



Stability Indicating RP-HPLC Method for Estimation of Fexofenadine Hydrochloride in Pharmaceutical Formulation

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Abstract: A stability-indicating HPLC method was developed and validated for the quantitative determination of fexofenadine in tablet dosage forms. An isocratic separation was achieved using a Zorbax, Eclipse XBD, C-8 Column having 150 x 4.6 mm i.d., 5 μ m particle size column with flow rate of 1.2 ml/min and using UV detector to monitor the eluate at 210 nm. The mobile phase consist of phosphate buffer: acetonitrile: methanol (60:20:20; v/v/v) with pH 3.7 adjusted with o-phosphoric acid. The drug was subjected to oxidation, hydrolysis, photolysis and thermal degradation. Fexofenadine was found to degrade in acidic, basic and oxidation condition. Complete separation of degraded product was achieved from parent compound. All degradation products in an overall analytical run time of approximately 60 min with the parent compound fexofenadine eluting at approximately 12.1 \pm 0.9 min. The method was linear over the concentration range of 1-100 μ g/ml ($r^2=$ 0.9970) with limit of detection and quantification of 0.2 μ g/ml and 0.6 μ g/ml, respectively. The method has the requisite accuracy, selectivity, sensitivity, precision and robustness to assay fexofenadine in tablets. Degradation products resulting from stress studies did not interfere with the detection of fexofenadine and the assay is thus stability indicating.

Keywords: Fexofenadine hydrochloride, stability indicating RP-HPLC, forced degradation.

Introduction

Fexofenadine Hydrochloride is chemically benzeneacetic acid, 4-[1-hydroxy-4-[4-(hydroxy diphenylmethyl)-1-piperidinyl]butyl]- α,α -Dimethyl-hydrochloride (Figure 1)^{1,2}.

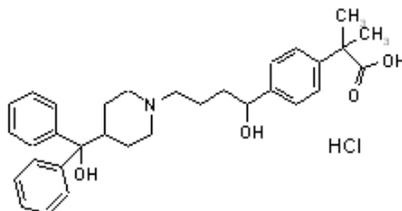


Figure 1. Structure of fexofenadine hydrochloride.

It is second generation long lasting H_1 receptor antagonist which has a selective and peripheral H_1 antagonist action. It is an active metabolite of terfenadine and like terfenadine it competes with histamine for H_1 receptor sites on effectors cells in gastrointestinal tract, blood vessels and respiratory tract, it appears that fexofenadine does not cross blood brain barrier to any appreciable degree resulting in a reduced potential for sedation.³ Literature survey revealed that methods have been reported for estimation of Fexofenadine in plasma using HPLC-MS.^{4,5} A few spectrophotometric methods has been reported for estimation of fexofenadine in pure form.⁶⁻⁹ Some methods have been reported for estimation of Fexofenadine with other combination using HPLC.¹⁰⁻¹² But the reported method was found to be more time consuming and more solvent consuming as it shows the long retention time for pure drug. The proposed validated method is more economical, precise, accurate and specific for quantitative determination of fexofenadine in pharmaceutical dosage form.

Experimental

Fexofenadine pure compound was kindly supplied by ISP Hongkong Ltd., Hyderabad, India and was used without further purification. Tablet formulation A is AllegraTM (Aventis Pharma Limited, Ankleshwar, India) and B is Telfast (Sanofi Aventis, India) containing labeled amount of 120 mg of fexofenadine were purchase from local market. All the chemicals used were of analytical grade. Purified HPLC grade water was obtained by double distillation and filtered was through filter (Millipore, Milford, MA) and was used to prepare all the solutions.

HPLC instrumentation and conditions

The HPLC system consists of a pump with injecting facility programmed at 10 μ l capacity per injection was used. A stability studies HPLC method was made on Water's Alliance 2690 system. The detector consists of a UV/Vis operated at a wavelength of 210 nm. The chromatographic separation was performed using a Zorbax, Eclipse XBD, C-8 Column having 150 x 4.6 mm i.d., 5 μ m particle size column separation was achieved using a mobile phase consisting of phosphate buffer: acetonitrile: methanol (60:20:20; v/v/v) with pH 3.7 adjusted with o-phosphoric acid at a flow rate of 1.2 ml /min. The eluent was monitored using UV detection at a wavelength of 210 nm. The column was maintained at ambient temperature and injection volume of 10 μ l was used. The mobile phase was filtered through 0.45 μ m filter prior to use.

Preparation of stock and standard solutions

A stock solution of fexofenadine (1 mg/ml) was prepared by accurately weighing approximately 10 mg of fexofenadine into 10 ml volumetric flask and dissolved and volume was made up to the mark with mobile phase. The stock solution is protected from light using

aluminum foil and stored for one week and was found to be stable during this period. Aliquots of the standard stock solutions of fexofenadine were transferred using A-grade bulb pipettes into 10 ml volumetric flasks and solutions were made up to the volume with mobile phase to give the final concentrations of 1-100 µg/ml.

Estimation of fexofenadine from pharmaceutical dosage form

To determine the content of fexofenadine in tablets (label claim: 120 mg fexofenadine) 20 tablets were opened and contents were weighed and mixed. An aliquot of powder equivalent to the weight of 1 tablet was accurately weighed and transferred to 100 ml volumetric flask and made up to volume with mobile phase. The volumetric flask was sonicated for 30 min to affect complete dissolution. The solutions were filtered through a 0.45 µm nylon filter. Suitable aliquots of the filtered solution was added to the volumetric flask and made up to the volume with mobile phase to yield the concentration of 1, 5, 10, 50 and 100 µg/ml. A 10 µl volume of each sample solution was injected into HPLC, six times, under the conditions described above. The peak areas were measured at 210 nm and concentrations in the samples were determined by comparing the area of sample with that of standard.

Forced degradation studies¹³

In order to determine whether the analytical method and assay were stability indicating, fexofenadine pure drug was stressed under various conditions to conduct forced degradation studies. A stock solution of 100 µg/ml was prepared by dissolving 10 mg of fexofenadine in 5 ml of mobile phase and volume was made up to with mobile phase. This solution was used for forced degradation studies to evaluate the stability indicating property and specificity of proposed method. In all forced degradation studies the average peak area of standard fexofenadine and degradation sample after application (20 µg/ml of HPLC) of six replicates were obtained.

Oxidation

To 2 ml stock solution, 2 ml of 3% hydrogen peroxide was added separately. The solutions were kept for 60 min at room temperature. For HPLC study, the resulting solution was diluted to obtain 20 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation studies

To 2 ml stock solution, 2 ml of 1 N hydrochloric acid was added. The solution was kept for 60 min at room temperature. The resultant solution was diluted to obtain 20 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies

To 2 ml of stock solution, 2 ml of 1 N sodium hydroxide was added. The solution was kept for 60 min at room temperature. The resultant solution was diluted to obtain 20 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug was placed in oven at 80° for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 20 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo stability studies

The photochemical stability of the drug was also studied by exposing the stock solution (1 mg/ml) to direct sunlight for 46 h on wooden plank and kept on terrace. For HPLC study, the resultant solution was diluted to 20 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Results and Discussion

The HPLC procedure was optimized with a view to develop a stability indicating assay method. Pure drug along with its degraded products were injected and run in different solvent systems. Initially water and methanol, acetonitrile and water in different ratios were tried. It was found that acetonitrile and methanol system was gives good results than water in combination as the drug was more soluble in methanol and acetonitrile than water. Acetonitrile: methanol (50:50 v/v) was not able to give good peak symmetry with acceptable retention time. An attempt to improve peak symmetry was made by adding phosphate buffer to the mobile phase. The presence of phosphate buffer in mobile phase resulted in excellent overall chromatography with appropriate peak symmetry and complete baseline resolution. Finally the mobile phase consisting of phosphate buffer: acetonitrile: methanol (60:20:20; v/v/v) with pH 3.7 adjusted with o-phosphoric acid was selected for validation purpose and stability studies. The method was validated with respect to the parameters including linearity, limit detection (LOD), limit of quantitation (LOQ), recovery, precision, accuracy, robustness and selectivity and a summary of validation parameters were presented in table 1.

Fexofenadine showed linearity in the concentration range of 1-100 µg/ml ($r^2 = 0.9967$) for HPLC. Linearity was evaluated by determining ten standard working solutions containing 1-100 µg/ml thrice in triplicate. Peak area of fexofenadine were plotted versus fexofenadine concentration and linear regression analysis performed on the resultant curve. For HPLC method the linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient and standard deviation for intercept value was less than 2%.

Table 1. Summary of validation parameters.

Parameters*	HPLC
Linearity range (µg/ml)	1-100
Correlation coefficient	0.9967
limit of detection (µg/ml)	0.2
limit of quantitation (µg/ml)	0.6
Recovery (%)	96.24-99.44
Intermediate Precision	0.501

*Average of six determinations.

The LOD and LOQ were determined based on a signal-to-noise ratios and were based on analytical responses of 3 and 10 times the background noise, respectively. The LOD was found to be 0.2 µg/ml. The LOQ was found to be 0.6 µg/ml.

Proposed method when used for extraction and subsequent estimation of fexofenadine from pharmaceutical dosage form after spiking with additional drug, afforded recovery of 96.34% to 99.44% and mean recovery of fexofenadine from the marked formulation are listed in Table 2.

Table 2. Recovery of Fexofenadine.

Level (%)	Actual concentration added (μg)	Observed concentration (μg)	% Recovery $\pm\text{SD}^*$
0	5	4.97	99.40 \pm 0.12
80	4	3.95	98.75 \pm 0.21
100	6	6.003	100.05 \pm 0.06
120	7	6.98	99.71 \pm 0.09

*SD: standard deviation.

The precision of assay was determined with respect to both repeatability and reproducibility. An amount of the product powder equivalent to 100 % of label claim of fexofenadine was accurately weighed and assayed. System repeatability was determined by six replicate applications and six times measurement of sample solution at the analytical concentration. The repeatability of sample application and measurement of peak area for active compound were expressed in terms of % RSD (Relative standard deviation).

Method repeatability was obtained from RSD value by repeating the assay three times in same day for intra-day precision. Inter-day precision was assessed by the assay of three sample sets on different days (inter-day precision). The intra-day and inter-day variation for determination of fexofenadine was carried out at three different concentration levels 3, 5, 7 $\mu\text{g}/\text{ml}$ (Table 3).

Table 3. Intra-day and Inter-day Precision.

Concentration($\mu\text{g}/\text{ml}$)	Intra-day ($\mu\text{g}/\text{ml}$)	% RSD*	Inter-day ($\mu\text{g}/\text{ml}$)	% RSD*
3	2.89	0.50	2.95	1.21
5	4.94	0.39	4.99	1.05
7	6.89	1.12	6.94	0.96

*% RSD: Percent relative standard deviation.

The accuracy of assay was determined by interpolation of replicates (n=6) peak areas of three accuracy standards (3, 5, 7 $\mu\text{g}/\text{ml}$) from a calibration curve prepared as previously described. In each case the accuracy was calculated. The resultant concentrations were 2.92 \pm 0.07, 4.98 \pm 0.06 and 7.02 \pm 0.06 (mean \pm standard deviation), respectively.

To evaluate HPLC method robustness a few parameters were deliberately varied. The parameters included variation of C_{18} columns from different manufacturers, flow rate, percentage of acetonitrile in mobile phase, column temperature and acetonitrile in different lots. Each parameters (except column from different manufacturers and solvents of different

lots) were changed at three levels (-1, 0 and 1) and examined. One factor at a time was changed to estimate the effect. Thus, replicate injections ($n=6$) of standard solution of three concentration levels were performed under small changes of six chromatographic parameters (factors). Results indicate that the selected factors remained unaffected by small variations of these parameters. The results from the two columns indicated that there is no significance difference. It was also found that acetonitrile of different lots from the same manufacturer had no significance influence on the determination. Insignificant difference in peak areas and less variability in retention time were observed.

The results of stress testing studies indicated a high degree of selectivity of this method of fexofenadine. Typical chromatograms obtained from the assay of pure sample and stressed samples are shown in figures 2, 3, 4, and 5, respectively. The average retention time \pm standard deviation for fexofenadine was found to be 12.1 ± 0.9 min for six replicates. The peaks obtained were sharp and have clear baseline separation.

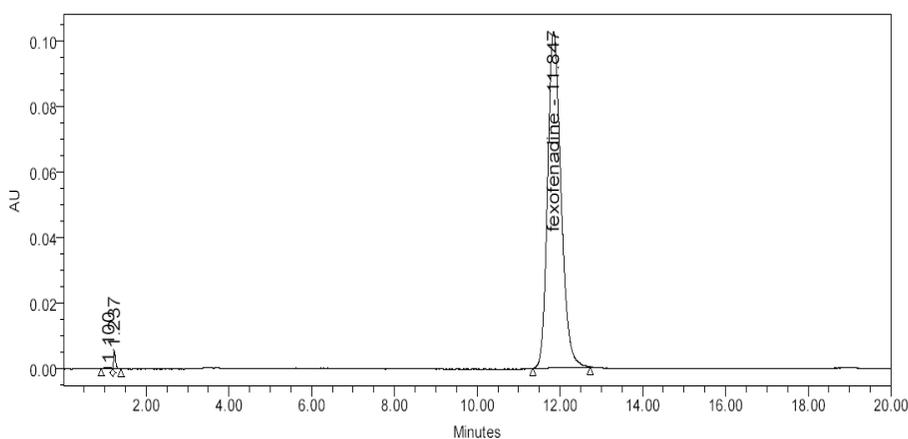


Figure 2. Chromatogram of standard drug. The mobile phase phosphate buffer: acetonitrile: methanol (60:20:20; v/v/v) with pH 3.7 adjusted with o-phosphoric acid. Peak of standard drug retention time $T_R=12.1 \pm 0.9$ min, 210 nm.

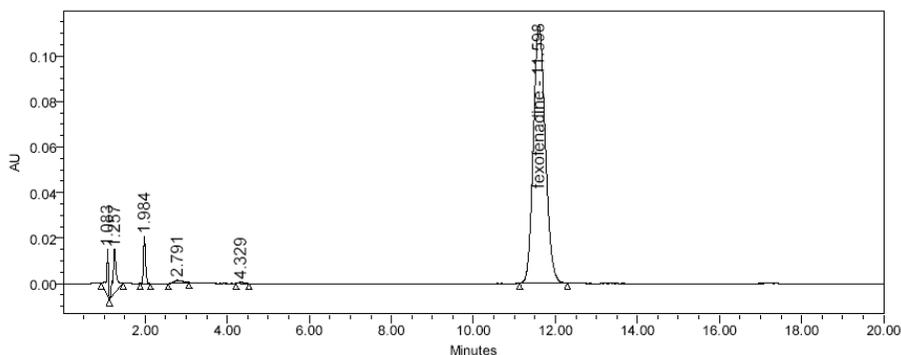


Figure 3. Chromatogram of base-treated fexofenadine. Chromatogram of base (1 N NaOH) hydrolyzed fexofenadine at 80° for 1 h showing degradation peaks at retention time (T_R) 1.237 min, 1.984 min, 2.791 min, 4.329 min.

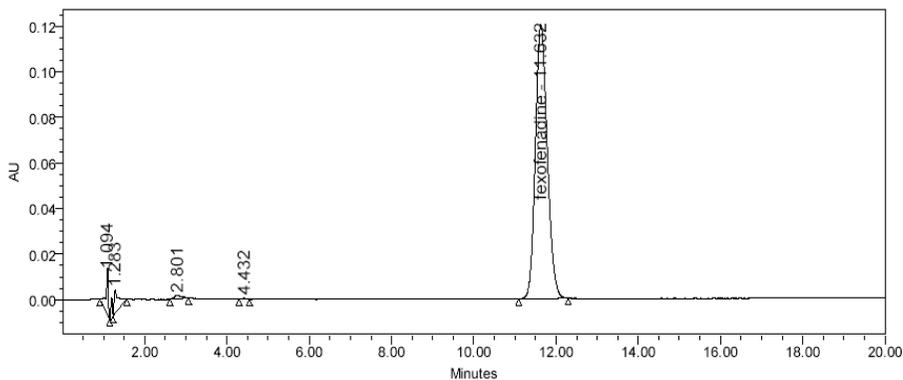


Figure 4. Chromatogram of acid-treated fexofenadine. Chromatogram of acid (1 N HCl) hydrolyzed fexofenadine at 80° for 1 h showing degradation peaks at retention time (T_R) 1.283 min, 2.801 min, 4.432 min.

Fexofenadine is characterized by a benzenecetic acid moiety with a side chain of carboxylic acid group, which was prone to hydrolysis. All the main degradation products were separated from the parent compound. Fexofenadine was found to be stable under dry heat conditions and also no decomposition was seen on exposure of solid drug powder to light, which was kept in day light for 46 h. The drug was unstable under basic stress conditions when kept for 1 h under room temperature. The drug was degraded approximately to 95.54%. Also it was unstable in acidic conditions when kept for 1 h at room temperature. The drug was degraded approximately to 96.76%. When kept under oxidative stress conditions with 3% H_2O_2 for 1 h at room temperature, the drug was degraded to around 97.63%. The stability of stock solution was determined by quantitation of fexofenadine and comparison to freshly prepared standard. No significant change was observed in the stock solution response, relatively to freshly prepared standard (Table 4).

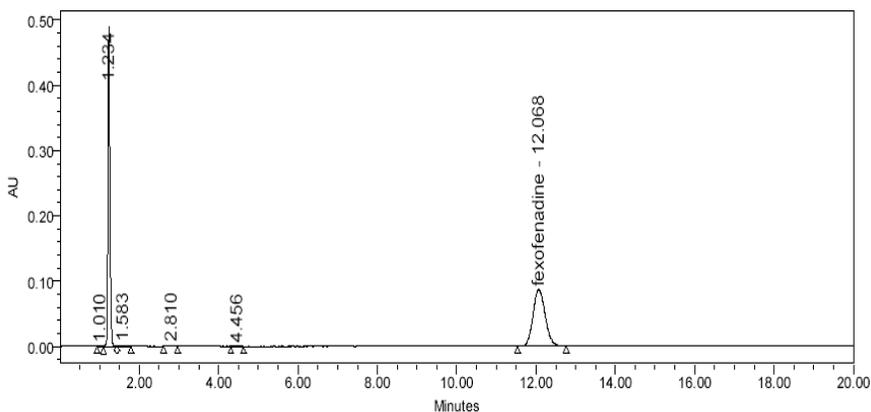


Figure 5. Chromatogram of H_2O_2 -treated fexofenadine. Chromatogram of chemically oxidized fexofenadine using 3% H_2O_2 showing degradation peak at T_R 1.234 min.

Table 4. Stress study data of Fexofenadine.

Condition	Time (h)	% Degradation	Retention time of degradation products (min)
Base 1N NaOH*	1	95.94%	1.237, 1.984, 2.791, 4.329
Acid 1N HCl*	1	96.76%	1.283, 2.801, 4.432
3% H ₂ O ₂ *	1	97.63%	1.234
Dry heat(80 ⁰)	1	--	--
Day Light (25 ⁰)	46	---	---

* room temperature.

The proposed method was applied to the determination of fexofenadine in tablets. The result of these assay yielded 120.14±0.170 (%RSD=0.285) and 119.88±0.079 ((%RSD=0.125) of label claim of the tablets. The results of the assay indicate that the method is selective for the assay of fexofenadine without interference from the excipients used in these tablets (Table 5).

Table 5. Assay results of tablet dosage form using proposed method.

Formulations	Label claim mg/ tablet	Drug content %± SD* ¹	% RSD* ²
Tablet A	120	120.14±0.170	0.285
Tablet B	120	119.88±0.079	0.125

*¹ Standard deviation. *²Relative standard deviation. Tablet formulation A is Allegra™ (Aventis Pharma Limited, Ankleshwar, India) and B is Telfast (Sanofi Aventis, India) containing label claim of 120 mg of FEX.

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