International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.3, pp 1376-1379, July-Sept 2010

Development and Validation of High Performance Thin Layer Chromatographic Method for Estimation of Brimonidine Tartrate as Bulk Drug and in Ophthalmic Solutions

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Abstract: A simple, specific and precise high performance thin layer chromatographic method was developed and validated for estimation of Brimonidine tartrate as bulk drug and in ophthalmic solutions. The chromatographic development was carried out on precoated silica gel 60 F_{254} aluminium plates using mixture of Methanol: Ammonia (8:0.2 v/v) as mobile phase and densitometric evaluation of band was carried out at 250 nm using Camag TLC Scanner-3 with win CAT 1.4.3 version software. The R_f value of drug was found to be 0.52 ± 0.01. The method was validated with respect to linearity, accuracy, precision and robustness. The calibration curve was found to be linear over a range of 200- 1200 ng/ band. The % assay (Mean ± S.D.) was found to be 101.3 ± 1.02. Thus the proposed HPTLC method was found to provide a faster and cost effective quantitative control for routine analysis of brimonidine tartrate as bulk drug and in ophthalmic solutions.
Key words: Brimonidine tartrate, HPTLC, Ophthalmic solutions.

Introduction

Brimonidine tartrate, chemically, 5-bromo-6-(2-imidazolidinylideneamino) quinoxaline L-tartrate is selective alpha-2 adrenergic receptor agonist used in the treatment of open-angle glaucoma or ocular hypertension[1].

Literature survey reveals few HPLC [2-4], LC-MS [5] methods reported for the estimation of brimonidine tartrate in blood serum and in ocular fluids. Stability-indicating assay method using hydrophilic interaction liquid chromatography (HILIC) has also been reported[6].

No HPTLC method reports were found for the estimation of brimonidine tartrate in pharmaceutical preparations. The present study describes the development and validation of a simple, specific, sensitive, accurate and precise HPTLC method for the determination of brimonidine tartrate in ophthalmic solutions. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines[7].

Experimental

Reagents and chemicals

Brimonidine tartrate was obtained as generous gift sample from Cipla Ltd. (Mumbai, Maharashtra). Methanol, Ammonia (all AR grade) were used for the method development. The pharmaceutical dosage form used in this study was Brimosun P 0.15 % ophthalmic solution (Sun Pharma Ltd., Turbhe, Navi Mumbai, India) labeled to contain 5 ml of brimonidine tartrate was procured from local market.

Instrumentation and chromatographic conditions

The samples were spotted in the form of bands of width of 8 mm with space between bands of 5 mm, with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F_{254} (10 ×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 6 mm × 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using mobile phase Methanol: Ammonia (8:0.2 v/v). The optimized chamber saturation time for mobile phase was 20 min. The length of chromatogram run was 9 cm and development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner 3 at 250 nm for all developments operated by WINCATS software version 1.4.3. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of Standard Stock Solution

Standard stock solution of brimonidine tartrate was prepared by dissolving 10 mg of drug in10 mL of methanol to get the concentration of 1 mg/mL from which 1 mL was further diluted to 10 mL with methanol to obtain a working standard having a concentration of 100 ng/µL.

Validation of method:

The method was validated as per the ICH guidelines in terms of linearity, accuracy and specificity, intra-day and inter-day precision, repeatability of measurement of peak area as well as repeatability of sample application.

Preparation of calibration curve

For the preparation of a calibration curve, aliquots 2, 4, 6, 8, 10, 12 µl of standard stock solution of brimonidine tartrate (100 ng/µL) were applied on the TLC plate under nitrogen stream. TLC plates were developed under above established conditions. Area under peak was recorded and plotted against concentration.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for drug was confirmed by comparing the R_f and spectra of the sample spots with that of standard drug.

Recovery Studies

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150 %. Chromatogram was obtained and the peak areas were noted. At each level of the amount, three determinations were carried out.

Intra-day and inter-day precision

The intra-day precision was determined by analyzing standard solutions of brimonidine tartrate in range 200-1200 ng/band for three times on the same day while inter-day precision was determined by analyzing corresponding standards on three different days over a period of one week.

Repeatability of measurement of peak area as well as repeatability of sample application

Repeatability of measurement of peak area was determined by applying 4 µL of standard drug solution on TLC plate. After developing the plate, band of drug was scanned six times without changing position of the plate and RSD value was calculated. Repeatability of sample application was assessed by applying 4 µL of standard drug solutions six times on a TLC plate by semiautomatic applicator, followed by development of plate and recording the peak areas for six spots. The RSD for the peak area values was calculated.

Robustness studies

Robustness studies were carried out by examining the effect of small, deliberate variation of the analytical conditions on the peak areas of the drug. Factors varied were volume of mobile phase (± 0.5 %), time from application to development (0, 10, 20, and 30 min) and from development to scanning (0, 30, 60, and 90 min). One factor at a time was changed to study the effect. The robustness of the method was checked at amount of 400 ng/band.

Assay of the marketed formulation

For the assay of marketed formulation, 1 mL of the marketed sample solution was pipetted out using a volumetric pipette and transferred to a 10 mL of

volumetric flask and diluted with methanol to get the concentration of 150 ng/µL. Two µL of this solution was applied on the plate. After chromatographic development peak areas of the bands were measured at 250 nm and the amount of drug present in sample was estimated from the calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Table 1. Recovery studies of brimonidine tartrate

Amount taken (ng/band)	Amount added (ng/band)	Total amount found (ng/band)	% Recovery	% Recovery (Mean ± S.D.)
300	150	452.97	100.66	99.72 ± 0.733
300	300	598.44	99.74	
300	450	744.08	99.21	

Table 2. Robustness Data in Terms of Peak Area

Sr. No.	Parameter Varied	% RSD
1	Volume of mobile phase	0.98
2	Time from application to development (Mins.)	1.55
3	Time from development to scanning (Mins.)	1.26

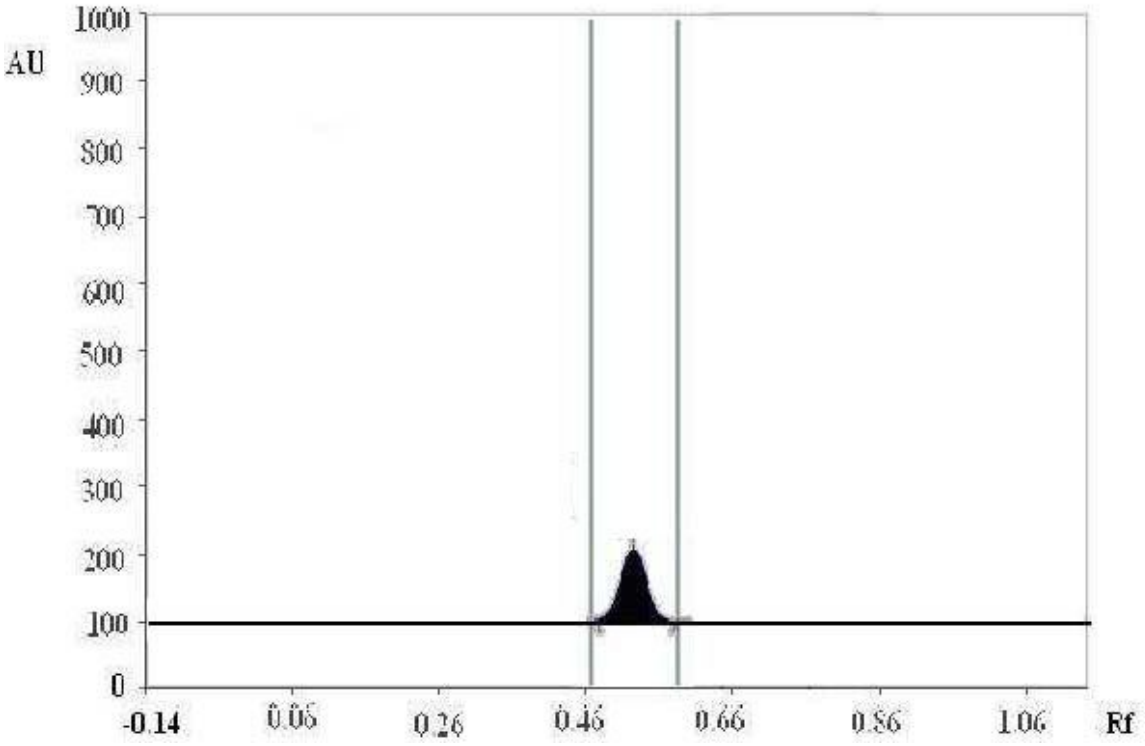

Figure 1: Representative densitogram of Brimonidine Tartrate (400 ng/ band, R_f = 0.52 ± 0.01)

Results and Discussion

Literature survey revealed that few HPLC and LC-MS methods have been reported for estimation of brimonidine tartrate which are sophisticated but costly and time consuming. As no HPTLC method has been reported so far for estimation of brimonidine tartrate, the present study was aimed at development of speedy and cost effective HPTLC technique for determination of brimonidine tartrate as bulk and in ophthalmic solutions.

The mixture of Methanol: Ammonia (8:0.2 v/v) as mobile phase gave better peak shape. The R_f value of drug was found to be 0.52 ± 0.01 (Figure 1).

The method was found to be linear in the range of 200 to 1200 ng/ band. The spectrum of brimonidine tartrate in ophthalmic solution compared with spectrum of standard brimonidine tartrate showed good correlation, confirm the specificity of the proposed method. The results of recovery study indicate that the proposed method is accurate for estimation of drug in ophthalmic solutions (Table 1).

The intra-day and inter-day relative standard deviations were found in the range 0.76-1.84 % and 0.89-1.79 % respectively. The smaller values of intra-day and inter-day variation in the analysis indicate that the method is precise. RSD for repeatability of measurement of peak area and repeatability of sample application were found to be 0.579 % and 1.592 %, respectively. The RSD values for measurement of peak area and sample application were both below the instrumental specifications (1 % and 3 %, respectively), ensuring proper functioning of HPTLC system. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% RSD < 2). The results are given in Table 2. The % assay (Mean ± S.D., n = 6) was found to be 101.30 ± 1.02.

The validated HPTLC method proved to be simple, less expensive, fast, accurate, precise and robust and thus can be used for routine analysis of brimonidine tartrate in ophthalmic solutions.

Acknowledgements

Authors are thankful to Cipla Ltd. (Mumbai, Maharashtra) for providing gift sample of brimonidine tartrate.

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