Nidhi NK. et al. / Vol 4 / Issue 7 / 2014 / 393-399.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF RUPATADINE FUMARATE AND MONTELUKAST SODIUM BY RP-HPLC

Nidhi NK*, Neelam I, Ajitha A, Uma Maheswara RaoV

Department of Pharmaceutical Analysis & Quality Assurance,
CMR College of Pharmacy, JNTU (H) University, Hyderabad, Andhra Pradesh, India.

ABSTRACT

A sensitive, selective and precise high performance liquid chromatographic method has been developed and validated for the simultaneous determination of Rupatidine fumarate and Montelukast sodium in tablet dosage form. The method employed like C18 column, Symmetry C18 (4.6 x 250mm, 5µm, Make: Waters) as the stationary phase while Phosphate buffer (pH 3.6), Methanol, Acetonitrile in proportion 30:65:5 v/v respectively. was used as mobile phase. The Retention time of Rupatadine fumarate and Montelukast sodium were observed to be 2.395 and 3.339 minutes, respectively. The flow rate was found to be 1ml/min and effluents were monitored at 230 nm. The linear regression analysis data for the calibration plots showed a good linear relationship for both Rupatadine fumarate and Montelukast sodium and over a concentration range of 10-50 µg/ml. with correlation co-efficient of 0.9989 for Rupatadine fumarate and 0.9999 for Montelukast sodium The LOQ was found to be 4.52 and 3.67µg/ml respectively for Rupatadine fumarate and Montelukast sodium. The method was validated as per ICH guideline and it was found to be accurate, precise and robust. Marketed formulation was analyzed successfully.

Keywords: Rupatadine fumarate, Montelukast sodium, HPLC, Validation etc.

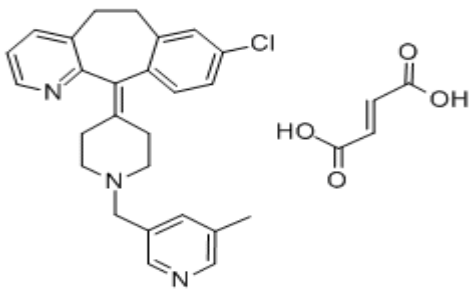
INTRODUCTION

Rupatadine is a non-sedating H_1-antihistamine (second generation) and platelet-activating factor inhibitor. It is potent and orally active that was developed as a therapeutic agent for the treatment of seasonal allergic rhinitis and chronic idiopathic urticarial [1].Montelukast is a specific cysteinyl leukotriene receptor antagonist belonging to a styryl quinolines series. It is developed as a therapeutic agent for the treatment of bronchial asthma [2-4].Fixed dose combination therapy of Rupatadine and Montelukast is indicated for the treatment of asthma, allergic rhinitis, and urticaria. Recent studies reveal that the treatment of` asthma with concomitant administration of antileukotriene (Montelukast) and an antihistamine(Rupatadine), shows significantly better symptom relief when compared with each of the treatments alone. and also to establish a simple, sensitive, precise, accurate, less time consuming and cost effective, RP-HPLC method for estimation of Montelukast and Rupatadine fumarate in bulk drug and dosage form [5-9].

Drug Profile

Rupatadine fumarate

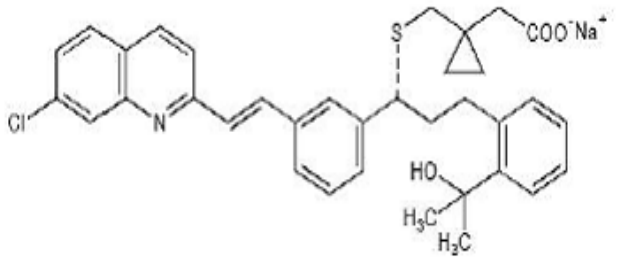
Chemical structure:



Corresponding Author:-Nidhi NK Email:- nidhichinnu.nk@gmail.com

Chemical name: 8-chloro-11-[1-[(5-methyl-3-pyridinyl)methyl]piperidin-4-ylidene]- 6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b] pyridine fumarate.
Molecular formulae: $C_{30}H_{30}ClN_3O_4$
Molecular Weight : 532.03
Category : Antihistamines

Montelukast Sodium
Structure



Chemical name: [R-(E)]-1-[[[1-[3-[2-(7-chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]cyclopropaneacetic acid, monosodium salt.
Molecular formulae: $C_{35}H_{35}ClNNaO_3S$
Molecular Weight : 608.18 g/mol
Category : It is a selective and orally active leukotriene receptor antagonist (LTRA) that inhibits the cysteinyl leukotriene CysLT1 receptor.

MATERIALS AND METHODS

Instrumentation

The separation was carried out on HPLC system with WATERS, software: Empower 2, 2695 separation module. 996 PDA detectors with binary HPLC pump and C18 column, Symmetry C18 (4.6 x 250mm, 5µm, Make: X-terra)

Chemicals

Rupanex M (10mg Montelukast sodium and 10mg Rupatadine fumarate) manufactured by Dr. Reddy's Laboratories Ltd. All chemicals and reagents used were of AR grade. Standard sample was taken from Sura Pharma training lab.

HPLC Conditions

The mobile phase consisting of Methanol, Phosphate buffer and acetonitrile (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 65:30:5 v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was 30°C. The detection was monitored at 230 nm and the run time was 6 min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 30 min with the mobile phase flowing through the system [10-13].

Preparation of standard solution

Accurately weigh 10 mg of Montelukast sodium and 10mg of Rupatadine fumarate into a 10ml of volumetric flask and dissolve the sample using diluent and sonicate it for 15min then finally make up the volume to 10 ml. Now pipette out 0.3ml of this solution into 10 ml of volumetric flask and make up the volume upto mark using same diluent.

Preparation of sample solution

Accurately weighed 10 tablets and calculated average weight of those tablets and crushed. Transfer the tablet powder weigh about 10mg of sample into 10ml of volumetric flask added with diluent and sonicated for 30 mins and make up the volume with diluent and filtered through the0.45µm millipore filter paper Transfer above solution 0.3ml into 10ml volumetric flask and make up the volume with diluent.

Method Validation

System Suitability Studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

Specificity

Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both Montelukast sodium and Rupatadine fumarate from impurities.

Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained in added recoveries of standard drugs were found to be accurate as shown in table 2(a) & 2(b).

Precision

Method Precision was determined by injecting six replicates of drug sample solution. The retention times and peak areas of six replicates are recorded. The precision is expressed as the % RSD of Peak areas and it should not be more than 2% shown in table 3.

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of Rupatadine fumarate and Montelukast sodium different concentration level (10ppm, 20ppm, 30ppm, 40ppm, 50ppm) were used for this purpose. Each measurement was carried out in six replicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentration of Rupatadine fumarate and Montelukast sodium to obtain the calibration curves. The five concentrations of the standard were subjected to regression analysis to calculate equation and correlation coefficients as shown in Fig4 (a) and (b).

Limit of detection and limit of quantitation

Limit of detection and limit of quantitation represent the concentration of analyte that would yield signal to noise ratio of 3 for LOD and 10 for LOQ respectively. To determine LOQ and LOD serial dilutions of mixed standard solution of Rupatadine fumarate and Montelukast sodium was made from standard solution. The samples were injected in the system and measured signal from the samples was compared with those of blank samples. LOD and LOQ was calculated from linear curve using formulae

LOD= 3.3 * σ / slope, LOQ= 10 * σ / slope

(Where σ = the standard deviation of the response and S = Slope of calibration curve) shown in table 5,6.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed, are rugged and robust shown in table 7(a) and 7(b).

RESULTS AND DISCUSSION

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig-1 are Rupatadine fumarate and Montelukast sodium standard chromatogram and other one is formulation, they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method accuracy passed for both Rupatadine fumarate and Montelukast sodium evaluated by recovery studies and the percentage mean recovery was found to be 100.47 and 100.31 for Rupatadine fumarate and Montelukast sodium respectively. The method precision was passed for both the drugs given in table 3 and 4. Linearity calibration curve was given below fig: 4the regression coefficient of Rupatidine fumarate is 0.9989 and Montelukast sodium is 0.9999. The LOD values of Rupatidine fumarate and Montelukast sodium are 1.46 and 1.22 respectively and LOQ values of Rupatidine fumarate and Montelukast sodium are 4.52 and 3.67 respectively.

Table 1. System Suitability parameters

S. No	Parameter	Rupatadine fumarate	Montelukast sodium
1	Retention time	1.891	2.851
2	Theoretical plates	4576	3552
3	Tailing factor	1.30	1.54
4	Resolution	-	6.197
5	Regression factor	0.9989	0.9999

Table 2(a). Accuracy observation of Rupatadine fumarate

Spike Level	Sample Weight	Sample Area	µg/ml added	µg/ml Found	% Recovery	% Mean
50%	5	1948862	1.5	1.52	101.33	
50%	5	1941133	1.5	1.49	99.33	
50%	5	1949927	1.5	1.51	100.66	100.44
100%	10	3887775	3.0	2.98	99.3	
100%	10	3888059	3.0	3.07	102.33	100.76
100%	10	3887192	3.0	3.02	100.66	
150%	15	5822928	4.5	4.49	99.77	
150%	15	5825696	4.5	4.53	100.66	100.21
150%	15	5827322	4.5	4.51	100.22	

Table 2(b). Accuracy observation of Montelukast sodium

Spike Level	Sample Weight	Sample Area	µg/ml added	µg/ml Found	% Recovery	% Mean
50%	5	1948862	1.5	1.49	99.33	
50%	5	1941133	1.5	1.53	102	
50%	5	1949927	1.5	1.51	100.66	100.66
100%	10	3887775	3.0	3.1	100.66	
100%	10	3888059	3.0	3.02	100.66	100.21
100%	10	3887192	3.0	2.98	99.33	
150%	15	5822928	4.5	4.51	100.22	
150%	15	5825696	4.5	4.48	99.55	100.07
150%	15	5827322	4.5	4.52	100.44	

Table 3. Results of precision for Rupatadine fumarate

S. No	Retention Time	Peak area	USP Resolution	USP Tailing
1	2.264	1010585	1.0	3802
2	2.246	1011075	1.1	3546
3	2.264	1011924	1.4	4633
4	2.246	1014299	1.1	4812
5	2.280	1022159	1.0	3802
Mean		1014008.4		
Std.dev		477460.5		
%RSD		0.5		

Table 4. Results of precision for Montelukast sodium

S. No	Retention Time	Peak area	USP Resolution	USP Tailing
1	3.132	1496209	1.2	4759
2	3.132	1507963	1.1	3695
3	3.129	1521163	1.1	4741
4	3.113	1522810	1.2	3793
5	3.113	1528916	1.1	4741
Mean		1515412.0		
Std.Dev.		13175.7		
%RSD		0.9		

Table 5. LOD results of the method

Drug	Amount (µg/mL)
Rupatadine fumarate	1.46
Montelukast sodium	1.22

Table 6. LOQ results of the method

Drug	Amount (µg/mL)
Rupatadine fumarate	4.52
Montelukast sodium	3.67

Table 7(a). Flow Rate observation of Rupatadine

Flow Rate (ml/min)		System Suitability Results		
		USP Plate Count	USP Tailing	Area
Low	0.9	4479	0.9	1104154
Actual*	1.0	4759	1.1	1245977
High	1.1	3072	1.1	1408920

Table 7(b). Flow Rate observation of Montelukast sodium

Flow Rate(ml/min)		System Suitability Results		
		USP Plate Count	USP Tailing	Area
Low	0.8	4508	0.9	2104921
Actual*	1.0	3695	0.9	1517199
High	1.2	3072	1.0	1408920

Table 8(a). Variation of Mobile phase composition of Rupatadine fumarate

Change in M.P organic composition	System Suitability Results		
	USP Plate Count	USP Tailing	Area
5%more	2028	0.9	1012763
Actual*	4759	0.9	1245977
5%less	3002	1.0	912635

Table 8(b). Variation of Mobile phase composition of Montelukast sodium

Change in M.P organic composition	System Suitability Results		
	USP Plate Count	USP Tailing	Area
5%more	3035	1.0	1501336
Actual*	3695	0.9	1517199
5%less	3002	1.0	1415632

Fig 1. Chromatogram of standard preparation

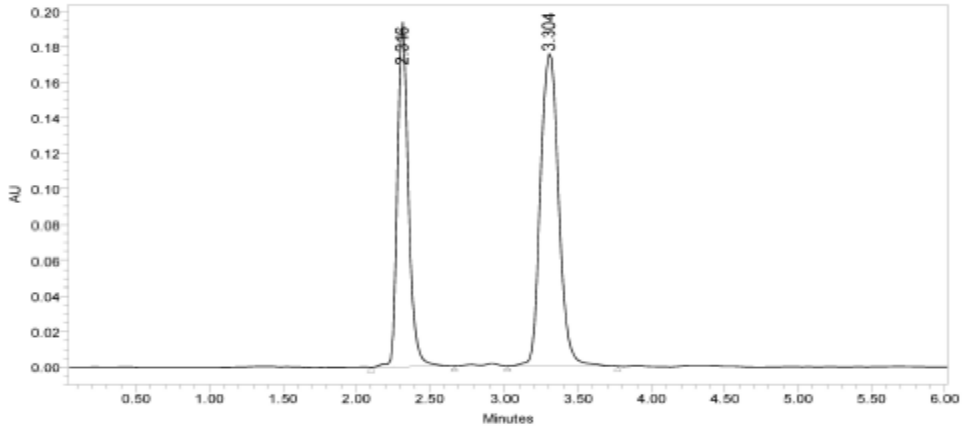


Fig 2. Chromatogram of sample preparation

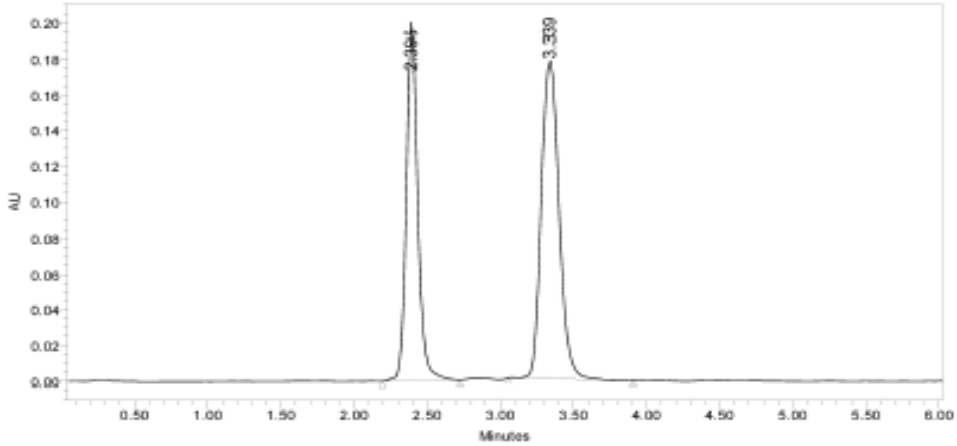


Fig 3. Blank chromatogram

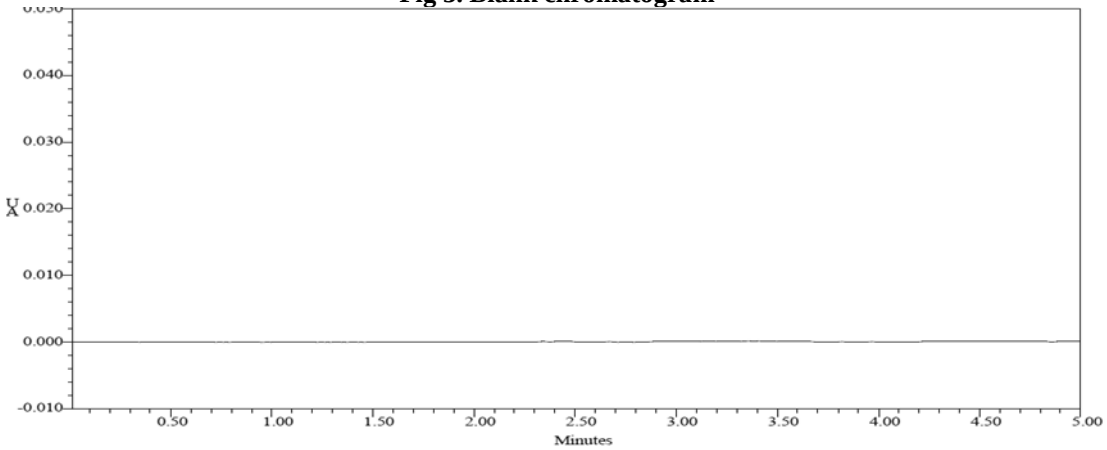


Table 4(a). Linearity curve of Rupatadine fumarate

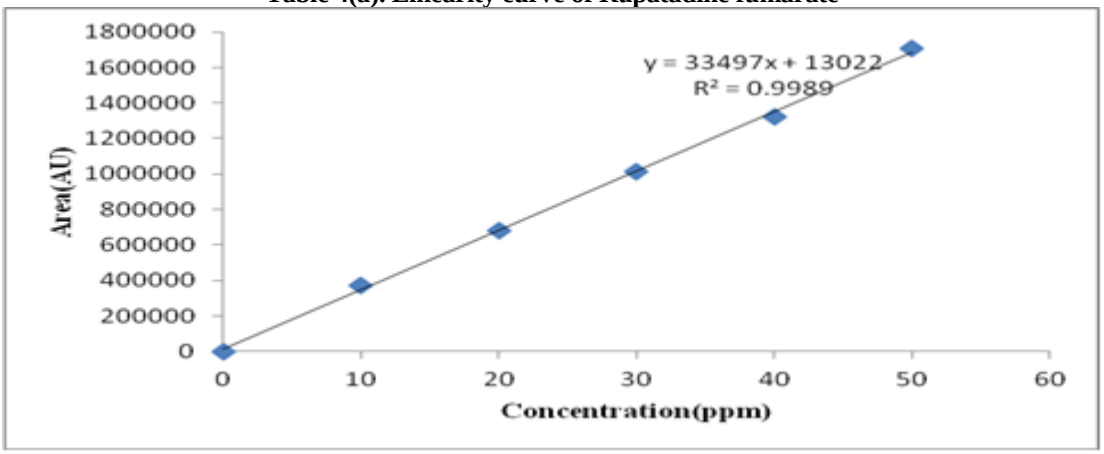
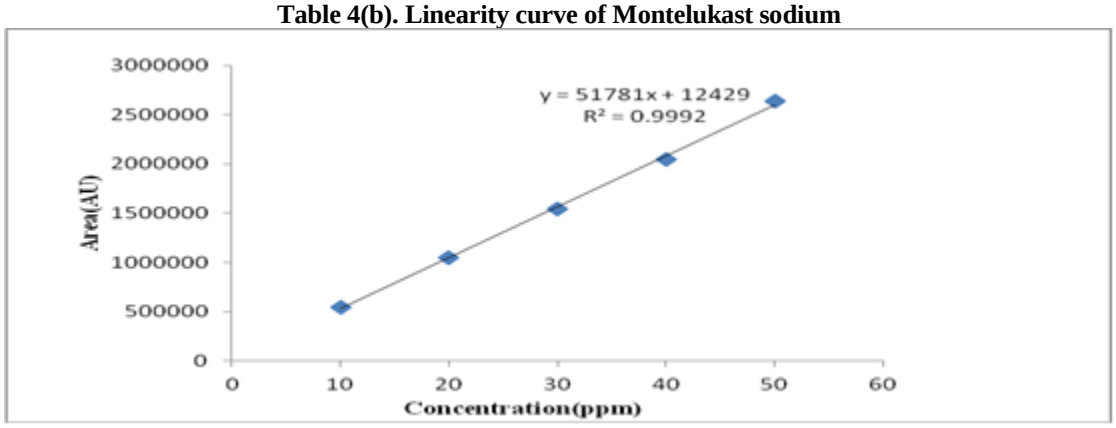


Table 4(b). Linearity curve of Montelukast sodium



CONCLUSION

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of Rupatadine fumarate and Montelukast sodium using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific. Hence, this method can easily and conveniently adopt for routine quality control analysis of Rupatadine fumarate and Montelukast sodium in its pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The authors are thankful to Sura Pharma Training Lab, Hyderabad.

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