HPTLC method development and validation for the estimation of Labatelol Hydrochloride in tablet dosage form

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ABSTRACT

A simple, selective, linear, precise and accurate HPTLC method was developed and validated for rapid assay of Labatelol Hydrochloride in tablet dosage form. The separation was achieved on aluminum plate 60F254, (10 × 10 & 20 × 10 cm) with 250 µm thickness as the stationary phase and the mobile phase consisted of chloroform: methanol: ammonia (8:2:0.2, v/v). The solvent system was found to give compact spot for Labatelol Hydrochloride (Rf values of 0.49). Densitometric analysis was carried out in the absorbance mode at 254 nm. The linear regression analysis data for the calibration plots showed good linear relationship with respect to peak area in the concentration range 0.50-1.50 µg spot-1 of Labatelol Hydrochloride (with r = 0.99774). The method was validated for limit of detection, limit of quantitation, accuracy, precision, robustness and recovery. The result and statistical analysis proves that the developed method is reproducible and selective for the estimation of said drug. The proposed method can be successfully applied for the estimation of Labatelol Hydrochloride in tablet dosage forms.

Keywords: Labatelol Hydrochloride; HPTLC; Densitometric analysis Validation.
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INTRODUCTION

Labatelol Hydrochloride (Fig. 1) is chemically 2- hydroxy- 5- [1- hydroxy- 2- (1- methyl - 3 phenyl propylamino) ethyl] benzamide hydrochloride is an adrenergic β-receptor blocking agent used in the treatment of hypertension, which exhibits both α- and β-adrenoceptor blocking activity 1, 2, and because of its use as doping agent in sports, this drug has been added to the list of forbidden substances issued by the International Olympic Committee. Therefore, the development of an analytical method sensitive and selective enough for determining Labatalol in both pharmaceutical and biological samples is of great importance.

Several analytical methods have been developed to determine the concentrations of Labetalol in biological fluids and pharmaceutical preparations based on spectrophotometry 3- 8 spectro-fluorometry 8, 9, potentiometry 10, thin-layer chromatography (TLC) 11, 12, high performance liquid chromatography (HPLC) with UV 13 and electrochemical detection (ED) 14, gas chromatography (GC) 15 and micellar liquid chromatography 16. Although these methods have been successfully employed, they require long and tedious steps for the sample pretreatment.

In many cases, highly specific mass spectrometric detection, especially using tandem mass spectrometry (MS–MS) just requires minimum separation on column. This will greatly shorten the assay time and make it possible to analyze large quantities of samples within a tight time frame. To date no LC–MS/MS method has been reported for quantitation of Labetalol in human plasma. In contrast, we report an LC– MS/MS method with an excellent

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sensitivity for 20µL injection volume corresponding to 3.1800 ng/mL on-column with a total run time of 2.5 min. This paper describes the development and validation of highly sensitive HPTLC method for the quantitation of Labetalol.
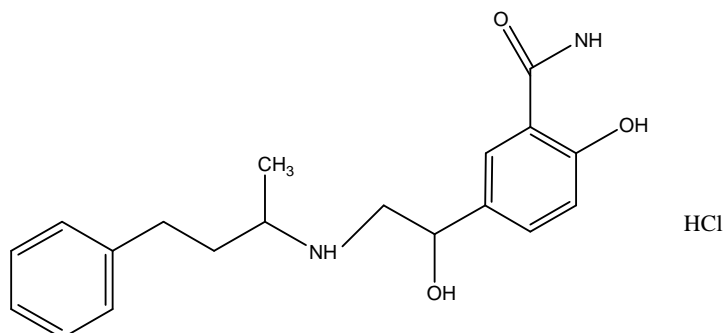

Fig. 1: Chemical structures of Labatelol Hydrochloride

MATERIALS AND METHODS

Instrumental and analytical conditions:
 Standard experimental conditions were optimized in view to develop an assay method to quantify Labatelol Hydrochloride as in its tablet dosage form. Samples was spotted in the form of band of 2 mm with Camag microlitre syringe on pre-coated silica gel aluminum plate 60F254, (10 × 10 & 20 × 10 cm) with 250 µm thickness; using CsAMAG LINOMAT 5 semiautomatic sample applicator and LINOMAT V automatic sample applicator with help of (Hamilton-100 µl Switzerland) syringe. The plates were prewashed with methanol so as to remove adhere impurity and activated at room 120°C for 5 min prior to chromatography. Samples were applied as band at a distance of 8 mm from lower edge and the distance between two bands was 4 mm. The mobile phase consisted of chloroform: methanol: ammonia (8:2:0.2v/v/v) was optimized for good resolution with compact spots. The length of chromatogram run was 80 mm. Subsequent to the development; TLC plate was dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorption mode at 254 nm. Reagents and chemicals: Analytically pure Labatelol Hydrochloride and tablet formulation was gifted by Cadila Zydus Pharmaceutical Limited, Thane. All chemicals and reagents used were of AR grade, from Merck Chemicals (Mumbai, India).

Preparation of Analytical solutions:
Preparation of mobile phase:
Mobile phase was prepared by mixing 8 ml chloroform, 2 ml of methanol and 0.2 ml of ammonia.

 Preparation of standard stock solution:
The stock solutions (1000 µg/ml) of Labetalol Hydrochloride was prepared by accurately dissolving 10 mg of the drugs with sufficient methanol in 10 ml volumetric flask and then the volume was made separately to 10 ml with methanol. Preparation of standard solution: 5.0ml of Labetalol Hydrochloride stock solution further diluted to 10 ml with methanol to get final concentration of 0.5 µg/µl of Labetalol Hydrochloride. Then further take 5 ml and diluted to 10 ml to get concentration 0.25 µg/µl.

Preparation of sample stock solution:
Twenty tablets were weighed and average weight was calculated. The tablets were triturated to a fine powder. Anaccurately weighed quantity of powder equivalent to 100 mg of Labetalol Hydrochloride was transferred to 10 ml volumetric flask. To it add 5 ml of methanol shake well and sonicated for 10 min. The resultant solution was filtered through 0.45µm membrane filter, diluted to volume with methanol to get stock sample solution containing 10 µg/µl of Labetalol Hydrochloride.

Preparation of sample solution:
 0.5 ml stock sample solution was further diluted to 10 ml with methanol to get concentration of 0.5 µg/µl of Labetalol Hydrochloride Then further take 5 ml and diluted to 10 ml to get concentration 0.25 µg/µl. Sample solution (2µl) was applied on TLC plate, developed and scanned under standard chromatographic condition.

Analysis of the marketed formulation:
To determine the content of commercial formulation the solution were prepared as described in preparation of sample solution. Mean peak area of the drug was calculated and the drug content in the tablets was quantified. Method development and validation of HPTLC

Linearity:
Standard solution of Labetalol Hydrochloride (0.25 µg/µl) was prepared in methanol. 2, 3, 4, 5, and 6 µl of standard solution was applied to TLC plate so as to give concentration 0.5, 0.75, 1.0, 1.25, 1.50, and 1.75 µg spot-1 for Labetalol Hydrochloride. The data of peak area plotted against corresponding concentration was treated by linear least-square regression analysis. (Fig. 3)

Precision:
Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions. Interday precision and intraday precision were determined both in terms of repeatability (injection and analysis). The intermediate precision of method was checked by repeating the study on different days. The repeatability of sample application and measurement of peak area was determined by performing six replicate measurements of the same band. The intermediate precision of method was checked by repeating the study on different days.

Accuracy:
The recovery studies were carried out by adding known amount of standard to samples at 80, 100 and 120% level and analyzed by the proposed method, in triplicate. This was done to check the recovery of the drug at different levels in the formulations by optimized method.

Limit of detection and limit of quantitation:
The limits of detection and quantitation of the developed method were calculated for Labetalol Hydrochloride using the formula as given below.

Limit of Detection=3.3 x σ/S
Limit of Quantitation=10 x σ/S

Where, "σ" is the standard deviation of the response, "S" is the slope of the calibration curve.

Specificity: The specificity of the method was ascertained by analyzing the standard drug and sample with respect to Rf value and spectra. The peak purity of Labetalol Hydrochloride was assessed by comparing the spectra of diluents, mobile phase, standard and sample.

RESULTS AND DISCUSSION

The present investigation reported a new HPTLC method development and validation of estimation of Labetalol Hydrochloride. The method developed was proceeding with wavelength selection. The optimized wavelength was 254nm. (Fig . 2) In order to get the optimized HPTLC method various mobile phases were used. The mobile phase consisted of an aqueous solution of chloroform: methanol: ammonia (8: 2: 0.2 v/v) was used and the Rf value was about 0.49. The specificity of the method was determined for presence of components that may be unexpected to be present. The absence of additional peaks in the chromatogram indicates non interference of the excipients in the tablet dosage form. The linearity was determined in analyte concentration range of 0.5-1.50 µg spot-1. The calibration curve obtained by plotting peak area versus concentration was linear and the correlation coefficient was found to be 0.99774 for Labetalol Hydrochloride. (Table 1, Fig. 4) The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The repeatability, interday and intraday were calculated for Labetalol Hydrochloride. (Table 3) The accuracy study was performed in 80%, 100% and 120%. The percentage recovery was determined for propafenone HCl and was found to be 99.12% (Tables 4). Assay of Labetalol Hydrochloride in its tablet dosages form was calculated. (Table 2). A typical chromatogram showing the separation of Labetalol Hydrochloride is shown in Fig. 3
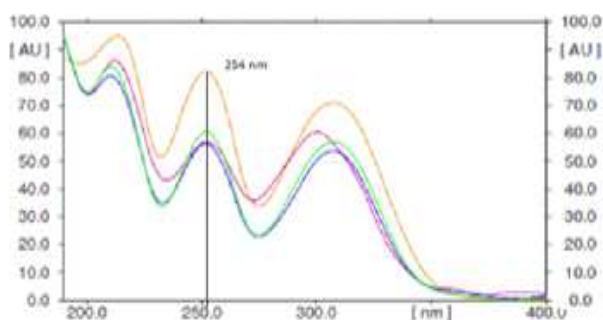
Fig. 2: Overlain spectra for selection of wavelength (254 nm) for Labetalol Hydrochloride

Table No. 1: Regression Statistics for analysis of Labetalol Hydrochloride

Range	R2	Slope	LOD	LOQ
0.50-1.50 µg/spot	0.99774	366.5 + 3.911	0.16 µg/spot	0.50 µg/spot

Table No. 2: For asssay of marketed formulation

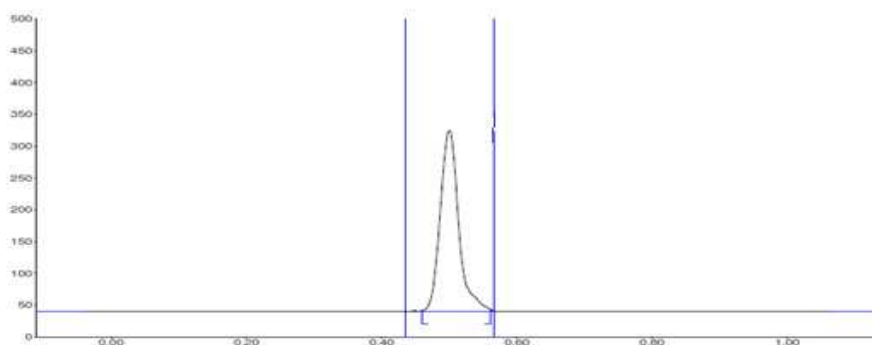| Drug	Area of standard	wt.of standard (mg)	Area of sample	wt.of sample (mg)	% purity	% lable claim
Labetalol HCl	8400	10 mg	7089	171.4	100	98.25

Fig. 3: Representative chromatogram of Labetalol Hydrochloride Standard
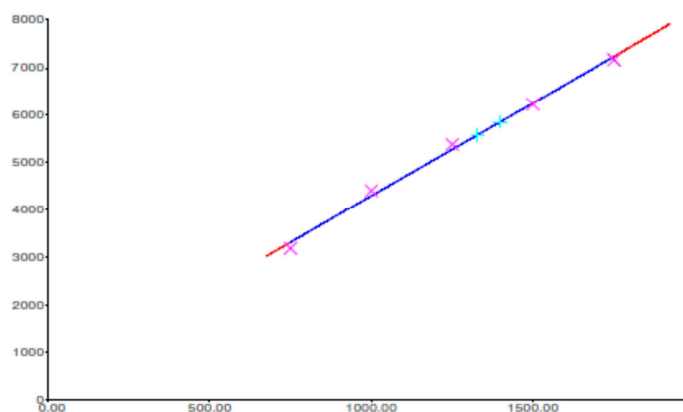

Fig. 4: Calibration curve of Labetalol Hydrochloride

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Table No. 3: Repeatability of Labetalol Hydrochloride

Rf value	Mean Rf value	Repeatability	Mean area	% CV
		Area		
0.49		6113.3		
0.49		6152		
0.49	0.49	6281.45	6295.62	2.729
0.5		6409.92		
0.5		6521.42		
		Intr-day repeatability		
0.5		6801.85		
0.5		6458.58		
0.51	0.51	6502.8	6541.4	2.518
0.51		6578.3		
0.51		6365.49		
		Inter-day repeatability		
0.5		6537.33		
0.51		6138.8		
0.5	0.51	6123.69	6180.03	3.558
0.51		6166.7		
0.51		5933.63		

Table No. 4: Recovery Analysis of Labetalol Hydrochloride

Drug	Level of addition %	Amt.of std.solution I applied (µl)	Amt.of pure drug added (µl)	% Recovery	% RSD
Labetalol HCl	80	1	3.2	99.02	1.28
	100	1	4	99.7	1.3
	120	1	4.8	98.65	0.82

* Each value corresponds to the mean of three determinations

CONCLUSION

The developed HPTLC method enables accurate, precise and specific for determination of Labetalol Hydrochloride. Statistical analysis proves that the method is reproducible and selective for routine analysis of Labetalol Hydrochloride in pharmaceutical dosage form without interference from excipients.

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