical University.

***Reagents and solvents:* 6.25 mg carvedilol tablets (PRANAPHARMJSC, Samara, series 20717), 12.5 mg Carvedilol Canon tablets (Canonpharma Production CJSC, Russia, series 060517), 25 mg Talliton tablets (EGIS Pharmaceuticals PLC, Hungary, series 473D0617), 25 mg Carvedilol-KV tablets (OJSC Kyiv Vitamin Plant, Kyiv, Ukraine, series EF 411017), diazol red 2G (NVF Sinbias), acetone, methanol, ethanol, isopropanol, water.**

***Analytical equipment:* SPECORD-200spectrophotometer (Analytic Jena AG, Germany), ULAB S131UV spectrophotometer, RADWAG XA 210.4Y electronic laboratory scales, Sonorex Digitec DT100H ultrasonic bath.**

RESULTS: The technique of spectrophotometric determination of the quantitative content of carvedilol based on its reaction with red diazole in water-methanol medium has been developed. The 1:1 stoichiometric ratios of the reactive components were obtained by the methods of continuous changes and the saturation method.

CONCLUSION: A spectrophotometric method of analysis for the quantitative determination of carvedilol in tablets has been developed. The method is simple, fast, accurate, and reliable. This has been proven by verification characteristics (linearity, precision, correctness and sustainability). The developed methodology is correct and can be used in the chemical and pharmaceutical industries.

Keywords: *carvedilol, diazonium salts, spectrophotometry, validation*

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INTRODUCTION

Carvedilol is in a class of medications called beta blockers. It works by relaxing blood vessels and slowing heart rate to improve blood flow and decrease blood pressure (1).

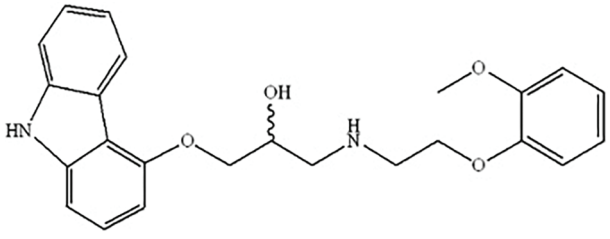

Fig. 1. Carvedilol—1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]-2-propanol (2).

Carvedilol is most often determined in drugs and biological fluids by chromatographic (3–5) and spectrophotometric methods (6). Determination of carvedilol in tablets by direct spectrophotometry of methanolic solutions at a wavelength of 241 nm has been proposed (7). When a mixture of acetonitrile and water is used as a solvent in a ratio of 60:40, the maximum absorption is observed at a wavelength of 332 nm (8). Determination of carvedilol in tablets by reaction with bromocresol purple in acetone (9), with bromothymol blue and bromocresol green in chloroform (10, 11), with p-dimethylaminobenzaldehyde and p-chloranil (12) are described. Determination of carvedilol by reaction with methyl orange and subsequent extraction with chloroform of the formed ionic associate is proposed by Wislous O.O. et. al. (13). Reaction of ionic associate formation with bromocresol green is the basis of another extraction-photometric method (14). From the literature data analysis, it follows that the existing methods of spectrophotometric determination of carvedilol are not always selective, sensitive, express, and satisfy the provisions of "green chemistry". So, the development of new methods is relevant.

AIM

The purpose of the present work was to develop a high-sensitivity, easy-to-execute, and valid spectrophotometric method for the quantitative determination of carvedilol in tablets based on the reaction with diazonium salts. The following tasks were set to achieve the goal:

- to establish the optimal conditions for the photometric reactions of carvedilol with diazole red 2G and calculate the analytical sensitivity parameters;
- develop a method for the quantitative determination of carvedilol in the pharmaceutical forms;
- validate the developed methodology.

MATERIALS AND METHODS

All reagents were analytical grade and included:6.25 mg carvedilol tablets (PRANAPHARMJSC, Samara, series 20717), 12.5 mg Carvedilol Canon tablets (Canonpharma Production CJSC, Russia, series 060517), tablets Talliton 25 mg (EGIS Pharmaceuticals PLC, Hungary, series 473D0617), 25 mg Carvedilol-KV tablets (OJSC Kyiv Vitamin Plant, Kyiv, Ukraine, series EF 411017), diazol red 2G (NVF Sinbias), acetone, methanol, ethanol, isopropanol, water.

SPECORD-200 spectrophotometer (Analytic Jena AG, Germany) and ULAB S131UV spectrophotometer were used to obtain the spectra and the absorbance measurements.

Other apparatuses included RADWAG XA 210.4Y electronic laboratory scales and Sonorex Digitec DT100H ultrasonic bath.

All studies were conducted on the basis of the experimental pharmaceutical research department of the scientific medical laboratory center (NMLC) of the Zaporozhye State Medical University.

Procedure for the Assay of Carvedilol

Preparation of carvedilol standard solution: 0.016 g of carvedilol, dissolved in methanol, was placed in a 100.00 mL volumetric flask, the flask was filled up to the mark with methanol, and then all was mixed.

Preparation of the compensating solution: 2.00 mL of 0.2% solution of diazole red 2G in methanol were transferred into a volumetric flask of 10.00 mL, 0.20 mL of 0.1% sodium carbonate solution, incubated for 10 min, were added,2.00 mL methanol were added, the flask was filled up to the mark with water, then all was mixed.

An aliquot of carvedilol (0.0544-0.5660 g) was placed in a 10.00 mL volumetric flask, 2.00 mL of 0.2% solution of diazole red 2Gin methanol were

added, 0.20 mL of 0.1% solution of sodium carbonate, incubated for 10 min, were added, 2.00 mL of methanol were added, the flask was filled up to the mark with water, then all was mixed. The absorption of the resulting solution was measured against the background of the compensation solution at an analytical wavelength of 385 nm.

Procedure for the Assay of Carvedilol in Pharmaceutical Preparations

The exact portion of a thoroughly ground tablet mass of 6.25 mg carvedilol (about 0.5 g), 25 mg Talliton (about 0.1 g), 12.5 mg Carvedilol Canon (about 0.2 g), and 25 mg Carvedilol-KV (about 0.1 g), was transferred to a volumetric flask of 100.00 mL, the flask was filled up to the mark with methanol, incubated in an ultrasonic bath for 2 minutes. The solution was then filtered through a paper filter ("Blue Ribbon"), discarding the first portions of the filtrate. A total of1.00 mL of solution from the following portions of the filtrate was transferred to a volumetric flask of 10.00 mL, 2.00 mL of 0.2% solution of diazole red 2Gin methanol were added, 0.20 mL of 0.1% sodium carbonate solution, incubated 10 min, were added, 2.00 mL of methanol were added, the flask was filled up to the mark with water, then all was mixed. The absorption of colored solutions was measured against the background of the compensation solution at an analytical wavelength of 385 nm. In parallel, the experiment was conducted with 1.00 mL of carvedilol standard solution. The calculation of the active substance content was carried out according to the conventional formula.

RESULTS AND DISCUSSION

Factors influencing the rate and completeness of the reaction (solvent, reagent amount, reaction time and stability of the analyzed solutions over time) were studied at the stage of the development of the method.

The solvent was chosen taking into account the solubility of carvedilol and diazole red 2G, as well as the maximum yield of the reaction product, i.e. the maximum value of optical density. The amount of reagent was also chosen according to the maximum value of the optical density.

It was found experimentally that the reaction took place in aqueous-methanol medium at room temperature using 0.2% solution of diazole red 2G as a color reagent with the formation of a colored reaction product with a maximum absorption at 385 nm (Fig. 2).

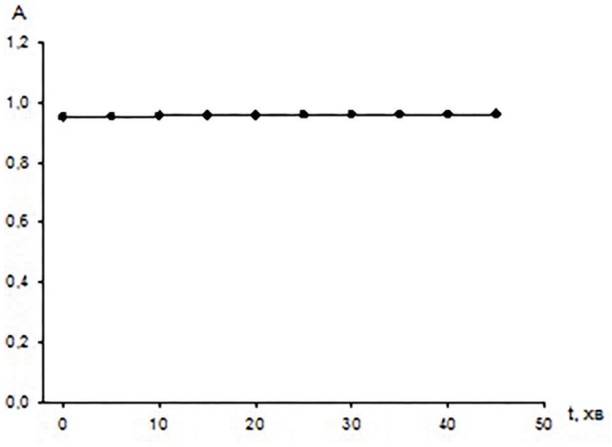

Fig. 2. Graph of the dependence of the optical density of the solution after the reaction of carvedilol with diazole red 2G

Determination of the amount of reagent required for the completeness of the reaction was set experimentally based on the maximum yield of the reaction product, i.e. the maximum value of optical density. To do this, 1.00 mL of the test drug solution and 0.50; 1.00; 1.50; 2.00, and 2.50 mL of reagent were added to a 10.00 mL volumetric flasks. The absorbance of the test solutions was measured against the background of compensating solutions at the above wavelength.

The external factors that can affect optical density (stability of the analyzed solutions in time, the

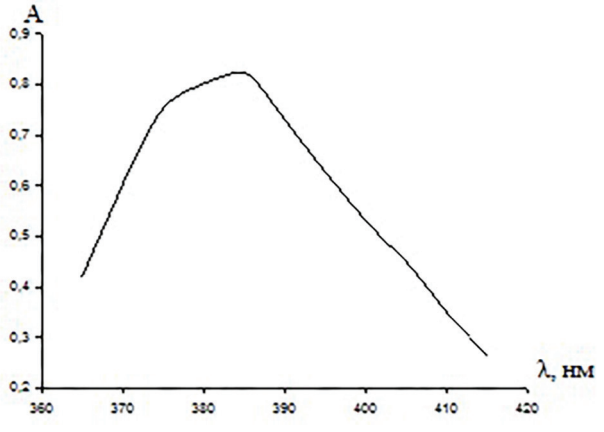

Fig. 3. The absorption spectrum of the reaction product of carvedilol with diazole red 2G

amount of added reagents and the temperature of the heated solutions of the analyzed solutions) were studied. It was found that analyzed solutions were stable at least 45 minutes (Fig. 3). The influence of other factors is described above.

Stoichiometry of the Reaction

Stoichiometric coefficients between carvedilol and diazole red 2G were established by the method of continuous changes (method of isomolar series) and by the method of saturation (method of molar ratios) (15).

The method of isomolar series is based on determining the ratios of isomolar concentrations of reactants, which corresponds to the maximum yield of compounds formed as a result of the reaction. To perform the assay, solutions of the reagent and test drug substance of the same molar concentration (0.0004 M) were prepared and mixed in anti-bath ratios, while the total volume of the solution remained unchanged. The reaction was carried out according to the developed method. Based on the obtained data, a graph of the dependence of the optical density on the ratio of the volumes of the components of the isomolar series was constructed (Fig. 4).

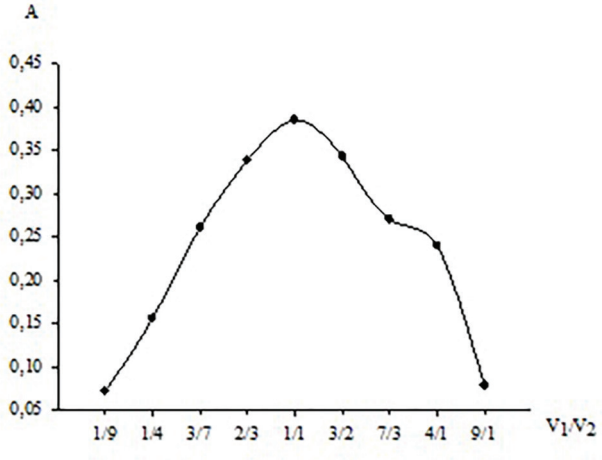

Fig. 4. Graph of the dependence of the optical density on the composition of the isomolar solution (V1—0.0004 M carvedilol solution, V2—0.0004 M solution of diazole red 2G) at 385 nm

The method of molar ratios determines the dependence of the optical density on the concentration of one of the components of the reaction mixture at a constant concentration of the second component and vice versa. The inflection point on the saturation curve is equal to the stoichiometric coefficient of the component whose concentration is varied (Figs. 5a, 5b).

As can be seen from Fig. 4 and Fig. 5a, b, the stoichiometric ratios of the carvedilol-diazole red 2G reacting components, obtained by both methods, agree with each other and are 1:1.

Determination of Validation Characteristics

In accordance with the requirements of the State Pharmacopoeia of Ukraine (16), the methods for quantitative determination of drugs should be validated. Validation of methods of quantitative analysis of drugs is the main condition for ensuring the reliability of the analysis results. Therefore, to verify the correctness of the proposed method, the main validation characteristics were determined, namely, linearity, precision, accuracy, and robustness (17).

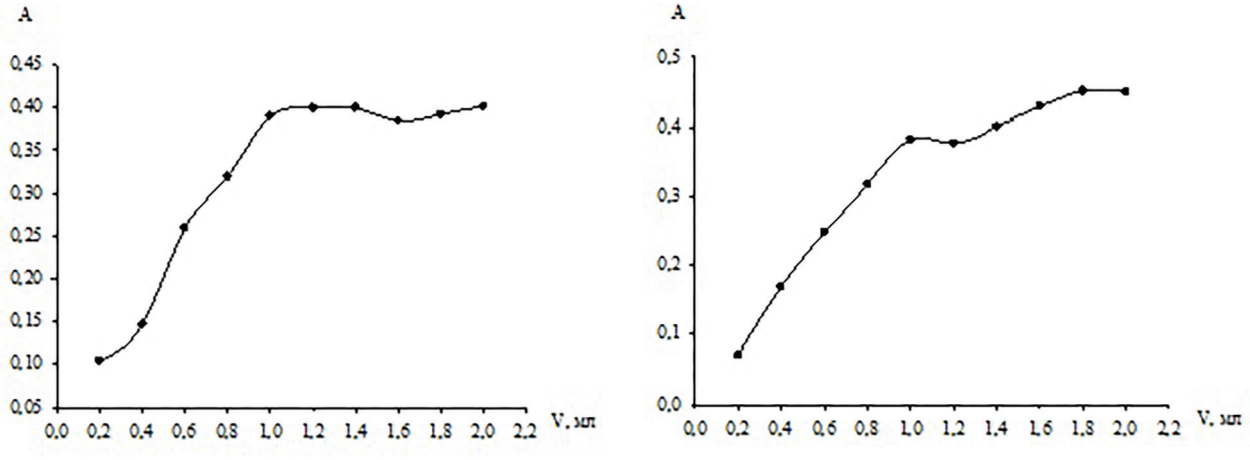

Fig. 5. a. Saturation curve of carvedilol solution at a constant concentration of the reagent solution; b. saturation curve of the reagent solution at a constant concentration of carvedilol solution

Linearity

The determination of the linearity was carried at the concentration range according to the main law of light absorption, namely 1–2 mg/100 mL. Standard solution of carvedilol was diluted for obtaining the solutions with known concentration, which were analyzed according to the general procedure. The dependence of the optical density on the concentration of the analyzed solution is described in Fig. 6.

The linearity of the method was confirmed throughout the concentration range indicated above (Table 1). Thus, the concentration range of application of the technique was 75–125% of the nominal content of carvedilol in the drug.

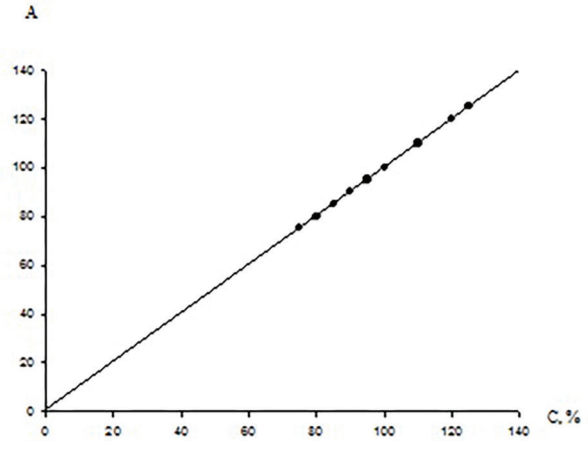

Fig. 6. Graph of the dependence of optical density on the concentration of carvedilol

Precision

The precision of the method was estimated by measuring nine replicate samples of carvedilol. The assays gave satisfactory results; the one-way confidence interval Δz did not exceed the maximum permissible uncertainty of the analysis Δ_{AS}, so the method was accurate at the level of convergence (Table 2). This level of precision of the proposed method was adequate for the quality control analysis of carvedilol in its pharmaceutical dosage forms.

Correctness

The correctness of the method has been determined via the method of additions. Different amounts of carvedilol solution were added to three equal sample dosage forms and analyzed three times. The estimated criterion of practical insignificance for all dosage forms did not exceed the maximum permissible uncertainty analysis (Table 3).

CONCLUSION

A validated spectrophotometric method for carvedilol assay in tablets has been developed. The method is simple, rapid, accurate, and reliable for the determination of carvedilol in tablets without inter-

Table 1. Spectrophotometric characteristics and statistical data of the regression equations

Parameter	Value
Molar absorption index, ε	21000
Sandel's ratio, WS	0.011275
Opening minimum, Cmin, (μg/mL)	0.97
Linear regression equation	Y = bX + a
Angular coefficient, b ± (Sb)	0.9947 (±0.0037)
Free term of linear regression, a ± (Sa)	0.5741 (±0.3653)
Residual standard deviation, Sx, 0	0.1835
Correlation coefficient, r	0.9999

Table 2. Evaluation of the accuracy and precision of the proposed procedure

Formulation Names	$\bar{Z}$% (n=9)	S_Z%	Δ_{AS}	Δ_Z
Carvedilol 6.25 mg	100.53	0.23	3.2	0.43
Carvedilol Canon 12.5 mg	100.01	0.36	3.2	0.67
Talliton 25 mg	100.23	0.66	1.6	1.23
Carvedilol-KV 25 mg	100.78	0.82	1.6	1.52

Table 3. Correctness of the results of quantitative determination of carvedilol

Formulation Names	$\bar{Z}$% (n=9)	S_z%	Δ_z	$\bar{Z}$ - 100
Carvedilol 6.25 mg	100.96	0.30	0.56	0.96
Carvedilol Canon 12.5 mg	100.23	0.28	0.52	0.23
Talliton 25 mg	100.18	0.33	0.61	0.18
Carvedilol-KV 25 mg	100.11	0.91	1.69	0.11

ference from the common excipients. It was proved by the validation characteristics (linearity, precision, correctness, and robustness) that the developed methodology is correct and can be applied in the chemical and pharmaceutical industry.

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