A Validated RP-HPLC Method and Force Degradation Studies of Fexofenadine Hydrochloride in Pharmaceutical Dosage Form

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ABSTRACT

A stability indicating HPLC method was developed and validated for the quantitative determination of fexofenadine hydrochloride. An isocratic separation was achieved using phenomenex (C_{18}) column (250×4.6 mm, 5 µm) with flow rate of 1.0 ml/min and UV detection at 254 nm. The mobile phase consists of 5Mm acetate buffer: acetonitrile (50:50; v/v) with pH 9.4 adjusted with acetic acid. The drug was subjected to oxidative, acidic, basic, neutral, photolytic and thermal degradation. All degradation products were eluted in an overall analytical run time of approximately 40 min with the parent compound fexofenadine hydrochloride at a flow rate of approximately 3.3±0.3 min. The method was linear over the concentration range of 31.5-500 µg/ml (r^2 = 0.999) with limit of detection and quantification of 3.5 µg/ml and 10.1 µg/ml, respectively. The method has the requisite accuracy, selective, precision and robustness to assay fexofenadine HCl in tablets.

Key words: Fexofenadine HCl, RP-HPLC, validation, force degradation.

INTRODUCTION

Fexofenadine, α,α-dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenyl-methyl)-1-piperidinyl]butyl]-benzene acetic acid (Figure 1), is used to relieve the allergy symptoms of seasonal allergic rhinitis ("hay fever"), including runny nose, sneezing, and red, itchy or watery eyes or itching of the nose, throat or roof of the mouth in adults.[1,2] It is carboxylic acid metabolite of terfenadine, a non-sedating selective histamine H_1 receptor antagonist.[3]

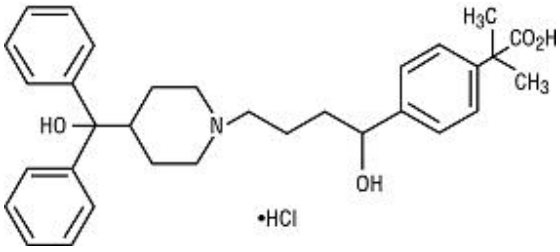

Figure 1. Structural Formula of Fexofenadine HCl.

Fexofenadine hydrochloride is a white to off-white crystalline powder. It is freely soluble in methanol and ethanol, slightly soluble in chloroform and water, and insoluble in hexane. Fexofenadine hydrochloride is formulated as tablet for oral administration. Each tablet contains 120 or 180 mg Fexofenadine hydrochloride (depending on the dosage strength). A simple, sensitive, reliable and cost-effective RP-HPLC method, validated in accordance with International Conference on Harmonization (ICH) guidelines for determination and force degradation of fexofenadine is described in this paper.[4] Some methods have been reported for estimation of fexofenadine with other combination using HPLC.[5,6] But the reported method was found to be more time consuming and required more solvent. In addition, it showed longer retention time for separation. Besides, some RP-HPLC methods were established using gradient method with prolong run

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time.[9] On the other hand, some of the validated methods were established using both polar and non polar mobile phase in combination which is contradictory.[10] Even though Fexofenadine itself is an acidic compound but some validated methods were run in acidic buffer.[10] The proposed validated method is more economical, precise, accurate and specific for quantitative determination of fexofenadine in pharmaceutical dosage form. In this method, pH was adjusted up to 9.4 (basic buffer of acetate) with isocratic method which can help to avoid the uses of excessive reagents. Besides, here mixture of polar and non-polar reagents was avoided to give the perfect chromatogram. We used phenomenex (C_{18}) column (250 × 4.6 mm, 5 µm) with a flow rate of 1.0 ml/min for the perfect separation of compounds and also for degradation products.

MATERIALS AND METHODS

Materials and reagents. Pure drug sample of fexofenadine hydrochloride was collected from Beximco Pharmaceuticals Ltd. Working standard of fexofenadine hydrochloride was collected from Aristopharma Ltd. The pharmaceutical tablets were purchased from different drug stores. Here, HPLC grade of acetonitrile and methanol were purchased from Sigma Aldrich, USA. Sodium acetate and acetic acid were purchased from Merck. Sodium hydroxide, hydrochloric acid and hydrogen peroxide were also of analytical reagent grade from Merck, Mumbai, India.

HPLC instrumentation and conditions. The HPLC system consisted of a binary pump (UV visible pump), auto-sampler (model 717 plus), column heater and PDA detector (SPD 20). Data collection and analysis were performed using LC data analysis software. Separation was achieved on phenomenex C_{18} column (250mm × 4.6mm, 5.0 µ) maintained at 35°C using column oven. Isocratic elution was carried out with sodium acetate buffer: acetonitrile (50:50; v/v) with pH 9.4 adjusted with acetic acid at the flow rate of 1.0 ml/min. The detection was monitored at 254 nm and injection volume was 20 µl. The peak purity was checked with the photodiode array detection.

Preparation of standard solutions and calibration curve. Standard stock solution of fexofenadine HCl containing 500µg/ml of fexofenadine HCl was prepared in buffer. To study the linearity range serial dilutions were accomplished to obtain the concentration of fexofenadine HCl at 32, 61, 125, 250 and 500 µg/ml in mobile phase and injected in to column. Calibration curves were plotted as concentration of drug versus peak area response. From the standard stock solution, 10 µg/20ml of fexofenadine HCl was injected in to column. The system test was performed from six replicate injections of standard solution.

Preparation of test sample from tablet. Twenty tablets were weighted accurately and crushed and powder equivalent to 50 mg of fexofenadine HCl was weighted and dissolved in 100ml of mobile phase with the aid of ultrasonication for 10 min and the solution was filtered through 0.45 µ Whatman paper No. 41 into a 100 ml volumetric flask.

Method validation. With respect to ICH guidelines, the HPLC method was validated in terms of precision, accuracy and linearity. Assay method precision was determined using six independent test solutions. The intermediate precision of the assay method was also evaluated as inter-day and intra-day precision. The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium & high) of the authentic standard were added to the placebo. The mixtures were extracted and analyzed using the developed HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) for analytes were estimated by injecting a series of dilute solutions of known concentration. Values of LOD and LOQ were calculated by using σ (standard deviation of response) and *b* (slope of the calibration curve) and by using equation, $LOD=(3.3\times\sigma)/b$ and $LOQ= (10\times\sigma)/b$. To determine the robustness of the method, final experimental conditions were purposely altered and results were examined. The parameters considered (±values) for the study were

flow rate, column temperature, measurement wavelength, % organic, buffer strength and effect of column. The drug solution stability was carried out for short-term stability by keeping at room temperature for 12 hrs, long-term by storing at 4°C for 30 days and by storing the samples for 24 hrs in the auto-sampler and then analyzing against freshly prepared solutions. For method development and optimization, retention factor (k) was calculated by using parameters t_R (retention time) and t_M (elution time of the solvent front) and by using the equation $K = (t_R-t_M)/t_M$.

Forced degradation studies of API and tablets

Acid hydrolysis. Solutions for acid degradation studies were prepared in methanol and 0.1 M hydrochloric acid (20 : 80, v/v) at room temperature (22ºC). It was observed that both acid and base hydrolysis proceeded fast for both drugs and almost completed within 10 min of the sample preparation. Therefore, the samples were analyzed after this period.

Base hydrolysis. Solutions for base degradation studies were prepared in methanol and 0.1 M sodium hydroxide (20 : 80, v/v) at room temperature (22ºC) and the resulting solutions were analyzed 10 min after preparation.

Neutral hydrolysis. Solutions for neutral degradation studies were prepared in methanol and water (20 : 80, v/v) and the resulting solutions heated on a water bath at 90ºC for 20 min. The mixture was then allowed to cool at room temperature, filtered using syringe filters and analyzed.

Oxidation. Solutions for use in oxidation studies were prepared in methanol and 6% hydrogen peroxide (20 : 80, v/v) at room temperature (22ºC) and the solutions were filtered using syringe filters and analyzed after 10 min.

Photostability. Solutions for photostability studies were prepared in methanol and water (20:80, v/v) and the solution was exposed to natural sunlight during the day time for 8 hours. The degraded sample was then filtered using syringe filters and analyzed.

Temperature stress studies. Tablets and API in powder form were exposed to dry heat (100º C) in an oven for 8 hrs. The API and tablet powder were then removed from the oven and an aliquot of tablet powder equivalent to the weight of one tablet was prepared for analysis as previously described.

RESULTS AND DISCUSSION

The HPLC procedures were optimized for the development of a stability-indicating assay method. Pure drug along with its degraded products were injected and run in different solvent systems. To determine significant values of fexofenadine, a preliminary screening must be carried out. It was observed that acetonitrile when used more than 40% separated the drug products with high resolution. Besides as an organic phase, methanol when used above 50% also gave a perfect peak and resolution. But here, methanol as an organic phase can lead the retention time after 15 minutes. Therefore, an optimum level of acetonitrile (50%) was used for identification of drug. Initially methanol and water in different ratio were tried. It was found that when methanol concentration was increased in the mobile phase, the degradants started to elute in dead volume. Hence, concentration of methanol was decreased and there was improvement in resolution. In case of selecting buffer, pH was an important factor for dissolving products. Buffers are most effective according to their pKa value which must be close to their pH value. pKa value for acetate buffer is around 4.76 which is very much close to our pH value. Besides, pH value must be maintained at 9.8 because higher pH can damage the column. It was found that mobile phase consisting of sodium acetate and acetonitrile (50 : 50, v/v), pH adjusted to 9.4 with acetic acid with a flow rate of 1.0 ml/min gave acceptable retention time of 3.1 ± 0.5 min. (t_R), theoretical plates and good resolution of drug and degradation products. Well defined symmetrical peaks were obtained upon measuring the response of eluent under the optimized conditions after thorough experimental trials. Two columns were used for performance investigations, including Phenomenex

C_{18} (4.6 × 150 mm, 5 micron) and Phenomenex C_{18} (4.6 × 250 mm, 5 micron). The UV detector response of fexofenadine HCl was studied and the best wavelength was found to be 254 nm showing highest sensitivity. Also, temperature variation and change of flow rate showed no major variation in retention time and peak shape.

Method validation. The method was validated, in accordance with ICH guidelines for linearity, ruggedness and robustness.

Here, placebo was the mixture of starch, cellulose and lactose. From the Figure 2 no acceptable peaks were observed. For the peak (b), internal standard cetrizine HCl, (c) working standard and (d) are the analyzing product of fexofenadine tablet (Figures 2 and 3).

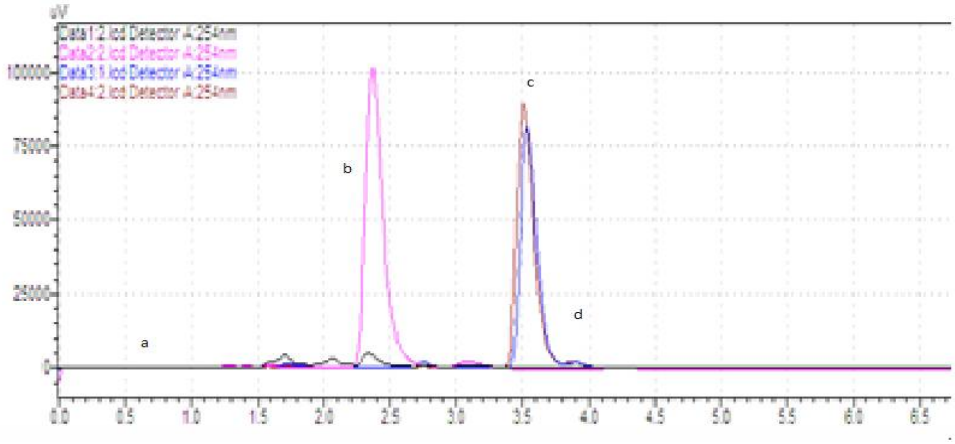

Figure 2. Chromatogram of a. placebo, b. internal std c. fexofenadine HCl API d. sample.

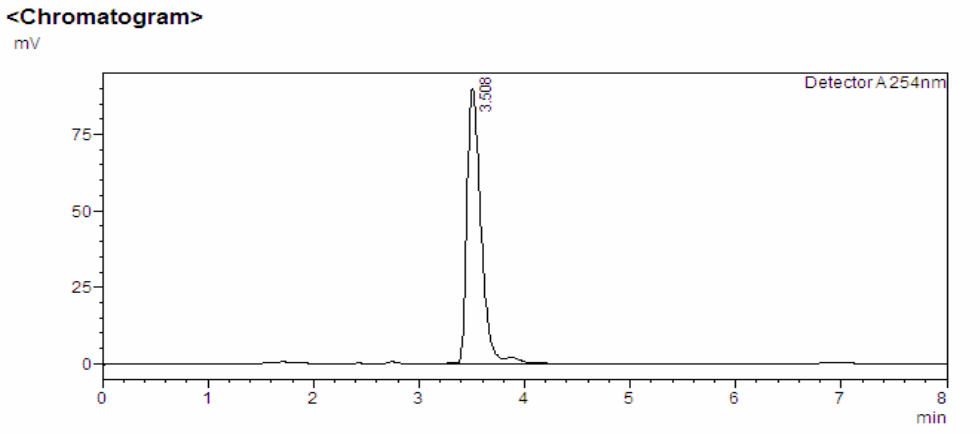

Figure 3: Chromatogram of fexofenadine HCl.

Linearity and range. For the construction of calibration curves, seven calibration standard solutions were prepared. Linearity was determined for fexofenadine HCl in the range of 32-500 µg/ml. The correlation coefficient (r^2) value was = 0.999 (n = 6). Typically, the regression equations for the calibration curve was found to be y = 1502 x + 27375

Table 1. System suitability parameters.

Parameter	Values ± SD
No of theoretical plates (SD)	4900 ± 20
USP tailing factor (SD)	1.030 ± 0.01
Capacity factor	0.3 ± 0.04
LOD in µg/ml	3.50
LOQ in µg/ml	10.01

Formulation analysis and accuracy. System suitability test was performed every time before formulations analysis (Table 1)

Formulation were analyzed as described in experimental section. Assay values (100 ± 8) % for both the formulations accuracy of the method were calculated by recovery studies at three levels by standard addition method. Results of formulation analysis and accuracy studies are presented in table 2.

Table 2. Results of tablets analysis and accuracy studies.

Tablet label claim	Formulation study (n=6)		Recovery (accuracy) study (n=3)	
	Tablet batch	% Assay found, % RSD	Recovery level Average of five samples	% Recovery, % RSD
Fexofenadine Hcl	1	92%, 1.5%	250	93%, 1.6%
120 mg	2	95%, 1.6%	500	96%, 1.4%
	3	98%, 1.5%	750	99%, 1.8%
	4	95.4%,1.7%		
	5	99.5%, 1.5%		

Table 3. Result of precision study.

Precision	Estimated amount in percentage		
	Injection of each sample (n=3)	Area	Assay
Inter-day	1	774415	95%
	2	776148	97%
	3	776954	98%
	4	761542	95%
	5	746985	95%
Intra-day	1	795465	105%
	2	786541	97%
	3	789524	98%
	4	764189	95%
	5	756985	96%

Table 4. Result of robustness study.

Parameter	Level	System suitability parameters n=3		
		Retention time	Capacity factor	Assay in %
Flow rate in ml/min	0.5	3.4	0.3 ± 0.04	96%
	0.8	3.3		
	1.0	3.4		
% of organic solvent	40	3.2	0.3 ± 0.04	96%
	50	3.2		
	60	3.4		
pH of mobile phase	7.4	2.9	0.3 ± 0.04	96%
	8.5	2.8		
	9.2	2.6		
Column	250 × 4.6	3.5	0.3 ± 0.04	96%
Wavelength	254	3.3	0.3 ± 0.04	96%
	230	3.4		
Column temperature	35	3.4	0.3 ± 0.04	96%
	40	3.2		

Precision. The precision of repeatability was studied by replicate (n=6) analysis of tablet solutions. The precision was also studied in terms of intra-day changes in peak area of drug solution on the same day and on three different days over a period of one week. The intra-day and inter-day variations were calculated in terms of percentage relative standard deviation and the results are summarized in table 3.

Here, five individual samples were analyzed for inter and intra days. Three consecutive days were observed for analysis. According to precision study, all results met the specifications. The potency was met to the range of 93-100% with sharp chromatographic peak and appropriate retention time (Table 4).

Table 5. Result of force degradation study.

Stress condition	Degraded products reported at retention time with recovery (API)		Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
	Time	Recovery	Time	Recovery	Time	Recovery	Time	Recovery	Time	Recovery	Time	Recovery
0.1N HCl, 3hr	3.2	92%	3.2	65.54%	3.2	70%	3.2	75.02%	3.2	72.14%	3.2	78.54%
0.1N NaOH, 3hr	3.3	94%	3.2	88.230%	3.1	89.21%	3.1	90.25%	3.1	91.25%	3.1	94.25%
6% of H_2O_2, 3hr	3.1	76%	3.1	50.12%	3.1	55.12%	3.1	65.24%	3.5	64.25%	3.1	59.35%
Long UV, 366nm, 8hr	3.2	90%	3.2	85.87%	3.5	95.87%	3.2	94.87%	3.2	96%	3.2	97.05%
Dry Heat, 80°C, 8hr	3.3	89%	3.4	74.65%	3.3	74.65%	3.5	78.65%	3.2	84.75%	3.3	87.%
Neutral	3.2	96%	3.0	91.45%	3.2	94.45%	3.2	98.45%	3.2	97.45%	3.2	93.45%

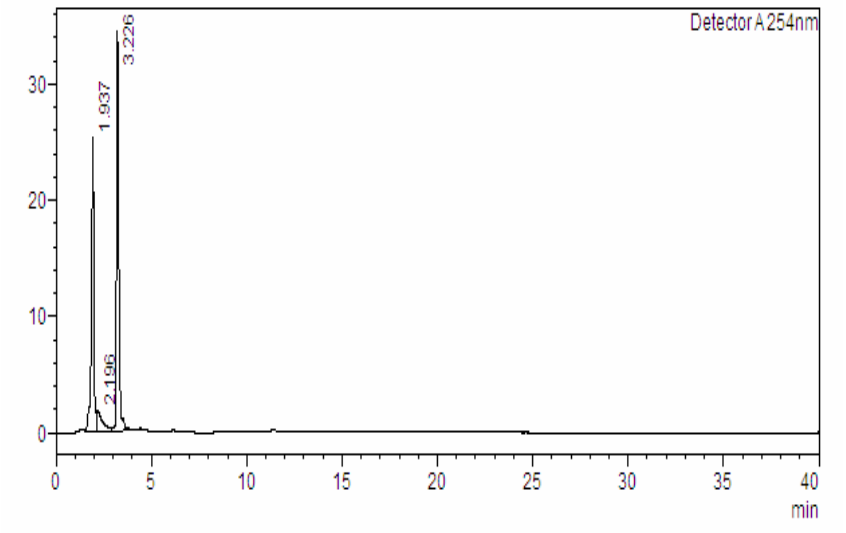

Figure 4. Acid degradation of fexofenadine hydrochloride.

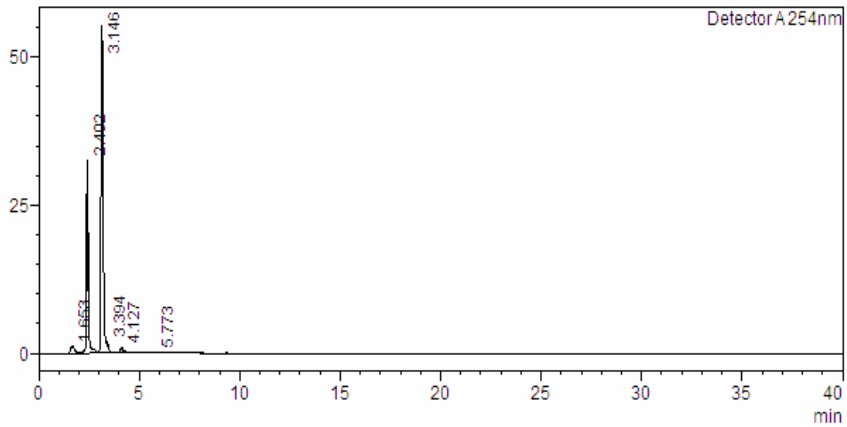

Figure 5. Basic degradation of fexofenadine hydrochloride.

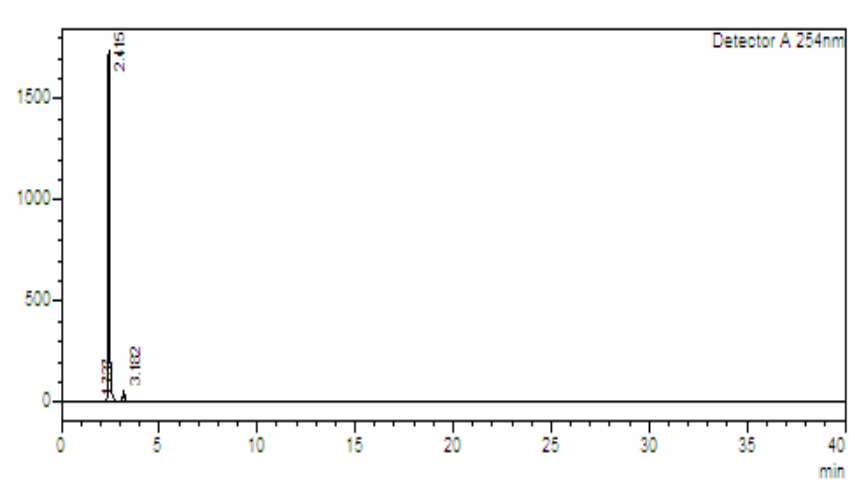

Figure 6. Neutral degradation of fexofenadine hydrochloride.

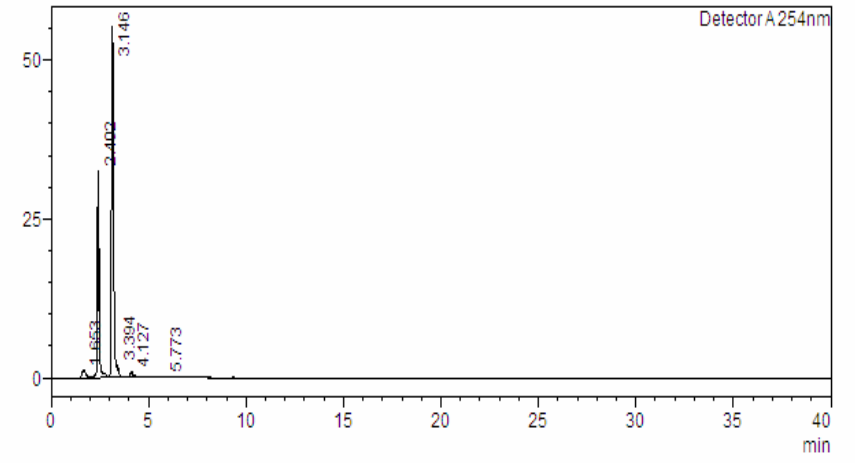

Figure 7. Oxidation degradation of fexofenadine hydrochloride.

According to table 4, robustness was examined by observing the change of small variations in different parameters such as flow rate (± 0.3 ml), mobile phase composition (± 10%), pH of inorganic solvent (± 0.5), temperature (± 5°C) and wavelength at 254 nm and 230nm. Here, all the parameters met the specification although small factors were changed.

Solution stability studies. The solution stability as described previously were observed in short-term and long-term basis. All the time results of the stability studies were within the acceptable limit (Figures 4-9).

According to table 5, the raw drug was found to degrade sufficiently in alkaline, basic and oxidative conditions, while negligible degradation was observed under neutral, hydrolytic and photolytic stress conditions. The peaks of the degradation products were not observed in the chromatogram due to their nonchromophoric nature. The developed RP-HPLC method was found to be simple, rapid, sensitive, accurate, precise and specific for the determination of fexofenadine HCl in bulk as well as stability study of samples of pharmaceutical dosage forms. All the analysis met the specifications with perfect retention time (Table 5).

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