An Insight on Analytical Profile on Bisoprolol Fumarate – A Selective Beta-1 Adrenoreceptor Blocker

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Abstract BF is Beta-adreno receptor antagonist and used as an Anti-Hypertensive Drug. BF gives the blocking action on β1-adrenergic receptors in the heart and vascular smooth muscle. The present review compiles the various approaches implemented for quantification of BF in bulk drug, pharmaceutical matrix and biological fluid. This review represents more than 50 analytical methods which include capillary electrophoresis, HPLC, HPTLC, UV-Spectroscopy, UPLC, impurity profiling and electrochemical methods implemented for estimation of BF as a single component as well as in multicomponent.

Keyword: BF; Bioanalytical; UPLC/LC-MS; capillary electrophoresis; impurity profile

1. INTRODUCTION

BF is an extremely discriminatory β1-adrenergic blocker [1]. BF is chemically: (RS)-1-[4-[[2-(1-Methylethoxy) ethoxy] methyl] phenoxy]-3-[(1 methyl ethyl) amino] propan-2-ol fumarate Figure 1. It is official in, USP. BF has similar structure to metoprolol, bopindolol, hydrochlorothiazide, atenolol [2]. Structure of BF, there is two substituents at para position of benzene provide the activity of β-selectivity, In which it has two substituents in para position of benzene which might be the activity of β-selectivity [3]. White crystalline powder of BF was soluble in water, methanol, ethanol, and chloroform. [4]. BF blocks catecholamine stimulus of β1-adrenergic receptors in the heart (cardio-selective) and
vascular smooth muscle, with decreasing the heart rate, cardiac output, systolic and diastolic blood pressure, and may be response orthostatic hypotension [5]. \(\beta\)-Blocker with calcium channel blocker mixture has efficacy in definite cardiovascular diseases like angina pectoris, myocardial infarction and hypertension [4]. For the decrease of workload on the heart and hence oxygen demands, so that the drug is pointed toward for secondary prevention of myocardial infarction, parallel therapy in patients with stable chronic heart failure, and for the treatment of hypertension and angina pectoris[5]. About 80% bioavailability given by BF after 10 mg oral dose[6]. The first pass metabolism of BF is about 20% and binding to serum proteins is approximately 30% [5]. The concentrations of plasma were taken in between 5 mg to 20 mg. It is contraindicated in person suffering from Psoriasis, Myasthenia Gravis, Sinus bradycardia, diabetes, depression and during Pregnancy. BF is available in combination with other drugs like HCT, AMD B, IRBE, CELI, METO T [7].

![Figure 1: Chemical structure of BF.](image)

2. ANALYTICAL ACCOUNTS ON BF

A variety of analytically urbanized methods like UV/Vis-Spectrophotometry, High-Performance Liquid Chromatography (HPLC), High-Performance Thin-Layer chromatography (HPTLC), Ultra Pressure Liquid Chromatography(UPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), Capillary Electrophoresis and Stability indicating methods have been studied for analysis of BF. The present papers described consolidate analytical methods published so far estimation of BF in bulk and pharmaceutical formulation as well as in biological samples. In literature reported method describe the analysis of BF in various dosage forms as single components as well as in combination with HCT, AMD B, IRBE, CELI, METO T, tropaeolin and Bromocresol green. Summary of these methods for determination of BF is shown in Figure 2.
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Figure 2: Analytical methods for BF.

Figure 3: % Utility of analytical Techniques for BF.
3. PHARMACOPOEIAL STATUS

BF is the official drug in the (USP29) united state of pharmacopeia (2004). USP reported HPLC assay method using 4.6 mm × 12.5 cm column that contain packing L7 as a stationary phase and a mobile phase consist mixture of (65:35 % \(v/v\)). Water-acetonitrile used as diluents to 1 L portion add 5 mL heptaflurobutyric acid, 5 mL diethyl amine and 2.5 mL formic acid with flow rate 1 mL/min, the column outflow was scan at 273 nm [8].

**Table 1:** Dosage form, route of administration and recommended dose of BF.

| Dosage forms | Dose | Route of administration | Indication / dose |
|--------------|------|-------------------------|-------------------|
| Tablet       | 2.5 mg | Oral                    | Adult Hypertension 5 mg/day and maxim. 20 mg. |
| Tablet       | 1 mg | Oral                    | **Patients with renal impairment:** Not exceed 10 mg once daily. |
| Tablet       | 5 mg | Oral                    | **Patients with severe liver impairment:** No dosage adjustment is required, however careful monitoring is advised. |
| Tablet       | 10 mg | Oral                    | The maximum recommended dose is 10 mg once daily. |
| Tablet       | 20 mg | Oral                    | **Elderly:** No dosage adjustment is normally required. It is recommended to start with the lowest possible dose. **Children:** There is no experience with bisoprolol in children, therefore its use cannot be recommended for children. |

4. UV/VIS-SPECTROPHOTOMETRIC METHODS OF BF [9 -20]

In article about nine UV-Spectrophotometric methods have been fixed for assurance of BF single and in combination of different dosage form. Also one spectrofluorometric method has been accounted for determination of BF. The detailed summary spectrophotometer and spectrofluorometer designating the basic principle, sample matrix, linearity and retention time in **Table 2**.
**Table 2:** Spectrophotometric methods used for determination of BF alone and in combined dosage form.

| Sr. No | Drugs                  | Methods          | Detection (nm) | Linearity (μg/mL) | Correlation coefficient ($r^2$) | LOD and LOQ (μg/mL) | Ref. |
|--------|------------------------|------------------|----------------|-------------------|---------------------------------|---------------------|------|
| 1      | BIS with bromocresol green | Zero order       | 402            | 7 - 80            | 0.9998                          | LOD- 1.78, LOQ- 5.41 | 9    |
| 2      | BF and HCT             | First order      | 223 & 274      | BIS 8 - 96 & HCT 4 - 48 | 0.999 & 0.998                      | –                   | 10   |
| 3      | BF                     | Zero order       | 412            | 5 - 30            | 0.9997                          | LOD- 0.67, LOQ- 2.23 | 11   |
| 4      | BIS using methyl orange | Zero order       | 427            | 0.8 - 9           | 0.9997                          | LOD- 0.20, LOQ- 0.66 | 12   |
| 5      | AMD B and BF           | Zero order       | 356 & 270      | 2 - 18 & 10-100   | 0.9966 & 0.9941                  | LOD- 0.4854, LOQ- 0.2013 | 13   |
| 6      | BF by using tropaeolin 00 | Zero order       | 412            | 5 - 30            | 0.9995                          | LOD- 0.67, LOQ- 2.23 | 14   |
| 7      | BF                     | Zero order       | 532 and 626    | 100 - 500 & 50 - 300 | 0.999 & 0.998                     | –                   | 15   |
| 8      | IRB and BHF            | Zero order       | 476 and 479    | 20 - 90 & 40 - 160 | 0.9998 & 0.9998                  | LOD- 1.37 and 3.98, LOQ- 4.17 and 12.06 | 16   |
| 9      | BF and HCT             | First order      | BF 285.5 & HCT 264.5 | 1.2 - 59 & 0.997 & 0.998 |                             | LOD- 0.27, LOQ- 0.89, LOD- 0.26, LOQ- 0.85 | 17   |
| 10     | BF with BPB and BCP    | First order      | BF 402         | In BPB 1.0-9.0 & in BCP 1.0-11.0 | In BPB for BIS 0.9976 & BCP for BIS 0.9999 | LOD in BPB and in BCP for BIS 0.1791 and 0.5868, and LOQ in BPB and in BCP 0.5964 and 1.9542 | 18   |
5. SPECTROFLUOROMETRIC METHOD OF BF

Hashem et al. (2016) reported IRB and BHF through spectrofluorometric method. It is depend on charge transfer reaction between the designed drugs and 7-Chloro-4-nitrobenzen-2-oxa-1; 3-diazole NBD-CI. Dilution was prepared by using specific volume of NBD-CI (0.1%, w/v). By using 5 mL with acetonitrile it get heated and after cooling the fusion of solution was attenuate to 10 mL with acetonitrile and methanol for IRB and BHF, respectively. The absorbance was recorded at 476 and 479 for IRB and BHF, at colored concentration respectively against the reagent blank treated similarly. The linearity was obeyed in the range of 2.5–8 µg/mL for IRB and 6–16 µg/mL for BHF. This method also gives detection limits of 0.18 and 0.39 µg/mL and a secondary quantification limit of 0.55 and 1.17 µg/mL for IRB and BHF. The statistical evaluation of the results with the results of reported methods reflected that there was no major differentiation [16].

6. CHROMATOGRAPHIC SYNOPSIS

6.1 High-Performance Liquid-Chromatography [21-42]

Distant from Pharmacopoeial methods many HPLC methods were accounted for assurance for BF in pharmaceutical formulation. The outlined of expressed HPLC methods specifically the mobile phase used for estimation, columns, wavelength, correlation coefficient and linearity range is shown in the Table 3.
| Sr. No | Name of drug | Columns | Mobile phase system | Discussion | Ref |
|-------|--------------|---------|---------------------|------------|-----|
| 2.    | BF and HCT   | RP Zorbax Eclipse XDB-C18 | Acetonitrile-water (25:75 % v/v) Containing 15mM phosphoric acid | Detection of BF and HCT was carried out at 225nm. Linearity range for BF and HCT 0.50–12.00 and 0.20–8.00 µg/mL. Correlation coefficient for BF and HCT 0.999 and 0.999. Retention time for BF and HCT 5.058 min and 2.783 min. | 22 |
| 3.    | BF and AMD   | Luna C18-2 | 25 mM ammonium acetate adjusted to pH 5.0 and methanol (65:35 % v/v) | Detection of BF and AMD was carried out at 230 nm. Linearity was established in the range of 8–33 µg/mL. Retention time was 1.45 min and 3.91 min for BF and AMD. Correlation Coefficient for BIS 0.999 and AMD 0.999. | 23 |
| 4.    | BF           | Hypersil ODS | mixture of buffer and acetonitrile in the ratio of (700:300 % v/v) | Detection of BF was carried out at 208nm. Retention time of 3.146 min. Linearity 5.00 - 17.5 µg/mL for BF. Correlation Coefficient 0.998. | 24 |
| 5.    | BIS F        | prontosil, chromo bond | buffer (pH 5.6) and acetonitrile in the ratio of (750:250 % v/v) | Detection of BIS F was carried out at 226nm. Linearity at 6 different levels from 25 µg/mL to 100 µg/mL. The retention time of BIS F was found to be 9.15min. Correlation coefficient 0.999917. | 25 |
| 6.    | BIS F and AMD B | C18Intersil | Methanol: Acetonitrile: 50mM Potassium dihydrogen phosphate buffer KH2PO4 (25:30:45% v/v) | Detection of BIS F and AMD B was carried out at 267nm. Correlation Coefficient For BIS 0.998 and AMD besylate 0.999. | 26 |
| 7.    | BF and HCT   | C18 column Kromasil 100-5C18 column | Acetonitrile-0.01 M KH2PO4 (40:60% v/v and pH 3.5) | Detection of BF and HCT was carried out at 232nm. Linearity: - 1-7 and 2.5-17.5µg/mL for BF and HCT. Amplitudes at 228.4 and 283nm of BF and HCT. Correlation Coefficient For BF 0.9999 and for HCT 0.9999. Retention Time:- BF and HCT were eluted at 3.38±0.04 and 4.03±0.02 min. | 27 |
| Sr. No | Name of drug | Columns | Mobile phase system | Discussion | Ref |
|--------|--------------|---------|---------------------|------------|-----|
| 8.     | BF enantiomer| Chiralcel OD column | methanol-glacial acetic acid-triethylamine, (100:0.020:0.025% v/v/v) | Detection of BF was carried out at excitation/emission 275/305 nm. Linearity was found to be 5 - 250 ng/mL. Correlation coefficient of 0.999. | 28 |
| 9.     | BF and HCT (tablet) | C18 column Kromasil | Acetonitrile and phosphate buffer (40:60% v/v, pH 3) | Detection of BF and HCT was carried out at 228. For both BF and HCT linearity was found to be 20-100 µg/mL. The retention time of BF and HCT were 3.3 min and 6.25 min. correlation coefficient 0.998 and 0.999. | 29 |
| 10.    | BF | C18 column Kromasil 100-5C18 | Phosphate buffer (pH 3.5) and acetonitrile (70:30% v/v) 1ml/min | Detection of BF was carried out at 225 nm. Linearity is 5-90µg/mL. Retention time 1.158 min. Correlation coefficient 0.9998 and regression coefficient 0.9996. | 30 |
| 11.    | BF (tablet) | Eclipse XDB C18 | Water / methanol / acetonitrile in a ratio of (50:30:20% v/v/v) | Detection of BF was carried out at 225nm. Two areas of linearity in the range of 0.8 - 80 g/mL and 80 - 1000 g/mL. Correlation coefficient 0.999 and intercept 0.4953 with a regression coefficient R2 = 0.999. LOD = 1.3 g/mL LOQ = 3.98 g/mL. | 31 |
| 12.    | BF and HCT and impurities (tablet) | BDS Hypersil C8 column | [acetonitrile-ammonium dihydrogen phosphate/orthophosphoric acid buffer solution (80:20% v/v)] | Detection of BF and HCT was carried out at 220nm. Linearity 1.50–46.20and 3.80–114.00 µg/mL. Correlation coefficient 0.9998 and 0.9998. Recovery: 98–102 % for active ingredients (B for HCT), 90–110 % for impurities (A, L, K For BF impurity). | 32 |
| 13.    | BF with potential impurity | LiChrosorb RP-18 | Acetonitrile- 0.050 M ammonium phosphate buffer (4:6% v/v). | Detection of BF and HCT was carried out at 226nm. Linearity: - 1 to 10 µg/mL. Regression coefficient of the linearity test was 0.9996. | 33 |
| Sr. No | Name of drug | Columns | Mobile phase system | Discussion | Ref |
|-------|--------------|---------|---------------------|------------|-----|
| 14.   | BF with HCT  | Zodiacsil-C18 | buffer solution (pH 3.60) containing 5 mM monobasic potassium phosphate in milliQ-water. Mobile phase B consists of a mixture of acetonitrile and methanol in the ratio (80:20 % v/v) | Detection of BF and HCT was carried out at 226 nm. Correlation coefficient obtained was 0.999. Stability indicating in the range of LOQ to 150%. The retention times was studied between ± 0.2 units. | 34 |
| 15.   | BF separated by chiral stationary column | Chiralpak IB | n-hexane/ethanol (95/5 % v/v), 0.2% DEA) | Detection of BIS was carried out at 223 nm. Correlation Coefficient was found to be 0.8635. Retention Time was over 100 min. | 35 |
| 16.   | BF with selected excipient | Hypersil BDS C18 | acetonitrile—potassium dihydrogen phosphate buffer (pH 3.0, adjusted with orthophosphoric acid; 20 mM) (50:50% v/v) | Detection of BF was carried out at 222 nm. Linearity in the range of 10–100 µg/mL. Correlation Coefficient: -0.999. LOD and LOQ values were found to be 0.03 and 0.1 g/mL. Retention Time: ±4.00 min. | 36 |
| 17.   | BF Tab | Chromolith RP18-e | phosphate buffer (pH3.5): acetonitrile (77.5: 22.5 % v/v) | Detection of BF was carried out at excitation/emission 232/320 nm. Linearity range is 3 to 200 ng/mL, correlation coefficient of 0.9998. LOQ was 3 ng/mL. Retention Time was detected 4.5 min and 7.24 min. | 37 |
| 18.   | BF | Zorbax SB-C18 Solvent Saver Plus | 0.1% formic acid solution – acetonitrile (50-50% v/v) | Detection of BF was carried out at 226 nm. Linearity in the range of 1 ng/mL and 100 ng/mL. Correlation Coefficient of 0.998599, LOQ is 1 ng/mL. Retention Time is 1.7 min and 1.9 min. | 38 |
| 19.   | BF | Kromasil C18 column | methanol and 0.05% phosphoric acid (40:60% v/v) | Detection of BF was carried out at exc./emi 275/305 nm. Linearity in the range of 10–100 ng/mL. Correlation Coefficient: - 0.994. LOD: 3 ng/mL, LOQ: 10 ng/mL. | 39 |
| Sr. No | Name of drug | Columns | Mobile phase system | Discussion | Ref |
|--------|--------------|---------|---------------------|------------|-----|
| 20.    | BF film coated tab | Nucleosil 100-5 C18 HD | water and formic acid as solvent A in the ratio (99% : 1% (v/v) and acetonitrile and formic acid as solvent B in the ratio (99% : 1% v/v) | Detection of BF was carried out at 225nm. The retention times are 16.2 min. | 40 |
| 21.    | BF and HCT | YMC Pack Pro C18 column | 0.1% orthophosphoric acid and acetonitrile (55:45% v/v) | Detection of BF and HCT was carried out at 259nm. Linearity range of 40-120 μg/mL (BF) and 50-150 μg/mL (HCT). Correlation Coefficient :- 0.9999 LOQ was 0.398 and 0.385 μg/mL for BIS and HCT. LOD (μg/ml) 0.398 and 0.385. | 41 |
| 22.    | BF and HCT | Zodiac C18 | phosphate buffer and acetonitrile in the ratio of (80:20 % v/v) | Detection of BF and HCT was carried out at 208nm. Linearity was obeyed in the range 2.5-75μg/ml and 3-90μg/ml of BF and HCT. The retention time of BF and HCT was found to be 2.253 min and 4.425 min. LOD and LOQ for BF 2μg/ml and 6μg/ml and for HCT 0.9 μg/ml and 1.8 μg/mL. | 42 |

6.2 High-Performance -Thin- Layer Chromatography [43-45]

About 3 HPTLC methods have been studied for simulation determination of BF with HCT and camphorsulphonic acid in different pharmaceutical dosage form.

Emanual M Patelia et al. (2013) investigated specific and precise method for quantitative estimation of BF and HCT in pharmaceutical dosage form. The separation of the BF and HCT was conceded on aluminum plate precoated with silica gel 60F, using mobile phase chloroform: ethanol: glacial acetic acid (5:1.5:0.2 v/v/v).The Rf value was found to be 0.62 and 0.40 for BF and HCT, respectively when the densitometry quantification was performed maximum at 225 nm. For analysis of BF and HCT, linearity was studied in the range of 200 - 1200 ng/band and 100 - 800 ng/band, respectively. Accurateness of the method was studied by % recovery and found to be 100.02 ± 1.14% for BF and 99.91 ± 0.96% for HCT [43]. Similarly; Rao et al. (2013) also developed and validated an
effortless manner for determination of BF and HCT. The separation was achieved on aluminum plates percolated silica gel 60 F$_{254}$ using mixture of ethyl acetate: methanol: ammonia 10:0.5:0.5 v/v/v as mobile phase with Rf of BF and HCT were 0.60 and 0.38, in that order and detection was monitored at 225 nm. For estimation of drugs, the linearity experiment was performed in the range of 150-900 ng/spot for BF and 100-600 ng/spot for HCT. Linear regression for BF and HCT was 0.999 [44]. The established methods were validated for correctness, robustness and specificity as per ICH guidelines.

Patel et al. (2011) reported an enantiomer separation of BF by TLC and HPTLC by means of (+)-10-camphorsulphonic acid as a chiral selector. Chromatographic separation of BF was performed with optically pure (+)-10-camphorsulphonic acid as a chiral selector. The mobile phase set for separation was triethyl amine–methanol–1-pentanol (0.14:9.9:0.18, %v/v/v). For TLC detection was executed at UV-chamber at short wavelength 254 nm and for HPTLC densitometry detection performed at 224 nm. The calibration ranges for both the isomers were 5-30 µg/mL [45].

7. STABILITY-INDICATING METHODS (SIM) FOR ESTIMATION OF BF [46-51]

With reference four stability indicating methods studied accordingly for persistence of BF in bulk substance and pharmaceutical dosage form implementing several analytical techniques. The reported stability indicating methods for BF illustrating dosage form, column, mobile phase and linearity and retention factor presented in [Table: 4].

**Table 4:** Stability indicating methods of BF by HPLC and UPLC.

| Sr. No | Drugs | Formulation | Column | Mobile Phase System | Detection | Discussion | Ref |
|-------|-------|-------------|--------|---------------------|-----------|------------|-----|
| 1     | BF    | Tablet      | Chromo band C18 | Buffer/ Acetonitrile (75:25% v/v, pH 5.6) | 226 nm | Linearity was found to be 25 and 100 µg/mL. Correlation Coefficient: - 0.9998. Retention Time:- 9.5 | 46 |
| 2     | BF and AMD | Aq.solution | C18 | Acetonitrile–water solution of 10 mM ammonium acetate (92:8 % v/v) | 230 nm | Retention Time is 4.042 min. | 47 |
| Sr. No | Drugs           | Formulation | Column              | Mobile Phase System                                                                 | Detection | Discussion                                                                                                                                                      | Ref  |
|--------|-----------------|-------------|---------------------|--------------------------------------------------------------------------------------|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 3      | BF and HCT      | Tablet      | YMC Pack Pro C18    | 0.1% Orthophosphoric acid and acetonitrile (55:45 % v/v)                             | 259 nm    | Linearity for both BF and HCT: - 40-120 and 50-150 μg/mL. Correlation Coefficient was found to be 0.9999. Retention Times 3.688 min and 5.824 min.                                                                             | 48   |
| 4      | BF Tablet       | Tablet      | HP1200              | Acetonitrile-water solution (10 mM ammonium-acetate, pH 4.0, adjusted with concentrated acetic acid) at a ratio of (92.8 % v/v) | 230 nm    | R-value for zero-order reaction had the highest value 0.9747.                                                                                                                                                            | 49   |
| 5      | BF and HCT      | Tablet      | ACQUITY BEH C18     | Mobile phase A consisting of a buffer solution (pH 3.60) containing 5 mM monobasic potassium phosphate in milliQ-water. Mobile phase B consists of a mixture of acetonitrile and methanol in the ratio 80:20 % v/v) | 226 nm    | Correlation Coefficient was found to be 0.999. A retention time was studied between ± 0.2 units.                                                                                                                         | 50   |
| 6      | BF and HCT      | Tablet      | Acquity UPLC BEH C18| Water : acetonitrile (50:50 % v/v)                                                   | 225 nm    | Linearity in the range of 0.5– 250 μg/mL for BF and 0.5–150μg/mL. Correlation Coefficient (r) was found to be 0.999. For human urine linear in the range between 0.5 and 10 μg / mL for HCT and 0.5–30 μg/mL for BF. LOD and LOQ 0.07– 0.21 μg/ mL; and 0.01–0.03 μg/ mL for BF and HCT | 51   |
8. CAPILLARY ZONE ELECTROPHORESIS METHOD [52-55]

Laszlo Gagyi et al. (2006) established a effortless capillary electrophoresis method for estimation of various stereoselective β1-blockers and H1-antihistamines by human serum transferrin. For the chiral separation of stereoselective β1-blockers and H1-antihistamines, pseudostationary protein zone was used. In that developed method about 15 compounds were screened and nearly all of them illustrate longer migration time, showed a communication with transferrin. Stereoselective interaction was observed only for five β1-blockers (CEL, TALINO, MEPIN, BOPIN, and OXPRE) and for one H1-antihistamine (bromopheniramine). A polyacrylamide-coated (3%, noncross-linked) capillary (34 cm effective length 650 mm id) was carried out [52].

Hong-Bing Duan et al. (2015) described a new routine estimation of METO T and BF. In that developed method, capillary electrophoresis fixed with tris (2, 2′-bipyridyl)-ruthenium (II) electrochemiluminescence for the estimation as well as illustrates relationship between the METO T and BF and human serum albumin. There are different parameters were selected for optimization of CZE separation; because they affect the CZE separation and ECL detection, the optimized parameters like pH, amount of running buffer, detachment voltage and potential exposures. Under enhanced condition METO T and BF were well separated and identified within 10 min [53].

Jingwu Wang et al. (2008) illustrate fast, selective, and responsive capillary zone electrophoresis (CZE) attached through tris (2,2′-bipyridyl) ruthenium(II)-based end-column electrogenerated chemiluminescence (ECL) was utilized to estimate BF in bulk and tablets subsequent to its separation from METO. Tetrahydrofuran were used as an additive in the running buffer to receive the absolute ECL peak of BF. It react with tris (2, 2′-bipyridyl) ruthenium (II) ECL system. Under the advanced experimental situation, BF was separated successfully and efficiently from METO and other co-existed materials in tablets and urine samples [54].

Laszlo Gagyiet al. (2008) reported the stereoselective detection of β-blockers by cyclodextrins within capillary zone electrophoresis. This category of medicinal agent was resolved by cardiovascular system disease and its derivative chiral aryloxy-propanolamine. In general, the S(−) enantiomer are more active than the R(+) enantiomer. Study understand the appliance of a choice of cyclodextrin derivatives, hydroxypropyl-β-cyclodextrins, at randommethylated β-cyclodextrin, sulphated β-cyclodextrin and sulphated α-cyclodextrins for the stereoselective examination of β-blockers. Separation was obtained for BOPI, CARV, MEPI, PIND and ALPR, while only partial separation was observed for SOT, PROP, OXPRE, ATEN, BIS, BUPRA and METO [55].
Bioanalytical methods are used for the quantitation of drugs and their metabolites and biological molecule in unnatural location or concentration) and biotic (macromolecule, large molecule drugs and metabolites) in organic systems[56]. Literature survey revealed that LC-MS/MS and HPLC and are predominantly used for the bioanalysis of BF. In Bioanalytical method validation sample is extracted from plasma with help of extraction techniques such as protein precipitation, liquid-liquid extraction and solid phase extraction techniques. In most of methods methanol was used as solvent for extraction of BF in biological fluids. Bioanalytical methods for determination of BF are summarized in [Table: 5].

Table 5: Bioanalytical determination of BF.

| Sr. No. | Drugs    | Biological fluid | Chromatographic conditions | Discussion                                                                 | Ref  |
|---------|----------|------------------|----------------------------|---------------------------------------------------------------------------|------|
| 1       | BF       | Human plasma     | RP-C18 column (Inertsil, 4 mm, 150 x 4.6 mm), Methanol: water (70:30, % v/v) | Sevgi Tatar Ulu et al. Described derivatization of BF with 4-chloro-7-nitro-2, 1, 3-benzoxadiazole in borate buffer at pH 9.5 to yield a fluorescent product. Plasma samples (BF and IS) were extracted employing a liquid–liquid extraction method. Ephedrine was used as internal standard. Linearity obeyed in the range of 10 –2,000 ng/mL Retention times was approximately 4.79 min for BF and 3.46 min for IS. | 57   |
| 2       | AMD and BF | Rat plasma      | Diamonsil C18 column (50 mm x 4.6 mm, 5 μm), Methanol: water: formic acid (75:25:0.01, % v/v/v) | Huichao Chang et al. Illustrated a sensitive, specific liquid chromatography–tandem mass spectrometry method for quantitative determination of AMD and BF. The analytes and IS were isolated plasma samples by liquid–liquid extraction. Linearity was followed in the range of 0.2–50 ng/mL and correlation coefficient 0.9961 for both BF and AMD. Retention time 2.12, 1.96 and 1.89 min for both. | 58   |
| 3       | BF       | Human plasma     | RP-C18 Column (3 x 100 mm, 3.5 μm), 0.1% formic acid solution – acetonitrile (50-50 % v/v) | Gabriela Peste et al. Developed specific liquid chromatography–tandem mass spectrometry method determination of BF. The analytes extracted using liquid-liquid extraction method and metoprolol was IS. Linearity obeyed in the range of 1 ng/mL and 100 ng/mL. Correlation Coefficient of 600.99. Retention time, 1.7 min and 1.9 min. result given by BF and METO. | 59   |
| Sr. No. | Drugs | Biological fluid | Chromatographic conditions | Discussion | Ref |
|--------|-------|------------------|----------------------------|------------|-----|
| 4      | BF    | Human plasma     | Kromasil C18 column (150 × 4.6 mm, 5 μm), Methanol and 0.05% phosphoric acid (40:60% v/v) | Ming Zhang et al. developed a three-phase solvent bar microextraction technique combined with high performance liquid chromatography fluorescence detection was for quantitative determination of BF. METO was used as the IS. Linearity obeyed in the range of 10–100 μg/mL. Correlation Coefficient was 0.994. | 60   |
| 5      | CEL, BF and IRB | Human plasma | Kromasil C18 column (150 x 4.6 mm, 5 μm) phosphate buffer 0.1 M adjusted to pH 3.4 ± 0.1 with hydrochloric acid | E. Caudron et al. described method for the simultaneous determination of cardiovascular drugs. Solid-phase extraction technique was used for determination of BF. PROP was used as the IS. Linearity 10–500 ng/ml for CEL is 5–250 ng/ml for BF and 20–1000 ng/mL for IRB. Retention time: BF: 7.31 CEL: 5.19 IRB: 16.32 Linear Regression Coefficient (r): CEL 0.9996, BF 0.9990, IRB 0.9994 | 61   |
| 6      | BF    | Human plasma     | Chromolith RP-18e (250 x 4 mm). Kaliumdihydrogen phosphate solution 0.01M, pH 3.5 acetonitrile (77.5:22.5% v/v/v) | Corneliu Oniscu et al. illuminated liquid-liquid extraction with diethyl ether, at alkaline pH, followed by back-extraction with phosphoric acid, and liquid chromatography analysis with fluorescence detection for BF. METO used as IS in this study. Linearity obeys in the range of 3 ng/mL and 200 ng/mL. Retention time was 4.5min and 7.24 min. Correlation coefficient was found 0.99981. | 62   |
| 7      | BF and METO | Human plasma | RP- C18 Nucleosil column acetonitrile–HPLC water with 1.2% (w/v) of triethylamine and the pH adjusted to 3 with 85% orthophosphoric acid (18:82, 20:80, % v/v) | A. J. Braza et al. was developed two different liquid–liquid extractions method. Fluorometric detection used for the identification of BF and METOP. Linearity was in the range of 6.25–200 ng/mL for both BF and METO. Retention times for BF and METO were 8.7 and 3.2 min. R 2 = 0.9857 and R 2 = 0.9959. | 63   |
| 8      | CEL and BF | Human skin | C18 Nucleosil column (5-mm 12.5 cm, 4 mm), acetonitrile and 67 mM Sorensen’s phosphate buffer (pH 5.0) (30/70 % v/v) | P. Modaio et al. Illustrated a predetermined procedure i.e. RP-HPLC with UV detection which is used for identification of CEL and BF. Phosphate buffer used as a standard solution. Linearity was followed in the range of 25–0.78 μg/mL. | 64   |
| Sr. No. | Drugs       | Biological fluid | Chromatographic conditions                                                                 | Discussion                                                                                           | Ref  |
|--------|-------------|------------------|-----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|------|
| 9      | BF          | Human plasma     | Zorbax SB-C18 (100 mm x 3.0 mm) mixture of methanol and (0.1% (v/v) acetic acid (40:60 % v/v) in water at 48 ºC | *Exp Clin Cardiol et al.* was developed multiple reaction monitoring (MRM) mode using an ion trap mass spectrometer equipped with an electro spray ion source for monitoring the BF Methanol was used as an internal standard. LOQ was found for BF 1.78 – 85.44ng/mL. Correlation coefficient greater than 0.993. Retention Time of BF in six different lots of blank plasma (1.9, 2.3, 4.68, 2.3, 3.8, 1.7 min). | 65   |
| 10     | ATEN, BF, HCT, CHLOR, SA, EP and its active metabolite, VAL and FLU. | Human plasma | Luna C18 (150 mm x 4.6 mm, 3 μm), Acetonitrile and water containing 0.01% formic acid and 10 mM ammonium formate at pH 4.1 | Oskar Gonzalez *et al.* reported simultaneous analysis of several drugs usually combined in cardiovascular therapy. Plasma samples were extracted employing a simple protein precipitation extraction with acetonitrile and pravastatin was used as IS. | 66   |
| 11     | BF          | Plasma serum     | stainless steel tube (125 x 5 mm ID) 1 mM camphorsulphonic acid in methanol | *R. J. Eastwood et al.* reported measurement of bisoprolol in plasma by using high performance liquid chromatography. Tris solution, benzimidazole which is aqueous internal standard and methyl t-butyl ether mixed with the sample vortex for 30 seconds. After the centrifugation at particular portion resulting extract is analyzed on a micro particulate silica column using 1 mM camphorsulphonic acid in methanol as the mobile phase. At 215 nm detection of limit and detection of quantitation can be calculated. Minimal interference from either commonly prescribed drugs or endogenous compounds can be determined. | 67   |

### 10. IMPURITY PROFILING ON BF [68-72]

The impurity profiling is designed with objectives to establish specific link between two or more samples, ascending drug distribution pattern, for identification of sources of drug samples and also for monitoring the process for drug manufacturing [68]. According to the ICH guidelines impurities are matter in the product which is not active pharmaceutical ingredients or the excipient used to manufacture it [69]. An impurity profile has been established for quantification of BF alone and in combined dosage form. There are a various types of impurities present in BF. Following explanation can be established the impurity present in BF and their combined dosage form.
Table 6: Impurities in Bisoprolol Fumarate.

| Sr. No. | Conditions | Number of impurities | Structure of impurities |
|---------|------------|----------------------|-------------------------|
| 1       | Evaluation on readymade impurities obtained from vendor | 2 | ![Image of molecule structure] |

Table 6: Contd...
Sr. No. | Conditions | Number of impurity | Structure of impurities | Ref |
--- | --- | --- | --- | --- |
2 | **1. Forced Degradation Study:** | 1 | ![Structure of impurities](image.png) | 71 |

1.1 Photo stability study: - light providing an illumination of 1.2 million lux-hours (7.1 h) and integrated near-ultraviolet energy of 200 Wh/m² (2.9 h) and then exposed to five times increased irradiance dose.

1.2 Oxidative Degradation Study: - Tablets were treated with hydrogen peroxide solution (2% in water, 5 mL).

2. **Thermal Degradation Study:** - Mixture directly exposed to 40°C/75%, for 2, 4, and 8 weeks.

2.1. Thermal Degradation Study A: - Mixture placed on an open quartz Petri dish and kept at 80°C for 5 hours, for 5 days.

2.2. Thermal Degradation Study B: - Mixture was placed in an open quartz Petri dish and kept at 80°C for 72 hours. A temperature of 80°C for 121 hours in an open Petri dish. The result for unidentified impurities was 1.07%.

**Table 6: Contd...**
### Table 6: Contd...

| Sr. No. | Conditions | Number of impurity | Structure of impurities |
|---------|------------|--------------------|-------------------------|
| 3       | Force degradation study: degradation parameter like acid, base, and peroxide, thermal, photolytic can be used with different conditions like 0.1NHCl at 60°C for 1 hour, 3% of H₂O₂, 60 °C for 72 hour and 0.01N NaOH at 60°C for 30 minutes. | 9       | 1.A |
|         |            |                    | ![Structure 1.A](image1) |
|         |            |                    | 2.B | ![Structure 2.B](image2) |
|         |            |                    | 3.E | ![Structure 3.E](image3) |
|         |            |                    | 4.K | ![Structure 4.K](image4) |
| Sr. No. | Conditions | Number of impurity | Structure of impurities | Ref |
|--------|------------|--------------------|-------------------------|-----|
| 5.N    |            |                    |                         | 72  |
| 6.G    |            |                    |                         |     |
| 7.L    |            |                    |                         |     |
| 8.Q    |            |                    |                         |     |

Table 6: **Contd...**
| Sr. No. | Conditions | Number of impurity | Structure of impurities | Ref |
|--------|------------|--------------------|-------------------------|-----|
|        |            |                    | 9.R                     |     |
|        |            |                    | 10.S                    |     |
Tijana Rakic et al. (2014) established hydrophilic inter face liquid chromatographic method for study of BF and its impurities A and C. The chemometric strategy was resolved to systems activities and establishing the mathematical association between acetonitrile content which was present in mobile phase, pH of the water phase and buffer concentration in the water phase and chromatographic responses. Investigation all studies of BF from beginning to end Chromatographic technique and its impurities was established on HILIC 100Å (100 mm x 4.5 mm, 2.6 µm particle size); using mobile phase mixture consist was acetonitrile – water phase (35 mM ammonium acetate, pH 4.9 manage with glacial acetic acid) (85:15 v/v) with flow rate 1 mL/min and analysis was performed at ambient temperature. Impurities of BF (impurity A and impurity C) are shown in Table 6 [70].

Ivana Mitrevska et al. (2017) established identification, structural interpretation and qualification of a degradation impurity RRT 0.95 of BF in film-coated tablets. The impurity of relative retention time gives at 0.95 was observed in the stress thermal degradation study of the BF film-coated tablets with identification, characterization and quantitation was performed using HPLC/DAD/ESI-MS method. The configuration of the embattled Impurity RRT 0.95 was shown in Table 6 with molecular mass of BF were 406 [71].

Venkata Narasimha Rao Ganipisetty et al. (2016) studied twelve impurities of BF and HCT and separated simultaneously using HPLC technique. Out of 12 reported impurities, five were found to be potential degradants. During the validation of stability indicating method, the focus was on the critical parameters in resolving the degradants from the main components. These parameters include Hand, temperature solvents because BF and HCT have different solubilities and polarities. The method was precise (RSD<1.0%), accurate, linear (r2>0.999), robust, and stability indicating in the range of LOQ to 150% [72].

11. ELECTROCHEMICAL METHODS:

11.1 Voltammetric methods for BF: [73-75]

Rajendra N. Goyal et al.(2011) recognized an voltammetric performance of BF by using graphite electrodes were completed with single wall carbon nanotubes. In comparison to BPPGE, EPPGE gives supplementary sharp peaks in oxidation of BF. In the variety 10 – 1000 mV/s in phosphate buffer solution of pH 7.2, the examination rate of repeated voltammogram was assorted. The limits of detection were found to be $2.8 \times 10^{-7}$ M and $7.3 \times 10^{-7}$ M [73].Bozal et al (2012) was reported Simultaneous estimation of BF and HCT in their pharmaceutical formulation by applying different voltammetric,
chromatographic, and spectrophotometric analytical methods. The level of difference pulse and square wave voltammetry techniques were used for the analysis of BF and HCT concurrently by measuring at with reference to 1400 and 1100 mv. By using different electrolytes including \( \text{H}_2\text{SO}_4 \), phosphate, acetate, and BR buffers with different pH values between 0.3 and 12.0 containing a constant amount of 20% methanol the voltammetric oxidation of BF and HCT were reported. BF was oxidized between pH 0.3 and 10.

Rajendra N. Goyal et al. (2007) studied a BF in pharmaceutical dosage form and urine using single-wall carbon nano tubes customized glassy carbon electrode. The SWNTs-modified GCE exhibited a sharp anodic peak at a potential of 950mV for the oxidation of BF. In good condition linearity was found in the range of 0.01–0.1mM in 0.5M phosphate buffer solution having pH 7.2 with a correlation coefficient of 0.9789 and limit of detection was reported at \( 8.27 \times 10^{-7} \) M.

11.2 Potentiometric method for BF: [76-77]

Grzegorzbazylak et al. (2002) reported execution of analytical and biopharmaceutical screening data for beta-adrenergic-drug simple menting many macro cycle in HPLC Systems. In the cation-exchange HPLC technique for the studies applying acetonitrile – 40 mM phosphoric acid (15: 85, % v/v,) as a mobile phase. By employing crossbreed polymer silica packets in RP-HPLC it can be considered that promising surrogate in high throughput drug control process for examination of beta adrenergic agonist in humans and animals recommend the Potentiometric recognition [76].

Saad S.M. Hassan et al. (2003) reported the used of polymeric medium membrane sensors for purpose of \( \beta \)-blockers. This sensor was depending on the cations with tungs to phosphate anion as electro active materials. In some dosage form sensors are implemented for direct potentiometry of \( \beta \)-blockers. for the construction of the sensor plastic membrane can be made by preparing composition 2:34:64% (w/ w) ion pair complex, PVC and DOP plasticizer. The sensor was uncomplicated for the purpose of b-blockers at a concentration level as low as \( 10^{-7} \) mol l\(^{-1} \) with an accuracy of 99.1 ±/1.3 %. [77].

12. CONCLUSION

The present review gives various analytical methods for the estimation of BF. A different analysis had perform which include, Bio-analytical, HPLC, HPTLC, UV/Vis-Spectroscopy, Spectroflurometry, capillary electrophoresis, stability indicating method, impurity profile and electrochemical method like voltammetric and Potentiometric method for validation of BF in bulk and in
its combined pharmaceutical formulations and in plasma. Through HPLC with UV detection has been found to be most studied for estimation of BF in bulk as well as pharmaceutical dosage forms, while hyphenated LS-MS, Bioanalytical, UPLC methods are reported for quantification of BF and its metabolite in plasma and other biological fluids. HPTLC and Stability-indicating by HPLC and HPTLC are also reported in literature survey. Certain Spectrophometric methods in UV-Visible along with spectrofluorometric are most often used for assessment for BF. Various types of stability indicating method and impurity profiling method have been estimated.

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CONFLICT OF INTEREST

Authors do not have conflict of interest for this manuscript.

ABBREVIATIONS

| Abbreviation | Full Form                          |
|--------------|-----------------------------------|
| UV           | Ultra Violet                      |
| VIS          | Visible                           |
| HPLC         | High-Performance Liquid Chromatography |
| HPTLC        | High-Performance Thin-Layer Chromatography |
| UPLC         | Ultra Pressure Liquid Chromatography |
| LC-MS        | Liquid Chromatography-Mass Spectrometry |
| IS           | Internal standard                 |
| Rf           | Retention factor                  |
| Rt           | Retention time                    |
| BF           | Bisoprolol Fumarate               |
| ML           | Mili Liter                        |
| MP           | Melting point                     |
| μg           | Microgram                         |
| IRB          | Irbesartan                        |
| BIS HEMI F   | Bisoprolol Hemifumarate           |
| AMD          | Amlodipine                        |
| AMD B        | Amlodipine Besylate               |
| CELI         | Celiprolol                        |
| Acronym | Full Form                                |
|---------|------------------------------------------|
| METO T  | Metoprolol Tartrate                      |
| USP     | United State Pharmacopeia                |
| Nm      | Nano Meter                               |
| TLC     | Thin Layer Chromatography                 |
| TALINO  | Talinolol                                |
| MEPIN   | Mepindolol                               |
| BOPIN   | Bopindolol                               |
| OXPRE   | Oxprenolol                               |
| Mm      | Mili Meter                               |
| CZE     | Capillary Zone Electrophoresis           |
| ECL     | Electrogenerated Chemiluminescence       |
| BOPIN   | Bopindolol                               |
| CARV    | Carvidilol                               |
| MEPI    | Mepindolol                               |
| PIND    | Pindolol                                 |
| ALPA    | Alprenol                                 |
| SOT     | Sotalol                                  |
| PROP    | Propranolol                              |
| OXPRE   | Oxprenolol                               |
| ATEN    | Atenolol                                 |
| BUPRA   | Bupranolol                               |
| DNA     | DNA                                      |
| RP-HPLC | Reversed-Phase High-Performance Liquid-Chromatography |
| SWNT/GCE| Single-Walled Carbon Nanotube/Modified Glassy Carbon Electrode |
| BUF     | Bufralol                                 |
| CAR     | Carazolol                                |
| CELEN   | Celenbuterol                             |
| MABU    | Mabuterol                                |
| CIM     | Cimaterol                                |
| ALP     | Alpenol                                  |
| TETR    | Tetertolol                               |
| BEV     | Bevanotolol                              |
| TCPB    | Tetrakis (P-Chlorophenyl)Borate          |
| CEX-HPLC| Cation-Exchange High Performance Liquid Chromatography |
| PVC     | Polyvinyl Chloride                      |
| BOR     | borate electrode                         |
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