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Development and Validation of TLC-Densitometry Method for Simultaneous Estimation of Bisoprolol Fumarate and Hydrochlorothiazide in Bulk and Tablets

Pradip N Bhoya, Emanual M Patelia* and Gautambhai

Department of Pharmaceutical Chemistry and Analysis, Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar–388121, Gujarat, India

Abstract

A new simple, precise, accurate and selective TLC-densitometry method has been developed for simultaneous determination of bisoprolol fumarate and hydrochlorothiazide in tablet dosage form. Chromatographic separation was performed on aluminum plate precoated with silica gel 60F254, using chloroform: ethanol: glacial acetic acid 5:1.5:0.2 (v/v) as mobile phase. Detection was carried out densitometrically at 225 nm. The R_f value of bisoprolol fumarate and hydrochlorothiazide were 0.62 and 0.40, respectively. The reliability of the method was assessed by evaluation of linearity which was found to be 200–1200 ng/spot for bisoprolol fumarate and 100-800 ng/spot for hydrochlorothiazide. Accuracy of the method was accessed by % recovery and found to be 100.02 ± 1.14% for bisoprolol fumarate and 99.91 ± 0.96% for hydrochlorothiazide. The method can be used for routine analysis of bisoprolol fumarate and hydrochlorothiazide in tablet dosage form.

Keywords: Bisoprolol fumarate; Hydrochlorothiazide; HPTLC; Simultaneous determination

Introduction

Bisoprolol fumarate (BF), (+/-) -1-(4-((2-(-methyl ethoxy) ethoxy) methyl) phenoxy)-3-((1-methylethyl) amino)-2- propanol (e)-2-butendioate is a competitive beta (1) - selective adrenergic antagonist [1]. Hydrochlorothiazide (HCTZ), is 6-chloro-3, 4-dihydro-7-sulfamoyl-2H-1, 2, 4-benzothiadizine 1, 1- dioxide (5) [e] is a benzothiadiazine diuretc [2]. The chemical structures are as shown in figure 1.

Literature survey reveals, many analytical methods such as HPLC [3,4], TLC-densitometry [5], fluorimetric determination [6], LC-MS [7], enantioanalysis human plasma [8] for estimation of BF in pharmaceutical formulation and biological fluids. Various analytical methods such as HPLC [9-12], differential pulse voltameter [13], stability indicating determination [14], photometric determination of HCTZ [15] were reported. BF is official in USP [16], whereas HCTZ is official in USP, IP [17] and BP [18].

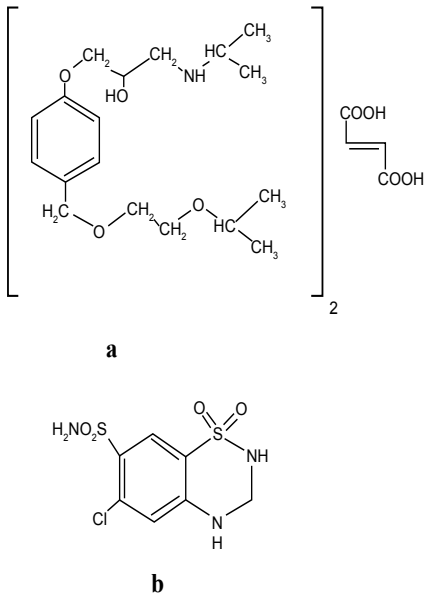

Figure 1: Chemical structures of BF (a) and HCTZ (b).

The purpose of this work is to establish and validate a simple accurate and reproducible procedure for quantitative TLC analysis of bisoprolol fumarate and hydrochlorothiazide in tablet dosage form as per ICH guidelines [19,20].

Experimental

Chemicals and reagents

Bisoprolol fumarate was supplied by Rusan Pharma Ltd., India and HCTZ was supplied by IPCA Lab, India as a gift sample. Qualiz 5 tablets containing 5 mg of bisoprolol fumarate and 6.25 mg of hydrochlorothiazide were obtained from commercial sources within their shelf life period. All the reagents used were of HPLC grade.

HPTLC instrumentation

The samples were spotted in the form of bands of width 6 mm with a camag 100 µl sample (Hamilton, Bonaduz., Switzerland) syringe on precoated silica gel aluminium plate 60 F_{254} (20 cm×10 cm with 0.2 mm thickness), supplied by Anchrom technologists, (Mumbai) using a camag Linomat 5 applicator (Switzerland). A constant application rate of 150 nl sec^{-1} was employed and space between two bands was 15

***Corresponding author:** Emanual M Patelia, Department of Pharmaceutical Chemistry and Analysis, Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar–388121, Gujarat, India, E-mail: ricky.emanual@gmail.com

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mm. The slit dimension was kept 6 mm×0.45 mm micro. The mobile phase consisted of chloroform: ethanol: glacial acetic acid 5: 1.5: 0.2 (v/v). The optimized chamber saturation time for mobile phase was 15 min at room temp (25°C ± 2) and relative humidity 60% ± 5. The length of chromatogram run was approximately 80 mm. Subsequent to the development; TLC plates were dried in current of air with the help of an air dryer. Densitometric scanning was performed using Camag TLC scanner 3 in the absorbance mode at 225 nm. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum in the range of 190-400 nm.

Preparation of standard solution and linearity study

An accurately weighed quantity 10 mg each of BF and HCTZ were transferred to two different 10 ml volumetric flasks, dissolved in methanol and volume was made up to mark with the same solvent to obtain concentration 1000 ng/µl each.

Aliquots of standard solution 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 µl of BF and 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 µl of HCTZ were applied on TLC plate with the help of microlitre syringe, using Linomat 5 sample applicator.

Method validation

Accurately weighed 10 mg of BF and 12.5 mg of HCTZ were transferred to 25 ml volumetric flask, dissolved in methanol and volume was adjusted to mark. This mix standard solution was used for validation study.

Precision: Repeatability of measurement of peak area was determined by spotting 400 ng/spot of BF and 500 ng/spot of HCTZ. Precision of the method was assessed by intra-day and inter-day variations. Intra-day variations were assessed by spotting 200, 400, 600 ng/spot of BF and 250, 500, 750 ng/spot of HCTZ on TLC plate on three different times within the same day. Inter-day variations were performed by analysing same concentrations described above for BF and HCTZ in three different days over a period of week.

Specificity: Specificity of the method was ascertained by analysing standard drug and sample. The mobile phase resolved both the drugs very efficiently, as shown in figure 2. The spot for BF and HCTZ was confirmed by comparing the R_f and spectra of the spot with that of standard. A typical absorption overlain spectrum of BF and HCTZ shown in figure 3; wavelength 225 nm was selected for densitometric scanning. Peak purity of BF and HCTZ was assessed by comparing the spectra of sample with that of standard at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions.

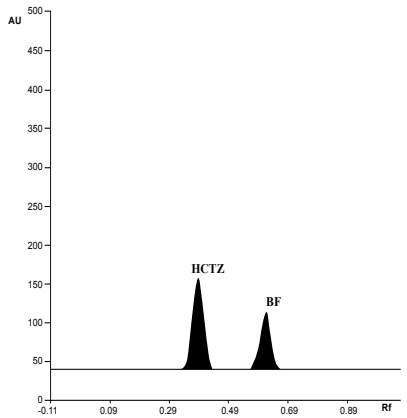

Figure 2: Densitogram obtained for BF and HCTZ in mobile phase chloroform: ethanol: glacial acetic acid (5:1.5:0.2 v/v).

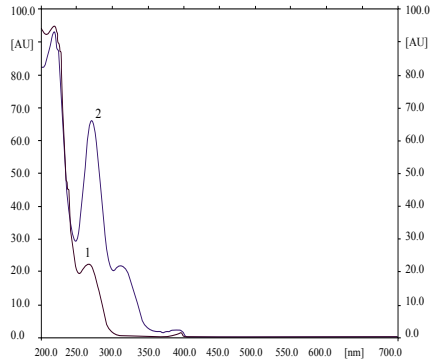

Figure 3: A typical overlain spectrum of standard BF (1) and HCTZ (2).

Accuracy: The pre-analysed samples were overspotted with extra 80, 100 and 120% of the standard drug solution of BF and HCTZ on TLC plate. The total concentrations of the drugs were determined. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in formulation.

Robustness: Robustness of the method was performed by spotting 400 ng of BF and 500 ng of HCTZ on TLC plate by making small deliberate changes in chromatographic conditions. Mobile phases having different composition like chloroform: ethanol: glacial acetic acid (5:1.5:0.2 v/v) and chloroform: ethanol: glacial acetic acid (4.5: 2: 0.2 v/v) were tried and chromatograms were run. The development distance was varied from 7, 7.5 and 8 cm. The amount of mobile phase (6.7 and 13.4) was tried and chromatograms were run. Temperature and relative humidity was varied in the range of ± 5%. The plates were prewashed by methanol and activated at 60 ± 5°C for 2, 5 and 7 min prior to chromatography. Duration of saturation time of chamber was varied as 10, 15 and 20 min. Time from spotting to chromatography and time from chromatography to scanning was varied from 0, 20, 40 and 60 min. Robustness of the method was done at three different concentration levels.

Ruggedness: Ruggedness of the method was performed by spotting 400 ng of BF and 500 ng of HCTZ, respectively by two different analyst keeping same experimental and environmental conditions.

Limit of detection (LOD) and limit of quantitation (LOQ): In order to determine detection and quantitation limit, concentrations in the lower part of the linear range of the calibration curve were used. Stock solutions of BF and HCTZ (1000 µg/ml) were prepared separately and different concentrations 200, 250, 300, 350 and 400 ng of BF and 100, 150, 200, 250 and 300 of HCTZ were separately spotted on TLC plates in triplicate. The LOQ and LOD were calculated using equation LOD=3.3×N/B and LOQ=10×N/B, where, N is 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 curve.

Application of proposed method to tablet formulation

Twenty tablets were weighed; average weight determined and crushed in to fine powder. An accurately weighed tablet powder equivalent to 5 mg of bisoprolol and 6.25 mg of HCTZ was transferred into 25 ml volumetric flask containing 15 ml methanol, sonicated for 10 min and volume was made up to mark with same solvent. The resulting solution was filtered using 0.41 µm filter (Millifilter, milford, MA).

The appropriate volume i.e. 2 µl containing 400 ng of BF and 500 ng of HCTZ was spotted on TLC plate.

Results and Discussion

HPTLC method development and validation

The TLC procedure was optimized with a view to develop method for simultaneous determination of BF and HCTZ. The mobile phase chloroform: ethanol: glacial acetic acid 5: 1.5: 0.2 (v/v) gave good resolution, sharp and symmetrical peak with R_f value of 0.62 for BF and 0.40 for HCTZ. It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 15 min ensure good reproducibility and peak shape of both the drugs.

Validation

Linearity: The linear regression data for the calibration curves showed good linear relationship over the concentration range 200–1200 ng/spot for BF and 100-800 ng/spot for HCTZ. Linear regression equation was found to be Y=2.5096X+262.47 (r^2=0.9991) for bisoprolol fumarate and Y=8.8624X+58.55 (r^2=0.9989) for hydrochlorothiazide.

Precision: The precision of the developed HPTLC method was expressed in terms of % relative standard deviation (% R.S.D.). The results depicted revealed high precision of the method is presented in table 1.

LOD and LOQ: Detection limit and quantitation limit was calculated by the method as described in the LOQ and LOD for BF were 104.54 ng and 34.59 and for HCTZ, LOQ and LOD were found to be 50.09 ng and 16.25 ng. This indicates that adequate sensitivity of the method.

Accuracy: The proposed method when used for extraction and subsequent estimation of both the drug from pharmaceutical dosage forms after over spotting with 80, 100 and 120% of additional drug; afforded recovery of 98-102% as listed in table 2.

Drugs	Conc. ng/ml	Intra-day		Inter-day	
		% Amount found*	% R.S.D.	% Amount found*	% R.S.D
BF	200	100.22	0.41	99.33	0.88
	400	99.20	0.74	100.93	0.79
	600	98.65	0.88	98.18	0.96
HCTZ	250	98.80	1.25	99.80	0.93
	500	100.89	1.89	101.26	1.69
	750	100.69	1.51	101.74	0.64

* mean of three estimations

Table 1: Intra-day and inter-day precision of HPTLC method.

Drugs	Label claim (mg/tablet)	Amount of standard drug added (%)	% Drug Recovered	% R.S.D.
BF	5	0	100.84	1.43
		80	100.97	1.57
		100	99.98	0.81
		120	98.31	0.76
HCTZ	6.25	0	101.64	1.46
		80	99.80	1.23
		100	100.18	0.84
		120	98.03	0.31

* mean of three estimations at each level

Table 2: Results of recovery studies.

Parameter	BF	HCTZ
Linearity range (ng/ spot)	200–1200	100–800
Correlation coefficient	0.9991	0.9989
Limit of detection (ng/spot)	34.59	16.2530.26
Limit of quantitation (ng/spot)	104.54326.62	50.0991.71
% Recovery (n=9)	99.71	100.08
Ruggedness (% R.S.D.)		
Analyst I (n=3)	1.19	0.95
Analyst II (n=3)	0.71	0.71
Precision (%R.S.D.)		
Repeatability of application (n=6)	1.63	0.910.84
Intra-day (n=3)	0.28–0.94	0.46–0.61
Inter-day (n=3)	0.45–1.19	1.19–0.83
Robustness	Robust	Robust
Specificity	Specific	Specific

Table 3: Summary of validation parameter.

Robustness: The standard deviation of peak areas was calculated for each parameter and % R.S.D. was found to be less than 2%.

Ruggedness of the method: Ruggedness of the method was performed by applying 400 ng and 500 ng for BF and HCTZ, respectively by two different analyst keeping same experimental and environmental conditions. The results summarized in table 3.

Conclusion

The developed HPTLC method is simple, precise, accurate and reproducible and can be used for simultaneous determination of BF and HCTZ in tablets. The method was validated as per ICH guidelines.

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