International Journal of Pharmaceutical Science and Research
www.pharmacyjournal.net
ISSN: 2455-4685
Received: 08-08-2026, Accepted: 07-09-2026, Published: 22-09-2026
Volume 11, Issue 3, 2026, Page No. 58-62

Simultaneous estimation of Bisoprolol Fumarate and Hydrochlorothiazide in pharmaceutical formulation by UV spectroscopic method

Harshitha K B[1*], Dr. S Vijaya Bhaskar[1], Indushree C[1], Udaykiran M S[1], Dr. Shachindra L Nargund[2], Dr. Shravan L Nargund[3]

[1] Department of Quality Assurance, Nargund college of Pharmacy, Karnataka, India
[2] Department of Pharmaceutical Chemistry, Nargund college of Pharmacy, Karnataka, India
[3] Department of Pharmaceutics, Dr Gurachar Nargund college of Pharmacy, Karnataka, India
Corresponding author: Harshitha K B
DOI: https://doi.org/10.66856/ijpsr.2026.11.3.11048

Abstract
A simple, accurate and reliable UV-visible spectrophotometric method was developed and validated for the simultaneous estimation of Bisoprolol Fumarate (BISO) and Hydrochlorothiazide (HCZ) in bulk drug samples and pharmaceutical dosage forms. 0.1N NaOH was selected as the solvent based on solubility studies of both drugs. Bisoprolol Fumarate showed maximum absorbance at 223nm, While Hydrochlorothiazide showed maximum absorbance at 274nm. The linearity range for both drugs was found to be 2-10µg/ml with correlation coefficients of 0.9991 for BISO and 0.9991 for HCZ, indicating excellent linearity. Various analytical validation parameters i.e., Accuracy, Precision, LOD and LOQ were calculated using standards. In order to check the performance assay was carried for the marketed formulation was found to be 101.49% and 101.42% respectively, LOD and LOQ for BISO were found to be 0.09191 and 0.27851 µg/ml, and for HCZ it was found to be 0.07954 and 0.24105µg/ml. The results % Recovery for BISO and HCZ were observed in the range of 99.00-102.06% and 99.393-101.77%. %RSD for the precision study was found to be less than 2% for both the drugs. The developed method can be used for the simultaneous estimation of BISO and HCZ from tablet formulation.

Keywords: BISO, HCZ, validation, UV-Visible spectrophotometry, simultaneous equation method

Introduction

Hypertension: Also known as high blood pressure (HBP), is a condition where the force of blood pushing against the walls of the arteries is excessively high. Generally, it is diagnosed when blood pressure reading exceeds 140/90mmHg, and it is considered as severe if the reading is above 180/120mmHg. This is a long-term chronic health condition characterized by persistently elevated pressure in the arteries. Often referred as the "SILENT KILLER" hypertension usually does not present noticeable symptoms. However, it significantly increases the risk of developing serious health issues such as coronary artery disease, stroke, heart failure, atrial fibrillation, and chronic kidney disease [1]. According to recent guidelines, starting treatment with two drugs is advised for patients whose systolic pressure exceeds the target by more than 20mmHg or whose diastolic pressure is over by more than 10mmHg, as well as for those at elevated cardiovascular risk [2].

In present research work the drug combination of Bisoprolol Fumarate and Hydrochlorothiazide is selected which is used in the treatment of hypertension in adult patient whose blood pressure is not adequately controlled by monotherapy.

Bisoprolol Fumarate: Is a selective beta 1 blocker used to treat hypertension and heart failure. It lowers heart rate and force of heart contractions, reducing the workload and oxygen demand of the heart. As a selective B1-blocker, Bisoprolol Fumarate decreases both the force (inotropic effect) and the rate (chronotropic effect) of heart contractions, which lowers myocardial oxygen demand and eases the heart's workload. Additionally, B1 receptors leads to reduced renin release, thereby inhibiting the renin-angiotensin system. This combined impact on both the heart and kidneys make Bisoprolol Fumarate beneficial for controlling high blood pressure and associated cardiovascular conditions [3].

The chemical name of Bisoprolol Fumarate is (E)-but-2-enedioic acid; 1-(propan-2-ylamino)-3-[4-(3-pro-2-yloxyethoxymethyl) phenoxy] propane-2-ol

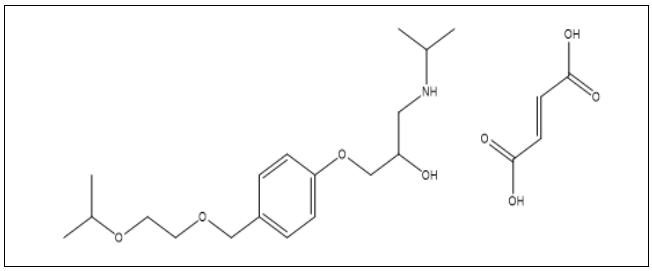

Fig1: structure of bisoprolol fumarate [4]

Hydrochlorothiazide: is an FDA approved medication used to manage high blood pressure and peripheral edema. As a thiazide diuretic, it works by blocking sodium reabsorption in the distal convoluted tubules of the kidneys. For more than six decades, thiazide diuretics have proven to be dependable agents for lowering blood pressure. They achieve this by directly inhibiting the sodium-chloride cotransporter, leading to increased sodium excretion (natriuresis) and urine production (diuresis) [5].

The chemical name of Hydrochlorothiazide is 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4- Benzothiadiazine-7-silfonamide

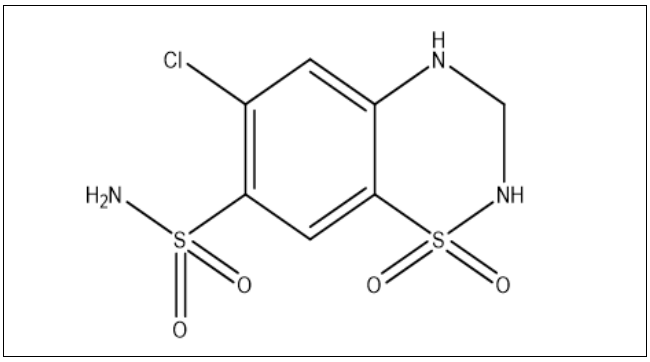

Fig 2: Structure of Hydrochlorothiazide [6]

Literature survey revealed that a number of methods have been reported for estimation of BISOPROLOL FUMARATE, HYDROCHLOROTHIAZIDE individually or in combination with other drugs. However, there is very few analytical methods including UV Spectroscopy [7], HPLC [8] methods reported for simultaneous analysis of these drugs in a combined dosage formulation.
So, in the present research work, the aim is to study and develop different, simple and economic method for the simultaneous estimation of Bisoprolol Fumarate and Hydrochlorothiazide by HPLC.

Materials and Methods

1. Instruments:

- UV-Visible Spectrophotometer (Shimadzu-1700, Software Version-UV Prob 2.32)
- Electronic Weighing Balance (Sartorious-TE-214S)
- Ultrasonicator (RC Systems- MU1700)

2. Chemicals:

Bisoprolol Fumarate API was procured as a gift sample from Supriya Life science Ltd, Maharashtra, India and Hydrochlorothiazide API were received as gift samples from CTX Life Sciences Pvt.Ltd., Surat, Gujarat, India. Marketed formulation CORBIS-H5 is manufactured by Torrent Pharmaceutical Ltd.

3. Preparation of Standard Stock Solutions

3.1 Standard stock solution of BISO

10mg of standard BISO was weighed and transferred to 10ml volumetric flask and dissolved in 5ml of 0.1N NaOH. The volume was made up to the mark with 0.1N NaOH to obtain a final concentration of 1000µg/ml and labelled as "Std Stock BISO-I". From stock-I, 1ml was pipetted out into a 10ml volumetric flask and the volume was made up to the mark with distilled water to obtain a final concentration of 100µg/ml and labelled as standard stock BISO-II.

3.2 Standard Stock solution of HCZ

10mg of standard HCZ was weighed and transferred to 10ml volumetric flask and dissolved in 5ml of 0.1N NaOH. The volume was made up to the mark with 0.1N NaOH to obtain a final concentration of 1000µg/ml and labelled as "Std Stock HCZ-I". From stock-I, 1ml was pipetted out into a 10ml volumetric flask and the volume was made up to the mark with distilled water to obtain a final concentration of 100µg/ml and labelled as standard stock HCZ-II.

3.1.1 Selection of Analytical Wavelength

From the 'Standard stock BISO-II (100µg/ml) solution, 0.4ml of the aliquot was transferred to a 10ml volumetric flask and the volume was made up to the mark with water to obtain the final concentration of 4µg/ml. The solution was scanned in the spectrum mode from 200nm to 400nm.
From the 'Standard stock HCZ-II (100µg/ml) solution, 0.2ml of the aliquot was transferred to a 10ml volumetric flask and the volume was made up to the mark with water to obtain the final concentration of 2µg/ml. The solution was scanned in the spectrum mode from 200nm to 400nm. Overlain spectra are shown in Figure 3.

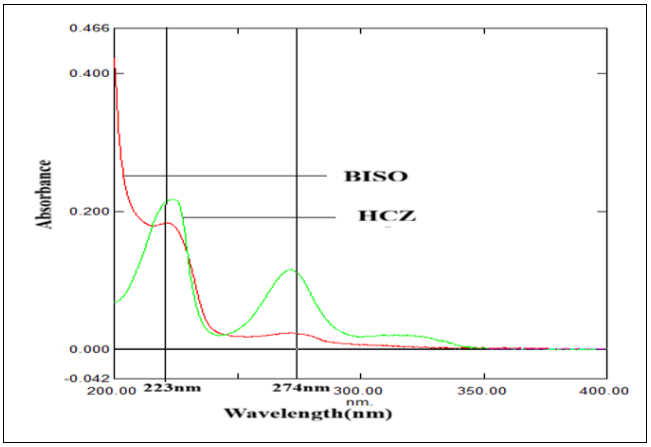

Fig 3: Overlay spectra od BISO (4µg/ml) and HCZ (2µg/ml)

3.1.2 Selection of Analytical Concentration Range

Calibration Curve for BISO and HCZ: The stock solution of BISO were diluted to get concentration range of 2-10µg/ml, absorbance of these solutions were measured at 223nm and 274nm respectively. While for HCZ the stock solution was diluted to get the concentration range of 2-10µg/ml and absorbance of these solutions were measured at 274nm and 223nm.Calibration Curves were plotted for these drugs as shown in the Figure 4,5.

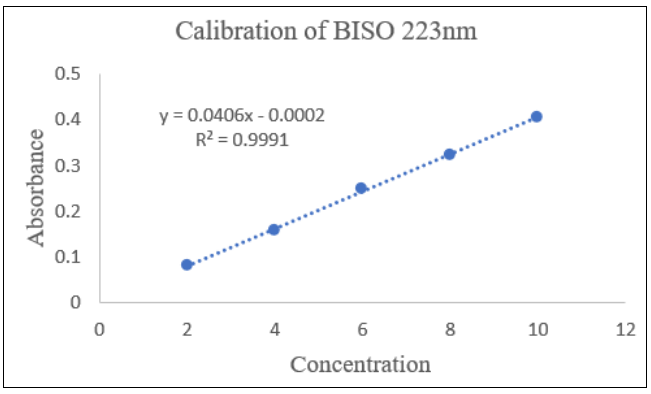

Fig 4: Calibration Curve of BISO 223nm

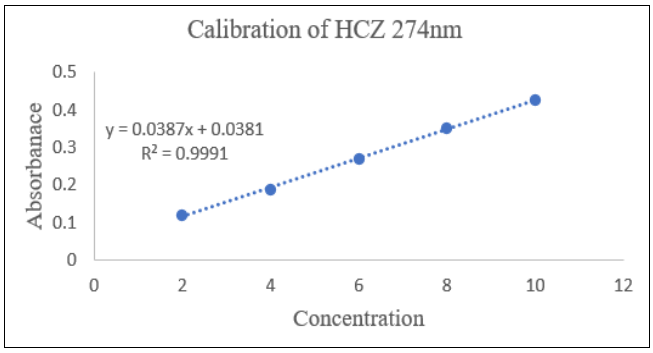

Fig 5: Calibration Curve of HCZ 274nm

3.1.3 Analysis of Tablet Formulation:

Twenty tablets of BISO and HCZ (CORBIS H5) were weighed and crushed to obtain fine powder. An accurately weighed 10mg of tablet powder equivalent to about 5mg of BISO and 6.25mg of HCZ was transferred to 10ml volumetric flask and sonicated for 10minutes in 5ml of 0.1N NaOH and made up to the volume with 0.1N NaOH to get the concentration of 500µg/ml BISO and 625µg/ml HCZ. The resulting solution was filtered through Whatman's filter paper no.41 and this solution was used as 'Sample Stock A'. From the 'Sample Stock A' solution 1ml of the aliquot was pipetted out and transferred into 10ml volumetric flask. The volume was made up to the mark with distilled water to obtain a solution with concentration of 50µg/ml BISO and 62.5µg/ml of HCZ and this solution was labelled as 'Sample Stock B'. From the 'Sample Stock B' solution 1ml of the aliquot was pipetted out and transferred into 10ml volumetric flask. The volume was made up to the mark with distilled water to obtain the working sample solution containing 5µg/ml of BISO and 6.25µg/ml of HCZ and this solution was labelled as 'Sample Stock C'. The prepared sample solution was scanned at 223nm and 274nm, and the corresponding absorbance values were recorded. Results are given in Table No:1

The concentration of two drugs (X and Y) in sample solution was calculated by using following equations:

$C_x = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2$

$C_y = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2$

Where, ax_1, ax_2, ay_1 and ay_2 are the absorptivity's of X and Y at λ_1 and λ_2 respectively. A_1 and A_2 are the absorbance of diluted sample at λ_1 and λ_2 respectively.

Table 1: Assay Results of Tablet Formulation

Sr. No.	Amount Present (mg/tab)		Amount Found (mg/tab)		% Assay	
	BISO	HCZ	BISO	HCZ	BISO	HCZ
1	5	6.25	5.052	6.383	101.00%	102.10%
2	5	6.25	5.210	6.372	104.20%	102.00%
3	5	6.25	5.044	6.300	100.90%	100.80%
4	5	6.25	5.052	6.383	101.05%	102.03%
5	5	6.25	5.036	6.216	100.73%	99.45%
6	5	6.25	5.052	6.299	101.05%	102.13%
Mean			5.074	6.325	101.49%	101.42%
±SD			0.066	0.066	0.013	0.010
%RSD			1.314	1.050	1.314	1.074

Validation of Spectrophotometric Method

1. Linearity:

The BISO stock solutions of 2-10µg/ml and HCZ stock solution of 2-10µg/ml were prepared and the absorbance measured at 223nm and 274nm respectively. After plotting graph between absorbance versus concentration and it was found to be linear. The %RSD was found to be under 2%. Results are in the Table No. 2,3.

Table 2: Results of Calibration Curve of BISO at 223nm and 274nm

Conc(µg/ml)	BISO at 223nm			BISO at 274nm		
	Mean Absorbance	±SD	%RSD	Mean Absorbance	±SD	%RSD
2	0.078167	0.001344	1.719032	0.021167	0.000372	1.760683
4	0.149333	0.001106	0.740318	0.022833	0.000372	1.632166
6	0.233833	0.000687	0.293878	0.030010	0.000577	1.924501
8	0.299000	0.001011	0.334448	0.035667	0.000471	1.321695
10	0.361333	0.000745	0.206279	0.051667	0.000471	0.912396

Table 3: Results of Calibration Curve of HCZ at 274nm and 223nm

Conc(µg/ml)	HCZ at 274nm			HCZ at 223nm		
	Mean Absorbance	±SD	%RSD	Mean Absorbance	±SD	%RSD
2	0.113500	0.000957	0.843548	0.232167	0.001067	0.459664
4	0.184833	0.001344	0.726984	0.373833	0.000687	0.183821
6	0.274167	0.004740	1.729053	0.527500	0.000957	0.181503
8	0.358167	0.001067	0.297958	0.656833	0.001067	0.162475
10	0.445333	0.002981	0.669481	0.812333	0.000942	0.116062

2. Accuracy study for BISO and HCZ

To determine the accuracy of the method, recovery study was performed by standard addition method at 80%, 100%, and 120% level of the assay concentration. For this study, 1ml of the pre-analysed 'sample stock-A' solution (5µg/ml of BISO and 6.25µg/ml of HCZ) was transferred to the three separate clean 10ml volumetric flask which was already containing 0.4ml, 0.5ml and 0.6ml of BISO and 0.5ml, 0.625ml and 0.75ml of HCZ respectively and made up to the mark with distilled water. These solutions were analysed in UV- Spectroscopy mode same as that of the sample solution analysis. Results are shown in Table No. 4&5.

Table 4: Results of Accuracy Study

Level of % Recovery	Sr. No	Amount of Standard Drug Added (µg/ml)		Total Amount Found (µg/ml)		Total Amount Recovered (µg/ml)		%Recovery	
		BISO	HCZ	BISO	HCZ	BISO	HCZ	BISO	HCZ
80%	1	4	5	9.066	11.23	4.066	4.98	101.6	99.68
	2	4	5	9.093	11.19	4.093	4.94	102.3	98.82
	3	4	5	9.093	11.23	4.093	4.98	102.3	99.68
100%	1	5	6.25	10.02	12.67	5.026	6.42	100.5	102.82
	2	5	6.25	9.973	12.67	4.973	6.42	99.47	102.82

	3	5	6.25	10.02	12.65	5.026	6.40	100.5	102.47
120%	1	6	7.5	10.89	13.86	5.897	7.61	98.29	101.57
	2	6	7.5	10.94	13.91	5.948	7.66	99.14	102.17
	3	6	7.5	10.97	13.86	5.974	7.61	99.57	101.57

Table 5: Statistical Validation Data for Accuracy Study

Level of % Recovery	Mean* (% Recovery)		± SD		% RSD	
	BISO	HCZ	BISO	HCZ	BISO	HCZ
80%	102.06	99.393	0.384	0.491	0.376	0.494
100%	100.15	102.70	0.610	0.204	0.609	0.198
120%	99.00	101.77	0.652	0.341	0.659	0.335

3. Precision

The Precision of an analytical method was studied by performing intermediate precision and repeatability

a. Intra-day Precision

Variation of results within the same day was analysed. Intra-day precision was determined by analysing the standard solution of 4, 6, 8µg/ml BISO and 4, 6, 8µg/ml HCZ at three different time intervals on same date. Results are shown in Table No: 6.

Table 6: Results of Intra-day Precision of BISO and HCZ

Drug& Wavelength	Conc µg/ml	Intra-Day			Mean ±SD	%RSD
		0hr	2hr	4hr		
BISO (223nm)	4	0.175	0.177	0.175	0.175±0.00094	0.5367
	6	0.256	0.260	0.261	0.259±0.00216	0.8340
	8	0.321	0.326	0.325	0.324±0.00216	0.6667
HCZ (274nm)	4	0.186	0.187	0.189	0.187±0.00125	0.6657
	6	0.265	0.263	0.264	0.264±0.00082	0.3092
	8	0.355	0.359	0.357	0.357±0.00163	0.4574

Inter-day Precision

Variation of results between the days was analysed. Inter-day precision was determined by analysing the standard solution of 4,6,8µg/ml BISO and 4,6,8µg/ml of HCTZ on three consecutive days. Results are shown in Table No:7

Table 7: Results of Inter-day Precision of BISO and HCZ

Drug& Wavelength	Conc µg/ml	Inter-Day			Mean ±SD	%RSD
		1	2	3		
BISO (223nm)	4	0.173	0.171	0.170	0.171±0.00125	0.7279
	6	0.254	0.259	0.259	0.257±0.00236	0.9159
	8	0.321	0.323	0.323	0.322±0.00115	0.3582
HCZ (274nm)	4	0.186	0.189	0.186	0.187±0.00141	0.7562
	6	0.265	0.262	0.262	0.263±0.00141	0.5377
	8	0.355	0.359	0.359	0.357±0.00189	0.5272

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

Detection limit and Quantification limit were determined from the standard deviation of y- intercepts of six calibration curves and average slope of six calibration curves.

The formula is as follows:-

$$LOD = 3.3 \times \frac{Standard\ Deviation\ of\ y-Intercepts\ of\ six\ Calibration\ Curves}{Average\ Slope\ of\ Six\ Calibration\ Curves}$$

$$LOQ = 10 \times \frac{Standard\ Deviation\ of\ y-Intercepts\ of\ Six\ Calibration\ Curves}{Average\ Slope\ of\ Six\ Calibration\ Curves}$$

Table 8: Summary of UV-Visible Spectrophotometric Method

Parameters	BISO	HCZ
Wavelength (nm)	**223nm**	**274nm**
Linearity Range (µg/ml)	2-10µg/ml	2-10µg/ml
Regression Equation (y = mx+c)	y = 0.0406x - 0.0002	y = 0.0387x + 0.0381
Correlation Coefficient (R^2)	0.9991	0.9991
LOD (µg/ml)	0.09191	0.07954
LOQ (µg/ml)	0.27851	0.24105
Analysis of Tablets (Mean % Assay)	101.49%	101.42%
%Recovery	100.10%	101.28%
Intra Day Precision (%RSD)	0.5367-0.8340	0.3092-0.6657
Inter Day Precision (%RSD)	0.3582-0.7279	0.5272-0.7562

Conclusion

A simple, precise, and sensitive UV- Spectrophotometric method utilizing the Simultaneous equation method has been developed for the simultaneous estimation of Bisoprolol Fumarate and Hydrochlorothiazide.

The developed method was validated according to ICH guidelines, and the results of the assay studies were satisfactory. Therefore, this method can be successfully applied for routine

Analysis of Bisoprolol Fumarate and Hydrochlorothiazide in marketed formulation of the pharmaceutical dosage forms.

Acknowledgment

I would like to thank the Dept. of Pharmaceutical Quality Assurance, Nargund college of Pharmacy, Bengaluru for providing the research facilities for conducting my research work. I would like to thank my principal and Guide for continuous encouragement and support.

Reference

1. Tripathi KD. Essentials of Medical Pharmacology. 8th ed. New Delhi: Jaypee Brothers Medical Publishers, 2019, 280-301.
2. Guerrero-García C, Rubio-Guerra AF. Combination therapy in the treatment of hypertension. Drugs in Context. 2018 Jun 6:(7):212531.
3. https://www.ncbi.nlm.nih.gov/books/NBK551623/ (accessed on 26th July 2026).
4. PubChem compound summary for [5281064]. https://pubchem.ncbi.nlm.nih.gov/compound/BISOPROLOL%20FUMARATE (accessed on 26th July 2026).
5. https://www.ncbi.nlm.nih.gov/books/NBK430766/ (accessed on 27th July 2026).
6. PubChem compound summary for [3639]. https://pubchem.ncbi.nlm.nih.gov/compound/Hydrochlorothiazide (accessed on 27th July 2026).
7. 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. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015 Apr 5:140:334-43.
8. Mostafa A, El-Gindy A, Emara S. Simultaneous spectrophotometric estimation of bisoprolol fumarate

and hydrochlorothiazide in tablet formulation using partial least-squares, principal component regression multivariate calibrations and RP-HPLC methods. J Anal Pharm Res,2017:4(6):00124.
9. Bozal B, Gumustas M, Topal BD, Uslu B, Ozkan SA. Fully validated simultaneous determination of bisoprolol fumarate and hydrochlorothiazide in their dosage forms using different voltametric, chromatographic, and spectrophotometric analytical methods. Journal of AOAC International. 2013 Jan 1:96(1):42-51.
10. Ganipisetty VN, Jalandhar D, Gnanadev G, Manoj P, Venkata Nadh R. A novel reversed-phase liquid chromatographic method for the simultaneous determination of potential impurities of bisoprolol fumarate and hydrochlorothiazide in a fixed dosage form. Separation Science and Technology. 2016 May 23:51(8):1362-9