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ORIGINAL ARTICLE **OPEN ACCESS**

RP-HPLC Analytical Method Development and Validation for Bisoprolol Fumarate and Perindopril Erbumine in Pharmaceutical Dosage Forms

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ABSTRACT

A robust and rapid analysis method was developed and validated for the simultaneous assay of Bisoprolol Fumarate (BF) and Perindopril Erbumine (PE) system using high-performance liquid chromatography (HPLC). The assay was performed using the C18 Inertsil- Analytical Column (5 µm, 150 × 4.6 mm) The mobile phase was composed of Acetonitrile and buffer (Adjust pH of purified water to 2.0 with 70% perchloric acid (25:75 V/V) with a flow rate of 1 mL/min and detection wavelength of 215 nm. The volume injected was 20 µL. The retention time of BF and PE Was obtained as 5.51 min and 7.99 min respectively. Over a wide concentration range of BT and ML (25 -200 µg/ml) and (20 -160µg/ml). The BF and PE All necessary validation parameters and system suitability tests were carried out in details. The analytical curve was linear (R2 = 1.000). The system shows adequate accuracy with relative standard deviation less than 2.0%. The method showed good duplicability and recovery with % RSD less than 2%. So, the proposed system was found to be simple, specific, precise, accuracy, linear, and rugged. Hence it can be applied for practice analysis of Bisoprolol Fumarate (BF) and Perindopril Erbumine (PE) in bulk drug.

Keywords: *RP-HPLC estimation,Validation, Bisoprolol Fumarate, Perindopril Erbumine*

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INTRODUCTION

Analytical chemistry termed as science of determining the components of materials in terms of the elements or compound contained. The approach of this science is used to recognize the substances which may be present in a material and to determine the exact amounts of the identified substances. Analytical chemistry is important in nearly all aspects of chemistry. Analytical techniques proved in assuring and maintaining the quality of substance and are critical components of QA and QC.

Analytical method should be,

1. Most productive, economical and convenient,
2. As accurate and precise as required,
3. As simple as possible,
4. Most specific

Should be fully optimized before transfer for validation of its characteristics such as precision, accuracy, sensitivity etc. [1-2]

The chemical name for bisoprolol fumarate is (±)-1-[4- [[2-(1-methylethoxy) ethoxy] methyl] phenoxyl-3-[(1-methylethyl) amino]-2-propanol (E)-2-butenedioate. Its empirical formula is (C18H31NO4)2 ·C4 H4 O4 and its structure is presented in (figure 1), Bisoprolol fumarate is a synthetic beta 1 – selective blocking agent. It is used in the treatment of cardiovascular diseases such as high blood pressure (hypertension). [3]

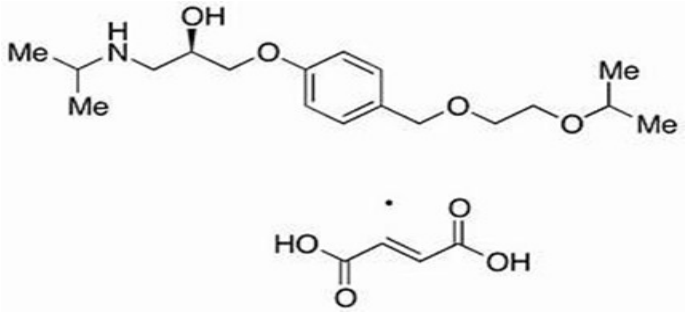

Figure 1: Chemical structure of Bisoprolol fumarate

Perindopril, Figure 1 is chemically: (2S,3aS,7aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1- oxopentan-2-yl]amino] propanoyl]-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic acid. Perindopril is a pro-drug type long acting angiotensin-converting enzyme inhibitor with a perhydroindole group and no sulfhydryl radical. It is widely used in the management of essential hypertension[1] and in stable coronary artery disease to reduce the risk of cardiovascular mortality or nonfatal myocardial infarction.(figure 2).[4]

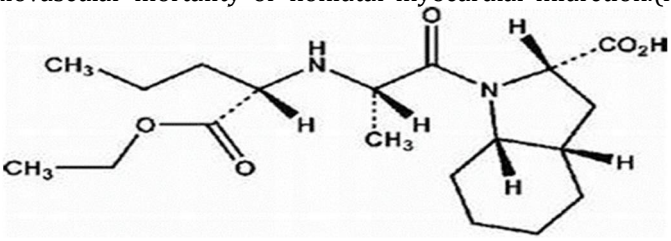

Figure 2: Chemical structure of Perindopril Erbumine

MATERIAL AND METHODS

Reagents and Chemicals:

Water, Acetonitrile, perchloric acid was used in the study.

Instrumentation

Agilent (1260series) with Auto sampler and UV detector with ez-chrome software were used.

Chromatographic condition:

A High-performance liquid chromatogram equipped with UV detector, the purity determination performed on an Inertsil C18 (150mm x 4.6ID, Particle size: 5 micron) at 50^0C temperature using mobile phase consisting of Acetonitrile: 70% perchloric acid (pH 2) (25:75) with the flow rate of 1 mL/min. Samples were monitored at the detection wavelength of 215 nm.

Preparation of standard solution BF and PE:

Weighed accurately about 50 mg of Bisoprolol (BF) and 40 mg of Perindopril (PE) standard and transferred into 100mL of volumetric flask, added about 70 mL of diluent, shaked to dissolved and volume was made up to the mark with diluent. (Concentration of BF and PE is 500µg/ml and 400 µg/ml)A-grade bulb pipette into 10 ml volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations of 25,50,75,100 and 100 µg/ml for BF 20,40,60,80, and 160 µg/ml for PE.

Preparation of Sample solution BF and PE:

Weigh and transfer 10 tablets into a 500 ml dry volumetric flask. Add 300 ml of diluents and sonicated for 5 minutes or till tablets gets dispersed completely. Stir this solution for 1 hr. using a magnetic stirrer. Cool to room temperature and complete to volume with diluents and mix well. Filter the Solution through 0.45µm PVDF filter by discarding 5 ml of initial filtrate.

Validation Procedure

Developed HPLC method was validated according to the International Conference on Harmonization of Technical Requirements for Registration of Pharma- ceuticals for Human Use. This method was validated regarding linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, specificity, and robustness.

Linearity

Linearity was analyzed at five different concentrations ranging from 25 µg/mL to 100 µg/mL for BF and 20 µg/mL to 160 µg/mL for PE. Each concentration was injected two times in order to obtain the area under the curve (AUC) which corresponded to each concentration. Accordingly, the AUC data were plotted versus BF and PE concentrations (µg/mL), separately. Linear regression analysis was assessed to determine the calibration equations. Calibration equations were expressed as y= mx+c, for which m and c coefficients represent the slope and intercept of the curves, respectively.

Accuracy

Two replicates of 50, 100 and 150 percentage concentrations were analyzed to determine the closeness of the obtained results with the actual amounts. Accuracy was reported as the percent relative error (RE %) for each concentration.

Precision

Intraday and interday precisions are regarded as the major parameters for a validated analytical method. Intraday precision was conducted by analyses of samples with concentrations of 100 µg/mL for BF and 80 µg/mL for PE in replicates of six. Two replicates for six consecutive Times. Both intraday and interday precisions were reported as the mean measured concentration along with the relative standard deviation (RSD).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The BF and PE binary standard mixture solution was diluted to determine the LOD and LOQ. The limit of detection (LOD) is defined as the minimum concentration which possesses a signal-to-noise ratio of three. The limit of quantification (LOQ) is defined as the minimum concentration that possesses a signal-to-noise ratio of ten.

Robustness

As recommended by ICH guidelines, a robustness assessment was performed for the development of the validated analytical method. Robustness indicates the ability of a method to tolerate small deliberate changes in the flow rate and wavelength. Briefly, the flow rate was set at +10% and -10% and wavelength was +3 and -3ml/min of the validated analytical method.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

For method validation and simultaneous analysis of BF and PE, various conditions such as different columns (C18) and mobile phase mixtures were tried. The C18 Inertsil Column (5 µm, 150 × 4.6 mm) at 50oc temperature was found to be appropriate for the separation of both drugs efficiently. Different mobile phase mixtures including perchloric acid with water and organic solvents including acetonitrile were tested. On the basis of preliminary experiments, a mobile phase composed of acetonitrile and water (perchloric acid) was selected for further analysis that showed good peak shape and resolution. Further attempts for mobile phase mixture composition showed that the mobile phase with a composition of 25% acetonitrile and 75% water (V/V) with the flow rate of 1 mL/min exhibited the appropriate separation of peaks [5-6].

Validation of the Method

Linearity

Linear calibration curves (n = 2) were obtained by plotting the peak areas (AUC) of BF and PE versus the concentration at five levels (25, 50, 75,100 and 200 µg/mL) for BF and (20, 40, 60, 80, and 160 µg/mL) for PE separately, each in duplicate (Fig no.3). Linearity was determined by least-squares linear regression analysis of the obtained calibration curves. Three correlation coefficients of R1 = 1 were obtained with the relative standard deviation (RSD %) values less than 2.00 for BF and correlations of R1= 1, with the relative standard deviation (RSD %) values less than 2 for PE. The equations for the calibration curve were typically calculated to be Y= 1800, 34,566.3927X+38445.0913 for BF (Fig no: 4) and Y=2762, 50,324.3151X+40241.8493 for PE (fig no: 5), in which Y is the area under the curve (AUC) and X corresponds to the concentration of each drug [7].

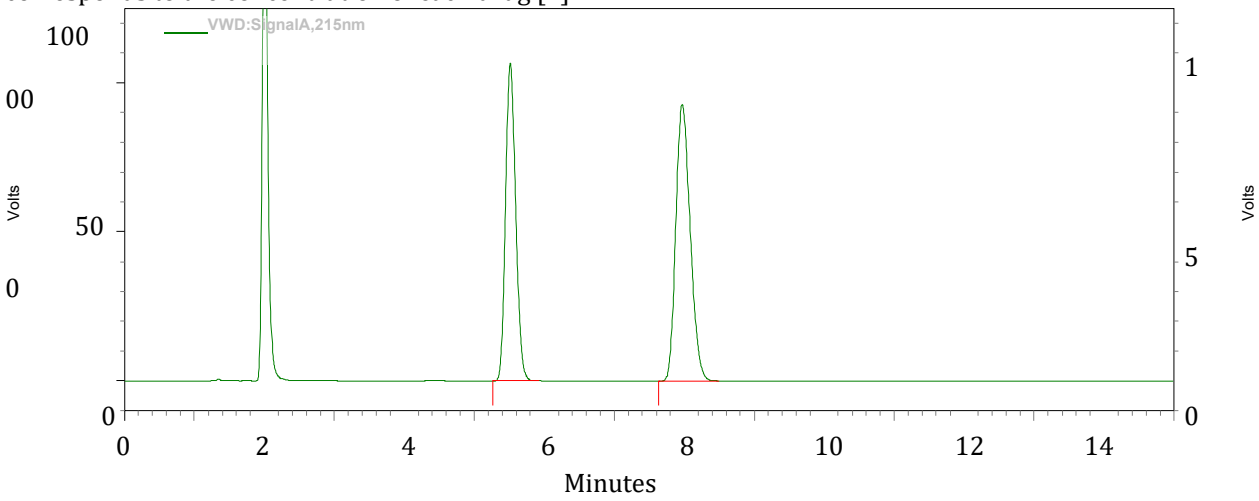

Figure 3: Atypical chromatogram of Bisoprolol and Perindopril Standard

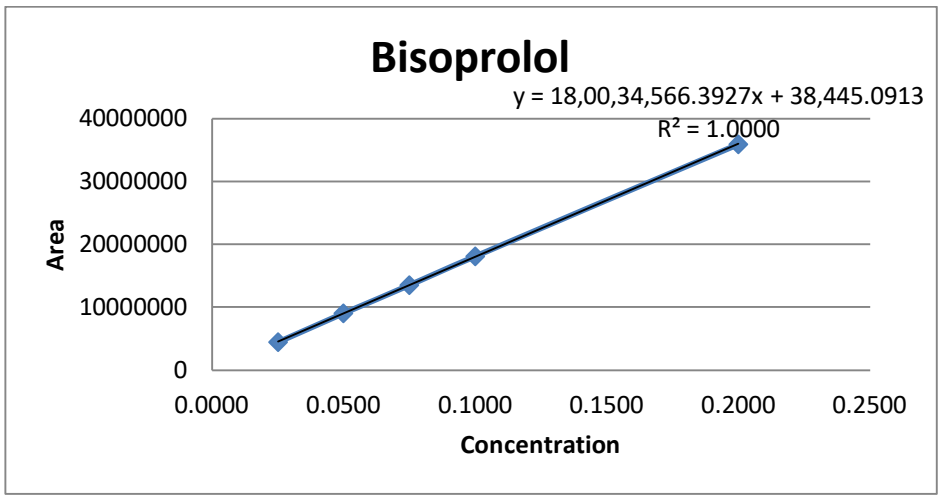

Figure 4: Calibration Curve of Bisoprolol

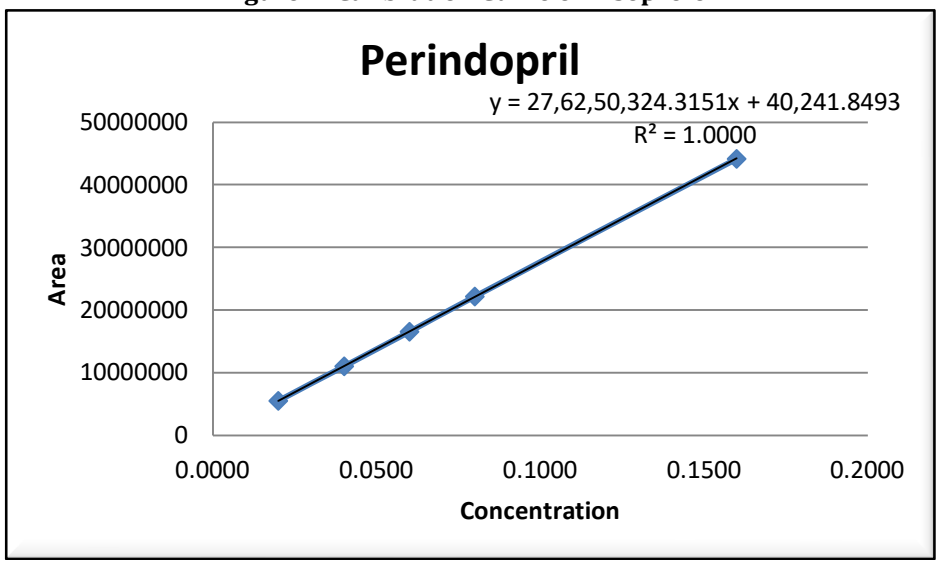

Figure 5: Calibration Curve of Perindopril

LOD and LOQ

The LOQ is defined as a signal-to–noise of more than ten-fold following three consecutive injections. The LOD amount is defined as a signal-to–noise of more than three.

Precision

Precision is an important factor in new method development and validation which indicates the repeatability of the obtained data on the same day (intraday precision) or other days (interday precision). Intraday precision was evaluated by injecting six independent BF/PE solutions at one concentration. Interday precision was carried out by injecting the same one samples over six consecutive Time. Table 1 shows inter- and intraday precision of the developed method. The maximum RSD% values for interday precision for BF and PE were found to be 0.3% and 0.2%, respectively. The maximum RSD% for intraday precision was 1% and 0.4% for BF and PE, respectively. Although in the present work, two drugs were validated simultaneously. However, none of these studies were for simultaneous detection. By applying the newly developed method, not only was a more precise analysis obtained [8].

Table 1: Results of intraday and interday precision

Drug	Concentration (µg/ml)	Intraday Precision			Interday Precision		
		%Assay	SD	%RSD	%Assay	SD	%RSD
Bisoprolol	100	98.3	0.334	0.3	96.7	0.924	0.380
Perindopril	80	99.36	0.1697	0.2	100.0	1	0.4

(N=6 and Concentrations are given in µg/ml)

Accuracy

Accuracy is defined as the closeness of obtained data from an analysis method to the actual value or reference value. The accuracy of the BF and PE concentration of three levels at 50, 100, and 150 mg/mL. Table 2 and 3 illustrates the accuracy data of BF and PE. In each series of data, the % RSD was calculated and found to be less than 2.0 for BF and PE, respectively. In a previous method which was developed for the validation of BF in pharmaceutical dosage form, the accuracy was explored; neither RE% nor the % RSD was reported [9].

Table 2: Results of Accuracy for Bisoprolol Fumarate (BF)

Level of Recovery (%)	Mean % Recovery	Standard Deviation*	% RSD
50%	99.3	0.52	0.5
100%	100	0.52	0.5
150%	99.0	0.527	0.5

(N=3 and Concentrations are given in µg/ml)

Table 3: Results of Accuracy for Perindopril Erbumine (PE)

Level of Recovery (%)	Mean % Recovery	Standard Deviation*	% RSD
50%	100.37	0.4	0.4
100%	100.20	0.44	0.4
150%	99.93	0.441	0.4

(N=3 and Concentrations are given in µg/ml)

Robustness Tests

Regarding the ICH guidelines, robustness is a substantial issue in the development of new analytical method validation. Robustness of an analytical method is determined by applying changes in analysis conditions such as flow rate, the wavelength composition and detection wavelength. The repeatability of the obtained data from these studies showed the robustness of the developed analytical method. % Assay values obtained by test solution for change in flow rate by (- 10%and +10) and change in wavelength (+3 and -3 nm) is NLT 90.0% and NMT 110.0% of labeled amount of BF and PE [10-11].

Analysis of Bisoprolol Fumarate (BF) and Perindopril Erbumine (PE) from marketed tablets

The percentage assay of tablet formulation was found to be 99.5% for BF and 99.4% for PE respectively.

CONCLUSION

A rapid, precise, and simple method was developed for simultaneous analysis of BF and PE by means of an HPLC method. The newly developed method showed acceptable precision and accuracy at least in the concentration range of 25 to 200 µg/mL for BF and 20 to 160 µg/mL. The developed method represented good resolution for both BF and PE. The validated analytical method is simple and reproducible which can be used in quality control departments. Moreover, by this method, the release profile of BF and PE from agilent system was analyzed precisely.

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