SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF BISOPROLOL FUMARATE AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORM

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ABSTRACT
Two analytical methods have been developed for simultaneous quantification of bisoprolol fumarate and hydrochlorothiazide in combined pharmaceutical dosage form using spectrophotometer. Excellent simplicity, accuracy, precision and economy were achieved by the assay. The 'method I' was based upon 'simultaneous equations' whereas the 'method II' was based upon 'multicomponent mode of analysis' of the instrument. For both these methods, 0.1 N NaOH was used as solvent. In this solvent, bisoprolol fumarate showed absorbance at 224 nm and hydrochlorothiazide at 273 nm. Linearity lies in the concentration range of 3 - 21 µg/ml for bisoprolol fumarate and 3 - 18 µg/ml for hydrochlorothiazide. The methods were validated statistically and by recovery studies.

Keywords: Bisoprolol fumarate; hydrochlorothiazide; spectrophotometry; simultaneous equations; multi-component mode of analysis.

INTRODUCTION

Recently, fixed-dose combination of a low-dose bisoprolol fumarate-hydrochlorothiazide was approved as initial once-a-day therapy for hypertension. Bisoprolol fumarate (BF), (+/-)-1-(4-((2-(1- methyl ethoxy) ethoxy) methyl) phenoxy)-3-((1-methylethyl) amino)-2-propanol (E)-2-butenedioate (2:1) (salt) is a competitive beta (1)-selective (cardioselective) adrenergic antagonist (Ding *et al*., 2007). Hydrochlorothiazide (HCTZ), 6-chloro-3, 4-dihydro-7-sulfamoyl-2H-1, 2, 4- benzothiadiazine 1, 1-dioxide (5) [e] is a benzothiadiazine diuretic (Flores, 2005). The chemical structures of BF and HCTZ are shown in fig. 1.

Literature survey reveals various analytical methods such as HPLC (Kintz *et al*., 1990; Eastwood *et al*., 1990)**,** TLC-Densitometric (Witek *et al*., 1997), LC-MS (Bhatt *et al*., 2007) for estimation of BF in biological fluids and in pharmaceutical formulations**.** Many analytical methods such as HPLC (Hsieh *et al*., 1994; Farthing *et al*., 1998), Non-aqueous titration (BP, 2002), differential pulse voltameter (Razak, 2004) have been reported for determination of HCTZ in pharmaceutical formulations and in biological samples. BF is official in USP (USP, 2005), whereas HCTZ is official in USP (USP, 2005), IP (IP, 1996), BP (BP, 2002).

Very few analytical methods such as ratiospectra derivative spectrophotometry (Sahu and Patel, 2006), HPTLC (Patel *et al*., 2006) have been reported for simultaneous determination of both these drugs in pharmaceutical formulations.

This prompted us to disclose our results, consisting of new and accurate methods for the simultaneous determination of both drugs in pharmaceutical formulations using simultaneous equations and multicomponent mode of analysis.

EXPERIMENTAL

Instruments

1. UV- visible spectrophotometer (2450 Shimadzu with UV probe 2.21 software)
2. UV-Visible spectrophotometer (1700 Shimadzu)
3. Micropipette, Variable volume 20-200 µL (Bio-system classic).

Reagents

1. 0.1N NaOH
2. Double Reverse Osmosis (R.O.) water

Procedure

Preparation of standard stock solution and selection of wavelength

The standard stock solutions of 100 µg/ml for BF and HCTZ were prepared separately in 0.1 N NaOH. From these stock solutions, appropriate dilutions were made and scanned in the UV range 400 - 200 nm. The absorbance of BF and HCTZ were recorded at 224 nm and 273 nm, respectively.

Method I: Simultaneous Equations

Different aliquots were taken from the stock solutions and diluted with the same solvent to prepare a series of concentrations. The absorbances of these solutions were measured at 224 nm and 273 nm for BF and HCTZ,

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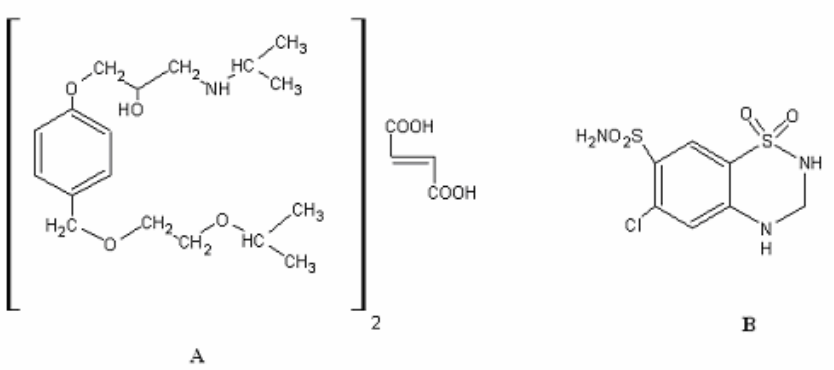

Fig. 1: Chemical structure of bisoprolol fumarate (A) and hydrochlorothiazide (B).

respectively and calibration curves were plotted at selected wavelengths; the optical characteristics and linearity data is shown in table 1. The E(1%, 1cm) of each drug at both wavelengths was determined; results are presented in table 2. The overlain spectra of BF and HCTZ are shown in fig. 2.

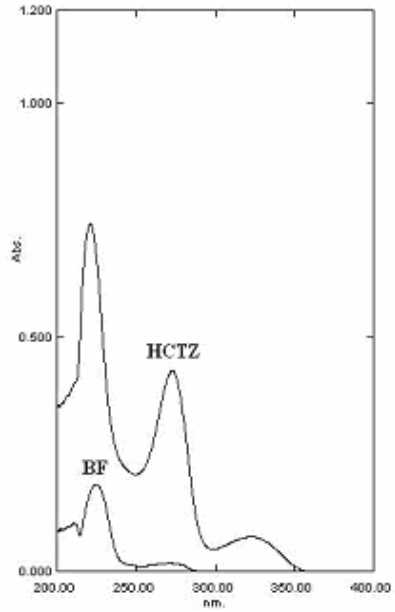

Fig. 2: Overlain spectra of bisoprolol fumarate (BF) and hydrochlorothiazide (HCTZ) in 0.1 N NaOH.

Table 1: Optical characteristics and linearity data

Parameters	BF	HCTZ
Absorption maximum (nm)	224	273
Beer's law limit (µg/ml)	3 - 21	3 - 18
Correlation coefficient	0.9997	0.9999
Regression equation (Y=m X+ C)	Y = 0.044 X + (-0.0009)	Y = 0.058 X + 0.0015
Intercept (C)	- 0.0009	0.0015
Slope (m)	0.044	0.058

Two simultaneous equations (in two variables C_1 and C_2) were framed by using E(1%,1cm) .

$A_1 = (410.66)\ C_1 + (887.33)\ C_2$ (I)

$A_2 = (43.16)\ C_1 + (547.37)\ C_2$ (II)

Where, C_1 and C_2 are the concentrations of BF and HCTZ measured in g /100 ml, in the sample solutions. A_1 and A_2 are the absorbances of the sample solutions, at selected wavelength i.e. 224 nm and 273 nm, respectively. By applying the Cramer's rule (Beckett and Stenlake, 2005) to equations I and II, the concentrations C_{BF} and C_{HCTZ} can be determined as follows:

$C_{BF} = A_2\,(887.33) - A_1\,(547.37) / - 186485.80$ (III)

and

$C_{HCTZ} = A_1(43.16) - A_2(410.66) / - 186485.80$ (IV)

Method II: Multicomponent Mode of Analysis

Seven mixed standard solutions with concentration of BF and HCTZ in the ratio of 14:0, 0:14, 2:2.5, 4:5, 6:7.5, 8:10, 10:12.5 (µg/ml) were prepared in 0.1N NaOH. All the mixed standard solutions were scanned over the range of 400 - 200 nm, in the multicomponent mode; using two sampling wavelength 224 (λ_{max} of BF) and 273 nm (λ_{max} of HCTZ). Overlain spectra of mixed standard solutions are shown (fig. 3). The spectral data from these scans was used to determine the concentration of two drugs in tablet sample solutions.

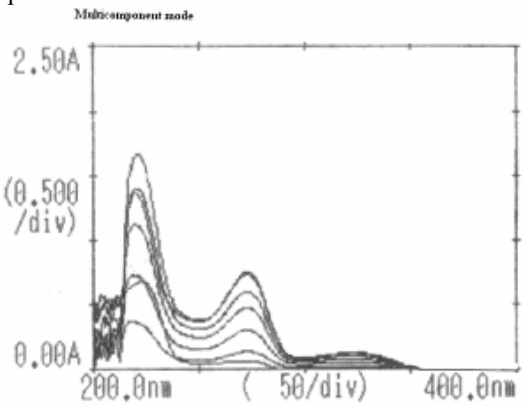

Fig. 3: Overlain spectra of mixed standard solutions of bisoprolol fumarate and hydrochlorothiazide.

Table 2: E(1%,1cm) for BF and HCTZ

* E(1%,1cm) at 224 nm ± SD		* E(1%,1cm) at 273 nm ± SD	
BF	HCTZ	BF	HCTZ
ax1 = 410.66 ± 0.52	ay1 = 887.33 ± 0.68	ax2 = 43.16 ± 0.33	ay2 = 547.37 ± 0.56

*mean of six estimations

Table 3: Analysis of tablet formulation (Brand I and II)

Brand		Method I (* % Amount found ± SD)	Method II (*% Amount found ± SD)
I BF 5 mg + HCTZ 6.25 mg	BF	99.80 ± 1.21	98.94 ± 0.15
	HCTZ	101.95 ± 0.29	99.68 ± 0.32
II BF 5 mg + HCTZ 6.25 mg	BF	99.62 ± 0.19	99.46 ± 0.18
	HCTZ	101.49 ± 0.16	100.35 ± 1.05

*mean of five estimations

Table 4: Results from Recovery Studies (for Method I and II)

Brand	Quantity of Drug Added		Method I		Method II	
			*% Recovery ± SD		*% Recovery ± SD	
	BF	HCTZ	BF	HCTZ	BF	HCTZ
I	4.8	6	101.66± 0.35	102.83± 0.78	99.83± 0.74	99.89± 0.37
	6	7.5	101.46± 0.19	102.66± 0.27	99.79± 0.53	100.27±0.76
	7.2	9	100.13± 0.67	102.77± 0.52	100.24±0.81	100.67±0.42
II	4.8	6	100.79± 1.29	101.32±0.83	98.71±1.06	99.49±0.19
	6	7.5	99.27±0.57	101.49±0.53	99.84±0.08	100.56±0.50
	7.2	9	101.39±1.49	102.34±0.73	99.69±0.05	99.82±0.24

*mean of three estimations at each level.

Table 5: Results from precision and ruggedness

Parameters	Method I		Method II	
	BF	HCTZ	BF	HCTZ
Precision (%RSD)				
Intra-day (n = 3)	1.60 - 1.67	0.31 - 0.19	0.74 - 1.25	0.40 - 1.09
Inter-day (n = 3)	1.00 - 1.08	0.31 - 0.52	0.36 - 0.82	0.29 - 0.58
Repeatability [%RSD (n = 5)]	0.78	0.40	0.56	0.38
Ruggedness (%RSD) (n = 3)				
Analyst 1	0.44	0.72	0.38	0.66
Analyst 2	0.42	0.64	0.45	0.69

Preparation and analysis of tablet formulations

Twenty tablets were accurately weighed, average weight determined and ground to fine powder. An accurately weighed quantity of powder equivalent to 5 mg of BF and 6.25 mg HCTZ was transferred into 100 ml volumetric flask containing 40 ml, 0.1N NaOH, shaken manually for 15 min, volume was adjusted to mark with same solvent and filtered through Whatmann filter paper no. 41. The resulting solution was further diluted to get concentration of 10 µg/ml of BF and 12.5 µg/ml of HCTZ. Absorbances of the sample solution were recorded at 224 nm and 273 nm and the amount of BF and HCTZ was determined using equation III and IV.

Also, the same solutions were scanned in multicomponent mode of instrument over the range of 400-200 nm and the concentrations of both the drugs were determined by analysis of spectral data of the sample solutions with the reference to the mixed standards.

The results of analysis of tablet formulation are presented in table 3.

Validation of Method (ICH guidelines, 2005)

The method was validated with reference to accuracy, precision, and ruggedness.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding

known amount of standard solution of BF and HCTZ to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods; the results are shown in table 4.

Precision

Precision of the methods was studied as intra-day, inter-day and repeatability. Intra-day study was performed by analyzing, the three different concentration of drug for three times in the same day. Inter-day precision was performed by analyzing three different concentration of the drug for three days in a week. Repeatability was performed by analyzing same concentration of drugs for five times. The results are shown in table 5.

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions. The results are shown in table 5.

RESULTS AND DISCUSSION

Two wavelengths 224 nm (λ_{max} for BF) and 273 nm (λ_{max} for HCTZ) were selected for analysis of the drugs in 0.1N NaOH. Linearity was observed in the range 3 - 21 µg/ml (r^2=0.9997) for BF and 3-18 µg/ml (r^2 =0.9999) for HCTZ. The amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. Both the methods were found to be precise as indicated by the repeatability, inter-day, intra-day analysis, showing %RSD less than 2. The results did not show any statistical difference between operators suggesting that methods developed were rugged. The results of precision and ruggedness are shown in table 5. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical formulations containing both these drugs.

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