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SIMULTANEOUS ESTIMATION OF FEXOFENADINE AND MONTELUKAST BY RP-HPLC AND ITS VALIDATION

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ABSTRACT

A simple, accurate, economical and precise reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous determination of Fexofenadine and Montelukast. The separation was achieved on Intersil C $_{18}$ column (250 x 4.6 mm, 5 µm) as stationary phase with a mobile phase comprising of Orthophosphoric acid buffer p^{H}(6.0):Acetonitrile: Methanol (30:50:20) in an isocratic mode, at a flow rate of 1 ml/min. The detection was monitored at 244 nm. The retention time of Fexofenadine and Montelukast were 2.65 min and 3.65 min respectively. The linearity was found to be in the range of 72-168 µg/ml and 6-14 µg/ml for Fexofenadine and Montelukast respectively with correlation coefficient of 0.999. The proposed method was validated according to ICH guidelines for parameters like linearity, accuracy, precision and specificity. All validation parameters were within the acceptable range. The developed method was successfully applied for the estimation of Fexofenadine and Montelukast in pure and pharmaceutical dosage form.

Keywords: Fexofenadine, Montelukast, RP-HPLC, Validation, Simultaneous estimation, ICH guidelines.

INTRODUCTION

Fexofenadine hydrochloride [Figure 1] (Molecular Formula $C_{32}H_{39}NO_4HCl$) 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 urticaria [1].

Fexofenadine is a second-generation selectively peripheral H1-blocker of the GI tract, large blood vessels and bronchial smooth muscle. Blockage prevents the activation of the H1 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 [3,4].White to off-white crystalline powder, odourless, Freely soluble in methanol and ethanol, Slightly soluble in chloroform and water, very slightly soluble in acetone [5,6].Montelukast Sodium [Figure 2] (Molecular Formula $C_{35}H_{35}ClNNaO_3S$) is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies [2]. 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 [3,4]. White to off-white crystalline powder, odourless,Freely soluble in methanol and ethanol, practically insoluble in acetonitrile [5,6].

Literature survey of Fexofenadine and Montelukast revealed few methods based on Chromatography [7-13] have been reported for determination of both drugs in single and combined dosage forms. The present work describes the development and validation as per ICH guidelines[14] of reverse phase high performance liquid chromatographic (RP-HPLC) method, which can quantify these components simultaneously.

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MATERIALS AND METHODS

Reagents required

Acetonitrile	: HPLC grade, Merck
Water	: HPLC grade, Merck
Methanol	:HPLC grade, Merck
Ortho phosphoric acid	: AR grade, Merck

Drugs used

The gift samples of Fexofenadine and Montlukast were kindly provided by Lara Drugs Hyderabad and the marketed formulations containing Fexofinadine (120 mg) and Montelukast (10 mg) were procured from local pharmacy (trade name: Acnofex MT).

Instrumentation and Chromatographic Conditions

The developed method HPLC system with UV detector data were acquired and processed by Empower software. The separation was carried out at ambient temperature by using a Intersil C_{18} (4.6 x 250mm, 5µm) .The mobile phase consisting of Ortho Phosphoric acid buffer (p^H 6.0) :Acetonitrile: Methanol (30:50:20v/v). The flow rate was 1 ml/min. The injection volume was 0.02 µL and detection at a wavelength of 244nm.

Preparation of Mobile phase

Mix the 300 ml of ortho phosphoric acid (30%) and 500ml of Acetonitrile (50%) and 200 ml of methanol (20%). Filter through 0.45 µ filter under vacuum filtration. The mobile phase liquid is also used for making working dilution of drugs.

Preparation of stock solutions

Stock Solution of Fexofenadine And Montelukast

The stock solution of Fexofenadine and Montelukast were prepared by weighing accurately 120 mg of Fexofenadine and 10 mg of Montelukast pure drug and transferred to a 100ml volumetric flask and dissolved in the mobile phase and made upto the mark with mobile phase

Linearity of pure standard solution:

The linearity of the samples of Fexofenadine and Montelukast was prepared by suitably diluting working solution and found to be linear response of drug over a range of 72-168 µg/ml concentration. for the Fexofenaine and 6-14µg/ml for Montelukast respectively. The three such linearity's of Fexofenadine and Montelukast were taken for correlation co-efficient and standard deviation calculation.

Preparation of Sample Solution

Acnofex MT of strength 120mg of Fexofenadine and 10mg of Montelukast respectively. Average weight of twenty tablets were taken and crushed to make powder, weighed powder containing 120 mg Fexofenadine was transferred to 100ml of volumetric flask and volume was made up to the mark with diluent (Ortho phosphoric acid buffer (P^H 6.0): Acetonitrile : Methanol) (30:50:20) and filtered through whatmann filter paper in to another 100ml volumetric flask and make up to mark with same diluent which gives the solution of 1.2 mg/ml concentration of Fexofenadine and 10 µg/ml of Montelukast, filter it with milli pore filter through syringe filter and used for further analysis. The same procedure as mentioned for the pure drug was followed for the formulation. The concentrations of both Fexofenadine and Montelukast were determined by measuring peak area at 244 nm.

Assay for Marketed formulation

The Assay performed by the marketed formulation of Fexofenadine and Montelukast (Acnofex MT). The prepared standard and sample solutions were injected into HPLC and peak areas were recorded. The amount of drug present and percentage purity was calculated by comparing the peak areas of the standards with that of samples.

VALIDATION OF HPLC METHOD

The HPLC method was validated in accordance with ICH guidelines.

Precision

System Precision for Fexofenadine and Montelukast: The system precision was evaluated by measuring the peak responses of Fexofenadine and Montelukast for five replicate injections of standard solution, prepared as the proposed method. The results shown in the table-3 indicate that the precision of the system is with in the limit. (Acceptance criteria: % rsd nmt 2.0%)

Method Precision for Fexofenadine and Montelukast was determined by preparing a sample solution of single batch Fexofenadine and Montelukast Tablet five times and analyzing as per the proposed method. The results shown in Table-3 indicate that the proposed method is precise. (Acceptance criteria: % RSD NMT 2.0%

Accuracy

To check the accuracy of the developed method and to study the interference of formulation excipients, recovery study was carried out by using standard addition method by adding 100% concentration to a fixed amount of the pre analysed sample and the amount of drug were analyzed by the proposed method. Results from the recovery studies are given in table 4,5,6&7.

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

Limit of detection and limit of quantification were estimated from signal to noise ratio. LOD is the lowest concentration resulting in a peak area of three times

the baseline noise and the equation is LOD = 3.3 x ASD/S. LOQ is the lowest concentration that provide signal to noise ratio more than 10 and the equation is LOQ = 10 x ASD/S, where 'ASD' is the average standard deviation and 'S' is the slope of the line.

Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The important parameter to be studied was the resolution factor between two peaks. Robustness of the method was carried out by deliberately made small variation in the flow rate, p^H of mobile phase, organic phase ratio and column oven temperature by using 72 µg mL^{-1} of Fexofenadine and 10 µg mL^{-1} solution of Montelukast, respectively.

Linearity

The linearity of the method was determined by comparing the known concentration Vs response, a series of calibration standards 72,96,120,144, 168 **µg/ml** of Fexofenadine and 6,8,10,12,14 **µg/ml** of Montelukast were prepared. The solutions were injected into the chromatographic system and peak area of each peak at each concentration was noted. The calibration curve was plotted using peak area versus concentration of the standard solution.

Degradation Studies

Acid degradation

About 120 mg of Fexofenadine and 20 mg of Montelukast was transferred to a 100 ml volumetric flask and dissolved in minimum quantity of diluent, 5 ml of 0.1 N HCL was added and the volume made up to the mark with diluent, the solution was heated at 60-70^{o}c for 1 hour. Cool the solution at room temperature and used for analysis. The same procedure as mentioned for the pure drug was followed for the formulation.

Base Degradation

About 120 mg of Fexofenadine and 20 mg of Montelukast was transferred to a 100 ml volumetric flask and dissolved in minimum quantity of diluent, 5 ml of 0.1 N NaoH was added and the volume made up to the mark with diluent, the solution was heated at 60-70^{o}c for 1 hour. Cool the solution at room temperature and used for analysis. The same procedure as mentioned for the pure drug was followed for the formulation.

Oxidative Degradation

About 120 mg of Fexofenadine and 20 mg of Montelukast was transferred to a 100 ml volumetric flask and dissolved in minimum quantity of diluent, 5 ml of 1% H_2O_2 was added and the volume made up to the mark with diluent, the solution was heated at 60-70^{o}c for 1 hour. Cool the solution at room temperature and used for analysis. The same procedure as mentioned for the pure drug was followed for the formulation.

Thermal Degradation

About 120 mg of Fexofenadine and 20 mg of Montelukast was placed in a china dish. The dish was covered by aluminium foil and kept in hot air oven at 60-70ºC for 1 hour and transferred to 100 ml volumetric flask and dissolved in minimum quantity of diluent and the volume made up to the mark with diluent, the solution was heated at 60-70^{o}c for 1 hour. Cool the solution at room temperature and used for analysis. The same procedure as mentioned for the pure drug was followed for the formulation. Results of degradation data was given in table no 9.

Table 1. Area Of Different Concentration Of Fexofenadine And Montelukast Obeying Beer's Law

S.No.	Fexofenadine (µg/ml)	Area (mV.s)	Montelukast (µg/ml)	Area(mV.s)
1	72	1089.102	6	570.015
2	96	1486.424	8	749.278
3	120	1921.086	10	968.671
4	144	2368.151	12	1129.34
5	168	2800.307	14	1312.58

Table 2. Assay of marketed formulation

Drug	Labeled amount (mg)	Amount found mg/tab	% Recovery	%RSD (n=5)
Fexofenadine	120 mg	119.79	99.83	0.053
Montelukast	10 mg	9.94	99.41	0.068

Table 3. Precision Data

Drug	Concentration (µg/ml)	System Precision % Recovery (%RSD, n=5)	Method Precision % Recovery (%RSD, n=5)
Fexofenadine	120	98.48 (0.42)	98.75 (0.24)
Montelukast	10	98.00 (0.44)	98.20 (0.14)

Table 4. Results of Accuracy study of Fexofenadine

S.No	Conc (µg/mL)	Drug		Calculated Conc (µg/mL)	Accuracy
		Peak area	Retention time		
1	120	1878.890	2.65	119.90	99.9.
2	120	1875.86	2.68	119.80	99.80
3	120	1875.037	2.67	119.90	99.90
Mean				119.87	99.87
STDEV				0.06	0.06
%CV				0.03	0.06

Table 5. Results of Accuracy study of Fexofenadine

S.No	Conc (µg/mL)	Drug		Calculated Conc (µg/mL)	Accuracy
		Peak area	Retention time		
1	144	2245.455	2.67	143.89	99.90
2	144	2250.486	2.68	144.90	100.90
3	144	2250.486	2.67	143.89	99.90
Mean				144.23	100.23
STDEV				0058	0.58
%CV				0.40	0.58

Table 6. Results of Accuracy study of Montelukast

S.No	Conc (µg/mL)	Drug		Calculated Conc (µg/mL)	Accuracy
		Peak area	Retention time		
1	10	940.049	3.62	9.89	99.80
2	10	922.840	3.62	9.99	99.90
3	10	897.761	3.63	9.89	99.80
Mean				9.92	99.83
STDEV				0.06	0.06
%CV				0.58	0.06

Table 7. Results of Accuracy study of Montelukast

S.No	Conc (µg/mL)	Drug		Calculated Conc (µg/mL)	Accuracy
		Peak area	Retention time		
1	12	1103.070	3.62	12.09	100.09
2	12	1118.209	3.62	12.09	100.09
3	12	1118.209	3.61	12.00	100.00
Mean				12.06	100.06
STDEV				0.05	0.05
%CV				0.43	0.05

Table 8. Optical Characteristics Of The Proposed Method For Fexofenadine And Montelukast

Parameter	Fexofenadine	Montelukast
λmax (nm) selected	244	244
Beer's law limits (µg/ml)	72-168	6-14
Correlation coefficient (r)	0.997	0.999
Relative standard deviation (%)	0.0146	0.0662
% Error at 99%(0.01 level)	1.256	0.321
% Error at 95%(0.05 level)	0.844	0.217
Limit of detection (µg/ml)	6.96	0.11
Limit of Quantification (µg/ml)	21.09	0.32

Table 9. Degradation Data

Drug	Degradation	Area (mV.s)	% Recovered	% Degraded
	Acid	1847.8205	97.92	2.08

Fexofenadine	Base	1863.9975	98.37	1.63
	Oxidative	1878.730	99.56	0.44
	Thermal	1895.000	100.42	-
Montelukast	Acid	892.283	96.48	3.52
	Base	910.360	99.74	0.26
	Oxidative	889.337	99.16	0.84
	Thermal	912.744	98.69	1.31

Fig1. Chemical structure of Fexofenadine

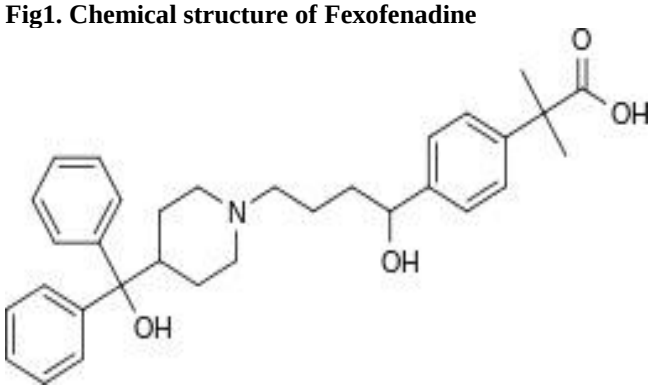


Fig 2. Chemical structure of Montelukast

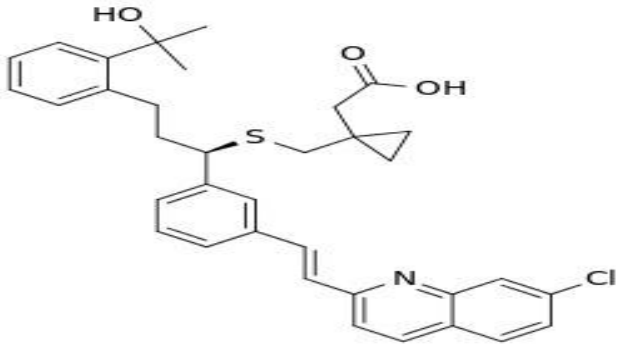


Fig 3. Graph showing Linearity of Fexofenadine

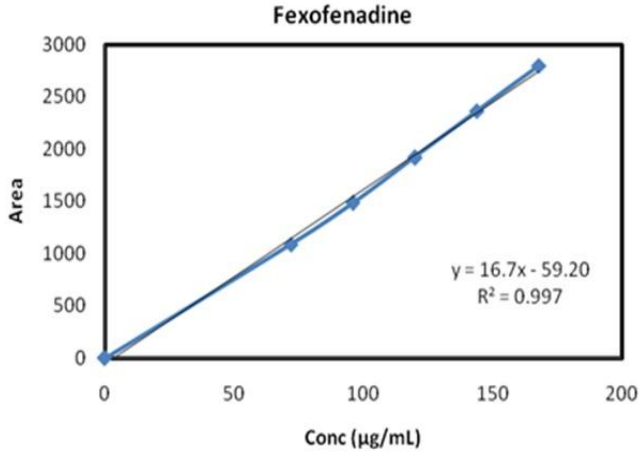


Fig 4. Graph showing Linearity of Montelukast

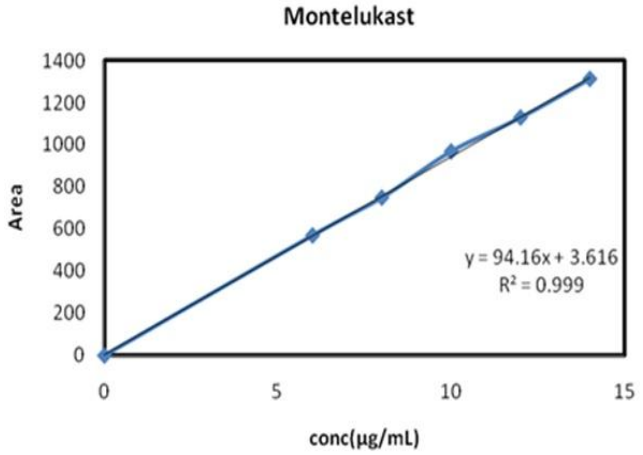


Fig 5. Chromatogram showing peaks of Fexofenadine and Montelukast

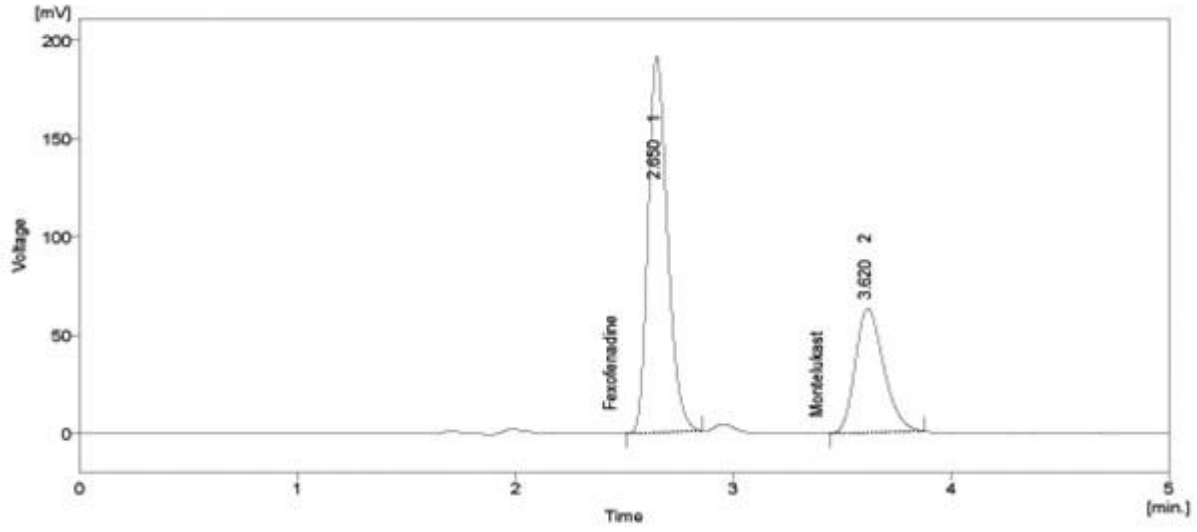
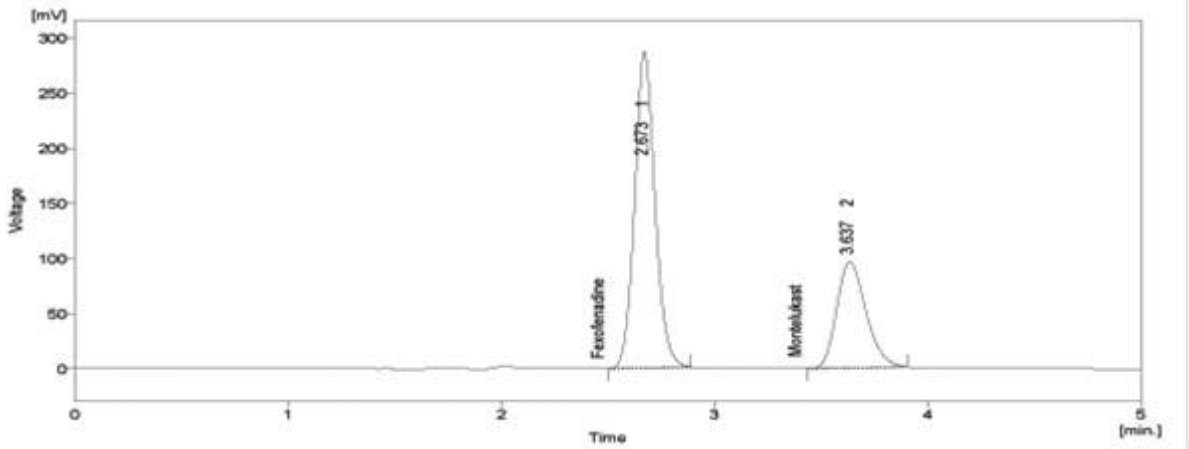


Fig 6. Chromatogram showing peaks of Fexofinadine and Montelukast of formulation



CONCLUSION

The proposed RP-HPLC method was found to be simple, accurate, precise, linear and specific for quantitative estimation of Fexofenadine and Montelukast 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 analysis of Fexofenadine and Montelukast in raw materials and in pharmaceutical formulations in the quality control laboratories.

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