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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC AND RP-HPLC METHODS FOR THE DETERMINATION OF BISOPROLOL FUMARATE IN PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The aim of the study was to develop UV-spectrophotometric and RP-HPLC methods for the analysis of Bisoprolol in marketed tablets and pure form.The methods were validated in terms of linearity, accuracy (%Recovery), precision (inter day, intraday and reproducibility) and robustness. Both the methods were linear (R2 =0.999 for UV method and 0.999 for RP-HPLC method) and accurate.The methods was also found precise (% RSD< 2%) and robust.All validation parameters were within the acceptable range as perInternational Conference on Harmonization (ICH). Parameters of validation prove the precision of the method and its applicability for the determination of Bisoprolol in pharmaceutical tablet formulations.

Key words: Bisoprolol, UV, RP-HPLC, Method validation.

INTRODUCTION

BisoprololFumarate is designated chemically (*RS*)-1-{4-[(2-Isopropoxyethoxy) Methyl] Phenoxy}-3-(Isopropyl amino) propan-2-ol.Literature survey reveals that the drug can be estimated by spectrophotometric methods, ionicspectrophotometric methodsand HPLCmethods[1-6]. In the present investigation, Water is used in spectrophotometric estimation of drug which is safe and inexpensive when compared with existing method with 0.1NNaoH.and RP-HPLC method is also carried out with simple solvent system.Bisoprolol is an Anti-hypertensive [7-11]. Bisoprolol is cardioprotective because it selectively and competitively blocks catecholamine (adrenalin) stimulation of β1 adrenergic receptors [12].

MATERIALSAND INSTRUMENTS

Materials and methods

Method-A (UV Method)

Instruments used:

Singlepanelectronicbalance-SartoriusGE412

UV-Visibledoublebeam spectrophotometer-Systronics 2203(smart)

Matched quartz cells corresponding to 1 cm path length

Reagents:

Distilled Water

Working Standard Bisoprolol Fumarate

Method-B (RP-HPLC)

Instruments used:

- HPLC – WATERS Model NO.486 series Compact System Consisting of Kromofil-C18 ODS column
- Electronic balance (SARTORIOUS)
- Digital pH meter(POLOMAN)
- Sonicator(FAST CLEAN)

Chemicals:

- Purified water HPLC Grade
- Triethylamine HPLC Grade
- Orthophosphoric acid HPLC Grade
- Methanol HPLC Grade
- Acetonitrile

PROCEDURE

Method A (UV Method)

Preparation ofstandard stock solutions:

The standard stock solution of drug was prepared by dissolving 50mg of the drug in 50 ml standard flask using water as a solvent to give a concentration of 1000 µg/ml. This stock solution on further dilutions is used for establishing following parameters.

Concentration of solvent and Wavelength selection:

Solutions of concentration of 9 µg/ml, 10 µg/ml, 20 µg/ml was prepared.

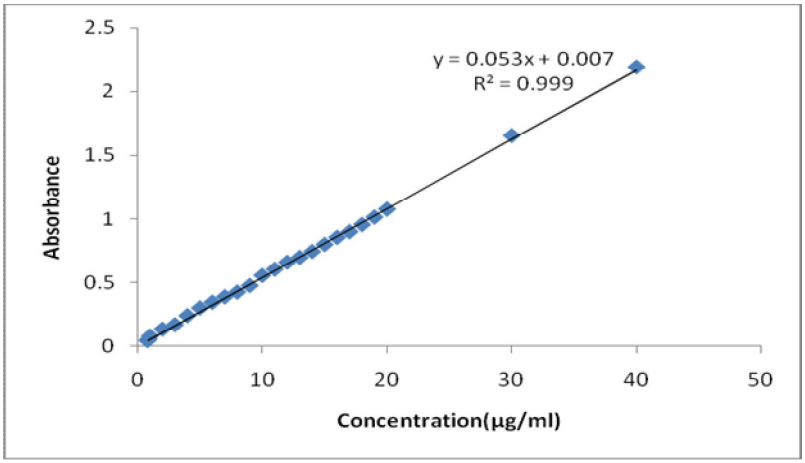
They were subjected to scanning from 200-400nm.

The dilutions were made using Water and scanned

From the different absorbance values obtained Maximum absorbance at224.5nm was selected for the present work.

Beer's law range:

The stock solution was suitably diluted with water to get concentration range from 1 to 1000 µg/ml. The solutions are scanned in UV regions between 200 to 300nm the absorption were measured at λ_{max} found. Using absorbance values against concentrations plotted the calibration curve and the linearity range can be found.



Analysis of formulation:

The proposed method is applied to the analysis of various marketed formulation

Method-B

Preparation of standard stock solutions and Wavelength selection:

The standard stock solution of drug was prepared by dissolving 50mg of the drug in 50 ml standard flask using Mobile phase(Acetonitrile(55): buffer(45)) as a solvent to give a concentration of 1000 µg/ml. This stock solution on further dilutions is used for establishing following parameters.

They were subjected to scanning from 200-400nm.

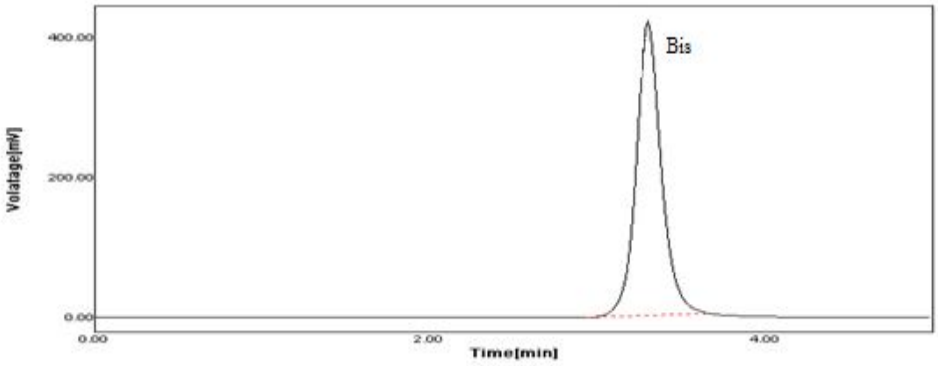
The dilutions were made using Mobile phase and scanned

From the different absorbance values obtained Maximum absorbance at226.5nm was selected for the present work.

Method Development and validation

The RP HPLC procedure was optimized with a view to develop an effective method for the estimation of Bisoprolol Fumarate in tablet dosage forms. A Kromofil-C18 ODS,

(250X4.6)mm, 5µ column(temp 35°C) was used as a stationary phase and the separation was achieved by usingmobile phase consisting of Buffer and Acetonitrilewere mixed in the ratio of 45:55v/v in isocraticmode at the flow rate of 1 ml/min with PDA detectionat 226.5 nm. Chromatogram of standard solution containing Bisoprolol Fumarate.



Chromatogram of standard Bisoprolol Solution

Validation of Method

The developed HPLC method for the estimation of Bisoprolol Fumarate was validated as per the ICHguideline in terms of specificity, linearity, accuracy, precision, ruggedness and robustness, limit of detection and limit of quantification

RESULTS AND DISCUSSIONS

It reports a UV Spectrophotometric method for the estimation of Bisoprolol in pure and pharmaceutical dosage form. In standardization of method initially, the solvent and wavelength are selected and different concentration solutions were prepared and scanned in the UV region between 200-400nm and the λmaxwas found at 224.8nm .Using the absorbance values against concentrations calibration curve was plotted .From the graph it was found that Bisoprolol obeys Beer's law between 6-40µg/ml.

The brand of formulation show the percentage purity values range from 99.09 to 101.01%w/w. The percentage deviation values were found to be between ± 0.13 to± 0.91.

The repeatability values vary from 99.71 to 100.11%w/w. The results obtained in repeatability test expresses the precision of the given method.

The stability of the drug was analyzed for 48hrs. The absorbance was recorded and the drug is stable up to 48hrs and sample is analyzed within that time.

Method-B

It reports a new RP-HPLC method for the estimation of Bisoprolol in pure and pharmaceutical dosage form.

In this method to optimize the mobile phase, various combinations of phosphate buffer, acetonitrile and were studied on an Kromofil- C18 ODS column .Initially, the combination of Ortho phosphoric acid buffer and pH adjusted(pH-2.5) to with tri ethylanmine and acetonitrile(50:50%v/v) was tried; however, very broad peak was eluted. The combination of the solvents were modified by using phosphate buffer (pH-2.5) and acetonitrile (60:40%v/v) and found that retention time is good but tailing was observed. Then the mobile phase containing a mixture of Ortho phosphoric acid buffer pH 2.5 adjusted with ortho phosphoric acid and ACN in the ratio of 50:50, 60:40, 30:70, 45:55, v/v was carried out and found that the ratio of buffer and acetonitrile 45:55 v/v resulted in peaks with good shape. A flow rate of 1.0mL/min was found to be optimum in the range of 0.8-1.2 mL/min resulting in short retention time, baseline stability and minimum noise and the retention times of Bisoprolol was found to be 3.2 min.

The developed method is validated in accordance with the ICH guidelines with all of the results within the limits. Quantitative linearity was obeyed in the concentration range of 25-150 µgs/mL of Bisoprolol the mean percentage recoveries for Bisoprolol was found to be 100.54%. The high percentage recovery indicates that the proposed method was highly accurate.

Precision of the method was studied by making the replicate injections of the standard solutions and standard deviation was determined. The % RSD values of Bisoprolol were found to be 1.02. The low % RSD value (below 2) indicates that the method was Precise.

Robustness of the method was performed by varying the flow rate of mobile phase. From that data it was found the asymmetric factor was less than 2.0 and theoretical plates were more than 2000 for B peak, which illustrates the good robustness of the developed method

Finally the developed HPLC method was applied for estimation of Bisoprolol Pharmaceutical dosage forms. No interfering peaks were found in the chromatogram indicating that excipients used in capsule formulations didn’t interfere with the estimation of the drugs by the proposed HPLC method.

CONCLUSION

The proposed methods of analysis is novel, simple,cost-effective,environment friendly, safe,

accurate andReproducible.This methods can be routinely employed in the analysis of Bisoprolo fumarate in tablet formulations

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