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DEVELOPMENT OF VALIDATED HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF FEXOFENADINE HYDROCHLORIDE AND MONTELUKAST SODIUM IN TABLET DOSAGE FORM

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Keywords:
High Performance Liquid Chromatography, Montelukast Sodium, Fexofenadine Hydrochloride, Validation, Simultaneous Estimation

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ABSTRACT

A simple, fast and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous determination of Fexofenadine hydrochloride (FEXO) and Montelukast Sodium (MONT). Efficient chromatographic separation was achieved on phenomenex C18 column (150mm x 4.6 mm, 5 µm) as stationary phase with a mobile phase comprising of 0.5% Orthophosphoric acid pH adjusted to 6 (tri ethyl amine): Acetonitrile(40:60 v/v) at a flow rate of 1.0mL min-1, column temperature of 25°C and UV detection at 240 nm. The retention time of and Fexofenadine hydrochloride and Montelukast Sodium were 2.7 min, and 4 min respectively .The linearity were found to be in the range of 72-120 µg/ml and 6-10 µg/ml Fexofenadine Hydrochloride and Montelukast Sodium respectively with correlation co efficient of 0.999. The proposed method was validated for linearity, accuracy, precision, sensitivity. All validation parameters were within the acceptable range. The developed method was successfully applied to estimate the amount of Fexofenadine hydrochloride and Montelukast Sodium in combined dosage forms.

INTRODUCTION: Fexofenadine hydrochloride (FEXO) (Figure 1) (*RS*)-2-[4-[1-Hydroxy-4-[4-(hydroxy-diphenylmethyl)-1-piperidyl]butyl]phenyl]-2-methyl-propanoic acid, is used to relieve the allergy symptoms of seasonal allergic rhinitis (hay fever), including runny nose; sneezing; and red, itchy, or watery eyes; or itching of the nose, throat, or roof of the mouth in adults [1].

It does not pass through the blood brain barrier and so causes less drowsiness than first generation histamine receptor antagonists. It is white to off-white crystalline powder. It is freely soluble in methanol and ethanol, slightly soluble in chloroform and water and in soluble in hexane. It is carboxylic acid metabolite of terfenadine, a non-sedating selective histamine H1 receptor antagonist. This drug contains an asymmetric carbon in its chemical structure and is administered clinically or is used as a P-glycoprotein probe as a racemic mixture of *R*- and *S*-enantiomers [2].

Montelukast sodium (MTKT) (Figure 2) is chemically (S, E)-2-(1-((1-(3-(2-(7-chloroquinolin-2yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl) cyclopropyl)acetic acid [3] is a leukotriene receptor antagonist used in the treatment of chronic asthma and allergic rhinitis [4]. Montelukast is hygroscopic and optically active white to off white powder. It is freely soluble in methanol ethanol and water.

Literature survey reveals that Fexofenadine hydrochloride is estimated individually or in combination with other drugs by UV spectrophotometry [5-6], RP-HPLC [7-8], HPTL [9-10], in biological fluid by RP-HPLC [11], LC/MS [12], LC/MS/MS [13]

and Stability indicating HPLC and TLC method [14]. Similarly for Montelukast sodium, UV spectro-photometery [15-16], spectrofluorometry [17], RPHPLC [18], HPTLC [19], plasma HPLC [20-21], LC/MS [22-23], and stability indicating HPLC methods [24-25] have been reported.

The aim of the present work to develop the simple, economical, accurate, reliable reverse phase HPLC method for the estimation of and in bulk and combined dosage form.This method was validated as per ICH guideline [26].

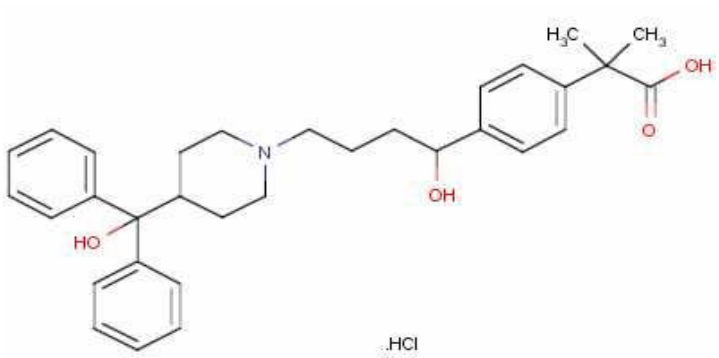

FIGURE 1: THE CHEMICAL STRUCTURE OF FEXOFENADINE HYDROCHLORIDE (FEXO)

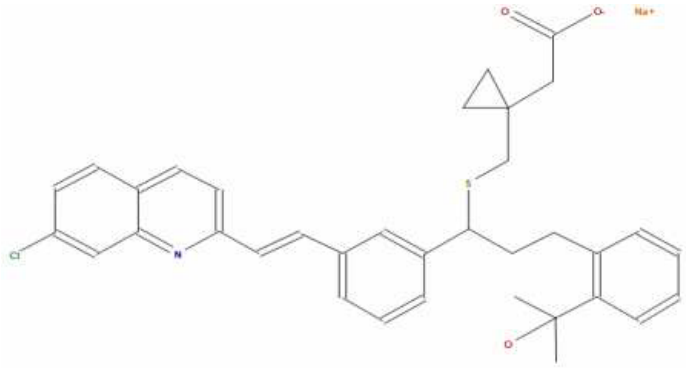

FIGURE 2: THE CHEMICAL STRUCTURE OF MONTELUKAST SODIUM (MONT)

EXPERIMENTAL:

Chemicals and Reagents: Working standards of pharmaceutical grade FEX (99.60 %, w/w) and MTKT (100.0 %, w/w) were obtained as gift samples from Hetero Pharmaceuticals, Hyderabad, India. Fixed dose combination tablets (MONTAIR FX, Cipla Ltd.) containing 120 mg FEX and 10 mg MTKT were purchased from local pharmacy, Coimbatore, India. HPLC-grade acetonitrile was from Merck. Ortho phosphoric acid and Tri ethyl amine were from Merck Chemicals, Mumbai, India. . Solvents were filtered through a 0.45µm nylon membrane filter and degassed by using ultrasonicator.

Instrumentation and Chromatographic Conditions: The developed method used a Shimadzu LC system consisting of a Model LC-20 AT pumps, an SPD-M20A Prominence Diode array detector, data were acquired and processed by LC solution software. The separation was carried out at ambient temperature by using a - Phenomenex Luna 5µ C18 (2) 100A, (250 X 4.6m.m x i.d, 5µ).The mobile phase consisting of 0.5% Ortho phosphoric acid pH adjusted to 6 (tri ethyl amine) : Acetonitrile (40:60v/v). The flow rate was 1.0 mL/min. The injection volume was 20 µL. Column temperature was maintained at 30°C and UV detection at 240 nm. For all standards and samples, triplicate injections were made..Before analysis both the mobile phase and sample solutions were degassed by use of ultrasonicator. External standards with measurement of peak areas were used for quantitation. Chromatography was performed in air conditioned room at ambient temperature.

Preparation of Standard Solutions: Each 120 mg of Fexofenadine Hydrochloride and 10 mg of Montelukast Sodium were taken in to a two separate 10 ml standard flasks and dissolved in few ml of mobile phase (4:6) and the contents were shaken for 1 min to get a clear solution and final volume was made up to 10 ml with mobile phase, 0.5% Orthophosphoric acid pH 6 adjusted with (TEA) : Acetonitrile (Stock solution A and B). Aliquots of mixed standard solutions were diluted in mobile phase to get a final concentration of 72, 84, 96, 108, 120 µg/ml for Fexofenadine Hydrochloride and 6, 7, 8, 9, 10 µg/ml for Montelukast Sodium. All the solutions were sonicated for 20 minutes before injection.

Preparation of Sample solution: Twenty tablets of Montair FX each containing Fexofenadine Hydrochloride- 120 mg, and Montelukast Sodium - 10mg were weighed, and crushed into fine powder. A quantity of powder equivalent to 120 mg of Fexofenadine was weighed and dissolved in 5 ml of mobile phase (4:6) and sonicated for 15 min. Then the volume was made up to 10 ml with mobile phase ratio and filtered through whatmann filter paper No: 41. The final sample solution was prepared, corresponding to 96µg/ml of Fexofenadine Hydrochloride and 8µg/ml of Montelukast Sodium.

Assay: The assays performed by the Marketed formulation of Fexofenadine and Montelukast (Montair-FX). The prepared standard and sample solutions were injected in HPLC. Results for assay showed in **Table 1.**

Method Validation: Precision was performed with six repeated sample preparation of nominal concentration, accuracy was performed 50% to 150% of five concentration level Fexofenadine Hydrochloride and Montelukast Sodium by the preparation sample was weighed as equivalent to 50%, 75%, 100%, 125% and 150% of the nominal concentration respectively. At low level concentration and highest level concentration accuracy proved with six replicate preparation and medium level triplicate preparations was done.

The linearity was performed for the standard solution of level Fexofenadine Hydrochloride and Montelukast Sodium from the level of 50% to 150% of about five different concentrations from nominal concentration of standard and sample solution. Range of the method has been captured from precision, accuracy and linearity section. Deliberate change of the chromatographic condition will affect the method reproducibility so the method accuracy was proved by ruggedness experiments and the system suitability was proved.

Formulation (Montair FX)	Labeled amount (mg)		Amount Found (mg)		Percentage assay (%)		%R.S.D*	
	FEXO	MONT	FEXO	MONT	FEXO	MONT	FEXO	MONT
	120	10	119.7	9.92	99.75	99.82	0.75	0.52

RESULTS & DISCUSSION: Developed analytical method was validated and system suitability was performed prior to each parameter and the result was found satisfactory for all the parameter. Refer chromatogram of standard and sample **(Figure 3 and Figure 4)**.

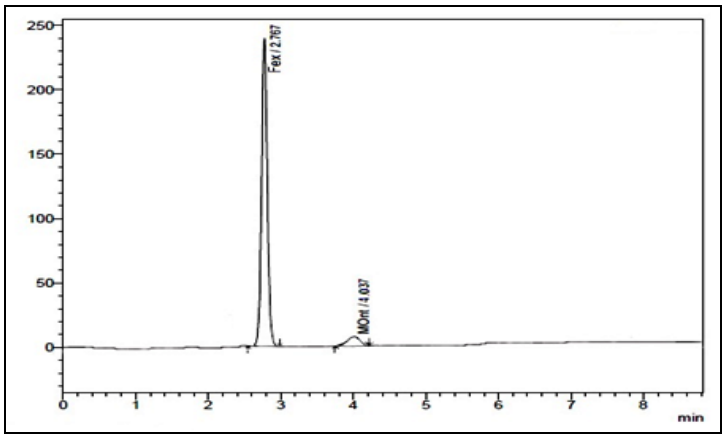

FIGURE 3: CHROMATOGRAM OF STANDARD

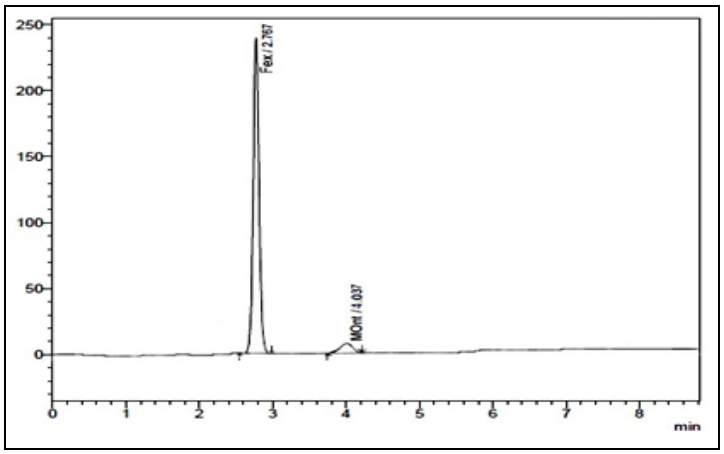

FIGURE 4: CHROMATOGRAM OF SAMPLE

Linearity: Linearity was evaluated by analysis of working standard solutions of Montelukast Sodium and Fexofenadine hydrochloride of five different concentrations. The range of linearity were from72 µg /ml to 120 µg/ ml for Fexofenadine hydrochloride and 6 µg/ ml to 10 µg/ ml for Montelukast Sodium **Table 2 and 3**. The peak area and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients**.**

Figure 4 and 5 represents the linearity plots of and Fexofenadine hydrochloride and Montelukast Sodium respectively. The result shows that within the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration.

TABLE 2: LINEARITY RANGE OF FEXOFENADINE HYDROCHLORIDE

S. NO.	Conc. of FEXO (µg/ml)	Peak area
1	72	1142313
2	84	1315266
3	96	1476383
4	108	1689305
5	120	1887522

TABLE 3: LINEARITY RANGE OF MONTELUKAST SODIUM

S. NO.	Conc. of MONT. (µg/ml)	Peak area
1	6	94302
2	7	108994
3	8	124729
4	9	137849
5	10	151041

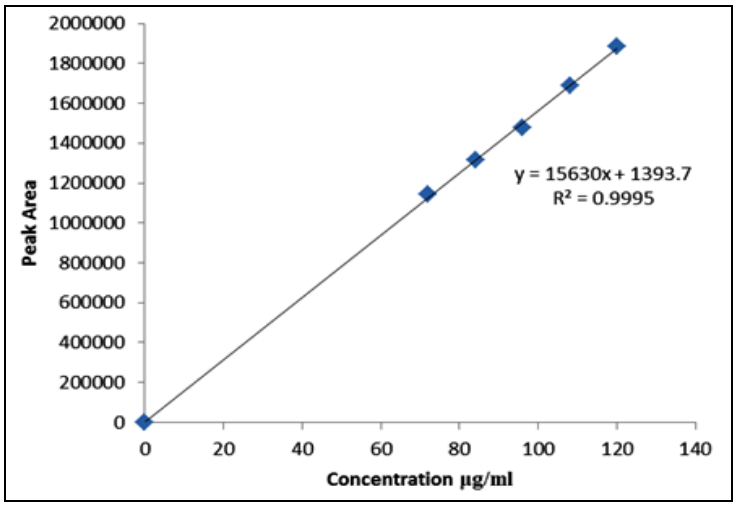

FIGURE 4: CALIBRATION CURVE FOR FEXOFENADINE HYDROCHLORIDE

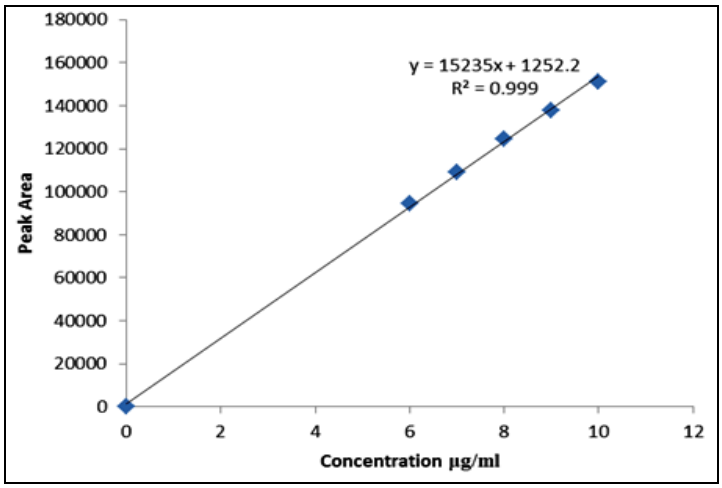

FIGURE 5: CALIBRATION CURVE FOR MONTELUKAST SODIUM

Precision:

1. **Intraday Precision:** Intraday precision was found out by carrying out the analysis of the standard drugs at three same concentrations 96 µg /ml for Fexofenadine hydrochloride and 8 µg /ml for Montelukast Sodium in the linearity range of drugs for six times on the same day. Each concentrations were applied in duplicate and percentage RSD was calculated **Table 4.**

2. **Interday Precision:** Inter day precision was found out by carrying out the analysis of the standard drugs at three same concentrations for 96 µg /ml FEXO and 8 µg /ml for MONT in the linearity range of drugs for three days for six times and the percentage RSD was calculated **Table 5**.

LOD and LOQ / Sensitivity: Sensitivity was determined by establishing the limit of detection (LOD) and limit of quantification (LOQ). The limit of detection (LOD)and limit of quantification (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively.The LOD and LOQ of Fexofenadine hydrochloride and Montelukast Sodium was experimentally determined by six injections of each drug. The LOD and LOQ of Fexofenadine hydrochloride was found to be 3.83 µg/ ml & 11.62 µg/ ml respectively. The LOD and LOQ of Montelukast Sodium was found to be 0.21 µg/ ml & 0.64 µg/ ml respectively.

TABLE 4: INTRADAY STUDIES

No of Injection	Conc. of FEXO (µg/ml)	Peak Area	% RSD*	Conc. of MONT (µg/ml)	Peak Area	% RSD*
6	96	1475262	0.48	8	123618	0.57

TABLE 5: INTER DAY STUDIES

Day	Conc. of FEXO (µg/ml)	Peak Area	% RSD*	Conc. of MONT (µg/ml)	Peak Area	% RSD*
DAY1	96	1475216	0.51	8	128667	0.61
DAY2	96	1469127	0.62	8	127975	0.64
DAY3	96	1470113	0.59	8	126959	0.70

Accuracy: Accuracy was determined over the range 50% to 150% of the sample concentration.Calculated amount of Fexofenadine hydrochloride and Montelukast Sodium from standard stock solution was added in placebo to attain 50%, 100% and 150% of sample concentration. Each sample was prepared in triplicate at each level. Blank and standard preparations were injected and chromatograms were recorded.Accuracy was expressed as the percentage of analytes recovered by the assay. **Table 6** lists the recoveries of the drug from a series of spiked concentrations.

The results indicate the method is highly accurate for simultaneous determination of Fexofenadine hydrochloride and Montelukast Sodium.

Specificity: Specificity of the method was evaluated by injecting diluents, placebo, individual Fexofenadine hydrochloride and Montelukast Sodium and sample solution in to the HPLC system to check any interference to the peaks. No peak was observed at the retention time of Fexofenadine hydrochloride and Montelukast Sodium in diluent and placebo chromatogram. Hence the method was specific.

Ruggedness: The sample was analyzed by a different chemist and same instruments on a different day have been performed. The deviation among the results obtained by two chemists on a different day is well within the limits. Hence the method is proved to be rugged **Table 7**.

Table 6: Accuracy (Recovery studies)

Day	Conc. of FEXO (µg/ml)	Peak Area	% RSD*	Conc. of MONT (µg/ml)	Peak Area	% RSD*
DAY1	96	1475216	0.51	8	128667	0.61
DAY2	96	1469127	0.62	8	127975	0.64
DAY3	96	1470113	0.59	8	126959	0.70

TABLE 7: RUGGEDNESS STUDIES

Drug name	Concentration (µg/ml)	Mean peak area	%RSD*
Day-1 analyst-1			
FEXO	84	1304133	0.53
MONT	7	110994	0.68
Day-2 analyst-2			
FEXO	84	1215362	0.62
MONT	7	104324	0.74

CONCLUSIONS: The method after being completely validated showed satisfactory data for all the method validation parameters. Method validation study showed that the method is specific, linear, accurate, easily reproducible and can be used for simultaneous determination of

Fexofenadine hydrochloride and Montelukast Sodium from pharmaceutical preparations.

The method seems to be suitable for quality control in the pharmaceutical industry because of its sensitivity, simplicity and selectivity.

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