

Bisoprolol Fumarate: An Exploration on its Properties and Analytical Methods

Manoj Verma¹, Swati Pandey², Bina Gidwani³, Ravindra Kumar Pandey⁴ & Shiv Shankar Shukla^{3*}

¹Research Scholar, Department of Pharmaceutical Quality Assurance, Columbia Institute of Pharmacy, Raipur (C.G.). ²Associate Professor, SIRT-Pharmacy, Sanjeev Agrawal Global Educational University, Bhopal. ³Professor, Department of Pharmaceutical Quality Assurance, Columbia Institute of Pharmacy, Raipur (C.G.). ⁴Professor, Department of Pharmacognosy, Columbia Institute of Pharmacy, Raipur (C.G.)

*Corresponding author: shivpharma007@gmail.com

Received: (27/12/2023), Accepted: (16/4/2024), Published: (1/12/2024)

ABSTRACT

Cardiovascular diseases stay ahead and are the zenith reason for global death, with a projected number of 17.9 million deaths in 2016 (i.e. 31% of global deaths). One of the third important threat cause of cardiovascular mortality is hypertension (high blood pressure). The US Food and Drug Organization (FDA) supported the use of Bisoprolol fumarate as another sub-atomic substance in July 1992. Bisoprolol fumarate is used as an anti-hypertensive agent which is a term that is used to diagnose that an individual's rhythm is above what is viewed as a standard. Beta-blockers obstruct the activity of epinephrine and nor-epinephrine also known as adrenaline and nor-adrenaline respectively belong to the chemical class of compounds called catecholamines. These drugs intervene in fight, flight and fight (3 Fs) reactions. Various analytical techniques that are accurate, simple and precise are presented for the analysis of bisoprolol fumarate. About five UV spectrophotometry, three HPLC, approximately three LC/MS/MS, and single HPTLC & HILIC methods have been used in the estimation of Bisoprolol in pure and bulk dosage forms. This review puts forward therapeutic, pharmacological, and analytical aspects regarding the drug bisoprolol fumarate and provides insights on several reported analytical and bioanalytical methods present for the estimation of the drug. It also aimed to provide a thorough outline of the bisoprolol covering the diverse areas in the study of a drug profile.

Keywords: Spectrophotometry, HPLC, Bisoprolol fumarate, Analytical method.

INTRODUCTION

The American Heart Association abbreviated as AHA is a key association for providing the statistics that need to be commonly cited everywhere along with the major statistic regarding stroke, heart disease, other cardiovascular diseases and their risk factors. The data cited here comes from the Association of Heart Disease and Stroke Statistics Update, 2021 compiled annually by AHA depicts that the age-adjusted death rate due to cardiovascular disease (CVD) is 219.4 per 100,000.[1]

Bisoprolol is cardio selective, beta1-specific, adrenoceptor blocking agent without any in-herent sympathomimetic action following its therapeutic index. The IUPAC name of bisoprolol fumarate is 1-(propane-2-ylamino)-3-[4-(2-propane-2-yloxyethoxy methyl) phenoxy] propane-2-ol. Bisoprolol is a chemically synthesized, sympatholytic drug that

blocks catecholamine synthesis at β_1 -adrenergic receptors present specifically in the heart and vascular smooth muscle, which reduces pulse rate hence the cardiovascular yield, systolic and diastolic circulatory pressure. This impact might be utilized to lessen pressure on the heart and consequently O₂ requests, so the medication is shown for optional additive treatment in patients with stable persistent cardiovascular breakdown, and for the surround of hypertension and angina pectoris. Analytical techniques are logically being taken into principal drug research, considering their accuracy, precision, selectivity, and affectability.[2]

CHEMISTRY OF BISOPROLOL FUMARATE

Bisoprolol fumarate has a molecular formula C₁₈H₃₁NO₄ consisting of a secondary alcohol and a secondary amine. It has a pK_a of 9.5 and melting point of 100-103°C. IUPAC

nomenclature of Bisoprolol is labelled as (\pm)-1-[p-[(2-isopropoxy ethoxy) methyl] phenoxy]-3-isopropyl-amino-2-propanol. It exists as a racemic mixture consisting of the R and S enantiomers. The specific rotation of (10 mg/mL) Bisoprolol Fumarate in methanol is found between -2° and $+2^\circ$. [2] Structure of Bisoprolol Fumarate is denoted as Figure No. 1 and FTIR Spectrum of Bisoprolol Fumarate is denoted in Figure No. 2.

Atenolol, metoprolol, betaxolol and other beta-selective blockers are compared with bisoprolol, it has proved to be the compound

with the highest beta-selectivity in all in vitro and in vivo experiments and all animal species investigated. [2] Hemi fumarate is a beta 1-selective (cardioselective) adrenoceptor blocking agent without major membrane stabilizing or intrinsic sympathomimetic actions in its therapeutic dose window. The beta-blocking activity is brought about by S(-) enantiomer alone. The most visible impact of bisoprolol fumarate is the lesser chronotropic impact, bringing about a decrease in latent and work out pulse.

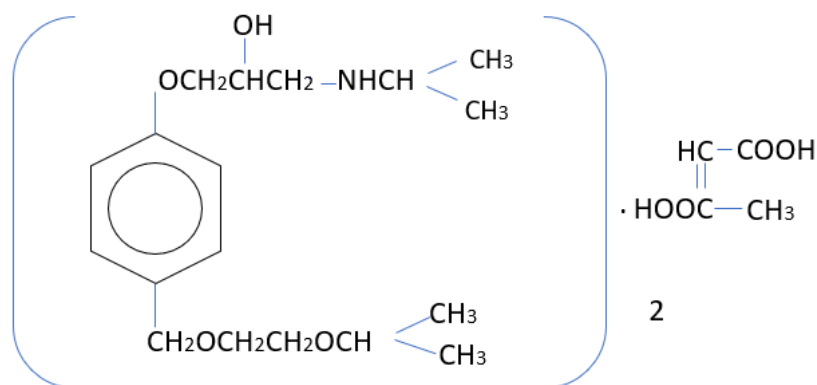


Figure (1): Structure of Bisoprolol Fumarate.



Figure (2): FTIR Spectrum of Bisoprolol Fumarate.

Method of Synthesis

The first step in the synthesis of Bisoprolol consists of reaction of Isopropyl amino propanediol with dimethylcarbonate to produce oxazolidinone sulphonate. The synthesised Oxalidinone sulphonate then reacts with

4-hydroxybenzaldehyde to give oxazolidinone benzaldehyde. Further Oxalidinone benzaldehyde is converted into oxazolidinone benzyl alcohol which is then reacted with isopropyl oxitol to form bisoprolol.[4]

Synthesis of Bisoprolol Fumarate is discussed in Figure No. 3.

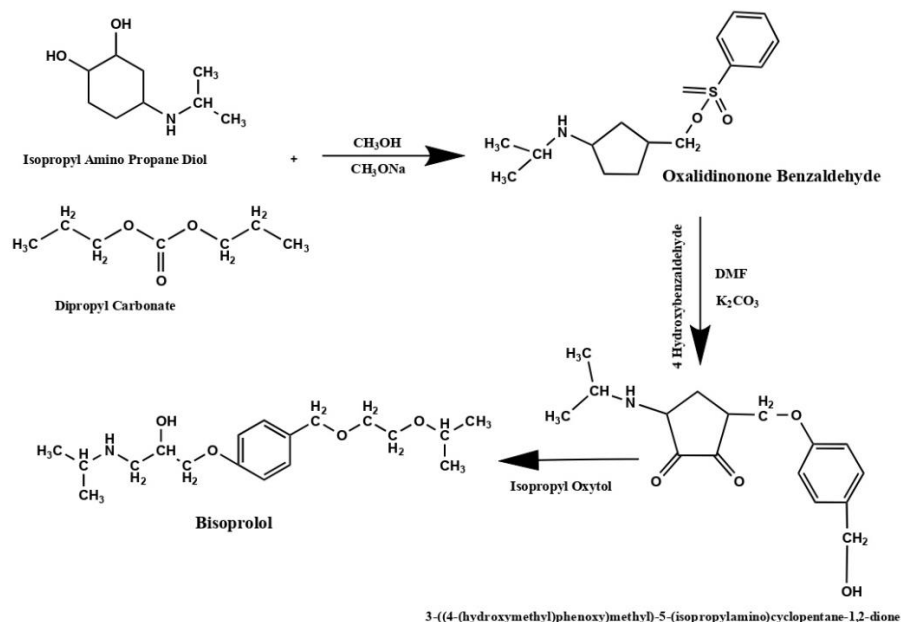


Figure (3): Synthesis of Bisoprolol Fumarate.

PHYSICAL PROPERTIES

The bisoprolol with molecular formula as C₁₈H₃₁NO₄ and a molecular mass 325.443 g/mol. The Pka value is 3.5 and the color is white. The bisoprolol fumarate nature is a bit hygroscopic powder and water soluble with free solubility in methanol and ethanol. The boiling point of bisoprolol fumarate is approximately 445 °C. [2]

Pharmacokinetic Properties

Bisoprolol has a bioavailability of 80%. The first-pass metabolic rate of bisoprolol is 20%. High peak plasma concentration of bisoprolol is achieved in 2-4 hours of the oral dosage form. Up to half of bisoprolol is discharged unaltered in urine, with the rest of inert metabolites.[3]

Absorption and Permeability

Bisoprolol is entirely retained from the oral route and has a level of bisoprolol of about 90% after administration, with around

90% of the part of 14C-checked bisoprolol released through urine and in the faeces is 1.4%.[4] Associative dietuse didn't affect its bioavailability. As such it might be taken with a little amount of food admission to increasing their affectability and bio-availability.[5] The peak plasma focus is reached in 1-3 hours after oral route intake. It was found after 2 hours of oral ingestion of 10 and 20 mg bisoprolol to eight coronary patients the inter-individual changeability of plasma concentration varied between 10.3-11.2 %.

As per WHO, EMA, and FDA's BCS models, seeing that the absorbtion of bisoprolol is about 90% after oral intake, bisoprolol can be considered tremendously penetrable. By its penetrability coefficient, it can be emphasized, which is higher than the range of 1-10*10⁻⁶ cm/s value that is commonly considered to infer high permeability. Further, bisoprolol isn't a substrate for P-glycol protein, that is an efflux protein.[6]

Distribution

3.5 litres/kg is the volume of distribution of bisoprolol and generally out of which 30% is bound to protein.[7] Any pathophysiological changes in the plasma proteins does not affect the pKa of bisoprolol. The pKa of bisoprolol is immediate, self-sufficient and shows lesser between and intraindividual variability.[8]

Metabolism

Bisoprolol was discovered to be principally processed through cytochrome P3A4 in vitro concentrates in human liver microsomes. Cytochrome P450 (2D6) isn't believed to be a part in its digestion. First-pass metabolism of Bisoprolol fumarate is about 20%. Restricting to blood proteins is around 30%. Peak plasma concentration of 5 to 20 mg happens within 2 to 4 hours of dosing and mean peak plasma ranges from 16 ng/ml at 5 mg to 70 ng/ml at 20 mg. [9]

Elimination

Bisoprolol is the release by two similarly effective routes in the body. About half of the concentration of drug breaks down in the liver which is further eliminated by the kidney. For patients with reduced liver function or renal insufficiency, considering this balanced clearance, an estimation change isn't required at the regular dose of 10 mg. Bisoprolol has a stretched plasma removal half-life of about 12 hours. Along with these daily once step by step dosing is satisfactory in inspiring a pharmacological effect, which in this manner usefully influences patient compliance.[10]

MECHANISM OF ACTION

β 1-specific blockers checks the arrival of renin, a biochemical made by the kidneys that cause contraction of veins. There is a decrease in receptor selectivity gradually starting with 20 mg, where bisoprolol shows a selective β 2-adrenergic receptors that are present at vascular smooth muscle and lungs. Not at all like propranolol and pindolol, bisoprolol doesn't show film balancing out or adrenergic movement. A single chiral center is present in Bisoprolol and the drug management is done by using its racemic mixture. The drug in its S-form shows enhanced selective β -Receptor dependent adrenergic activity.

Adverse Effects

Findings related to the use of bisoprolol bring upon arthralgia, confusion, hypoesthesia, bradycardia, lack of sleep, detachment of the bowels, affliction, dyspnea, asthenia, migraine and fatigue. Bradycardia and asthenia are seldom related. The unexpected withdrawal of bisoprolol may speed up myocardial restricted rot and ventricular arrhythmias in those with coronary artery disease, or hyperthyroid in patients with thyrotoxicosis. [13]

MARKETED PRODUCT

Marketed as a beta-adrenergic blocking agent, bisoprolol fumarate is used to treat cardiac disorders. The marketed tablets are available in various dosage ranges that have a drug content of 5 mg, 10 mg, and 20 mg. The shelf-life is 10 hrs and shows bioavailability of above 80%. The drug has fairly high bioavailability and half-life. For improving the onset of action, release characteristics, and reducing the side effects the controlled release formulation can be given much significance. Nanotechnology has at last given away to us with to rearrange and restructure matter on an atomic scale, allowing us to reach down to the root establishments of any issue.[14]

ANALYTICAL AND BIO-ANALYTICAL ANALYSIS OF BISOPROLOL FUMARATE

Drug dosing proposes affecting rational improvements, strategies, or methods to analysis, or to choose purity, prosperity, and effect of the drug. Thus, drug valuation has an essential effect in selecting the nature of the drug or finished product. Widely, it is the technique to analyze a compound moiety or about a drug to separate it qualitative or quantitatively in single or double dose, in its medication portion structure, or any model. Validation analytical methods are used hence, which are precise, accurate, and selectivity for the drug to determine and estimate the drug and its formulated current in any drug dosage form. This analytical validation method accepts a part in the regular investigation in the pharmaceutical industry.[15-16]

Analytical Methods for Bisoprolol

Analytical methods of Bisoprolol by different chromatographic & spectroscopic techniques are summarized in Table No 1 and 2 respectively. Different methods are performed on the bisoprolol fumarate that includes both analytical and bio-analytical methods.

1. Swati Pandey *et al.*, 2021 described and established HPLC method that was validated according to the ICH guidelines. Degradation studies were performed on Bisoprolol fumarate as per different method like (hydrolytic, photolytic, thermal, and oxidative stress). The outcome was formation of one degradation product and one fragmented product of the degradation product due to heat due to nucleophilic substitution reaction. The formed degradation product was identified by ESI-QTOF-Mass Spectroscopy. The determination of routine qualitative analysis and stability studies developed with the help of the HPLC method.[17]
2. Performed by Dina Elshaprawy, *et al.*, and published on 15 January 2020; the objective of the introduced study was to identify the excess using as an oxidant the Ceric Ammonium Sulfate in the sulphuric acid medium. It is noticed that the unreacted Ce⁺⁴ have been utilizing various colors indigo carmine and methyl orange. The methyl orange and indigo carmine have absorbance at 504 nm and 610 nm. the linearity was observed in the method for levobunolol hydrochloride and bisoprolol with indigo carmine is (8.8–88) µg ml⁻¹ and (19.6–45.7) µg ml⁻¹ and (8.8–66) µg ml⁻¹ is observed for bisoprolol as well as for levobunolol hydrochloride with molar absorptivity measure the (19.6–45.7) µg ml⁻¹ whereas the for beside methyl orange and Sandell's is observed 1.261 x 10⁴ L mol⁻¹ cm¹ and 0.0350 µg cm⁻² for bisoprolol, it also measured for levobunolol 8.166 x 10³ L mol⁻¹ cm¹ and 0.040 µg cm⁻². [18]
3. Performed by Shubhada Pawar, *et al.*, published on 10 Aug 2020. identify the RP-HPLC method was performed on the HPLC method using the column (C18) Inertsil ODS 3V column (150*4.6mm, 5µm) in the method and the solvent used as mobile phase the ratio of buffer: methanol (20:80 %v/v) and the observed flow rate was 1.0 ml/min as well as the determination of by UV at 231nm. The retention time observed was 2.84 min and 0.518 min for Bisoprolol Fumarate and Cilnidipine drug used in the method. During the process used the concentration between 5-25 µg/ml for bisoprolol fumarate and 10-50 µg/ml of Cilnidipine sample drug were analyzed. The limit of detection for both bisoprolol fumarate and cilnidipine drug was found to be 2.0252 µg/ml and 1.0121 µg/ml respectively and similarly the limit of quantification was 6.1370 µg/ml and 3.0948 µg/ml. [19]
4. Almesyah, Melinda Mustika, *et al.*, (31 Nov 2020), The purpose of the review technique for the assurance of bisoprolol fumarate (BF) with bromothymol blue (BTB) using the spectrophotometric method, define the interaction depends on the impact of color in a solution containing KCL-HCl buffer (pH 2.2) to form an ion-pair complex (1:1 drug/color) at a frequency of 412 nm which can be removed with chloroform and then estimated spectrophotometrically. In the method observed impact factor is 25 which is very fast and their absorbance is unchanged between the 24 hours. For the BF-BTB range, 0.50 – 40.88 µg/ml is accepted in Beer's Law and the recovery percentage found to be in the process is 100.00-104.00 %. Impedance with unlike products and other excipients was not observed.
5. Nilam B Jadhav, Savita S Yadav (2019). The objective of the HPTLC method for Amlodipine besylate and bisoprolol fumarate for the simultaneous method determination from tablet formulation. In the method were used the precoated silica gel on the HPTLC aluminum plate 60 F254 using glacial acetic acid: chloroform: ethanol in the ratio (0.1: 2: 8 v/v/v). Observation detection was observed scanning at 231 nm. Amlodipine besylate and Bisoprolol fumarate linearity were observed at the range between 200-1200 ng/spot. Bisoprolol fumarate was found to be 50 ng/spot and 100 ng/spot of the

- limits of detection and quantitation and same as for Amlodipine besylate 40 ng/spot and 120 ng/spot. The percent recoveries were found to in the method is $99.20 \pm 0.41\%$ for Amlodipine besylate $99.11 \pm 0.13\%$ for Bisoprolol fumarate.[20]
6. Liliya Logoyda et.al.,2018, demonstrated a proposed method of the study was to identify with HPLC Chromatography was experimented on the samples in the mobile phase (eluent A [formic acid: acetonitrile: water] within a ratio 0.1: 5: 95 and eluent B [acetonitrile: formic acid] in the ratio 100:0.1 v/v. The flow rate is 0.400 ml/min, on the column used the C18, 50mm \times 2.1 mm 5 μ m in the chromatography, and their recovery rate is observed at 97.69%. [21]
 7. Performed by A 'da'm Detrich, et al., (2018). The introduced a study to recognize the thermodynamical stability of the drug. Solvency of the introduced polymorphs were additionally researched. FT-IR and XRPD techniques were observed to be appropriate for the representation of the various crystal structures. Thermo-analytical estimations showed that Form I & II own diverse melting points. Form II and hydrate structures together can change into Form I at higher temperatures. After effects of the DVS estimations demonstrate that both Forms I and Form II became metastable under incredibly humid conditions (80% RH) and changed over into hydrate. Thermodynamic stability studies presented that Form I & II polymorphs are in an enantiotropic relationship with an enantiotropic point at around 40–45 °C. Solvency concentrates on demonstrating that every one of the prepared structures is exceptionally dissolvable, and no distinction was found between them.[22]
 8. Barge V U, et.al., published on 25th August 2018. A major, fast, and express HPLC technique for determination of the Telmisartan and Bisoprolol fumarate using a solvent system as a mixture of the water and methanol in the ratio between 25:75 v/v using a column in the method is Waters X Bridge RP C18 (4.6 x 250 mm) parcel and observed the flow rate of the column is 1ml/min. Linearity was found for Bisoprolol fumarate in the concentration between 5-25 μ g/ml and for Telmisartan found in the concentration between 40-200 μ g/ml. Developed the percentage recovery is found for the Bisoprolol fumarate is 99-101% and 99-100% found for the telmisartan drug.[23]
 9. Alina Diana Panainte, et al, (2018). The introduced study was to identify the HPLC separation was done by using the column in the method as an Agilent Eclipse XDB-C18 column (150 mm, 4.6 mm, 5 μ m) and a mobile phase mixture of acetonitrile 10 mm and pH 4.5 phosphate buffer solution (10:90, v/v) and the flow rate is observed in the 1.0 ml/min. Bisoprolol fumarate was isolated within 4 minutes with good resolution and with no following impact or excipient obstruction. The limit of detection and the limit of quantification were 2.79 and 3.07 μ g/ml. The relative standard deviation for accuracy was lower than 1%. [24]
 10. Ivana Mitrevska, et.al, published on 23 March 2017. The objective of the research was the finding the Impurity with an RRT 0.95 that was identified to be phosphomonoester of bisoprolol, got a relative sub-atomic mass of 406 (positive ionization mode). The impurity characterized was the result of calcium hydrogen phosphate in the bisoprolol film-coated tablet during the thermal degradation. define the structure of the identified impurity HPLC/DAD/ESI-MS was applied and further in- silico studies were completed.[25]
 11. Elżbieta Dąbrowska-Maś, et al. (2017). The method says that impurity A may be formed as a by-product during the method used for the bisoprolol fumarate synthesis as well as active substance degradation with the hydrolytic degradation. Impurity A was formed from para hydroxy benzyl alcohol and uninterrupted epichlorohydrin reactions and isopropylamine. The process of purification involved, its isolation of fumarate salt of Impurity 'A', its crystallization was obtained at 95.5 %.[26]

12. Shahinaz A. Mohammed, *et al.*, (2017). The objective of the introduced study was developed for the bisoprolol fumarate (BF) estimation method in bulk and tablet dosage form. The wavelength at 271nm, depended on the estimation of absorbance of BF aqueous solution. The concentration between range 5-25 µg/ml was observed the linearity in the method. 0.22 µg/ml is the limit of detection and 0.66 µg/ml is the limit of quantification. Intraday RSD% values are 1.19 and intermediate is 0.854. the recovery percentage is obtained in the method is $105.0 \pm 1.3\%$, $n=3$. [27]
13. Irena Kasagiü-Vujanoviü1 *et al.*, published in 2018. The purpose of the research work was to observe the stress that causes activity that is impacting its product stability. The investigation recently enhanced and approved HILIC strategy was utilized. It was exhibited that oxidizing stress causes have the biggest impact degradation on the bisoprolol fumarate, afterward, the alkaline and acidic stress causes progressively. Water is a neutral solvent, was no important impact on the stability of bisoprolol fumarate. Till degradation performs under acid phase impurity, A confirmed by UPLC/MS/MS techniques. The method of degradation more clearly, dynamic investigations to degradations of bisoprolol fumarate was done. The degradation and degradation half-time order of the reaction rate, which gives better results perform of the degradation mechanism. [28]
14. Stefania Corina Mahu *et al.* (2016). The purpose of the chromatographic separation analysis work on the method done by using the column as an Eclipse XDB C18 (150 mm x 4.6 mm, 5 µm). The solvent system of bisoprolol is as a water: methanol: acetonitrile in the ratio between 50:30:20 (v/v/v) and column rate of flow measured was 1 ml per min. 225 nm UV wavelength detection 0.8 - 80 g per ml and 80 -1000 g/ml is measured linearity range in the method as well as 1.3 g per ml and 3.98 g per ml obtained as LOD and LOQ. [5]
15. Marothua *et al.* 2015. The objective of the study defines the similarity of bisoprolol fumarate and their excipients are Ascorbic acid with Citric acid anhydrous, as well as Butylated hydroxyanisole, Polyvinyl pyrrolidone, Glycerol, Mannitol, and Sorbitol using as a mixture ratio at 1:10 which are investigated by isothermal stress conditions to the 90°C at 48 hours. HPLC showed the drug degradation was determined with butylated hydroxyanisole (89.4%), as well as citric acid anhydrous (89%), mannitol (77%), and glycerol (61.9%). [29]
16. Hong-Bing Duan, Jun-Tao Cao *et al.* (2015). The purpose of the work was to recognize by capillary electrophoresis combined with tris (2, 2'- bipyridyl) - ruthenium (II) 10 electro chemiluminescence (ECL) for separation of metoprolol tartrate and bisoprolol fumarate (BF) detection. Day intraday the RSD peak area of ME ($n = 3$) is 2.1% and BF peak area is 3.8%, and interday of ME is 3.1% and BF 5.5% in 3 repeated days. RSD of the relocation time of metoprolol tartrate (ME) are 7.4 for interday and 4.5 for intraday, bisoprolol fumarate (BF) are 7.9% for interday, 6.7% for intraday. The limits of quantification (LOQ $S/N = 10$) in the human urine test are 3.3×10^{-7} mol L⁻¹ for metoprolol tartrate and 1.4×10^{-6} mol L⁻¹ for bisoprolol fumarate. The recoveries ($n = 3$) of metoprolol tartrate (ME) and bisoprolol fumarate (BF) in human urine are from 89.0 to 126.0% with under 7.4% in RSD. The quantity of metoprolol tartrate (ME) and bisoprolol fumarate (BF) with HSA is 1.2 and 1.1, and the limiting factors are 2.8×10^3 and 2.7×10^3 L mol⁻¹, respectively. [30]
17. Performed by Róbert Kormány *et al.* Published on 20 January 2014. The purpose of the work is establish a quality by design principle using design of experiment (DOE) taking in account. 3 experimental factors that are the gradient time (TG), temperature (T), and mobile phase. The mobile phase was used as acetonitrile and 30 mm phosphate buffer at pH 2.0, 2.6, 3.2. the column was used in the method as the Acquity column (50×2.1 mm, 1.7µm BEH C-18 shield RP-18, BEH C-18, and the observed flow rate is 0.5ml/min. [31]

18. Performed by Marcin Skotnicki, et al. Distributed on 24 July 2014. The introduced work of DSC and TMDSC establishing the thermal effect of bisoprolol fumarate. The crystal-like bisoprolol fumarate defined the XRPD, solid-state NMR, and solution, and phase transitions from thermal techniques solid-state NMR spectra determined by interaction with it all. Solid associations between bisoprolol fumarate and valsartan were seen over 60°C, bringing about the development of new amorphous material. Arrangement and solid-state NMR gave knowledge in the sub-atomic behavior of the inconsistency. The thermal techniques of bisoprolol fumarate analyzed, arrangement, and solid-state NMR and XRPD tests permitted the investigation of the conformational and dynamic properties.[32]
19. Performed by Irena Kasagić-Vujanović et al (2014). This review based on ideal chromatographic conditions as far as sufficient separation and investigation duration were set by graphical improvement by the user of mobile phase in the process as a water-acetonitrile (10 mm ammonium acetate and pH 4.0 adjusted with glacial acetic acid) in the ratio of 92: 8 v/v using the column as a column Luna HILIC 200 A, 150 mm x 4.6 mm, and the molecular size of the product 5 µm and maintain the temperature between 30°C. The flow rate of the method used is 1 ml min⁻¹ with an absorbance of the UV is 230 nm.[33]
20. Performed by Charlene Galea et al. Distributed on 27-12-2014. The article dependent on Supercritical liquid chromatography is taking significant advantage as a separate procedure in the pharmaceuticals company. In the SFC method were use the different types of columns for the 64 drugs were estimated using 27 columns for the retention factors. In the process maintain 25°C temperature and conveyed run at 150 bar back pressing factors. The mobile phase used are CO₂ and methanol with 0.1% isopropylamine (5-40% more than 10 minutes) and the flow rate is measured 3 ml per minute.[30]
21. Performed by Eman S. Elzanfaly, et al, distributed on 28 December 2014. The purpose of the introduced study was to recognize the five signal handling techniques that were applied to proportion spectra for the quantitative assurance of bisoprolol (BIS) and hydrochlorothiazide (HCT) in their binary mixture. The ratio spectra are used as a Numerical Differentiation of Ratio Spectra (ND-RS), Savitsky-Golay of Ratio Spectra (SG-RS), Continuous Wavelet Transform of Ratio Spectra (CWT-RS), Mean Centering of Ratio Spectra (MC-RS), and Discrete Fourier Transform of Ratio Spectra (DFTRS). The linearity in the scope of 2-40 and 1-22 µg/ml for BIS and HCT, individually. The proposed strategies were applied effectively for the assurance of the drug in research facility arranged combinations and commercial drug arrangements and the standard deviation was under 1.5. [31]
22. Performed by Tijana Rakić, et al. Distributed in 2014. The study was to perform the hydrophilic interaction liquid chromatographic analysis for bisoprolol. the chromatographic column is used in the method is Kinetex HILIC 100 Å (100 mm x 4.5 mm, their molecule size is 2.6mm and mobile phase made out of acetonitrile-water phase (35 mm ammonium acetic acid derivation, pH 4.9 changed with glacial acetic acid) (85:15 v/v). The flow rate was 1 ml per min⁻¹ and the column temperature was 30°C. The UV observation at 254 nm was observed.[36]
23. Performed by Savita S Yadav, Janhavi R Rao, published on 28 Feb 2013. the HPTLC separation method of bisoprolol fumarate and hydrochlorothiazide for developing and validating with the simultaneous method. The silica gel HPTLC aluminum plate 60 F254 with as a mobile phase use ethyl acetic acid derivation: methanol: smelling salts 10:0.5:0.5 (v/v). Absorption of the drug was detected at 225nm. The RF value of bisoprolol fumarate and hydrochlorothiazide were identify at 0.60 and 0.38. Linearity was found for bisoprolol fumarate is 150-900 ng/spot and for hydrochlorothiazide 100-600 ng/spot identifying 99.45 ± 0.06% for

- bisoprolol fumarate and hydrochlorothiazide is 100.25 ± 1.20 % found the recovery percentage in the validation method.[37]
24. Performed by Sevinc Kurbanoglu et al. Published on 4 December 2013. The introduced study was to perform the identity the separation of the drug by using the column as Acquity UPLC BEH C18 1.7 μm (2.1×50 mm) in the method and the temperature is maintained between 40°C . the using mobile phase is used as acetonitrile: phosphate buffer (20 mm) at the pH 3.0 solution and observed the wavelength 225nm. The concentration is used in the linearity between 0.5–150 $\mu\text{g/ml}$ for hydrochlorothiazide and the Limit of detection and limit of quantification is 0.01 and 0.03 $\mu\text{g/ml}$ and 0.5–250 $\mu\text{g/ml}$ for bisoprolol fumarate and Limit of detection and limit of quantification is 0.03 $\mu\text{g/ml}$ to be found.[34]
 25. Performed by Pradip N Bhoya, et al passed in 2013. The study was TLC-densitometry Chromatographic segment performed on aluminum plate precoated with silica gel 60F254 in the mobile phase as chloroform: ethanol: hard acidic risky in the ratio between 5:1.5:0.2 (v/v). the absorbance of the drug is 225 nm. The R₂ value of the bisoprolol fumarate drug obtained was 0.62 and hydrochlorothiazide is 0.40. 200–1200 ng/spot for bisoprolol fumarate and 100-800 ng/spot for hydrochlorothiazide linearity observed. 100.02 ± 1.14 % percentage recovery for bisoprolol fumarate and 99.91 ± 0.96 % for hydrochlorothiazide.[35]
 26. Performed by Huichao Chang et al. Published on 19 August 2012. The goal of the study was to establish a sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantitative assurance of amlodipine and bisoprolol, with clenbuterol as the inward standard (IS). The separation was performed on a Diamonsil C18 section (50 mm 4.6 mm, 5 m) with a mobile phase of methanol-water-formic corrosive (75:25:0.01, v/v/v) flowing at 0.3 ml/min. The chromatographic absolute run season for the strategy was 3 minutes. To assess amlodipine, bisoprolol, and IS, different responding observing (MRM) transitions of m/z [M+H] + 409.1237.9 (amlodipine), m/z [M+H] + 326.2116.0 (bisoprolol), and m/z [M+H] + 277.0203.0 (clenbuterol, IS) were used. For amlodipine and bisoprolol, the quantification limit was 0.2 ng/ml, and the linearity range was 0.2–50 ng/ml ($R^2 > 0.9961$).[36]
 27. Performed by Alina Diana Panainte et al. Published in September 2012. A research method performed on the Visible spectrophotometric technique was analyzed for the bisoprolol assay in pharmaceutical preparation using bromo-cresol green (BCG) in an HCL medium. The absorbance of the drug at 402nm. Linearity was found at 7-80 $\mu\text{g/ml}$. The limit of quantification was 5.41 μg per ml limit of detection was 1.78 μg per ml. The mean recovery percentage was 100.11% in the 98.35-101.57 % concentration range. [20]
 28. Performed by Sevgi Tatar Ulu et al, published on May 16, 2011. The proposed technique relied on the charge transfer reactions of bisoprolol fumarate with 7,7,8,8 tetra cyano-quomodimethane (TCNQ) and 2,3 dichloro 5,6 dicyano 1,4 benzoquinone (DDQ) as a bond to produce very high colored complexes using the N-electron donor method. The ICH criteria for linearity, the limit of detection, the limit of quantification, accuracy, precision, recovery and specificity are used to validate the parameter. With TCNQ and DDQ, Beer's rule is followed over concentration ranges of 10–60 and 10–80 g/ml bisoprolol respectively.[37]
 29. Performed by Clarice Maddalena Bueno Rolim et al. Published on Nov 2011. The purpose behind the review was to analysis of the HPLC techniques was worked isocratically at controlled-surrounding temp. With a reversed-phase C18 column (150 mm 4.6 mm i.d.; 5 mm molecule size), utilizing a mobile phase methanol: phosphate buffer (pH-3.5; 0.01 M) (55:45, v/v) at a 1.0 ml min flow rate. Absorbance was recognized at 225 nm and their LOD and LOQ are 0.05 $\mu\text{g/ml}$ -1 and 0.12 $\mu\text{g/ml}$ was observed.[38]

30. Performed by Gabriela peste, et.al, published on March 30, 2010. In the present work are performed Liquid chromatography coupled with mass spectrometry detection were used 0.1 formic acid-acetonitrile (50-50 % v/v) as mobile phase. The flow rate is 0.3 ml/min. In the method was used column zorbax SB 18 solvent saver plus, 3×100mm, 3.5µm. The LOQ was obtained 0.99 ng/ml. [39]
31. Experimented by Maha F. Tutunji, et.al. in 2009, where they had developed a simultaneous HPLC Tandem Mass Spectroscopy method for determination of Bisoprolol and hydrochlorothiazide in human plasma. The mobile phase deployed in the study was solvent mixture of ammonium acetate & Formic acid: Methanol: Acetonitrile in the ratio (65:17.5:17.5 % v/v/v) where the flow rate was 0.65mL/min. The linearity range lied between 0.10-30.0 (ng/mL) for Bisoprolol and 1.00-80.00 ng/mL for hydrochlorothiazide. The LOD for the bisoprolol and hydrochlorothiazide was found to be 0.100 & 1.00 ng/mL respectively. The developed and validated method was applied effectively on healthy volunteers at a dose of 5 mg & 12.5 mg Bisoprolol & hydrochlorothiazide respectively. [44]

Table (1): Bisoprolol analytical methods by Chromatographic Techniques.

S. No.	Method	Mobile phase	Flow rate	Column	Wave length	LOD, LOQ	Reference
1.	LC/MS/MS	Eluent A [Acetonitrile: water: formic acid (5:95:0.1v/v)] Eluent B [acetonitrile: formic acid (100:0.1v/v)]	0.4 ml/min.	C18, 50mm ×2.1mm, 5µm			[21]
2.	RP-HPLC	Methanol: phosphate buffer (55:45 v/v) [pH 3.5, 0.01]	1.0ml/min 21	C18(150 mm, 4.6mm, I.d 5mm particle size	225 nm	0.05µg/ml/ 0.12µg ml	[42]
3.	Robust UHPLC	Acetonitrile and 30mm phosphate buffer (pH2.0, 2.6, 3.2)	0.5ml/min	Acquity column (50×2.1mm, 1.7) µm BEH C18			[31]
4.	LC Hydrophilic interaction	Acetonitril -water phase (10mm ammonium acetate, pH 4.0 adjusted with glacial acetic acid) 92:8 v/v	1 ml/min	Luna HILIC 200 (50mm×4.6mm, 5µm)	230 nm		[33]
5.	LC/MS/MS	Methanol: water: formic acid (75:25:0.01 v/v/v)	0.3 ml/min	Diamonsil C18 (50mm×4.6mm, 5m)	277nm	0.2ng/ml/0.2ng /ml	[40]
6.	HPTLC	Chloroform: ethanol: glacial acetic acid (2:8:0.1 v/v/v)			231nm	40ng/spot/120ng/spot	[45]
7.	UPLC Stability indicating	Acetonitrile: phosphate buffer (20mm)	5µl/ 2min	Acquity UPLC BEH C18 1.7µM(2.1×50mm)	225nm	0.01µgml 1/0.3µg ml 1	[38]
8.	TLC	Chloroform: ethanol: glacial acetic acid(5:1.5:0.2 v/v) silica gel-60F254			225nm	104.54ng /34.59ng	[39]
9.	LC/MS/MS	0.1 Formic acid: acetonitrile (50:50 v/v)	0.3ml/min	Zorbax SB-C18 Solvent saver plus, 3×100mm, 3.5µm		LOQ- 0.99ng/ml	[15]

Table (2): Bisoprolol analytical methods by UV Vis Spectroscopy.

S No.	Method	Soft-ware model	Wave length	Solvent	LOD	LOQ	RSD Value	% Recovery	Reference
1.	UV-Vis absorbance	Hewlett Packard 8453	402 nm	Bromocresol green in HCL acidic medium (methyl orange reagent)	1.7µg/ml	5.41µg/ml	0.67%	100.11%	[46]
2.	UV-VIS absorbance	Jenway 7315 single beam	271 nm	Distilled water	0.22µg/ml	0.66µg/ml	1.19 and 0.854	105.0 ± 1.3%	[27]
3.	Ratio spectra for spectro photometry resolution	UV-VIS 1650 PC double beam shimadzu	210-300nm	methanol					[35]
4.	UV- VIS Absorbance	T 80 uv-vis double beam spectrophotometry	610 nm (indigo carmine, ic) 504nm (methyl orange, mo)	Ceric ammonium sulphate in sulphuric acid Dye indigo carmine Methyl orange	0.250 (ic) 0.250 (mo)	0.76 (ic) 0.66 (mo)	2.41 (ic) 2.10 (mo)		[18]
5.	Charge transfer reaction UV-VIS Spectrophotometry	Shimadzu uv 160	842.5nm (TCNQ-Bisoprolol) 587.5nm(DDQ-Bisoprolol)	Methanol TCNQ and DDQ w/v in acetonitrile	0.07µg/ml	0.21µg/ml		98.64%+0.21(TCNQ) 98.85%+0.32	[41]

CONCLUSION

Bisoprolol fumarate (BF), a strong beta-blocker approved by the FDA in 1992, selective to Beta 1receptor and is competitive antagonist of adrenergic receptors. It works well in the prevention of angina pectoris and hypertension. Maintaining the highest levels of quality, safety, and efficacy is critical for delivering a fully potent dose form to patients. Maintaining the safety of the drugs in this category that are given regularly for the maintenance of cardiovascular disease is the need of the hour. Numerous analytical and bioanalytical methods including the SIM (stability-indicating methods) are available for estimation of Bisoprolol in solid and finished dosage forms. Most of the available methods are using sophisticated analytical instruments that are highly accurate and precise but altogether when seen are not cost-effective in sense of the instrument cost and use of costlier solvents. The solvents used are also not environmentally friendly and produce toxicities. Thus, we suggest a need of such a method for Bisoprolol's analytical estimation that would have an equilibrium of cost-effectiveness and can be ecofriendly.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Ethics approval and consent to participate

Not applicable

Consent for publication

The authors give the Publisher the Author's permission to publish the work.

Availability of data and materials

All data generated for this study are included in the article.

Author's contribution

Manoj Verma: Data curation, writing original draft, methodology, investigation. **Swati Pandey:** Draft formal analysis, project administration, software, validation, visualization, and writing review editing. **Bina Gidwani:** resources, supervision, and validation, **Ravindra Kumar Pandey:** supervision, validation, visualization, funding acquisition. **Shiv Shankar Shukla:** Conceptualization, supervision, validation, visualization, project administration, funding acquisition.

Competing interest

There are no competing interests to declare.

Funding

The authors are grateful to the Department of Science and Technology (DST-FIST) Letter no-SR/FST/COLLEGE/2018/418, New Delhi for providing financial assistance.

Acknowledgement

Authors would also like to acknowledge the invaluable assistance provided by Columbia Institute of Pharmacy; Raipur whose contributions made this review possible.

REFERENCES

- Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al. heart disease and Stroke Statistics - 2021 Update: A Report from the American Heart Association. *Circulation*. 2021; 143(8): e254-e743.

- 2] Bakheit AH, Ali R, Alshahrani AD, El-Azab AS. Bisoprolol: A comprehensive profile. *Profiles Drug Subst Excip Relat Methodol.* 2021; 46: 51-89.
- 3] Lithell H, Weiner L, Vessby B, Nordström ML. Effects of small doses of bisoprolol on blood pressure and lipoprotein concentrations in hypertensive patients. *Eur J Clin Pharmacol.* 1993; 44: 19–22.
- 4] John A.O’neill, Helmut Schickaneder, Aggelos Nikolopoulos GJ. Manufacture of bisoprolol and intermediates therefor. 2010.
- 5] Mahu SC, Spac AF, Ciobanu C, Hancianu M, Agoroaei L, Butnaru E. Quantitative Determination of Bisoprolol Fumarate by HPLC Method validation. *Rev Chim.* 2016; 67: 414–417.
- 6] Leopold G. Balanced pharmacokinetics and metabolism of bisoprolol. *J Cardiovasc Pharmacol.* 1986;8 Suppl 11: S16-20.
- 7] Leopold G, Pabst J, Ungethüm W, Bühring KU. Basic pharmacokinetics of bisoprolol, a new highly beta 1-selective adrenoceptor antagonist. *J Clin Pharmacol.* 1986 Nov-Dec;26(8):616-21.
- 8] Charoo NA, Shamsheer AAA, Lian LY, Abrahamsson B, Cristofolletti R, Groot DW, et al. Biowaiver monograph for immediate-release solid oral dosage forms: Bisoprolol fumarate. *J Pharm Sci.* 2014; 103: 378–91.
- 9] Logoyda L, Kovalenko S, Abdel-Megied AM, Zhulkevych I, Drapak I, Demchuk I, et al. HPLC method development for the analysis of bisoprolol in combined dosage form containing bisoprolol and enalapril and in vitro dissolution studied. *Int J Appl Pharm.* 2019; 11: 186–94.
- 10] Hong. Ultra-high-performance liquid chromatography as an assay method for the investigation of conditions of captopril extraction by organic solvents. *Asian J Pharm.* 2018; 12: S111–4.
- 11] Horikiri Y, Suzuki T, Mizobe M. Stereoselective metabolism of bisoprolol enantiomers in dogs and humans. *Life Sci.* 1998;63(13):1097-108.
- 12] Hong. Polymorphic forms of bisoprolol fumarate: Preparation and characterization. *J Therm Anal Calorim.* 2019; 135: 3043–55.
- 13] Schuchert A; BISEX Investigators. Effects of bisoprolol treatment for chronic heart failure initiated and followed up by primary care physicians. *Eur J Heart Fail.* 2005 Jun;7(4):604-11.
- 14] Panainte AD, Gafitanu C, Stoleriu I, Tarțau LM, Popescu MC, Lisa G, et al. New modified release tablets of bisoprolol fumarate for the treatment of hypertension: characterization and in vitro evaluation. *J Drug Deliv Sci Technol.* 2019;50: 402–9.
- 15] Hong. Experimental research for determination of bisoprolol fumarate in human plasma samples using liquid chromatography-tandem mass spectrometry (LC-MS / MS) technique. 2010;15: 5140–5.
- 16] Logoyda, L. S. Analysis of approaches to the development and validation of the methods of analysis of Bisoprolol in drugs and biological liquids. *Medical and Clinical Chemistry*, 2019; 3:111–118.
- 17] Pandey S, Pandey R, Shukla SS. Spectroscopic Substantiation for the Identification of Degradants by Q-TOF Micromass (ESI-MS) in Bisoprolol Fumarate with an Inventive Validation Approach for Stability Indicating HPLC Method. *Indian J Pharm Educ Res.* 2022; Jan 1;56(1):272-80
- 18] Elshaprawy DS, Elmosallmy MF, Elgendy K. Spectrophotometric Determination of Bisoprolol Fumarate and Levo-bunolol Hydrochloride in Bulk and Pharmaceutical Forms. *Eurasian J Anal Chem.* 2020; 15: 20–31.
- 19] Pawar S, Tamboli A, Patil S. RP-HPLC method development and validation for simultaneous estimation of Bisoprolol fumarate and Cilnidipine in pharmaceutical dosage form. *J Pharm Sci Biosci Res.* 2020; 10 (2):149-155.

- 20] Jadhav NB, Yadav SS. Analysis of bisoprolol fumarate and amlodipine besylate in tablet dosage form by using HPTLC. *Int J Pharm Sci Rev Res.* 2019; 58: 75–78.
- 21] Logoyda L, Korobko D. A High-Performance Liquid Chromatography – Mass Spectrometry / Mass Spectrometry Method Development for The Quantitative Determination of Bisoprolol From CACO-2 Cell Monolayers. 2018;11: 386-389.
- 22] Detrich Á, Dömötör KJ, Katona MT, Markovits I, Vargáné Láng J. Polymorphic forms of bisoprolol fumarate. *J Therm Anal Calorim.* 2019; 135: 3043–3055.
- 23] Barge VU, Gaikwad RB, Chaudhari FM, Kande TR. Development and Validation of Analytical Method for Simultaneous Estimation of Bisoprolol Fumarate and Telmisartan by Using RP-HPLC Method. *Int J Pharm Clin Res.* 2018;10: 219–23.
- 24] Panainte AD. RP-HPLC Analysis Method of Bisoprolol Fumarate in a New Tablet Formulation. *The Medical-Surgical Journal.* 2018; 122: 634–9.
- 25] Mitrevska I, Kikovska-Stojanovska E, Petrusovski G, Chachorovska M, Memed-Sejfulah S, Ugarkovic S. Identification and Structural Characterization of Unidentified Impurity in Bisoprolol Film-Coated Tablets. *Adv Chem.* 2017:1-16. doi: 10.1155/2017/3047517.
- 26] Dąbrowska-Maś E. Preparation of Analytical Standard of Bisoprolol Impurity A. *Int Res J Pure Appl Chem.* 2017; 14: 1–8.
- 27] Mohammed SA, Adam ME, Shantier SW. Development and validation of UV spectrophotometric method for determination of bisoprolol fumarate in bulk and pharmaceutical dosage forms. *Mediterr J Chem.* 2017; 6: 196–9.
- 28] Kasagić-Vujanović I, Jančić-Stojanović B, Ivanović D. Investigation of the retention mechanisms of amlodipine besylate, bisoprolol fumarate, and their impurities on three different HILIC columns. *J Liq Chromatogr Relat Technol.* 2018; 41: 523–31.
- 29] Marothu VK, Yerramothu P, Gorrepati M, Majeti S, Mamidala SK, Nellutla A. Application of HPLC to assess the compatibility of bisoprolol fumarate with selected excipients in mixtures by isothermal stress testing. *Ann Pharm Fr.* 2015;73(6): 442-451.
- 30] Duan HB, Cao JT, Wang H, Liu YM. Determination of metoprolol tartrate and bisoprolol fumarate by capillary electrophoresis coupled with tris (2,2'-bipyridyl)-ruthenium (ii) electrochemiluminescence detection and study on the interaction between the drugs and human serum albumin. *Anal Methods.* 2015; 7, 3946-3951.
- 31] Kormány R, Molnár I, Fekete J, Guilmarme D, Fekete S. Robust UHPLC separation method development for multi-API product containing amlodipine and bisoprolol: The impact of column selection. *Chromatographia.* 2014; 77: 1119–27.
- 32] Skotnicki M, Aguilar JA, Pyda M, Hodgkinson P. Bisoprolol and Bisoprolol-Valsartan Compatibility Studied by Differential Scanning Calorimetry, Nuclear Magnetic Resonance and X-Ray Powder Diffractometry. 2015 Feb;32(2):414-29
- 33] Jancic-Stojanovic B, Rakić T, Ivanović D, Jancic-Stojanovic I. Design of experiments in optimization and validation of a hydrophilic interaction liquid chromatography method for determination of amlodipine besylate and bisoprolol fumarate. *J Liq Chromatogr Relat Technol.* 2015; 38(8): 919-928.
- 34] Galea C, Mangelings D, Heyden Y Vander. Method development for impurity profiling in SFC: THE selection of a dissimilar set of stationary phases. *J Pharm Biomed Anal.* 2015; 111: 333–43.
- 35] Elzanfaly ES, Hassan SA, Salem MY, El-Zeany BA. Different signal processing techniques of ratio spectra for spectrophotometric resolution of binary mixture of bisoprolol and hydrochlorothiazide; A comparative study. *Spectrochim Acta - Part A Mol Biomol Spectrosc.* 2015; 140: 334–43.

- 36] Rakic T, Jovanovic M, Tumpa A, Jancic-Stojanovic B, Medenica M. Optimization of HILIC method for the analysis of bisoprolol and its impurities considering uncertainties in peak position. *Arh Farm (Belgr)*. 2014;
- 37] Yadav SS, Rao JR. Simultaneous HPTLC Analysis of Bisoprolol Fumarate and Hydrochlorothiazide in Pharmaceutical Dosage Form. *Int J Pharm Pharm Sci*. 2013; 5: 286–96.
- 38] Kurbanoglu S, Rodriguez San Miguel P, Uslu B, Ozkan SA. Stability-indicating UPLC method for the determination of bisoprolol fumarate and hydrochlorothiazide: Application to dosage forms and biological sample. *Chromatographia*. 2014; 77: 365–71.
- 39] N Bhoja P. Development and Validation of TLC-Densitometry Method for Simultaneous Estimation of Bisoprolol Fumarate and Hydrochlorothiazide in Bulk and Tablets. *J Chromatogr Sep Tech*. 2012; 04: 1–4.
- 40] Chang H, Li J, Li J, Guan X, Sun F, Qian Z, et al. Simultaneous determination of amlodipine and bisoprolol in rat plasma by a liquid chromatography/tandem mass spectrometry method and its application in pharmacokinetic study. *J Pharm Biomed Anal*. 2012; 71: 104–10.
- 41] Ulu ST, Kel E. Spectrophotometric determination of bisoprolol in pharmaceutical preparations by charge transfer reactions. *Opt Spectrosc (English Transl Opt i Spektrosk)*. 2012; 112: 864–867.
- 42] Bueno Rolim CM, Brum L, Fronza M, Malesuik MD, Bajerski L, Dalmora SL. Development and validation of an RP-HPLC method for the dissolution studies of bisoprolol in pharmaceutical dosage forms. *J Liq Chromatogr Relat Technol*. 2005; 28:3, 477–486.
- 43] Peste G, Bibire N, Apostu M, Vlase A, Oniscu C. A new liquid chromatography-tandem mass spectrometry method for determination of bisoprolol in human plasma samples. *J Biomed Biotechnol*. 2009; Volume 2009, Article ID 736327, 8 pages doi:10.1155/2009/736327.
- 44] Tutunji MF, Ibrahim HM, Khabbas MH, Tutunji LF. Simultaneous determination of bisoprolol and hydrochlorothiazide in human plasma by HPLC coupled with tandem mass spectrometry. *J Chromatogr B Anal Technol Biomed Life Sci*. 2009; Jun 1;877(16-17):1689-97.
- 45] Jadhav NB, Yadav SS. Analysis of bisoprolol fumarate and amlodipine besylate in tablet dosage form by using HPTLC. *Int J Pharm Sci Rev Res*. 2019; 58:75–8.
- 46] Gudruman AD, Murărau A, Dorneanu V. Spectrophotometric determination of bisoprolol using methyl orange as reagent. *Farmacia*. 2012; 60: 634–41.

The Palestinian Medical and Pharmaceutical Journal (Pal. Med. Pharm. J.) © 2024 by An-Najah University, Nablus, Palestine is licensed under CC BY-NC 4.0 