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Stability Indicating Analytical Method Development and Validation of Fexofenadine by RP-HPLC Method.

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KEYWORDS:

Fexofenadine Method Development, Validation, Stability Study.

ABSTRACT:

Introduction: Fexofenadine in pharmaceutical dosage form can use to treat and control s used for the treatment of allergic rhinitis and chronic urticaria through the antagonism of histamine receptors H1.

Objective: The objective is to develop a novel, easy, fast, accurate RP-HPLC method and validate it as per the ICH guidelines. This method aims to determine both Fexofenadine in pure and pharmaceutical formulation.

Method: Separation of analyte was carried out using an Agilent C18 (5µm; 4.6 x 250 mm ID) column connected to a DAD detector. The moving phase consists of methanol and Formic acid in (40:60) with flow rate 0.8 ml per minute. Analytes were detected at 240 nm wavelength.

Result: Under optimized condition the retention time was found to be 4.523for Fexofenadinewith sharp peak. The linearity of a method was found at 24-120µg/ml for Fexofenadinehaving a coefficient of correlation (R2) 0.999. Fexofenadine Limit of Quantification and Limit of Detection were determined to be 0.7185 and 0.2371. The procedure is accurate, as demonstrated by the founded good percent recovery.

Conclusion: The method was developed and validated as per guidelines of International Conference on Harmonization. Forced degradation study ofthe drug was done in controlled acidic, basic, peroxide and hydrolysis condition. The develop method shows its suitability for the regular quantitative analysis Fexofenadine in their pure form and in its pharmaceutical formulation. This application of method serves for the purpose of quality control.

INTRODUCTION

Fexofenadine hydrochloride(FEX)(figure1)(±)-4-[1-hydroxy-4-(4hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-∝,∝-dimethyl benzene acetic acid hydrochloride relieves the symptoms of seasonal allergic rhinitis, often known as hay fever, which include runny nose, sneezing, red, watery, or itchy eyes, as well as adult-onset itching of the nose, throat, or roof of the mouth. Terfenadine is acarboxylic acid metabolite of Fexofenadine also nonsedating selective histamine H1

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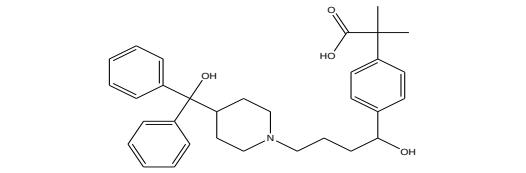
receptor antagonist. This drug, which is supplied therapeutically or used as a P-glycoprotein probe in the form of a racemic combination of R- and S-enantiomers, has an asymmetric carbon in its chemical structure.[1]It is freely soluble in methanol, ethanol and slightly soluble in water, chloroform and practically insoluble in hexane. The molecular weight is 538.13 and the empirical formula is $C_{32}H_{39}NO_4$.HCl. Recent research has shown that simultaneously administering an anti-leukotriene and an antihistamine to treat allergic rhinitis results in much improved symptom relief as compared to a slight improvement in rhinitis symptomatology with only the individual treatments.[2]

Literatures are available for the determination of Fexofenadine in individual and in combined form byRP-HPLC[3-9], UV spectrophotometry[10-11], HPTLC[12-13], in biological fluid by RP-HPLC[14-15], LC/MS[16], LC/MS/MS[17-18], and stability indicating method[19].



(±)-4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-a, a-dimethyl benzeneacetic acid
Caution: Stereochemical terms discarded: +

Figure 1. Structure of Fexofenadine

MATERIALS AND METHODS

Reagent and Reference Sample

The standard samples of Fexofenadine obtained as a gift sample from Swapnroop Drugs and Pharmaceuticals, Aurangabad. The chemicals, reagents, double distilled water and Mili-Q water which are used in analysis are of HPLC grade. Calibrated glassware's and analytical balance were used during the study. For UHPLC study,were utilized.

Instrumentation

Agilent RP C-18 (5µm; 4.6 x 250 mm ID.) column was used in the high-performance liquid chromatography (HPLC) of Agilent 1100 inbuilt with reciprocating pump (HP-1100) equipped with UV DAD detector throughout the analysis. The chemstation software was used for controlling and analysing data from chromatography system. Digital pH meter (EQ-610, Lab line) was used to check the PH of the sample. Digital weighing balance for weighing and ultra-sonicator of Labman used to dissolve the undissolved particle of drug.

Determination of working Wavelength

The spectrophotometric was used to identify the wavelengths at which the drug shows significant absorption in figure 2. The wavelength where the drug exhibits the highest absorbance or a strong absorption peak is typically chosen for HPLC analysis.

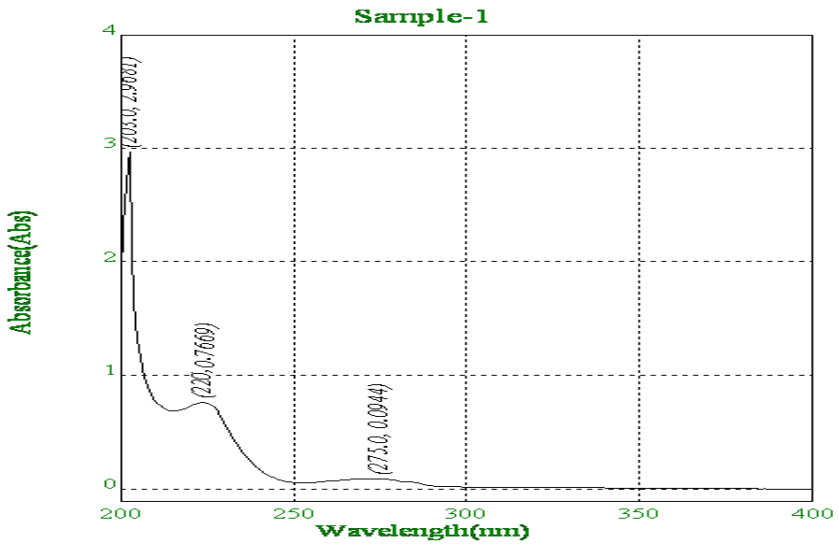


Figure 2. Spectrum of Fexofenadine.

Methodology

Selection of Mobile Phase

The mobile phase selection process involved consideration of buffer type, buffer pH, solvent selection and ratio of buffer and solvent. Fexofenadine standard solution was separately injected into the HPLC system using various mobile phase ratio ofMethanol: Formic acid.Among these, Methanol: 0.05%Formic acid in a ratio of 40:60 showed promising results for the separation.

After testing different ratios, the optimized mobile phase for the accurate determination for the drug was

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found a mixture Methanol: 0.1 % Formic Acid (pH 3.5) 40:60 %v/v. This specific combination provided the ideal conditions for effective separation and precise analysis of Fexofenadine in the HPLC system.

Preparation of Standard Solution

Fexofenadine 120 mg was precisely weighed and dissolved in 50 ml of methanol to achieve concentrations of 2400µg/ml, respectively. The solution is sonicated for 2-5 min. The solution containing Fexofenadine 2400µg/ml is prepared for assessing robustness, accuracy, repeatability, and other validation parameters.

Preparation of Sample Solution

About 20 tablets were initially weighed to establish their average weight. Then the tablets were ground into a fine powder using a mortar and pestle. The resultant powder weighed carefully and transferred into a 100 mL volumetric flask, equivalent to 120 mg of Fexofenadine. Then 100 mL methanol is added as a diluent and sonicating the sample for 15 min to remove air bubble. The volume was further adjusted by adding additional diluent to the solution to attain a concentration of 2400 µg/mL of Fexofenadine.

Preparation of Buffer

100 ml HPLC-grade water and 0.1ml of Formic acid are mixed to make 0.1% Formic acid buffer. PH is adjusted to 3.5.

Optimized Chromatographic Conditions:

The optimized chromatographic conditions yielded results as presented in table 1. All system suitability attributes, including theoretical plates, tailing factor and retention time, have to meet the specified acceptance standards.

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	Methanol: 0.1 % Formic Acid (pH 3.5) 40:60%v/v.
Column	Agilent RP C-18 (5µm; 4.6 x 250 mm ID
Flow Rate	0.8ml/min
Runtime	10 min
Injection Volume	20µL
Detection Wavelength	240
Temperature	28^0C
Retention Time	4.523for FEX

Table 1: Optimized chromatographic conditions.

Validation Parameters

System Suitability Studies

Six injections of a working standard solution containing Fexofenadineat a volume of 20µg/ml was injected and analysed under optimized chromatographic conditions.This was conducted to assess the consistency of results with respect to the relative standard deviation (RSD), which should consistently remain below 2%. Furthermore, various system suitability parameters, such as retention or capacity factor (k'), resolution (Rs), theoretical plates (N), tailing factor/peak asymmetry (As), and separation factor, were examined and assessed and showed in table 2

Table 2: Summary of system suitability parameters.

Parameters	Fexofenadine	Acceptance criteria
Tailing factor	0.79	≤2
Retention time	4.523	≥2
Theoretical plates	4699	≥2000

Linearity

Linearity denotes the capacity of a method or instrument to yield results that exhibit consistent relationship with the varying concentrations or amount of the analyte being measured across a range of concentrations. In these five different concentrations are used to determine the linearity.Drawing a graph where the y-axis represent the peak areas and X-axis represent

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the concentrations over the concentration ranges of 24-120µg/ml for Fexofenadineproduced the calibration curve plot. It is desirable for the correlation coefficient to exceed 0.99.Figure 3 shows the linearity curve for Fexofenadine

Table 3: Linearity values of Fexofenadine

Parameters	Fexofenadine
Linearity range (µg/mL)	24-120
Regression coefficient ± SD	0.999± 5.11
Slope ± SD	78.717 ±5.11
Intercept ± SD	282.61± 5.11

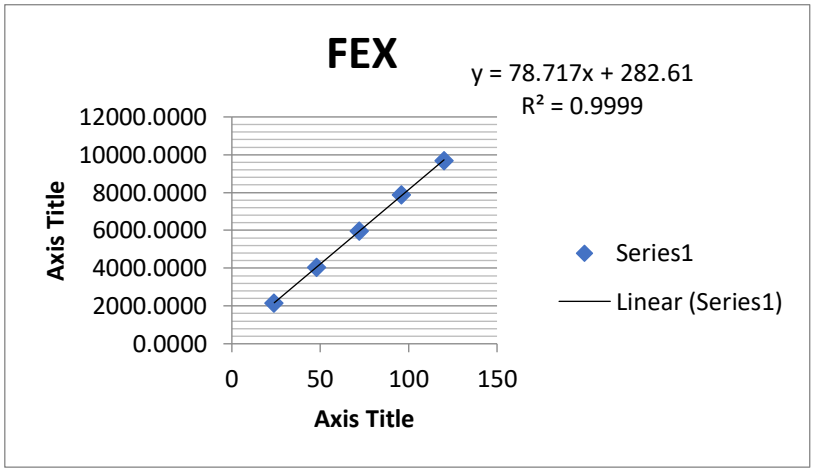

Figure 3. Linearity curve of Fexofenadine

Accuracy

The accuracy of the method was assessed by conducting recovery experiment, where a precise quantity of the drug was introduced into the sample solution. The recovery was assessed by measuring the drug's recovered amount based on the peak areas. The sample mix with standard drug at concentrations of 80%, 100% and 120% of the original concentrations. The mixed sample was analysed in triplicate. The expected range for percentage recovery at each level was set between 98% and 102%.Recovery of Fexofenadine evaluated in the range of 99.48 to 102.63. The results are illustrated in table 4. From results the process said to accurate.

Table 4: Recovery values of Fexofenadine.

Drug	Level	Analyte amount (mg)	Recovery amount (mg)	Mean % recovery	RSD %
Fexofenadine	80%	19.20	19.10	99.48	0.24
	100%	24	23.89	99.57	0.10
	120%	28	28.73	102.63	0.10

Method precision and Intermediate Precision

Method precision represents the capacity of an analytical method or instrument to generate consistent and reproducible results through multiple measurements under similarconditions, emphasizing the method's reliability.

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The peak areas of three replicates of the sample solutions were measured the same day they were injected under ideal circumstances. The three replicate injection results peak areas RSD% shouldn't be more than 2. Table 5 shows the result of study.

Table 5. Intraday and Interday Precision

Concentration (µg/ml)	Fexofenadine %RSD	
	Intraday	Interday
48	0.03	0.03
72	0.06	0.07
96	0.00	0.02

Robustness

Robustness of a chromatographic method by deliberately altering parameters like flow rate, wavelength, and mobile phase pH, it's common to observe impacts on various chromatographic parameters. These alterations can affect different aspects of the chromatographic separation, and monitoring parameters like retention pattern, theoretical plates (N), peak area, tailing factor (Tf), resolution (Rs), capacity/retention factor (k'), and separation factor allows for a comprehensive assessment of the method's robustness.

Robustness values of Fexofenadine illustrated in table 6.

Table 6. Robustness values of Fexofenadine

Sr. No	Chromatographic Condition	Changes	%RSD of FEX
1.	Flow (-0.1ml/min)	0.7	0.08
2.	Flow(+0.1ml/min)	0.9	0.11
3.	M.P (-0.1ml/min)	52+48	0.11
4.	M.P (+0.1ml/min)	50+50	0.08
5.	Wavelength (-1)	247	0.09
6.	Wavelength (+1)	249	0.04

LOQ:

LOQ is the lowest amount of an analyte that can be accurately quantified with acceptable accuracy and reliability. LOQ typically involves a higher signal-to-noise ratio than LOD and indicates the point where quantitative measurements can be made with a defined level of precision and accuracy.

LOD:

The limit of detection represents the minimum concentration of an analyte that is detectable although not necessarily quantifiable. It's evaluated by measuring the signal-to-noise ratio, where the signal from the analyte is compared to the background noise level. Table 7 shows the LOD and LOQ value of Fexofenadine.

Table 7. LOD and LOQ Values of FEX

Sample	LOD	LOQ
Fexofenadine	0.237169	0.718536

Forced Degradation Study

Force degradation study involves introducing the sample to the various stress conditions for potential degradation that may occur during the storage conditions. The aim is that to understand the how

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subject reacts, changes or degrades under various stress conditions.

Force degradation study mainly conducted in pharmaceutical research to evaluate the stability and degradation pathway of drug and drug products. In this study sample undergoes various stress conditions such as acid, base, oxidative and neutral degradation.

Acid Degradation

0.3 ml ofFexofenadine stock solution was mixed with 5ml of 0.1N HCL was added followed by makeup volume upto 10 ml with mobile phase. The resulting stock solution allowed to remain exposed for 24 hr. After 24 hrs the Sample was neutralized with 0.1 N NAOH before injection.

Base Degradation

Mix 0.3 ml of Fexofenadine stock solution with 5ml of 0.1N NAOH and adjust the volume upto 10ml with mobile phase. Let the stock solution exposed for 24 hr. Afterward, neutralized the sample with 0.1 N HCL prior injection

Oxidative (H_2O_2)Degradation

0.3 ml of stock solution of Fexofenadine is added in 10ml volumetric flask with 5ml of 3% H_2O_2. Then volume is adjusted to 10ml with mobile phase and stock solutions is exposed for 24 hr. Then analysis was carried out.

Neutral

Combine the 0.3 ml of stock solution of Fexofenadine with 5ml of water and 10 ml volume is made up with mobile phase in volumetric flask. After exposing the stock solutions for 24 hr proceed for the analysis.

The Fexofenadine degradation was performed in Acidic, Basic, oxidative and in Neutral Condition. Figure 4 and figure 5 shows Acidic Degradation 5.73% degradation while in basic condition it is 4.20%. Oxidative condition and Neutral condition show the 0.61% and 0.77% degradation respectively showed in figure 6 and figure 7.Degradation results of Fexofenadine illustrates in table 8.

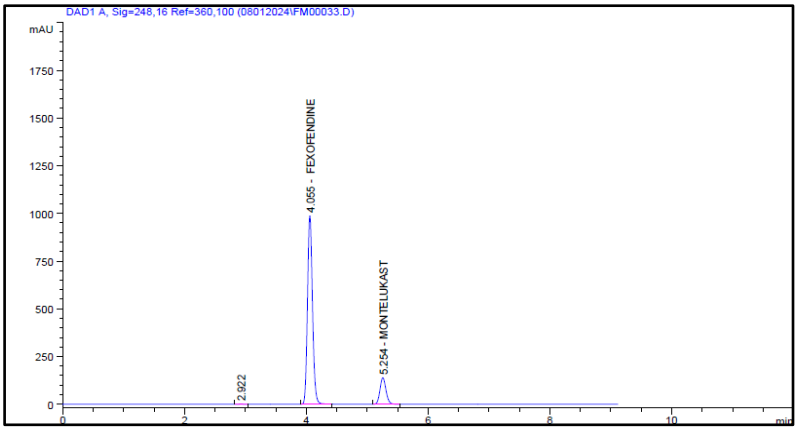

Figure 4. Acidic Degradation of Fexofenadine

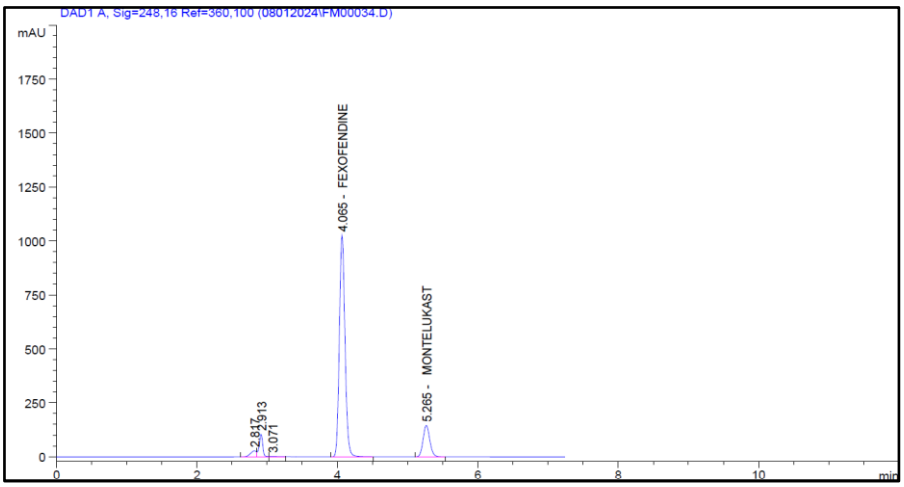

Figure 5. Basic Degradation of Fexofenadine

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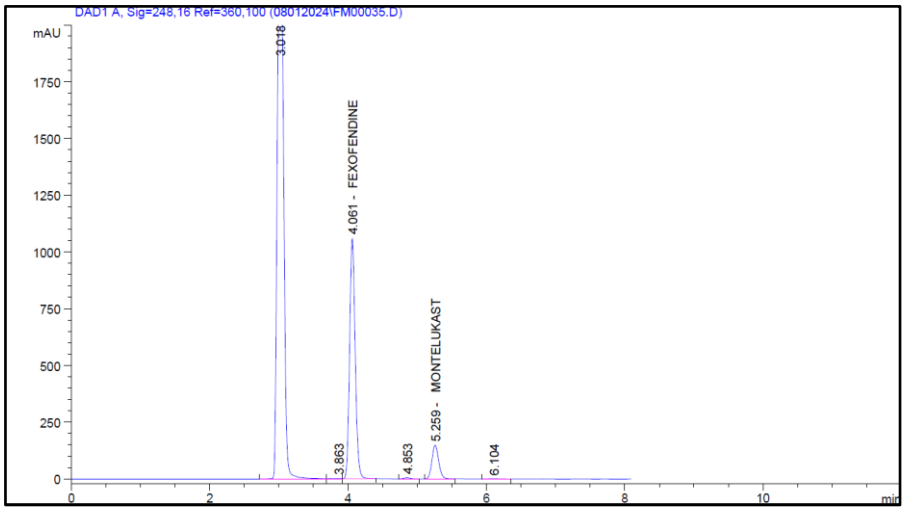

Figure 6. Oxidative Degradation of Fexofenadine

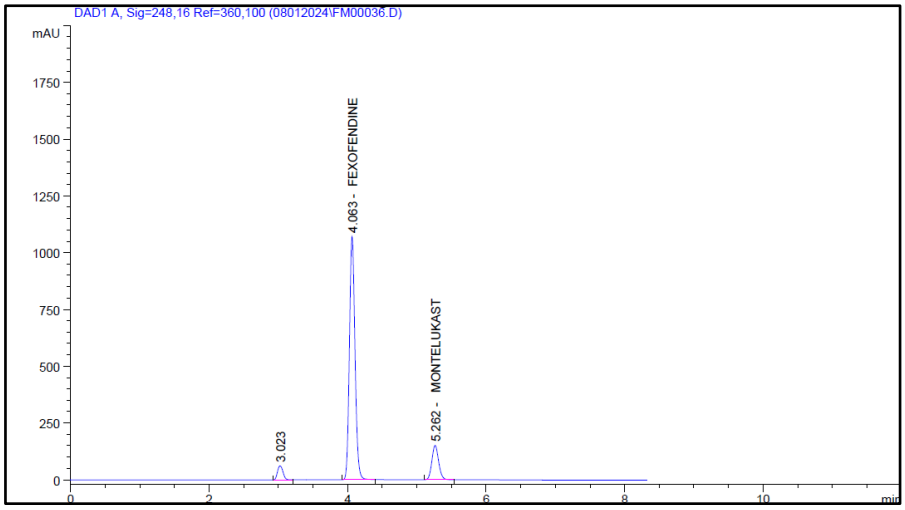

Figure 7. Neutral

Table 8. Degradation study of Fexofenadine

Degradation Condition	Retention Time	% Recovery	%Degradation
0.1N HCL	4.055	94.27	5.73
0.1N NaOH	4.065	95.80	4.20
3%H_2O_2	4.061	99.39	0.61
Neutral	4.063	99.23	0.77

DISCUSSION:

This linearity of this RP-HPLC method demonstrated between the range of 24-120µg/ml for Fexofenadine. The method validation is carried out successfully in the optimized conditions and validation results for various parameters were within the acceptable limits. Sample preparation done with the dilution and filtration of sample. The determination was achieved by using column Agilent RP-C18, 4.6 x 250mm;5µm consisting an isocratic mobile phase of Methanol: 0.1 % Formic Acid (pH 3.5) 40:60 %v/v at a rate of flow 0.8ml/min. Retention time of Fexofenadine is4.523min. The lower quantification limit (LOQ)and lower limit of detection (LOD)for Fexofenadine were found to be0.718536µg/ml and 0.237169µg/ml. Validated was conducted by following the ICH guidelines.

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Determination of Fexofenadinehas been successfully done by this method.

CONCLUSION:
The RP-HPLC method was developed and validated for the determination of Fexofenadineoffering the speed, convenience, precision and accuracy. This method considered more cost effective as compared to other reported method. It is suitable for the tablet analysis, making it a viable option routine quality control of Fexofenadinein the pharmaceutical formulations.

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CONFLICT OF INTEREST:
The authors declare that there is no conflict of interest.

ABBREVIATIONS:
ICH: International Council for Harmonisation; FEX: Fexofenadine; RP-HPLC: Reverse Phase High Performance Liquid Chromatography; FDA: Food and Drug Administration; HPLC: High Performance Liquid

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