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RP-HPLC Method Development and Validation of Bisoprolol Fumarate and Hydrochlorothiazide in Combined Dosage Forms.

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ABSTRACT

A simple, rapid, accurate, precise, and economical RP-HPLC method was developed and validated for the simultaneous estimation of Bisoprolol Fumarate (BIS)and Hydrochlorothiazide (HCT) in pharmaceutical dosage forms. Chromatographic separation was achieved using a Shim-pack C8 column with acetonitrile: water (80:20, v/v) as the mobile phase at a flow rate of 1.0 mL/min and UV detection at 227 nm. The method showed good linearity over 2-10µg/mL for BISand 2-10µg/ml for HCT, with R^2 of 0.9909 and 0.9944. Accuracy was confirmed by recovery studies, with recoveries of 99.8-101.34% for BIS and 99.64-99.09% for HCT. Precision showed below 2%RSD. The method was also validated for LOD, and LOQ according to ICH guidelines and was suitable for routine analysis.

KEYWORDS: Bisoprolol Fumarate, Hydrochlorothiazide, RP-HPLC, Hypertension, ICH Guidelines.

I. 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

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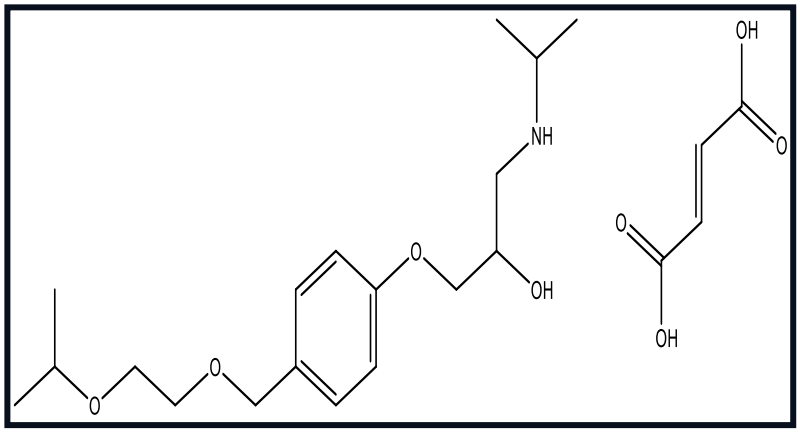

Figure 1: Structure of Bisoprolol Fumarate [4]

HYDROCHLOROTHIAZIDE is a medication approved by the US Food and Drug Administration (FDA) to treat hypertension and peripheral edema associated with congestive heart failure and cirrhosis. Hydrochlorothiazide is a thiazide-type diuretic that inhibits sodium resorption in the distal convoluted tubules (DCTs) of the kidney. For more than 60 years, thiazide diuretics have been a well-established class of antihypertensive diuretics that reduce sodium reabsorption and promote natriuresis and diuresis through direct inhibition of the sodium–chloride cotransporter.[5].

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

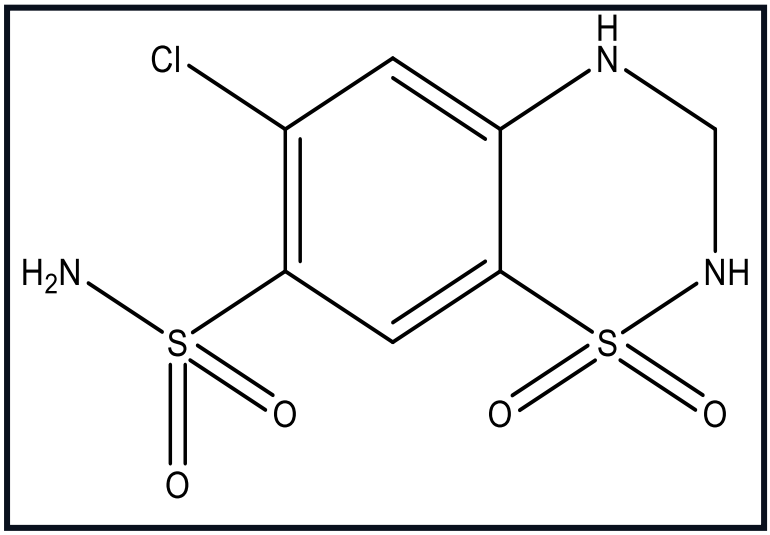

Figure 2: Structure of Hydrochlorothiazide [6]

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 [7-8], HPLCmethods [9-10] reported for simultaneous analysis of these drugs in a combined dosage formulation.

So, in the present research work, the aim is to develop and validate a RP-HPLC method for the simultaneous determination of Bisoprolol Fumarate, Hydrochlorothiazidein active pharmaceutical ingredients (APIs) and pharmaceutical dosage

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forms. The method was developed and validated in accordance with the ICH guidelines. Method development and Validation parameters including solubility, selection of mobile phase, selection of wavelength, linearity, range, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) were evaluated to establish the reliability and suitability of the proposed analytical method

II. MATERIALS AND METHODOLOGY:

2.1 Materials:

Bisoprolol Fumarate API was procured as a gift sample from Supriya Life science Ltd, Maharashtra, India and Hydrochlorothiazide API was received as gift samples from CTX Life Sciences Pvt.Ltd., Surat, Gujarat, India. Marketed formulation CORBIS-H5 is manufactured by Torrent Pharmaceutical Ltd. HPLC-grade methanol and acetonitrile, procured from Avantor Performance Materials India Pvt. Ltd., Thane, Maharashtra, India, were used as solvents throughout the study. Double-distilled water was used for all analytical procedures.

Chromatographic analysis was performed using a Shimadzu Liquid Chromatograph (LC-20AT) equipped with a binary pump, UV–Visible detector (Shimadzu SPD-20A), Rheodyne injector (Model 7725i, 20 µL loop), and LabSolutions software (Version 5.90). Separation was carried out using a Shimadzu Shim-pack C8 column (4.6 × 250 mm, 5 µm). A Hamilton syringe (25 µL) was used for sample injection. The mobile phase and sample solutions were filtered through Nylon membrane filters (25 mm, 0.2 µm) and degassed using an ultrasonic cleaner (RC Systems MU1700). A Shimadzu UV-1700 UV–Visible spectrophotometer with UV Probe software (Version 2.32) was used for absorbance measurements, while a Value High Reliability Vacuum Pump was used for filtration. All glassware used was made of borosilicate glass and calibrated before use. Chemicals were weighed using a calibrated Sartorius TE-214S digital analytical balance (maximum capacity 200 g).

2.2 Methodology:

2.2.1 Selection of Analytical Wavelength:

To determine the appropriate analytical wavelength for the estimation of BIS and HCT, the standard solutions of BIS(6µg/mL) and HCT (2µg/mL) were prepared by using HPLC-grade methanol and water. The solutions were scanned individually over the UV range of 200–400 nm, and the overlay spectra were evaluated to select the analytical wavelength. The overlay spectra are shown in Figure 3.

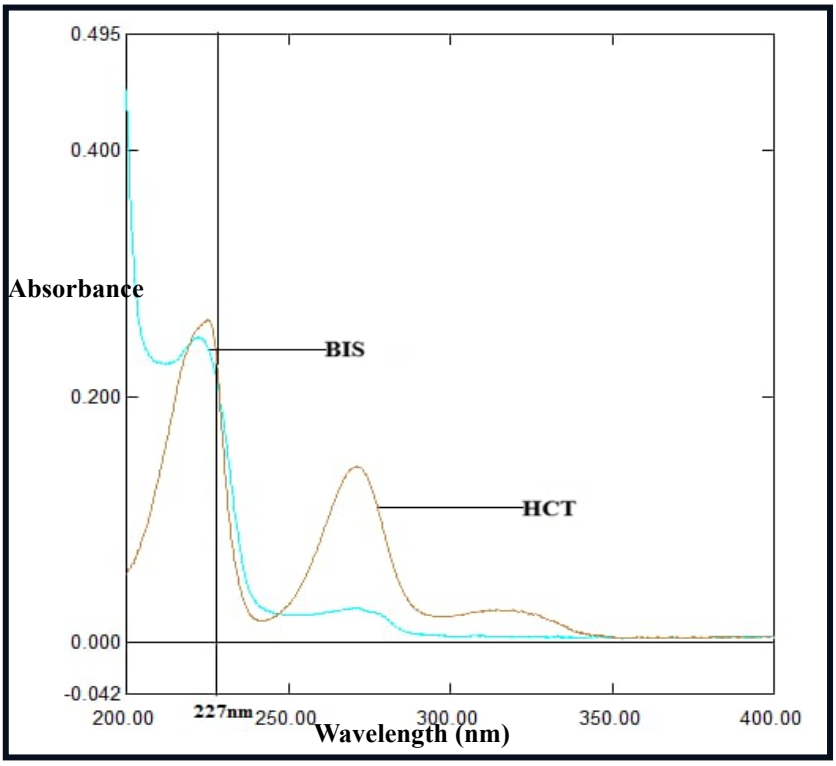

Figure No: 3:Overlay spectra of BIS (6µg/ml) andHCT (2µg/ml)

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2.4.2. Finalized Chromatographic Conditions

- ✓ **Analytical Column:** Shim pack C8(250 mm×4.6 mm, 5 µm)
- ✓ **Mobile Phase:** ACN: Water (80:20 % v/v)
- ✓ **Injection volume:** 20 µl
- ✓ **Flow rate:** 1 ml/min
- ✓ **Detection Wavelength:** 227nm
- ✓ **AUFS:** 0.1000
- ✓ **Pressure** ~11.2MPa

2.4.3. Preparation of Mobile Phase

The mobile phase was prepared by mixing 800 mL of HPLC-grade acetonitrile with 200 mL of HPLC grade water. The prepared mobile phase was filtered through a 0.2 µm membrane filter under vacuum and sonicated for 10 minutes before use.

2.4.4. Preparation of Standard Stock Solution

I. Preparation of Standard Solution of Bisoprolol Fumarate:

An accurately weighed quantity of 10 mg of Bisoprolol Fumarate was transferred into a 10 ml volumetric flask and dissolved in HPLC-grade methanol. The volume was made up to the mark with the same solvent to obtain a standard stock solution containing 1000 µg/ml, labelled as Std Stock BIS-A. Subsequently, 1 ml of this stock solution was transferred into a 10 ml volumetric flask and diluted up to the mark with HPLC-gradewater to obtain a working standard solution of 100 µg/ml, designated as Std Stock BIS-B.

II. Preparation of Standard Solution of Hydrochlorothiazide:

An accurately weighed quantity of 10 mg of Hydrochlorothiazide was transferred into a 10 ml volumetric flask and dissolved in HPLC-grade methanol. The volume was then made up to the mark with the same solvent to obtain a standard stock solution containing 1000 µg/ml, labelled as Std Stock HCT-A. FromStd Stock HCT-A, 1ml of this stock solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with HPLC-grade water to obtain a working standard solution of 100 µg/ml, designated as Std Stock HCT-B.

2.4.5. Preparation of Calibration Curve for BIS and HCT

Aliquots of 0.2, 0.4, 0.6, 0.8 and 1.0 ml were pipetted from Std Stock BIS-B into five separate 10 ml volumetric flasks. The flasks were labelledas B1, B2, B3, B4, and B5and the volume was made up to the mark with HPLC-grade water to obtain final concentrations of 2-10µg/ml for BIS.

Similarly, 0.2, 0.4, 0.6,0.8 and 1.0 ml aliquots were transferred from Std Stock HCT-Binto another set of volumetric flasks. The flasks were labelledas H1, H2, H3, H4, and H5and the volume was made up to the mark with HPLC-grade water to obtain final concentrations of 2-10µg/ml for HCT.

The prepared solutions were filtered through a 25 mm, 0.25 µm nylon syringe filter and 20 µl of each solution was injected into the Rheodyne injector of the HPLC system. Chromatograms were recorded under the optimized chromatographic conditions after achieving a stable baseline. The peak areas of both drugs were measured for each concentration.

Theoverlay chromatograms of the calibration solutions for BIS and HCTare presented in Figure No. 4 and Figure No. 5.Calibration curves for BIS and HCT were constructed by plotting Area versus concentration**,** as shown in Figure No. 6 and Figure No 7**.** The results of the calibration study are summarized in Table No. 1and Table No. 2.

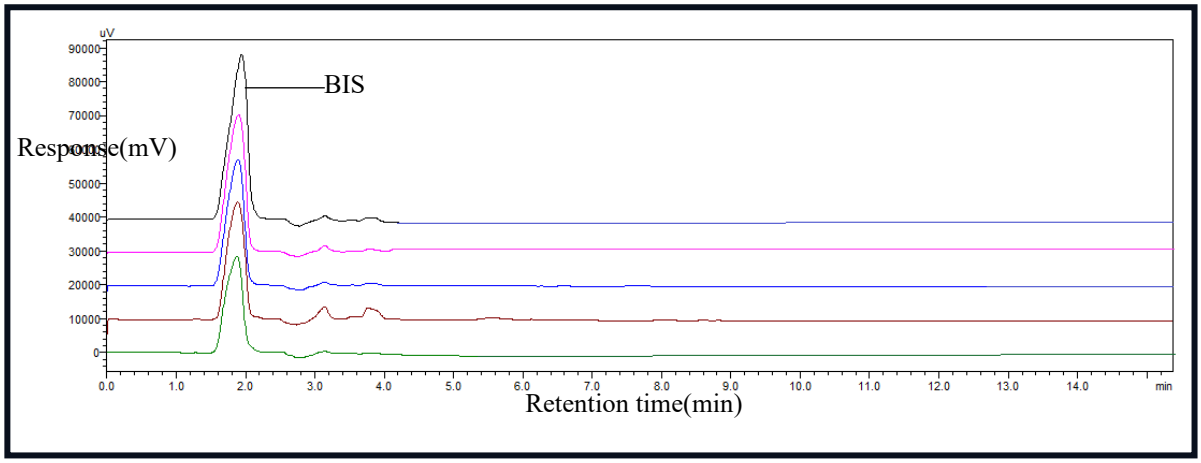

Figure No: 4. Overlain Chromatograms of BIS (2-10µg/ml)at 227 nm.

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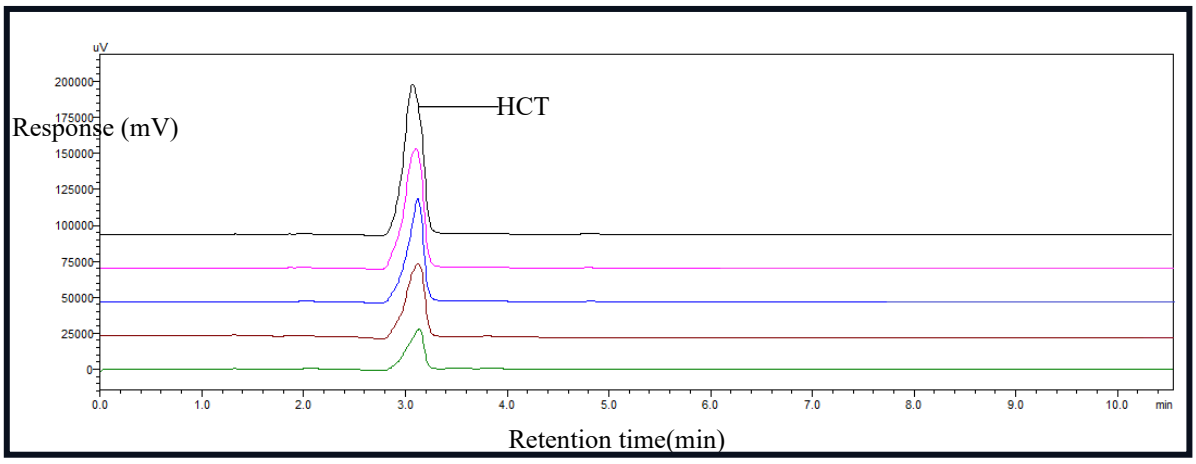

Figure No: 5. Overlain Chromatograms of HCT (2-10µg/ml) at 227 nm.

2.4.6 Analysis of Tablet Formulation

Twenty tablets of (CORBIS H5)were weighed and crushed to obtain fine powder. An accurately weighed tablet powder equivalent to 5mg of Bisoprolol Fumarate and 6.25 mg of Hydrochlorothiazide was transferred to 10 ml volumetric flask and 5ml of HPLC-grade methanol was added and sonicated for 10 min. The volume was made up to the mark with the same solvent and sonicated for 10 min to obtain 500 µg/ml of BIS and 625µg/ml of HCT. The resulting solution was filtered through Whatman filter paper No. 41 and designated as 'Sample Stock A'. From the above 'Sample Stock A' solution, 1ml of the aliquot was pipetted out and transferred to a 10 ml volumetric flask and it is labelled as 'Sample Stock B' (50µg/ml of BIS, 62.5µg/ml of HCT). From the 'Sample Stock B' 1ml of the aliquot was pipetted out and transferred to a 10 ml volumetric flask to get the final concentration (5µg/mlof BIS and6.25µg/ml of HCT).

The final sample solution was filtered through a 25 mm, 0.2 µm nylon syringe filter before analysis. A 20 µL aliquot of the solution was injected into the Rheodyne injector of the HPLC system using a Hamilton syringe. Chromatograms of sample solution was recorded under the optimized chromatographic conditions after obtaining a stable baseline. The peak areas of Bisoprolol Fumarate and Hydrochlorothiazide were measured, and the drug contentwas determined using the single-point calibration method. The assay results are presented in Table No. 4, and the corresponding chromatograms are shown in Figure No. 6.

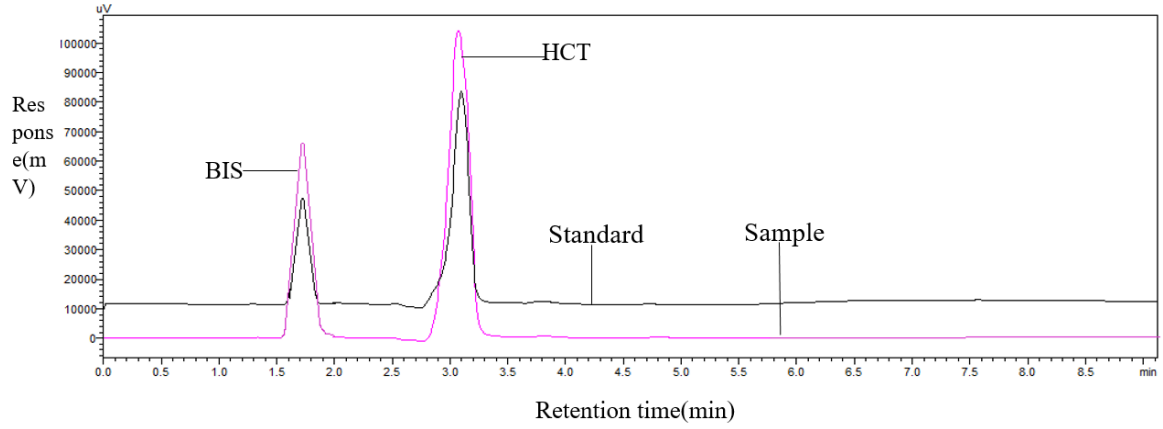

Figure No: 6 Overlain Chromatograms of Sample and Standard Solution of BIS and HCTat 227 nm.

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2.5 Validation of RP-HPLC Method

2.5.1. Linearity and Range

The linearity of the method was evaluated by preparing and analysing BIS standard solutions in the concentration range of 2-10µg/ml and HCT standard solutions in the concentration range of 2–10µg/ml. The linearity of the method was established based on the correlation coefficient (R^2) obtained from the calibration curves.

2.5.2. Precision

The precision of the developed analytical method was evaluated by assessing repeatability.

2.5.3. Accuracy

The accuracy of the developed method was evaluated using the recovery study. Twenty tablets were accurately weighed, powdered, and used for analysis. Recovery studies were performed by spiking the pre-analysed sample with known concentrations of standard BIS (4, 5, and6µg/mL) and standard HCT (5, 6.25, and 7.5µg/mL) at three concentration levels, namely 80%, 100%, and 120%. The percentage recovery was calculated for each level, and the results are presented in Table No. 5 and 6.

2.5.4. Repeatability

Repeatability of the developed method was evaluated by preparing standard solutions of BIS (2µg/mL) and HCT(6µg/mL) and analysing each solution six times under identical experimental conditions. The standard deviation (SD) and percentage relative standard deviation (%RSD) were calculated to assess the repeatability of the method. The results are presented in Table No 7**.**

2.5.5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the standard deviation of the y-intercepts of six calibration curves and the average slope of the corresponding calibration curves. The values were calculated using the following equations:

$$\text{LOD} = 3.3 \times \frac{\text{Standard Deviation of y–Intercepts of Six Calibration curves}}{\text{Average Slope of Six Calibration Curves}}$$

$$\text{LOQ} = 10 \times \frac{\textit{Standard Deviation of y–Intercepts of Six Calibration curves}}{\textit{Average Slope of Six Calibration Curves}}$$

The calculated LOD and LOQ values are presented in Table No. 3**.**

III. RESULTS

Table No: 1. Results of Calibration Curve of BIS at 227 nm

Conc (µg/mL)	Average Peak area	SD	%RSD
2	527731	5822.491	1.103307
4	611487	4155.42	0.67956
6	688706	6188.383	0.898552
8	790924	4947.842	0.625577
10	904503	6908.039	0.763739

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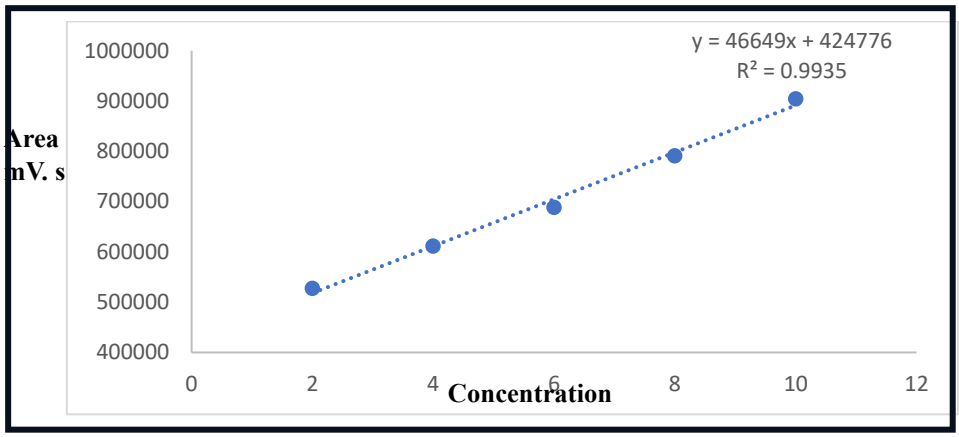

Figure No: 7. Calibration Curve of BIS at 227nm.

Table No: 2. Results of Calibration Curve of HCT at 227nm.

Conc(µg/mL)	Average Peak area	SD	%RSD
2	345040	3143.376	0.911016
4	625301	6720.635	1.074783
6	883379	8877.186	1.004913
8	1189315	9957.511	0.837248
10	1415980	15440.57	1.090451

Figure No: 8. Calibration Curve of HCT at 227 nm.

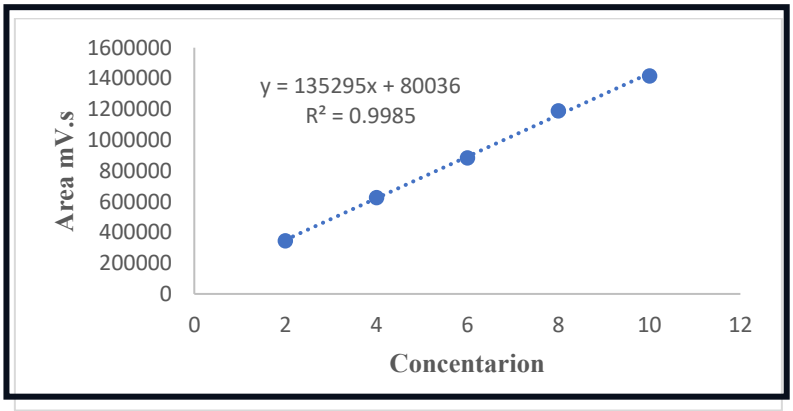

Table No: 3. Linear Regression Analysis of BIS and HCT at 227nm.

Parameters	BIS	HCT
Beer's law limit (µg/ml)	2-10	2-10
Wavelength (nm)	227	227
(Correlation Coefficient) R^2	0.9909	0.9944
Slope	9330.4	134319
Intercept	46725	104252

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LOD (µg/ml)	5.934889	0.115183
LOQ (µg/ml)	17.98451	0.349038

Table No: 4. Assay Results of Tablet Formulation

Sr. No.	Amount Present (mg/tab)		Amount Found (mg/tab)		% Assay	
	BIS	HCT	BIS	HCT	BIS	HCT
1	5	6.25	4.97992	6.255726	99.59%	100.09%
2	5	6.25	5.059604	6.353481	101.19%	101.65%
3	5	6.25	5.075606	6.431612	101.51%	102.91%
4	5	6.25	5.019384	6.332169	100.38%	101.31%
5	5	6.25	4.987736	6.255245	99.75%	100.08%
6	5	6.25	5.003572	6.338129	100.07%	101.41%
Mean			5.02097	6.327727	100.42%	101.24%
±SD			0.038917	0.060582	0.007121	0.009697
%RSD			0.77508	0.957406	0.709184	0.957842

Table: 5. Results of Accuracy for BIS and HCT

Level of % Recovery	Label Claim	Amount of Standard Drug Added (µg/ml)		Total Amount Found (µg/ml)		Total Amount Recovered (µg/ml)		%Recovery	
		BIS	HCT	BIS	HCT	BIS	HCT	BIS	HCT
80%	5:6.25	4	5	8.999	11.216	3.999	4.966	99.99	99.33
	5:6.25	4	5	9.001	11.281	4.001	5.031	100.0	100.62
	5:6.25	4	5	8.984	11.198	3.984	4.948	99.62	98.96
100%	5:6.25	5	6.25	10.049	12.685	5.049	6.435	100.9	102.96
	5:6.25	5	6.25	10.096	12.681	5.096	6.431	101.9	102.90
	5:6.25	5	6.25	10.070	12.569	5.070	6.319	101.4	101.11
120%	5:6.25	6	7.5	11.019	13.665	6.019	7.415	100.3	98.86
	5:6.25	6	7.5	11.136	13.733	6.136	7.483	102.2	99.77
	5:6.25	6	7.5	11.086	13.830	6.086	7.580	101.4	101.07

Table: 6. Statistical Validation Data for Accuracy Study

Level of % Recovery	Mean* (% Recovery)		± SD		% RSD	
	BIS	HCT	BIS	HCT	BIS	HCT
80%	99.87	99.64	0.218	0.869	0.219	0.872

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100%	101.44	102.33	0.465	1.052	0.458	1.028
120%	101.34	99.90	0.979	1.108	0.966	1.109

Table No: 7. Results of Repeatability Study for BIS and HCT

Sr. No.	Peak Area	
	BIS (2 µg/ml)	HCT (6 µg/ml)
1	524579	885740
2	525148	886149
3	512487	879598
4	514987	881454
5	526578	879128
6	534789	885486
Mean	523094.7	882925.8
±SD	8167.831	3241.206
% RSD	1.561444	0.367098

IV. CONCLUSION

A simple, accurate, precise, reproducible, and cost-effective RP-HPLC method was successfully developed and validated for the simultaneous estimation of Bisoprolol Fumarate and Hydrochlorothiazide in pharmaceutical dosage forms. The method was validated in accordance with ICH guidelines and demonstrated satisfactory results for parameters including linearity, assay, accuracy, precision, limit of detection, and limit of quantitation. Owing to its reliability and reproducibility, the developed method can be effectively employed for routine quality control analysis of these drugs in pharmaceutical formulations.

REFERENCE

[1]. Tripathi KD, Essentials of Medical Pharmacology. 8th edition, Jaypee brothers Medical Publishers: New Delhi; 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 20th September 2026).
[4]. PubChem compound summary for CID:5281064 https://pubchem.ncbi.nlm.nih.gov/compound/BISOPROLOL%20FUMARATE(accessed on 20thSeptember 2026)
[5]. https://www.ncbi.nlm.nih.gov/books/NBK430766/ (accessed on 20thSeptember 2026).
[6]. Pubchem compound summary for CID:3639https://pubchem.ncbi.nlm.nih.gov/compound/Hydrochlorothiazide(accessed on 20thSeptember 2026).
[7]. Harshitha KB, Vijaya BS, Indushree C, Udaykiran MS, Shachindra LN, Sharvan LN. Simultaneous estimation of Bisoprolol Fumarate and Hydrochlorothiazide in pharmaceutical formulation by UV spectroscopic method. Int J of Pharm Sci and Res.2026 Sep 22;11(3):58-62.
[8]. 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.
[9]. 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.
[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.

[11]. 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. J of AOAC International. 2013 Jan 1;96(1):42-51.