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Stability indicating thin-layer chromatographic determination of fexofenadine hydrochloride as bulk drug: Application to forced degradation study

Pallavi M. Sutar, Deepali A. Bansode and Suneela S. Dhaneshwar*

Department of Pharmaceutical Chemistry, Bharati Vidyapeeth University, Poona College of Pharmacy, Pune, Maharashtra, India

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ABSTRACT

The objective of present work was to develop a validated stability indicating high performance thin layer chromatographic method (HPTLC) for estimation of fexofenadine hydrochloride. 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) gave a resolved peak at (Rf value of 0.27 ± 0.02). The densitometric quantification was carried out at 220 nm. Fexofenadine hydrochloride was subjected to acid and alkali hydrolysis, oxidation, dry heat treatment and photo degradation. The drug was found to degrade in acidic, alkaline, oxidative and dry heat conditions, but was found to be stable in photolytic conditions. All the peaks of degraded products were separated from the standard drug with significantly different (R_f values as 0.03± 0.02 in acid, 0.06± 0.02 and 0.84± 0.02 in base, 0.06± 0.02 , 0.62± 0.02, 0.64± 0.02 and 0.82± 0.02 in hydrogen peroxide, 0.02± 0.02, 0.68± 0.02 in dry heat conditions). The developed method can effectively separate the drug from its degradation products under accelerated degradation studies, it can be routinely employed as stability indicating method for fexofenadine hydrochloride.

Key words: Fexofenadine hydrochloride, Thin layer chromatography, Densitometric determination, Stability indicating method.

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^{-1}. It is a white to off-white, odorless, crystalline powder, freely soluble in methanol, ethanol, slightly soluble in chloroform, water and insoluble in hexane [1-4].

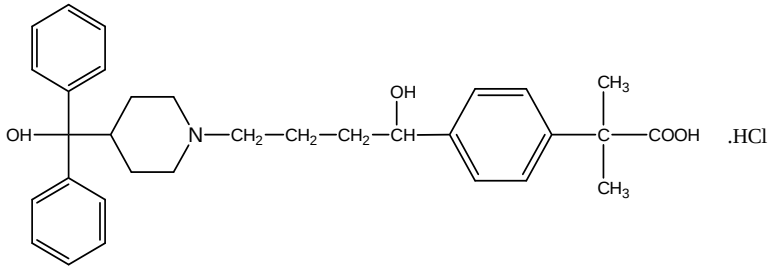

Figure 1: Chemical structure of fexofenadine hydrochloride

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].

Stability-indicating HPLC method for simultaneous determination of montelukast and fexofenadine hydrochloride [8], RP-HPLC method for simultaneous estimation of montelukast sodium and fexofenadine hydrochloride in combined dosage form [9], HPTLC method for simultaneous estimation of fexofenadine hydrochloride with montelukast sodium [10] and stability indicating RP-HPLC method for the estimation of montelukast sodium and fexofenadine hydrochloride in pharmaceutical preparations [11] are reported in the literature.

Stability indicating chromatographic method for fexofenadine hydrochloride and related impurities and stability indicating HPLC method for fexofenadine hydrochloride in pharmaceutical formulation has also been documented in the literature [12-13]. However to the best of our knowledge, stability indicating HPTLC method has not been reported for fexofenadine hydrochloride so far.

The International Conference on Harmonization (ICH) guidelines entitled stability testing of new drug substances and product requires the stress testing of the drug substance should be carried out to elucidate the inherent stability characteristics of the active substance. An ideal stability indicating method is one that quantifies the drug per se and also resolves its degradation product. HPTLC has become a part of routine analytical techniques in many product development and analytical laboratories due to its advantages. The major advantage of HPTLC is that several samples can be simultaneously using a small quantity of mobile phase unlike HPLC thus lowering the analysis time and cost per analysis with high sample throughput. The uniform particle size (7 µm) of precoated HPTLC plates enables achievement of a greater resolution and an easy reproducible separation. The method of detection does not place any restriction on the choice of the mobile phase and unlike HPLC mobile phases having pH 8 and above can be employed [14-15].

The aim of the present work was to develop an accurate, specific, reproducible and stability indicating TLC method for fexofenadine hydrochloride as bulk drug in presence of its degradation products.

MATERIALS AND METHODS

2.1. Chemicals and reagents

Gift sample of pure drug fexofenadine hydrochloride was procured from ZIM Laboratories Ltd. Kalmeshwar, Nagpur, Maharashtra, India. All chemicals and reagents used for the analysis were of analytical grade and were purchased from Merck, Mumbai, India.

2.2. HPTLC 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, 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 with data resolution of 100 μm^{-1}

step and the scanning speed was 20 mm s^{-1}. 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.

2.3. HPTLC Method and Chromatographic Conditions

2.3.1. Preparation of standard stock and working standard solutions

For preparation of standard stock and working standard solutions fexofenadine hydrochloride (5 mg) was weighed accurately and transferred into a 10 mL volumetric flask. Methanol 10 mL was added and the flask was sonicated for 20 min. Volume was made up to the mark with methanol to give the concentration of (500 ng $spot^{-1}$).

2.3.2. Prewashing of plates

Densitometric estimation was carried out on (20 cm × 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.

2.3.3. Selection of solvent

Methanol was selected as a solvent for preparing drug solution.

2.3.4. 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 (250 µm thickness E. Merck, Darmstadt, Germany).

2.3.5. 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, at 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 150 nL s^{-1}.

2.3.6. 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 detection wavelength was selected at 220 nm and the spectrum of the drug is in (Fig.2).

2.3.7. Optimization of the 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 = 0.27 ±0.02. Pre-saturation of TLC chamber with mobile phase for 20 min assured better reproducibility in migration of fexofenadine hydrochloride and better resolution.

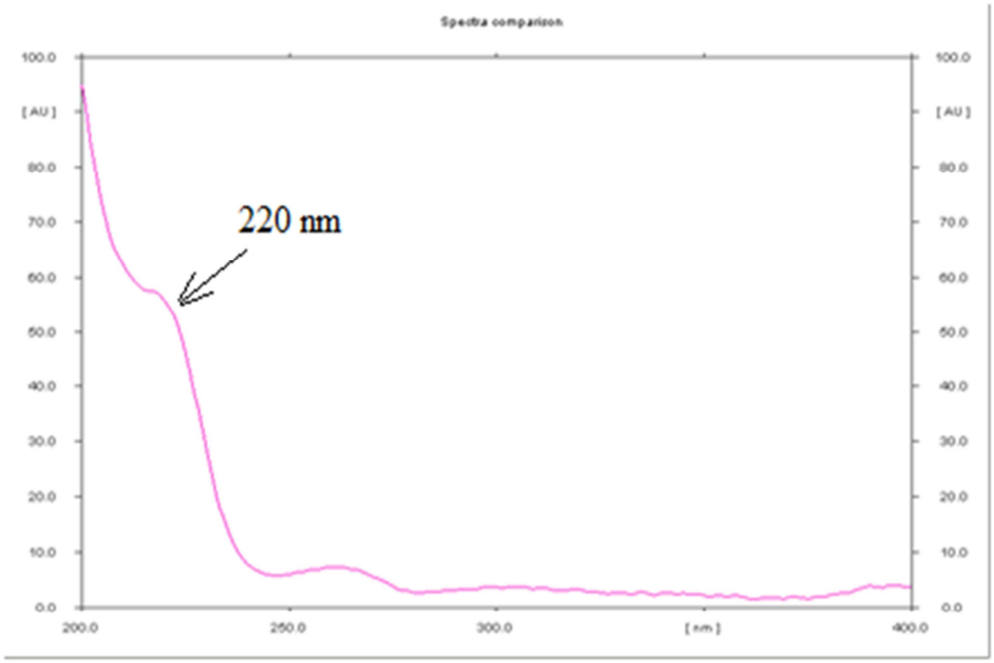

Figure 2: Spectrum for selection of wavelength (220 nm)

2.4. METHOD VALIDATION

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

2.4.1. Linearity (calibration curve)

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 (1000 to 10000 ng spot^{-1}). 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 100 nm s^{-1}. 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.

2.4.2. Precision

The intra-day precision was determined by analyzing standard solutions in the concentration range of (2000, 3000 and 4000 ng spot^{-1}). 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.

2.4.3. Repeatability

It is also termed as intra-assay precision. Repeatability of sample application was assessed by spotting (8000 nL spot^{-1}) 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.

2.4.4. Recovery and specificity studies

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 (500 ng nL^{-1}) with the sample formulation and the contents were analyzed by the proposed method. The percent recovery and percent RSD were calculated respectively.

The specificity of the method was ascertained by analyzing standard drug and formulation (Allegra 120 mg, manufactured by Sanofi Aventis India limited). 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.

2.4.5. Robustness

Robustness was performed by spotting the standard and samples by small variation in the chromatographic conditions and found to be unaffected by small variations like ± 0.1 mL min^{-1} in volume of mobile phase composition. It was observed that there were no marked changes in the densitogram.

2.4.6. Limit of detection and limit of quantitation

To estimate the LOD and LOQ blank methanol was spotted six times. Spotting for LOD was done by taking different concentrations as 50, 100, 150, 200 and 250 ng $spot^{-1}$ and the values were considered with a signal-to-noise ratio of 3:1 and 10:1 respectively.

2.5. Accelerated degradation of fexofenadine hydrochloride

Accelerated degradation was carried by exposing the drug to different stressed conditions.

A drug stock solution of fexofenadine hydrochloride (5mg) was prepared in 10 mL methanol. This drug solution was used for forced degradation to provide an indication of the stability indicating property and specificity of the proposed method.

2.5.1. Acid and base induced degradation

To 5 mL solution of fexofenadine hydrochloride, 5 mL 0.9 N HCl and 0.7 N NaOH were added separately. These mixtures were refluxed separately for 2 h and 24 h at 80°C respectively. Two micro liters (2000 ng $spot^{-1}$ of fexofenadine hydrochloride) of resultant solutions were applied on TLC plate and developed as described in section 2.2.

2.5.2. Hydrogen peroxide induced degradation

To 5 mL solution of fexofenadine hydrochloride, 5 mL (H_2O_2) (3% v/v) was added. This solution was heated in boiling water bath for 10 min to remove completely the excess of hydrogen peroxide and refluxed for 2 h at 80°C. Two micro liters (2000 ng $spot^{-1}$ of fexofenadine hydrochloride) of resultant solutions was applied on TLC plate and developed as described in section 2.2.

2.5.3. Dry heat induced degradation

Dry heat degradation of fexofenadine hydrochloride was carried out by placing the bulk drug into a hot air oven at 60ºC for 2 h. Two micro liters (2000 ng $spot^{-1}$ of fexofenadine hydrochloride) of resultant solutions was applied on TLC plate and developed as described in section 2.2.

2.5.4. Photochemical degradation

5 mL solution of fexofenadine hydrochloride was studied by exposing to direct sunlight for 48 h. Two micro liters (2000 ng $spot^{-1}$ of fexofenadine hydrochloride) of resultant solution was applied on TLC plate and developed as described in section 2.2.

RESULTS AND DISCUSSION

3.1. Optimization of chromatographic conditions

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 = 0.23±0.02 for fexofenadine hydrochloride (Fig 3).

The developed plates were evaluated densitometrically in absorbance mode at 220 nm. Well defined spot was obtained when chamber was saturated with mobile phase for 10 min at temperature 20 ±5ºC and relative humidity 60± 5ºC

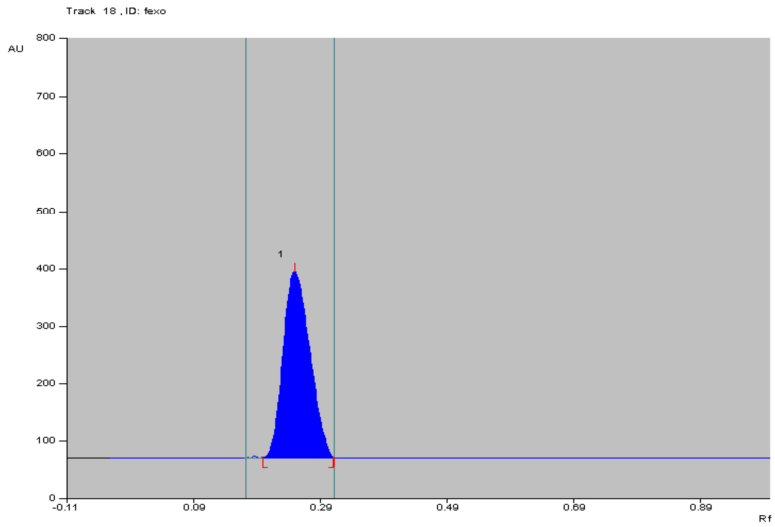

Figure 3: Chromatogram of standard (1) fexofenadine hydrochloride (R_f = 0.23)

3.2 Calibration curve

The linearity was evaluated by linear regression analysis which was evaluated by least square analysis. The regression data (Table 1) showed a good linear response over the concentration range (1000-10000 ng spot^{-1}) of fexofenadine hydrochloride with respect to peak area. The linearity of calibration graphs and adherence of the system to Beer's law was validated by correlation coefficient.

No significant difference was observed in the slopes and standard curves (ANOVA, p< 0.05).

Table 1 Summary of linear regression and validation data

Parameters	HPTLC
Linearity range (ng spot-1)	1000-10000
Correlation coefficient (r2) (± SD)	0.997 ± 0.015
Slope (± SD)	1.892 ± 0.018
Y –intercept (± SD)	1028 ± 0.121
Inter-day precision (n=3)	7046.6 ± 22.97, 0.336
Intra-day precision (n=3)	6653.± 22.68, 0.340
Limit of detection (ng spot-1)	50
Limit of quantification (ng spot-1)	200
Recovery (n=3) (%)	100.04%
Robustness	Robust
Specificity	Specific

SD= Standard deviation

Table 2 Precision study (n= 3)

Precision	Conc. (ng spot-1)	Rf	Area	S. D.*	S E	%RSD
Intra-day	2000	0.23	6080.6	20.42	11.78	0.335
	3000	0.23	7099	22.6	13.04	0.318
	4000	0.23	6780.6	25.02	14.44	0.369
Inter-day	2000	0.23	6305.6	20.81	12.11	0.33
	3000	0.23	7063.6	23.54	13.59	0.333
	4000	0.23	7770.6	24.58	14.19	0.347

3.3. Validation of the method

3.3.1. Precision

The repeatability of sample application and measurement of peak areas at 2000 ng spot^{-1} of fexofenadine hydrochloride was expressed in terms of % R.S.D. and S.E. and was found to be <2.The measurement of peak areas

at three different concentration levels showed low value of % R.S.D. (<2) and low value of S.E. (<2) for intra and inter-day variation, which suggested the method is precise (Table 2).

3.3.2. Repeatability

The % RSD for repeatability of the drug was found to be (<2). The measurement of peak areas at three different concentration levels showed low value of % R.S.D. (<2) and low value of S.E. (Table 3). Hence the proposed method for estimation is proved to be repeatable in nature.

Table 3 Repeatability study (n=6)

Concentration (ng spot-1)	Area	S.D	%RSD
4000	7520.01	21.5	0.284
4000	7501.3	21.85	0.286
4000	7681.21	22.24	0.289
4000	7751.11	20.51	0.272
4000	7821.84	21.95	0.289
4000	7888.37	20.41	0.264

3.3.3. Recovery and specificity studies

The proposed method was checked for the accuracy and was carried out by standard addition of drug solution to pre-analyzed sample solution at three different levels 80, 100, and 120 %. The percent recoveries were found to be 99.13-101.63 % (Table 4).

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 which indicated non interference of the sample matrix and indicates specificity of the method.

Table 4 Recovery study (n=3)

Levels (%)	Conc. Added (ng spot-1)	Conc. found (ng spot-1)	% Recovery
80	3600	3568.7	99.13
100	4000	3975.3	99.38
120	4400	4471.8	101.63

3.3.4. Robustness

The % R.S.D. and SE of the peak areas was calculated for change in mobile phase composition, mobile phase volume, temperature, relative humidity, time from spotting to chromatography and time from chromatography to scanning in triplicate at concentration level of (2000 ng spot^{-1}) of fexofenadine hydrochloride.

The deviation obtained by deliberate changes in various parameters % R.S.D (<2) (Table 5) which indicated that the developed HPTLC method was robust.

Table 5 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

3.3.5. LOD and LOQ

Detection limit and quantitation limit was described in section 2.4.6. The signal to noise ratio of 3:1 and 10:1 were considered for LOD and LOQ respectively. LOD was found to be (50 ng spot^{-1}) and LOQ was found to be (200 ng spot^{-1}) for fexofenadine hydrochloride which indicates the proposed method was sensitive in nature and detects the drug at very low level (Table 1)

3.4. Stability indicating property

The number of degradation products with their R*f* values at % recovery of fexofenadine hydrochloride was calculated and listed in Table 6.

Table 6 Summary of forced degradation study

S.N.	Stressed condition	Fexofenadine HCl		Degradation product		Figure
		%	R_f	%	R_f	
1	Acid, 5 mL (0.9 N HCl reflux for 2 h at 80ºC)	93.9±0.24	0.27	6.1±0.29	0.03	Fig. 4
2	Base, 5 mL (0.7 N NaOH reflux for 24 h at 80ºC)	91.2±1.01	0.27	8.8±1.12	0.06, 0.84	Fig. 5
3	Hydrogen peroxide, 5 mL, 3% v/v H_2O_2 (reflux for 2 h at 80ºC)	87.1±2.15	0.27	12.9±2.7	0.06, 0.62, 0.64, 0.82	Fig. 6
4	Dry heat (2 h at 60ºC)	90.8±1.53	0.27	9.2±1.42	0.02, 0.68	Fig. 7
5	Photochemical stability (Daylight, 48 h)	100±0.36	0.27	--	Not detected	--

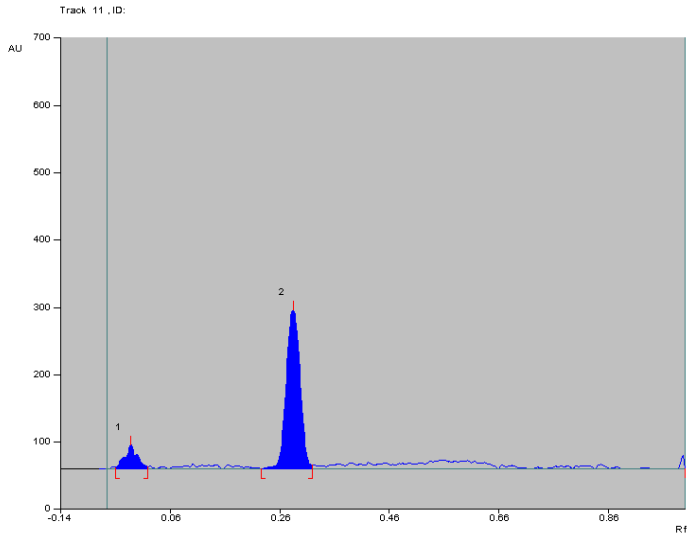

Figure 4: Chromatogram of acid [0.9 N HCl (reflux for 2h at 80ºC)] treated sample
Peak 1- degradant [R_f=0.03], Peak 2- fexofenadine hydrochloride [R_f= 0.27]

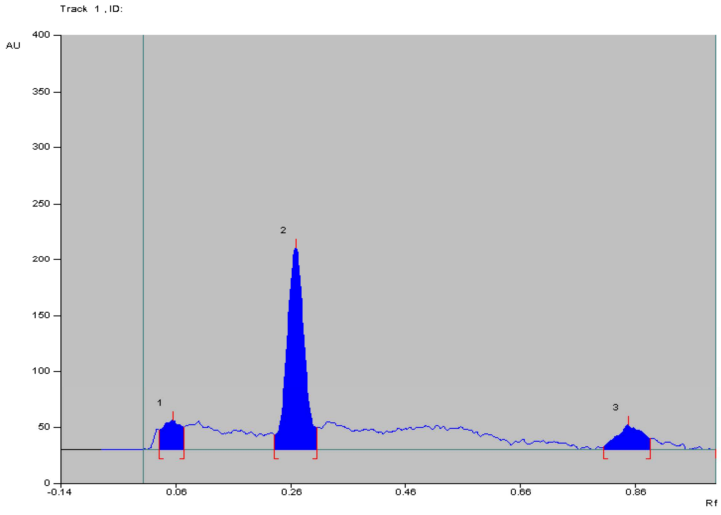

Figure 5: Chromatogram of base [0.7 N NaOH (reflux for 24h at 80ºC)] treated sample
Peak 1- degradant [R_f=0.06], Peak 2- fexofenadine hydrochloride [R_f=0.27], Peak 3- degradant [R_f= 0.84]

3.4.1. Acid and base induced degradation

The chromatograms of acid and base degraded products showed additional peaks at R_f value 0.03 in acid induced degradation and 0.06, 0.84 in base induced degradation. The % recoveries of fexofenadine hydrochloride at the level of 93.9% in acid and 91.2% in basic condition suggested that fexofenadine hydrochloride undergoes mild degradation under acidic (Fig. 4) and basic conditions (Fig. 5).

3.4.2. Hydrogen peroxide induced degradation

The chromatogram of hydrogen peroxide showed four additional peaks at R_f value 0.06, 0.62, 0.64 and 0.82 (Fig. 6) other than the standard peak of fexofenadine hydrochlorid. The % recovery of fexofenadine hydrochloride at the level of 87.1% suggested that fexofenadine hydrochloride showed mild degradation under oxidative condition.

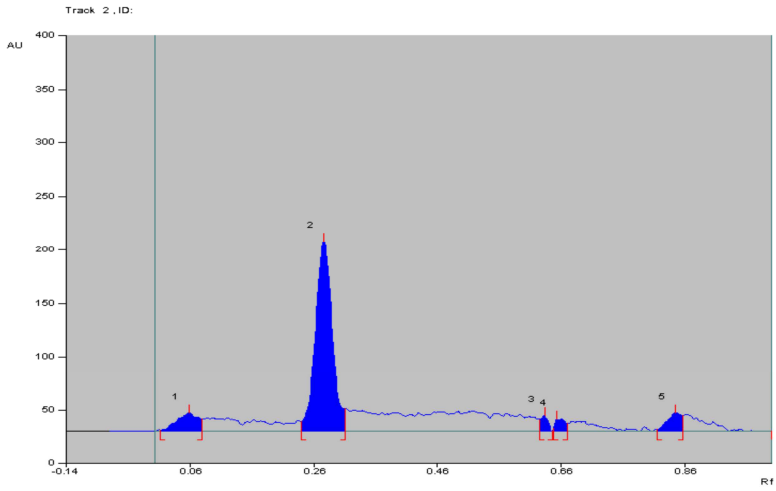

Figure 6: Chromatogram of hydrogen peroxide [3% v/v H_2O_2 (reflux for 2h at 80°C)] treated sample

Peak 1- degradant [R_f=0.06], Peak 2- fexofenadine hydrochloride [R_f=0.27], Peak 3- degradant [R_f=0.62], Peak 4- degradants [R_f=0.64], Peak 5- degradants [R_f= 0.82]

3.4.3. Dry heat degradation

The sample degraded under dry heat showed two additional peaks at R_f value 0.02 and 0.68 (Fig. 7) other than the standard peak of fexofenadine hydrochloride. The % recovery of fexofenadine hydrochloride at the level of 90.8% suggested that fexofenadine hydrochloride undergoes mild degradation under dry heat condition.

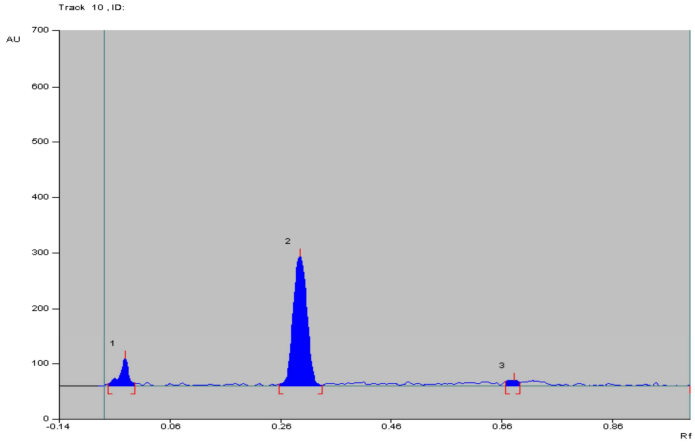

Figure 7: Chromatogram of dry heat [2h at 60°C] treated sample

Peak 1- degradant [R_f=0.02], Peak 2- fexofenadine hydrochloride [R_f=0.27], Peak 3- degradant [R_f=0.68]

3.4.4. Photochemical degradation

The sample exposed to photochemical degradation showed no additional peak other than fexofenadine hydrochloride which suggests that fexofenadine hydrochloride is stable towards photochemical degradation.

CONCLUSION

The developed HPTLC method enables accurate, precise, specific and stability indicating TLC method for determination of fexofenadine hydrochloride. Statistical analysis proves that the method is reproducible for routine analysis of fexofenadine hydrochloride in pharmaceutical dosage form without interference from excipients. This work will further be extended to study the degradation kinetics of the drug in plasma and other biological fluids. The developed method was able to separate the drug from its degradants hence can be successfully applied as a stability indicating one.

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