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Research Article

Method development and validation of Rupatadine fumarate and Montelukast sodium by RP- HPLC

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Abstract

A simple, sensitive, rapid and selective isocratic reversed phase High Performance Liquid Chromatographic method has been developed for simultaneous estimation of Rupatadine fumarate & Montelucast Sodium from pharmaceutical dosage form using a mobile phase consisting mixture methanol: acetonitrile: buffer 40:30:30, (pH adjusted to 3.2 using ortho phosphoric acid) at the flow rate of 1.0 mL/min. A Hypersil BDS C_8, (250 mm X 4.6 mm, 5µ particle diameters) column was used as stationary phase. The retention time of Rupatadine Fumarate and Montelucast Sodium was 3.97 min. and 2.79 min. respectively. The eluent were detected at 270 nm. The proposed method is precise, accurate, selective and rapid for the simultaneous determination of Rupatadine Fumarate & Montelucast Sodium.

Keywords: Rupatadine fumarate , Montelucast Sodium , RP-HPLC Method, Mobile phase.

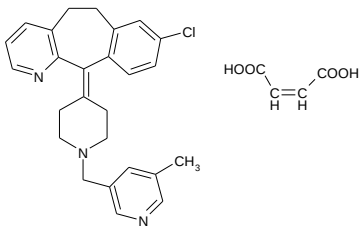
1. Introduction

Rupatadine is a second generation antihistamine and PAF antagonist used to treat allergies. Rupatadine fumarate has been approved for the treatment of allergic rhinitis and chronic urticaria in adults and children over 12 years. The defined daily dose (DDD) is 10 mg orally. Montelukast is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies.[1,2] Montelukast is a $CysLT_1$ antagonist; it blocks the action of leukotriene D4 (and secondary ligands LTC4 and LTE4) on the cysteinyl leukotriene receptor $CysLT_1$ in the lungs and bronchial tubes by binding to it. Literature review reveals that few analytical methods were evoked for the estimation of rupatadine fumarate and montelukast sodium. We here in report a simple and reliable RP-HPLC for the estimation of rupatadine fumarate and montelukast sodium in bulk and pharmaceutical dosage forms.

1.1 Drug Profile

Rupatadine fumarate

Chemical structure:



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.

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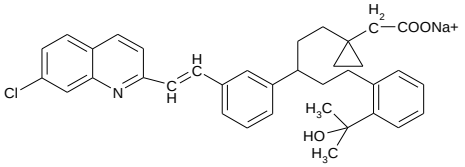
Molecular formulae: $C_{30}H_{30}ClN_3O_4$
Montelukast Sodium
Structure



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: $C_{35}H_{35}ClNNaO_3S$

2. Materials and methods

2.1 Chemicals: Acetonitrile, Methanol HPLC grade, Merck, India. Milli Q Water, Ortho phosphoric acid AR grade, Merck, Hydrogen peroxide solution (30% w/v), AR grade, Qualigens Sodium hydroxide pellets, GR grade, Merck. All other chemicals used were of pharmaceutical or analytical grade.

2.2 Chromatographic conditions

The HPLC system Waters HPLC 2695 separation module. Using waters 2996 Photo diode array detector winchrome 2004 is software used. Xttera C_{18} (5 µm, 250 mm X 4.6 mm i.d.) column is used for chromatographic analysis. Methanol: Acetonitrile: buffer 40:30:30 was used as mobile phase. 270 nm was used as a detection wavelength. Flow rate maintained at 1ml/min.

2.3 Experimental

2.3.1 Preparation of the standard

Weigh accurately standard 50mg of Rupatadine fumarate and 125mg of Montelucast sodium. Dissolve in 100ml of Mobile phase. Sonicate for 15 to 20 min. Filter through 0.45 µm membrane filter. Pipette 1ml from this solution and dilute to 100 ml mobile phase.

2.3.2 Preparation of Sample

Weigh accurately sample equivalent to standard. Dissolve it in 100 ml of mobile phase. Sonicate it for15 min. Filter the solution through 0.45 µm membrane filter. Pipette 1ml from this solution and dilute to 100 ml mobile phase. The absorbance of standard and sample was taken at 270nm.

2.3.3 Validation of the developed RP-HPLC method

2.3.3.1 Specificity

Specificity is the ability to measures accurately and specifically the analyte of interest in the other components that may be expected to be present in the sample matrix. Distinguish an analyte from known impurities, synthetic precursors, metabolite or degradation products and other inactive. It should be shown by its resolution with these compounds. Check the interference of placebo by injecting into the equilibrated system. There should not be any peak at the R.T. of main peak.

2.3.3.2 Preparation of specificity solution

Specific amount of placebo and API standard was accurately weighed and transferred it into a 100 ml volumetric flask, 30 ml of diluents was added & sonicated for 5 to 10 min to dissolve the drug completely and finally the 100ml volume was made with diluents. The amount of placebo and API added were summarized in following table.

2.3.3.3 Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte.

Determination: The accuracy of an analytical method is determined by applying the method to analyzed samples or placebo sample to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay.

2.3.3.4 Precision

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample.

Determination

Intra-day precision: Standard solutions containing 500µg/ml RUPATADINE FUMARATE and MONTELUCAST SODIUM were analyzed three times on the same day and % RSD was calculated.

Inter-day precision: Standard solutions containing 1250µg/ml for RUPATADINE FUMARATE and MONTELUCAST SODIUM were analyzed three times on different days and % RSD was calculated. It is usually expressed as standard deviation or relative standard deviation.

2.3.3.5 Linearity

Linearity of an analytical method is its ability to elicit test results that are directly or by a well defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Determination:

The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration. Percentage curve fittings are calculated.

2.3.3.6 Ruggedness

It is the degree of reproducibility of the test result obtained by analysis of samples, under a variety of condition such as different lab, analyst, instrument, lots of reagents, elapsed time, different time, temp, days etc.

Determination:

The ruggedness of an analytical method is determined by analysis of Aliquots from homogenous lots by different analysts using operational and Environmental conditions that may differ but are still within the specified Parameters of the assay. The degree of reproducibility of test results is then Determined as a function of the assay variables.

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

$$\textbf{LOD} = 3.3 * \sigma / \text{slope}, \quad \textbf{LOQ} = 10 * \sigma / \text{slope}$$

(Where σ = the standard deviation of the response and S = Slope of calibration curve)

2.3.3.7 Robustness

It is the measure of capacity of the method to remain unaffected by small but deliberate variation in method parameter and provides an indication of its reliability under normal usage.

Determination:

The robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. For example change in physical parameters like P^H of mobile phase and its ratio. Standard preparation, placebo preparation and sample preparation in triplicate were prepared.

3. Results and Discussion

System suitability results were given by table no. 01 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. 01 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 no. 02 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 99.83 and 99.98 for Rupatadine fumarate and Montelukast sodium respectively. The method precision was passed for both the drugs given in table no. 03 and 04. Linearity calibration curve was given below fig: 4the regression coefficient of Rupatadine fumarate is 0.9989 and Montelukast sodium is 0.9999. The LOD were 0.141 and 0.167 µg/mL for Rupatadine fumarate and Montelucast sodium, respectively. For Rupatadine fumarate and Montelucast sodium, the LOQ were found to be 0.429 and 0.507 µg/mL respectively.

Fig. 01: Chromatogram for sample solution

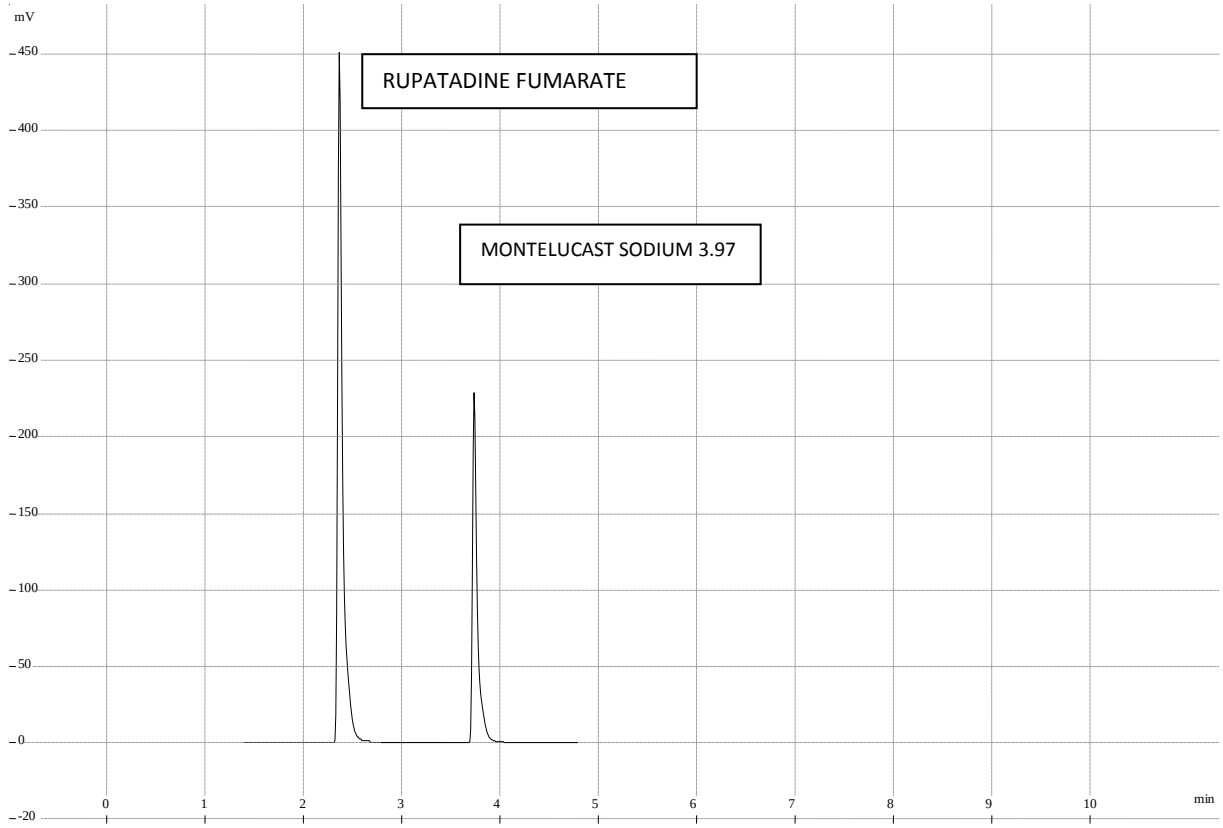


Fig. 02: Linearity graph for Rupatadine fumarate

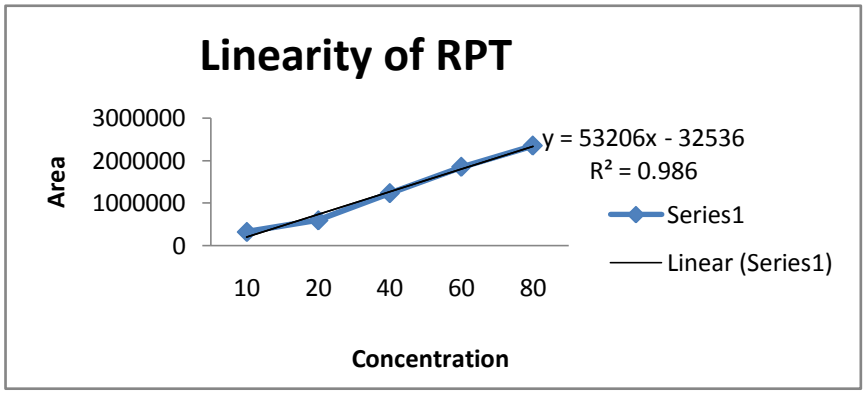
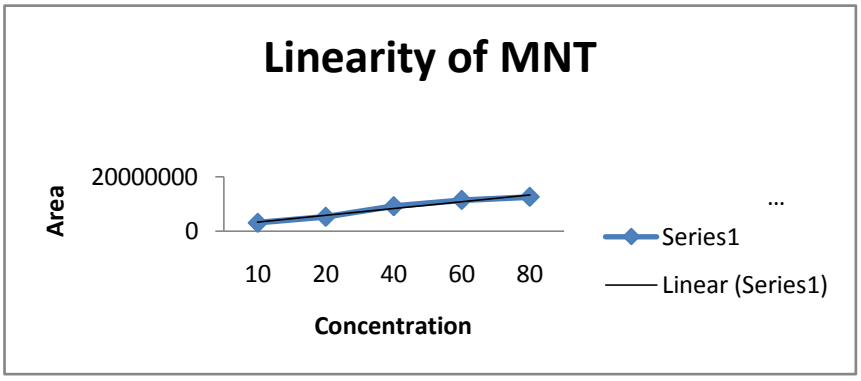


Fig. 03: Linearity graph for Montelukast sodium



Results for Inter-day Precision:

Figure No.4. Chromatogram for Inter-day Precision study; Day-1

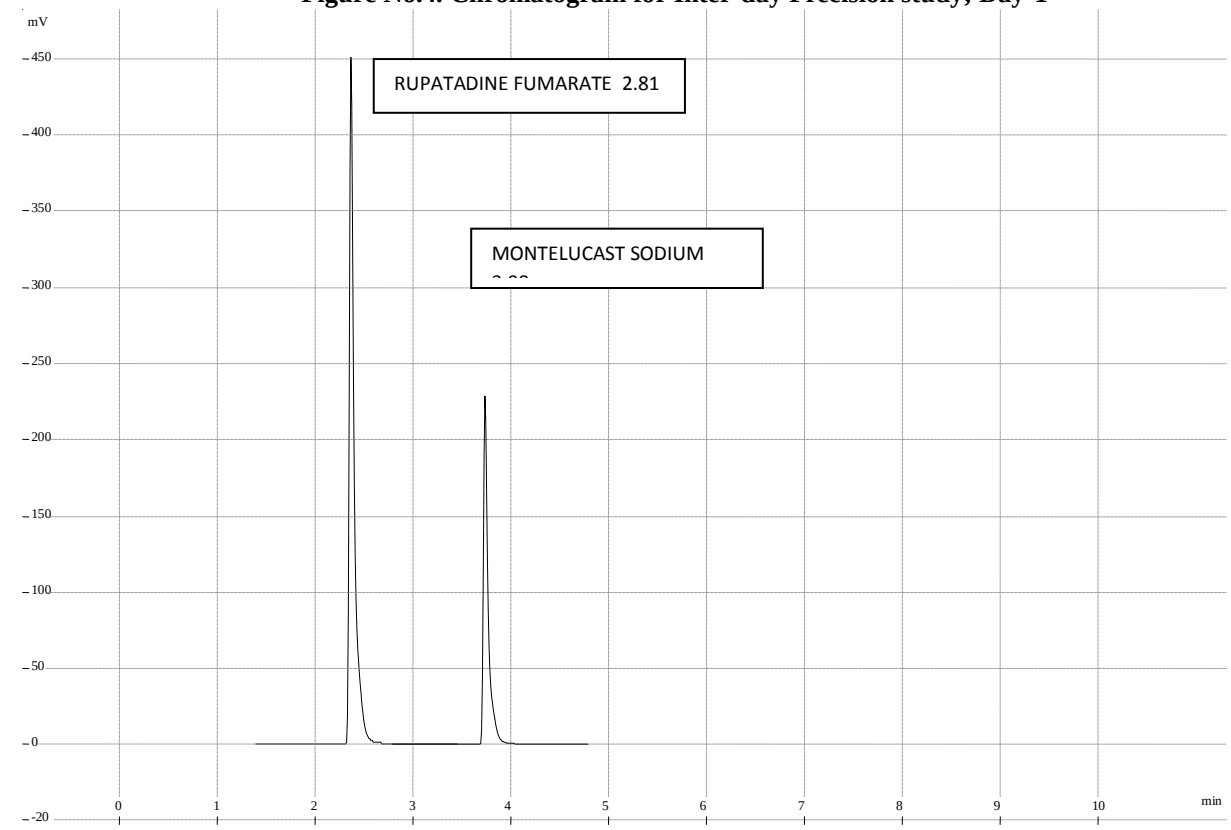


Figure No.5: Chromatogram for Inter-day Precision study; Day-2

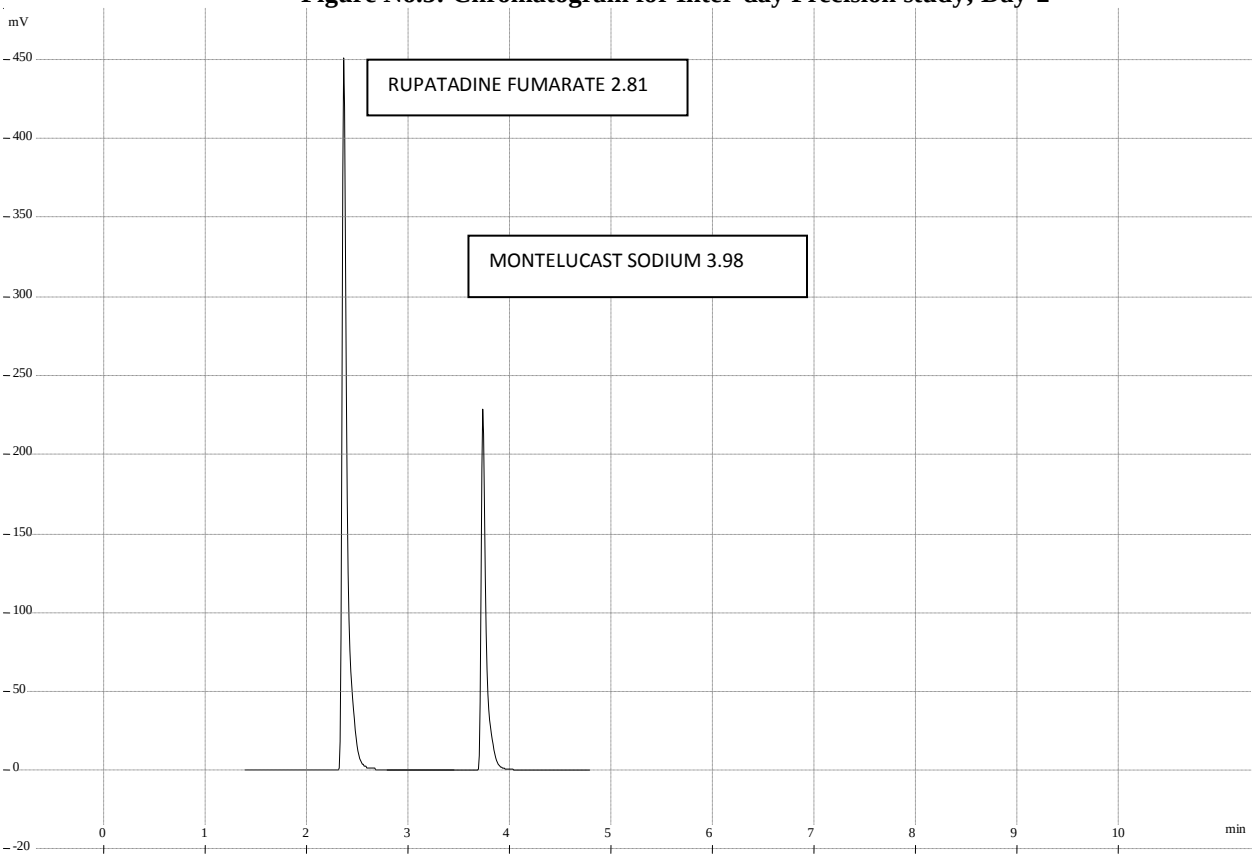
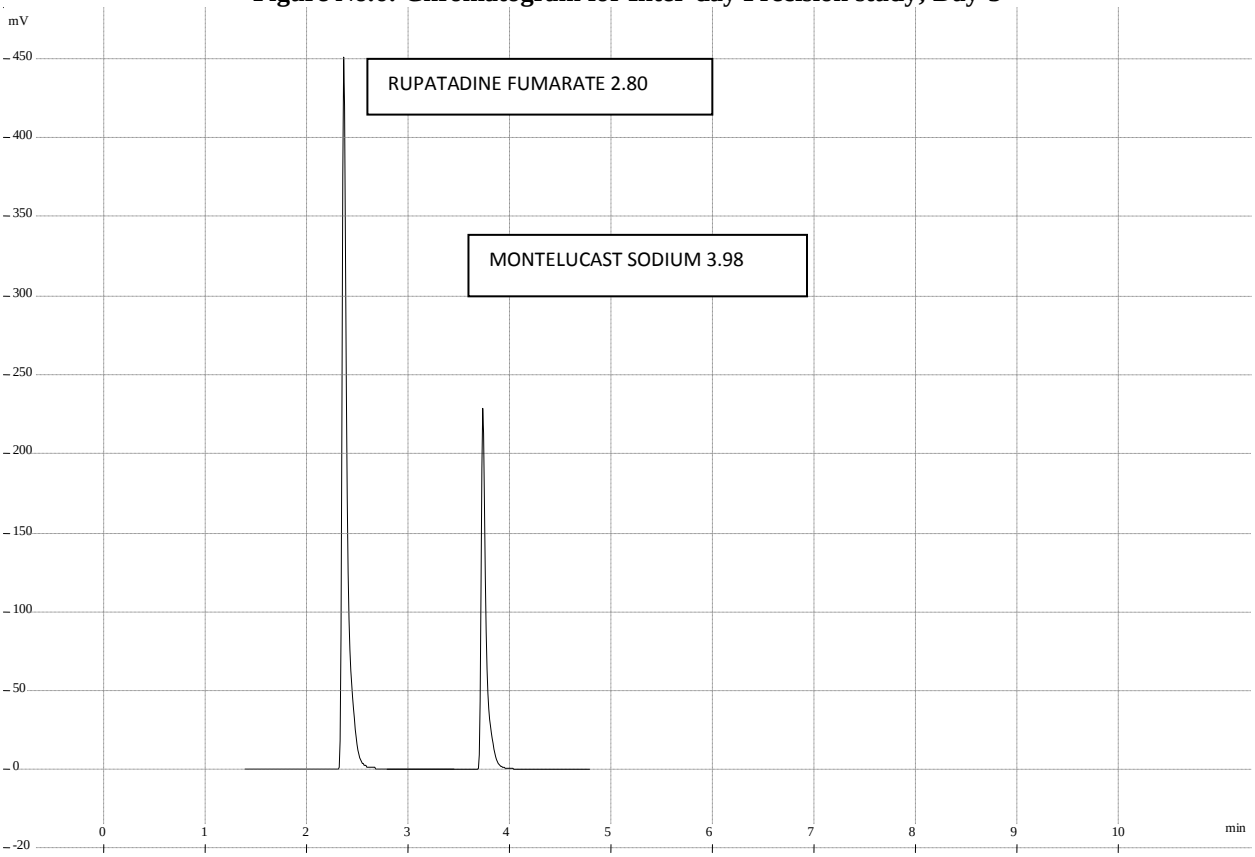


Figure No.6: Chromatogram for Inter-day Precision study; Day-3



Results for Intra-day precision: Figure No.7 Chromatogram for Intra-day Precision study; Trial-1

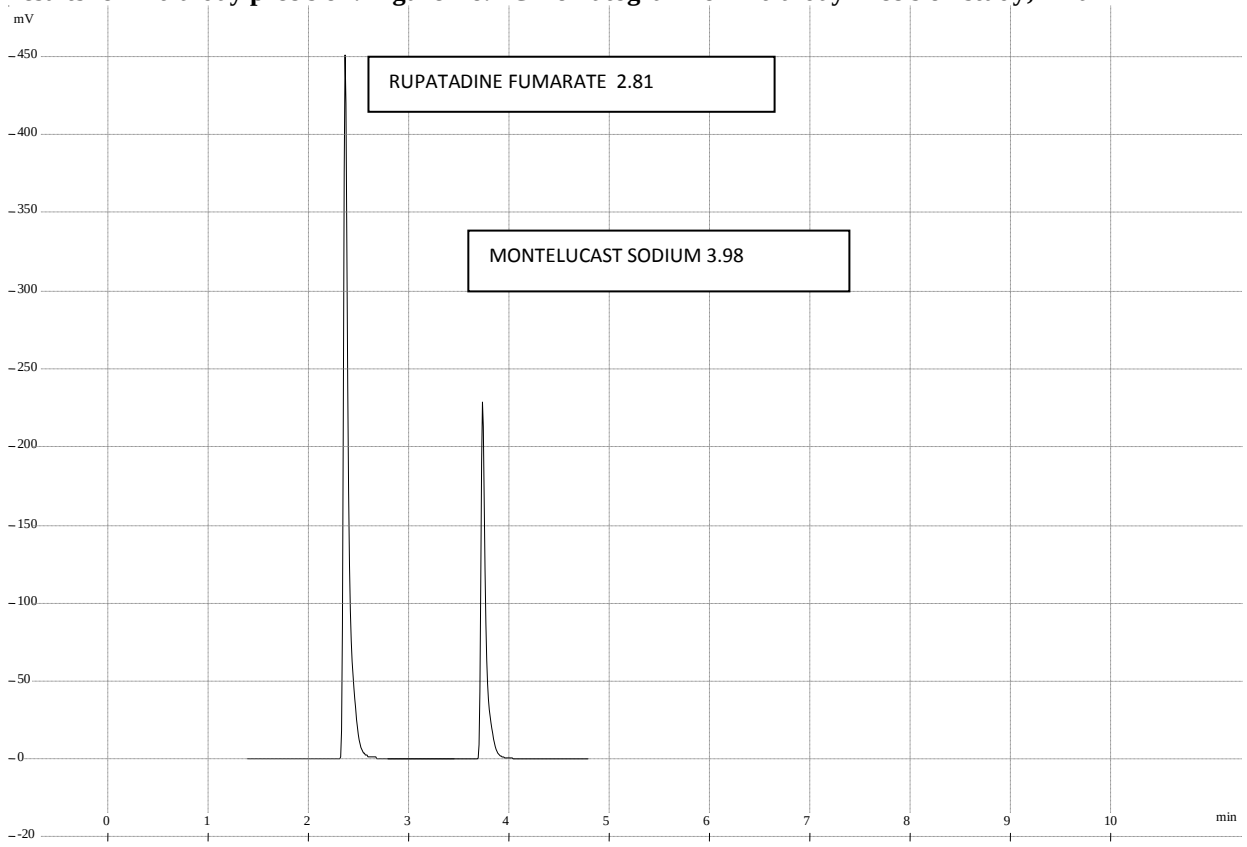


Figure No.8: Chromatogram for Intra-day Precision study; Trial-2

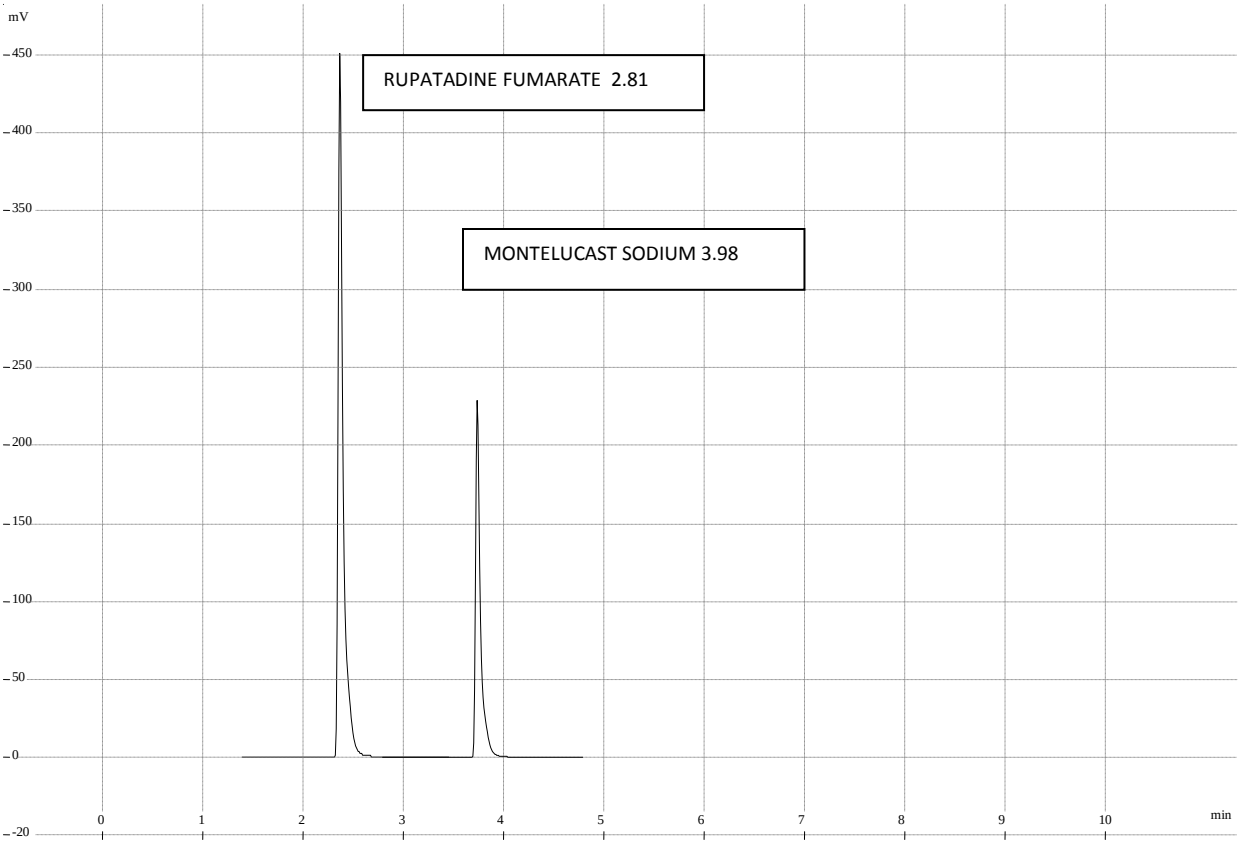


Figure No.9: Chromatogram for Intra-day Precision study; Trial-3

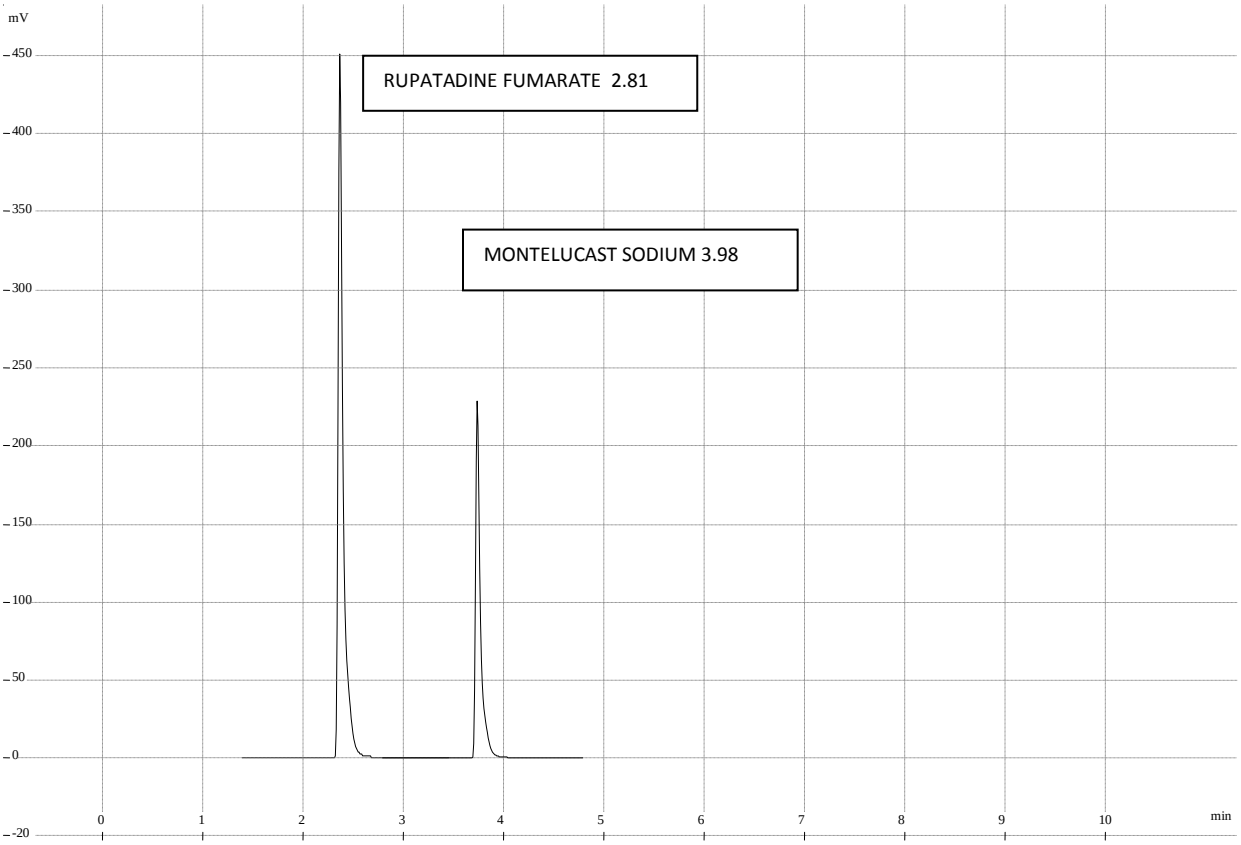


Table no. 01. System Suitability parameters

Sr. no.	Parameter	Rupatadine fumarate	Montelukast sodium
1	Retention time	2.79	3.97
2	Theoretical plates	7775.68	6958.94
3	Tailing factor	1.43	1.66
4	Resolution	4.37	4.37

Table no. 2. Accuracy observation of Rupatadine fumarate and Montelukast sodium

Drug	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	RSD
Rupatadine fumarate	400654.00	62.5	62.06	99.29	1.07	1.08
	322259.31	50	46.91	99.83		
	236065.71	37.5	36.56	97.49		
Montelucast sodium	3381243.60	156.25	156.23	99.98	1.54	1.55
	2700920.17	125	124.79	99.83		
	1964414.97	93.75	90.76	96.81		

Table no. 3. Results of Intra-Day variability for Rupatadine fumarate and Montelucast sodium

Drug	Trial No	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	RSD
Rupatadine fumarate	1	322259.31	50	49.91	99.83	0.09814	0.09843
	2	321697.09		49.83	99.66		
	3	321713.03		49.83	99.66		
Montelucast sodium	1	2700920.17	125	124.79	99.83	0.07505	0.07518
	2	2698897.26		124.70	99.76		
	3	2703009.03		124.89	99.91		

Table no. 4: Results of Inter-Day variability for Rupatadine fumarate and Montelucast sodium

Drug	Trial No	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	RSD
Rupatadine Fumarate	1	321552.69	50	49.80	99.60	0.16165	0.16
	2	321554.06		49.80	99.60		
	3	322413.77		49.94	99.88		
Montelucast sodium	1	2698165.03	125	124.67	99.73	0.04041	0.041
	2	2699370.97		124.72	99.78		
	3	2700199.66		124.76	99.81		

Table no.5: LOD results of the method

	Rupatadine Fumarate	Montelucast Sodium
LOD	0.141 µg/mL	0.167 µg/mL

Table no. 6: LOQ results of the method

	Rupatadine Fumarate	Montelucast Sodium
LOQ	0.429 µg/mL	0.507 µg/mL

Table no. 07: Analysis Data for Tablet Formulation

Drug	Label claim (mg/tab)	Amount found (mg/tab)	Percent label claim	% RSD
Rupatadine fumarate	50	50.01	100.02	0.39
Montelucast Sodium	125	125.15	100.12	0.22

4. Conclusion

The modalities adopted in experiment were successfully validated as per ICH guidelines analytical procedures laid down in routine analysis. The proposed method was validated by preliminary analysis of standard sample and by recovery studies. Based on the results obtained, it can be concluded that the proposed RP-HPLC method for the simultaneous determination of Rupatadine fumarate and Montelucast sodium is simple, linear, precise, accurate, robust, and rugged. The utility of the developed methods have been demonstrated by analysis of combined dose tablet formulation. Hence, the proposed method can be used for quantitative determination of these ingredients in combined dose tablet formulation.

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