Research Article

Stability indicating RP-HPLC Method for Determination of FexoFenadine Hydrochloride and its Related Substances in Active Pharmaceutical Substance

Abhay Gupta*[1], Dr. Birendra Srivastava, Rajkiran Rajgor[2]
[1]Department of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India.
[2]Group Leader, Analytical R&D, Jubilant Organosys Ltd., Rajasthan, India.
*Corresponding author's E-mail: guptabhay78@gmail.com

ABSTRACT

An RP-HPLC method for the determination of fexofenadine hydrochloride and its related substances in Active pharmaceutical substance was developed and validated as per ICH & FDA guidelines. The separation was achieved with a 150 mm x 4.6 mm, 5 µm C18 column, by using a simple linear gradient. The mobile phase A was 0.01M Potassium di-hydrogen orthophosphate, pH 6.0 and mobile phase B was Methanol. The flow rate was 1.0 mL/min and column temperature was maintained at 30°C. The gradient separation was achieved within 70 minutes. The retention time of Fexofenadine its impurities were found 18 minutes (Fexofenadine), 21.4 minutes (Impurity-B), 23.8 minutes (Impurity-A), 36.4 (Impurity-D) and 38.4 minutes (Impurity-C). The linearity was established in the range 0.001-0.03 mg/mL (r2= 0.999) for Fexofenadine and its impurities. The method found specific for fexofenadine and its impurities and accuracy for impurity-A. B, C and D was established 103.09, 96.90, 102.69 and 99.26%. The limit of quantification and limit of detection was found 0.001 mg/mL and 0.0003 mg/mL.

Keywords: RP-HPLC, ICH, Validation, Fexofenadine hydrochloride, HPLC, impurity-A, Impurity-B, impurity-C, Impurity-D.

INTRODUCTION

Fexofenadine hydrochloride, the major active metabolite of terfenadine, is an antihistamine with selective peripheral H1-receptor antagonist activity. Fexofenadine hydrochloride inhibited antigen-induced bronchospasm in sensitized guinea pigs and histamine release from peritoneal mast cells in rats. In laboratory animals, no anticholinergic or alpha1-adrenergic-receptor blocking effects were observed. Moreover, no sedative or other central nervous system effects were observed. Radio labeled tissue distribution studies in rats indicated that fexofenadine does not cross the blood-brain barrier. Fexofenadine hydrochloride tablets are contraindicated in patients with known hypersensitivity to Fexofenadine and any of the ingredients of Fexofenadine hydrochloride tablets. Rare cases of hypersensitivity reactions with manifestations such as angioedema, chest tightness, dyspnea, flushing and systemic anaphylaxis have been reported. Chemically drug is (±)-4-[1-hydroxy-4-[4-(hydroxyl diphenyl methyl)-l¾piperidinyl] ¾butyl]-a,a¾dimethyl benzene acetic acid hydrochloride.

Literature survey revealed that there are other methods available for determination of Fexofenadine, the method is linear in the concentration range 8-20 µg per mL for these drugs.[8] However, none of the method available has been reported for the simultaneous determination of Fexofenadine hydrochloride and its related substances (process and degradation impurity) pharmaceutical active compound.

The method was validated as per the present ICH guideline on validation of analytical procedure Q2A (R1).[9-10] Quantitation was achieved with UV detection at 210 nm based on peak area with linear calibration curves at concentration ranges. The method was linear over wide concentration range of 0.0001-0.03 mg/mL for Fexofenadine and its related substances.

The accuracy of the method was evaluated in triplicate at five concentration level i.e. LOQ to 120% of specification limit concentration.

MATERIALS AND METHODS

Chemicals and Reagents

Fexofenadine hydrochloride, Fexofenadine reference standard, impurity-A, impurity-B, impurity-C, impurity-D were available from Jubilant Organosys Ltd., Noida, India.

HPLC grade, methanol and acetonitrile were obtained from Merck Chemicals (Mumbai, India). HPLC grade potassium hydrogen orthophosphate, potassium hydroxide was from Spectrochem (Mumbai, India). Distilled water was prepared using a Milli-Q system (Millipore). Nylon syringe filters (0.45 µm) were from Millipore (Mumbai, India). All the other used reagents were of analytical grade.

Selection of UV Wavelength

50 ppm solution of Fexofenadine hydrochloride was prepared separately in methanol. UV scan of the above solutions were carried out over a wavelength range of 200–400 nm by using the Shimadzu UV spectrophotometer, Model-UV-1800.

The detection wavelength was set at 254 nm because all the components had higher responses. An overlaid UV absorption spectrum is shown in Figure 1.
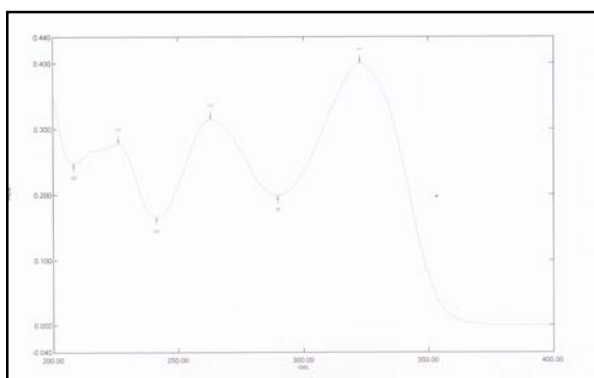
Figure 1: UV absorption spectrum of Fexofenadine hydrochloride.

HPLC Instruments and Analytical Conditions

Chromatographic separation was achieved using HPLC System (Waters Alliance 2695 Separation Module) containing quaternary solvent manager, an autosampler and UV and PDA detector. The output signal was monitored and processed using Empower software.

An Kromacill C18 column (150 mm X 4.6 mm id and 5 µm particle size) was used as the stationary phase. Mobile phase consisting of Mobile phase A: buffer (0.01 M Potassium dihydrogen orthophosphate pH 6.0 with potassium hydroxide) and Mobile Phase B: Methanol with simple gradient program (0-5 min :: MP-A : 60; 5-30 min :: MP-A : 60-40; 30-35 min :: MP-A : 40-20, 35-40 min :: MP-A : 20, 40.1-45 min :: MP-A : 60) was delivered at a flow rate of 1.0 mL/min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicate for 5 min. The column temperature was kept at 30°C. The detector was set at the wavelength of 254 nm. Injection volume kept was 10 µL.

Solutions and Sample Preparation

For the system suitability test, the solution containing 0.02 mg/mL Impurity-A, B, C and D., 2.5 mg/mL of Fexofenadine hydrochloride were prepared in diluent containing methanol and water (1:1 v/v).

For the linearity studies, a standard stock solution containing 0.1 mg/mL Fexofenadine and its related substances was prepared by diluent and diluted with the same solvent to yield solutions at different concentration.

For test sample solution, 25mg of drug was weighed and dissolved in 10 mL of volumetric flask. Then volume was made up to 10 mL with diluent. For spiked test solution, weighed and transferred 25.08 mg of sample to 10 mL of volumetric flask.

Added 0.5 mL of each impurity stock solution (0.05 mg/mL) and sonicate to dissolve. 5 mL of diluent was added and sonicate to dissolve. Volume was adjusted up to the mark with diluent.

10 µL of these solutions were injected into the HPLC system and the peak area was recorded from the respective chromatogram.

Calculation

For known impurity:

$$\% \text{ of impurity} = \frac{Au}{As} \times \frac{W1}{W2} \times \frac{2.5}{50} \times \frac{P}{100} \times 100$$

Where,

Au = Peak area due to known impurities obtained with test Solution

As = Peak area due to known impurity obtained with standard solution

W_1 = Weight of known impurity standard in mg

W_2 = Weight of substance being examined in mg

P = Potency of impurity standard

For unknown impurities:

$$\% \text{ of impurity} = \frac{Au}{As} \times \frac{W1}{W2} \times \frac{2.5}{50} \times \frac{P}{100} \times 100$$

Where,

% Total Impurities = Total known impurities + sum of total unknown impurities

RESULTS AND DISCUSION

Literature survey revealed that, only one method is available in the official compendia using HPLC for analyzing Fexofenadine hydrochloride and its related impurities. The present proposed method was compared with the reported method in the literature and comparison is shown in Table 1. The complete separation of the analytes was accomplished in 45 min and the method can be successfully applicable to perform routine analysis of Fexofenadine hydrochloride and its related impurities.

The method is developed to elute the process impurity for the used synthetic process. Forced degradation study was carried out using different condition i.e. acid, base, oxidation, thermal and photolytic pathways. Accuracy for all impurities was established from LOQ to 120% of specification level.

Method Validation

The developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guideline, Validation of Analytical Procedures: Q2 (R1), for the parameters like system suitability, linearity and range, precision (repeatability), intermediate precision (ruggedness), specificity, accuracy and robustness.

System Suitability

The system suitability test performed according to USP36.[11] The standard solution was injected six times and results were recorded to find the adequate peak separation (resolution), percentage relative standard deviation for area and retention time, peak asymmetry and theoretical plates. The results obtained were compiled in Table-2.

Table 1 Comparison of the performance characteristics of the present method with the published methods

S. No.	Method	Reagents	Detection Wavelength / Runtime	Linearity (mg/mL)	Remark	Reference
1	HPLC	TEA, Acetonitrile	220 nm / 30 min	-	USP method	[8]
2	HPLC	Sodium perchlorate, Acetonitrile	254 nm / 45 min	0.001-0.03 mg/mL	Wide linearity range	Present work

Table 2: System Suitability

Reference solution Peak Area, for n=6					
	Fexofenadine	Impurity-A	Impurity-B	Impurity-C	Impurity-D
%RSD	0.68	1.18	0.59	0.46	0.45
Acceptance Criteria	Not more than 2.0%				
Reference solution Peak retention time (min) for n=6					
%RSD	0.43	0.54	0.18	0.31	0.23
Acceptance Criteria	Not more than 1.0%				
Reference solution Peak resolution, for n=6					
Resolution	-	-	2.8	-	3.2
Acceptance Criteria	Not less than 2.0				
Reference solution Peak Symmetry factor, for n=6					
Symmetry Factor	1.31	1.06	1.41	1.14	1.23
Acceptance Criteria	Should be between 0.8 – 1.2				
Reference solution Peak Theoretical plates, for n=6					
Theoretical plates	15383	24939	19974	16543	18829
Acceptance Criteria	Not less than 10000				

Results

It was observed that limits for percentage standard deviation for peak area's and retention time for individual analyte, as well as resolution, symmetry factor and theoretical plates for all individual analytes are within the limit, which shows that the method have good system suitability.

Specificity

Specificity was performed to detect the presence of interference peak (blank) at the retention time of the analyte peak and its related substances.

The specificity of the method was checked by comparison of chromatograms obtained from test sample solution and the corresponding spiked test solution.

The interference of spiked test solution prepared by spiking impurities in test and was injected into the HPLC system.

The interference of blank was detected by injecting diluent as per the test method.

The representative chromatogram obtained for spiked test solution is shown in Figure-2.

Results

No interference from diluent, excipients or any other peak was found at the retention time of Fexofenadine hydrochloride and its related impurities.
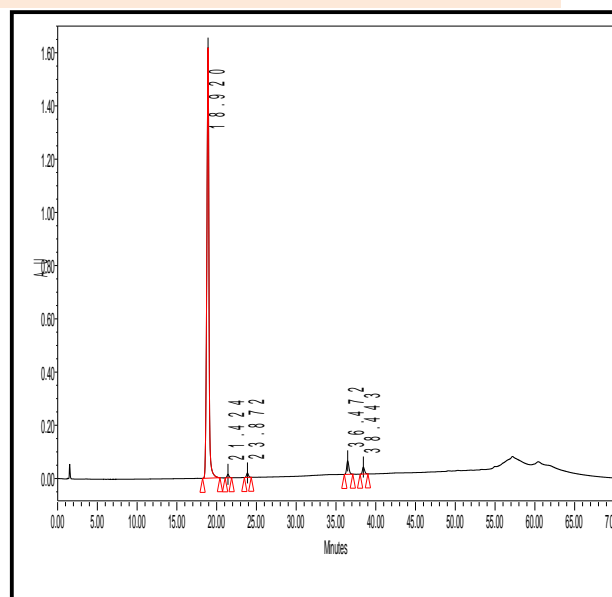

Figure 2: Typical Chromatograms of Spiked Solution containing Fexofenadine and impurities.

Precision and Ruggedness (Intermediate Precision)

Method precision was evaluated by carrying out six different test sample solution preparation. Different analyst from the same laboratory evaluated the intermediate precision of the method.

The Impurities of samples were determined. Precision and intermediate precision of the method was evaluated by calculating the %RSD. The values were given in Table 3.

Table 3: Precision and Intermediate Precision results

Sample No.	Imp – A		Imp – C		Total RS	
	Set-I	Set-II	Set-I	Set-II	Set-I	Set-II
1	0.015	0.014	0.025	0.026	0.038	0.039
2	0.015	0.013	0.025	0.026	0.040	0.040
3	0.015	0.014	0.025	0.025	0.040	0.039
4	0.014	0.013	0.025	0.026	0.040	0.039
5	0.014	0.014	0.025	0.025	0.039	0.039
6	0.015	0.013	0.025	0.026	0.039	0.039
Mean	0.015	0.014	0.025	0.026	0.040	0.039
SD	0.0005	0.0005	0.0001	0.0005	0.040	0.039
% RSD	3.33	3.57	0.40	1.92	0.0005	0.0004
Overall Mean	0.014		0.025		0.039	
Overall SD	0.0008		0.0004		0.0005	
Overall % RSD	5.71		1.60		1.28	

Results

Percentage Relative standard deviation (%RSD) obtained was found to be less than 10% for day – 1 and day – 2.

Linearity and Range

The linearity of detector response was determined by preparing a series of solution of the working standards (mixture of all active ingredients) over the range of LOQ to 120% of specification level targeted concentration. These solutions were injected into the chromatographic system and response area was recorded.

Calibration curve was constructed by plotting area against concentration and regression equation was computed. The linearity plots with values were shown in Figure 3.

Linearity of Fexofenadine

$y = 52511x - 6222.8$
$R^2 = 0.9997$

Linearity of Imp - A

$y = 61689x - 6378.1$
$R^2 = 0.9997$

Linearity Plot of Imp - B

$y = 50812x - 5226$
$R^2 = 0.9998$

Linearity Plot of Imp - C

$y = 70361x - 6406$
$R^2 = 0.9997$

Linearity Plot of Imp - D

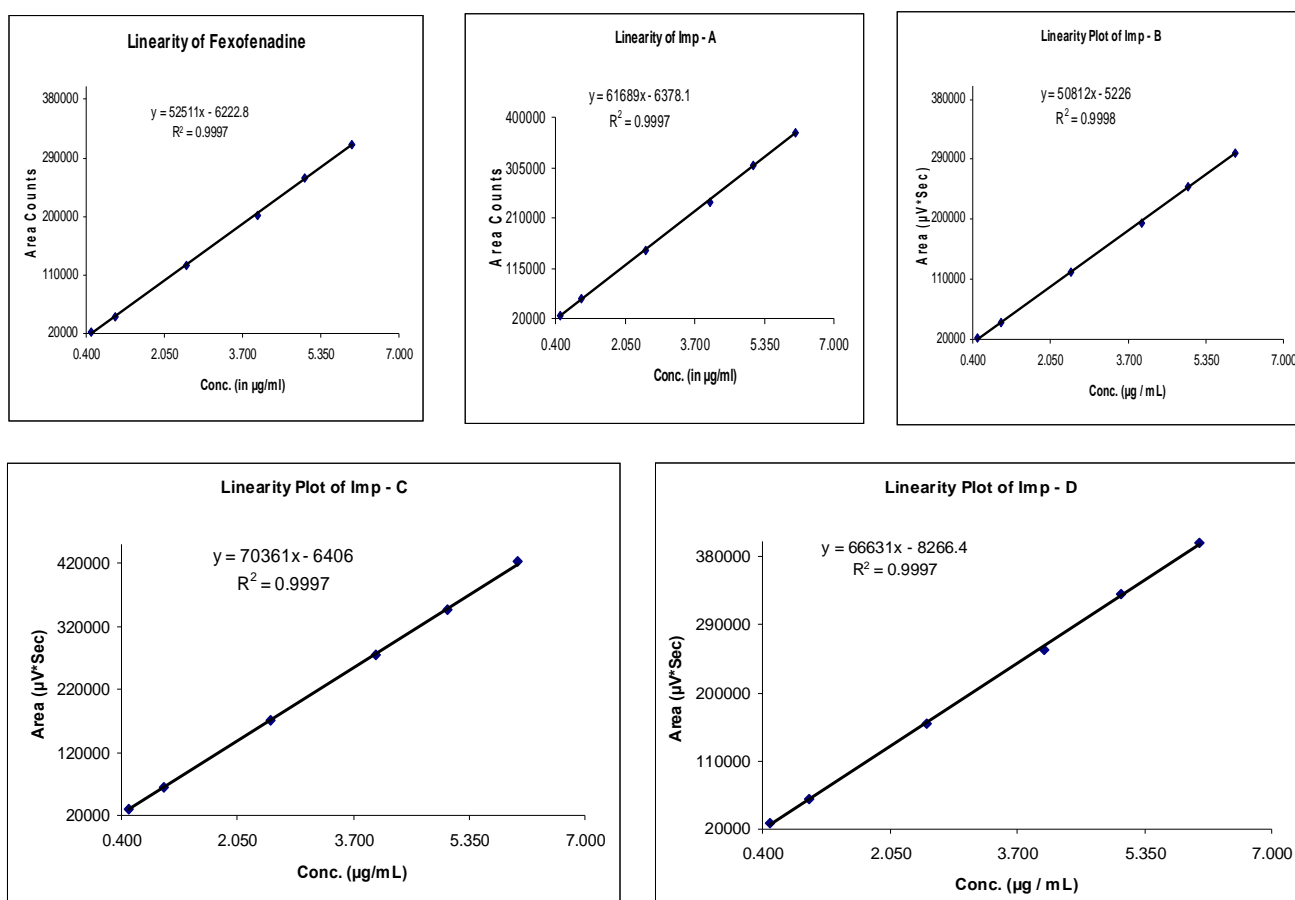$y = 66631x - 8266.4$
$R^2 = 0.9997$

Figure 3: Calibration curves of Fexofenadine hydrochloride, Impurity-A, Impurity-B, Impurity-C, Impurity-D showing linearity.

Results

The correlation coefficient values were within the limit 0.995 and Y-intercept values were within ± 2 %.

Accuracy (Recovery)

To study the accuracy of the method recovery experiments were carried out. The accuracy of the test method was determined by spiking impurities in drug substance at the level of LOQ, 50%, 100% and 150% of targeted concentration.

The recovery samples were prepared in triplicate at each level. The contents were determined from the respective chromatograms. The samples at different levels were chromatographed and the percentage recovery for the amount added was calculated. The values were given in Table 4.

Table 4: Accuracy (Recovery)

Active Ingredient Name	Concentration (%)	Amount Added (mg/mL)	Amount Found (mg/mL)*	Mean Recovery (%)**	Average Recovery (%)
Impurity-A	LOQ	0.010	0.0099	99.0	100.9
	80	0.050	0.0508	101.5	
	100	0.1004	0.1021	101.7	
	120	0.1203	0.1218	101.3	
Impurity-B	LOQ	0.0099	0.0099	100.7	99.9
	80	0.0494	0.0480	97.1	
	100	0.0979	0.1007	101.4	
	120	0.1189	0.1194	100.5	
Impurity-C	LOQ	0.0099	0.0107	106.6	102.9
	80	0.0480	0.0512	101.9	
	100	0.1007	0.1033	102.4	
	120	0.1194	0.1216	100.7	
Impurity-D	LOQ	0.099	0.0095	96.3	100.7
	80	0.0495	0.0501	101.2	
	100	0.0992	0.1024	103.2	
	120	0.1189	0.1216	102.3	
Acceptance criteria	The mean and individual recoveries should be within 85.0 – 115.0%				

* mean of 3 readings for individual level

** Average recovery for all levels

Results

Accuracy results obtained shows that the mean and individual recoveries were in range of 98.0 – 102.0%.

Robustness - Effect of variation in Temperature and variation in Flow Rate

To study robustness of the test method, small, deliberate changes were made to the chromatographic condition. A study was performed by changing the temperature and flow rate.

Standard solution prepared as per the test method and was injected into the HPLC system at 35°C and 45°C temperature. Flow rate change was done by varying flow rate at from 1.1 mL/min to 0.9 mL/min.

The system suitability parameters were evaluated. The values were given in Table 5.

Table 5: Robustness Results

Summary of system suitability Parameters						
Variations	Resolution between Imp-D and Imp-C	Resolution between Fexofenadine and Imp-B	Symmetry Factor			
			Imp-A	Imp-B	Imp-C	Imp-D
0.9 mL/min	2.7	2.5	1.21	1.36	1.26	1.41
1.1 mL/min	2.5	2.3	1.31	1.42	1.11	1.31
35°C	2.6	2.6	1.29	1.39	1.19	1.25
45°C	2.3	2.7	1.34	1.32	1.24	1.51
pH minus 5.9	2.7	2.7	1.35	1.22	1.09	1.33
pH plus 6.1	2.8	2.5	1.12	1.26	1.20	1.28
Acceptance Criteria	Not less than 2.0	Not less than 2.0	Should be less than 2.0			

Results

From variation in Temperature and flow rate, it was observed that there were no marked changes in the chromatograms, which demonstrated that the method developed is robust. Resolution, symmetry factor and Theoretical plate limits for flow rate variation and temperature variation are within the acceptance criteria, which show that the method exhibit good system suitability under given set of conditions.

Solution Stability

To assess the solution stability, reference standard and test solutions were kept at 25°C (laboratory temperature) for 26 hours, and injected in HPLC system at predetermined time interval.

The percentage change with respect to initial of test and reference standard solutions were evaluated. The values were given in Table 6.

Table 6: Solution Stability Results

Interval	% Difference in standard area counts				
	Imp – A	Imp – B	Imp - C	Imp - D	Fexofenadine
155	0.30	0.91	0.67	0.68	1.46
439	0.66	0.47	1.42	1.08	2.90
722	1.32	0.96	0.95	1.37	0.01
1006	0.71	0.05	0.65	1.83	0.62
1290	0.29	0.94	0.21	0.47	1.10
1573	1.05	0.19	0.05	2.07	0.68

Interval	Imp – A		Imp – B		Imp – C		Imp - D	
	Area	% RSD	Area	% RSD	Area	% RSD	Area	% RSD
-	342406	-	218876	-	437389	-	327579	-
155	340755	0.34	218543	0.11	433425	0.64	325091	0.54
439	340416	0.31	218399	0.11	437013	0.50	325366	0.42
723	341328	0.26	217272	0.32	437340	0.44	325636	0.35
1006	341128	0.22	218198	0.28	438670	0.45	329185	0.54
1290	341754	0.21	218813	0.27	438205	0.43	329803	0.63
1573	339601	0.27	217098	0.33	431233	0.63	325283	0.61

Results

Both Test and reference solution was found to be stable up to 26 hours, at 25°C (laboratory temperature).

CONCLUSION

A gradient RP-HPLC method has been developed and validated for the analysis of Fexofenadine and its related substances by RP-HPLC in Active pharmaceutical ingredient.

The results of the method validation revealed that the assay method is specific, selective, linear, accurate and robust.

The validation performed further gives documented evidence, that the analytical method for the simultaneous estimation of Fexofenadine and its related substances by RP-HPLC in Drug substance consistently analyze these drugs quantitatively in drug substance and can be used for routine analysis in quality control and R&D laboratory.

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