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Development and Validation of RP-HPLC-UV Method for Simultaneous estimation of Rupatadine and Montelukast in Bulk and its Tablets

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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 Waters C18 column (250×4.6 mm, 5 µ particle size) using a mobile phase, (Methanol: Phosphate buffer pH adjusted to 3.0 with orthophosphoric acid in the ratio of 70:30 v/v. The flow rate was 1 ml/min and effluent was detected at 245 nm and 20µl of sample was injected. The retention time of Rupatadine and Montelukast were 2.3 and 3.9 min respectively. Linearity was observed in the concentration range of 50-150 µg/ml for Montelukast and Rupatadine. Percent recoveries obtained for both the drugs were 100.00-101.00%. 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, LOD and LOQ. The method developed was successfully applied for the analysis of simultaneous estimation of Rupatadine and Montelukast tablets.

Key words: Rupatadine, Montelukast, Methanol, Phosphate buffer, orthophosphoric acid.

Contents

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1. Introduction

Rupatadine fumarate is 8-Chloro-6, 11-dihydro-11- [1- [(5-methyl-3-pyridinyl) methyl]-4-piperidinylidene] -5H-benzo [5, 6] cyclohepta [1, 2-b] pyridine fumarate. Rupatadine is a non-sedating H1-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 urticaria [1]. The structure of Rupatadine

is shown in Figure1.Montelukast sodium is [R-(E)]-1-[[[1-[3-[2-(7-chloro-2quinolinyl)ethenyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl] propyl] thio] methyl] cyclopropane aceticacid, monosodium salt. 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]. The structure of Montelukast is shown in Figure 2.

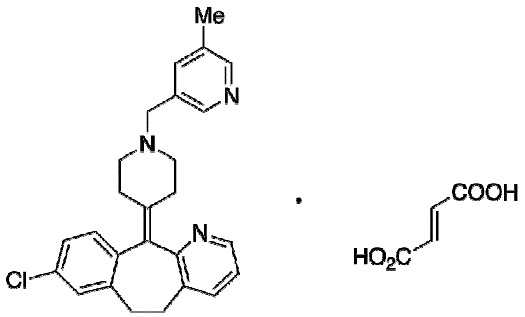

Figure 1: Chemical structure of Rupatadine fumarate

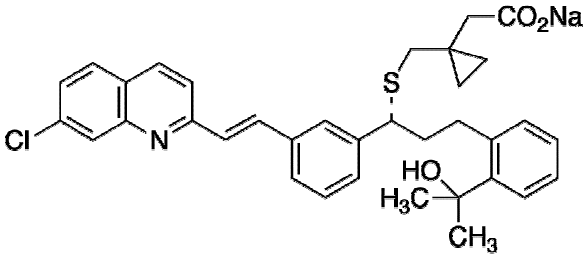

Figure 2: Chemical structure of Montelukast sodium

Some analytical methods for the quantitative determination of rupatadine in pharmaceutical formulations are described in the literature like stability indicating the high-performance liquid chromatography (HPLC) method [3, 4]. Method available for the determination of montelukast sodium include HPLC,[5] protein precipitation,[6] liquid chromatography/tandem mass spectrometry(LC-MS/MS),[7] liquid-liquid extraction using HPLC with the fluorescence detector,[8] its *S*-enantiomer in human plasma by stereoselective high performance liquid chromatography (HPLC) using column-switching [9] and its determination in human plasma by the column-switching HPLC method,[10] derivative spectroscopy, HPLC,[11] microwave-assisted extraction (MAE) technique,[12] method for the application of pressurized liquid extraction technology for pharmaceutical solid dosage form[13] Residual acetate analysis in bulk drug.[14-16]

No HPLC-UV method has been reported in the literature for the simultaneous determination of montelukast and rupatadine in their commercial formulations. It would therefore be beneficial to provide accurate, precise, and reliable methods for simultaneous determination of montelukast and rupatadine. The present work describes an analytical procedure for the quantitation of montelukast in co-formulation with rupatadine using reversed phase HPLC.

2. Materials and Methods

Chemicals

Rupatadine and Montelukast were obtained as a gift sample from Zydus Cadila Healthcare Limited (Ahmedabad, India) and Vent Unimark Remedies with the percentage purity of 99.90% and 100.00%, respectively. A commercial tablet formulation Rupanex-M from Dr. Reddy, s Laboratories Ltd, (Hyderabad, India) containing 10mg of RUPA and 10mg of MONT was purchased from local market and used within their shelf life period. HPLC grade methanol was obtained from Merck Limited. Analytical grade Dipotassium Phosphate buffer was obtained from SD Fine (Mumbai, India). HPLC grade water was obtained by distilling deionizer water produced by a Milli-Q Millipore water system (Milford, MA, USA). All other chemicals used were of pharmaceutical or analytical grade.

Chromatographic conditions

The HPLC system (LC Waters, Milford, MA, USA) consisted of quaternary gradient system (600 Controller), in-line degasser (Waters, model AF), photodiode array detector (Water, 2998 model) and auto sampler (Waters, model 717 plus). Data was processed using Empower Pro software (Waters, Milford, MA, USA). Isocratic elution of the mobile phase Methanol: Phosphate buffer pH adjusted to 3.0 with orthophosphoric acid in the ratio of 70:30 v/v.

with the flow rate of 1 ml/min. Separation was performed on a Waters C_{18} (250 x 4.6 mm i.d, 5 µ particle size) analytical column and a pre-column to protect the analytical column from strongly bonded material. Integration of the detector output was performed using the Waters Empower software to determine the peak area. The contents of the mobile phase were filtered through a 0.45 µm membrane filter and degassed by sonication before use. Mobile phase was used as diluents. The flow rate of the mobile phase was optimized to 1 ml/min which yields a column back pressure of 110–112 kg/cm. The run time was set at 6 min and a column temperature was maintained at 30°C. The volume of injection was 20 µl, prior to injection of the analyte, the column was equilibrated for 30–40 min with the mobile phase. The eluent was detected at 245 nm. The developed method was validated in terms of specificity, linearity, accuracy, limit of detection (LOD), limit of quantification(LOQ), intra-day and inter-day precision and robustness for the assay of Tapentadol and Paracetamol as per ICH guidelines[17].

Preparation of standard stock solutions of rupatadine and montelukast:

Stock solutions of rupatadine and montelukast were prepared by accurate weighing of 50 mg in each case and dissolving in mobile phase up to 50 ml in volumetric flasks. For simultaneous quantitative studies of both drugs, a series of standard working solutions containing both the drugs were prepared by an appropriate dilution of a mixture of stock solutions. All the volumetric flasks were wrapped with an aluminum foil.

Preparation of test Solution (Analysis of rupatadine and montelukast in tablets):

Ten tablets of Rupanex-M were weighed and finely powdered. A powder equivalent to 50mg was accurately weighed, transferred into a 50 ml volumetric flask containing mobile phase. The above mixture was sonicated for about 10 min. for complete mixing. This solution was filtered through Whatman No.1 filter paper. From the filtrate different aliquots were taken in separate 10 ml volumetric flasks and diluted with mobile phase up to the mark so as to get a concentration ranging from 50-150 µg ml^{-1} each of rupatadine and montelukast. Each of these solutions (10 µl) was then injected three times into the column. The mean peak areas of each drug were calculated and the drug contents in the tablets were quantified using the respective regression equations.

3. Results and Discussion

The present research work was designed at developing a rapid, sensitive, precise and accurate HPLC method for the simultaneous estimation of rupatadine and montelukast in pharmaceutical dosage forms. In order to affect analysis of the component peaks under isocratic conditions, mixtures of water and methanol in different combinations with different pH were tested as mobile phase on a Waters C18 stationary phase. A binary mixture of Methanol: Phosphate buffer pH adjusted to 3.0 with orthophosphoric acid in the ratio of 70:30 v/v was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and free from tailing. A flow rate of 1.0 ml/min of the mobile phase was found to be suitable.

Method development

After various trials, the following chromatographic conditions were finally optimized for the simultaneous estimation of rupatadine and montelukast in a tablet dosage form. Mobile phase constitutes of Methanol: Phosphate buffer pH adjusted to 3.0 with orthophosphoric acid in the ratio of 70:30 v/v. Detection wave length 245 nm flow rate1.0 ml/min, after a steady baseline the standard solution were injected and chromatograms were recorded until the reproducibility of the peak areas were found and finally 100 µg/ml of the standard solution of the individual samples of rupatadine and montelukast and mixed standard solutions were injected and the chromatograms were recorded. The separation of rupatadine and montelukast with retention times of 2.3 and 3.9 min respectively. The typical chromatograms of the standard solutions were recorded for the repeatability and the respective chromatogram was given in Figure 3.

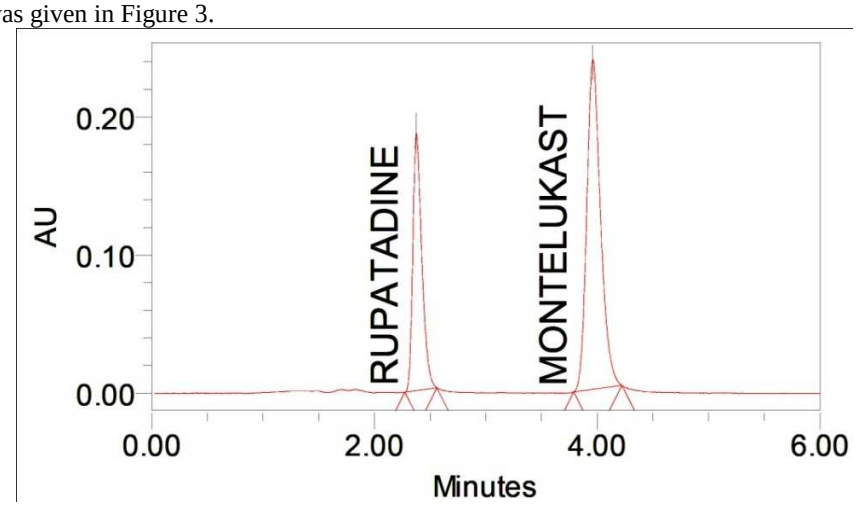

Figure 3: Typical chromatogram of rupatadine and montelukast with detection at 245 nm.

Method validation After development of method, validation of the method for simultaneous estimation of rupatadine and montelukast was performed in accordance with ICH guidelines (International Conference on Harmonization (ICH) 2000) which include System suitability, Linearity, Accuracy, Precision, LOD and LOQ, Specificity and Robustness.

Linearity

Calibration graphs were constructed by plotting peak area vs. concentration of rupatadine and montelukast and the regression equations were calculated. The calibration graphs were plotted over 5 different linear concentrations in the range of 50-150 µg/ml for all the drugs. Aliquots (20 ml) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n = 6)]. The method was found linear over the concentration range of 50-150 µg/ml for rupatadine and montelukast. Linearity curves of rupatadine and montelukast were shown in figure 4 & 5 respectively.

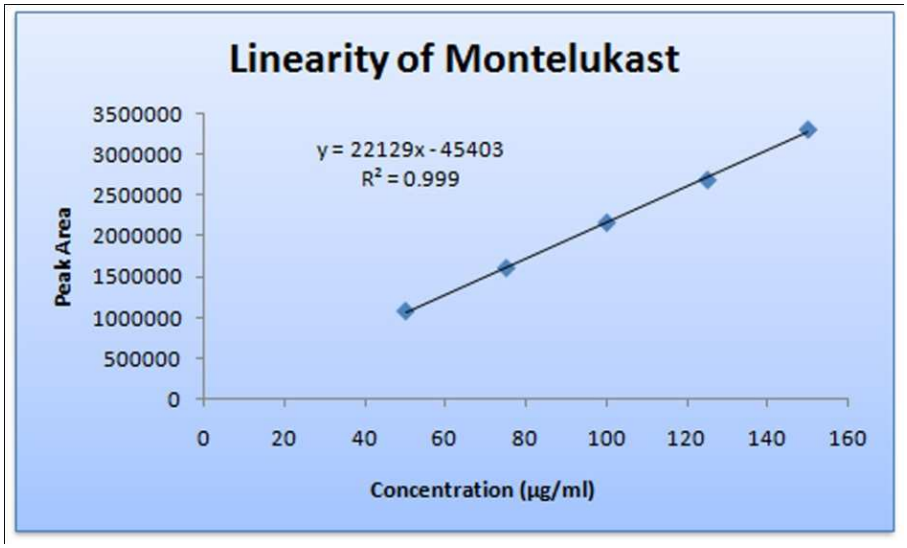

Figure 4: Linearity of Montelukast

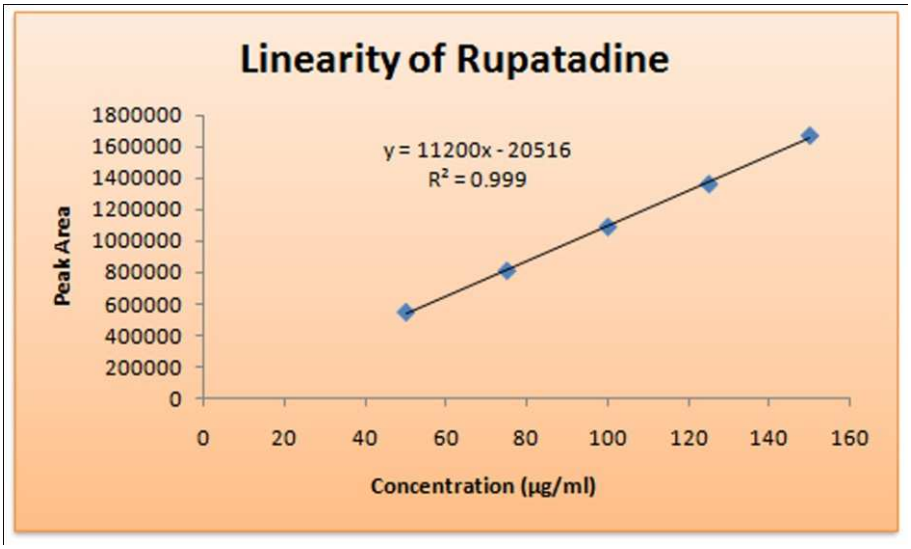

Figure 5: Linearity of Rupatadine

Accuracy

The accuracy of the method was established by recovery studies i.e., external standard addition method. The known amount of standard was added at three different levels to preanalyzed sample. Each determination was performed in

triplicate. The mean recoveries obtained were 98.00% and 100.00% for Rupatadine and montelukast. The results of accuracy were tabulated in table 2.

Precision

The intraday and interday precision of the proposed method was determined by analyzing mixed standard solution of Rupatadine and montelukast at concentration 100 µg/ml 3 times on the same day and on 3 different days. The results are reported in terms of relative standard deviation. The % RSD values for Rupatadine and montelukast were found to be 0.55% and 0.74% respectively.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) of Rupatadine and montelukast were determined by calculating the signal-to noise (S/N) ratio of 3:1 and 10:1, respectively according to International Conference on Harmonization guidelines. LOD values for Rupatadine and montelukast were found to be 0.07 ng ml^{-1} and 0.14 ng ml^{-1} respectively. LOQ values for Rupatadine and montelukast were found to be 0.33 ng ml^{-1} and 0.66 ng ml^{-1} respectively.

Table 2: Summary of validation parameters

S. No	Validation Parameters	Results	
		Rupatadine	montelukast
1	Accuracy (% Recovery)	100-101	100-101
2	Precession (% RSD)	0.74	0.55
3	Interday precession (%RSD)	0.70	0.33
3	LOD	0.07 ng ml^{-1}	0.14 ng ml^{-1}
4	LOQ	0.33 ng ml^{-1}	0.66 ng ml^{-1}

Robustness

The robustness of the method was evaluated by assaying the test solutions after slight but deliberate changes in the analytical conditions like flow rate (0.1 ml min^{-1}), and pH of the mobile phase (± 0.2). Stability of standard and test solution (prepared from the dosage form) was established by storage at 25 °C and 15 °C for 48 h. During the storage period, the test solutions were reanalyzed at intervals of 6, 12, 24, 36 and 48 h and assay was determined against appropriate fresh standard preparations.

Assay of the tablet dosage form

The proposed validated method was successfully applied to determine Rupatadine and Montelukast in tablet dosage form. The result obtained was comparable with corresponding labeled amounts (Table 3).

Table 3: Assay of the tablet dosage form

Sample No.	MONTELUKAST			RUPATADINE		
	Labeled amount (mg/tablet)	Amount Found (mg/tablet)	% Assay	Labeled amount (mg/tablet)	Amount Found (mg/tablet)	% Assay
1	10	9.960	99.60	10	10.000	100
2		10.104	101.04		10.100	101
3		10.072	100.72		10.000	100
4		10.099	100.99		10.000	100
5		10.070	100.70		10.000	100
6		10.105	101.05		10.100	101
Average Assay:			**101**	**Average Assay:**		100
STD			**0.55**	**STD**		0.74
% RSD			**0.55**	**% RSD**		0.74

The accuracy of the proposed method was assessed by recovery studies. All solutions were prepared and analysed in triplicate. The above procedure is adopted for both the drugs and a high recovery values obtained (Table-2) indicate that the proposed method is highly accurate. The method specificity was assessed by studying the chromatograms (Figure 3) obtained for a mixture of the drugs and the common excipients. As none of the excipients interfered with the analytes of interest, the method was found to be suitable for analyzing the commercial formulation of these drugs.

4. Conclusion

The present results provide clear evidence that the proposed method can be successfully used for simultaneous determination of drug content in marketed formulations.

5. Acknowledgement

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