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Simultaneous determination of bisoprolol fumarate and rosuvastatin calcium in a new combined formulation by validated RP-HPLC

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RESEARCH ARTICLE

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ABSTRACT

A simple, specific, and precise RP-HPLC method was developed and validated for simultaneous determination of Bisoprolol fumarate (BIS) and Rosuvastatin calcium (ROS) in new formulated tablets. The developed RP-HPLC method depended on chromatographic separation using C_{18} column (150×4.6 mm, 0.5 µm) with mobile phase consisted of acetonitrile and 0.05 aqueous solution of orthophosphoric acid at the ratio of 65:35 % (*v:v*) with a flow rate of 1 mL/min and UV detection was carried out at 230 nm. Factors affecting the developed methods were studied and optimized and the retention times for BIS and ROS were found to be 2.758 and 4.974 min, respectively. Linearity of the proposed method was observed over a concentration range 0.2-50 µg/mL for each of BIS (r = 0.9999) and ROS (r = 0.9998). The proposed method was successfully applied for the determination of the studied drugs in their bulk powder, laboratory prepared mixtures and in the formulated tablets. The developed method is the first chromatographic method for determination of those drugs and showed no significant difference when compared with the reported methods.

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1. Introduction

Bisoprolol, (*RS*)-1-[4-[[2-(1-methylethoxy)ethoxy]methyl] phenoxy]-3-[(1-methylethyl)amino]propan-2-ol (Figure 1), is a cardioselective beta blocker used mainly for treatment of hypertension [1,2]. Rosuvastatin, (3*R*,5*S*,6*E*)-7-[4-(4-fluoro phenyl)-2-(*N*-methylmethanesulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid (Figure 1), working as lipid lowering drug by inhibition of hydroxyl methyl glutaryl coenzyme A (HMG CoA) reductase [3].

The combination of BIS and ROS is not official in any of the commonly known pharmacopoeias. While this combination is fruitful for the treatment of patients with multiple cardio-vascular diseases, such as hypertension with/or susceptible to atherosclerosis, or hypercholesterolemia. This combination was the invention related to Bondjers *et al.* [4] as United States Patent Application Publication No.: US 2003/0060477A1 Pub. Date: Mar. 27, 2003.

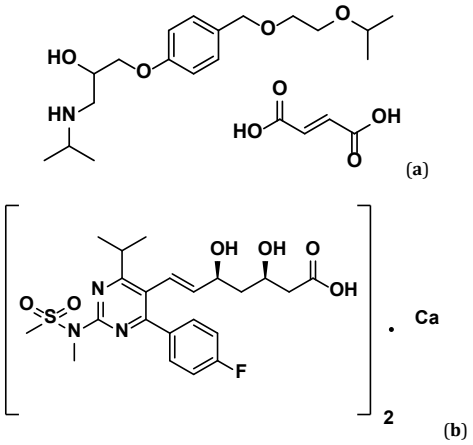

Figure 1. Chemical structure of (a) Bisoprolol fumarate and (b) Rosuvastatin calcium.

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The literature survey revealed that no any chromatographic method was reported for the simultaneous estimation of BIS and ROS in combination. While only one spectrofluorimetric method was found for their determination in those tablets [5]. On the other hand, determination of each drug was reported either alone or in other combinations. BIS was selectively determined by several methods like non aqueous potentiometric titration [1], spectrophotometric [6-9], spectrofluorimetric [9], and voltammetric methods [10]. Also, it was analyzed by different chromatographic methods such as HPTLC [11-13], HPLC [14-17], UPLC [18,19], and LC-MS/MS methods [20,21]. Rosuvastatin calcium was determined either alone or in its different combined dosage forms by several methods including titrimetric [22], different spectrophotometric [23-29], spectrofluorimetric [30], HPTLC [31-34] and HPLC [35-38] methods.

This research paper presents, for the first time, RP-HPLC method for the separation and quantification of BIS and ROS. The developed method was successfully applied for resolving the both drugs in a single run using a single detection wavelength which promotes application of the developed method in further quality control studies of the proposed drugs.

2. Experimental

2.1. Apparatus

The instrument used was an HPLC Agilent 1260 Infinity (Germany), equipped with an Agilent 1260 infinity preparative pump (Model No. G1361A), Agilent 1260 infinity diode array detector VL (Model No. G131SD), Agilent 1260 infinity thermostated column compartment (Model No. G1316A), and Agilent 1260 infinity preparative autosampler (Model No. G2260A). The stationary phase was a Zorbax Eclipse plus C18 column (150×4.6 mm id, 5 µm particle size, United States). A Sonix TV SS-series ultrasonicator (United States) was also used. Nylon 66 membrane syringe filter for filtration of the formulation solution, (Npore, Ghaziabad, India) was used.

2.2. Materials

2.2.1. Chemicals and reagents

All chemicals and solvents used throughout this work were of analytical grade and were used without further purification such as methanol; acetonitrile were HPLC grade (SDS, France), while orthophosphoric acid and deionized water were purchased from El-NASR Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt. For tablets formulation, the used Avecil PH 101, pharmaburst, aerosil, magnesium stearate, and lactose monohydrate were purchased from Sigma-Aldrich Company, Egypt [5].

2.2.2. Pure standard

Bisoprolol fumarate (BIS) was kindly supplied by AMOUN Pharmaceutical Co. Egypt. While, Rosuvastatin calcium (ROS) was kindly supplied by Astrazeneca Co., Cairo, Egypt.

2.2.3. Standard solutions

The stock standard solutions of BIS and ROS were prepared in the concentration of 1000 µg/mL in methanol. Then, two working standard solution of each BIS and ROS (100 then 10 µg/mL) were prepared from their respective stock standard solutions (1000 µg/mL) in methanol. All stock standard solutions were freshly prepared on the day of analysis and stored in the refrigerator to be used within 24 h.

2.3. Procedures

2.3.1 Laboratory prepared mixtures

Different laboratory prepared mixtures containing different ratios of BIS and ROS were prepared by accurately transferring different volumes of each from their respective working solutions of each drug into 10 mL-glass volumetric flasks and diluting to volume using the mobile phase

2.3.2. Tablets formulation

The tablets were formulated using, the pure drugs, Avecil PH 101, Pharmaburst, Aerosil, magnesium stearate, and lactose monohydrate with suitable ratios to get the final tablet weight 150 mg according to reference [5]. By using mortar and pestle, the calculated amounts of the drug and excipients were mixed then the calculated amount of magnesium stearate was added. Single punch machine was used for the preparation of tablets by concave 8 mm punch and die set.

2.4. Method validation

2.4.1. Linearity and range

Accurately measured aliquots equivalent to 2-500 µg/mL of each drug were separately transferred from their respective working standard solutions (100 and 10 µg/mL) into two series of 10 mL glass volumetric flasks and diluted to volume with mobile phase. Triplicate 20 µL injections were made for each concentration. Chromatographic separation was performed on the C18 column at 25 °C, and the effluent was UV-scanned at 230 nm. Isocratic elution consisting of acetonitrile and 0.05% aqueous solution of orthophosphoric acid at the ratio of 65:35 % (*v*:*v*) was used with a flow rate of 1 mL/min and UV detection at 230 nm. The peak areas were recorded, and calibration curves relating the obtained integrated peak areas to corresponding concentrations were constructed.

2.4.2. Accuracy

The accuracy of the proposed method was assessed by analyzing the samples with different concentrations of BIS and ROS within their linearity ranges by the developed method. The concentrations of both drugs were calculated from their corresponding regression equations, and the mean recoveries were calculated.

2.4.3. Precision

Repeatability was evaluated by assaying three concentrations of each drug (1, 10, and 20 µg/mL) three times intraday. Intermediate precision was evaluated by assaying the three chosen concentrations of each drug in triplicate on 3 successive days using the procedure stated in the Linearity and range section and SD values were calculated.

2.4.4. Specificity

The specificity of the chromatographic method was ascertained by application of the developed method to the laboratory-prepared mixtures containing different ratios of BIS and ROS, following the procedure stated in the Linearity and range section. Also, specificity was confirmed by calculating system suitability testing parameters, such as capacity factor, resolution, and selectivity factor for the separated peaks.

Table 1. Data of average weight, thickness, diameter, friability, and hardness of ten formulated tablets.

Parameters	Mean *	S.D.
Uniformity of weight (mg)	150.50	1.24
Tablet thickness (mm)	3.03	0.005
Tablet diameter (mm)	8.06	0.01
Friability % fine	0.370	0.104
Hardness (Kg)	8.02	0.227

* Average of ten determinations.

Table 2. Results of analysis of laboratory prepared mixtures and assay of the formulated tablets content by applying the proposed method and application of standard addition technique.

Sample	BIS	ROS
Mean±SD, Lab. prepared mixtures [a]	99.74±0.563	100.11±0.727
%Recovery±SD, Formulated tablets [b]	100.20±0.934	100.65±0.856
Standard addition [a]	98.50±0.424	99.80±0.704

[a] Average of 3 determinations.
[b] Average of 10 tablets determinations.

2.4.5. Sensitivity

Sensitivity of the method was established with respect to LOD and LOQ for both drugs. The LOD and LOQ were established by the slope method using the lower part of the calibration curves and the slope of the regression equations as mentioned below:

$$\text{LOD} = 3.3\times\frac{\text{Standard deviation of the response}}{\text{Slope of the calibration curve}} \quad (1)$$

$$\text{LOQ} = 10\times\frac{\text{Standard deviation of the response}}{\text{Slope of the calibration curve}} \quad (2)$$

2.4.6. Robustness

Robustness is the capacity of a method to remain unchanged with small changes in method parameters, e.g., changes in acetonitrile (±1%) and mobile phase flow rate (±0.1 mL/min). The effect of these changes on retention time (t_R) values were recorded and expressed as RSD.

2.4.7. System suitability testing parameters

Parameters such as resolution (RS), peak asymmetry, selectivity factors (α), and capacity factor (k') were calculated to test the overall system performance.

2.4.8. Application to the formulated tablets

The content of 20 formulated tablets were powdered and mixed well. An amount of the powdered tablets equivalent to 100 mg of each drug was accurately weighed and transferred to 100 mL glass volumetric flask, 75 mL methanol was added and the prepared solution was ultra-sonicated for about 30 minutes then cooled well; the volume was completed with methanol to get 1000 µg/mL stock solution and, then filtered. Calculated dilutions were made to obtain concentrations of both BIS and ROS in their linearity ranges. Then, the procedure illustrated under linearity was followed.

3. Results and discussion

Utilization of drug combinations to treat cardiovascular conditions that does not correspond well with availability of combination of fixed-dose products containing these agents. Chronic conditions such as dyslipidemia and hypertension are frequently coexisting and also they are common causes of coronary or ischemic heart diseases [39].

The aim of our work is to assist in further quality control and clinical studies for the use of the new combination that improve the patient compliance.

3.1. Evaluation of the formulated tablets

The formulated tablets were evaluated according to the quality control criteria [40-42]. Table 1 summarizes the acceptable results values concerning weight uniformity, thickness and diameter, friability, and hardness for ten randomly chosen tablets. The content uniformity of those tablets was tested, and the results summarized in Table 2 were found to be within the official acceptable range for the tablets content analysis [1,14].

3.2. Method developments and optimization

Our main goal of establishing this RP-HPLC method was to provide rapid and specific tool for further quality control analysis of BIS and ROS. Various isocratic mobile phase systems were tried on many reversed phase columns C8 and C18 with different length and successfully attempts were reached upon using C18 column (150×4.6 mm, 0.5 µm). Several trials were applied beginning with methanol and water at first with different ratios to separate the two studied drugs with reasonable retention time with sharp peaks but it was noticed they took long time to be completely eluted especially ROS, even after increasing methanol ratios. So methanol had to be replaced by acetonitrile: water (40:60), (50:50), (60:40), and (65:35) which somewhat enabled to elute both of the drugs in earlier time upon increasing the ratio of acetonitrile but with broad peaks which affected peaks symmetry. In order to enhance the separation and peaks shapes for each drug the effect of pH was tested and it was found that both drugs response well to acidic medium, so acetic acid, formic acid, and orthophosphoric acid were tested. Successful attempts were obtained by using 0.05% aqueous solution of orthophosphoric acid in place of using neutral water to reach the optimum mobile phase consisting of acetonitrile: 0.05% aqueous solution of orthophosphoric acid (65:35, *v*:*v*) with flow rate 1 mL/min. Several wavelengths were tested (220, 230, 242, and 254 nm); the most suitable wavelength for detection was 230 nm, at which high sensitivity of both cited drugs with minimum detector noise was obtained. Upon using those optimum chromatographic conditions, satisfactory separation of BIS and ROS were obtained and eluted at 2.758 and 4.974 min, respectively, as shown in Figure 2.

3.3. Method validation

Validation of the proposed method was performed according to ICH guidelines [43]. The linearity of the proposed methods was evaluated, and linearity was proved in the range of 0.2-50.0 µg/mL for each drug. Regression and analytical parameters are shown in Table 3.

Table 3. Assay and validation parameters obtained by applying the proposed methods.

Parameters	RP-HPLC	
	BIS	ROS
Range (µg/mL)	0.2-50	0.2-50
Slope	379.28	578.74
Intercept	245.40	139.75
Correlation coefficient	0.9999	0.9998
Accuracy (Mean recovery %)	99.50	100.02
Precision (SD)		
Repeatability	0.325	0.195
Intermediate Precision	0.645	0.425
LOQ (µg/mL)	0.20	0.20
LOD (µg/mL)	0.07	0.07
Robustness (RSD%) *		
Acetonitrile ratio ±1%	0.548	0.452
Flow rate ±0.2 mL/min	0.348	0.445

* Change in retention time.

Table 4. System suitability testing parameters of the HPLC method.

Parameters	BIS	ROS
Resolution	4.94	4.94
selectivity (α)	2.08	2.08
Tailing factor (T)	1.05	1.00
Capacity factor (K')	2.940	6.106
Column efficiency (n)	1452.10	2456.52
HETP *	0.010	0.006

* HETP = Height equivalent to the theoretical plate (cm/plate).

Table 5. Statistical comparison between the results obtained by the proposed method and the reported methods.

Item	BIS, Found%		ROS, Found%	
	HPLC	Reported [15]	HPLC	Reported [35]
Pure drug	101.00±1.200	100.11±1.140	100.09±0.750	99.96±1.020
N	6	6	6	6
Student t-test (2.228) *	0.943	-	0.108	-
F-Value (5.050) *	1.107	-	1.857	-

* The values in the parenthesis are corresponding theoretical value at degree of freedom $p = 0.05$.
[15] Reported method for determination of BIS by HPLC on cyano column (4.6×250 mm, 5 µm) with the isocratic mobile phase of 0.1 M aqueous phosphate buffer, acetonitrile and tetrahydrofuran (85:10:5, *v:v:v*) at a flow rate of 1.0 mL/min. The UV detection was carried out at 225 nm.
[35] Reported method for determination of ROS by HPLC on C_{18} column (250×4.6 mm, 0.5 µm) using acetonitrile and 1 % acetic acid in water (80:20, *v:v*) with a flow rate of 1 mL/min and UV detection at 252 nm.

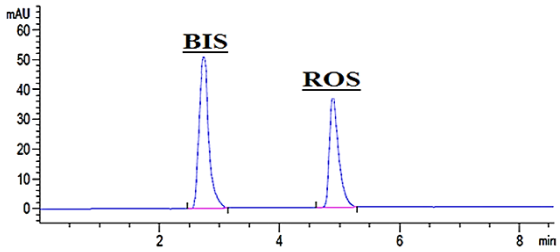

Figure 2. HPLC chromatogram of bisoprolol fumarate and rosuvastatin calcium using acetonitrile: 0.05% aqueous solution of orthophosphoric acid (65:35, *v:v*) at 230 nm.

Good percentage recoveries were obtained when testing method accuracy and the results are given in Table 3. Accuracy was further assessed by applying the standard addition technique on the formulated tablets for which good results were obtained, revealing the good accuracy of the proposed method and proving that excipients did not interfere Table 2. The proposed method provided acceptable intra- and inter-day variation, indicating their acceptable precision, and that they are suitable for the quality control (QC) of the suggested components. Good standard deviation (SD) values were obtained, Table 3.

Specificity of the proposed method was evident from the HPLC chromatograms in Figure 2. Also, specificity of the methods was proven from the good recovery percentages obtained when they were applied for the determination of BIS and ROS laboratory-prepared mixtures, Table 2. Moreover, the good results obtained when this method was applied for analysis of the formulated tablets, Table 2, confirmed that there was no interference from excipients. Low values of LOD and LOQ, as shown in Table 3, proved the high sensitivity of the developed method.

The method was found to be robust, and small changes in the studied parameters did not lead to significant changes in t_R values or the area or symmetry of the peaks, Table 3. When system suitability testing parameters were evaluated, acceptable values were obtained, Table 4.

Finally, when the statistical comparison of the results obtained by the proposed method and the reported methods [15,35] were carried out, the values of the calculated t- and F-revealed that there was no significant difference with respect to both accuracy and precision between the proposed method and the reported ones, Table 5.

4. Conclusion

The successful combination of two drugs has been formulated in tablets for treating coexisting cardiovascular problems.

The new formulation has passed all quality control criteria with acceptable results regarding the mean and standard deviation values. Also, the presented work afford rapid, accurate, and specific RP-HPLC method for simultaneous determination of the cited drugs in pure forms and in lab prepared mixture with high sensitivity which was proved by the small values of LOD 0.07 µg/mL for each drug. Moreover, it was applied on the formulated tablet and it was no interference from excipients this assures the accuracy of the method with average mean recovery of 99.5 and 100.02% for BIS and ROS, respectively. Very good separation of the drugs with reasonable retention times which will be the corner stone for further routine quality control procedure or any clinical studies on those drugs.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

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