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Method Development And Stability Indicating RP-HPLC Method For The Estimation Of Montelukast And Fexofenadine For Bulk And Pharmaceutical Dosage Form

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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 Montelukast Sodium (MONT) and Fexofenadine hydrochloride (FEXO). The chromatographic separation was achieved on ACE C8 column (250 mm x 4.6 mm, 4µ particle size) as stationary phase with a mobile phase comprising of ortho phosphoric acid (pH 6.2): methanol (40:60) with flow rate of 1.0 mL/min, column temperature of 28±2ºC at a wavelength of 290nm. The retention time of Montelukast Sodium and Fexofenadine hydrochloride were 5.0 min, and 3.2 min respectively. The linearity were found to be in the range of 2-6µg/mL and 24-72mg/mL for Montelukast Sodium and Fexofenadine hydrochloride with correlation coefficient greater than 0.999. The proposed methods were validated as per ICH guidelines and successfully applied for the determination of investigated drugs in tablets.
Keywords: Montelukast, Fexofenadine, RP-HPLC, Method development, forced degradation.

INTRODUCTION[1-14]

Montelukast is chemically designed as 2-[1-({[(1R)-1-{3-[(E)-2-(7-chloroquinolin-2yl)ethenyl]phenyl}-3-[2-(2-hydroxypropane 2yl)phenyl]propyl]sulfanyl}methyl)cyclopropyl] acetic acid is a leukotriene receptor antagonist(LTRA) it is freely soluble in ethanol, methanol, and water and practically insoluble in acetonitrile. It is used in the treatment of allergic rhinitis with concomitant administration of an anti-leukotriene and an antihistamine shows significantly better symptom relief compared with the modest improvement in rhinitis symptomatology with each of the treatments alone

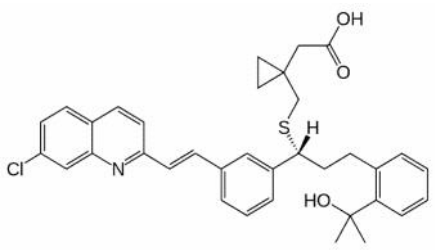

Figure 1 Structure of Montelukast

Fexofenadine HCl (FEXO), chemically designated as 2-(4-{1-hydroxy-4-[4-(hydroxydiphenylmethyl) piperidin-1- yl]butyl}phenyl)-2-methylpropanoic acid is a histamine H1 receptor antagonist used in patients with allergic rhinitis. It is freely soluble in methanol, ethanol and slightly soluble in water, chloroform and practically insoluble in hexane.

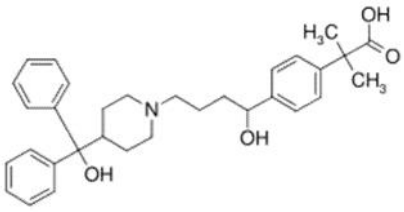

Figure 2 Structure of Fexofenadine

Both the drugs MONT and FEXO are official in IP 2010. Detailed survey of literature for MONT revealed several reported methods based on different technique like HPLC, UPLC, HPTLC, UV spectrophotometry, voltammetric, and LC-ESI-MS/MS for its determination from pharmaceuticals. Forced degradation of drug substances and drug product was performed under different stress conditions (thermal, photolytic, UV exposure, acid and basic hydrolytic and oxidative), and stressed samples were analyzed by the proposed method. The proposed LC method was able to separate both drugs from degradants generated during forced degradation studies.

EXPERIMENTAL[1-14]

Chemical and reagents

Montelukast and Fexofenadine were obtained as a gift sample from Hetero laboratories, Hyderabad, India. The formulation MONTAIR-FX (label claim: Fexofenadine 120mg and Montelukast 10mg) manufactured by CIPLA.Ltd was purchased from market. All the chemicals used like Methanol, and ortho- phosphoric acid are of analytical grade and mobile phase solvents of HPLC grade were purchased from MERCK Chem. Ltd., Mumbai.

Instrumentation and Chromatographic conditions

HPLC device Waters model 2695 with Empower software version 2.0.Detector waters 2996 PDA Detector. Elico Ph meter, Sartorius – Digital balance (0.1 mg – 205 gm).separation was achieved on a ACE C8 column (250 mm x 4.6 mm, 4µ particle size) as a stationary phase in which mobile phase consisted of Methanol and orthophosphoric acid buffer in 40:60 ratios was pumped at a flow rate of 1ml/min. The elution is observed using a PDA detector at 290 nm and the injection volume was 15 µL. The validation of the method was done following the ICH guidelines.

Preparation of standard solution

An accurately weighed sample 120mg and 10mg of Fexofenadine and Montelukast are dissolved in 50ml of mobile phase in volumetric flask to give standard stock solution. The working standard solutions were obtained by diluting the stock solution. 48µg/ml and 4µg/ml solution

Of FEXO and MONT was obtained by dilution of the stock solution in mobile phase. All the volumetric flasks containing solution were stored at room temperature.

Preparation for marketed formulation

Ten tablets were weighed and average weight was calculated. Tablets were crushed to a fine powder. 304mg is weighed which is one tablet equivalent weight containing 120mg of Fexofenadine and 10mg of Montelukast and transferred to 50mL volumetric flask. Then, 25 mL of mobile phase was added, placed for 20 min in

ultrasonicator and more mobile phase was added until the solution reached 50mL. 5mL of the above solution is transferred in 25mL of volumetric flask and was made up to the mark .It was filtered through a 0.45 µm membrane filter (Milli-pore). The peak purity was checked with the PDA.

Method Development and method optimization and experimental condition

Several trials have been taken for the proper optimization of RP HPLC method by changing different mobile phase with different ratio. And finally the mobile phase for optimized condition was selected and given follows. And the optimized parameter for MONT and FEXO was given in Table 1.

Table 1 A optimized chromatographic condition of Met & Sit

Chromatographic conditions	Mode of separation
Separation	Waters 2695
Column	ACE C8(250x 4.6 mm,4µm particle size)
Flow rate	1.0ml/min
Solvent	Water
Column temperature	28±2ºC
Sample temperature	25^0C
Wavelength selected	290 nm
Injection volume	15µl
Run time	10 minutes
Mobile phase	Methanol: OPA(60:40v/v)

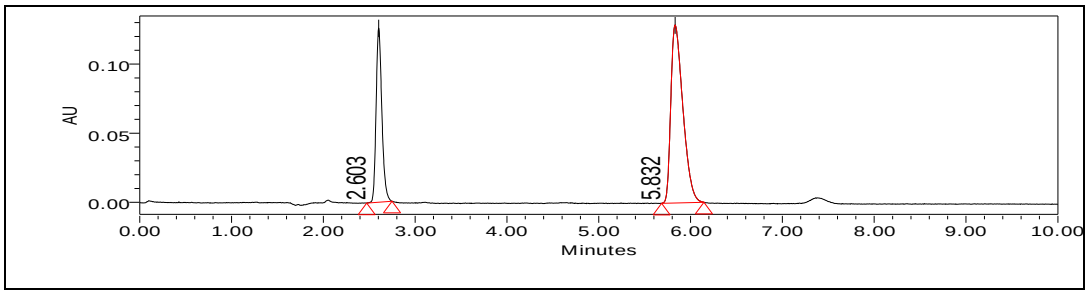

Figure 3 Optimized chromatogram of FEXO AND MONT

Method validation

The method of analysis was validated as per the recommendations of ICH and USP for the parameters like accuracy, linearity, precision, detection limit, quantitation limit, and robustness. The accuracy of the method was determined by calculating percentage recovery of MONT and FEXO. For both the drugs, recovery studies were carried out by applying the method to drug sample to which the known amount of MONT and FEXO corresponding to 50, 100, and 150% of label claim had been added (standard addition method). At each level of the amount three determinations were performed and the results obtained were compared.

Intra- and inter-day precision study of MONT and FEXO was carried out by estimating the corresponding responses three times on the same day and on three different days for the concentration of 4 µg/ml and 48 µg/ml for MONT and FEXO, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following formula.

LOD=3.3(SD)/S and LOQ=10(SD)/S,

Where SD is standard deviation of response (peak area) and S is average of the slope of the calibration.

System suitability tests are an integral part of any chromatographic analysis method which is used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level of 4 and 48 µg/ml for MONT and FEXO.

For robustness evaluation of HPLC method a few parameters like flow rate and pH of mobile phase were deliberately changed. Each factor selected was changed at three levels (−1, 0, +1) with respect to optimized parameters. Robustness of the method was done at the concentration level of 4 and 48 µg/ml for MONT and FEXO, respectively.

RESULTS AND DICUSSIONS

System suitability

Theoretical plates, Tailing factor and resolution between MONT and FEXO were determined for each drug. The results were within acceptable limits and are summarized in table 2.

Table 2 Optimized parameters

	Retention Time	Area	% Area	Height	Resolution	USP Tailing	USP Plate Count
MONT	5.832	1174457	68.07	128692	18.326	1.593	8983
FEXO	2.603	550894	31.93	125757		1.228	8339

Linearity

Appropriate amounts of Montelukast and Fexofenadine stock solution were diluted with mobile phase to give concentrations of 2-6µg/mL for MONT and 24-72µg/mL for FEXO. The calibration curves were drawn by plotting the peak areas against the corresponding concentration. The slope and Y-intercept of the calibration curve was calculated from the linearity graphs (figures 4 & 5). The data for slope, intercept and regression was mentioned in table 4.

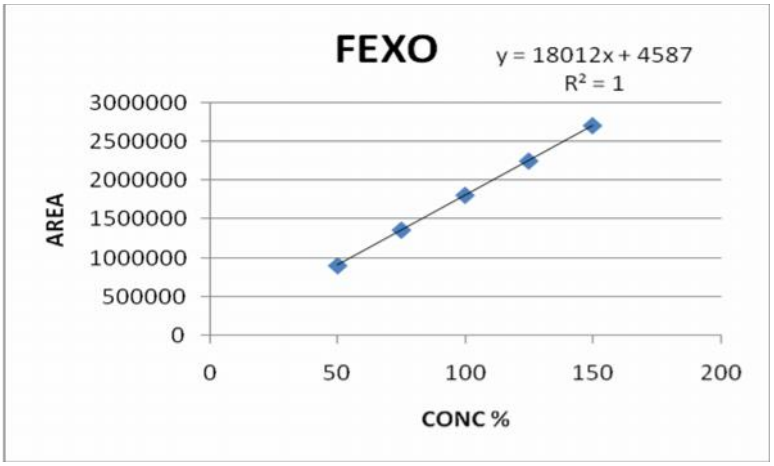

Figure 4 Linearity of FEXO

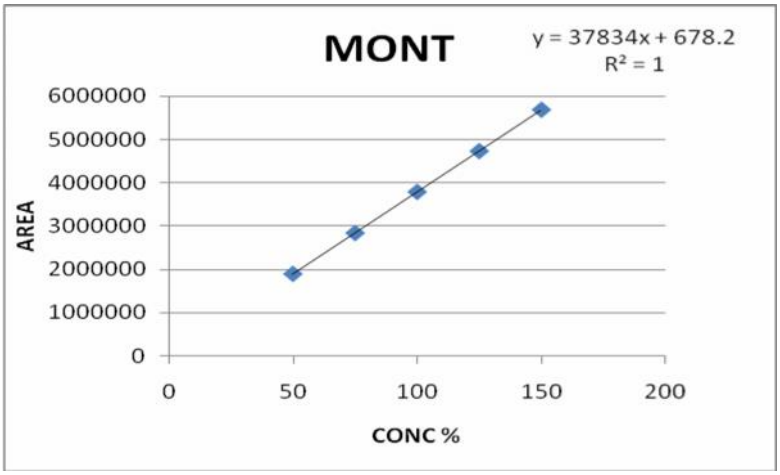

Figure 5 Linearity of MONT

Table 3 Parameters of system suitability

Parameters	MONT	FEXO
Retention time (minutes±SD)	2.6±0.07	5.8 ± 0.05
Repeatability (% RSD)	0.018	0.013
Theoretical plates per meter	8983	8339
Tailing factor	1.59	1.22
Resolution	18.32	

Table 4 Linearity parameters

Parameters	FEX	MON
Linearity range	24-72 µg/ml	2-6 µg/ml
Regression equations	y=18012x-4587	y = 37834x-678.2
Slope	18012	37834
Intercept	4587	678.2
Correlation	1	1

Precision

Repeatability was evaluated by assaying samples, at the same concentration 48µ g/mL and 4µg/ml for FEXO and MONT during the same day. The standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated. The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and found to be 0.018 and 0.013 for six replicate determinations (Table 5).

Accuracy

Accuracy of the method was observed at three different concentration levels i.e. 80%, 100, 120%. To the pre-analyzed sample solution a known quantity of standard drug was added at three different levels and pre-analyzed by proposed method. Accuracy was evaluated by the simultaneous determination of the analytes in solutions prepared by the standard addition method. The %Recoveries of the added analytes were found to be in below (Table 6).

Table 5 Precision

DRUG	CONC (%)	Peak area(n=6)	% Assay (n=6)	STD	%RSD
MONT	100	1803240	100.0	0.11	0.018
FEXO	100	3785440	100.1	0.08	0.013

Table 6 Accuracy

Conc of Spiked level %	Amount added µg/ml		Total amount found µg/ml		% Recovery µg/ml(n=3)		%RSD	
	FEX	MON	FEX	MON	FEX	MON	FEX	MON
50 50	24	2	23.79	1.99	99.12	99.5	0.020	0.20
100 100	48	4	47.66	3.99	99.29	99.75	0.201	0.14
150	72	6	71.33	5.98	99.06	99.66	0.036	0.09

Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The robustness was studied by analyzing the same samples of MONT and FEXO by deliberate variation in the method parameters. Robustness of the method was studied by changing the extraction time of MONT and FEXO from tablet dosage forms by ± 2 min, flow rate by ± 0.2 ml/min and column oven temperature by ±2^{o}C(Table 7).

Table 7 Robustness

Flow rate (ml/min)	RT FEX(min)	RT MON(min)	Mean ± SD	
			FEX	MON
0.8ml/min	3.991	6.614	3.339±0.92	5.389±1.7
1.2 ml/min	2.687	4.153		
Temp				
Temp(25^{o}c)	3.96	6.614	5.38±0.90	5.51±1.5
Temp(30^{o}c)	2.68	4.419		

Forced Degradation

Forced degradation of the drug product was carried out under hydrolytic, oxidative, photolytic, and thermolytic conditions. After degradation these solutions were diluted with mobile phase to achieve a concentration of 4µg/ml of MONT and 48µg/ml of FEXO (on label claim basis for marketed formulation). Then 15 µl portions of degraded solutions were injected into the HPLC system and analyzed under the chromatographic analysis condition described earlier.

Acid hydrolysis in solution state was performed in 0.1N hydrochloric acid for 2 h. Base hydrolysis was performed in 1.0 N sodium hydroxide solution for 2 h. For oxidation, sample solutions of drug product in 3%

hydrogen peroxide were kept at room temperature for 4 h. For thermal, samples of drug product were placed in a hot air oven at 80° for 24 h. For photolytic, samples of drug product were placed in UV light for 24h.The degradation study indicated that MONT was susceptible to acid, base, oxidation, photo and thermal degradation. FEXO was found to be susceptible to acid, base, oxidation and photo while it was stable to thermal degradation conditions (Table 8).

Table 8 Forced Degradation

Degradation conditions	%Assay MON	% Degradation MON	Peak purity	%Assay FEX	% Degradation FEX	Peak purity
1.0 NaOH	86.20	13.5	passes	87.32	11.78	passes
0.1N HCl	88.12	11.58	passes	97.01	2.09	passes
3% H_2O_2	84.52	15.18	passes	93.80	5.3	passes
Photo/UV	93.86	5.84	passes	97.25	1.85	passes
Thermal	97.30	2.4	passes	95.00	4.1	passes

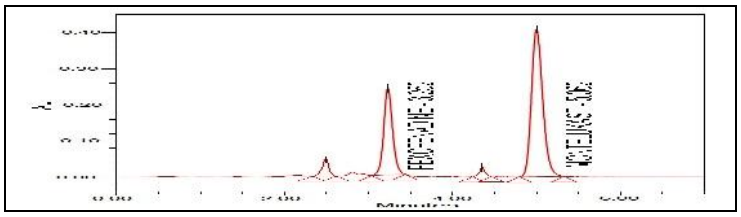

Figure 6 1.0 NaOH

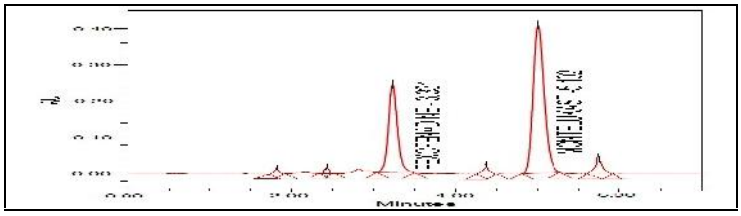

Figure 7 0.1N HCL

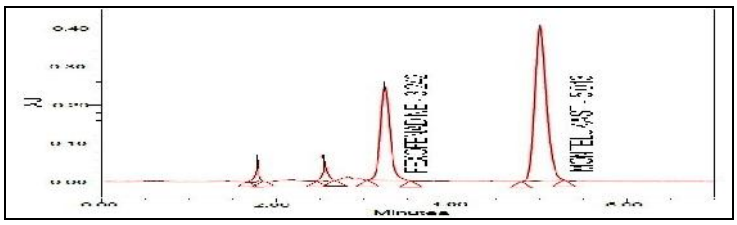

Figure 8 3% Hydrogen Peroxide

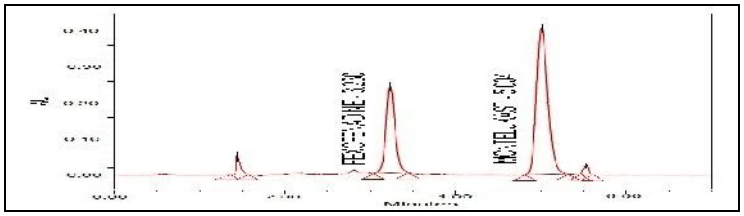

Figure 9 Photo/UV

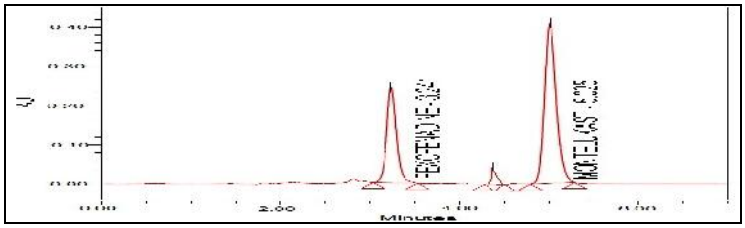

Figure 10 Thermal

Limit of detection (LOD) & Limit of quantification (LOQ)

Calibration curve was repeated five times and the standard deviation of the intercepts of regression equations was calculated. The LOD and LOQ were calculated using equation:

LOD = 3.3 * SD/ S and LOQ = 10 * SD/ S

Where; SD = standard deviation of intercepts

S = mean slope of calibration

LOD & LOQ of Fexofenadine was found to be 0.139 and 0.395µg/ml and Montelukast was 0.140 and 0.410µg/ml.

Conclusion

A simple specific stability-indicating HPLC method has been developed for the estimation of Montelukast and Fexofenadine simultaneously. This method has been validated and found to be specific, precise, accurate, linear, robust, and linear for the detection and quantification of MONT and FEXO. This method exhibited an excellent performance in terms of sensitivity and speed. The major advantage of this technique is that it is less time consuming and also eco-friendly because of its low consumption of organic solvents as compared to other analytical techniques.

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