Research Article

Development and Validation of Novel RP- HPLC Method for Simultaneous Estimation of Fexofenadine Hydrochloride and Montelukast Sodium in Pharmaceutical Dosage Forms

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ABSTRACT

A simple reversed phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Fexofenadine Hydrochloride and Montelukast sodium in tablet dosage form. Chromatographic analysis was performed on a Hypersil ODS C18 (150x 4.6 mm, 5µm) with a mixture of Acetonitrile: 0.01 M Phosphate buffer (pH was adjusted to 3.0 with o-phosphoric acid) in the ratio 60:40 as mobile phase, at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 230 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The retention times of Fexofenadine Hydrochloride and Montelukast sodium were 2.127 and 5.45min respectively. Calibration plots were linear over the concentration ranges 4.8-33.6 µg mL^{-1} and 0.4-2.8 µg mL^{-1} for Fexofenadine Hydrochloride and Montelukast sodium respectively. The Limit of detection was 0.42 µg mL^{-1} and 0.03µg mL^{-1} and the quantification limit was 1.23 µg mL^{-1} and 0.11µg mL^{-1} for Fexofenadine Hydrochloride and Montelukast sodium respectively. The accuracy of the proposed method was determined by recovery studies and found to be 100.11% to 100.65% for Fexofenadine Hydrochloride and 100.76% to 101.36% for Montelukast sodium. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of simultaneous determination of Fexofenadine Hydrochloride and Montelukast sodium in tablet dosage form.

Keywords: Fexofenadine Hydrochloride and Montelukast sodium, RP-HPLC, ICH guidelines.

1. INTRODUCTION

Fexofenadine Hydrochloride (FEX) [Figure 1] is an antihistamine drug used in the treatment of hay fever, allergy symptoms, and urticarial. FEX is a second-generation selectively peripheral H_1-blocker of the GI tract, large blood vessels and bronchial smooth muscle. Blockage prevents the activation of the H_1 receptors by histamine, preventing the symptoms associated with allergies from occurring. FEX cannot cross the blood–brain barrier and therefore does not cause drowsiness. It also exhibits anticholinergic, anti-dopaminergic, alpha1-adrenergic or beta-adrenergic receptor blocking effects.

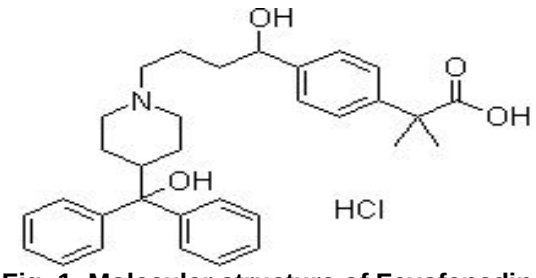

Fig. 1: Molecular structure of Fexofenadine Hydrochloride

Montelukast Sodium (MON) [Figure 2] is a potent and selective cysteinyl leukotriene receptor antagonist that is being investigated in the treatment of asthma. MON selectively antagonizes leukotriene D4 (LTD4) at the cysteinyl leukotriene receptor, CysLT1, in the human airway. MON inhibits the actions of LTD4 at the CysLT1 receptor, preventing airway edema, smooth muscle contraction and enhanced secretion of thick, viscous mucus.

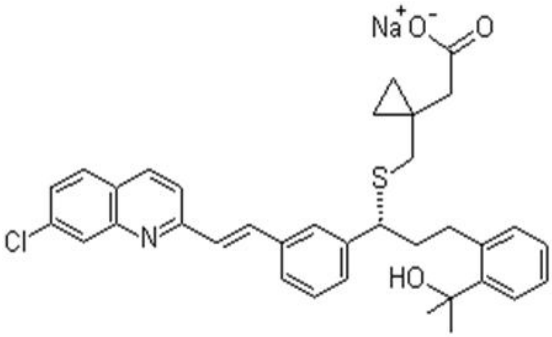

Fig. 2: Molecular structure of Montelukast Sodium

Literature survey of FEX and MON revealed few methods based on UV Spectrophotometry[1-4] and Chromatography[5-16] have been reported for determination of both drugs in single and combined dosage forms. The present work describes the development and validation of reverse phase high performance liquid chromatographic (RP-HPLC) method, which can quantify these components simultaneously

2. EXPERIMENTAL

2.1. Materials and Methods

Reference standard samples of FEX and MON were gifted by Dr. Reddy's Laboratories Limited, Hyderabad. A commercial sample (HISTKIND-M tablets) containing FEX -120 mg and MON -10 mg were purchased from local market.

2.2. Instrumentation and Chromatographic Conditions

The author had developed a rapid, precise and accurate RP-HPLC method for the simultaneous determination of FEX and MON in tablet dosage forms. The separation was carried out using a Shimadzu High Performance liquid Chromatographic instrument on a Hypersil ODS C18 (150 x 4.6mm, 5µ) column. A mobile phase consisting of Acetonitrile and 0.01M Phosphate Buffer (pH was adjusted to 3.0 with o-phosphoric acid) (60:40 v/v) was pumped through the column with a PU 2080 isocratic pump at a flow rate of 1.0 mL/min. Sample injection was performed by using Rheodyne 7725 injection valve using a 20 µL Hamilton syringe. Data acquisition was done by using spin chrome software. Degassing of the mobile phase and other solutions was done by using a Loba ultrasonic bath sonicator. The UV spectrum of all the drugs was taken with a Elico UV-Visible spectrophotometer. A Shimadzu electronic balance was used for weighing the materials.

2.3. Preparation of mobile phase

A mobile phase mixture of Acetonitrile and 0.01M Phosphate Buffer in the ratio of 60:40 v/v was prepared by diluting 600 mL of Acetonitrile, and 400 mL of 0.01M Phosphate Buffer in a one liter flask. pH of the mobile phase was adjusted to 3.0 with o-phosphoric acid. The mobile phase liquid was also used for making working dilutions of the drugs.

2.4. Standard solution

About 240 mg of FEX and 20 mg of MON were weighed and transferred into a 100 mL volumetric flask containing 25 mL of methanol. The solution was sonicated for 5 min and then volume was made up with a further quantity of the mobile phase to get 2.4 mg/mL and 0.2 mg/mL of FEX and MON respectively.

2.5. Assay in formulation

Twenty tablets were weighed and powdered into uniform size in a mortar. From this the average weight of a tablet was calculated. An accurately weighed portion from this powder equivalent to 240 mg of FEX and 20 mg of MON was transferred to a 100mL volumetric flask containing 25 mL of the methanol. The contents of the flask were sonicated for about 20min for complete solubility of the drug and the volume was made up to 100 mL with mobile phase. Then the mixture was filtered through 0.45µ membrane filter. Above solution was further diluted and 20 µL was then injected six times into the column. The mean peak areas of the drugs were calculated and the drug content in the formulation was calculated.

3. RESULTS AND DISCUSSIONS

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatogram of FEX and MON were shown in (Fig.3). There was clear resolution between FEX and MON with retention time of 2.127 and 5.45 minutes, respectively.

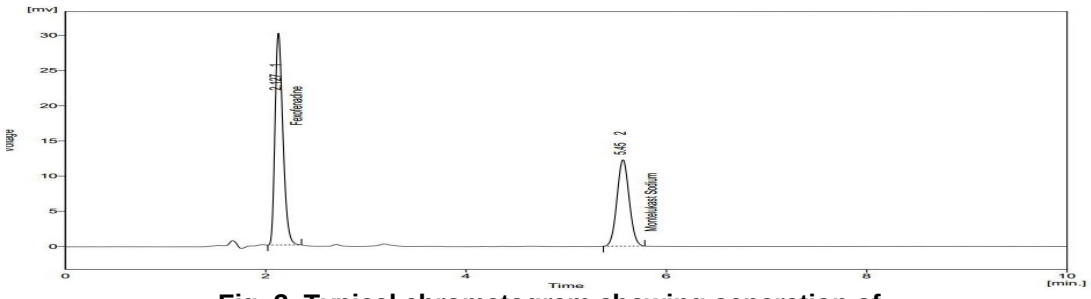

Fig. 3: Typical chromatogram showing separation of Montelukast sodium and Fexofenadine Hydrochloride

3.1. Linearity

Linearity performed by preparing mixed standard solutions of FEX and MON at different concentration levels. Twenty micro liters of each concentration was injected into the HPLC system. The response was read at 230 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method.

Table 1: Linearity study of Fexofenadine Hydrochloride

Level	Concentration of Fexofenadine HCl (µg/mL)	Mean peak area
Level -1	4.8	38.992
Level -2	9.6	68.726
Level -3	14.4	97.098
Level -4	19.2	123.074
Level -5	24	152.782
Level -6	28.8	180.437
Level -7	33.6	211.021

Table 2: Linearity study of Montelukast Sodium

Level	Concentration of Montelukast Sodium (µg/mL)	Mean peak area
Level -1	0.4	24.287
Level -2	0.8	40.598
Level -3	1.2	57.9
Level -4	1.6	75.093
Level -5	2	93.186
Level -6	2.4	110.266
Level -7	2.8	129.351

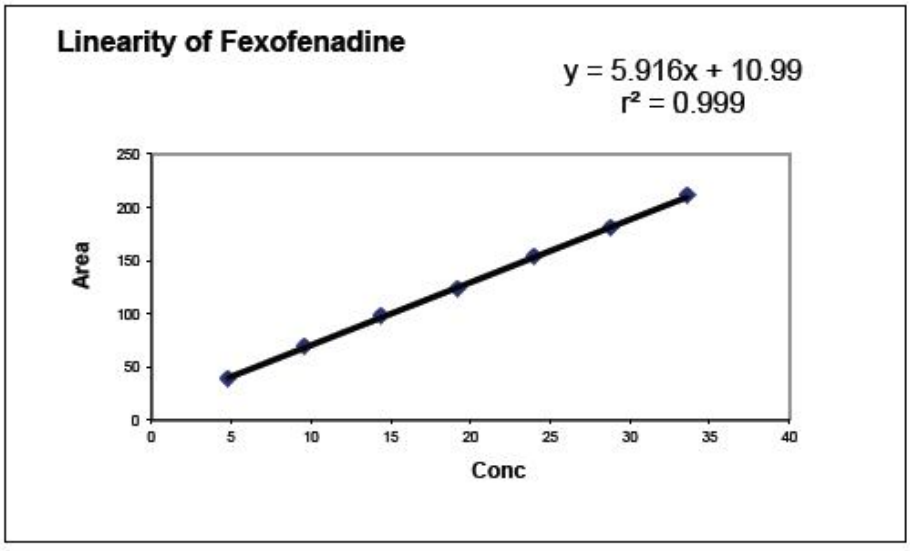

Fig. 4: Calibration curve for Fexofenadine Hydrochloride

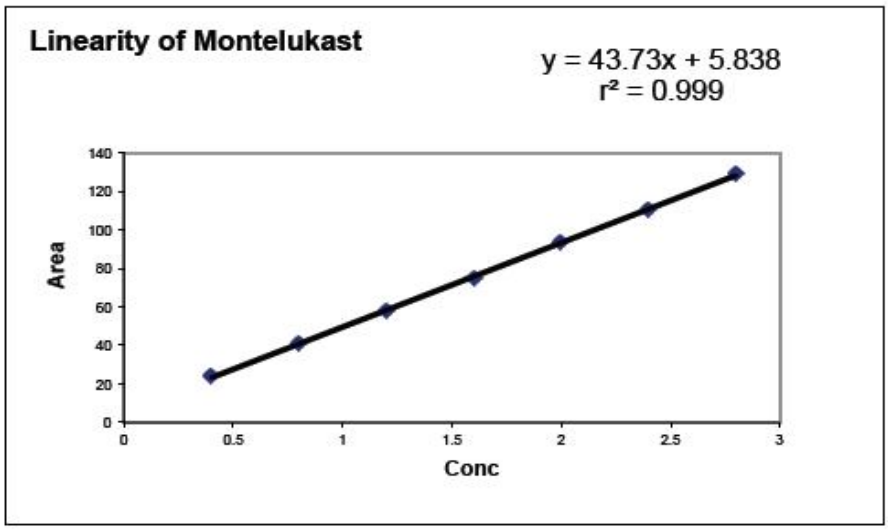

Fig. 5: Calibration curve for Montelukast Sodium

3.2. Accuracy

Accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 80%, 100% and 120% level. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD at each level was calculated and results are presented in Table 3& 4. Satisfactory recoveries ranging from 100.11 to 100.65 for FEX and 100.76 to 101.36 for MON respectively were obtained by the proposed method. This indicates that the proposed method was accurate.

Table 3: Recovery for Fexofenadine Hydrochloride

Amount taken (µg)	Area	Amount Found (µg)	Mean % recovery	% RSD
19.2+2.4	137.904	21.62	100.65 %	0.529
19.2+2.4	138.733	21.74		
19.2+2.4	138.733	21.85		
24+2.4	168.667	26.42	100.11 %	0.417
24+2.4	168.257	26.54		
24+2.4	168.357	26.32		
28.8+2.4	224.661	31.32	100.35 %	0.178
28.8+2.4	224.122	31.25		
28.8+2.4	224.226	31.36		

Table 4: Recovery for Montelukast Sodium

Amount taken (µg)	Area	Amount Found (µg)	Mean % recovery	% RSD
1.6+0.2	88.014	1.81	101.11 %	0.549
1.6+0.2	89.093	1.82		
1.6+0.2	88.093	1.83		
2.0+0.2	110.466	2.21	101.36 %	0.933
2.0+0.2	109.902	2.24		
2.0+0.2	106.821	2.25		
2.4+0.2	127.73	2.61	100.76 %	0.382
2.4+0.2	125.497	2.62		
2.4+0.2	125.275	2.63		

3.3. Limit of Detection and Quantification

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S)

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response, S = Slope of the calibration curve The slope S may be estimated from the calibration curve of the analyte.

The LOD was found to be 0.03 µg mL^{-1} and 0.11 µg mL^{-1} and LOQ was found to be 0.42 µg mL^{-1} and 1.23 µg mL^{-1} for MON and FEX respectively which represents that sensitivity of the method is high.

3.4. Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as system precision and method precision.

System Precision

To study the system precision, six replicate mixed standard solutions of FEX and MON were injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.8 and 0.6 for FEX and MON respectively, which are well within the acceptable criteria of not more than 2.0.

Table 5: Results of System Precision

Injection number	Area of Fexofenadine HCl	Area of Montelukast Sodium	Acceptance criteria
1	38.992	24.287	The %RSD of peak areas of Fexofenadine Hydrochloride and Montelukast sodium should not be more than 2.0
2	38.254	24.521	
3	38.524	24.623	
4	38.623	24.354	
5	38.754	24.257	
6	38.251	24.457	
Mean	**38.56**	**24.41**	
%RSD	**0.8**	**0.6**	

Method Precision

The method precision study was carried out on six preparations from the same tablet samples of FEX and MON and percent amount of both were calculated. The % RSD of the assay result of six preparations in method precision study was found to be 0.8 and 0.7 for FEX and MON respectively, which are well within the acceptance criteria of not more than 2.0.

Table 6: Results of Method Precision

Sample number	% Assay	
	Fexofenadine Hydrochloride	Montelukast sodium
1	99.42	99.73
2	100.04	99.80
3	101.60	100.82
4	99.92	101.69
5	100.90	100.25
6	99.72	100.32
Mean	**100.26**	**100.43**
%RSD	**0.8**	**0.7**

3.5. Robustness

The robustness of the method was determined by deliberate changes in the method like alteration in pH of the mobile phase, percentage organic content, changes in the flow rate. The robustness of the method shows that there were no marked changes in the chromatographic parameters, which demonstrates that the developed method was robust.

3.7. Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the

various components of a mixture and detect the analyte qualitatively the method is called selective. It has been observed that there are no peaks of diluents and placebo at main peaks. Hence, the chromatographic system used for the estimation of FEX and MON is very selective and specific .Specificity studies indicating that the excipients did not interfere with the analysis. The specificity of method was performed by comparing the chromatogram of blank, standard and sample.

3.8. System Suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time, number of theoretical plate, tailing factor and resolution was evaluated for five replicate injections of the drug.

Table 7: Results of system suitability parameters

Parameters	Fexofenadine HCl	Montelukast Sodium	Acceptance Criteria
Tailing factor	1.52	1.08	NMT 2.0
No. of theoretical plates	3597	9057	NLT 2000
Retention time (min)	2.127	5.42	
Resolution	18.15		NLT 2.0

4. CONCLUSION

The proposed RP-HPLC method was found to be simple, accurate, precise, linear and specific for quantitative estimation of FEX and MON in bulk and its formulation. The proposed RP-HPLC method is cost effective and less time consuming. Hence the proposed HPLC method is suitable for routine assay of FEX and MON in raw materials and in pharmaceutical formulations in the quality control laboratories.

ACKNOWLEDGEMENT

The authors thanks to Management of Hindu College of Pharmacy, Guntur for providing necessary facilities to carry out the present work.

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