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Original Article

Establishing Pharmaceutical Brand Variability for Bisoprolol Fumarate and Hydrochlorothiazide Combinations: As an applied Q-absorbance Spectrophotometry

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ABSTRACT

The amalgamation of a unique spectrophotometric technique and principle concept of studying variations in pharmaceutical brands as a contrivance for simultaneous determination of the highly marketed combination of Bisoprolol fumarate and Hydrochlorothiazide is established hereby. The commercial purpose of marketing same drugs or drug combinations by different names creates scope for such analysis. Hence an accurate, precise and sensitive Q-Absorbance ratio UV-spectrophotometric method has been developed and validated for the simultaneous estimation of Bisoprolol Fumarate (BF) and Hydrochlorothiazide (HCT) in combined pharmaceutical dosage form. The analysis was performed using 0.1 N NaOH as solvent. Q-Absorbance ratio method involves formation of Q-absorbance equation at two wavelengths 274 nm (λ max of HCT) and 238.4 nm (iso-absorptive point). Calibration curves were linear in range of 8-96 µg/mL (r^2=0.999, 0.999) and 4-48 µg/mL (r^2= 0.998, 0.999) for BF and HCT respectively. The method was found to be rapid, economical and rugged as indicated by low values of percent RSD, can successfully be applied for the routine analysis of BF and HCT in bulk and combined pharmaceutical dosage form.

Key words: Bisoprolol fumarate, Hydrochlorothiazide, Q-Absorbance Ratio Method, UV-Spectrophotometry, Brand Variability.

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INTRODUCTION

Bisoprolol Fumarate (BF) and Hydrochlorothiazide (HCT) tablets are being widely used for treatment hypertension pertaining to children, adolescents, and pregnant women.[1] These tablets consist of two anti-hypertensive agents; a β1- adrenoceptor blocking agent (BF) and an added one is a Benzothiadiazine diuretic (HCT). BF; Figure 1 (**A**) is chemically described as (RS)-1-[4-[[2-(1-methylethoxy) eth-oxy] methyl] phenoxy]-3-[(1-methylethyl) amino]-2-propanol (E)-2-butenedioate. The presence of an asymmetric carbon atom in its structure needs it to be provided as a racemic mixture. Most of the β-blocking activities are due to the S (-) enantiomer.[2,3] HCT Figure 1 (B) is 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadia zine-7-sulfonamide 1, 1-dioxide. HCT is a white, practically odourless crystalline powder. It is slightly soluble in water, sparingly soluble in dilute sodium hydroxide solution, freely soluble in n-butylamine and dimethylformamide, sparingly soluble in methanol, and insoluble in ether, chloroform, and dilute mineral acids.[4,5]

The Bisoprolol fumarate (BF) and Hydrochlorothiazide (HCT) are official in the United States Pharmacopeia and European Pharmacopeia as a monotherapy and co-therapies.[6,7] The United States Pharmacopeia (USP) prescribes two separate chromatographic purity methods for the two actives and tablets dosage form. In the European Pharmacopoeia, two separate HPLC methods are available for BF and HCT. But these methods uses more than one chromatographic procedure to determine BF and HCT, which seems to be lengthy, time-consuming, expensive, cumbersome and tedious. A thorough literature survey revealed very rare methods for simultaneous UV-spectroscopic determination of BF and HCT in combined dosage form which are, ratio spectra derivative and simultaneous equation method using methanol as solvent,[8] simultaneous equation method using multi component mode of analysis employing alkaline solutions with sodium hydroxide;[9] first order derivative UV-spectrophotometric method using methanol as solvent.[10] As a result of recent developments several chromatographic methods have been reported to facilitate fast and more efficient evaluation for simultaneous determination of BF and HCT; such as HPLC coupled with UV,[11-13] Simultaneous UPLC determination,[13-15] simultaneous HPTLC analysis.[16]

None of these reports mentioned above provide application of the Q-absorbance Spectrophotometry for simultaneous determination of BF and HCT in combined dosage forms. Further, as the Indian market, today is busy with Pharmaceutical companies marketing same drugs combination under different brand names concurrent evaluation of different brands of commercially available BF and HCT dosage forms creates scope for such analysis. Hence to add a; simple, novel, quantitative method for simultaneous estimation of the aforementioned combination present investigation was carried out which is focused on development of a single, selective, sensitive UV-spectroscopic method for the determination of BF and HCT simultaneously in three tablet dosage forms; using simple and inexpensive chemicals, solvents and timely determination.

EXPERIMENTAL

MATERIALS AND METHODS

Chemicals

Pharmaceutically pure samples of Bisoprolol fumarate and Hydrochlorothiazide standards were gifted by Unichem Laboratory Ltd. Mumbai, India. Sodium hydroxide pellets were procured from LOBA Chemie Pvt. Ltd. Mumbai, India. The formulated dosage form of Bisoprolol fumarate and Hydrochlorothiazide: CORBIS®-H5, LODOZ®5 and ZABESTA®-XLO tablets were purchased from the local market.

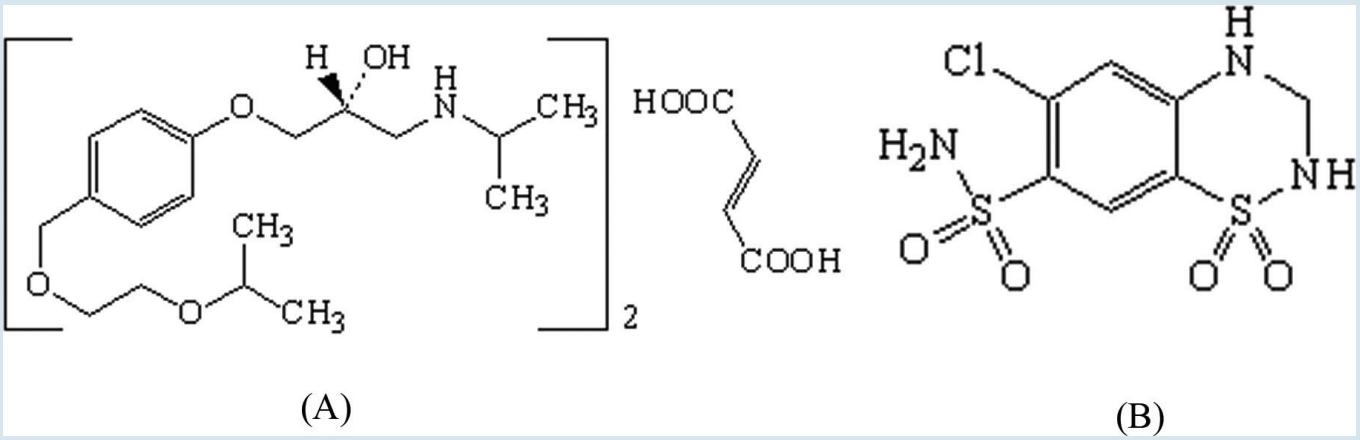

Figure 1: Chemical structures for Bisoprolol Fumarate (A) and Hydrochlorothiazide (B)

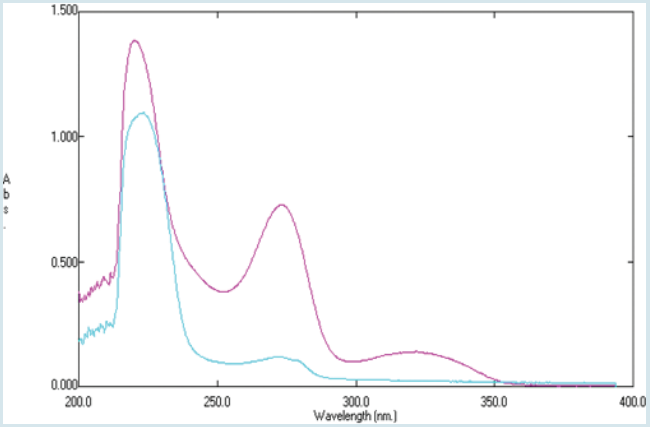

Figure 2: Overlain UV-spectrum of BF and HCT depicting λ_{max} of HCT at 274 nm and Isobestic Point at 238.4 nm for Absorbance Ratio Methodin 0.1 N NaOH.

Instrumentation

Spectrophotometric analysis was carried out on a double beam UV-Visible spectrophotometer Model: UV2401 PC, All weighing operations were performed using Analytical Balance AW-220 and BX-620S, by Shimadzu Corporation, Kyoto, Japan.

Preparation of standard solution

Standard solutions were prepared by dissolving 125 mg BF and 62.5 mg HCT in 50 mL 0.1 N NaOH to obtain concentration 2500 mg/mL of BF and 1250mg/mL of HCZ. From 50 mL of each standard solution 5.0, mL solution was pipetted out which contain 12.5 mg of BF and 6.25 mg of HCT was transferred separately to 50 mL volumetric flasks and volume was made up to the mark by using 0.1 N NaOH. Then further dilution was made obtain final conc. 10 µg/mL for BF and 5 µg/mL for HCT and scanned in the UV-region of 400-200 nm. The overlain spectrum (Figure 2) was obtained to determine the maximum absorbance (λ max) and iso-absorptive point.

Study of linearity curves

Appropriate volumes of the aliquot from each standard stock solution were transferred to different volumetric flasks of 10 mL capacity. The volume was made up to mark with 0.1 N NaOH to obtain concentrations of 8 - 96 µg/mL for BF and 4 - 48 µg/mL for HCT. Absorbances of each solution against 0.1 N NaOH as blank at both wavelength 274 and 238.4 nm were measured and calibration curves were plotted as concentrations *versus* absorbances. The optical characteristics and results of linearity study are as shown in Table 1.

Q-ABSORBANCE RATIO METHOD[18]

Q-Absorbance method uses the ratio of absorbance at two selected wavelengths, one at isoabsorptive point and other being the λ max of one of the two drugs. BF and HCT have λ max at 223 nm and 274 nm, respectively and iso-absorptive point 238.4 nm. The wavelengths selected for analysis were 274 nm and 238.4 nm, respectively. E (1%, 1cm) values of BF and HCT were determined at selected wavelengths.The concentration of two drugs in mixture was calculated by using following equations:

$$Cx = \frac{Qm - Qy}{Qx - Qy} \times \frac{A}{ax} \quad \ldots\ldots\ldots (1)$$

$$Cy = \frac{Qm - Qx}{Qy - Qx} \times \frac{A}{ay} \quad \ldots\ldots\ldots (2)$$

Where,

Qm = Ratio of absorbance of mixture at 274 nm and 238.4 nm

Qx = Ratio of absorptivity of BF at 274 nm and 238.4 nm

Qy = Ratio of absorptivity of HCT at 274 nm and 238.4 nm

A = Absorbance of mixture at 238.4 nm.

ax and **ay** = Absorptivities E (1 %, 1 cm) at 274 nm and 238.4 nm

$$\% \text{ Drug estimated} = \frac{c \times d}{w} \times 100 \quad \ldots\ldots (3)$$

Where,

C = **Cx** or **Cy**.

d = Dilution factor.

W = Weight of respective drug in laboratory mixture

Analysis of Marketed Tablets by Standard Addition Method

The content of twenty tablets was accurately weighed and crushed into fine powdered.For CORBIS®-H and LODOZ® an accurately weighed quantity of tablet powder equivalent to about 5 mg and for ZABESTA®-XLO 2.5 mg of Bisoprolol fumarate (equivalent to 6.25 mg Hydrochlorthiazide) was transferred to 50 mL volumetric flasks. Add 7.5 mg of pure BF in CORBIS®-H and LODOZ® and 10 mg of pure BF in ZABESTA®-XLO to make the ratio BF: HCT, 2:1then to each flask sufficient amount of 0.1 N NaOH was added and sonicated for 15 min. and volume was made up to the mark with 0.1 N NaOH and filtrated through Whatmann filter paper (No. 41). Then required dilutions were made to get the final concentration containing 10 µg/mL for BF and 5 µg/mL for HCT with 0.1 N NaOH. The absorbance of this solution was measured at 274 nm and 238.4 nm and concentrations of these two drugs in the sample were calculated using equation (1) and equation (2). The analysis procedure was repeated six times for tablet formulations. The results of analysis are as represented in Table 2.

Validation of methods

The Absorbance ratio method was validated for accuracy, sensitivity, precision and ruggedness as per the guidelines prescribed by International Council on Harmonization (ICH).[17]

Recovery experiments

The accuracy of the method was ascertained by recovery studies performed at different levels of concentrations. Quantitative recovery calculated from samples at concentration levels 80 %, 100 %, 120 %. The result of recovery study was found to be within the range of 98 - 100% prescribed by USP, indicating that the method is free from interference from excipients.

Sensitivity

The sensitivity of the method was determined as Limit of Detection (LOD) and Limit of Quantification (LOQ). To determine the limits of detection and quantification, concentrations at the lower end of the linear range of the calibration plot were analyzed. The LOD and LOQ were calculated with application of equations; LOD = 3.3 × *N/B* and LOQ = 10 × *N/B;* where, 'N' is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and '*B*' is the slope of the corresponding calibration plot.

Ruggedness

Proposed method was evaluated for ruggedness by analyzing fixed concentration of 10 µg/mL of BF and 5 µg/mL of HCT of sample solution; by two different analysts keeping operational and environmental conditions identical and the results are reported in terms of % RSD.

RESULTS AND DISCUSSION

BF and HCT obeyed the linearity in the concentration range of 8 - 44 µg/mL and 4 - 22 µg/mL In 0.1 N NaOH, respectively at their respective λ max with the correlation coefficient (r^2> 0.99) in both the case. Marketed brands of the tablets were analyzed. The percent amount of BF and HCT in three different brands as estimated by Absorbance ratio method was found to be BF- 98.94 % and HCT-97.92 % for CORBIS®-H, BF- 99.71 % and HCT- 99.14 % for LODOZ® and BF- 99.60 % and HCT- 99.11% for ZABESTA®-XLO, respectively. In these three brands precision was studied as repeatability (% RSD < 2) and inter and intra-day variations (% RSD < 2). The accuracy of the method was determined by calculating mean percentage recovery. It was determined at 80%, 100 %, and 120 % level. The ruggedness of the methods was studied by two different analysts using the same operational and environmental conditions. The results of method validation studies pertaining to the Accuracy as Percentage Recovery, Repeatability as Intraday and Interday Precision and Ruggedness are presented in Table 3, 4, 5. respectively.

CONCLUSION

The developed Absorbance ratio spectrophotometric method was found to be rapid, economical, and for the simultaneous estimation of the BF and HCT in their combined tablet dosage forms. Accuracy and precision are found within the acceptable range (ICH Guideline Q2 (R1), 2005). Thus developed methods can be used for routine analysis of BF and HCT in bulk and in its tablet formulation effectively.

From the results, it can be concluded that brand LODOZ®5 and ZABESTA®-XLO are more superior to CORBIS®-H in respect of % drug content. Brand CORBIS®-H is more superior to LODOZ® **and ZABESTA®**-XLO in respect of accuracy and repeatability showing less SD and RSD values.

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Table 1: The results for optical characteristics and linearity Studies for BF and HCT

Sr. No.	Parameters BF HCT	BF	HCT
1	Absorption Maxima (nm)	223	274
2	Wavelength for Absorbance Measurement	238.4 274	238.4 274
3	Coefficient of Correlation (r^2)	0.999[a] 0.999[b]	0.998[c] 0.999[d]
4	Beer's Law Limit (µ/mL)	8 - 96	4 - 48
5	Regression Equations	y^a= 0.056x + 0.006 y^b = 0.019x + 0.001	y^c = 0.022x + 0.016 y^d = 0.012x + 0.001

Table 2: Regression data for the analysis of marketed tablet combinations of BF and HCT

Brand	Drug	Label claim (mg/tablet)	% Label Amount*	%RSD
CORBIS®-H	BF	05	98.94 %	0.002052
	HCT	6.25	97.92 %	0.003511
LODOZ®	BF	05	99.71 %	0.004091
	HCT	6.25	99.14 %	0.005450
ZABESTA®-XLO	BF	2.5	99.60 %	0.005104
	HCT	6.25	99.11%	0.005384

*Values represent the mean of six determinations

Table 3: Experimental Outcomes for therecovery studies during Q-absorbance analysis

Recovery		Brands					
		CORBIS®-H		LODOZ®		ZABESTA®	
		BF	HCT	BF	HCT	BF	HCT
*Amount (%)		98.92	98.12	99.39	99.05	99.74	98.89
	±SD	0.2306	0.3601	0.51797	0.6672	0.5102	0.4266
	RSD	0.2312	0.3642	0.52114	0.6735	0.5156	0.4356

*Mean of three estimations at each level.

Table 4: Precision studies for the brand variability analysis of BF and HCT combinations

Sr. No.	Precision		Brands					
			CORBIS®-H		LODOZ®		ZABESTA®	
			BF	HCT	BF	HCT	BF	HCT
1	Interday	*Amount (%)	98.0	97.82	99.73	99.34	99.66	99.37
		±SD	0.5342	0.5342	0.5666	0.5651	0.5992	0.5776
		RSD	0.5451	0.5461	0.5681	0.5689	0.6012	0.5812
2	Intraday	*Amount (%)	97.93	97.89	99.76	99.43	99.67	99.40
		±SD	0.4534	0.5499	0.4742	0.7029	0.6444	0.7081
		RSD	0.4629	0.5617	0.4753	0.7069	0.6465	0.7123

*Denotes mean of three determinations

Table 5: Results from Ruggedness Studies

Sr. No.	Ruggedness	Brands					
		CORBIS®-H		LODOZ®		ZABESTA®	
		BF	HCT	BF	HCT	BF	HCT
		Percent Amount Found					
1	Analyst I	97.84	97.65	99.92	98.70	99.35	98.82
2	Analyst II	97.92	98.48	99.13	99.65	100.41	99.35

*All values represents mean of three determinations

CONFLICT OF INTEREST

Authors declare no conflict of interest in respect of content and publication of the manuscript in Pharmaceutical Methods.

ABBREVATIONS USED

BF: Bisoprolol Fumarate; **HCT:** Hydrochlorothiazide; **SD:** Standard Deviation; **RSD:** Relative Standard Deviation.

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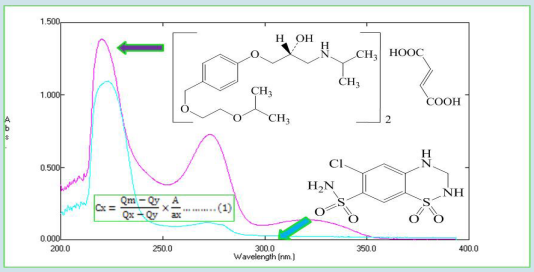
PICTORIAL ABSTRACT



SUMMARY

- Assesment of Pharmaceutical Brand Variability for the combination of BF and HCT was performed with aplication of Q-absorbance ratio Spectrophotometry. Simultaneous UV-Spectrophotometric analysis of BF and HCT was carried out. The develped method was validated as per ICH guideline. The use of ideal solvent system, rapidity of method and simplicity will add to the pharmaceutical determination of BF and HCT in combined dosage form in bulk and various pharmaceutical dosage forms.

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Ms. Preeti S Bobade: Has passed her graduation with first class and post graduation with distinction. Assessed pharmaceutical brand variability of drugs using UV and HPLC analysis. She has a teaching experience of about two years at undergraduate level at Government College of Pharmacy, Amravati and NMIM's School of Pharmacy Technology and Management (SPTM), Shirpur Campus.

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