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Validated High Performance Thin Layer Chromatographic Method for Estimation of Olopatadine Hydrochloride 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 olopatadine hydrochloride as bulk drug and in ophthalmic solution. The chromatographic development was carried out on precoated silica gel 60 F_{254} aluminium plates using mixture of Methanol: Water: Glacial Acetic acid (8:2:0.2 v/v/v) as mobile phase and densitometric evaluation of band was carried out at 247 nm using Camag TLC Scanner-3 with win CAT 1.4.3 version software. The R_f value of drug was found to be 0.37 ± 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 to 1200 ng/ band. The method has been successfully applied for the analysis of drug in ophthalmic solutions. The % assay (Mean ± S.D.) was found to be 100.51 ± 1.01 for olopatadine hydrochloride. Thus the proposed HPTLC method was found to provide a faster and cost effective quantitative control for routine analysis of olopatadine hydrochloride as bulk drug and in ophthalmic solutions.

Key Words: Olopatadine hydrochloride, High performance thin layer chromatography, Ophthalmic solution.

Introduction

Olopatadine hydrochloride, chemically, 11-[(Z)-3-(Dimethylamino) propylidene]-6-11-dihydro dibenz [b,e] oxepin-2-acetic acid hydrochloride is a dibenzoxipine derivative used for systemic treatment of allergic rhinitis, urticaria, and bronchial asthma. It is a selective inhibitor for the release of histamine and other pro-inflammatory mediators from the mast cell[1].

Literature survey reveals that several LC-MS methods have been reported for the estimation of Olopatadine hydrochloride in human plasma[2-4]. No HPTLC method reports were found for the estimation of Olopatadine hydrochloride 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 Olopatadine hydrochloride in ophthalmic solutions. The proposed method is optimized and validated as per the

International Conference on Harmonization (ICH) guidelines[5].

Experimental

Reagents and chemicals

Olopatadine hydrochloride was obtained as generous gift sample from Intas Pharma, (Ahmedabad, Gujrat). Methanol, Ammonia, Glacial Acetic acid (all AR grade) were used for the method development. The pharmaceutical dosage form used in this study was OLOPINE 0.1 % ophthalmic solution (Ajanta Pharma, India) labeled to contain 5 ml of Olopatadine hydrochloride was procured from local pharmacy.

Instrumentation and chromatographic conditions

The samples were spotted in the form of bands of width of 6 mm with space between bands of 8 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. 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 247 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 olopatadine hydrochloride was prepared by dissolving 10 mg of drug in 10 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, 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 olopatadine hydrochloride (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 spot 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 developed 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 olopatadine hydrochloride 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 development distance (± 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 100 ng/µL. Four µL of this solution was applied on the plate. After chromatographic development peak areas of the bands were measured at 247 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 Olopatadine hydrochloride

Amount taken (ng/band)	Amount added (ng/band)	Total amount found (ng/band)	% Recovery	% Recovery (Mean ± S.D.)
400	200	598.527	99.75	99.81 ± 0.189
400	400	797.420	99.67	
400	600	1000.382	100.03	

Table 2. Robustness Data in Terms of Peak Area (% RSD)

Sr. No.	Parameter Varied	% RSD
1	Development distance	0.96
2	Time from application to development (Mins.)	0.87
3	Time from development to scanning (Mins.)	1.42

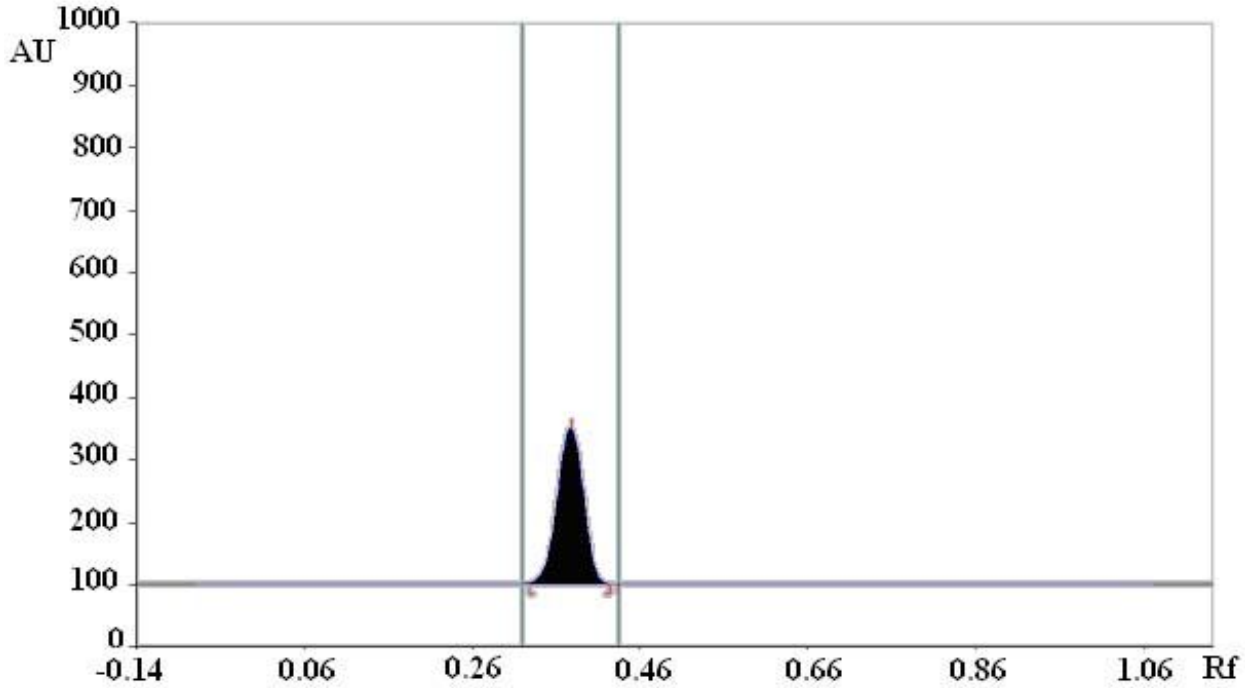

Figure 1: Densitogram of Olopatadine hydrochloride (1200 ng/ band, R_f = 0.37 ± 0.01)

Results and Discussion

Literature survey revealed that several LC-MS methods have been reported for estimation of olopatadine hydrochloride which are sophisticated but costly and time consuming. As no HPTLC method has been reported so far for estimation of olopatadine hydrochloride, the present study was aimed at development of a speedy and cost effective HPTLC technique for determination of olopatadine hydrochloride as bulk and in ophthalmic solutions. The mixture of Methanol: Ammonia: Glacial Acetic acid (8:2:0.2 v/v/v) as mobile phase gave better peak shape. The R_f value of drug was found to be 0.37 ± 0.01 (Figure 1).

The method was found to be linear in the range of 200 to 1200 ng/ band. The spectrum of olopatadine hydrochloride in ophthalmic solution compared with spectrum of standard olopatadine hydrochloride 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 0.98-1.96 % and 0.75-1.14 %, 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 0.137 % and 1.813 %, 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 area of peak 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 100.51 ± 1.01.

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

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