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Der Pharma Chemica, 2012, 4(5):1819-1825
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Simultaneous RP-HPLC method for estimation of rupatadine fumarate and montelukast sodium in tablet dosage form

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ABSTRACT

A simple, selective, linear, precise, and accurate RP-HPLC method was developed and validated for the simultaneous estimation of Rupatadine and Montelukast from bulk and formulations. Chromatographic separation was achieved isocratically on a Hiber@ Lichrosphere ® C18 column (250×4.6 mm, 5 µ particle size) using a mobile phase, Methanol and Potassium Di Hydrogen Phosphate buffer and Acetonitrile (adjusted to pH 3.0 with 1% orthophosphoric acid) in the ratio of 50:30:20v/v/v. The flow rate was 1 ml/min and effluent was detected at 226 nm and 20µl of sample was injected. The retention time of Rupatadine and Montelukast were 2.48 min and 6.38 min. respectively. Linearity was observed in the concentration range of 5-30 µg/ml for both Rupatadine and Montelukast. Percent recoveries obtained for both the drugs were 99.53-100.16% and 97.63-98.95%, respectively. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. The method developed can be used for the routine analysis of Rupatadine and Montelukast from their combined dosage form.

Key words: RP-HPLC Method; Rupatadine Fumarate and Montelukast sodium; Combined Dosage Form.

INTRODUCTION

Rupatadine is a new drug chemically 8-chloro-6, 11-dihydro-11-[1-[(5-methyl3-pyridinyl) methyl] -4-Piperidinylidene]-5H-benzo [5, 6]-cyclohepta [1,2b] pyridine (fig.01).second generation antihistamine and PAF antagonist used to treat allergies. It is a second generation, non-sedating, long-acting histamine antagonist with selective peripheral H_1 receptor antagonist activity. It further blocks the receptors of the platelet-activating factor (PAF) according to in vitro and in vivo studies. Rupatadine possesses anti-allergic properties such as the inhibition of the degranulation of mast cells induced by immunological and non-immunological stimuli, and inhibition of the release of cytokines, particularly of the TNF in human mast cells and monocytes.

Montelukast Sodium (1-(1R)-1-[3-[(1E)-2-(7-chloro-2- quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl] -propyl] thio] methyl] cyclopropaneacetic acid, monosodium salt is a white colored powder and it is freely soluble in ethanol, methanol, and water and practically insoluble in acetonitrile. Molecular weight of Montelukast Sodium is 608.2 g/mol and formula is C35H35ClNO3S.Na. For structure refer Figure 02.

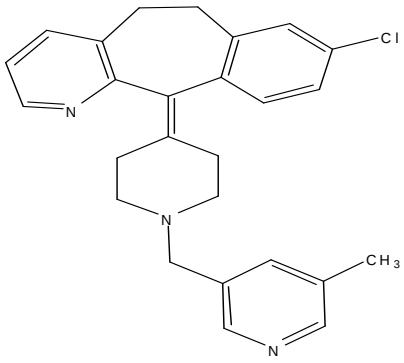

Fig.01.Chemical structure of Rupatadine

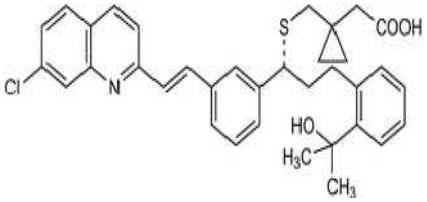

Fig.02. Chemical structure of Montelukast

Literature survey reveals separate HPLC method of analysis for Rupatadine fumarate in bulk as well as from tablet is available. Furthermore HPLC assay methods for Montelukast in human plasma were established. Several methods also have been reported for simultaneous determination of Rupatadine fumarate Hydrochloride and Montelukast Sodium from various formulations which include TLC, Ratio Derivative Spectroscopy, HPTLC as well as HPLC. The objective of this work is to develop an accurate, specific, repeatable and validated HPLC method for simultaneous determination

Hence, the aim was to develop validated HPLC method, which can simultaneously determine both components in marketed pharmaceutical dosage forms with better accuracy, precision and sensitivity.

MATERIALS AND METHODS

Experimental

Instruments and Apparatus

The chromatography was performed on a Waters 2487 RP-HPLC instrument equipped with PDA detector and EMPOWERS software, Hiber @ Lichrosphere ® C18 column (250×4.6 mm id, 5µm particle size) was used as stationary phase. Shimadzu analytical balance and ultrasonicater were used during the research work.

Reagents and materials

Standard samples of MLT and RPN were obtained from Sun Pharmaceutical Pvt Ltd, (Vadodara, Gujarat).Combination tablet formulation containing Montelukast sodium equivalent to Montelukast 10 mg and Rupatadine 10 mg was procured from local pharmacy. Triple distilled water, methanol, acetronitrile, phosphate buffer pH 3 and 0.45 membrane filters (Millipore) used were of HPLC grade.

Preparation of Standard Solution

Accurately weighed MLT (100 mg) and RPM (25 mg) standards were transferred to a 25 ml volumetric flask, dissolved in 25ml diluent and diluted up to the mark with methanol to obtain a standard stock solution (1000µg/ml)

of MLT and RPM, each. From the above stock solution, an aliquot (100 µg/ml) of the solution was transferred to 10 ml volumetric flask, and diluted up to the mark with mobile phase of MLT and RPM, each.

Preparation of Calibration Curve

Aliquots (0.5, 1.0, 1.5, 2, 2.5, 3.0 ml) of mixed working standard solution (equivalent to 5, 10, 15, 20, 25 and 30 µg/ml for MLT and RPM respectively were transferred in a series of 10 ml volumetric flasks and the volume was made up to the mark with mobile phase. An aliquot (20 µl) of each solution was injected under the operating chromatographic conditions as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentrations, and the regression equations were calculated. Each response was average of three determinations.

Preparation of Sample Solution

For determination of the content of MLT and RPM in tablets; twenty tablets were weighed and average weight was determined. The accurately weighed powder equivalent to 100 mg MLT and 100 mg of RPM was transferred in a 25 ml volumetric flask and methanol (10 ml) was added. The solution was sonicated for 15 min. The flask was allowed to stand for 5 min at room temperature and the volume was diluted up to the mark with methanol to obtain the sample stock solution of MLT (15µg/ml) and RPM (15µg/ml).The solution was filtered through 0.45µm-47mm membrane filter. An aliquot (2.5 ml) was transferred to a 50 ml volumetric flask and diluted up to the mark with mobile phase used for HPLC, to obtain working sample solution of MLT (100µg/ml) and RPM (100 µg/ml). An aliquot (1 ml) of the working test solution was transferred to a 100 ml volumetric flask and diluted up to the mark with mobile phase to obtain the sample solution of MLT (1 mg/ml) and RPM (1 mg/ml).

Method Validation: The methods were validated in compliance with ICH guidelines.

Accuracy

The accuracy of the methods was determined by calculating recoveries of MLT and RPM by the standard addition method.

Intermediate Precision (Reproducibility) The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of MLT (5, 10 and 15µg/ml) and RPM (10, 20 and 30µg/ml).

Method Precision (Repeatability)

The repeatability was checked by repeatedly injecting (n = 6) solution of MLT (15µg/ml) and RPM (15 µg/ml, each).

LOD and LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) of the MLT and RPM were calculated using the standard deviation of responses (N) and slopes (S) of respective calibration curves using signal-to-noise ratio.

$$LOD = 3.3 \times N/S$$
$$LOQ = 10 \times N/S$$

Robustness

The robustness was studied by analyzing the same samples of RPN and MLT by deliberate variation in the method parameters. The change in the responses of RPN and MLT were noted. Robustness of the method was studied by changing the extraction time of RPN and MLT from tablet dosage forms by ± 2 min, composition of mobile phase by ± 2 % of organic solvent, flow rate by ± 0.2 ml/min and column oven temperature by ± 2 ^{0}C. The parameters used in system suitability test were asymmetry of the chromatographic peak, peak resolution, theoretical plates and capacity factor, as RSD of peak area for replicate injections.

RESULTS AND DISCUSSION

The responses of sample solutions were measured at 226 nm (fig.03) for quantitation of RPN and MLT by the proposed methods. The amount of RPN and MLT present in the sample solutions were determined by fitting the responses into the regression equations of the calibration curve for RPN and MLT, respectively. The mobile phase consisting of Methanol and Potassium Di Hydrogen Phosphate buffer and Acetonitrile (adjusted to pH 3.0 with 1%

orthophosphoric acid) in the ratio of 50:30:20v/v/v , at a flow rate of 1.0 ml/min was found to be satisfactory to obtain good peak symmetry, better reproducibility and repeatability for RPN and MLT. Quantification was achieved with PDA detector at 226 nm based on peak area. The retention times were found to be 6.3 and 2.4 min for MLT and RPM, respectively (Figure 4). Linear correlation was obtained between peak area and concentration for RPN and MLT, each, in the range of 5-30µg/ml. (Table 1&Fig.05 and 06.). The method was found to be specific as no significant change in the responses of RPN and MLT was observed after 24 h. The percent mean recoveries obtained for RPN and MLT were100.16 ± 0.49 % RSD and 98.95 ± 0.57 % RSD (Table1), which suggest accuracy of the method. The values of % RSD for intraday and interday variations were found to be in range of 0.34-0.82 and 0.53-1.07 for MLT, and 0.47-0.98 and 0.65-1.19 for RPM, respectively (Table 1). % RSD for repeatability was found to be 0.24 and 0.31 for MLT and RPM, respectively. Low RSD values for precision suggest that the method is precise. The LOD and LOQ were found to be 9.99 and 3.01µg/ml for MLT, 6.447 and 1.446µg/ml for RPM, respectively (Table 1), suggest sensitivity of the method. Results of system suitability testing are given in Table 2. The results obtained for RPN and MLT were comparable with the corresponding labeled claim (Table 3).

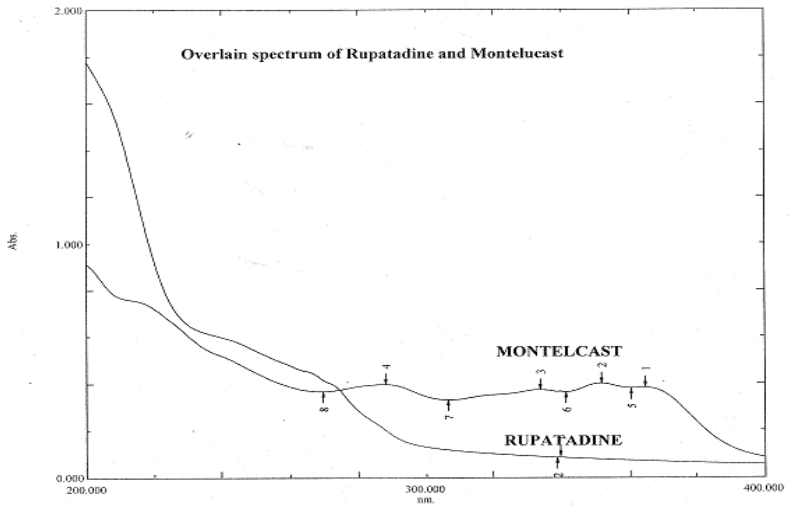

Fig.03.Overlain spectra of Rupatadine and Montelukast

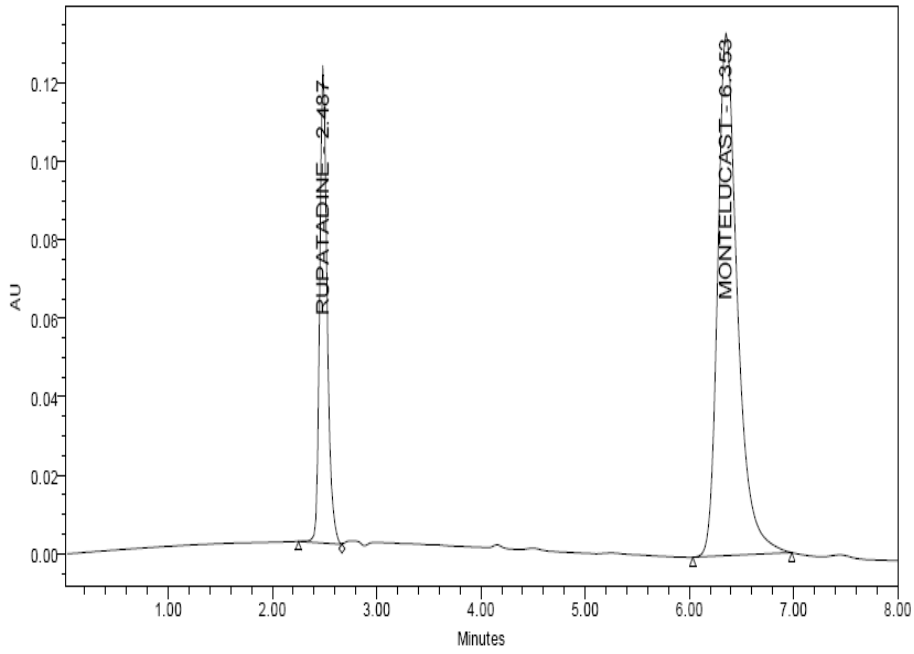

Fig.04.Typical chromatograms of Rupatadine and Montelucast.

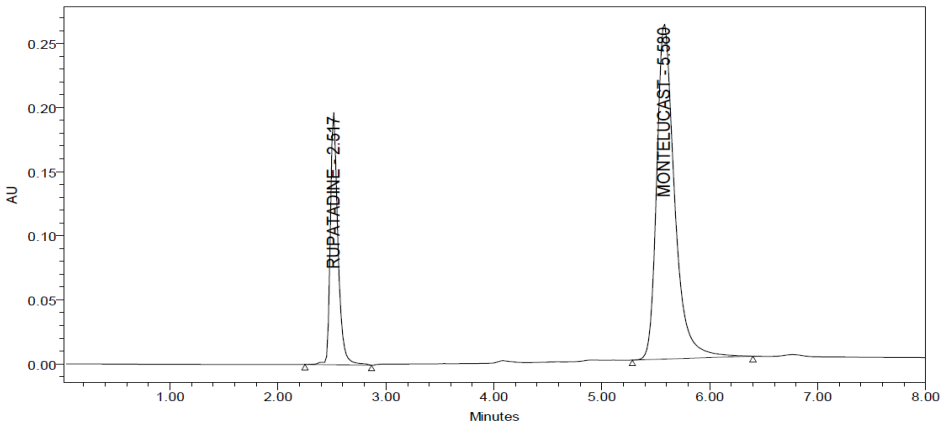

Fig.05.Precision chromatogram 15 For RPN and 15 µg/ml for MLT concentration

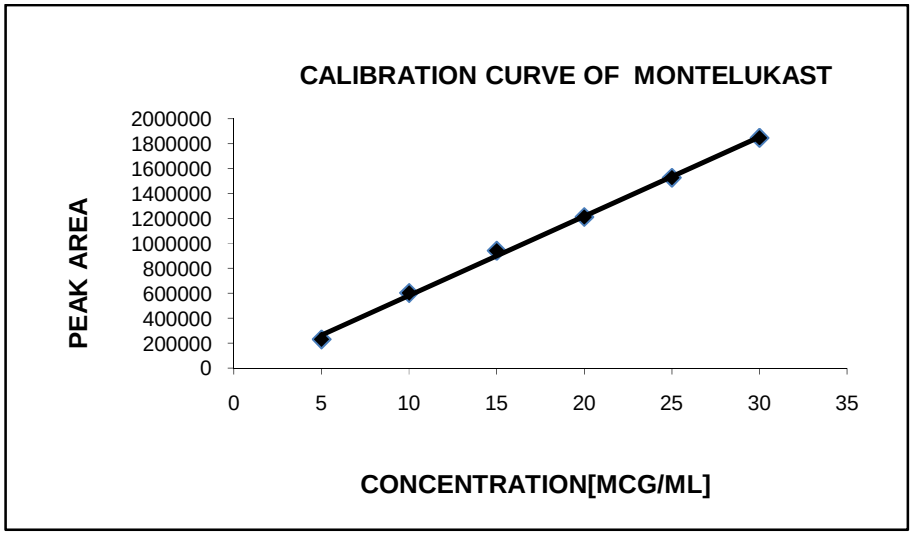

Fig.06.Calibration graph of Montelukast

Table 1: Regression analysis data and summary of validation parameter for the proposed method

Parameters of RP-HPLC method	**MLT**	**RPM**
Concentration range (µg/ml)	5-30	5-30
Slope	18210.05	6247.980714
Intercept	22527.18	28171.96429
Correlation coefficient	0.9994	0.9997
LOD(µg/mL)	1.08	1.56
LOQ(µg/mL)	1.301	1.4465997
% recovery (Accuracy, n = 6)	100.16 ± 0.32	98.95 ± 0.57
Repeatability (% RSD, n = 6)	1.85	0.8723
Precision (%RSD)	0.37	0.56
Interday (*n* = 6)	0.42	0.97 0.53
Intraday (*n* = 6)	0.35	0.82 0.61
Standard Error	0.75526	0.3816695

a RSD is a Relative standard deviation, b n is number of determinations, MLT is Montelukast Sodium, RPM is Rupatadine

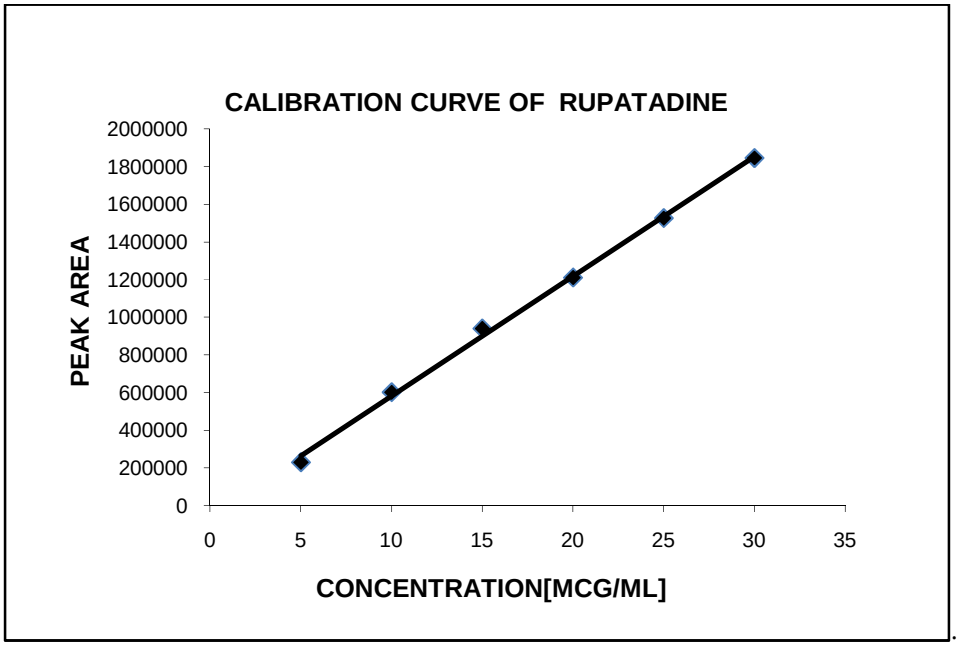

Fig.07.Calibration graph of Rupatadine

Table 2: Assay Results for Tablets Using the Proposed Method

Formulation Amount of drug taken (mg)Amount of drug found (mg)% Amount found (na=3) ±SDb						
Tablets	MLT	RPM	MLT	RPM	MLT	RPM
1	10	10	10.14	9.97	101.40± 0.26	99.70± 0.43

an is number of determinations, bSD is a Standard deviation

Table 03.Recovery studies of Rupatadine and Montelukast

Drug	**Amount present in (μg/ml)**	**Amount added %**	**% Recovery +_SD***
RPN	**10**	**50**	**97.87+0.34**
	10	**100**	**98.23+0.15**
	10	**150**	**98.54+0.80**
MLT	**10**	**50**	**98.63+0.65**
	10	**100**	**99.68.+0.16**
	10	**150**	**99.17+0.63**

Table 4: system suitability test parameters for MLT & RPM for Proposed method

Parameters	**RP-HPLC method**	
	RPN ± % RSD a	**MLT ± % RSD a**
Retention time, min	2.48	6.35
Tailing factor	0.97	1.56
Asymmetry factor	0.97 ± 0.93	1.36 ± 0.37
Theoretical plates	6243.5 ± 1.13	4986.9 ± 1.33
Repeatability of measurement (nb = 6)	0.67	0.48

a RSD is a Relative standard deviation; b n is number of determinations

CONCLUSION

The developed RP-HPLC method was validated and the system suitability studies were performed and all parameters combined with the simplicity and ease of operation ensures that the validated method can successfully used for routine analysis of RPN and MLT in bulk and tablet dosage formulation.

Acknowledgements
The authors wish to thank Dr. Reddy's Laboratories, Hyderabad for providing the gift sample of RPN and MLT. The authors are thankful to D. Manohar Reddy, Chairman of Trinity College of Pharmaceutical Sciences for their kind help and providing all necessary facilities.

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