

SIMULTANEOUS HPTLC ANALYSIS OF BISOPROLOL FUMARATE AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: The present study was designed with an objective of a simple, fast, precise, selective and accurate HPTLC method was developed and validated for the simultaneous determination of bisoprolol fumarate and hydrochlorothiazide from bulk and formulations.

Methods: High performance thin layer chromatography (HPTLC) method was developed and validated for rapid analysis of simultaneous determination of bisoprolol fumarate and hydrochlorothiazide. Chromatographic separation was achieved on precoated silica gel HPTLC aluminum plate 60 F₂₅₄ using ethyl acetate: methanol: ammonia 10:0.5:0.5 (v/v) as mobile phase. Detection was performed at 225 nm wavelength densitometrically.

Results: The R_F of bisoprolol fumarate and hydrochlorothiazide were 0.60 and 0.38, respectively. Linearity was observed in the concentration range of 150-900 ng/spot for bisoprolol fumarate and 100-600 ng/spot for hydrochlorothiazide. Percent recoveries obtained for both the drugs were 99.45 ± 0.06% for bisoprolol fumarate and 100.25 ± 1.20 % for hydrochlorothiazide, respectively. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

Conclusion: The method developed can be used for the routine analysis of bisoprolol fumarate and hydrochlorothiazide from their combined dosage form.

Keywords: Bisoprolol fumarate, Hydrochlorothiazide, HPTLC, Validation

INTRODUCTION

Bisoprolol fumarate (BISO) is a synthetic, beta₁-selective (cardioselective) adrenoceptor blocking agent. The chemical name for bisoprolol fumarate is (±)-1-[4-[[2-(1-Methylethoxy) ethoxy] methyl] phenoxy]-3-[(1-methylethyl) amino]-2- propenol (E)-2-butenedioate (2:1) (salt). It possesses an asymmetric carbon atom in its structure and is provided as a racemic mixture. The S (-) enantiomer is responsible for most of the beta-blocking activity. Bisoprolol fumarate is official in USP [1]. Bisoprolol fumarate can be determined by UV [2-4], RP-HPLC [5-8], LC-MS/MS [9] and HPTLC

[10-12] methods have been reported for analysis of bisoprolol fumarate either alone or in combination with other drugs in pharmaceutical formulations. Hydrochlorothiazide (HCTZ) is 6-Chloro-3,4-dihydro-2H-1,2,4-benzothiazine-7-sulfonamide 1,1-dioxide. Hydrochlorothiazide is a diuretic/ antihypertensive agent. The diuretic drug hydrochlorothiazide (HCTZ) is used mainly for treatment of mild to moderate hypertension and is usually administered with other drugs. Spectrophotometric [13-17], HPTLC [18-19], HPLC [20-23] and LC-MS [24-25] methods have been reported for its determination in pharmaceutical formulations and biological fluids. The structures of the drugs are shown in Fig. 1.

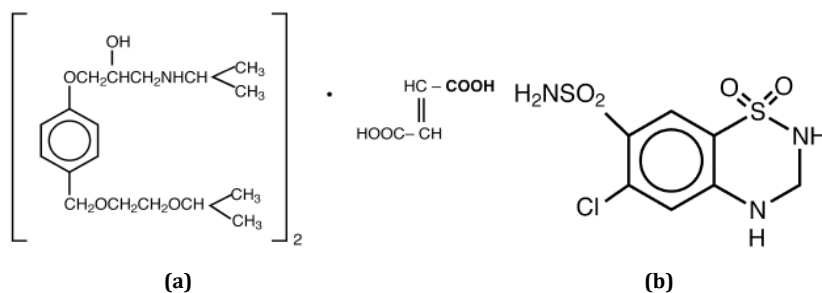


Fig. 1: The chemical structures of Bisoprolol fumarate (a) and Hydrochlorothiazide (b)

Although the combinational use of bisoprolol fumarate and hydrochlorothiazide continuously increasing and HPTLC assays offer significant economic advantages over the techniques cited above, the aim of the present investigation was to develop simple and sensitive method for simultaneous determination of bisoprolol fumarate and hydrochlorothiazide in combined dosage form. The developed method is simple, precise, selective, and rapid and can be used for routine analysis.

MATERIALS AND METHODS

Chemicals and Reagents

Bisoprolol fumarate and Hydrochlorothiazide were supplied, as a gift sample, by Intas Pharmaceuticals Ltd, Ahmedabad, India and

Encure Pharmaceuticals Ltd., Pune, India. Lodoz 2.5 tablet containing 2.5 mg bisoprolol fumarate and 6.25 mg hydrochlorothiazide were obtained commercially within their shelf life. All chemicals and reagent used were of AR grade and were purchased from Merck Chemicals, Mumbai, India.

Preparation of Standard and Sample Solutions

A standard mixed stock solution of bisoprolol fumarate and hydrochlorothiazide was prepared by accurately weighing 10 mg bisoprolol fumarate and 10 mg hydrochlorothiazide into a 10-mL volumetric flask. The drugs were dissolved in methanol and the solution was diluted to volume. Twenty tablets of the pharmaceutical formulation Lodoz 2.5 tablet containing 2.5 mg bisoprolol fumarate and 6.25 mg hydrochlorothiazide were assayed.

They were crushed to a fine powder and an amount of the powder corresponding to approximately 2.5 mg bisoprolol fumarate and 6.25 mg hydrochlorothiazide was weighed in a 25 mL volumetric flask. After addition of 15 mL methanol and sonication (30 min) the solution was diluted to volume with methanol and filtered through a Whatman no. 41 filter paper.

Chromatographic conditions

TLC was performed on aluminium foil plates coated with 0.2-mm layers of silica gel 60F254 (Merck). Before use plates were prewashed with methanol then dried and activated. Samples were applied to the plates, as 6-mm bands, by means of a Camag Linomat 5 sample applicator used at a constant application rate of 0.1 μ L/s. Plates were developed with ethyl acetate: methanol: ammonia 10:0.5:0.5 (v/v) as mobile phase in a Camag twin-trough chamber previously saturated with mobile phase vapour for 20 min at room temperature ($25 \pm 2^\circ\text{C}$). The development distance was approximately 80 mm. After development the plates were scanned in absorbance mode at 225 nm by use of a Camag TLC Scanner 3 controlled by winCATS software. The slit dimensions were 5 mm \times 0.45 mm and the source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190–400 nm.

Sample Analysis

From the filtered sample solution 1 μ L was applied to a TLC plate followed by development and scanning. The analysis was repeated five times.

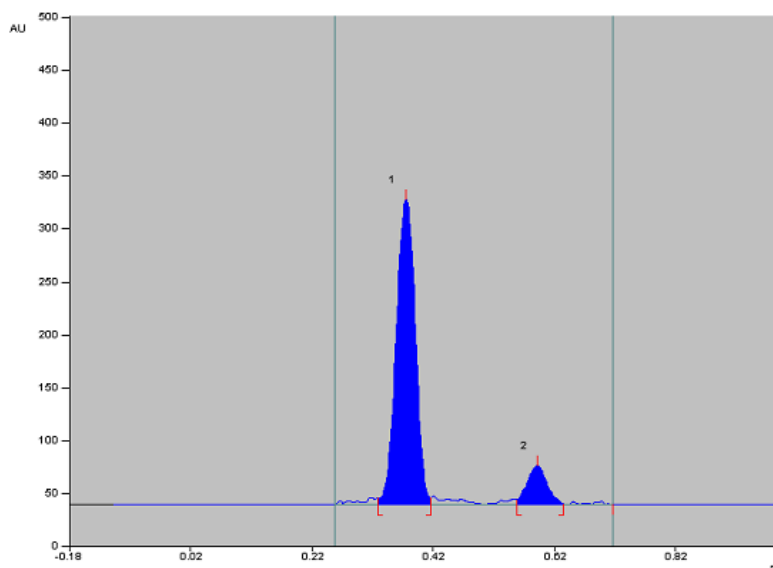


Fig. 2: Densitogram obtained from peak 1 hydrochlorothiazide and peak 2 bisoprolol fumarate

Accuracy

Analysed samples were overapplied with an extra 80, 100, and 120% of the drugs from standard solutions of bisoprolol fumarate and hydrochlorothiazide and the mixtures were reanalyzed by use of the method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulation.

Robustness

Robustness was assessed by deliberately changing the chromatographic conditions and studying the effects on the results obtained.

RESULTS AND DISCUSSION

Method Development

The TLC procedure was optimized for simultaneous determination of bisoprolol fumarate and hydrochlorothiazide. The mobile phase

Method Validation

Precision

The intra-day precision (RSD, %) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (RSD %) was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week.

Limits of Detection and Quantitation

To determine the limits of detection (LOD) and quantitation (LOQ), solutions of concentration in the lower part of the linear range of the calibration plot were used. LOD and LOQ were calculated using the equations $\text{LOD} = 3.3 \times N/B$ and $\text{LOQ} = 10 \times N/B$, 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.

Specificity

The specificity of the method was ascertained by analysis of drug standards and samples. The mobile phase resolved both the drugs very efficiently, as shown in Fig. 2. The identities of the bands for bisoprolol fumarate and hydrochlorothiazide were confirmed by comparing the R_f and spectra of the bands with those of standards.

ethyl acetate: methanol: ammonia 10: 0.5:0.5 (v/v) resulted in good resolution, and sharp and symmetrical peaks of R_f 0.60 for bisoprolol fumarate and 0.38 for hydrochlorothiazide. 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 20 min (optimum chamber saturation time) ensured good reproducibility and peak shape of both drugs.

Validation

Linearity

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 150–900 ng/spot for bisoprolol fumarate and 100–600 ng/spot for hydrochlorothiazide. The linear regression equations were $Y = 3.611X - 236.6$ ($r^2 = 0.999$) for bisoprolol fumarate and $Y = 3.215X + 326$ ($r^2 = 0.999$) for hydrochlorothiazide. The plots obtained from linear regression and residuals analysis are given in Fig 3a and 3b for bisoprolol fumarate and 4a and 4b for hydrochlorothiazide.

Precision

The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table 1 reveal the high precision of the method.

Limits of Detection and Quantitation

The limits of detection and quantitation, calculated as described above, were 50 and 100 ng, respectively, for bisoprolol fumarate and 25 and 50 ng for hydrochlorothiazide. This indicates the method is sufficiently sensitive.

Accuracy

When the method was used for extraction and subsequent analysis of both drugs from the pharmaceutical dosage forms, and the extract was over applied with 80, 100, and 120% of additional drug, the recovery was listed in Table 2.

Robustness

The relative standard deviation of peak areas was less than 2%. The RSD shown in Table 3 indicate the robustness of the method.

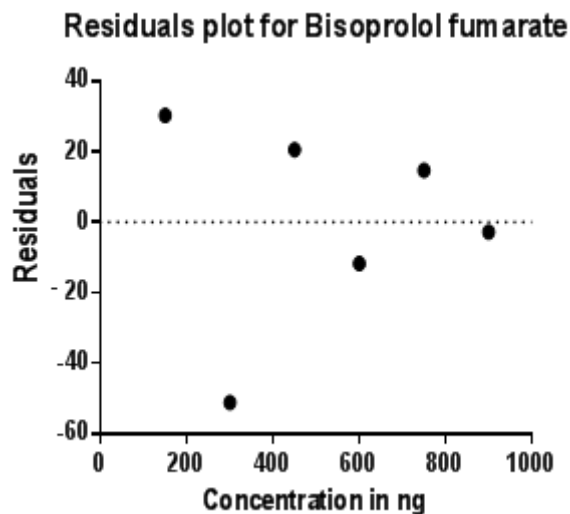
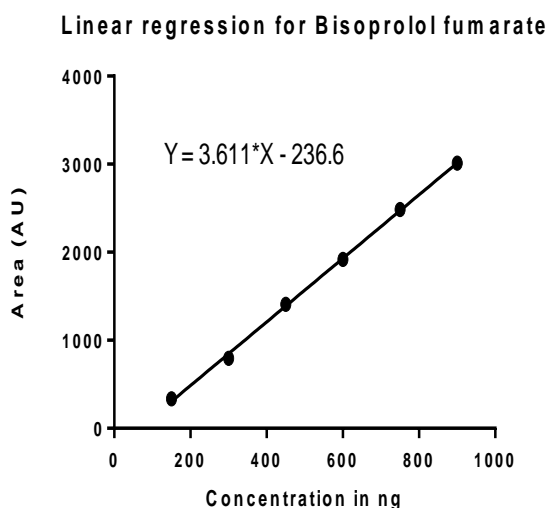


Fig. 3a & 3b: Linear regression for Bisoprolol fumarate and Residual plot for Bisoprolol Fumarate

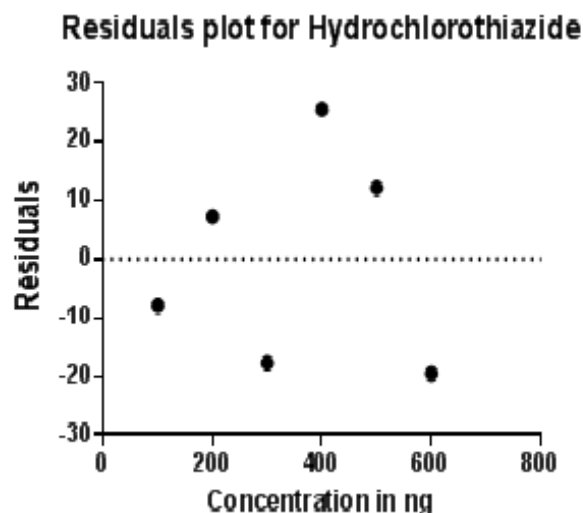
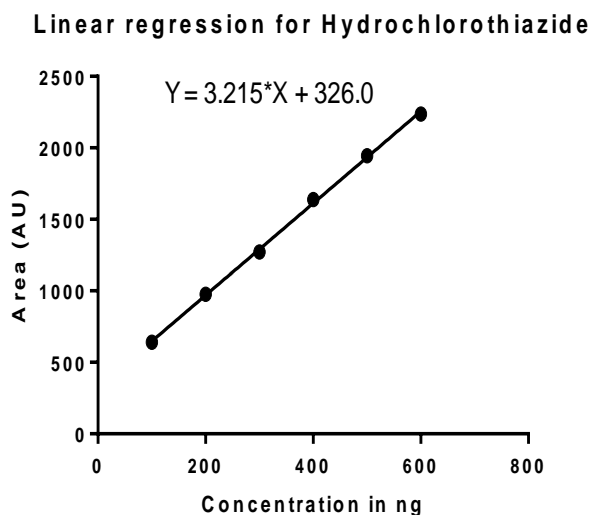


Fig. 4a & 4b: Linear regression for Hydrochlorothiazide and Residual plot for Hydrochlorothiazide

Table 1: Intra-day and inter-day precision of the method

Drugs	Conc. (ng/spot)	Intra-day precision		Inter-day precision	
		Amount found (%)*	% RSD	Amount found (%)*	% RSD
Bisoprolol fumarate	300	99.50	0.47	99.57	0.21
	600	99.45	0.81	99.10	0.71
	900	99.39	1.01	99.67	0.53
Hydrochlorothiazide	200	100.85	1.05	99.32	0.96
	400	101.77	0.31	99.43	0.79
	600	98.94	0.18	99.71	1.36

*Mean from three analyses

Table 2: Results from recovery studies

Drugs	Label claim (mg/tablet)	Amount Added (%)	Total Amount (mg)	Amount Recovered (mg)	Recovery ^a (%)
Bisoprolol fumarate	2.5	80	4.5	4.47	99.33
		100	5	4.96	99.20
		120	5.5	5.47	99.45
Hydrochlorothiazide	6.25	80	11.25	11.21	99.64
		100	12.50	12.45	99.60
		120	13.75	13.69	99.56

^aMean from three estimates at each level

Table 3: Robustness of the method^a

Condition	Bisoprolol fumarate		Hydrochlorothiazide	
	SD of peak area	RSD (%)	SD of peak area	RSD (%)
Mobile phase composition				
ethyl acetate: methanol: ammonia 10: 1: 0.5 (v/v)	5.78	0.85	7.41	1.16
ethyl acetate: methanol: ammonia 9.5: 0.5: 0.5 (v/v)	7.80	0.75	8.30	0.85
Development distance				
7 cm	6.43	1.32	8.00	1.20
7.5 cm	6.87	0.86	8.34	0.97
8 cm	5.30	0.76	7.20	0.64
Duration of saturation				
20 min	5.26	0.72	7.43	0.52
25 min	6.54	1.09	7.98	1.28
30 min	6.98	1.45	8.37	1.13
Time from application to chromatography + 20 min.	6.75	1.20	8.91	1.43
Time from chromatography to scanning + 20 min	7.10	0.95	8.53	1.39

^aMean from three estimates

Sample Analysis

When the Lodoz 2.5 tablets were analysed, sharp and well defined peaks for bisoprolol fumarate and hydrochlorothiazide were obtained at R_f 0.60 and 0.38, respectively, when scanned at 225 nm. The amount of the label claim measured were 99.45 ± 0.06% for bisoprolol fumarate and 100.25 ± 1.20 % for hydrochlorothiazide.

CONCLUSION

The results of the analysis of pharmaceutical dosage forms by the proposed HPTLC method are highly reliable and are in good agreement with the labeled claim of the drug. The percent recoveries obtained was 99.20 to 99.64 indicates none interference from the common excipients in the tablet formulations. The proposed HPTLC method is found to be simple, sensitive, accurate, precise, specific and robust can be used for the routine simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in pharmaceutical tablet formulation.

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