STABILITY INDICATING HPTLC METHOD DEVELOPMENT FOR FEXOFENADINE HYDROCHLORIDE

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ABSTRACT
A highly selective, sensitive and robust stability indicative HPTLC method was developed and validated for Determination of Fexofenadine Hydrochloride in bulk for degradation studies, stability studies as well as in formulation. The chromatography was performed on pre-coated silica gel 60F254 aluminum plate using the mobile phase Hexane: Methanol: Tri-Ethyl amine (6:4:0.1 v/v/v) with UV detection at 234 nm with RF 0.68 ± 0.02. The method was demonstrated to be precise, accurate and specific with no interference from the tablet excipients and was able to separate the drug from the degradation products produced under Acidic, Alkaline, Oxidative, Photolytic and Thermal Degradation condition. The method was validated for Linearity, Specificity, Precision, Accuracy and Robustness as per ICH guidelines. The Linearity of peak area responses was demonstrated within the concentration range of 1000-6000 ng/spot with R² 0.9904. The Limits of Detection and the Limits of Quantitation were 623.59 and 1889.66 ng/spot respectively. The Accuracy determined at 80, 100 and 120% levels was found to be within 99.81 % ± 0.76 and 101.01 % ± 0.99. Fexofenadine Hydrochloride was found to be degraded 11.1% in Acidic Condition, 7.88% Alkaline Condition, 14.55% Oxidative Stress Condition, 2.51% Photolytic Condition and 5.78% Thermal Condition. It was found that oxidative condition is more susceptible to degradation of drug, whereas photolytic condition is least susceptible to degradation of drug. The results indicated that the proposed method can be used as a Stability Indicating Method for estimation of Fexofenadine Hydrochloride.

KEYWORDS: Fexofenadine Hydrochloride, HPTLC, Method Validation, Stability Indicating Method.

INTRODUCTION
Fexofenadine is a selective peripheral H1-blocker. It prevents the activation of the H1 receptors by histamine, preventing the allergy symptoms such as sneezing, runny nose, itchy throat, or itchy, watery eyes. It is also used to treat hives and skin itching. Fexofenadine hydrochloride is a second generation antihistamine drug used in the treatment of hay fever, rhinitis, urticaria and similar allergy symptoms. 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.

Fexofenadine hydrochloride is a histamine H1-receptor antagonist having chemical name (±)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-α, α-dimethyl benzene acetic acid hydrochloride, molecular weight 538.13 empirical formula C32H30NO2•HCl and the following chemical structure.

Figure 1: Structure of Fexofenadine Hydrochloride.

Fexofenadine is the non-toxic, pharmacologically active metabolite of terfenadine. It has been introduced as a substitute of terfenadine free of arrhythmogenic potential. It is non-sedative type drug so can be used in day time also. It does not cure but rather prevents the aggravation of rhinitis and urticaria and reduces the severity of the symptoms associated with those conditions, providing relief from repeated sneezing, runny nose, itchy eyes and general body fatigue. It is use in form of acidic salt for better pharmacokinetic performance of drug for its pharmacological action.
Fexofenadine Hydrochloride (60mg) has been approved by FDA for marketing from July 1996 for treatment of seasonal allergic rhinitis. Fexofenadine Hydrochloride is official in the Indian Pharmacopeia, British Pharmacopoeia, United States Pharmacopoeia, Japanese Pharmacopoeia and HPLC is the official method for the estimation of Fexofenadine hydrochloride in tablets dosage form.

Stability indicating method development forms an important part of the process of drug product development. The purpose of stability testing is to provide the evidence on how the qualities of the drug substance or drug product will be varied with time under influence of a variety of environmental condition such as temperature, humidity and light and enables recommendation for storage conditions. The two main aspects of drug product that play importance role in self-life determination are assay of active drug as well as degradants generated, during the stability study. The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by ICH guidelines and USP.

Several other techniques for the estimation of Fexofenadine hydrochloride have been described in the literature which includes UV-Visible Spectrophotometric [8-10] RP-HPLC [11-13] and RP-UPLC [14]. To the best of our knowledge very few scientific literatures has been published for stability indicating HPTLC method for determination of Fexofenadine Hydrochloride.

HPTLC is a simple, rapid, cost effective technique that offers the advantages of high sensitivity, several analysis simultaneously, lower analysis time and less cost per analysis, lower maintenance cost, simple sample preparation, easy handle samples of divergent nature and no prior treatment like filtration and degassing for solvent.

Therefore, it was thought of interest to develop and validate a simple, accurate, precise and specific Stability Indicating HPTLC method for estimation of Fexofenadine Hydrochloride in its pharmaceutical tablet dosage form.

Objective of the study was to develop a stability indicating HPTLC method for the estimation of fexofenadine hydrochloride in presence of its degradation products, to perform forced degradation study of Fexofenadine Hydrochloride in various stress conditions such as acid, alkali, oxidative, thermal and photolytic degradation conditions and to validate the developed HPTLC method and to apply the developed method to analyze Fexofenadine Hydrochloride in marketed tablet formulations.

MATERIALS AND METHODS

Materials
Fexofenadine Hydrochloride API was provided as a gift sample from DOLPHINE PHARMACEUTICAL LTD, Surat, Gujarat, India. All Chemicals and Reagents used were of Analytical Grade and HPLC Grade. Marketed Formulation used for assay was Allegra 120 having Label Claim 120mg of Fexofenadine Hydrochloride.

Instruments
FT-IR (Shimadzu); Double Beam UV Spectrophotometer (Shimadzu UV1800); HPTLC system with Linomat V semi-automatic sample applicator (Camag Linomat V, Muttenz, Switzerland), TLC scanner IV (Camag), Flat bottom, Twin through developing chamber (10 x 10 cm2)(Camag), UV cabinet with dual wavelength (254/366)UV lamp (Camag), Software: Win-CATS, version 1.4.6 and Digital camera; Silica Plates used were Pre coated silica gel aluminum plate 60F254, (10 x 10cm2with 250 μm thickness; E. Merck, Darmstadt, Germany, supplied by Anchron Technologies, Mumbai, India).

Optimization of HPTLC Method

Selection of Detection Wavelength
The standard solution of Fexofenadine Hydrochloride (20μg/ml) in methanol was recorded using double beam UV spectrophotometer. Wavelength maxima values were compared with reported UV spectra of the drug.

Optimization of Mobile Phase
Pre coated silica gel aluminum plate 60F254 was washed with methanol and activated in oven at 60°C for 5 min. The standard stock solution of Fexofenadine Hydrochloride and Degraded Drug solutions were spotted separately on Pre Coated Silica Gel Aluminum Plate by using glass capillary tube and allowed to dry for few minutes. Different mobile phases (10 ml) were taken in 100 ml glass beaker and allowed to saturate for 30 minutes. The spotted plate was developed in mobile phase to about ¾ height of the plate. The plate was removed and allowed to dry. Spots were observed in U.V cabinet lamp.
Optimization of Chromatographic Condition

Table 1: Optimization of Chromatographic Condition.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Chromatographic conditions</th>
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<tbody>
<tr>
<td>Stationary phase</td>
<td>Aluminum plates pre-coated with silica gel 60 F254 (10 × 10 cm)</td>
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<td>Mobile phase</td>
<td>Chloroform : Hexane: Methanol (5:4:1 v/v/v)</td>
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<tr>
<td>Chamber saturation time</td>
<td>30 min</td>
</tr>
<tr>
<td>Temperature</td>
<td>Room Temperature (25±2°C)</td>
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<td>Migration distance</td>
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<th>Application Parameter</th>
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<tr>
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<tr>
<td>Distance from the plate edge</td>
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<td>Distance from the bottom of the Plate</td>
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<td>Scanning speed</td>
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<td>Detection wavelength</td>
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<tr>
<td>Lamp</td>
<td>D2</td>
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<tr>
<td>Measurement mode</td>
<td>Absorption</td>
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</table>

Validation of proposed HPTLC Method
Preparation of Standard Stock Solution of Fexofenadine Hydrochloride

Accurately weighed 100 mg of standard Fexofenadine Hydrochloride was transferred to 50 ml volumetric flask, dissolved in 5 ml methanol and diluted up to the mark with same to get stock solution having strength 2000 μg/ml.

Calibration Curve

Standard stock solution (2000μg/ml) of Fexofenadine Hydrochloride was spotted on pre-coated TLC plate under nitrogen stream using Linomat V semi-automatic sample applicator. The plate was dried in the air and developed up to 80 mm using mixture of Chloroform : Hexane: Methanol: (5:4:1v/v/v) as mobile phase in a twin trough chamber previously saturated with mobile phase for 30 minutes. The plate was removed from the chamber, dried in hot air oven and scanned and quantified at 234 nm in absorbance mode. The calibration curve was constructed by plotting area versus respective concentration (ng/spot).

Linearity
The linearity range was determined by analyzing 6 independent levels of calibration curve in the range of 2000-12000 ng/spot for Fexofenadine Hydrochloride. A volume of 1, 2, 3, 4, 5, and 6 μl of standard stock solution (2000 μg/ml) were applied on TLC plate and analyzed as per the proposed method. The calibration curve was prepared by plotting peak area vs. concentration and correlation coefficient was calculated.

Specificity
The spot of Fexofenadine Hydrochloride from dosage form were confirmed by comparing its RF and absorbance-reflectance spectrum with that of standard Fexofenadine hydrochloride. The peak purity of Fexofenadine hydrochloride from each sample was determined by comparing the spectra scanned at peak start, peak apex, and peak end positions of the spot.

Precision

Working standard solution of Fexofenadine hydrochloride (1 μl of 2000 μg/ml) was spotted on a TLC plate seven times, and Repeatability was analyzed by the proposed method. The area of seven spots was measured and percent RSD of peak area was calculated. Intra-day precision and Inter-day precision of the proposed method was evaluated by analyzing the range of Fexofenadine hydrochloride (6000, 8000 and 10000 ng/spot), three times on same day and three different days respectively.

Accuracy

Accuracy of the method was confirmed by Recovery study from Marketed Formulation at three level of standard addition. Known amount of standard Fexofenadine hydrochloride was added at 80%, 100% and 120% level to pre-analyzed sample of Fexofenadine hydrochloride. The quantity of tablet powder equivalent to 50 mg of Fexofenadine hydrochloride was transferred to four individual 25 ml volumetric flasks. Known amount of standard Fexofenadine hydrochloride was spiked to pre-analyzed sample of Fexofenadine hydrochloride. 10 ml methanol was added to dissolve powder drug sample and volume was made up to mark with methanol. A volume of 2 μl of all the solutions were spotted and analyzed.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of detection was calculated using following equation as per ICH guidelines.
Forced Degradation Study
Forced degradation of Fexofenadine Hydrochloride was carried out under acidic, alkaline, oxidative, photolytic and dry heat conditions.

Acidic Degradation
Accurately weighed 100 mg of fexofenadine hydrochloride was transferred to a 50 ml volumetric flask add 10 ml methanol. To the above solution 10 ml of 1M HCl was added. The solution was reflux for 2 hours at 60°C in water bath. After that cooled and neutralized by 1M NaOH and diluted up to mark with methanol.

Alkaline Degradation
Accurately weighed 100 mg of fexofenadine hydrochloride was transferred to a 50 ml volumetric flask add 10 ml methanol. To the above solution 10 ml of 0.5M NaOH was added. The solution was reflux for 2 hours at 60°C in water bath. After that cooled and neutralized by 0.5N HCl and diluted up to mark with methanol.

Oxidative Degradation
Accurately weighed 100 mg of fexofenadine hydrochloride was transferred to a 50 ml volumetric flask add 20 ml methanol. To the above solution add 5ml of 3% H2O2. The solution was reflux for 1 hour at 70°C in water bath. After that cooled and diluted up to mark with methanol.

Thermal Degradation (Dry Heat Degradation)
Accurately weighed 100 mg of Fexofenadine Hydrochloride was transferred to Petri plate with close lead. Plate was heated in previously heated hot air oven at 0°C for 6 hrs. Remove petri plate and cooled it. Add 20 ml methanol petri plate and transfer it to 50 ml volumetric flask. Wash petri plate with small aliquots of methanol. Add it in volumetric flask and make up volume to 50 ml with methanol.

Photolytic Degradation
Accurately weighed 100 mg of Fexofenadine Hydrochloride was transferred to Petri plate with close lead. Plate was exposed to direct sunlight for 24 hrs. Add 20 ml methanol in petri plate and transfer it to 50 ml volumetric flask. Wash petri plate with small aliquots of methanol. Add it in volumetric flask and make up volume to 50 ml with methanol.

A volume of 3μl of each degraded solution was applied to TLC plate. The plate was dried in air and developed up to 80 mm using mixture of Chloroform: Hexane: Methanol (5:4:1 v/v/v) as mobile phase in a Camag twin through chamber previously saturated with mobile phase for 30 minutes. The plate was removed from the chamber, dried in hot air oven and standard zones were scanned and quantified at 234 nm.

RESULTS AND DISCUSSION
Optimization of HPTLC Method
Selection of Detection Wavelength
UV spectrum Fexofenadine Hydrochloride of shows maximum absorbance at 220 nm.

Optimization of Mobile Phase
The TLC procedure was optimized with a view to develop a Stability Indicating Method to quantify the Fexofenadine Hydrochloride. Both the Fexofenadine Hydrochloride Standard Solutions and the Degraded Solutions prepared by Forced Degradation in different conditions were spotted on the TLC plates and were run in different solvents. Mobile Phase consisting of Chloroform: Hexane: Methanol (5:4:1 v/v/v) showed

LOD= 3.3 x SD/S
Where SD is the standard deviation of the Y- intercepts of the five calibration curves and S is mean slope of the five calibration curves.

Limit of quantitation was calculated using following equation as per ICH guidelines.
LOQ = 10 x SD/S
Where SD is the standard deviation of the Y- intercepts of the five calibration curves and S is mean slope of the five calibration curves.

Solution Stability
Freshly prepared standard stock solution of Fexofenadine hydrochloride (2000 µg/ml) was analyzed and stored at 27±2°C. The standard solution was analyzed after 24 hours and area obtained was compared with the initial area.

Robustness
Robustness of the method was determined by subjecting the method to slight change in the mobile phase composition, mobile phase volume and duration of chamber saturation time and the effects on the results were examined. Mobile Phases having different compositions of Chloroform: Hexane: Methanol (6:3:1 and 4:5:1 v/v/v) were tried and chromatograms were run. Mobile phase volume and duration of saturation were varied at 20±2ml (18, 20 and 22 ml) and 30±10 min (20, 30 and 40 min), respectively. Robustness of the method was studied in triplicate at a concentration level of 4000 ng/spot.

Assay
Twenty tablets of each formulation of Fexofenadine hydrochloride were weighed and finely powdered. The tablet powder equivalent to 50 mg of Fexofenadine hydrochloride was accurately weighed and transferred to a 100 ml volumetric flask, about 25 ml of methanol was added and the flask was sonicated for 15 min. The volume was made up to 50 ml with methanol mixed well and filtered through whatman paper no. 41. From the filtrate, 5μl was applied on the TLC plate and analyzed as per the developed method.

Forced Degradation Study
Forced degradation of Fexofenadine Hydrochloride was carried out under acidic, alkaline, oxidative, photolytic and dry heat conditions.

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Accurately weighed 100 mg of fexofenadine hydrochloride was transferred to a 50 ml volumetric flask add 10 ml methanol. To the above solution 10 ml of 1M HCl was added. The solution was reflux for 2 hours at 60°C in water bath. After that cooled and neutralized by 1M NaOH and diluted up to mark with methanol.

Alkaline Degradation
Accurately weighed 100 mg of fexofenadine hydrochloride was transferred to a 50 ml volumetric flask add 10 ml methanol. To the above solution 10 ml of 0.5M NaOH was added. The solution was reflux for 2 hours at 60°C in water bath. After that cooled and neutralized by 0.5N HCl and diluted up to mark with methanol.

Oxidative Degradation
Accurately weighed 100 mg of fexofenadine hydrochloride was transferred to a 50 ml volumetric flask add 20 ml methanol. To the above solution add 5ml of 3% H2O2. The solution was reflux for 1 hour at 70°C in water bath. After that cooled and diluted up to mark with methanol.

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Accurately weighed 100 mg of Fexofenadine Hydrochloride was transferred to Petri plate with close lead. Plate was heated in previously heated hot air oven at 0°C for 6 hrs. Remove petri plate and cooled it. Add 20 ml methanol petri plate and transfer it to 50 ml volumetric flask. Wash petri plate with small aliquots of methanol. Add it in volumetric flask and make up volume to 50 ml with methanol.

Photolytic Degradation
Accurately weighed 100 mg of Fexofenadine Hydrochloride was transferred to Petri plate with close lead. Plate was exposed to direct sunlight for 24 hrs. Add 20 ml methanol in petri plate and transfer it to 50 ml volumetric flask. Wash petri plate with small aliquots of methanol. Add it in volumetric flask and make up volume to 50 ml with methanol.

A volume of 3μl of each degraded solution was applied to TLC plate. The plate was dried in air and developed up to 80 mm using mixture of Chloroform: Hexane: Methanol (5:4:1 v/v/v) as mobile phase in a Camag twin through chamber previously saturated with mobile phase for 30 minutes. The plate was removed from the chamber, dried in hot air oven and standard zones were scanned and quantified at 234 nm.

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good resolution with forced degradants and compact spot of Fexofenadine Hydrochloride was observed with Rf value 0.68 ± 0.02. This mobile phase was able to separate maximum the degradation products of Fexofenadine Hydrochloride in different stress conditions.

**Validation of proposed HPTLC Method**

**Linearity**

Linearity parameters for the Fexofenadine Hydrochloride peak area response versus the Fexofenadine Hydrochloride concentrations were studied in the concentration range range of 2000, 4000, 6000, 8000, 10000 and 12000 ng/spot. The calibration curve was found to be linear in this range. The correlation coefficient was found to be 0.9973. Using the least square method, the regression equation between the peak area (Y) and the concentration (X) was found to be y = 0.755x + 124.99.

<table>
<thead>
<tr>
<th>Concentration (ng/spot)</th>
<th>Peak Area (Mean ± S.D.); (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>1463.5 ± 18.23</td>
</tr>
<tr>
<td>4000</td>
<td>3186.4 ± 28.42</td>
</tr>
<tr>
<td>6000</td>
<td>4778.1 ± 45.02</td>
</tr>
<tr>
<td>8000</td>
<td>6278.3 ± 27.47</td>
</tr>
<tr>
<td>10000</td>
<td>7768.6 ± 34.99</td>
</tr>
</tbody>
</table>

**Table 2: Linearity of Fexofenadine Hydrochloride.**

![Figure 2: The overlain 3D chromatogram shown in linearity of Fexofenadine Hydrochloride (2000-12000 ng/spot).](image)

**Specificity**

From the overlain spectrum of Fexofenadine Hydrochloride standard and sample, identity of the drug was confirmed. Comparison of spectra of Fexofenadine Hydrochloride from each sample scanned at peak start (s), peak apex (m) and peak end (e) position of individual spots showed a high degree of correlation which confirmed the purity of the corresponding spots. The excipients and other components present in tablet formulation did not interfere in the separation and resolution of Fexofenadine Hydrochloride. The method can be considered to be specific.

**Table 3: Peak Purity Data.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>r (S,M)</th>
<th>r (M,E)</th>
<th>Correlation R-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fexofenadine Hydrochloride</td>
<td>0.9999</td>
<td>0.9997</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

**Precision**

The %RSD value of Fexofenadine Hydrochloride for Repeatability of Measurement (n=7) was found to be 1.16% and Repeatability of sample application (n=7) was found to be 1.04%, for Intra-Day Precision was found to be in range 0.92-0.97% and for Inter-Day Precision was found to be in range 0.86-1.01%. The %RSD value of Fexofenadine Hydrochloride was found to be less than 2%, which indicates that the developed method is precise.

<table>
<thead>
<tr>
<th>Concentration (ng/spot)</th>
<th>Intra-day Precision</th>
<th>Inter-day Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area (Mean ± SD) (n=3)</td>
<td>% RSD</td>
</tr>
<tr>
<td>6000</td>
<td>4762.76 ± 43.68</td>
<td>0.92</td>
</tr>
<tr>
<td>8000</td>
<td>6149.40 ± 57.79</td>
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<tr>
<td>10000</td>
<td>7684.36 ± 74.27</td>
<td>0.97</td>
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</table>

**Table 4: Intra-Day and Inter-Day Precision.**

Figure 3: Calibration Curve for Fexofenadine Hydrochloride (2000-12000ng/spot).
Accuracy
The percentage recovery of Fexofenadine Hydrochloride was found to be in range 99.81 ± 0.76% – 101.01 ± 0.99%.

Table 5: Results for Recovery of Fexofenadine Hydrochloride

<table>
<thead>
<tr>
<th>Level</th>
<th>Amount from sample (ng)</th>
<th>Amount of Std. Spiked Fexofenadine Hydrochloride (ng)</th>
<th>Total amount (ng)</th>
<th>Total area (Mean)</th>
<th>Recovered amount (ng) ± SD (n=3)</th>
<th>% Recovery of spiked amount ± S.D (n=3)</th>
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<tbody>
<tr>
<td>0%</td>
<td>4000</td>
<td>-</td>
<td>4000</td>
<td>3156.17</td>
<td>3198.18 ± 38.48</td>
<td>99.81 ± 0.76</td>
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<tr>
<td>80%</td>
<td>4000</td>
<td>3200</td>
<td>7200</td>
<td>5567.79</td>
<td>4040.57 ± 14.39</td>
<td>101.01 ± 0.99</td>
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<td>100%</td>
<td>4000</td>
<td>4000</td>
<td>8000</td>
<td>6206.81</td>
<td>4807.96 ± 35.16</td>
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<td>120%</td>
<td>4000</td>
<td>4800</td>
<td>8800</td>
<td>6786.18</td>
<td>4807.96 ± 35.16</td>
<td>100.16 ± 0.73</td>
</tr>
</tbody>
</table>

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The LOD and LOQ of Fexofenadine Hydrochloride were found to be 623.59 ng/spot and 1889.66 ng/spot, respectively, which indicates the adequate sensitivity of the method.

Robustness
The % R.S.D. of the peak areas were calculated for change in mobile phase composition, mobile phase volume and chamber saturation time at a concentration level of 4000 ng/spot in triplicate. The low values of % R.S.D. (<2) obtained after introducing small deliberate changes in the developed HPTLC method indicated the robustness of the method.

Solution Stability
Solutions were kept at room temperature (27±2 C) for 24 hours and analyzed by the developed HPTLC method. No significant change was observed in area of chromatogram (%RSD < 1.5%) implies that solutions were stable at room temperature.

Assay of Marketed Formulation
A single spot at Rf value 0.68 was observed in the chromatogram of the Fexofenadine Hydrochloride samples extracted from tablets. There was no interference from the excipients present in the tablets. The drug content was found to be 101.73% with a % R.S.D. of 0.87%. The low % R.S.D. value indicated the suitability of this method for routine analysis of Fexofenadine Hydrochloride in pharmaceutical novel drug delivery systems.

The proposed method was successfully applied to the marketed tablet dosage form Allegra 120 having label claim 120mg of Fexofenadine Hydrochloride. The % Assay was found to be 98.09 ± 1.22%.
Table 6: Summary of Validation Parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
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<tr>
<td>Linearity range</td>
<td>2000-12000 ng/spot</td>
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<td>Regression line equation</td>
<td>Y= 0.755x + 124.99</td>
</tr>
<tr>
<td>Correlation co-efficient(R^2)</td>
<td>0.9973</td>
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<tr>
<td>Precision (%RSD)</td>
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<tr>
<td>Repeatability of measurement (n=7)</td>
<td>1.16</td>
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<tr>
<td>Repeatability of sample application (n=7)</td>
<td>1.04</td>
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<tr>
<td>Intra-day precision (n=3)</td>
<td>0.92-0.97%</td>
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<tr>
<td>Inter-day precision (n=3)</td>
<td>0.86-1.01%</td>
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<tr>
<td>% Recovery(n=3)</td>
<td>99.81 ± 0.76-101.01 ± 0.99</td>
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<tr>
<td>Limit of Detection (LOD)(ng/spot)</td>
<td>623.59</td>
</tr>
<tr>
<td>Limit of Quantitation (LOQ) (ng/spot)</td>
<td>1889.66</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
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Force Degradation Studies

Degradation under Acidic Condition
After heating drug solution with 1M HCl at 60°C for 2 hr, the percentage degradation of Fexofenadine Hydrochloride in acidic condition was found to be 11.7%. The chromatogram of acid treated Fexofenadine Hydrochloride showed two additional peaks at Rf = 0.55, 0.65.

Degradation in Alkaline Condition
After heating drug solution with 1N NaOH at 60°C for 2 hr, the percentage degradation of Fexofenadine Hydrochloride in alkaline condition was found to be 7.88%. The chromatogram of alkali treated Fexofenadine Hydrochloride showed one additional peak at Rf = 0.87.

Oxidative Degradation
After heating drug solution with hydrogen peroxide at 70°C for 1 hr, the percentage degradation of Fexofenadine Hydrochloride in oxidative condition was found to be 14.55%. The chromatogram of hydrogen peroxide treated Fexofenadine Hydrochloride showed one additional peak at Rf = 0.80, 0.66.
Thermal Degradation (Dry Heat Degradation)
Drug sample was exposed to dry heat 80°C for 6 hr in hot air oven, the % degradation of Fexofenadine Hydrochloride in thermal degradation was found to be 5.76%. The chromatogram of Thermal Degraded Fexofenadine Hydrochloride showed one additional peak at Rf = 0.72.

Photolytic Degradation
Drug sample was exposed to direct sunlight for 24 hr, the percentage degradation of Fexofenadine Hydrochloride in photolytic condition was found to be 2.51%. The chromatogram of direct sunlight treated Fexofenadine Hydrochloride showed one additional peak at Rf = 0.72 and fronting of peak was observed.

<table>
<thead>
<tr>
<th>Force Degradation Condition</th>
<th>Stress Condition</th>
<th>Rf value of degradants</th>
<th>Fexofenadine Hydrochloride remained Undegraded (%)</th>
<th>Degradation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic Degradation</td>
<td>(1NHCl/60°C/2hrs)</td>
<td>0.55</td>
<td>88.9</td>
<td>11.1</td>
</tr>
<tr>
<td>Alkaline Degradation</td>
<td>(1NNaOH/60°C/2hr)</td>
<td>0.65</td>
<td>92.12</td>
<td>7.88</td>
</tr>
<tr>
<td>Oxidative Degradation</td>
<td>(3% v/v H2O2/70°C/1 hrs)</td>
<td>0.80</td>
<td>85.45</td>
<td>14.55</td>
</tr>
<tr>
<td>Thermal Degradation</td>
<td>(80°C/6 hr)</td>
<td>0.66</td>
<td>94.24</td>
<td>5.76</td>
</tr>
<tr>
<td>Photo Degradation</td>
<td>24 hours in Sunlight</td>
<td>0.72</td>
<td>97.49</td>
<td>2.51</td>
</tr>
</tbody>
</table>

CONCLUSION
The developed HPTLC method was simple, accurate, precise, and reproducible and stability indicating for quantitative analysis for determination of Fexofenadine Hydrochloride in pharmaceutical tablets, without any interference from the excipients and in the presence of its Acidic, Alkaline, Oxidative, Thermal and Photolytic Degradation Products.

ICH guidelines were followed throughout the study for Method Validation and Stress Testing, and the suggested methods can be applied for Quality Control and Routine Analysis.

It was found that oxidative condition was more susceptible to Degradation of Drug, whereas Photolytic condition least susceptible to Degradation of Drug.

It was one of the rare studies where forced decomposition was done under all different suggested conditions and the degradation products were resolved. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities and also in stability studies. It is proposed for the analysis of the drug and degradation products in stability samples in industry.
ACKNOWLEDGEMENT
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5. Indian Pharmacopoeia, Government of India Ministry of Health and Family.