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Simultaneous Determination of Montelukast Sodium and Fexofenadine Hydrochloride in Combined Dosage Form by Using RP-HPLC Method

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Abstract: A simple, accurate, economical and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of montelukast sodium and fexofenadine hydrochloride in bulk and pharmaceutical formulations. The separation was achieved on a phenomenex C_{18} column (150 × 4.6 mm i.d, particle size of 5µ) using a mixture of 0.1M potassium dihydrogen orthophosphate buffer (pH 5.0) and methanol in the ratio of 60:40 v/v as mobile phase in an isocratic elution mode, at a flow rate of 1 ml/min. The detection was monitored at 220 nm. The retention time of montelukast sodium and fexofenadine hydrochloride was found to be around 2.17 ± 0.12 min and 6.24 ± 0.14 min respectively. Excellent linearity range was found between 5-15 µg/ml for montelukast sodium and 10-100 µg/ml for fexofenadine hydrochloride. The method was validated with respect to linearity, robustness, precision and accuracy and was successfully applied for the simultaneous determination of montelukast sodium and fexofenadine hydrochloride from the combined dosage formulation.

Key words: Montelukast Sodium · Fexofenadine Hydrochloride · RP-HPLC Method · Validation

INTRODUCTION

Montelukast Sodium (MON) (Molecular Formula $C35^{H}35^{Cl}{}^{NNaO}3^{S)\ is\ a}$ leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies [1]. It is usually administered orally. Montelukast is a CysLT1 antagonist; that is it blocks the action of leukotriene D4 on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation. Fexofenadine hydrochloride (FEX) (Molecular Formula $^{C}32^{H}39^{NO}4^{HCl}$) is 4-[1-Hydroxy-4-[4-(hydroxyl diphenyl methyl)-1-piperidinyl] 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 urticaria [2]. The structure of the drug is shown in Figures 1 and 2. One such combination contains 10 mg of Montelukast Sodium and 120 mg of Fexofenadine hydrochloride.

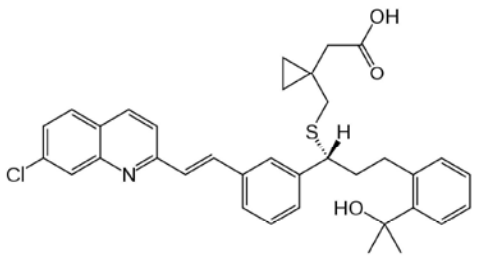

Fig. 1: Structure of montelukast sodium (MON)

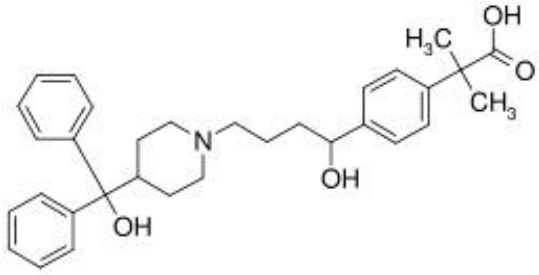

Fig. 2: Structure of fexofenadine hydrochloride (FEX)

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Several methods were reported for quantitative estimation of Montelukast Sodium such as voltametric [3], capillary electrophoresis [4], spectrophotometry [5-6] and HPLC method using various detectors [7-15]. The voltametric, capillary electrophoresis and spectrophotometry is very complicated and lengthy.

A few HPLC [16-18] and spectrophotometry [19-20] methods have been reported for the estimation of Fexofenadine hydrochloride. But there is no method for the simultaneous estimation of Montelukast sodium and Fexofenadine hydrochloride till now.

MATERIALS AND METHODS

Apparatus: A SHIMADZU (Japan) HPLC instrument (LC-20AD) equipped with a UV-Visible detector, rheodyne injector with 20 µL loop, phenomenex C18 column (150 mm x 4.6 mm i.d, 5µ particle size) and LC-Solution software were used. Other instruments included are SHIMADZU electronic balance, BL-220H (SHIMADZU corp. Japan), fast clean ultrasonic cleaner and value 1 stage vaccum pump (model: VE115).

Materials: MON and FEX pure powder were gift samples supplied from Aurabindo Pharma Ltd, India. Acetonitrile (HPLC grade) was purchased from Merck Ltd, India. Potassium di-hydrogen orthophosphate from S.D. Fine Chem. Ltd, India. Water for HPLC was prepared by triple glass distillation and filtered through a 0.45 µ membrane filter (Gelman Laboratory, India).

Pharmaceutical Formulation: Formulation, Montair FX® (120 mg FEX + 10 mg MON per capsule), manufactured by Cipla Pharmaceutical Ltd, India, was purchased from the local pharmacy in Hyderabad.

Chromatographic Conditions: Chromatographic separation was performed on ii HPLC with phenomenox C18 column (150 x 4.6 mm i.d, particle size of 5 µ) and constant flow pump. Rheodyne injector with 20 µl loop. The composition of the mobile phase was in the ratio of 0.01M potassium di-hydrogen orthophosphate buffer (pH 5.0) and acetonitrile (60:40 v/v) and was delivered at a flow rate of 1 ml /min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 5 min. Analysis was performed at ambient temperature. Chromatogram of standard drugs was shown in fig 3. Optimized chromatographic conditions are listed in Table 1.

Preparation of Mobile Phase: The selected mobile was prepared by dissolving 0.136 g of potassium di-hydrogen orthophosphate in 100 ml of tripled distilled water, mixed thoroughly and pH of the solution was at 5.0. The buffer and acetonitrile were mixed in the ratio of 60:40 v/v.

Preparation of Standard Fex and Mon Solutions: MON and FEX 10 mg were dissolved in 100 ml of mobile phase to obtain a standard stock solution of (100 µg/ml) of each drug. Further working standard solutions of FEX & MON, 10-100 µg/ml and 5-15µg/ml respectively, were prepared by suitable dilution of the stock solution with mobile phase.

Preparation of Sample Solution: For analysis of commercial formulations, 20 tablets were weighed, powdered and weight equivalent to 5 mg and 60 mg of MON and FEX respectively was taken and transferred into 100 ml volumetric flask and dissolved in 100 ml mobile phase, filtered through a Whatmann filter paper and the solution was further diluted stepwise with mobile phase to get the concentration within the linearity range.

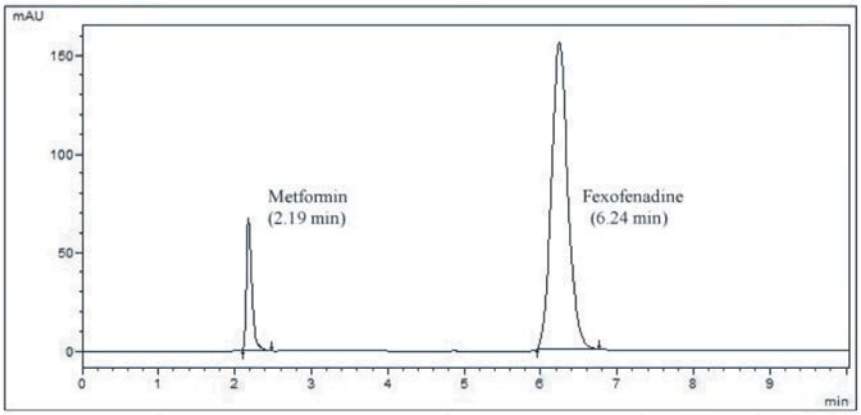

Fig. 3: Chromatogram of marketed formulation of MON and FEX

Table 1: Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (Column)	Phenomenex C18 (150 x4.6 mm i.d, 5 µ size)
Mobile phase	0.01M Potassium dihydrogen orthophosphate (pH 5.0 ± 0.05) & Acetonitrile (60:40 v/v)
Flow rate (ml/min)	1
Pressure (kgf)	194
Run time (min)	15
Column temperature(^{0}c)	Ambient
Detection wavelength (nm)	220
Drugs Retention time (R_t in min)	2.19 ± 0.12 (MON) and 6.24 ± 0.14 (FEX)

Table 2: Analysis Of Formulation

S. No.	Drug name	Amount labeled Mg	Amount estimated mg	% Label claim	% deviation
1	MON	5mg	4.93	98.68	(-)1.32
2	FEX	60mg	60.06	100.1	(+)0.1

Analysis of Formulation: The amount of drug present in the pharmaceutical formulation was calculated through peak area by making use of the standard calibration curve (Concentration in µg/ml on x-axis and peak area on Y-axis) the results were shown in table 2.

RESULTS AND DISCUSSIONS

Once the HPLC method development was over, the method was validated in terms of parameters like linearity, precision, LOD, LOQ, recovery studies etc. The proposed HPLC method was validated as per ICH guidelines [19].

The linearity measurement was evaluated by analysing different concentrations of the standard Solutions of MON and FEX. The Beer lamberts concentration was found to be between 5-15 µg/ml for MON and 10-100 µg/ml for FEX. Calibration curve was constructed by plotting peak area against concentration and regression equation was computed. The slope, intercept and correlation coefficient values were shown in table 3.The precision of the method was ascertained separately from the peak areas obtained by actual determination of three replicates of a fixed amount of drug. The intra and inter-day variation in the peak areas of the drug solution was calculated in terms of percent RSD and the results are presented in table 4.

To determine the accuracy of proposed method recovery studies (50%, 100% and 150%) were carried out by taking different amounts of bulk sample of MON and FEX within the linearity range were taken and added to the pre-analysed formulation. From that percent recovery values were calculated and results are presented in Table 5.

Table 3: Regression Analysis Of The Calibration Curves

S. No.	Parameters	FEX	MON
1	Concentration range	10-100 µg/ml	5-15 µg/ml
2	Slope	34392	64815
3	Intercept	136063	2190.4
4	Correlation coefficient (r^2)	0.998	0.998

Table 4: Precision Studies

			Intra-day conc. Measured* (µg/ml)		Inter-day conc. Measured* (µg/ml)	
S.No.	Drug	Concentration µg/ml	Mean	% RSD	Mean	%RSD
1	MON	9	8.91	0.5	8.86	0.35
		10	9.87	0.98	9.93	0.87
2	FEX	40	39.58	0.35	38.86	0.53
		50	48.81	0.87	49.54	1.23

Table 5: Recovery Studies

S. No.	Drug	% Standard drug added	% Recovery	% RSD
1	MON	50	99.41	0.38
		100	99.24	
		150	99.97	
2	FEX	50	99.70	0.74
		100	99.97	
		150	98.76	

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3).The LOD values were found to be 0.1 µg/ml and 1 µg/ml for MON and FEX respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ values were found to be 1 µg/ml and 10 µg/ml for MON and FEX respectively.

The ruggedness of the method was assessed by comparison of the intra-day and inter-day assay results for MON and FEX that has been performed by two analysts. The % RSD values for assays performed in the same laboratory by two analysts did not exceed 2, indicating the ruggedness of the method.

CONCLUSION

A convenient and rapid RP- HPLC method has been developed for estimation of MON and FEX in combined dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day % RSD coupled with excellent recoveries. Hence, this method can be conveniently adopted for routine analysis of MON and FEX in pure form and its dosage forms and can also be used for dissolution or other similar studies.

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