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Development and Validation of RP-HPLC Method for the Quantitative Determination of Fexofenadine Hydrochloride in Tablet Dosage form

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Abstract

Fexofenadine hydrochloride is a non-sedative and selective peripheral H1 receptor antagonist used for allergic rhinitis and chronic urticaria. This paper deals with a simple, feasible and sensitive reverse-phase high-performance liquid chromatographic method for the quantitative determination of fexofenadine hydrochloride in bulk drug and in pharmaceutical dosage forms. The chromatography was carried out by using HPLC system (Shimadzu LC2010HT) with UV- Visible dual absorbance detector (PDA), Inertsil 250 x 4.6 mm 5-µm packing L11 column. The mobile phase consisting of Acetonitrile and Buffer in the ratio of (9:16) [pH 5.25 adjusted with ortho phosphoric acid], detection was made at 220 nm and the mobile phase flowed at 2 ml min−1. Validation parameters included system suitability, specificity, linearity, accuracy and precision (repeatability & reproducibility) over a linearity range 50–150 µg/ml according to the ICH guidelines (r > 0.9999). The retention time of fexofenadine hydrochloride was 3.45 min. Hence, the method could be successfully applied for routine analysis of fexofenadine hydrochloride in pharmaceutical dosage forms.

Keywords: Fexofenadine hydrochloride, Solid dosage form, RP-HPLC, Validation.

Contents

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1. Introduction

Fexofenadine hydrochloride (Fig.1) chemically, (±)-4-[1-hydroxy-4-(4-hydroxydiphenylmethyl)-1-piperidinyl] -butyl]-α, α -dimethyl benzeneacetic acid hydrochloride [1] is a histamine H1 receptor antagonist used to relieve the allergy symptoms of seasonal allergic rhinitis, including runny nose and sneezing. Fexofenadine hydrochloride is

rapidly absorbed with a long duration of action, making it suitable for once daily administration. Thus, it fulfils the essential and desirable characteristics of an ideal antihistamine, being responsible for the improvement in quality of life of the patients with allergic diseases [2].

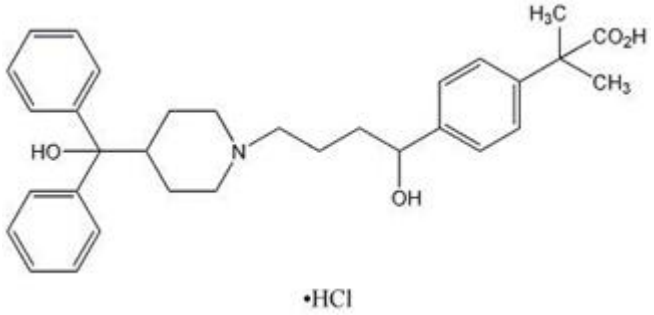

Figure 1: Structure of Fexofenadine hydrochloride

Literature survey reveals that Fexofenadine hydrochloride is estimated individually or in combination with other drugs by UV spectrophotometry [3], HPTLC [4], LC/MS [5], capillary electrophoresis [6] and Stability indicating HPLC and TLC method [7]. In this present work, an attempt was made to develop a simple, feasible and sensitive reverse-phase high-performance liquid chromatographic method for the quantitative determination of fexofenadine hydrochloride in bulk drug and in pharmaceutical dosage forms.

2. Materials and Methods

Chemicals and reagents

Acetonitrile of HPLC grade, Triethylamine and Glacial acetic acid were purchased from E.Merck (India) Ltd., Mumbai. Orthophosphoric acid of AR grade was obtained from Qualigens Fine Chemicals Ltd., Mumbai. Fexofenadine hydrochloride was a gift sample by The Madras Pharmaceuticals, Chennai – 600 096, Tamil Nadu, India. The commercially available tablets containing Fexofenadine hydrochloride were procured from the local market.

Instrumentation and chromatographic conditions

The chromatographic separation was carried out on HPLC system (Shimadzu LC2010HT) with UV- Visible dual absorbance detector (PDA), Inertsil 250 X4.6 mm 5-µm packing L11 column. The mobile phase consisting of Acetonitrile and solution B in the ratio of (9:16) was mixed (pH 5.25 adjusted with Ortho phosphoric acid) and filter through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 20:20 of Acetonitrile and solution B was pumped into the column at a flow rate of 2.0 ml/min. The detection was monitored at 220 nm. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 15 min. with the mobile phase following through the system.

Preparation of solution A: 17ml of glacial acetic acid was diluted with 983ml of water. 100ml of this solution was further diluted with water to 1000ml.

Preparation of solution B: 15ml of a solution containing Acetonitrile and Triethylamine (1:1) was mixed with solution A to 1000ml. pH to 5.25 was adjusted with Phosphoric acid.

Preparation of Diluent: Acetonitrile and Solution A was mixed in the ratio of (3:1).

Preparation of Mobile phase: Acetonitrile and solution B was mixed in the ratio of (9:16).

Preparation of standard stock solution:

Accurately weighed about 50 mg of Fexofenadine hydrochloride and transferred in to a 200ml volumetric flask, 100ml of diluent was added and allowed to dissolve then make up to the volume with diluent. (0.25mg/ml).

Preparation of standard solution:

3ml of standard stock solution was pipette out and transferred in to 50ml volumetric flask and make up to the volume with mobile phase. (0. 015mg/ml).

Preparation of sample stock solution:

Randomly selected 10 intact tablets were weighed and powdered then it was transferred in to 1000ml volumetric flask, 200ml of solution A was added and shacked by mechanical means at high speed for 30 minutes or until the tablets were fully disintegrated and finely dispersed. 800ml of Acetonitrile was added and shacked by mechanical means for 60minutes. The above prepared solution was filtered through polytetrafluoro-ethylene having 0.45 µm before use.

Preparation of sample solution:

5ml from sample stock solution was taken in to 500ml volumetric flask and made up to the Volume with mobile phase. (0.018mg/ml).

Calculation: Amount of Fexofenadine hydrochloride present in the each tablet was found to be

$$\frac{A}{B} \times \frac{WS}{200} \times \frac{3}{50} \times \frac{1000}{N} \times \frac{500}{5} \times \frac{P}{100} \times \frac{AV\,WT}{L.C} \text{ X } 100$$

3. Results and Discussion

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines [8].

System Suitability

It is essential for the assurance of the quality performance of chromatographic system. Five injections of standard drug solutions, fexofenadine hydrochloride was given separately to the system. The mean area, standard deviation and %RSD were calculated for the standard drug solutions and mentioned in Table 1. It was observed that all the values are within the limits.

Table 1: System suitability for Fexofenadine Hydrochloride

S.No	Standard	Concentration (µg/ml)	Area
1.	Standard -1	15	393742
2.	Standard -2	15	392075
3.	Standard -3	15	392705
4.	Standard -4	15	391338
5.	Standard -5	15	391568
		Mean	**392184**
		Standard deviation	**902**
		RSD %	**0.203**

Specificity

The specificity of the HPLC method is illustrated in Fig. 2, where a complete separation of fexofenadine hydrochloride was noticed in presence of other inactive excipients used in tablets. In addition, there was no any interference at the retention time of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data were presented in the Table 2.

Table 2: Specificity for Tadalafil

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	477301
4.	Sample	1	475846

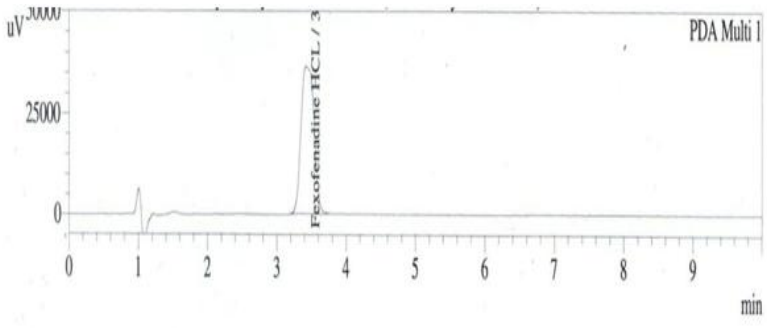

Figure 2 : Typical HPLC Chromatogram of Sample Tablets (Fexofenadine hydrochloride)

Linearity and Range

The Linearity of this method was determined at five levels from 50%– 150% of operating concentrations for fexofenadine hydrochloride and it was shown in Table 3. The plots of peak area of each sample against respective

concentrations of fexofenadine hydrochloride were found to be linear (Figure 3) in the range of 50%– 150% of operating concentrations. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The regression equations were found to be Y= 4250.9x +2066.4 for fexofenadine hydrochloride and correlation coefficient of the standard curves were found to be 0.9999 for fexofenadine hydrochloride. It observed that correlation coefficient and regression analysis are with in the limits.

Table 3: Linearity of response for Fexofenadine Hydrochloride

S.No	Linearity Level	Concentration (µg/ml)	Volume of stock solution (ml)	Volume made up to (ml)	Area
1.	Linearity -1	50	5.0	100	216546
2.	Linearity -2	75	7.5	100	319160
3.	Linearity -3	100*	10.0	100	425973
4.	Linearity -4	125	12.5	100	533241
5.	Linearity -5	150	15.0	100	640870

*****Operating concentration

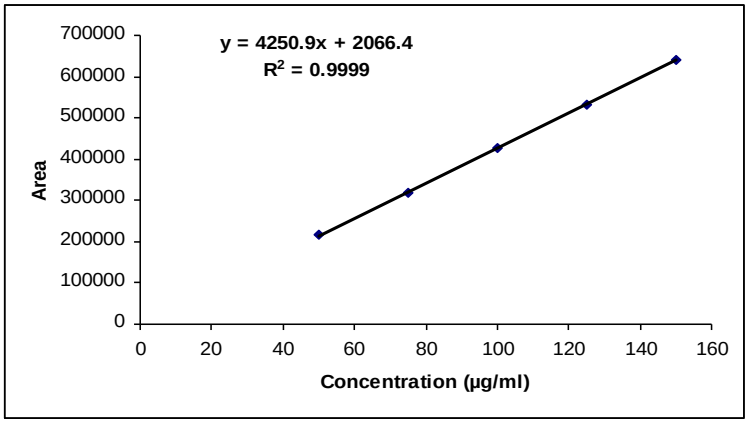

Figure 3: Linearity of response for Fexofenadine Hydrochloride

Accuracy

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standard, fexofenadine hydrochloride was added to pre-analysed samples at a level from 80% up to 120% and then subjected to the proposed HPLC method individually. The results of recovery studies were shown in Table 4. It was observed that the mean percentage recoveries were found to be for fexofenadine hydrochloride which demonstrated that the method was highly accurate.

Table 4: Accuracy for Fexofenadine Hydrochloride

S.No.	Target level	Area	Drug Recovery (%)
1.	80%	386366	100.5
2.	80%	369795	98.6
3.	80%	370207	98.8
4.	100%	474325	100.3
5.	100%	475335	100.9
6.	100%	476666	100.5
7.	120%	561878	99.7
8.	120%	564521	100.2
9.	100%	566465	100.7
		Mean	**100.02**
		Standard deviation	**0.8227**
		RSD %	**0.82**

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

Reproducibility

Examines the precision between laboratories and is often determined in collaborative studies. Reproducibility data for fexofenadine hydrochloride was shown in Table 5. This indicated that method was highly precise.

Table 5: Precision - Reproducibility for Fexofenadine Hydrochloride

S.No.	Sample Name	Area	Drug Recovery (%)
1.	Sample -1	467906	99.9
2.	Sample -2	468684	100.4
3.	Sample -3	476871	101.9
4.	Sample -4	474971	101.5
5.	Sample -5	465081	99.4
6.	Sample -6	469621	100.3
		Mean	**100.56**
		Standard deviation	**0.9542**
		RSD %	**0.95**

Repeatability

Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the same conditions. Repeatability data for Fexofenadine Hydrochloride were shown in Table 6. This indicated that method was highly precise.

Table 6: Precision – Repeatability for Fexofenadine Hydrochloride

S.No	Sample Name	Area	Drug Recovery (%)
1.	Sample -1	475201	100.1
2.	Sample -2	476638	100.4
3.	Sample -3	474671	100.0
4.	Sample -4	475592	100.2
5.	Sample -5	484240	102.0
6.	Sample -6	479584	101.2
		Mean	**100.65**
		Standard deviation	**0.7893**
		RSD %	**0.78**

4. Conclusion

The Proposed study describes a simple, feasible and sensitive reverse-phase high-performance liquid chromatographic method for the quantitative determination of fexofenadine hydrochloride in bulk drug and in pharmaceutical dosage forms. The method was validated as per ICH guidelines and found to be simple, sensitive, accurate and precise. Therefore the proposed method can be successfully used for the routine analysis of fexofenadine hydrochloride in solid dosage form without interference.

5. Acknowledgements

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