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RP HPLC METHOD DEVELOPMENT & VALIDATION OF MONTELUKAST & FEXOFENADINE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, accurate and precise RP-HPLC method was developed and validated for determination of Montelukast Sodium (MONT) and Fexofenadine hydrochloride (FEXO) in pharmaceutical dosage form. The chromatographic separation was achieved on (Cosmosil) C18 column (4.6mm x 250mm) as stationary phase with a mobile phase comprising of Acetonitrile: Water(0.1% with OPA) 70:30 adjust pH 3 at a flow rate of 1.0 mL/min, column at Ambient temperature and UV detection at 241 nm. The retention time of Montelukast Sodium and Fexofenadine hydrochloride were 5.3666 min and 6.8333 min respectively. The linearity were found to be in the range of 1-5 µg/mL and 12-60µg/ml for Montelukast Sodium and Fexofenadine hydrochloride with correlation coefficient greater than 0.999. The precision of the method was demonstrated with % RSD while the % recovery was found in between 99.69 -100.17%. The proposed methods were validated as per ICH guidelines and successfully applied for the determination of investigated drugs in tablets.

KEYWORDS: Montelukast Sodium, Fexofenadine hydrochloride, RP-HPLC, validation.

INTRODUCTION

Montelukast is chemically designed as 2-[1-({[(1R)-1-{3-[(E)-2-(7-chloroquinolin-2yl)ethenyl]phenyl}-3-[2-(2hydroxypropane2yl)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.[1] It is a leukotriene receptor antagonist used in the treatment of chronic asthma and allergic rhinitis.[2] It works by blocking the action of leukotriene D4 on the cysteinyl leukotriene the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. Montelukast is hygroscopic and optically active white to off white powder. It is freely soluble in methanol ethanol and water.[2-3] Fexofenadine hydrochloride is 4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1piperidinyl] butyl]-α,α-dimethylbenzeneacetic acid of hydrochloride. Fexofenadine is indicated for the relief from physical symptoms associated with seasonal allergic rhinitis and treatment of chronic urticarial. The structure of the drug is shown in Figure 1 and 2. One such combination contains 10 mg of Montelukast Sodium and 120 mg of Fexofenadine hydrochloride.[4-5] Both the drugs are official in IP 2010. Detailed survey of literature revealed several reported methods for their determination from pharmaceutical preparations.[6]

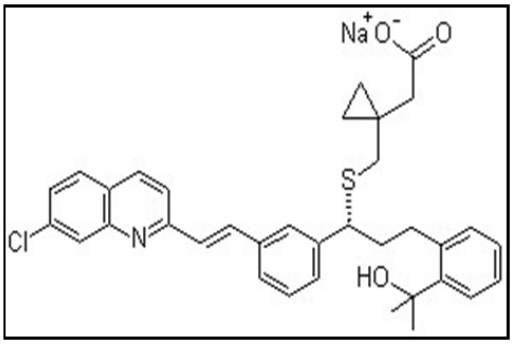

Figure 1: Structure of MONT.

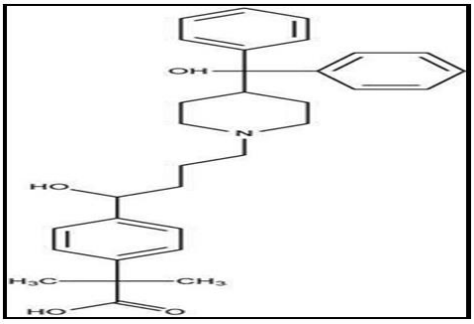

Figure 2: Structure of FEXO.

MATERIALS AND METHODS

Chemical and Reagents

The **MONTE** and **FEXO** APIs are collected from the company R.S.I.T.C. Ltd. Jalgaon., Maharashtra India as a gift sample. Orthophopsphoric acid (OPA) HPLC grade shall be obtained from Avantor Performance material India Ltd. Thane, Maharashtra. Acetonitrile and Water HPLC grade shall be obtained from Merck Specialities Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai. Pharmaceutical tablet dosage form containing 10 mg + 120mg of Montair-FX was purchased from local pharmacy Dhule Maharashtrta, India.

Instrumentation

The HPLC experiment is performed on a Younglin (S.K.) Gradient System UV Detector. Equipped with Reverse Phase (Cosmosil) C18 column (4.6mm x 250mm; 5µm), a SP930Dpump, a 20µl injection loop and UV730D Absorbance detector and running autochro-3000 software, UV-Spectrophotometer Analytical Technologies Limited. Other equipment's used were ultra-sonicator, pH meter and Balance (WENSAR™ High Resolution Balance.

Solution preparation

Preparation of standard stock solution

Preparation of std. Montelukast solution: (Stock I)

From the freshly prepared standard stock solution (100ug/ml), 0.1ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration of 1ug/ml.

Preparation of std. Fexofenadine solution: (Stock II)

From the freshly prepared standard stock solution (1200ug/ml), 0.1 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 12ug/ml.

Preparation of std. Montelukast and Fexofenadine solution: (Stock III)

From the freshly prepared standard stock solution (1000ug/ml), 0.1 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 10 ug/ml.

Preparation of mobile phase

Acetonitrile and Water (0.1% OPA) was selected since it gave sharp, well resolved peaks with symmetry within the limits and significant reproducible retention time for Montelukast and Fexofenadine.

Diluent Preparation: Mobile phase was used as Diluent.

Chromatographic conditions

The following chromatographic conditions were established by trial and error and were kept constant throughout the analysis.

Column	: C18 (250 mm× 4.6mm)
Particle size packing	: 5µm
Detection wavelength	: 241nm
Flow rate	: 1 ml/min
Temperature	: Ambient
Sample size	: 20 µl
Mobile phase	: Acetonitrile: Water (0.1% OPA) (70: 30)

Determination of λmax

UV absorption of 10 µg/mL solution of Montelukast and Fexofenadine in ACN was generated and absorbance was taken in the range of 200-400 nm. λmax of Montelukast and Fexofenadine in Acetonitrile was found to be 286nm and 220 nm respectively.[7] **(Figure No.03, 04, 05)**

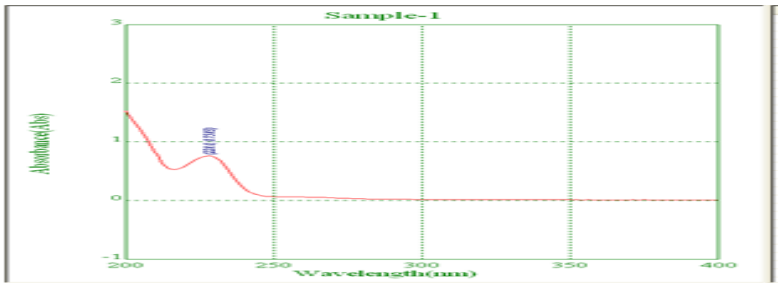

Figure No. 03: UV Spectrum of Montelukast.

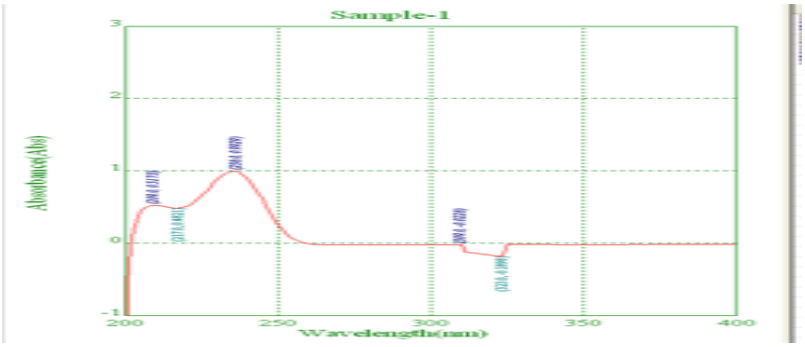

Figure No. 04: UV Spectrum of Fexofenadine.

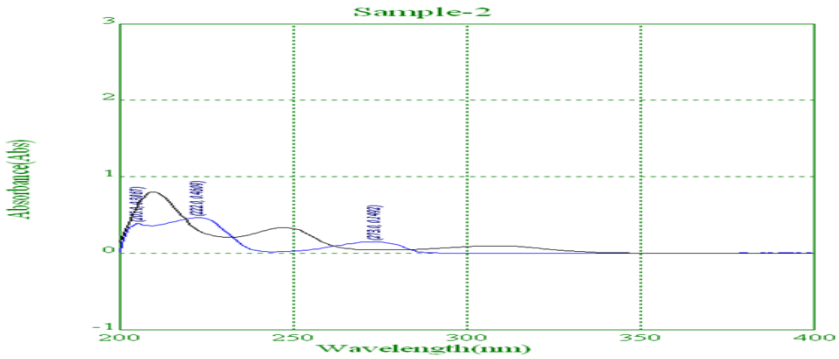

Figure 05: Iso-absorptive point of Montelukast and Fexofenad.

Method Validation

The optimized chromatographic method was validated for different parameters like system suitability, specificity, linearity, accuracy, precision, robustness, LOD and LOQ as per ICH guidelines.[8-9]

System suitability

It was carried out for the assessment of system suitability of the equipment for the analysis. The test was carried out by injecting six replicate injections of standard solution. The results were validated for theoretical plates (N), tailing factor, % RSD and peak height. **(Table No.06)**

Accuracy

Studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed **(Table No.01).** Statistical validation of recovery studies shown in. The percentage recovery and percentage relative standard deviation [% RSD] were taken into consideration for testing accuracy.

Precision

The method was established by analyzing various replicates standards of Montelukast and Fexofenadine. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday is shown in **(Table No. 02)** respectively.[10-11]

Linearity

From Montelukast standard stock solution, different working standard solution (1-5µg/ml) were prepared in mobile phase Likewise from Fexofenadine standard stock solution different working standard solution (12-60µg/ml) were prepared in mobile phase 20 µl of sample solution was injected into the chromatographic system using fixed volume loop injector. Chromatograms were recorded. The area for each concentration were recorded**.** The Calibration curves are shown in **Figure No.07, 08.**

Specificity

For the simultaneous determination of Montelukast and Fexofenadine potassium, the specificity requires that the method should not be affected by the presence of other components. Solutions of mobile phase, sample solution, standard solution were injected into liquid chromatography. Retention times of samples and standard were compared.[12-14]

F. Limit of detection (LOD)

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope The limit of detection (LOD) may be expressed as:

LOD = 3.3 (SD)/S

where, SD = Standard deviation of Y intercept

S = Slope

Limit of detection = 3.3 X 5.02/60.12= 0.2755(µg/mL)

Limit of Quantitation = 10 X 5.02/60.12= 0.8349(µg/mL)

The LOD and LOQ of Montelukast was found to be 0.2755 (µg/mL) and 0.8349(µg/mL), analytical method that concluded.

Limit Quantification

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:

LOQ = 10 (SD)/ S

where, SD = Standard deviation Y intercept

S = Slope

Limit of detection = 3.3X1.57/118.5= 0.04372(µg/mL)

Limit of Quantitation = 10 X1.57 / 118.5= 0.1324µg/mL)

The LOD and LOQ of Fexofenadine was found to be 0.04372 (µg/mL) and 0.1324 (µg/mL), analytical method that concluded. The detection and quantification limits for the Montelukast and Fexofenadine were performed and calculated using S/N ratio method.

G. Robustness

Robustness is the measure of the ability of an analytical method to remain unaffected by small but deliberate variations in method parameter. The effect of change in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied. The mobile phase composition was changed in (±1 ml/min^{-1}**)** proportion and the flow rate was varied by (±1ml/min^{-1}**)**, and wavelength change (±1 ml/min^{-1}**)** of optimized chromatographic condition. (**Table No. 4 & 5)** For a specific method, the robustness can be determined by performing analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay.

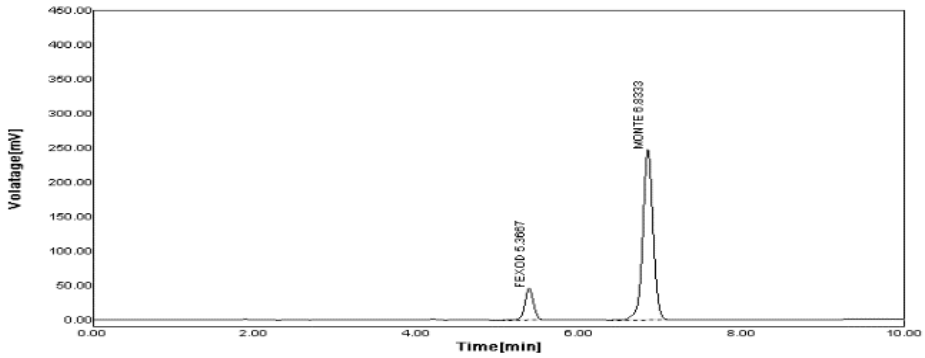

Figure No. 06: Chromatogram of standard Combination of Montelukast and Fexofenadine.

Table No. 02: Result of Intraday and Inter day Precision studies on RP-HPLC and UV method for Montelukast and Fexofenadine.

Method	Drug	Conc^{n} (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
RP-HPLC METHOD	MONTELUKAST	1	98.03 ±1.10	101.54	96.72 ±0.69	100.00
		3	332.69± 0.73	99.61	333.05 ±3.05	99.67
		5	574.45 ±6.91	100.60	572.63± 0.80	100.40
	FEXOFENADINE	12	633.43 ±7.90	101.67	624.86 ±0.89	100.42
		36	2074.74 ±9.16	100.47	2071.68± 3.62	100.33
		60	3544.08 ±4.19	101.02	3544.57 ±3.42	101.03

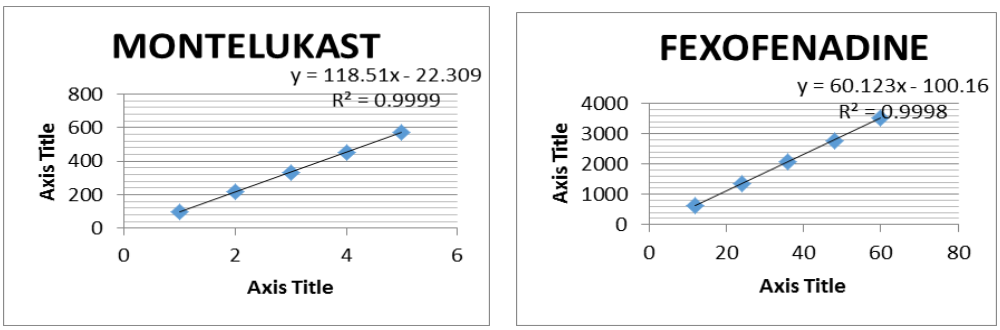

Figure No. 07 and 08: Calibration curve of Montelukast and Fexofenadine.

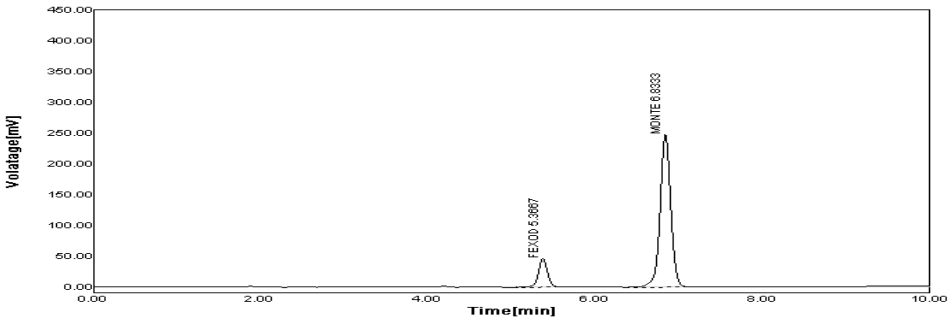

Figure 09: Representative Chromatogram of Montelukast and Fexofenadineusing Acetonitrile+ Water OPA 0.1% (70:30 % v/v) 1 ml 241 nm.

Table No. 03: Chromatogram result of Montelukast and Fexofenadine using Acetonitrile+ Water OPA 0.1% (70:30 % v/v) 1 ml 241 nm.

Sr.No.	Name	RT[min]	Area%	TP	TF	Resolution
1	Fexofenadine	5.3667	13.77	8983.6	1.0625	0.0000
2	Montelukast	6.8333	86.23	11508.0	1.0556	5.1765

Table No. 04 Result of Robustness Study of Montelukast.

Sr. No.	Parameters	Conc.(µg/ml)	Amount of detected (mean ±SD)	%RSD
1	Chromatogram of flow change 0.9ml	4+48	463.5 2.81	0.61
2	Chromatogram of flow change 1.1 ml	4+48	419.16 1.51	0.36
3	Chromatogram of comp change 69 ACN+31 WATER	4+48	449.9 3.47	0.77
4	Chromatogram of comp change 71ACN+ 29 WATER	4+48	476.01 0.60	0.13
5	Chromatogram of comp change wavelength change 240 nm	4+48	469.4 3.15	0.67
6	Chromatogram of comp change wavelength change 242 nm	4+48	466.14 1.28	0.27

Table No. 05 Result of Robustness Study of Fexofenadine

Sr. No.	Parameters	Conc.(µg/ml)	Amount of detected (mean ±SD)	%RSD
1	Chromatogram of flow change 0.9ml	4+48	2932.70 ±3.46	0.12
2	Chromatogram of flow change 1.1 ml	4+48	2611.71± 4.06	0.16
3	Chromatogram of comp change 69 ACN+31 WATER	4+48	3628.8 ±23.31	0.64
4	Chromatogram of comp change 71ACN+ 29 WATER	4+48	3562.41 ±51.13	1.44
5	Chromatogram of comp change wavelength change 240 nm	4+48	2889.4 ±5.74	0.20
6	Chromatogram of comp change wavelength change 242 nm	4+48	2793.12± 3.57	0.13

Table No. 06: Data for system suitability test for MONT and FEXO.

Sr. No.	System suitability parameter	Observed value		IP standards
		MONT	FEXO	
1	Number of theoretical plates (N)	8676.3	6269.6	Not less than 2000
2	Resolution (Rs)	5.2353	0.0000	Greater than 2.0
3	Tailing factor (Tf)	1.0500	0.9444	Not greater than 2.0

Table No. 7: Analysis of Marketed Formulation.

Tablet	Concentration of Formulation **MONT : FEXO**	Concentration found **MONT: FEXO**	%Mean Recovery **MONT: FEXO**
Montair-FX	**10:120**	09.932±0.41 : 120.1891±0.38	99.69 : 100.17

Table No. 8: Summary of validation parameters for MONT and FEXO.

Sr. No.	Parameters	Results	
		Montelukast Sod.	**Fexofenadine HCl**
1	**Linearity range**	1-5 µg/mL	12-60 µg/mL
2	**Regression equation**	y = 118.51x - 22.309	y = 60.123x - 100.16
3	**Correlation coefficient (R2)**	0.9999	0.9998
4	**%Recovery ± SD**	99.69± 0.45	99.50 ±0.71
5	**Precision (Mean ± SD)** **Interday** **Intraday**	 572.63± 0.80 574.45 ±6.91	 624.86 ±0.89 633.43 ±7.90
6	**LOD (mg/mL)**	0.2755	0.04372
7	**LOQ (mg/mL**	0.8349	0.1324
8	**Robustness (%RSD)**	0.64	0.77

RESULTS AND DISCUSSION

Aim of this study was to develop a rapid, easy accurate, precise, reliable and least time consuming HPLC method for the analysis of from the combined pharmaceutical formulation. The system suitability parameters and system precision are evaluated and found within the limits in Table No.06. A plot is drawn between concentration of the component and the instrument response; The respective linear equation for Montelukast was y = 118.51 x – 22.309 and Fexofenadine equation y = 60.123 x – 100.16 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. Precision and accuracy of the developed method are expressed in %RSD and % of recovery of the active pharmaceutical ingredient respectively. Low %RSD value and high percent of recovery indicate that the method is highly precise and accurate. All system suitability parameters were found within the standard limit as shown in Table 8.

CONCLUSIONS

Simple, rapid, accurate and precise RP-HPLC as well as spectrophotometric methods have been developed and validated for the routine analysis of Montelukast and Fexofenadine in API and tablet dosage forms. Both methods are suitable for the simultaneous determination of Montelukast and Fexofenadine in multi-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

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