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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ANALYSIS OF FEXOFENADINE HYDROCHLORIDE AS A BULK DRUG AND IN TABLET DOSAGE FORM

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ABSTRACT

The objective of present work was to develop a validated simple, precise, accurate and specific high performance thin layer chromatographic method for estimation of fexofenadine hydrochloride as a bulk drug and in tablet dosage form. The stationary phase used was precoated silica gel aluminium plates 60 F_{254} with 250 µm thickness. The mobile phase used for separation was toluene: ethyl acetate: methanol: ammonia (30%) (0.5: 7: 3: 0.6; v/v/v/v). The densitometric quantification was carried out at 220 nm. The calibration curve was found to be linear between 1-10 µg/spot. The method was validated in terms of linearity, precision, repeatability, accuracy, LOD, LOQ, robustness and specificity. The proposed method can be successfully applied for routine analysis of fexofenadine hydrochloride in bulk and tablet dosage form.

KEYWORDS: Fexofenadine hydrochloride, HPTLC, Method development, Densitometric determination, Method validation.

INTRODUCTION

Fexofenadine hydrochloride (Fig.1) is chemically known as 4-(1-hydroxy-4-(4-(hydroxydiphenylmethyl)-1-piperidinyl) butyl) - α, α - dimethylbenzene acetic acid hydrochloride. Its chemical formula is $C_{32}H_{39}NO_4$ with a molecular weight 538.13 g/mol. It is white to off-white, odourless, crystalline powder, freely soluble in methanol, ethanol, slightly soluble in chloroform, water and insoluble in hexane.[1-4]

Fexofenadine hydrochloride is an antihistaminic drug used in the treatment of hay fever and similar allergy symptoms. It was developed as an alternative to terfenadine. Fexofenadine,

like other second and third-generation antihistamines, does not readily pass through the blood-brain barrier, and so causes less drowsiness than first-generation histamine-receptor antagonists.[5-7]

HPTLC method for simultaneous estimation of fexofenadine hydrochloride with montelukast sodium[8], development and validation of RP-HPLC method for simultaneous estimation of montelukast sodium and fexofenadine hydrochloride in combined dosage form[9], stability-indicating HPLC method for simultaneous determination of montelukast and fexofenadine hydrochloride[10] and stability indicating RP-HPLC method for the estimation of montelukast sodium and fexofenadine hydrochloride in pharmaceutical preparations[11], are reported. Stability indicating chromatographic method for fexofenadine hydrochloride and related impurities and stability indicating HPLC method for fexofenadine hydrochloride in pharmaceutical formulation have also been reported in the literature.[12-13] However, to the best of our knowledge, no HPTLC method has been reported for estimation of fexofenadine hydrochloride in bulk and tablet dosage form so far. Hence the aim of present work was to develop and validate HPTLC method for determination of fexofenadine hydrochloride in bulk and tablet dosage form as per ICH guidelines.[14-15]

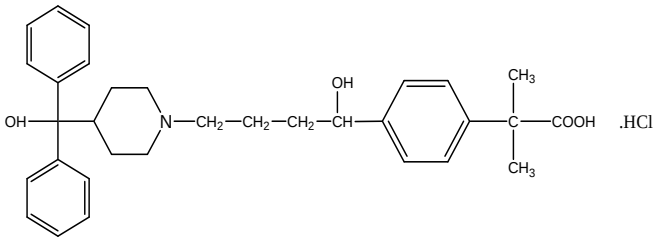

Fig. 1: Structure of fexofenadine hydrochloride

MATERIALS AND METHODS

Instrumentation

A Camag HPTLC system equipped with Linomat V applicator (Switzerland), TLC Scanner III and integrated software Win-Cats (v 3.15, Camag) was used for the analysis. The standard and the sample solutions were spotted in the form of bands of width 6 mm with a Camag 100 µl sample (Hamilton, Bonaduz, Switzerland) syringe, on silica gel pre-coated aluminum plate $60F_{254}$ plates (20×10cm) with 250 µm thickness; (E. Merck, Darmstadt, Germany), supplied by Anchrom Technologist, Mumbai. The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. The slit dimension was kept at 5 mm × 0.45 mm, data resolution of 100µm/step and the scanning speed was 20 mm/s. The monochromatic

band width was set at 220 nm; each track was scanned three times and baseline correction was used.

The mobile phase consisted of toluene: ethyl acetate: methanol: ammonia (30%) (0.5: 7: 3:0.6; v/v/v/v) and 11.1 ml of mobile phase was used per chromatographic run. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature (25°C ± 2) at relative humidity of 60% ± 5. Each chromatogram was developed over a distance of 80 mm. Following the development, the TLC plates were dried in a stream of air with the help of hair dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed at 220 nm. The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample analysis.

Chemicals

All solvents used for analysis were of analytical grade. Gift sample of pure drug was procured from Zim Laboratories Ltd. Kalmeshwar, Nagpur, Maharashtra.

HPTLC Method and Chromatographic Conditions

Preparation of standard stock and working standard solutions

For preparation of standard stock and working standard solutions, an accurately weighed quantity of fexofenadine hydrochloride (5 mg) was taken in 10 ml volumetric flask and dissolved in methanol. Volume was made up to the mark with methanol to give the concentration of 0.5µg/µl.

Procedure for analysis of tablet formulation

Tablets (10) were weighed and the average weight of each tablet was calculated. Then the weight equivalent to 5 mg was transferred into a 10 ml volumetric flask, 5 ml methanol was added and sonicated for 20 min, further the volume was made up to the mark with methanol, mixed sample solution was allowed to settle down. The solution was filtered using Whatmann filter paper. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was spotted, chromatogram was obtained and the peak areas were recorded.

Prewashing of plates

Densitometric estimation was carried out on 20cm × 10 cm pre-coated silica gel 60F_{254} plates from E. Merck. The plates were pre-washed with methanol, dried and activated for 30 min at 110°C.

Selection of solvent

Methanol was selected as a solvent for preparing drug solutions.

Selection of stationary phase

Identification and separation of fexofenadine hydrochloride was carried out on 20 cm × 10 cm, pre-coated silica gel aluminium plates 60 F_{254} with 250 µm thickness (E. Merck, Darmstadt, Germany).

Sample application

The standard and working standard solution of fexofenadine hydrochloride were spotted on pre-coated TLC plates in the form of narrow bands of length 6 mm, with 10 mm from the bottom and left margin and 10 mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150nl/s.

Selection of wavelength

Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample analysis at 220 nm using methanol as a blank solution. The selection of wavelength display is as shown in (Fig.2)

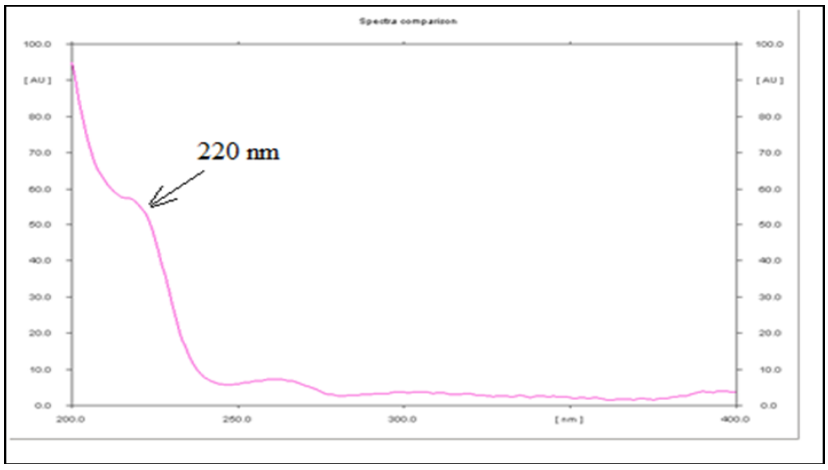

Fig.2: Display of detection wavelength

Optimization of mobile phase

Various solvent systems like mixture of (a) toluene: methanol (7:3; v/v) (b) triethylamine: methanol (6: 3; v/v) (c) toluene: chloroform: methanol (1: 5: 3; v/v/v) and (d) toluene: ethyl

acetate: methanol (0.5: 4: 2; v/v/v) were tried to separate and resolve spot of fexofenadine hydrochloride from its impurities and other excipients of formulation. The mixture of toluene: ethyl acetate: methanol (0.5: 7: 3; v/v/v) resolved fexofenadine but there was tailing in the peaks. To improve peak symmetry, 30% ammonia was added. Finally, the mixture of toluene: ethyl acetate: methanol: 30% ammonia (0.5: 7: 3: 0.6; v/v/v/v) showed well resolved peak with better peak shape. The drug was resolved with R_f value of 0.23 ±0.02. Pre-saturation of TLC chamber with mobile phase for 20 min assured better reproducibility in migration of fexofenadine hydrochloride and better resolution (Fig. 3).

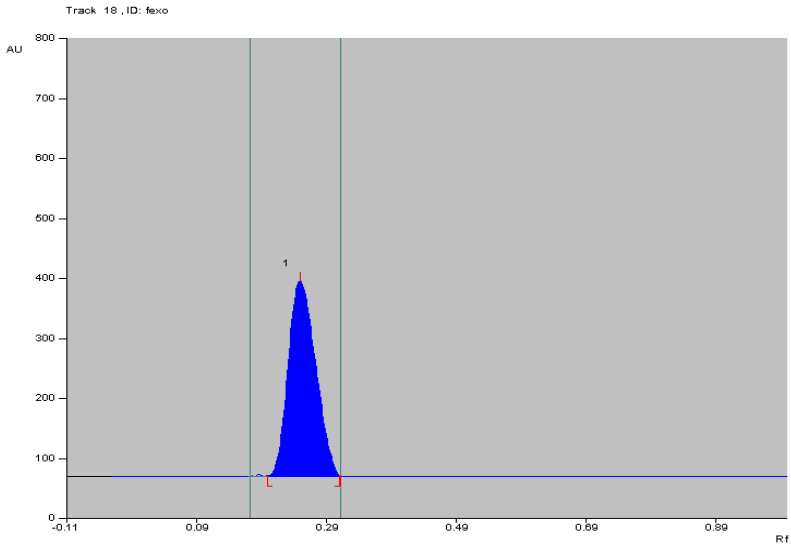

Fig. 3: Densitogram of standard fexofenadine hydrochloride

METHOD VALIDATION

The developed HPTLC method was validated as per the ICH guidelines Q2 9(R1)[14-15] for linearity, precision, repeatability, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and specificity.

Linearity (calibration curve)

The linearity of an analytical procedure is, its ability to obtain test results that are directly proportional to the concentration of the analyte in the sample. It is usually demonstrated by the analysis of various concentrations of the analyte across the intended range and represented graphically.

Linearity of the method was evaluated by constructing calibration curves at 10 concentration levels. Aliquots of standard working solution of fexofenadine hydrochloride were applied to the plate, to obtain concentration in the range 1 to 10 µg/spot. The calibration curves were

developed by plotting peak area *Vs* concentration with the help of Win-Cats software. The plate was developed in a twin trough glass chamber, using 20 min chamber saturation time. The length of the run was 80 mm. The developed plates were air-dried. Scanning was performed in UV mode at 220 nm. The slit dimension was kept at 5 × 0.45 mm at scanning speed of 100nm/s. After completion of scanning, peak areas were noted. Peak areas were plotted against corresponding concentration and least square regression analysis was performed to generate the calibration equation.

Precision

The precision of an analytical method is the closeness of agreement between series of measurements obtained from multiple sampling of the same sample. The intra-day precision was determined by analyzing standard solutions in the concentration range of 2, 3 and 4 µg/spot. While, inter-day precision was determined by analyzing standards daily for 3 days over a period of 1 week. Each concentration was spotted 3 times on the plate.

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. It is also termed as intra-assay precision. Repeatability of sample application was assessed by spotting 8 µl/spot of standard drug solution six times on TLC plate at different times on same day by sample applicator, followed by development of plate and recording of the peak areas for six spots.

Accuracy

Recovery studies were carried out to determine accuracy of the developed method at 80-120% levels. It was done by mixing known quantity of standard drug (0.5µg/µl) with the sample formulation and the contents were analyzed by the proposed method. The percent recovery and percent RSD were calculated respectively.

Limit of detection and limit of quantitation

LOD of an individual analytical procedure is the lowest amount of an analyte in a sample which can be detected but not necessarily quantitated as an exact value and LOQ of an individual analytical procedure is the lowest amount of an analyte in a sample which can be quantitatively determined. LOD and LOQ value represent the sensitivity of the proposed analytical method.

To estimate the LOD and LOQ, blank methanol was spotted six times. Spotting for LOD was done by taking different concentrations as 0.05, 0.1, 0.15, 0.20 and 0.25 µg/spot. No spot was detected up to concentration 0.04 µg/spot. The peak was detected at 0.05µg/spot with a signal-to-noise ratio of 3:1. The LOQ was done by taking different concentrations as 0.05, 0.1, 0.15, 0.20 and 0.25 µg/spot. The peak was detected with quantifiable area at 0.20µg/spot with a signal-to-noise ratio of 10:1.

Robustness

As defined by ICH, the robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed to injected the standard and samples by small variation in the chromatographic conditions and found to be unaffected by small variations like ± 0.1 ml/min in volume of mobile phase composition. It was observed that there were no marked changes in the densitogram.

Specificity

The specificity of the method was ascertained by analyzing standard drug and formulation (Allegra 120 mg). The spot for fexofenadine hydrochloride in formulation was confirmed by comparing the R_f and densitogram of the spot with that of standard. The peak purity of fexofenadine hydrochloride was assessed by comparing the densitogram at three different levels i.e., peak start, peak apex and peak end positions of the spot.

RESULT AND DISCUSSION

Linearity (calibration curve)

The calibration curve was constructed ranging from 1-10 µg/spot. The linearity was evaluated by linear regression analysis which was evaluated by least square analysis and is depicted in Table 1. It showed good correlation between regression coefficient and concentration of the drug (Fig. 4).

Table.1: Results of linearity curve

Sr.No.	Volume (µl/spot)	Conc. (µg/spot)	R_f ± S.D.*	Area
1	2	1	0.23 ± 22.72	3965.6
2	4	2	0.23 ± 21.22	4667.3
3	6	3	0.23 ± 20.30	6689.6
4	8	4	0.23 ± 21.54	7733.3
5	10	5	0.23 ± 22.11	9666.0

6	12	6	0.23 ± 21.00	11747.0
7	14	7	0.23 ± 22.50	13697.3
8	16	8	0.23 ± 20.00	16109.3
9	18	9	0.23 ± 20.81	18272.6
10	20	10	0.23 ± 21.37	20276.0

*Mean of 3 estimations

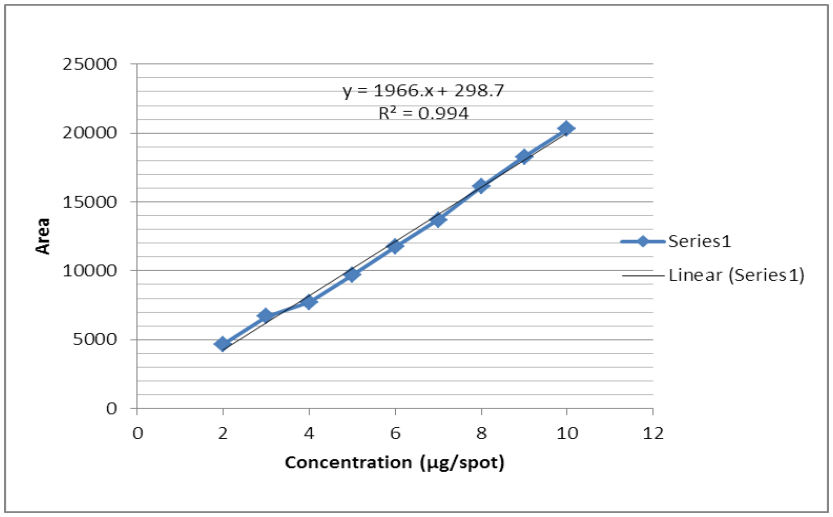

Fig. 4: Calibration curve of fexofenadine hydrochloride

Precision

The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were within the acceptance criteria which indicated that the method was precise. The results of intra and inter-day precision are shown in Table 2 and 3.

Table.2: Results of intra-day precision (Mean of 3 estimations)

Sr.No.	Volume (µl/spot)	Conc. (µg/spot)	R_f	Area	Std. deviation	Std. error of mean	%RSD
1	4	2	0.23	6080.6	20.42	11.78	0.335
2	6	3	0.23	7099.0	22.60	13.04	0.318
3	8	4	0.23	6780.6	25.02	14.44	0.369

Table.3: Results of inter-day precision (Mean of 3 estimations)

Sr.No.	Volume (µl/spot)	Conc. (µg/spot)	R_f	Area	Std. deviation	Std. error of mean	%RSD
1	4	2	0.23	6305.6	20.81	12.11	0.330
2	6	3	0.23	7063.6	23.54	13.59	0.333
3	8	4	0.23	7770.6	24.58	14.19	0.347

Repeatability

The % RSD for repeatability of the drug was found to be less than 2. So, it was concluded that the proposed method for estimation was repeatable in nature; the data for the same is shown in Table 4.

Table.4: Results of repeatability

Sr.No	Volume (µl/spot)	Concentration (µg/spot)	Area	S.D	% RSD
1	8	4	7520.01	21.50	0.284
2	8	4	7501.30	21.85	0.286
3	8	4	7681.21	22.24	0.289
4	8	4	7751.11	20.51	0.272
5	8	4	7821.84	21.95	0.289
6	8	4	7888.37	20.41	0.264

Accuracy

To check the accuracy of the method, recovery studies were carried out by standard addition of drug solution to pre-analyzed sample solution at three different levels 80, 100, and 120 %. The percent of recoveries were calculated and the results are represented in Table 5.

Table.5: Results of recovery study (Mean of 3 estimations)

Sr. no.	Levels (%)	Conc. added (µg/spot)	Conc. found (µg/spot)	% Recovery
1	80	3.6	3.50	98.08
2	100	4.0	3.94	99.02
3	120	4.4	4.47	101.05

LOD and LOQ

The LOD for fexofenadine hydrochloride was found to be 0.05µg/spot (Fig. 5) and the LOQ was found to be 0.20µg/spot (Fig. 6). The proposed method was sensitive in nature and can detect the drug at very low level as depicted in Table 6.

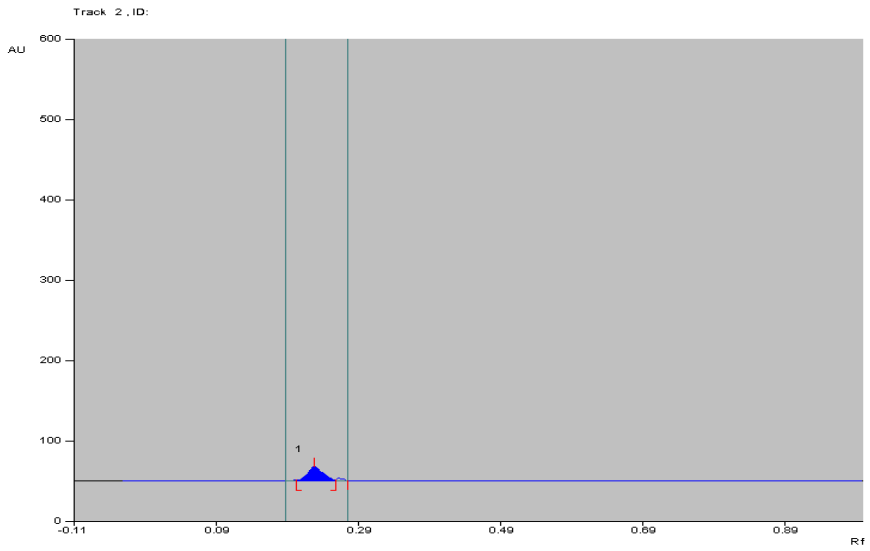

Fig.5: Densitogram of LOD for fexofenadine hydrochloride

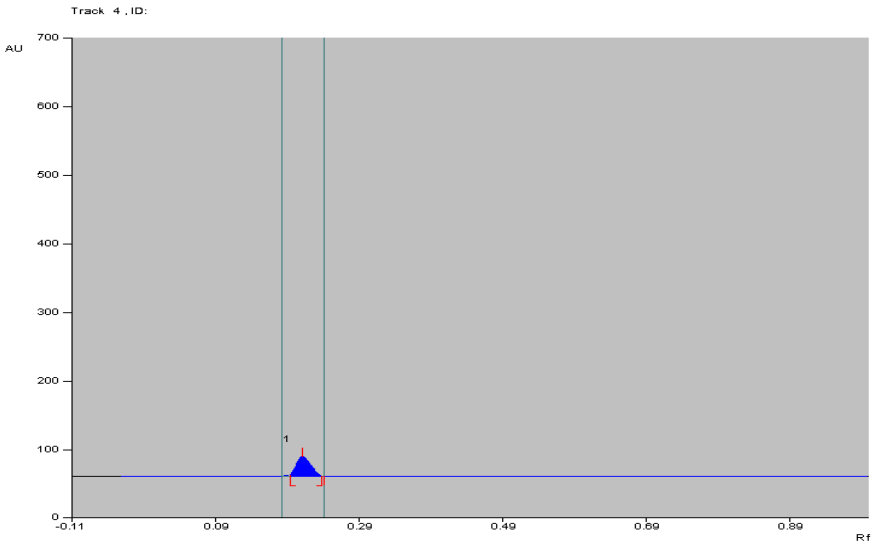

Fig.6: Densitogram of LOQ for fexofenadine hydrochloride

Table 6: Results of limit of detection and limit of quantification

LOD	LOQ
0.05 µg/spot	0.20 µg/spot

Robustness

The experimental conditions were deliberately altered to evaluate the robustness of the method. The deviation obtained by deliberate changes in various parameters was below 2% RSD which proved that the developed HPTLC method was robust. The data is depicted in Table 7.

Table.7: Results of robustness testing

PARAMETERS	S. D.	%RSD
Mobile phase composition ± 0.1ml	20.62	0.311
Amount of mobile phase ± 0.1ml	22.97	0.352
Temperature ± 5ºC	22.73	0.381
Relative humidity ± 5%	22.21	0.333
Time from spotting to chromatography ± 5 min	23.11	0.362
Time from chromatography to scanning ± 5 min	22.68	0.371

Specificity

The peak purity of fexofenadine hydrochloride was assessed by comparing their respective densitograms at peak start, peak apex and peak end positions of the spot i.e., r (start, middle) = (0.20 -0.23) and r (middle, end) = (0.23 - 0.27). Good correlation was obtained between standard and sample densitograms of fexofenadine hydrochloride. The densitogram is shown in Fig.7.

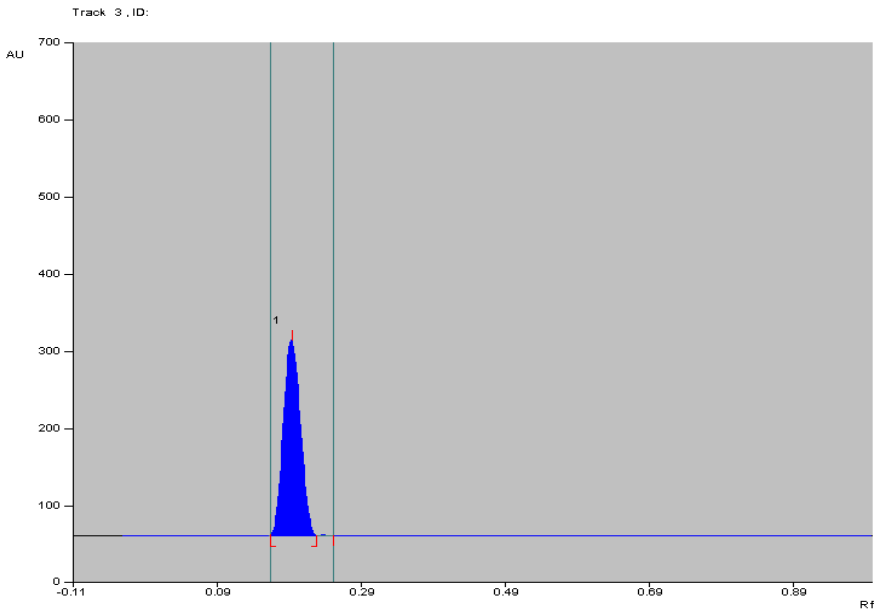

Fig. 7: Densitogram of tablet (Allegra 120 mg) of fexofenadine hydrochloride

CONCLUSION

The validated HPTLC method for quantitative analysis of fexofenadine hydrochloride in pharmaceutical formulations was simple, precise, accurate, specific, reproducible and more economic than the reported methods without interference from the excipients. The method was validated in accordance with ICH guidelines. The method reduced analysis time

compared with reported methods and seems to be suitable for routine analysis of pharmaceutical formulations in quality-control laboratories, where economy and speed are essential.

ACKNOWLEDGEMENT

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