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Research Article

DEVELOPMENT AND SIMULTANEOUS VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE ASSAY OF MONTELUKAST AND RUPATADINE IN SOLID DOSAGE FORM

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ABSTRACT

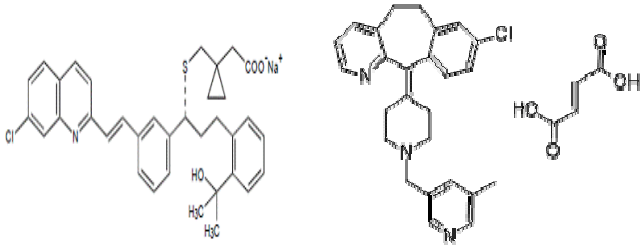
This paper emphasizes on a new simple, accurate, rapid and precise Isocratic Reverse Phase HPLC method for Simultaneous Estimation and Validation of Montelukast sodium and Rupatadine fumarate in Tablet formulation.The Method was performed on Shimadzu-10AT HPLC system using YMC Pack pro-C_4 butyl column-3u particle size(150x4.6mm id) and flow rate of 1ml/min with a load of 10ul. The mobile phase consists of organic phase as Acetonitrile and aqueous phase i.e buffer as Ammonium acetate adjusted to pH-3±0.2 with Acetic acid in the ratio of 80:20. Tailing modifier 1-Octane sulphonic acid was used in the experiment. The detection was carried out at 252nm. Retention time for Montelukast and Rupatadine were 3.4min and 4.1min respectively. Linearity was calculated over the range of 1-100ug/ml for Montelukast and Rupatadine respectively. The % regression for both drugs found to be at 0.999. Validation parameters like accuracy, precision, linearity, robustness, ruggedness were done according to ICH guidelines.

Keywords: - Montelukast, Rupatadine, Simultaneous Estimation, Isocratic RP-HPLC, Tablet formulation.

INTRODUCTION

Montelukast sodium is chemically 2-(1-((1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl)cyclopropyl)acetic acid. It is a cysteinyl leukotriene receptor antagonist and used in the treatment of chronic asthma.The individual determination of Montelukast or in formulation has been carried out by HPLC, LC-MS/MS, HPTLC, SPECTROSCOPY. Rupatadine fumarate is chemically 8-chloro-6,11-dihydro-11-[1[(5-methyl-3-pyridinyl)methyl]-4-piperinylidene]-5H-benzo[5,6]cyclohepta[1,2-b]pyridine fumarate. It is a second generation antihistamine and Platelet Activating Factor(PAF) antagonist, used in the treatment of allergies. There is only individual determination of rupatadine in formulation by HPLC, HPTLC, TITRIMETRY, LC-MS/MS, SPECTROSCOPY and no simultaneous estimation within formulation has been reported. Literature survey reveals that there is only UV- spectroscopic determination of Montelukast and Rupatadine in tablet form, and no HPLC determination so far, hence it is convenient to develop the method for Simultaneous Estimation and Validation for the combined Tablet formulation. The method describes simple, accurate, reproducible and economic isocratic RP-HPLC for Montelukast and Rupatadine combined tablets[1-3].

Montelukast Sodium Rupatadine Fumarate



Chemical structures of Montelukast and Rupatadine

MATERIALS & METHODS

Instrumentation

The Chromatographic system consisted of a Shimadzu Class VP Binary pump LC-10ATVP, SIL-10ADVP Auto sampler, CTO-10AVP Column Temperature Oven, SPD-10AVP UV-Visible Detector. All the components of the system are controlled using SCL-10AVP System controller. Data acquisition was done using LC Solutions software Ver 1.23 SP 1. [3-11]

Materials Required

Methanol (HPLC grade, Merck ltd), Acetonitrile (HPLC grade, merck ltd), Milli-Q water, Ammonium acetate, Acetic acid (AR Grade, SD Fine chem ltd), Octane sulphonic acid (AR Grade) Montelukast and Rupatadine (API purchased from Cosmacls. pharma. Ltd)

Optimized conditions

The Mobile phase consisted of 80:20 %(v/v) of Acetonitrile & Ammonium acetate buffer adjusted to pH-3+0.2 with acetic acid, Octane sulphonic acid used as tailing modifier. Operated on Isocratic mode. The flow rate is 1.0 ml/min. chromatographic separation of Montelukast and Rupatadine was performed on YMC PACK PRO C4 Column (150 x 4.6 mm id, 3µm), the wavelength of detection is 252 nm. The injection volume is 10µL.

Standard Stock Solution:

The stock concentrations of Montelukast and Rupatadine are 6.54mg/ml, 2.5mg/ml respectively using Methanol: water (70:30) as diluents. From the above solution, Mont-0.765ml diluted to 5ml and Rup-2.0ml diluted to 5ml to get the standard stock of 1000ug/ml each. And finally 50ug/ml each were prepared and used as standard solutions in the experiment. Stock and dilution preparations are well sonicated for dissolution and filtered. The standard chromatogram of Montelukast and Rupatadine shown in figure 1.

Analysis Of Marketed Formulation:

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 20mg of Montelukast sodium and Rupatadine fumarate into a 100ml volumetric flask and about 70ml of diluents and sonicate for 15min. with intermittent shaking. Dilute to the volume, and transfer 1.25ml of this solution to 25ml volumetric flask and dilute to volume with diluents and mix. Filter through 0.45u nylon filter paper. And filtrate was used under the chromatographic conditions. Area of each peak was measured at selected wavelength. The amount of each drug

present in the sample was determined by comparison with the peak areas that of the standard solutions. The results shown in table 1.

VALIDATION RESULTS

System Suitability (SST)

SST is commonly used to verify resolution, column efficiency, and repeatability of the chromatographic system to ensure its adequacy for a particular analysis. It is performed by performing 6 injections on a sample mixture containing Montelukast and Rupatadine stds. The precision of the run is calculated for the peak area & retention times of Montelukast and Rupatadine separately. Parameters such as tailing factor, theoretical plates and resolution etc. were determined. Results shown in table 2. The system suitability parameters for the method are listed below in table 3. [12-18]

Linearity & Range

Linearity is the method to obtained tests that are directy proportional to the analyte concentration within a given range. Range of analytical procedure is the interval between the upper and lower concentrations of the analyte in the analytical procedure has a suitable level of precision, accuracy, linearity.

Calibration curve standards were prepared in the range of 1-100ug/ml of Montelukast and Rupatadine respectively. The calibration curves of peak areas against concentration were found to be linear and correlation coefficient (r) found to be 0.999 for Montelukast and Rupatadine respectively. The results and the calibration graphs are shown below in the tables 4 and figures 2,3 and inter and intra day precision for linearity is shown in tables 5,6.

Acceptance Criteria: The Correlation Coefficient should be not less than 0.999.

Result: The Correlation Coefficient obtained was 0.999 for both drugs which compiles the required acceptance criteria.

Accuracy

The Accuracy is determined as the closeness of the calculated concentration to the nominal concentration. The Precision is the reproducibility of the data. The mean recoveries were calculated at three different levels i.e at 50%, 100%, 150% solutions made from the standard solution. The % mean recoveries found to be 95.263 for Montelukast and 94.9 for Rupatadine results shown in table 7.

Acceptance criteria: the % mean recovery should be within the range of 85-115.

Result: Results comply with acceptance criteria, and are well within the limits. The observed mean % recovery is between 89.49-106.25 for Montelukast and 90.15-102.94 for Rupatadine.

Method Precision

Precision is calculated by 6 repeated injections of test formulation. In this method results are reported in the form of % RSD values for peak areas of all components and are within limits. Results shown in table 8.

Acceptance criteria: In this precision method % RSD or coefficient of correlation should be ≤ 4

Specificity

The Specificity of the method is done by injecting 4 samples namely: blank sample, sample with Montelukast Std, sample with Rupatadine std, sample containing mixture of Montelukast and Rupatadine. There is no interference in the retention time of the Montelukast and Rupatadine. specificity chromatograms are shown in figures 4, 5, 6.

Acceptance criteria:-An interference in the peak area of $\leq$ 20% at the retention time of the drug in the blank sample. Result: There is no interference in the retention time of the Montelukast and Rupatadine.

Room Temperature Stability

1ml stock solution taken and placed on a bench top allowing it to stand at room temperature for a period of atleast 12hrs. Stability of this diluted solution is known from sample freshly injected. The results are shown in tables 10, 11. The % stability (after 12 hrs) = (mean peak area ratio of stability sample / mean peak area ratio of fresh sample) * 100. Validation of proposed methods shown in table 9.

Result: % stability (after 12 Hrs) = 96.14 for Montelukast and 89.93 for Rupatadine

Robustness & Ruggedness

The Ruggedness of the method is observed by evaluating the influence of Analyst variation & Column variation. Robustness is observed by evaluating the influence of Flowrate, Mobile phase composition on the developed method. Flowrate variation, mobile phase composition chromatograms shown in figures 7,8,9,10.

Result:

a) At Flow rate of 0.9ml/min – late elution of drugs, Rt increases and tailing factor of Rupatadine increases across the USP limits.
b) At flow rate of 1.1ml/min -fast elution of drugs, Rt decreases and tailing factor of Rupatadine is very low.
c) Mobile phase composition: (85:15)- Rt of Montelukast decreases, and Rupatadine increases. At (75:25)- Rt of Montelukast decreases and Rupatadine with normal elution.
d) With column and analyst variation, there is no change in Retention time or peak intensities. Hence the method is said to be robust and rugged.

Stress Degradation Studies

For Stress degradation analysis, aliquots of stock are treated separately with 100 µL of 0.1N HCl (Acid Stability), 0.1N NaOH (Alkaline Stability), 5% v/v Hydrogen Peroxide (Oxidative Stress), for 24 Hrs. Samples for Photo-degradation are placed in a transparent glass vial & placed in a UV chamber for 24 Hrs. Samples are then diluted suitably to obtain a sample within the linear calibration range. Stability of these samples are compared with fresh sample on the day of analysis. Photolytic and alkaline stress degradation chromatograms shown in figures 11,12

Acceptance criteria: - The active peak of drug should not have flag in the results. And should be stable enough in all the above stress conditions.

Result:- Montelukast is unstable with light producing very low peak area, and split in peak appeared in presence of alkali. Rupatadine is stable , and low peak area observed in Alkaline state.Hence, Montelukast is photosensitive and alkaline instable drug whereas Rupatadine is only alkaline instable.

Table 1: Analysis of marketed formulation data of Montelukast and Rupatadine

Drug Name	Label claim(mg)	Amount Found(mg)	% Assay
Montelukast	10	10.21	102.10
Rupatadine	10	10.18	101.77

Table 2: System suitability data of Montelukast and Rupatadine

Sample ID	Montelukast peak retention time	Montelukast peak area	Rupatadine peak retention time	Rupatadine peak area
1	3.47	1106764	4.05	2110734
2	3.47	1069245	4.07	2010549
3	3.46	1095721	4.07	2037658
4	3.48	1091476	4.1	2107638
5	3.48	1075051	4.11	1999715
6	3.49	1087024	4.13	2075484
Mean	3.475	1087546.83	4.088	2056963
SD	0.0105	13732.6702	0.0299	48180.0362
% CV	0.3	1.26	0.73	2.34

Table 3: System Suitability Parameters of Montelukast and Rupatadine

S.No	Parameters	Montelukast	Rupatadine
1	Tailing factor	1.411	1.816
2	Retention time	3.48	4.1
3	Resolution	0.00	6.85
4	Theoretical plates	17206	8974

Table 4: Linearity Data of Montelukast and Rupatadine

Sample ID	Montelukast Conc(ug/ml)	Montelukast peak area	Rupatadine conc(ug/ml)	Rupatadine peak area
1	1	10769	1	23985
2	5	99826	5	224869
3	10.01	208044	10	462247
4	20.01	428259	20	963631
5	40.02	888754	40	1912261
6	60.04	1382032	60	2847194
7	80.05	1770479	80	3882653
8	100.06	2199642	100	4894059

Table 5: Summary of Montelukast linear calibration curve showing inter and intraday Precision

Sample ID	1	5	10.01	20.01	40.02	60.04	80.05	100.06	y-intercept	slope	r^2
PA-1	1.23	5.11	10.11	20.19	40.04	60.1	81.09	100.11	7974	22302	0.999
PA-2	1.22	5.12	10.17	20.11	40.16	60.14	81.12	100	11583	23009	0.999
PA-3	1.21	5.14	10.15	20.16	40.11	60.01	80.99	100.22	11931	23699	0.999
Mean	1.22	5.123	10.143	20.153	40.103	60.083	81.067	100.11	10496	23003.33	0.999
SD	0.01	0.0153	0.0306	0.0404	0.0603	0.0666	0.0681	0.11			
% CV	0.82	0.3	0.3	0.2	0.15	0.11	0.08	0.11			

Table 6: Summary of Rupatadine linear calibration curve showing inter and intra day Precision

Sample ID	1	5	10	20	40	60	80	100	y-intercept	slope	r^2
PA-1	1.12	5.04	10.01	20.08	40.03	60.06	80.08	100.02	30030	48896	0.999
PA-2	1.15	5.09	10.09	20.1	40.05	60.11	80.14	100.15	32309	48854	0.999
PA-3	1.17	5.06	10.05	20.12	40.09	60.09	80.02	100.09	46640	51511	0.999
Mean	1.147	5.063	10.05	20.1	40.057	60.087	80.08	100.087	36326.3	49753.667	0.999
SD	0.0252	0.0252	0.04	0.02	0.0306	0.0252	0.06	0.0651			
% CV	2.19	0.5	0.4	0.1	0.08	0.04	0.07	0.07			

Table 7: Accuracy data of Montelukast and Rupatadine

	Montelukast		Rupatadine	
Spike level	% recovery	Mean % recovery	% recovery	Mean % recovery
50%	105.77	106.25	98.12	102.94
50%	106.06		105.47	
50%	106.92		105.24	
100%	89.06	90.05	89.04	90.15
100%	89.06		91.16	
100%	89.06		90.26	
150%	90.33	89.49	92.54	91.61
150%	89.35		91.83	
150%	88.8		90.47	

Table 8: Method precision data of Montelukast and Rupatadine

S.No	% Assay of Montelukast	% Assay of Rupatadine
1	102.51	101.52
2	101.60	101.82
3	102.20	101.97
4	102.51	101.82
5	102.50	101.52
6	101.60	101.97
average	102.10	101.77
% RSD	0.45	0.23

Table 9: Validation of the proposed methods

	Montelukast	Rupatadine
Linearity & range	1-100ug/ml	1-100ug/ml
Intercept	7974	300300
Slope	22302	48896
Correlation coefficient (r)	0.999	0.999

Table 10: RT stability data of Montelukast

	Fresh sample		Stability sample	
SR NO	Retention time	Peak Area	Retention time	Peak Area
1	3.5	1166761	3.49	1105631
2	3.47	1193889	3.49	1092083
3	3.47	1192773	3.45	1103114
4	3.47	1184391	3.46	1201998
5	3.47	1182095	3.47	1006380
6	3.46	1144855	3.5	1167335
Mean		1170447		1125237

Table 11: RT stability data of Rupatadine

	Fresh sample		Stability sample	
SR NO	Retention time	Peak Area	Retention time	Peak Area
1	4.32	2267007	4.29	2281257
2	4.29	2478154	4.3	2164340
3	4.3	2501007	4.27	2184958
4	4.3	2477441	4.28	2514492
5	4.3	2431677	4.3	1879722
6	4.29	2476353	4.32	2247171
Mean		2461823.6		2213795

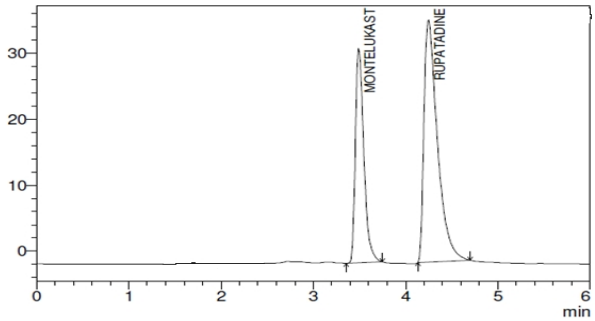


Figure 1: Chromatogram of Montelukast and Rupatadine standards
Montelukast-Rt-3.42 Rupatadine-Rt-4.12

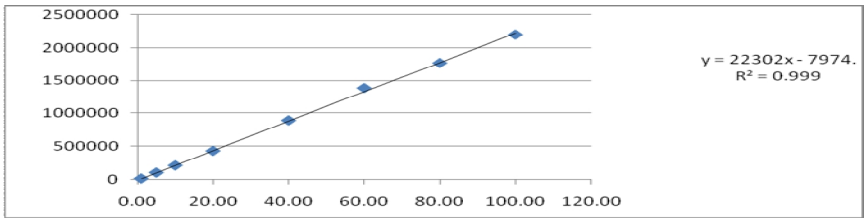


Figure 2: Linearity plot of Montelukast

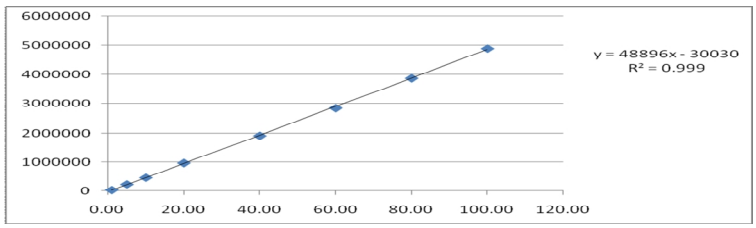


Figure 3: Linearity plot of Rupatadine

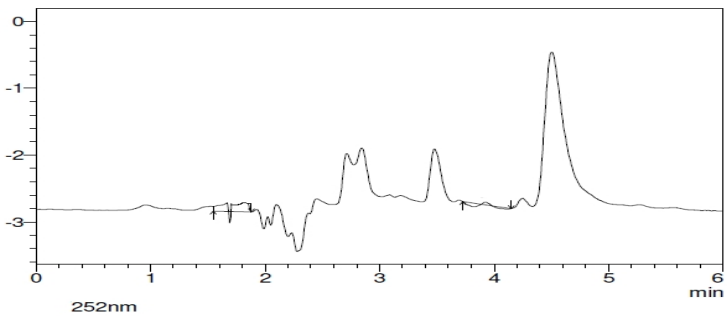


Figure 4: Specificity chromatogram of Blank sample

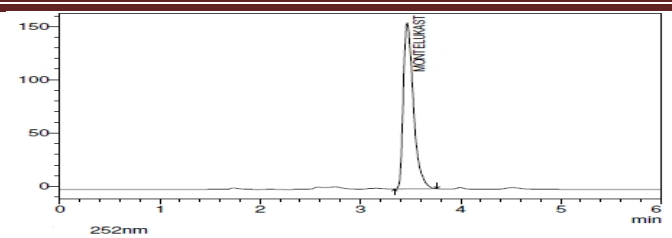


Figure 5: Specificity Chromatogram of Montelukast

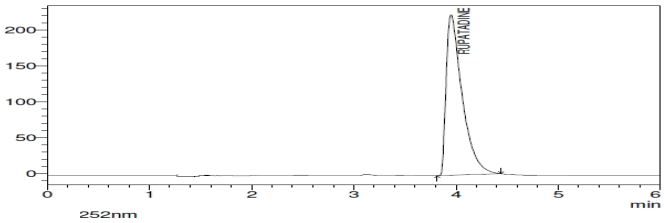


Figure 6: Specificity Chromatogram of Rupatadine

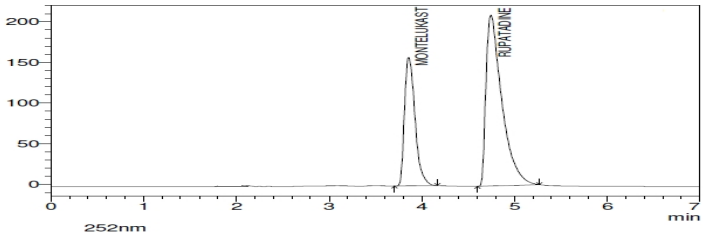


Figure 7: Flow Rate Variation (0.9ml/Min) Of Montelukast and Rupatadine

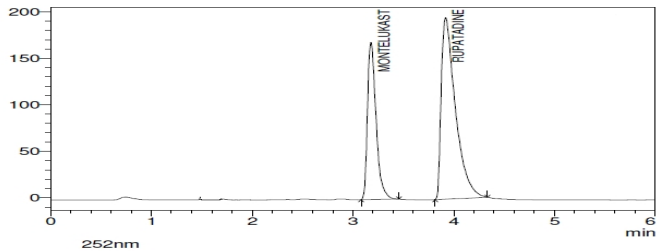


Figure 8: Flow Rate Variation (1.1ml/Min) Of Montelukast and Rupatadine

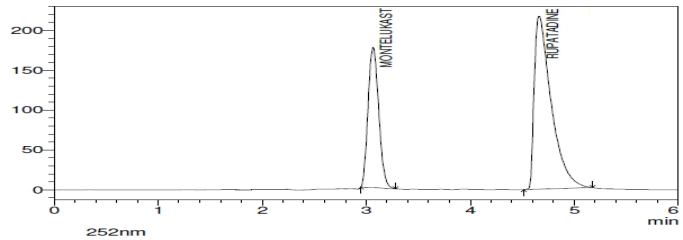


Figure 9: Mobile Phase Variation (85:15) of Montelukast and Rupatadine

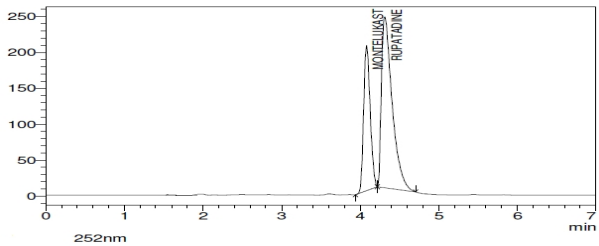


Figure 10: Mobile Phase Variation (75:25) of Montelukast and Rupatadine

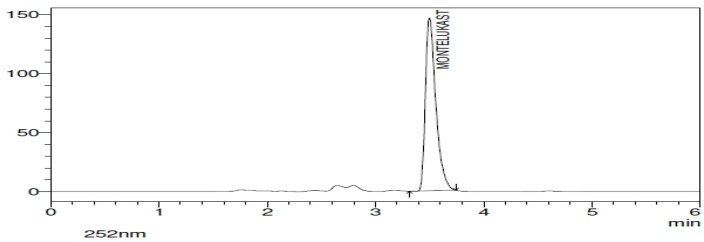


Figure 11: Photolytic Stress Degradation of Montelukast

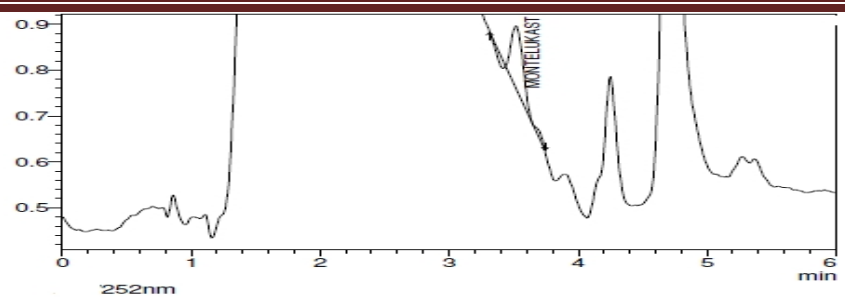


Figure 12: Alkaline Stress Degradation of Montelukast

DISCUSSION

A new, Reversed-phase HPLC method has been developed for Simultaneous Estimation of Montelukast sodium and Rupatadine fumarate in a Tablet formulation. It was shown that the method was accurate, reproducible, repeatable, linear, precise and selective, proving the reliability of the method. The run time is relatively short , i.e. 6 min, which enables rapid quantitation of many samples in routine and Quality Control analysis of Tablet formulations. The method employed is isocratic and the results show the method could find practical application as a quality-control tool for simultaneous estimation of two drugs, Montelukast and Rupatadine from their combined dosage forms in Quality-control laboratories[16-21].

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