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A new stability-indicating RP-HPLC method for the simultaneous determination of fexofenadine hydrochloride and montelukast in combined dosage form

K. Padmavathi and M. Subba Rao*

Department of Chemistry, Acharya Nagarjuna University, Guntur, A.P, India

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ABSTRACT

A new stability-indicating RP-HPLC method was developed for the simultaneous determination of fexofenadine hydrochloride and montelukast in combined dosage form, using a Aligent, Zorbax (Make: 150 mmx4.6 mm I.D; particle size 5µm and a mobile phase composed of phospahte buffer (pH 4.0): Acetonitile (60:40 v/v) at a flow rate of 1.0mL/min. The retention times of fexofenadine hydrochloride and montelukast were found to be 10.16 and 12.03 min, respectively. Linearity was established for fexofenadine hydrochloride and montelukast in the range of 10-30µg/ml and 5.0-15µg/ml, respectively. The percentage recoveries of fexofenadine hydrochloride and montelukast were found to be in the range of 99.80 to 99.90 and 99.50 to 99.93 respectively. Both the drugs were subjected to acid and base hydrolysis, oxidation, photolytic, and thermal degradation conditions. The degradation products of fexofenadine hydrochloride and montelukast were well resolved from the pure drug with significant differences in their retention time values. This method can be successfully employed for simultaneous quantitative analysis of montelukast and fexofenadine hydrochloride in various combined formulations.

Keywords: Fexofenadine hydrochloride, Montelukast, stability-indicating method.

INTRODUCTION

Fexofenadine hydrochloride (FEXO)[1] (**Fig.1**) (RS)-2-[4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidyl]butyl]phenyl]-2-methyl-propanoic acid is, a non-sedating selective histamine H1 receptor antagonist, 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.

Montelukast sodium (MTKT)[2] (**Fig.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.

Fexofenadine hydrochloride and montelukast combination tablets were recently introduced as tablet formulation in indian market that is used as anti-asthmatic, anti-allergic drug.

Literature survey revealed that many HPLC methods [3-12] were reported for determination of fexofenadine hydrochloride and montelukast in combined dosage forms that suffer from drawbacks of long run times and good sensitivity. The present research paper describes the development and validation of stability indicating reverse phase high performance liquid chromatographic (RP-HPLC) method, for the assay of these components simultaneously with has less run time and with extremely low LOD & LOQ values.

MATERIALS AND METHODS

INSTRUMENTATION: The analysis of the drug was carried out on a waters LC system equipped with 2695pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Aligent,Zorbax(150 mmx4.6 mm I.D; particle size 5 µm)) was used. The output of signal was monitored and integrated using waters Empower 2 software.

CHEMICALS AND REAGENTS: Pure standard samples of Fexofenadine HCL and Montelukast Sodium were obtained as gifted samples from Cipla Ltd. and its marketed formulations in the brand name of Histakind-M [Label claim containing Montelukast 10mg and Fexofenadine 120 mg] were procured from local pharmacy (MEDPLUS). Milli-Q water, methanol (HPLC Grade), orthophosphoric acid (GR Grade), Potassium dihydrogen phosphate monohydrate (GR Grade) was obtained from Qualigens Ltd., Mumbai. Sodium hydroxide, Hydrochloric acid, and Hydrogen peroxide used were of Analytical grade and purchased from Merck Specialties Pvt. Ltd., Mumbai. All dilutions were performed in standard class-A, volumetric glassware.

BUFFER PREPARATION: Dissolve 2.72g of Potassium dihydrogen Phosphate in 1000mL of Milli-Q Water, adjust pH to 4.0 with dilute ortho phosphoric acid and Filter the solution through 0.45µm membrane filter.

MOBILE PHASE PREPARATION: Prepare a filtered and degassed mixture of phosphate buffer(pH-4.0) and acetonitrile in the ratio of 60:40 %v/v respectively.

DILUENT PREPARATION: Mobile phase is used as diluent.

STANDARD PREPARATION: About 100mg of fexofenadine hydrochloride and montelukast were accurately weighed and taken separately in 100ml volumetric flasks separately and dissolved in the mobile phase. Solutions were sonicated for 5mins. The volume was adjusted to the mark with diluent to obtain stock solution of concentration 1.0mg/ml of fexofenadine hydrochloride and montelukast separately. Calibration standards were prepared using the stock solutions [10-30µg/ml of fexofenadine hydrochloride and 2.0- 10µg/ml of montelukast].

SAMPLE PREPARATION: Twenty tablets of Histakind-M [Label claim containing Montelukast10mg and Fexofenadine 120 mg] were weighed and finely powdered in a pestle and mortar. Tablets powder equivalent to 100mg of fexofenadine hydrochloride and montelukast was transferred to 100ml volumetric flask and dissolved in about 50ml of mobile phase. The solutions were sonicated for 15min., diluted to the mark with mobile phase and then filtered through 0.45µm membrane filters (Millipore, USA). Aliquots of the sample solution were transferred to 50 ml volumetric flasks and diluted with diluent to obtain 10-30 µg/ml of fexofenadine hydrochloride and 2.0-10µg/ml of montelukast respectively.

CHROMATOGRAPHIC CONDITIONS: Aligent, Zorbax (Make: 150 mmx4.6 mm I.D; particle size 5µm) Column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 1.0mL/min. The sample injection volume was 10µL. The photodiode array detector was set to a wavelength of 225nm for the detection and Chromatographic runtime was 6 minutes.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT: In the initial trials the following mobile phases were used: phosphate buffer (pH 4.0) and Acetonitrile (40:60, v/v) (mobile phase 1) and phosphate buffer (pH 4.0) and Acetonitrile (50:50 v/v) (mobile phase 2) as the mobile phases. Mobile phase 1 has been rejected due to a lack of fexofenadine hydrochloride and montelukast signal on chromatogram.

When the sample of fexofenadine hydrochloride and montelukast was analyzed using mobile phase 2, peak shape was not good and retention time was ~10 min, therefore organic modifier concentration was changed but no improvement was observed. Subsequent attempts were made by lowering the pH of the mobile phase with various buffers including phosphate buffer but the peak shape was disturbed and therefore finally phospahte buffer (pH 4.0) was chosen and marked improvement was observed. Eventually, a mobile phase composed of phospahte buffer (pH 4.0): Acetonitile (60:40 v/v) gave the best results. During the course of these studies the injection volume and the mobile phase flow rate was constant (10µL and 1.0mL.min^{-1} respectively). The analytical wavelength was 343nm.
A typical chromatogram for simultaneous estimation of fexofenadine hydrochloride and montelukast obtained by using the aforementioned mobile phase from 10µL injection volume of the assay preparation is illustrated in **Fig.3.**

DEGRADATION STUDIES: Various stressed degradation studies were conducted for the study of stability indicating method in tablets containing fexofenadine hydrochloride and montelukast. The degradation samples were prepared by transferring powdered tablets, equivalent to 50mg of fexofenadine hydrochloride and 20mg of montelukast into a 100ml round bottom flask.and diluted with the diluent, Then prepared samples were employed for acidic, alkaline and oxidant media and thermal stress conditions.

After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature and diluted with mobile phase.

ACIDIC CONDITION: In acidic degradation the drug was heated under reflux with 1 N HCl at 80° C for 1 h and the mixture was neutralized with 1 N NaOH solutions.

ALKALINE CONDITION: In alkaline degradation the drug was treated with 0.1 N NaOH at room temperature for 100 min and the mixture was neutralized with 0.1 N HCl solutions.

OXIDATIVE CONDITION: Oxidation degradation study was performed by taking the drug content in 3% v/v H_2O_2 at room temperature for 1 hour.

THERMAL CONDITION: Thermal degradation was performed by exposing solid drug at 90˚ C for 48 hour. Resultant chromatogram of thermal degradation study indicated that fexofenadine hydrochloride and montelukast is found to be stable under thermal degradation condition.

In all degradations the decreased area in the peaks of the drugs were compared with peak area of the same concentration of the nondegraded drug and the percent degradation was calculated. Summary of the degradation studies of fexofenadine hydrochloride and montelukast was shown in **Table-1**. The degradation studies indicated that fexofenadine hydrochloride and montelukast was stable to acid, base, oxidation and photo degradation making the proposed method stability indicating.

METHOD VALIDATION: The developed RP-HPLC method is validated in accordance with ICH guidelines using the following parameters.

a.SYSTEM SUITABILITY: System performance parameters of the developed HPLC method were determined by analyzing standard working solutions. The chromatographic parameters, such as number of theoretical plates (N), resolution (Rs), capacity factor (k) and selectivity factor (α) were determined. The results are shown in **Table-2**, indicating the good performance of the system.

b.SPECIFICITY:

BLANK AND PLACEBO INTERFERENCE: The interference of blank and placebo with the elution of the present cited drugs solutions of diluent and placebo were injected into the chromatographic system with the mentioned chromatographic conditions and their respective chromatograms were recorded. From the reported chromatograms it was observed that the placebo and blank showed no peaks at the retention time of fexofenadine hydrochloride and montelukast peak indicating that the diluent and placebo solutions used in standard and sample preparations did not interfered in estimation of fexofenadine hydrochloride and montelukast in formulations respectively.

c.LINEARITY & DETECTOR RESPONSE: The linearity of the proposed method was accessed by calculating slope, intercept and correlation coefficient [r2] of standard curve. The results revealed that there was an excellent correlation between the peak area and analytes concentration. The slope and intercept of the calibration plot of fexofenadine hydrochloride and montelukast were 422925.32x-17499 and 1302954x - 5504 respectively. The linearity curves of fexofenadine hydrochloride and montelukast were depicted in **Fig.4.a & 4.b** and the linearity results of both the drugs were given **Table-3**.

The LOD values for fexofenadine hydrochloride and montelukast are 0.003 and 0.012µg/mL respectively. The LOQ values for fexofenadine hydrochloride and montelukast for method precision are 0001 and 0.006µg/mL respectively and the results were given in **Table-3** respectively.

d.PRECISION: The precision of fexofenadine hydrochloride and montelukast by proposed method was ascertained by replicate analysis of homogeneous samples of capsule powder. Intermediate precision of the method was studied by intra- and inter-day variation of the method was carried out. The results were given in **Table-4** and the low %

RSD values of within a day for fexofenadine hydrochloride and montelukast revealed that the proposed method is highly precise.

e.ACCURACY: The accuracy of the proposed method for fexofenadine hydrochloride and montelukast was assessed by recovery studies at three different levels i.e. 50%, 100%, 150%. The recovery studies were carried out in triplicate by adding known amount of standard solution of fexofenadine hydrochloride and montelukast to preanalysed tablet solutions. The resulting solutions were then reanalysed by proposed method and the results are represented in **Table- 5**. The percentage recoveries were found in the range of 99.80 to 99.90 for fexofenadine hydrochloride and 99.50 to 99.93 for montelukast respectively revealing that the developed RP-HPLC method was found to be accurate.

f.ROBUSTNESS: The robustness of the developed method was evaluated by altering few experimental conditions and evaluating the resolution between two adjacent peaks of fexofenadine hydrochloride and montelukast. The altered experimental conditions carried out in this study are given below.

Change in Flow Rate: The flow rate of the mobile phase was 1.0 ml/min. To study the effect of the flow rate on the resolution, the flow rate was changed by 0.2 units (0.8 and 1.2 ml/min).

Change in temperature: The effect of the column temperature on the resolution of fexofenadine hydrochloride and montelukast was studied at 32°C and 38°C instead of 35°C.

In all the above said varied chromatographic conditions (flow rate and column temperature) insignificant differences in peak areas and in retention time were observed for fexofenadine hydrochloride and montelukast illustrating the robustness of the developed method (**Table- 6**.)

g.SOLUTION STABILITY AND MOBILE PHASE STABILITY: No significant change in the amounts of fexofenadine hydrochloride and montelukast was observed during solution stability experiments performed using the proposed method. The %RSD of assay of fexofenadine hydrochloride and montelukast during solution stability experiments was less than 2.0 confirming that sample solutions and mobile phase used in the present study was stable at room temperature for more than 24hrs for both the drugs respectively.

h.APPLICATION OF THE METHOD: The analysis of commercial formulation [Histakind-M] indicated that the developed RP-HPLC method is specific and selective for their determination in formulation (**Table-8**).

TABLE-1: Results of degradation studies of fexofenadine hydrochloride and montelukast

DEGRADATION PARAMETERS	%FEXOFENADINE HYDROCHLORIDE	% MONTELUKAST
ACID	93.4 ± 0.7	97.5 ± 1.1
BASE	98.5 ± 1.4	93.3 ± 1.8
PEROXIDE	97.5 ± 1.4	94.7 ± 1.6
PHOTOLYTIC	97.6 ± 1.9	98.6 ± 2.3

TABLE-2: System suitability parameters of fexofenadine hydrochloride and montelukast

NAME OF THE COMPOUND	RETENTION TIME	THEORETICAL PLATES	ASYMMETRY
FEXOFENADINE HYDROCHLORIDE	3.745	20616	1.241
MONTELUKAST	4.484	5152	1.283

TABLE-3: Results of linearity studies of fexofenadine hydrochloride and montelukast

FEXOFENADINE HYDROCHLORIDE			MONTELUKAST		
% LEVEL (APPROX.)	CONC. µg/mL	AREA	% LEVEL (APPROX.)	CONC. µg/mL	AREA
50	10	4216487	50	5	6505942
75	15	6329135	75	7.5	9759905
100	20	8437190	100	10	13036373
125	25	10536067	125	12.5	16290280
150	30	12686154	150	15	19527679
Slope		422925.34	Slope		1302954
RSQ(r2)		0.9999	RSQ(r2)		1.000
LOD (µg/mL)		0.003	LOD (µg/mL)		0.001
LOQ (µg/mL)		0.012	LOQ (µg/mL)		0.006

TABLE-4: Results of method precision (inter and intraday) studies for fexofenadine hydrochloride and montelukast

SET	FEXOFENADINE HYDROCHLORIDE		MONTELUKAST	
	Intraday(n=6)	Interday(n=6)	Intraday(n=6)	Interday(n=6)
1	100.17	100.7	100.5	99.79
2	99.85	100.2	98.89	99.92
3	99.97	99.98	99.97	99.94
4	99.95	99.79	99.94	100.08
5	99.90	99.98	100.12	99.99
6	100.05	99.89	99.95	99.94
Mean	99.98	100.09	99.89	99.94
SD	0.114	0.328	0.536	0.094
%RSD	0.114	0.327	0.536	0.094

**Average of six determinations*

TABLE-5: Results of accuracy studies for fexofenadine hydrochloride and montelukast

DRUGS	%LEVEL	THEORETICAL CONCENTRATION (mcg/mL)	OBSERVED CONCENTRATION*	%RECOVERY
FEXOFENADINE HYDROCHLORIDE	50%	10.0	9.98	99.80
	100%	20.0	19.96	99.80
	150%	30.0	29.97	99.90
MONTELUKAST	50%	5.0	4.98	99.60
	100%	10.0	9.95	99.50
	150%	15.0	14.99	99.93

**Average of three determinations*

TABLE-6: Robustness studies of fexofenadine hydrochloride and montelukast

ROBUST CONDITIONS		FEXOFENADINE HYDROCHLORIDE	MONTELUKAST
		PEAK AREA	PEAK AREA
FLOW RATE	0.8mL/min.	4.979	5.938
	1.0mL/min.*	**3.745**	**4.484**
	1.2mL/min.	2.995	3.590
TEMP	32°C	3.748	4.472
	35°C*	**3.745**	**4.484**
	38°C	3.705	4.398

**Optimized conditions mentioned in the developed method*

TABLE-7: Results of ruggedness studies of fexofenadine hydrochloride and montelukast

FEXOFENADINE HYDROCHLORIDE			MONTELUKAST	
S.No.	**RT**	**Area**	**RT**	**Area**
1	3.742	8405681	4.489	12965300
2	3.743	8460947	4.481	13062276
3	3.728	8418436	4.456	13013704
4	3.727	8404422	4.461	12958892
5	3.746	8465940	4.487	13021429
***Average**	3.737	8431085	4.47	13004320
%RSD	**0.240**	0.356	**0.341**	**0.329**

**Average of five determinations*

TABLE-8: Assay of fexofenadine hydrochloride and montelukast in formulations

Drug name [Histakind-M]	**Quantity label claim(mg)**	***Quantity found ± SD**	**% Assay ± SD**
FEXOFENADINE HYDROCHLORIDE	120mg	119.97 ± 0.87	99.97± 0.79
MONTELUKAST	10mg	9.99 ± 0.69	99.99 ± 0.783

**Average of three determinations*

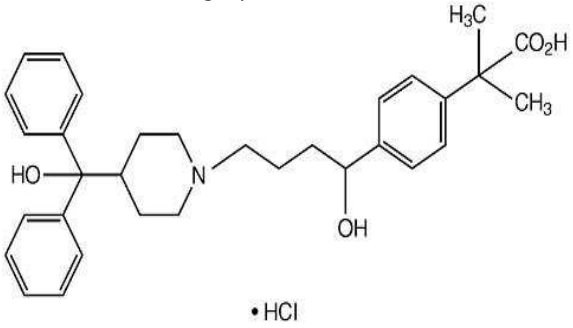

Fig-1.MOLECULR STRUCTUTRE OF FEXOFENADINE HYDROCHLORIDE

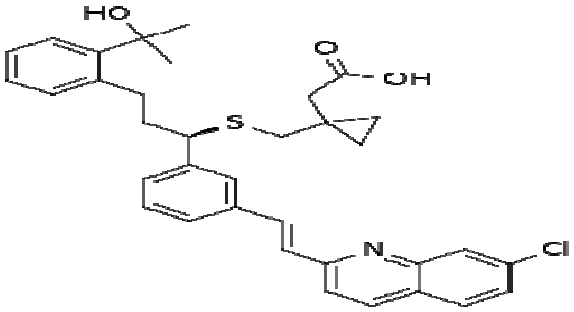

Fig-2.MOLECULR STRUCTUTRE MONTELUKAST

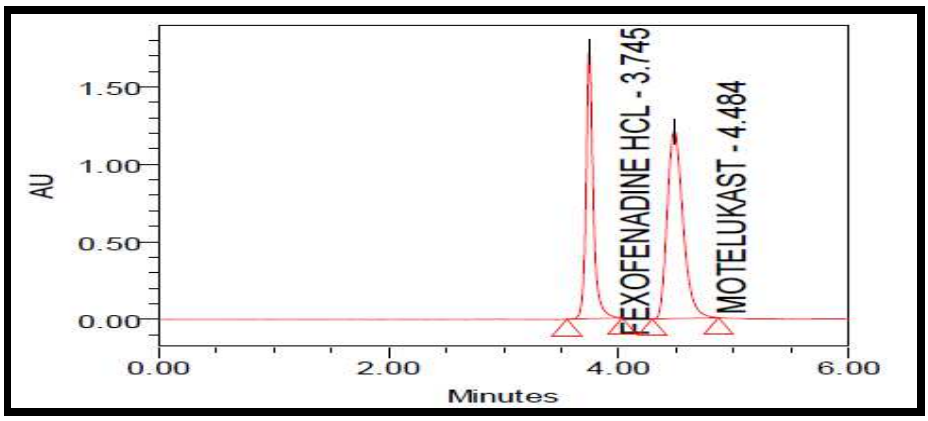

Fig.3.VALIDTED CHROMATOGRAM OF FEXOFENADINE HYDROCHLORIDE AND MONTELUKAST

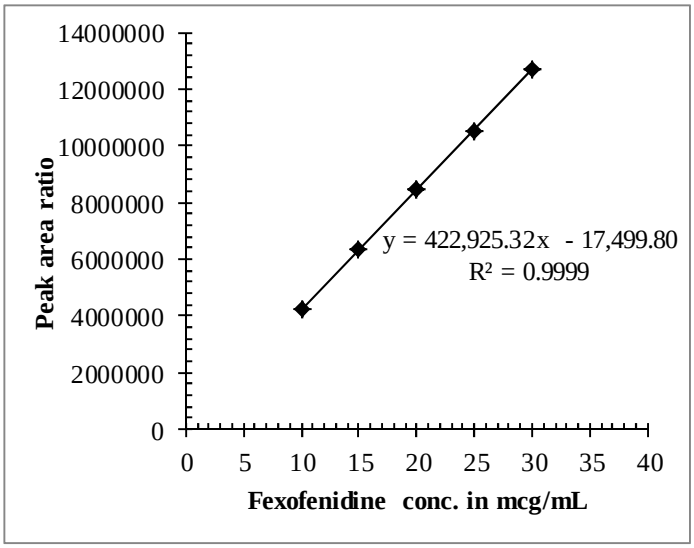

Fig.4.a: LINEARITY SPECTRAOF FEXOFENADINE HYDROCHLORIDE

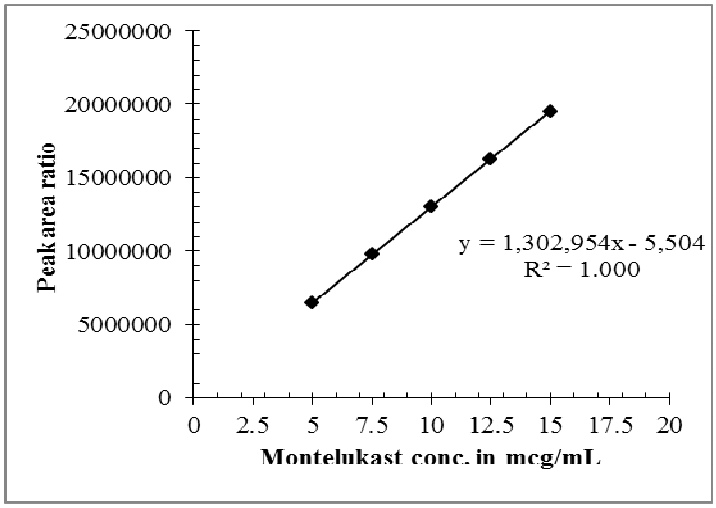

Fig.4.a: LINEARITY SPECTRAOF MONTELUKAST

CONCLUSION

An RP-HPLC method for simultaneous estimation of fexofenadine hydrochloride and montelukast and was developed and validated as per ICH guidelines. The developed method offered several advantages in terms of simplicity in mobile phase, isocratic mode of elution and sample preparation steps and comparative short run time makes the method specific, repeatable and reliable for its intended use in simultaneous determination of fexofenadine hydrochloride and montelukast in combined dosage forms. From the above citations it is concluded that this proposed method can be used for the routine analysis of formulations containing any one of the above drugs or their combinations without any alteration in the assay.

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