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Advances in quality control through the development of a more sustainable and eco-friendly direct spectrophotometric method than pharmacopoeial methods for the determination of bisoprolol fumarate

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A sustainable eco-friendly direct spectrophotometric method was developed and validated for the estimation of bisoprolol fumarate (BF) in the marketed dosage form. BF in an aqueous medium of 0.10 N NaOH exhibits a maximum absorption at 273 nm that permits its direct estimation by zero-order spectrophotometric method without any interference in a linear range of 1.66–190.0 µg mL^{-1} (r = 0.9999, n = 5) with detection and quantitation limits were 0.211 and 0.638 µg mL^{-1}, respectively. The suggested method was successfully adopted to evaluate BF in bulk and tablets. The technique showed adequate precision, with a relative standard deviation value lower than 1.78%. Excellent values of accuracy were obtained, with a recovery mean value of 100.37%. The results indicated that the proposed method is highly efficient in determining the amount of BF in the tablets, which helps improve quality control and environmental improvement. Furthermore, the greenness and whiteness profile of the method was evaluated using different evaluation metrics; AES, AGREE, AGREEprep, GAPI, BAGI, and RGB12. The proposed spectrophotometric method was more sustainable, eco-friendly, efficient, productive, and practical than the reported HPLC-UV methods (BP and USP), as confirmed by the absence of consumption of chemical reagents and polluting organic solvents, reduced analysis stages, energy consumption, analysis time and low cost, making it a safer alternative to be considered.

Keywords: Spectrophotometry; bisoprolol fumarate; eco-friendly; tablets.

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Introduction

Bisoprolol fumarate (BF) is chemically known as (2RS)-1-[4-[[2-(1-methylethoxy)ethoxy]methyl] phenoxy]-[(1-methylethyl)amino]propan-2-ol fumarate, as shown in Figure 1. Bisoprolol fumarate belongs to the drug class of selective 1 adrenergic receptor blockers. It is primarily used to treat cardiovascular disease, has a half-life ($t_{1/2}$) of 9-12 hours, and has a bioavailability of more than 80% [1]. British Pharmacopoeia [2] describes from long ago to the present a non-aqueous titration using perchloric acid and HPLC-UV methods for the determination of bisoprolol fumarate in bulk and tablets, respectively. While United States Pharmacopoeia [3] describes HPLC for the assay of bisoprolol fumarate in bulk and tablets. Bisoprolol fumarate has been determined in pharmaceutical preparations using various analytical techniques including the acid-base titrimetric method [4], potentiometry [5-8], voltammetry [9,10], and high-performance liquid chromatography (HPLC) using UV detection [11-17]. Spectrophotometric methods for the determination of bisoprolol fumarate in formulations have been reported using various reagents to form a colored product between bisoprolol fumarate and picric acid in chloroform [18], 1,2-naphthoquine-4-sulphonate (NQS) in alkaline medium [19], synthesized gold nanoparticles (Au NPs) [20], urea and sodium citrate solution in methanol [21] and ceric ammonium sulphate in sulphuric acid medium [22]. UV-spectrophotometry in water [23], 0.1 N HCl [24], or methanol [25,26] was also reported to the determination of bisoprolol fumarate in dosage forms. Extractive spectrophotometry based on the formation of an ion pair complex between bisoprolol fumarate and methyl orange or eriochrome black T [27], tropaeolin 00 [28] and bromothymol blue [29] in an acidic medium, extractable in dichloroethane or dichloromethane have been published for the evaluation bisoprolol fumarate in formulations. These above spectrophotometric methods were burdened with several drawbacks, such as the requirement of long waiting times, complicated procedures, the need for non-aqueous media [18–22,25–29] or poor accuracy and sensitivity of the method [23–26,28]. Therefore, it was necessary to develop an economical and accurate spectrophotometric method for the direct determination of bisoprolol fumarate.

The importance of green chemistry is evident in reducing the environmental impact of chemical procedures in general. The interest of green analytical chemistry (GAC) in increasing analytical performance and reducing the environmental impact of analytical procedures has been demonstrated using several tools to evaluate the greenness of improved analytical methods [30-32]. The AGREEprep scale is a tool for assessing the greenness and environmental impact of analytical sample preparation processes since ensuring sample preparation ensures the reliability and validity of the analytical method. This tool includes ten criteria covering different aspects that

contribute to the overall greenness of sample preparation, selection and use of solvents, materials and reagents, waste generation, energy consumption, sample volume, and productivity. The efficiency of each section is rated from 0 to 1, where 1 is the ideal level of performance [33].

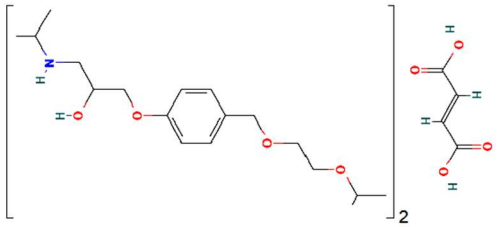


Figure 1. The structure of bisoprolol fumarate $(C_{18}H_{31}NO_4)_2C_4H_4O_4$ [104344-23-2].

When using the Analysis Eco-Scale (AES), penalty points (PPs) are calculated that relate to the amount of chemicals, energy consumed, hazardous quantities, and waste generated during the analytical procedures. Finally, the PPs subtracted from the total green score of 100. A total score greater than 75 indicates excellent greenness; a total score of 50 to 75 indicates acceptable greenness while a total score of less than 50 indicates insufficient greenness of the analytical method [34,35]. Using the AGREE tool, a twelve-part color scale scheme is generated, representing all twelve principles of green analytical chemistry. In the final result, green analytical procedures are given a score of 1, with twelve parts being completely green, and deviations from the ideal green color standards result in lower scores and lower color scales (light green, orange, yellow, red) [36]. The Green Analytical Procedure Index (GAPI), a fifteen-part diagram representing different aspects of the analytical process, also assesses greenness. Although the GAPI tool is widely used, it does not allow for the evaluation of processes that occur before the analytical methodology; synthesis, other reactions, preparation of stationary phases, etc. [37,38]. The Blue Application Grade Index (BAGI) is a proposed measure as a means of assessing the practicality of an analytical technique (e.g., conventional, modern, newly developed, etc.) and is complementary to the Green Scales, focusing on the applied features of white analytical chemistry. The BAGI assesses ten key parameters. The color scheme used to represent levels of compliance ranges from dark blue for excellent compliance to white for non-compliance through blue and light blue. The minimum threshold for a procedure to be considered "practical" is 60 points [39].
White analytical chemistry (WAC) refers to the red-green-blue color model of light, where red indicates the quality of the analytical process, such as accuracy and sensitivity, and blue indicates the practicality and economics of the analytical method. The overall quality of the analytical process is expressed through whiteness. A method is not necessarily greener if it is whiter and more comprehensive. RGB12 algorithm analysis is a semi-quantitative approach that classifies analytical methods into three color-coded groups based on their environmental impact [40-42]. The present work deals with the development of an eco-friendly direct spectrophotometric method, which has been proven to be a novel, stable, inexpensive, accurate, reliable, time-saving, and alternative to pharmacopoeial methods for the determination of bisoprolol fumarate in pure state and dosage forms.

Experimental section

Chemicals and samples

Analytical reagent grade sodium hydroxide, anhydrous acetic acid, glacial acetic acid, perchloric acid, formic acid, heptaflourobutyric acid, diethylamine, sodium acetate trihydrate, ethanol and HPLC grade methanol, acetonitrile, and water were purchased from Merck (Germany). Double distilled water was also used. Working reference standard of bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4]$, and bulk BF powder were supplied by Rarecrown Pharmachem CO., LTD. (China), their purities were 99.92 and 99.87 %, respectively, as calculated by official method [2], Concor® tablets (Merck-Germany) labeled to contain 5 mg/tablet bisoprolol fumarate.

Standard solutions

1 mg mL^{-1} standard stock solution of BF was prepared by dissolving the appropriate weight of the working reference standard BF in double distilled water. Working standard solutions were prepared by diluting the standard stock solution with 0.10 N NaOH (≈ pH 13). The stock solution was stable for one month when stored in the dark at 2–8 °C.

Sample solutions

Stock solution 1 mg/mL of bulk BF powder was prepared by dissolving the appropriate weight of the powder in double distilled water, sonicated, and then filtered through a 0.45 µm membrane filter (Millipore Corp). Different aliquots from the standard stock solution and bulk solution of BF were transferred to a set of 10 mL volumetric flasks. The solutions were diluted with 0.10 N NaOH and mixed well. Absorbance values corresponding to BF were measured concurrently and the corresponding concentrations were obtained from a calibration curve or regression equation. The same sample was tested by pharmacopoeial methods [2,3].

Commercial tablet solutions

The average weight of a tablet containing BF was calculated by weighing twenty tablets of the preparation. The tablets were finely ground and a portion of the powder equivalent to 25 mg of BF was accurately weighed and dissolved in 25 mL of methanol, ultrasonicated for 5 min, and filtered through a 0.45 µm membrane filter (Millipore Corp). Methanol was evaporated to near dryness. The remaining portion was diluted in a 25 mL volumetric flask with bidistilled water. The resulting solution (~1 mg mL^{-1}) was used for analysis by the recommended procedure in the

indicated concentration range. The concentration of BF in the samples was calculated from the regression equation of the corresponding calibration graph.

Calibration graph

To prepare diluted standard solutions of BF, aliquots of standard stock solutions (0.0166-1.90 mL, 1 mg mL^{-1}) were transferred into a series of 10 mL volumetric flasks and diluted with 0.10 N NaOH. The zero-order absorbance spectra were scanned at 240–400 nm against the blank solution. The calibration curve was constructed by plotting the linear relationship between the absorbance (an average of five determinations) and the concentration.

Instruments and analytical conditions

UV-1650PC spectrophotometer (Shimadzu, Japan) was used for all absorbance measurements under the following operating conditions; scan speed 400 nm min^{-1} and slit width 2 nm using 1.0 cm quartz cell. pH measurements and automatic potentiometric titration were made with Metrohm Compact Titrator Model 794 Basic Titrino (Switzerland) with a glass pH electrode. A Thornton sonicator (model T-14) was also used. All measurements were made at room temperature 25.0±1 ºC. An integrated Hitachi (Japan) HPLC system Model L-2000 was used, equipped with a binary pump, degasser, and column oven. All samples were injected using a Hitachi L-2200 autosampler. Each chromatogram was analyzed and integrated automatically using the Ezchrom Elite Hitachi Software.
Separation with the BP method [2] was achieved on a reversed-phase octylsilyl silica column C8 (25 cm×4.6 mm, 5 µm particle size,). The mobile phase consisted of glacial acetic acid and sodium acetate trihydrate in methanol, pumped at a flow rate of 1.0 mL min^{-1}. The column temperature was 45 ºC and the injection volume was 10 µL. The elution of the analyte was monitored at 225 nm. Separation with the USP method [3] was achieved on a reversed-phase Nucleodur column C8 (12.5 cm×4.6 mm, 5 µm particle size,). The mobile phase consisted of water containing heptaflourobutyric acid, diethylamine and formic acid-acetonitrile (65/35, *v/v*) and was pumped at a flow rate of 1.0 mL/min. The injection volume was 10 µL. The elution of the analyte was monitored at 273 nm.

Method validation

The validation of the derivative spectrophotometric method was conducted following the guidelines provided by the International Conference on Harmonization (ICH), specifically the Q2 (R2) guidelines [43].

Linearity range

The linearity range was tested by plotting the peak amplitude as a function of concentration; at least eleven concentrations of BF were tested in five replicates.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) of the BF assay were determined experimentally after getting the regression equation. LOD and LOQ were calculated by the following equations: $LOD_{(k=3.3)} = k \times S_a/b$ and $LOQ_{(k=10)} = k \times S_a/b$ (where S_a is the standard deviation of the intercept and b is the slope of the calibration curve) [44].

Accuracy

Dilutions of working solutions of BF corresponding to (1.66, 10, 40, 100, 140 and 190 µg mL^{-1}) were analyzed in five replicates. Mean percentage recoveries were calculated from the corresponding linear regression equation by applying the following relation.

$$\text{Recovery \%} = C_{found} / C_{taken} \times 100\%$$

where C_{found} is the concentration calculated from the calibration curve and C_{taken} is the theoretical concentration based on the weight.

Precision

Precision was tested by analyzing dilutions of working solutions corresponding to six different concentrations (within linearity range) in five replicates, within the same day (intra-day precision) or in three consecutive days (inter-day precision), subsequently relative standard deviation (RSD %) values were calculated.

Selectivity

The selectivity was ascertained by analyzing standard BF in the presence of common excipients such as lactose, magnesium stearate, cellulose, and silicon dioxide. For this purpose, a powder blend using typical tablet excipients was prepared along with the drug and then analyzed. The concentration was calculated using the corresponding linear regression equation.

Results and Discussion

The primary aim of this work was to evaluate separation-free and reagent-less spectrophotometry as an attractive green alternative to pharmacopoeial methods (non-aqueous titration and HPLC-UV) for the quality control testing of BF. This was achieved through two main steps, first, we developed a platform suitable for the quality control testing of BF, second, we compared the platform in terms of selectivity, sensitivity, and most importantly, their impact on the environment.

Optimization of UV- spectrophotometric method

Different dilution solvents of varying impact on the environment were evaluated based on absorbance intensity. Bi-distilled water, methanol, ethanol, and 0.10 N NaOH were all tested. The absorption spectra were

recorded for standard solutions containing studied active pharmaceutical ingredients in the mentioned media. An appropriate blank was used as a blank test and spectrophotometric measurements were done. The results are presented in Figure 2.

Absorption maxima for all four spectra are at 273 nm. The analysis of the spectrum allows for the observation of a similar spectrum of BF in the mentioned media with different intensities. To avoid the use of environmentally unfriendly organic solvents and to achieve high sensitivity, a solution of 0.10 N NaOH was the solvent of choice, which resulted in high absorbance, in addition, no shift in absorbance wavelength was observed.

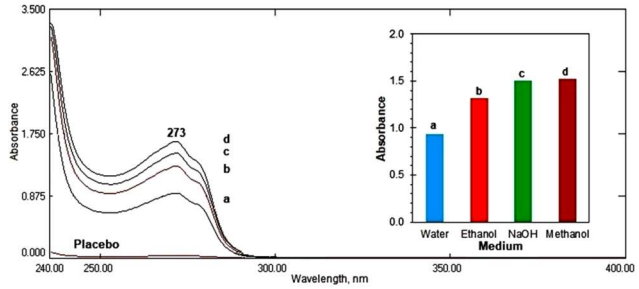


Figure 2. Zero-order absorption spectra and effect of diluting solvents on absorbance for BF (150 µg mL^{-1}) in (a) water, (b) ethanol, (c) 0.10 N NaOH, (d) methanol.

Since the main aim was to develop a green platform for quality control quantification of BF, the obtained results using NaOH as a diluting solvent were in full agreement with the main objective. UV spectrum of BF in the range of 1.66 to 190.0 µg mL^{-1} in 0.10 N NaOH gives one broad-shouldered peak at 273 nm (Figure 3).

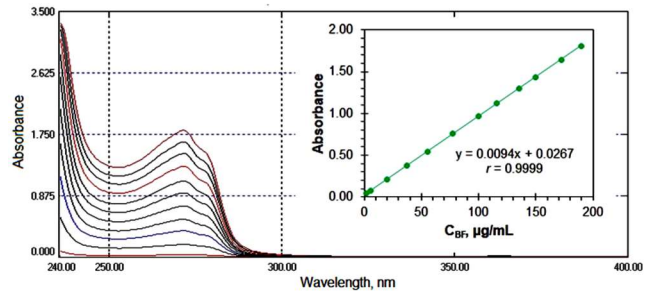


Figure 3. Absorption spectra and calibration curve of BF standards in the range of 1.66-190 µg mL^{-1} in 0.1 N NaOH.

Validation of the spectrophotometric method

Linearity range and sensitivity

The linear regression equation was derived at 273 nm from the correlation between absorbance and the concentration of the standard solution (Figure 3). Linearity was confirmed in the range of 1.66–190 µg mL^{-1} with a correlation coefficient value indicates excellent linearity (r=0.9999). Table 1 shows the calibration data, statistical parameters, and low values of LOD and LOQ, which confirm the good sensitivity of the proposed method.

Table 1. Regression and validation data for the determination of BF with the developed method.

Parameters	BF
λ_{max} (nm)	273
Linear range (µg mL^{-1})	1.66–190.0
Intercept (*a*)	0.0267
Standard deviation of the intercept (S_a)	6.00×10^{-4}
Slope (*b*)	0.0094
Standard deviation of the slope (S_b)	1.70×10^{-4}
Standard deviation of residuals ($S_{x/y}$)	5.78×10^{-4}
RSD% of the slope	1.81
Correlation coefficient (*r*)	0.9999
Ringbom concentration range (µg mL^{-1})	6.40-150
Sandell's sensitivity (µg cm^{-2} per 0.001 Abs. unit)	0.115
Molar absorptivity (L mol^{-1} cm^{-1})	0.90×10^{4}
LOD (µg mL^{-1})	0.211
LOQ (µg mL^{-1})	0.638
Number of experiments (n)	5

Accuracy and precision

The proposed method was evaluated regarding accuracy through its application in the estimation of standard solutions of BF at concentrations of 1.66, 10, 40, 100, 140, and 190 µg mL^{-1}. Mean percentage recoveries were calculated in view to justify the accuracy of the proposed and listed in Table 2, they ranged from 99.40 to 101.20 % for the developed method, this close agreement of our results to the true values is indicative of the high accuracy of the developed method. Intra and inter-day precision of the proposed method were determined by performing replicate (*n*=5) analyses of standards. Intra–day assay variation was evaluated by measuring these standard solutions on the same day. Inter-day assay variation was evaluated by measuring these solutions on 7 different days from day 1 to 30 after preparation. The RSD % of the assay values was calculated in view to justify the precision of the proposed method. The values of RSD % were less than 2 % for spectrophotometry, this confirms the high precision of the developed method (Table 2).

Selectivity

The selectivity of the method was ascertained by analyzing standard BF in the presence of common excipients such as lactose, cellulose, and silicon dioxide. For this purpose, a powder blend using typical tablet excipients was prepared along with the drug and then analyzed. Mean % recoveries were 100.88 % indicating excellent selectivity and ensuring the feasibility of its determination in tablets.

Table 2. Accuracy and precision of within and between run analysis for determination of BF.

Concentration	Inter-day (*n*=5)			Intra-day (*n*=5)		
(µg mL^{-1})	Mean±SD (µg mL^{-1})	% RSD	% Recovery	Mean±SD (µg mL^{-1})	% RSD	% Recovery
1.66	1.68±0.03	1.78	101.20	1.65±0.02	1.21	99.40
10.00	9.98±0.11	1.10	99.80	9.97±0.10	1.00	99.70
40.00	40.15±0.39	0.97	100.37	40.21±0.34	0.84	100.52
100.00	100.23±0.75	0.75	100.23	100.19±0.66	0.65	100.19
140.00	140.52±0.77	0.55	100.37	140.39±0.82	0.58	100.28
190.00	191.04±0.81	0.42	100.55	190.87±0.72	0.38	100.46

Robustness

The robustness of an analytical approach is described as its ability to withstand slight purposeful changes to method parameters without affecting findings. Three factors from the methodology were chosen to be tested: pH, NaOH concentration, and temperature. Under the varied conditions sample, solutions of BF were analyzed at a concentration of 100.0 µg mL^{-1}. Results prove that the proposed method is robust and acceptable with excellent recoveries. Table 3 presented all of the conclusions.

Table 3. Results of robustness study of BF.

Parameter variation	Recovery %±SD[a]
No Change	100.57±0.21
Change in pH	
13.10	99.83±0.20
12.90	99.62±0.21
Change in NaOH concentration	
0.11 N	99.94±0.22
0.09 N	99.63±0.20
Change in temperature	
27 ºC	100.25±0.26
23 ºC	100.08±0.24

[a]Average of five determinations.

Application to bulk powder

The proposed spectrophotometric, non-aqueous titration [2], and HPLC [3] methods were applied to determine BF in bulk powder samples. Four levels of BF concentrations (2, 40, 100, and 190 µg mL^{-1}) were prepared and analyzed by the proposed spectrophotometric and reported HPLC methods. Figure 4 shows an example of HPLC chromatogram of BF.

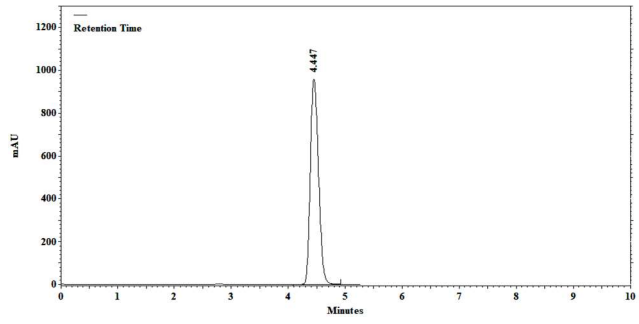


Figure 4. HPLC chromatogram of BF (100 µg mL^{-1}) under optimal conditions.

While, in the non-aqueous titration method, we analyzed several samples of the bulk powder by dissolving the taken weight in 50 mL of anhydrous acetic acid, and then applied the automation potentiometric titration of the resulting solution with a standard reagent solution of 0.10 N perchloric acid. Figure 5 shows an example of a non-aqueous potentiometric titration curve of 415 mg BF, the end-point was at 10.82 mL of reagent. The results obtained by the proposed and reported methods are given in Table 4.

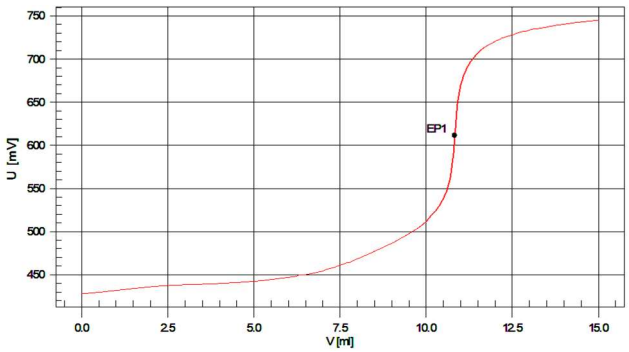


Figure 5. Non-aqueous potentiometric titration curve of BF (415 mg) with 0.10 N perchloric acid.

Application to tablets (Concor®)

The proposed and HPLC [2,3] pharmacopoeial methods have been successfully applied for the determination of BF in tablet dosage form under optimum conditions. Table 5 shows the results of applications of the proposed and pharmacopoeial methods for the quantitative analysis of BF in Tablets. These data were statistically assessed utilizing Student's t-test and variance ratio F-test [45]. In all cases, the average results obtained by the proposed and official methods were statistically identical, as the difference between the average values had no significance at 95% confidence level. Compared to pharmacopoeial [2,3], electrochemical [5–10], and separation [11–17] methods reported for the analysis of BF, the UV spectrophotometric method described in this manuscript has the advantages of being simpler and can analyze BF in its bulk powder and tablets without complex calculations or multi-step procedures. In addition, does not require sample extraction and preparation, buffers, or sophisticated techniques.

Table 4. Application of the proposed and pharmacopoeial methods for determination of BF in bulk.

Conc. taken ($\mu g\ mL^{-1}$)	Proposed method	USP HPLC method [3]	BP titration method [2]	
	Found[a] %	Found[a] %	Taken (mg)	Found[a] %
2.00	99.99	100.07	300	99.94
40.00	100.07	100.14	350	100.22
100.00	100.11	99.94	370	100.34
190.00	101.32	102.03	415	102.22
Mean±SD	100.37±0.94	100.54±0.81	100.68±1.04	
RSD %	0.93	0.80	1.03	
Variance	0.89	0.66	1.08	
t-test	0.88*			
F-value	1.22*			

[a] The average of 5 separate results. * The tabulated **t** and **F** values were **2.776, 6.26,** respectively at $P = 0.05$.

Table 5. Statistical analysis of the proposed and official methods for BF in tablets (Concor®, 5 mg)

Parameters	BF		
	UV proposed method	BP HPLC [2]	USP HPLC [3][a]
% Recover*±SD	100.17±0.42	100.12±1.04	100.09±0.91
RSD %	0.41	1.03	0.90
Variance	0.18	1.08	0.83
t-test[a]		0.91	
F-value[a]		1.47	

*Five independent analyses. [a]At 95% confidence level t-value is 2.776 and F-value is 6.26.

Greenness and whiteness evaluation for the developed method

Both the proposed spectrophotometric method and HPLC pharmacopoeial methods were efficiently applicable for the assay of BF in its tablets. As mentioned in the introduction, GAC is primarily concerned with reducing energy and solvent consumption, which in turn reduces the amount of waste generated. In addition, replacing hazardous solvents with less hazardous ones is one of the most important goals of GAC. To emphasize the importance of green analysis of pharmaceutical drugs, we investigated the proposed method using ASE, AGREEprep, AGREE, GAPI, and BAGI tools.

To ensure that the proposed method is eco-friendly, it has become assessing the greenness of the proposed analytical procedure is essential to understand its environmental impact and its potential health risks. The detailed criteria for the AES scale are shown in Table 6 by calculating penalty points revealing the harmful effects of the method on the environment.

Table 6. Penalty points (PPs) of the proposed spectrophotometric method according to the Analytical eco-scale in comparison with pharmacopoeial methods to determine BF.

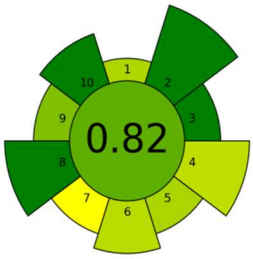
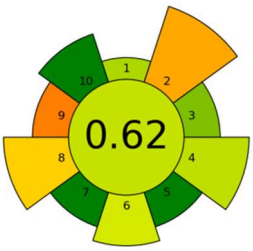
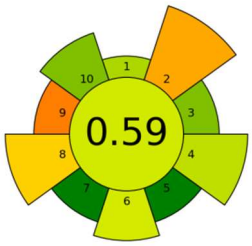
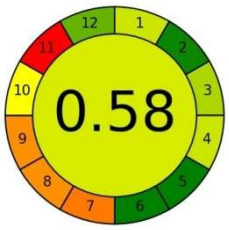
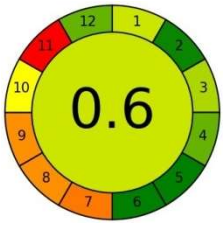
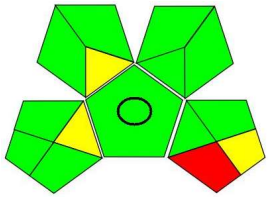
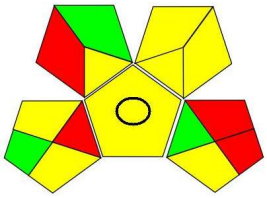
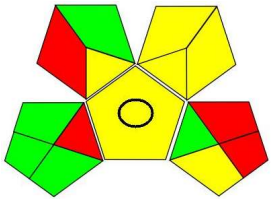
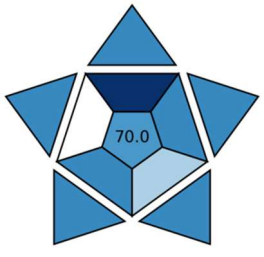
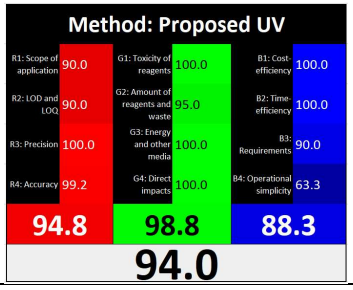
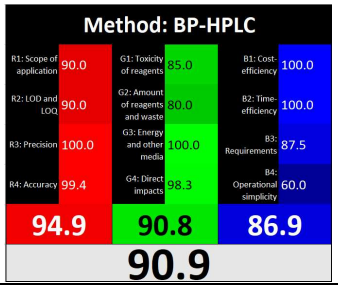
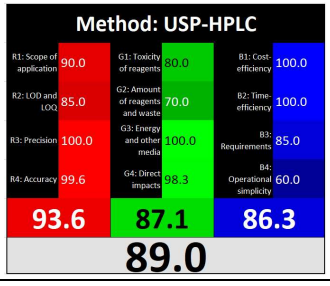
Evaluation Points	Penalty Points (PPs)		
	The proposed method	BP HPLC method [2]	USP HPLC method [3]
Reagents			
Methanol	6	12	6
NaOH 0.1 N	1	-	-
perchloric acid 0.1 N	-	1	-
Anhydrous acetic acid	-	4	-
Acetonitrile	-	-	8
Formic acid	-	-	2
Heptaflourobutyric acid	-	-	4
Diethylamine	-	-	2
Glacial acetic acid	-	4	-
Sodium acetate trihydrate	-	2	-
Instrument			
Energy consumption	0 (≤0.1 kWh per sample)	0 (≤0.1 kWh per sample)	1 (≤1.5 kWh per sample)
Waste generation	1 (<1 mL (g))	5 (>10 mL (g))	3 (1-10 mL (g))
Waste treatment	3	3	3
Occupational hazard	0	0	0
Total PPs	Σ 11	Σ 28	Σ 29
Eco-Scale score	**89**	**72**	**71**
Assessment	Excellent greenness	Acceptable greenness	Acceptable greenness

After the total analytical eco-score is calculated, the UV proposed method has higher eco scores compared to the HPLC pharmacopoeial methods [2,3]. It can be concluded from Table 6 that the proposed spectrophotometric method has the highest Eco-Scale score compared to other formal chromatographic methods which consume higher energy and a greater amount of solvents and wastes.

Table 7 demonstrates the greenness and performance profiles of the proposed method using the mentioned metrics and their comparison with reported formal HPLC ones. The practical application of AGREEprep showed a value of 0.82 for our method. The greenness of sample preparation of the proposed method was compared with the previously mentioned methods in the Pharmacopoeia for BF determination.

The proposed method was found to be greener and safer. Using AGREE, our method showed a score (0.86) that ranked first ahead of the formal HPLC methods. This distinctive visual indicator confirms the eco-friendly features of the recommended methodology. In conclusion, the application of the AGREE analytical greenness scale approach reinforces the idea that the proposed method is well aligned with the principles of green analytical chemistry

Table 7. Assessment tools to evaluate the greenness and performance profile. Comparative study of the proposed method with pharmacopoeial methods for determining the amount of BF in tablets.

Tool	Proposed UV method	BP HPLC method [2]	USP HPLC method [3]
AES	89	72	71
AGREEprep			
AGREE			
GAPI			
BAGI			
RGB12			

The GAPI index indicates that the proposed method exhibits three yellow steps in its GAPI index, with one red-colored step indicating the area of no treatment of waste, while the result revealed the greenness of most. So, this newly developed method is considered to be eco-friendly. On the other hand, the official methods approved by the British and American Pharmacopoeias obtain less green and more yellow steps, but it has four red-colored steps, suggesting potential environmental drawbacks. The superiority of the recommended green methodology is due to the absence of solvents, shortened sample preparation and analysis stages, speed of analysis, and the absence of hazardous wastes of the proposed method, in addition to energy savings. While pharmacopoeial methods use toxic solvents in large quantities in addition to some chemicals which results in untreated wastes, also to consuming more energy [2] due to heating the column oven to 45 °C. Complementing the green assessment tools, BAGI is a tool used to evaluate the performance of the analytical method by following the blue principles of White Analytical Chemistry. Finally, the RGB12 tool expresses the most comprehensive evaluation of the method stages, especially the required application related to the analyzers used in each evaluation method as shown in Table 7. As for the comparison between the proposed spectrophotometric method and the reported HPLC methods in terms of analytical efficiency, it gave almost similar results, while the higher whiteness of the proposed method resulted from the fact that the proposed method is greener and more practical compared to HPLC methods.

All applied greenness assessment tools have demonstrated that there is a significant potential for environmental harm from pharmacopoeial technologies. Yellow indicates a disturbing effect and frequent red indicates a risky effect that should be avoided.

Conclusions

The prestigious pharmaceutical industries are those that strive for safety, quality and proven efficacy. This makes the development of appropriate analytical methods essential for the quality control of drugs. Therefore, this research proposed to develop an eco-friendly, easy, efficient and reliable UV spectrophotometric method for the determination of bisoprolol fumarate in tablets with only a simple sample preparation step in an aqueous medium. This allowed for direct spectrophotometric quantification of BF at 273 nm, with a linear range of 1.66–190 µg mL^{-1} (r = 0.9999) with LOD and LOQ of 0.211 and 0.638 µg mL^{-1}, respectively. The green method was evaluated and the tested greenness and whiteness evaluation parameters (AES, AGREEprep, AGREE, GAPI, BAGI and RGB12) of the proposed analytical method showed that the developed method is green, white, feasible and efficient by all the obtained data and it is superior to the pharmacopoeial analytical methods due to its simplicity, non-use of organic solvents and environmentally polluting chemical reagents and low cost. In addition, the method does not produce any hazardous waste to public health and environment. Hence, the proposed method is very suitable for use in the quality control of bisoprolol fumarate in tablets.

Conflict of interest

The author declares no conflicts of interest.

References

1. Martindale, The Complete Drug Reference, Sweetman SC (editor), 36th Ed., USA, 2009. ISBN 978 0 85369 840 1.
2. British Pharmacopeia. "Bisoprolol Fumarate Monograph" and "Bisoprolol Fumarate Tablets Specific Monograph". Medicines and Healthcare Products Regulatory Agency, London; 2024.
3. United States Pharmacopeia. USP 43–NF 38. Rockville: The United States Pharmacopoeial Convention; 2020.
4. Mohammed FS, AbdAlkareem SA, AbdAlrhman SM, Osman HM, Abdelwahid MAS, Gaily MM. Development of acid–base titrimetric method for determination of bisoprolol fumarate in raw material and tablet dosage form. Int J Multidisciplin Curr Res. 2016; 4: 972–975. http://ijmcr.com/
5. Ramadan NK, Zaazaa HF. Membrane electrodes for determination of some β-blocker drugs. J AOAC Int. 2007; 90(4): 987-994. https://doi.org/10.1093/jaoac/90.4.987
6. Hassan SSM, Abou-Sekkina MM, El-Ries MA, Wassel AA. Polymeric matrix membrane sensors for sensitive potentiometric determination of some beta-blockers in pharmaceutical preparations. J Pharm Biomed Anal. 2003; 32(1): 175-180. https://doi.org/10.1016/S0731-7085(03)00015-3
7. Frag EY, Mohamed ME, El-Boraey HA, EL-Sanafery SS. Carbon potentiometric sensors modified with beta-cyclodextrin as a carrier for the determination of bisoprolol fumarate. International Journal of Electrochemical Science 2019; 14: 6603–6616. https://doiorg/10.20964/2019.07.40
8. Elgendy K, Elmosallamy MAF, Soltan MK, Amin AS, Elshaprawy DS. Novel potentiometric methods for the estimation of bisoprolol and alverine in pharmaceutical forms and human serum. Rev Anal Chem. 2021; 40: 127–135. https://doi.org/10.1515/revac-2021-0129
9. Goyal RN, Tyagi A, Bachheti N, Bishnoi S. Voltammetric determination of bisoprolol fumarate in pharmaceutical formulations and urine using single-wall carbon nanotubes modified glassy carbon electrode. Electrochim Acta. 2008; 53(6): 2802–2808. https://doi.org/10.1016/j.electacta.2007.10.057
10. Goyal RN, Chatterjee S, Singh SP, Rana ARS, Chasta H. The electrocatalytic activity of bare pyrolytic graphite and single wall carbon nanotube modified glassy carbon sensors is same for the quantification of bisoprolol fumarate. Am J Anal Chem. 2012; 3: 106–112. http://dx.doi.org/10.4236/ajac.2012.32015
11. Wang WX, Gao LJ, Shi TS. HPLC determination of the content of bisoprolol fumarate tablets. Chin J Pharm Anal. 1999; 19(5): 308–310.

https://www.ingentaconnect.com/content/jpa/cjpa/1999/00000019/00000005/art00006
12. Induri M, Raju BM, Prasad RY. Validated and stability indicating liquid chromatography method for quantification of bisoprolol fumarate in tablet dosage form. Int J Pharm. 2012; 2(1): 64–70. http://www.pharmascholars.com/
13. Kondratova Y, Logoyda L, Voloshko Y, Abdel-Megied A, Korobko D, Soroka Y. Development and validation of HPLC-DAD method for the determination of bisoprolol in tablet dosage forms. Int J Appl Pharm. 2017; 9(6): 54–59. http://dx.doi.org/10.22159/ijap.2017v9i6.21616
14. Todevska EL, Piponski M, Stefova M. Development and validation of an analytical method for the determination of related substances in bisoprolol fumarate in dosage forms by HPLC-UV-DAD. Macedonian J Chem Chem Eng. 2021; 40(20): 263–276. https://doi.org/10.20450/mjcce.2021.2430
15. Bhavyasri K, Goud JS, Sewthasri R, Sumakanth M. Development and validation of stability indicating Rp-Hplc method for the estimation of bisoprolol fumarate in bulk and pharmaceutical dosage form. Int J Pharm Phytopharmacol Res. 2020; 10(4): 49–70. https://eijppr.com/7q-NQjh
16. Gibril ABM, Rudwan EH. Design of optimized RP-HPLC method for quantitative analysis of bisoprolol fumarate in bulk and pharmaceutical dosage form. Schol Int J Chem Mater Sci. 2023; 6(4): 91–99. http://dx.doi.org/10.36348/sijcms.2023.v06i04.005
17. Aade MM. Development and validation of analytical method for estimation of bisoprolol fumarate in bulk and solid dosage form by RP-HPLC. Int J Pharm Res Appl. 2023; 8(6): 1712–1719. https://doi.org/10.35629/7781-080617121719
18. Panainte (Gudruman) AD, Bibire N, Ţântaru G, Apostu M, Vieriu M. Validation of a spectrophotometric assay method for bisoprolol using picric acid. Revista medico-chirurgicala a Societatii de Medici si Naturalisti, Iaşi 2013; 117(2): 520–524. https://www.researchgate.net/publication/259347577
19. Ulu ST, Elif Kel E. Spectrophotometric determination of bisoprolol in pharmaceutical preparations by charge transfer reactions1. Opt Spectrosc. 2012; 112(6): 864–867. http://doi.org/10.1134/s0030400x12060197
20. Ayad MM, Abdellatef HE, Hosny MM, Kabil NA. Aggregation of gold nanoparticles for spectrophotometric determination of bisoprolol hemifumarate, buspirone HCl and doxazosin mesylate. Nano Biomed Eng. 2019; 11(1): 1–10. http://dx.doi.org/10.5101/nbe.v11i1.p1-10
21. Sharma S, Sharma MC. Novel method for spectrophotometric analysis of simultaneous estimation of bisoprolol fumarate tablet formulations using hydrotropy solubilization agents. J Optoelec Biomed Mater. 2010; 2(4): 223–225. http://chalcogen.ro/223_Sharma.pdf
22. El-Shaprawy DS, Elmosallmy MF, Elgendy K. Spectrophotometric determination of bisoprolol fumarate and levobunolol hydrochloride in bulk and pharmaceutical forms. Eurasian J Anal Chem. 2020; 15(1): 20–31. https://www.researchgate.net/publication/339045593
23. Mohammed SA, Adam ME, Shantier SW. Development and validation of UV spectrophotometric method for determination of bisoprolol fumarate in bulk and pharmaceutical dosage forms. Mediterran J Chem. 2017; 6(5): 196–199. http://dx.doi.org/10.13171/mjc65/01710181149-shantier
24. Tripathy SN, Swain S, Jena BR, Sahu A. New validated UV spectrophotometric method for the quantification of bisoprolol fumarate in its pharmaceutical dosage form. Acta Sci Pharm Sci. 2023; 7(2): 4-9. http://dx.doi.org/10.31080/ASPS.2023.07.0927
25. Patil S, Tamboli A, Jokar S, More S. Development and validation of UV spectrophotometric method for bisoprolol fumarate in bulk and tablet dosage form. World J Pharm Res. 2020; 9(7): 1908–1915. https://doi.org/10.20959/wjpr20207-17908
26. Prabhakar M, Vamshi RM, Shirisha RN, Neha B, Venkateswara RP, Akiful HM, Vasudha B, Narender B. Development and validation of UV spectrophotometric method for the determination of bisoprolol in bulk material and in tablets. Int J Biol Pharm Allied Sci. 2023; 12(6): 2822-2834. https://doi.org/10.31032/IJBPAS/2023/12.6.7214
27. El-Didamony AM, Shehata AM. Spectrophotometric determination of β-adrenergic antagonists drugs via ion-pair complex formation using MO and EBT. Optics and Spectroscopy 2014; 117(3): 492–499. https://link.springer.com/article/10.1134/S0030400X14090069
28. Panainte AD, Bibire N, Ţântaru G, Apostu M, Vieriu M, Dorneanu V. Spectrophotometric method for estimation of bisoprolol fumarate in tablets. Revista medico-chirurgicala a Societatii de Medici si Naturalisti, Iaşi 2014; 118(2): 558–563. https://pubmed.ncbi.nlm.nih.gov/25076731/#linkout
29. Ashour S, Al-khalil R, Alfares B. Hydrochlorothiazide used as diuretic with antihypertensive agents in pharmaceutical preparations. estimation by first-order derivative and extractive spectrophotometry. Canad Chem Trans. 2014; 2(2): 190-200. http://canchemtrans.ca/uploads/journals/CCT-2014-0085.pdf
30. Tobiszewski M. Metrics for green analytical chemistry. Anal Meth. 2016; 8: 2993–2999. http://dx.doi.org/10.1039/C6AY00478D
31. Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE—Analytical GREEnness metric approach and software. Anal Chem. 2020; 92: 10076–10082. https://dx.doi.org/10.1021/acs.analchem.0c01887
32. Yenduri S, Koppuravuri NP, Varalakshmi HN. Assessment and comparison of sustainability aspects of UV-spectroscopy methods for simultaneous determination of antihypertensive combination. Green Anal Chem. 2024; 9: 100108, http://dx.doi.org/10.1016/J.GREEAC.2024.100108

33. Wojnowski W, Tobiszewski M, Pena-Pereira F, Psillakis E. AGREEprepe Analytical greenness metric for sample preparation. Trends Anal Chem. 2022; 149: 116553. https://doi.org/10.1016/j.trac.2022.116553
34. Gałuszka A, Migaszewski ZM, Konieczka P, Namieśnik J. Analytical Eco-Scale for assessing the greenness of analytical procedures. Trends Anal Chem. 2012; 37: 61–72. http://dx.doi.org/10.1016/j.trac.2012.03.013
35. Shi M, Zheng X, Zhang N, Guo Y, Liu M, Yin L. Overview of sixteen green analytical chemistry metrics for evaluation of the greenness of analytical methods. Trends Anal Chem. 2023; 166: 117211. https://doi.org/10.1016/j.trac.2023.117211
36. Sajid M, Płotka-Wasylka J. Green analytical chemistry metrics: A review. Talanta 2022; 238: 123046. https://doi.org/10.1016/j.talanta.2021.123046
37. Płotka-Wasylka J. A new tool for the evaluation of the analytical procedure: green analytical procedure index. Talanta 2018; 181: 204–209. https://doi.org/10.1016/j.talanta.2018.01.013
38. Płotka-Wasylka J, Wojnowski W. Complementary green analytical procedure index (ComplexGAPI) and software. Green Chem. 2021; 23: 8657–8665. https://doi.org/10.1039/D1GC02318G
39. Manousi N, Wojnowski W, Płotka-Wasylka J, Samanidou V. Blue applicability grade index (BAGI) and software: a new tool for the evaluation of method practicality. Green Chem. 2023; 25: 7598–7604. https://doi.org/10.1039/D3GC02347H
40. Nowak PM, Kościelniak P. What color is your method? Adaptation of the RGB additive color model to analytical method evaluation. Anal Chem. 2019; 91: 10343–10352. http://dx.doi.org/10.1021/acs.analchem.9b01872
41. Nowak PM, Wietecha-Posłuszny R, Pawliszyn J. White analytical chemistry: an approach to reconcile the principles of green analytical chemistry and functionality. Trends Anal Chem. 2021; 138: 116223. http://dx.doi.org/10.1016/J.TRAC.2021.116223
42. Saleh SS, Obaydo RH, El Hamd MA, Rostom Y, Mohamed D, Lotfy HM. Guidelines for accurate application of green and white analytical concepts: merits versus demerits with insights of significant milestones of assessment tools applied for antiviral drugs. Microchem J. 2024; 199: 109917. http://dx.doi.org/10.1016/j.microc.2024.109917
43. ICH, Q2 (R2). Validation of analytical procedures- An Overview of the Revised Guideline, 2024. https://database.ich.org/sites/default/files/ICH_Q2%28R2%29_Guideline_2023_1130.pdf
44. Long GL, Winefordner JD. Limit of detection, A closer look at the IUPAC definition. Anal Chem. 1983; 55: 712A–724A. http://dx.doi.org/10.1021/ac00258a001
45. Miller JN, Miller JC. Statistics and Chemometrics for Analytical Chemistry, Pearson Education Limited, Edinburgh Gate, Harlow, Essex CM20 2JE, England, 6th ed., 2010.