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Research Article

METHOD DEVELOPMENT AND VALIDATION OF MONTELUKAST AND RUPATADINE IN TABLET DOSAGE FORMS BY RP-HPLC

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ABSTRACT:
RP-HPLC method was developed for the simultaneous estimation of Montelukast and Rupatadine in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using a Sunfire C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of Na_2HPO_4 buffer and acetonitrile in the ratio 60:40 % v/v, pumped at a flow rate of 1.0 mL/min. The detection wavelength was optimized at 235.0 nm and the column temperature was maintained at 30°C. The retention times of Montelukast and Rupatadine were found to be 2.280 min and 2.976 min, respectively. %RSD values of 0.5% for Montelukast and 0.4% for Rupatadine confirmed the precision of the method. Accuracy results demonstrated excellent recovery, with 99.64% for Montelukast and 99.54% for Rupatadine. LOD and LOQ for Montelukast were 0.03 µg/mL and 0.10 µg/mL, while for Rupatadine they were 0.01 µg/mL and 0.04 µg/mL, respectively, indicating the sensitivity of the method. The %Assay was obtained as 99.28% for Montelukast and 99.37% for Rupatadine. The regression equations were y = 64680x + 6030 for Montelukast and y = 66027x + 547.57 for Rupatadine, showing excellent linearity. Overall, the proposed RP-HPLC method was validated successfully and can be reliably applied for routine quality control analysis of Montelukast and Rupatadine in combined dosage forms.

Key Words: Montelukast, Rupatadine, RP HPLC, Validation.

INTRODUCTION

Montelukast and Rupatadine are widely prescribed therapeutic agents used in the management of allergic disorders. Montelukast is a selective leukotriene receptor antagonist that inhibits the cysteinyl leukotriene receptor (CysLT1), thereby preventing airway edema, bronchoconstriction, and inflammatory responses associated with asthma and allergic rhinitis[1]. It has demonstrated proven clinical efficacy in acute and chronic asthma, exercise-induced bronchoconstriction, and seasonal allergic rhinitis due to its anti-inflammatory profile and oral bioavailability[2]. Montelukast has also shown emerging therapeutic potential in chronic urticaria and pediatric asthma management owing to its favorable safety index[3,4]. Montelukast is a leukotriene receptor antagonist used as part of an asthma therapy regimen, to prevent exercise-induced bronchoconstriction, and to treat seasonal allergic rhinitis. Montelukast is a leukotriene receptor antagonist that binds to the CysLT type 1 receptor with high affinity and selectivity. This helps to prevent any physiological activities of CysLTs, such as LTC4, LTD4, and LTE4, at the receptor that could exacerbate allergic rhinitis or asthma.[5]

It is also written as 2-[1-({[(1R)-1-{3-[(1E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl}-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfanyl}methyl) cyclopropyl] acetic acid. Rupatadine is a second-generation antihistamine with dual antagonistic activity against histamine H1 receptors and platelet-activating factor (PAF), offering potent anti-allergic and anti-inflammatory effects[6]. It has been effectively utilized in the management of chronic urticaria, allergic rhinitis, and other hypersensitivity-related conditions with minimal sedation and improved tolerability compared to earlier antihistamines.[7] Rupatadine demonstrates rapid onset of action and prolonged duration due to its receptor selectivity and pharmacokinetic behavior[8]. It is known as 13-chloro-2-{1-[(5-methylpyridin-3-yl)methyl]piperidin-4-ylidene}-4-azatricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3(8),4,6,11,13-hexaene.[9] Recently, the combination of Montelukast and Rupatadine has gained attention due to their complementary mechanisms of action, yielding enhanced symptom relief in allergic rhinitis and chronic urticaria patients compared to monotherapy.[10]

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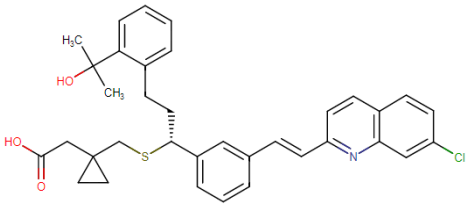

Figure 1: Structure of Montelukast

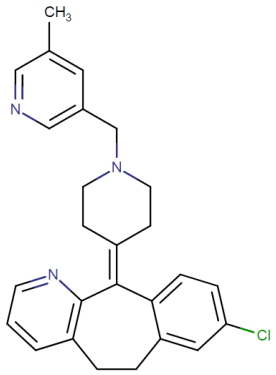

Figure 2: Structure of Rupatadine

Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently few documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Montelukast, Rupatadine, and their medicinal dose form using RP-HPLC [11 - 15] must be validated and developed as per ICH guidelines

Materials and Methods: Spectrum pharma Research Solution provided with Montelukast and Rupatadine pure drugs (API) gift samples and Combination Montelukast and Rupatadine tablets (**Rupanex M**). The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation: The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective: In order to fulfill ICH standards, we need to design and test an HPLC technique that can detect Rupatadine and Montelukast in pharmaceutical formulations at the same time.

Table 1: Chromatographic Conditions

Mobile phase	Acetonitrile and Na_2HPO_4 (40:60 v/v)
Flow rate	1 ml/min
Column	Sunfire C18 (4.6 x 250mm, 5µm)
Detector wave length	235 nm
Column temperature	30°C
Injection volume	10 mL
Run time	5.0 min
Buffer	Na_2HPO_4

Buffer Preparation: 0.01N Na_2HPO_4 Buffer: Accurately weighed 1.41gm of Disodium phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.8 with dil. Acetic acid solution

API Preparation:

Preparation of Standard stock solutions: Accurately weighed 5 mg of Montelukast, 5mg of Rupatadine and transferred to 50ml volumetric flasks and 3/4 th of diluents was added to these flasks and sonicated for 6 minutes. Flask was made up with diluents and labeled as Standard stock solution. (100µg/ml of Montelukast and 100µg/ml Rupatadine). From this 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (10µg/ml of Montelukast and 10µg/ml Rupatadine)

Formulation Preparation:

Preparation of Sample stock solutions: 10 Tablets were accurately weighed and average weight equivalent[(Rupatadine (10mg) Montelukast (10mg)] to 1 tablet was transferred into a 50ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (200µg/ml of Montelukast and 200µg/ml of Rupatadine) from this 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (10µg/ml of Montelukast and 10µg/ml Rupatadine)

System suitability parameters: Montelukast (10 ppm) and Rupatadine (10 ppm) standard solutions were prepared, injected six times, and metrics such as peak tailing, resolution, and USP plate count were measured in order to evaluate the system suitability parameters. The region of six standard injection results should have an RSD of no more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Precision: The procedure and system accuracy were demonstrated by creating a standard solution with a known concentration and providing six repeating injections to ensure the suggested approach's consistency. Intermediate precision was also attained by producing six working sample solutions and injecting them six times. The area was measured, and the mean, standard deviation, and % RSD were calculated. The results were favourable, falling below the 2% limit.

Linearity: To test the drug's linearity, serial dilutions from 25%- 150% were prepared. A graph was used to demonstrate the link between peak area response and medication concentration. It was found to be linear at the indicated drug concentration. Dilution were as follows.

1. 25 µg/mL: Take 0.25 mL of stock solution and dilute to 10 mL
2. 50 µg/mL: Take 0.5 mL of stock solution and dilute to 10 mL
3. 75 µg/mL: Take 0.75 mL of stock solution and dilute to 10 mL
4. 100 µg/mL: Take 1.0 mL of stock solution and dilute to 10 mL
5. 125 µg/mL: Take 1.25 mL of stock solution and dilute to 10 mL
6. 150 µg/mL: Take 1.5 mL of stock solution and dilute to 10 mL

Accuracy: Accuracy was performed in triplicate for various concentrations equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. Dilution were as follows.

1. 50 µg/mL: Take 0.1 mL of stock solution and dilute to 10 mL
2. 100 µg/mL: Take 0.2 mL of stock solution and dilute to 10 mL
3. 150 µg/mL: Take 0.3 mL of stock solution and dilute to 10 mL

Sensitivity

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. Based on the response’s standard deviation and calibration curve’s slope, the LOD and LOQ can be estimated.

Assay

The assay and % purity were calculated for brand Rupanex M with label claim Rupatadine 10g and Montelukast 10mg. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form.

Degradation studies:

Oxidation: To 1 ml of stock solution of Rupatadine and Montelukast, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 10µg/ml & 10µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock s solution Rupatadine and Montelukast, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 10µg/ml &

10µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies: To 1 ml of stock solution Rupatadine and Montelukast, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 10µg/ml & 10µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 10µg/ml & 10µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 100µg/ml Rupatadine &100µg/ml Montelukast solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 10µg/ml & 10µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 10µg/ml & 10µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Table 2: System suitability results

S.no	Montelukast			Rupatadine			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.236	6759	1.53	2.950	11757	1.27	6.2
2	2.244	6782	1.53	2.954	12282	1.28	6.3
3	2.256	7985	1.53	2.989	11333	1.37	6.4
4	2.257	6826	1.52	2.989	11935	1.31	6.3
5	2.257	6756	1.52	2.994	12256	1.32	6.3
6	2.260	6578	1.52	2.997	11681	1.28	6.4

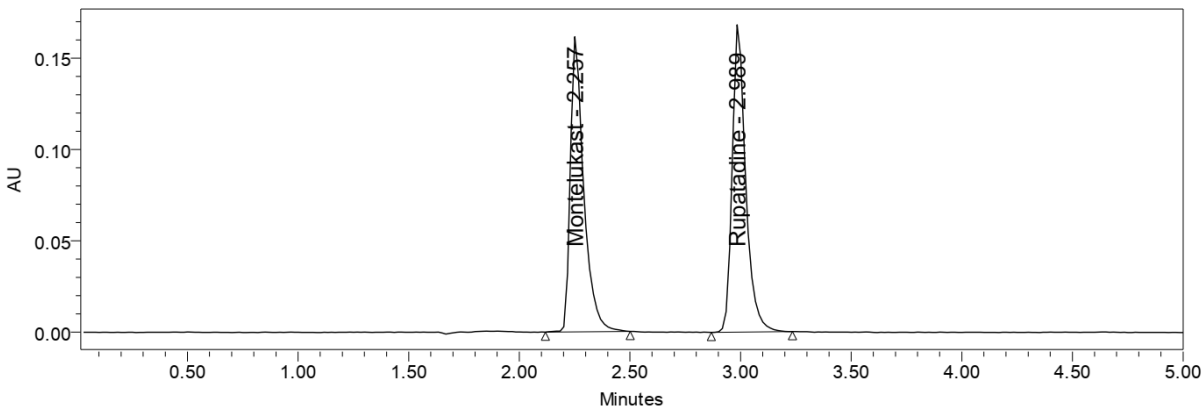

Figure 3: system suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time	Area	Plate count	Tailing	Resolution
Montelukast	2.280	652689	6457.3	1.2	
Rupatadine	2.976	653571	10398.3	1.3	6.3

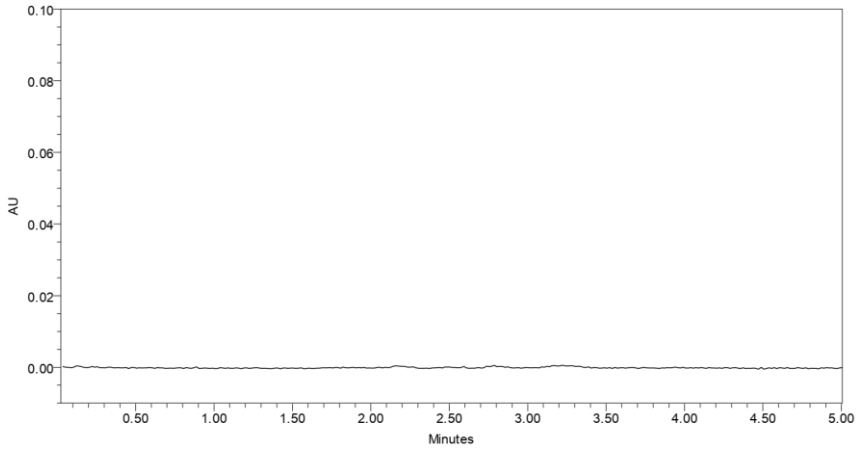

Figure.4 Specificity of Montelukast and Rupatadine

Linearity:

Calibration data is given in table 4 and regression data in table 5 and calibration curve in figure 5, 6

Table 4: Calibration data of Montelukast and Rupatadine

Montelukast		Rupatadine	
Conc (µg/mL)	**Peak area**	**Conc(µg/mL)**	**Peak area**
0	0	0	0
2.5	163929	2.5	163471
5	331177	5	339297
7.5	498270	7.5	489021
10	661693	10	662137
12.5	823539	12.5	822981
15	959277	15	993342

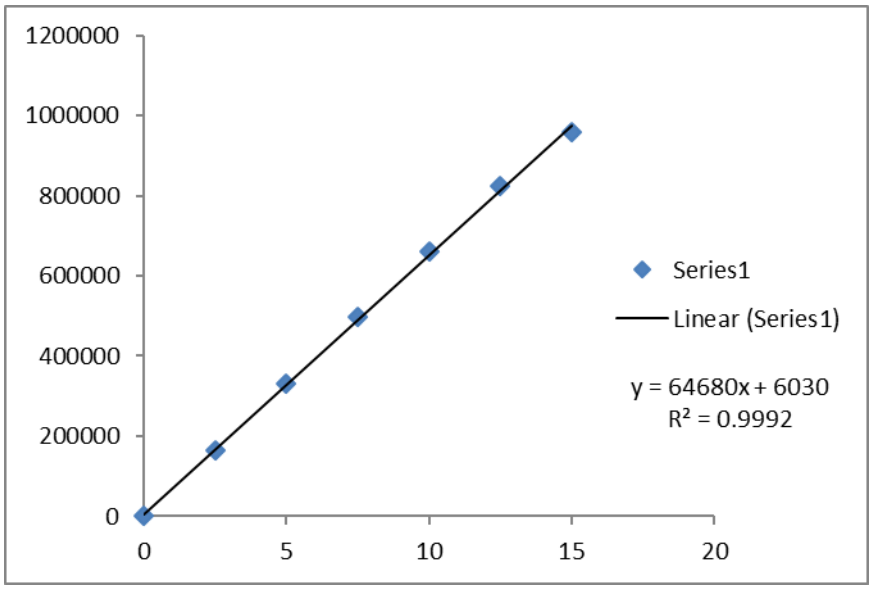

Figure 5 Calibration curve of Montelukast

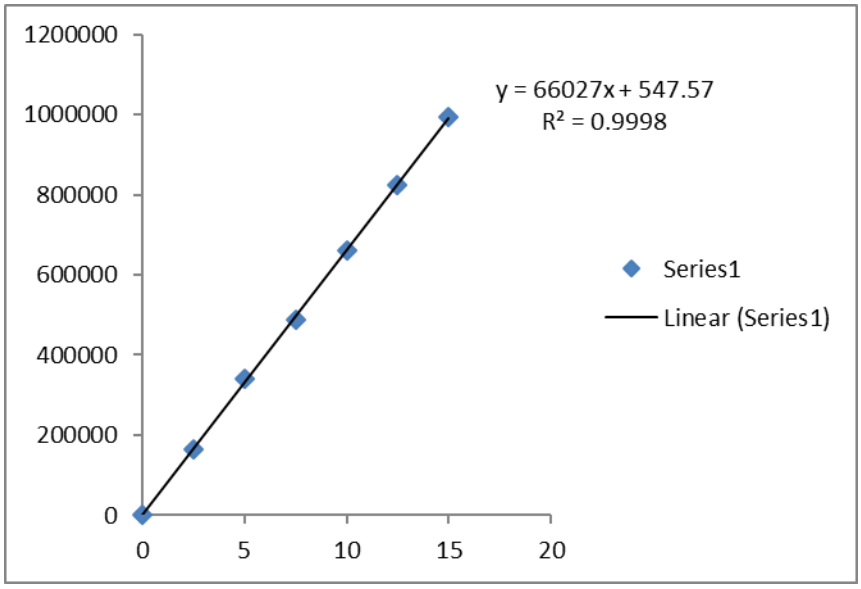

Figure 6 Calibration curve of Rupatadine

Table 5: regression data

Parameter	Montelukast	Rupatadine
Conc range (µg/mL)	2.5 – 15 µg/ml	2.5-15 µg/ml
Regression Equation	y = 64680x + 6030	y = 66027x + 547.57
Co-relation	0.999	0.999

Accuracy:

Recovery data shown in table 6

Table 6: recovery data of Montelukast and Rupatadine

	Montelukast			Rupatadine		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	5	4.99	99.81	5	4.98	99.67
		4.96	99.21		4.99	99.79
		4.99	99.82		4.98	99.61
100%	10	9.908	99.08	10	9.95	99.51
		9.996	99.96		9.93	99.31
		9.969	99.69		9.99	99.94
150%	15	14.92	99.47	15	14.96	99.71
		15.00	99.99		14.87	99.10
		14.96	99.70		14.88	99.23
% recovery	**99.64**			**99.54**		

System precision was performed and the data was shown in table 7

Table 7: System precision of Montelukast and Rupatadine

S. No	Area of Montelukast	Area of Rupatadine
1.	661685	664190
2.	660480	665590
3.	662123	664864
4.	660434	661971
5.	662616	660632
6.	669192	659538
Mean	662755	662798
S.D	3273.0	2449.7
%RSD	0.5	0.4

The % RSD for the peak areas of Montelukast and Rupatadine obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Montelukast and Rupatadine and shown in table 8.

Table 8: method Precision

S. No	Area of Montelukast	Area of Rupatadine
1.	660220	661872
2.	659622	659616
3.	660080	659543
4.	659120	658979
5.	659975	654755
6.	652959	661054
Mean	658663	659303
S.D	2822.0	2474.3
%RSD	0.4	0.4

From the above results, the % RSD of method precision study was within the limit for Montelukast and Rupatadine.

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.0ml/min), mobile phase minus (65A:35B), mobile phase plus (55A:45B), temperature minus (27°C) and temperature plus(33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 9: Robustness data for Montelukast and Rupatadine.

Condition	%RSD of Montelukast	%RSD of Rupatadine
Flow rate (-) 0.9ml/min	0.2	0.5
Flow rate (+) 1.1ml/min	0.8	0.8
Mobile phase (-) 65A:35B	0.5	0.9
Mobile phase (+) 55A:45B	0.6	0.7
Temperature (-) 27°C	0.2	0.2
Temperature (+) 33°C	0.3	0.2

Sensitivity:

Table 10: sensitivity of Montelukast and Rupatadine

Molecule	LOD	LOQ
Montelukast	0.03 µg/ml	0.10 µg/ml
Rupatadine	0.01 µg/ml	0.04 µg/ml

Force Degradation Studies: table 11 shows degradation conditions and table 10 shows the obtained degraded data and purity plot chromatogram in figure 8, 9.

Table 11: degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	60 mins
Base	2N NAOH	60^{0}c	60 mins
Oxdation	20% H_2O_2	60^{0}c	60 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	60 mins

Table 12: degradation data

Conc of degradation study	Montelukast		Rupatadine	
	% drug Undegraded	% drug degraded	% drug Undegraded	% drug degraded
Acid	96.71	3.29	97.91	2.09
Base	96.87	3.13	97.95	2.05
Peroxide	95.68	4.32	96.38	3.62
Thermal	98.10	1.90	98.10	1.90
UV	98.55	1.45	98.11	1.89
Water	99.47	0.53	99.46	0.54

Acid degradation chromatogram

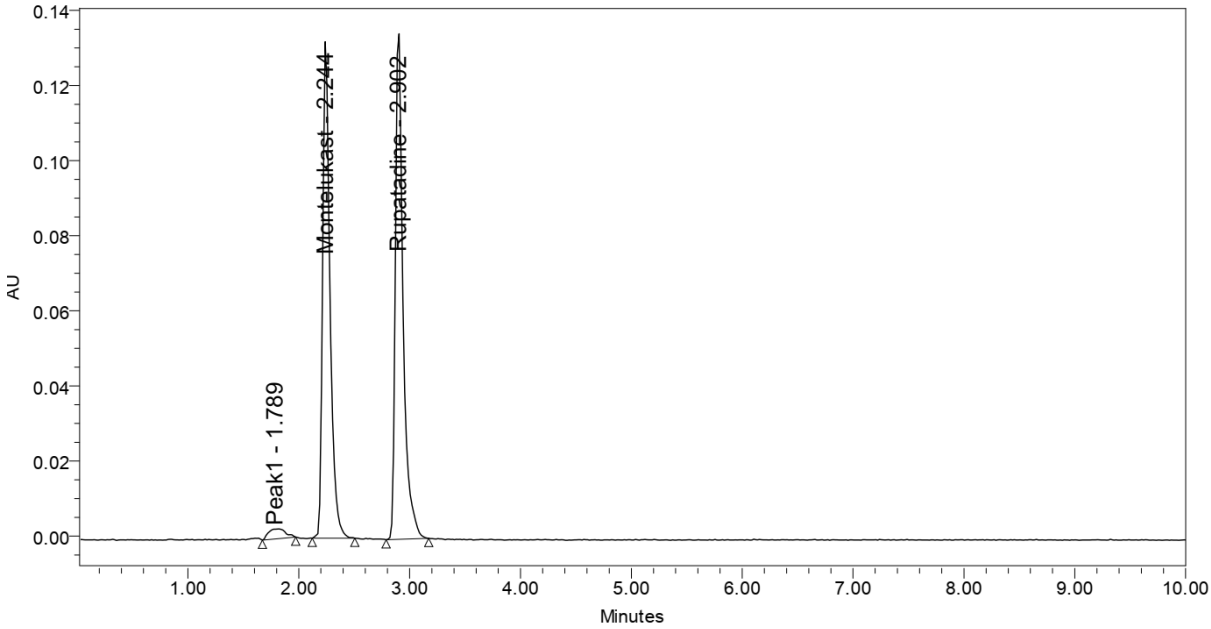

Fig 7 acid

Base degradation chromatogram

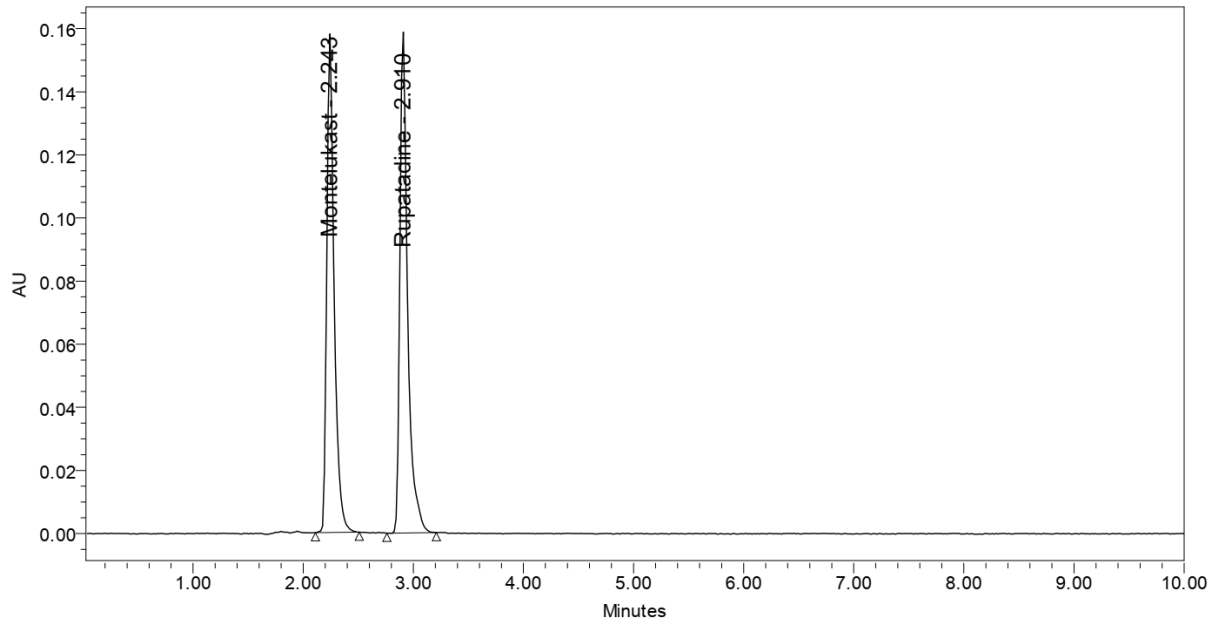

Fig 8 base

Peroxide degradation chromatogram

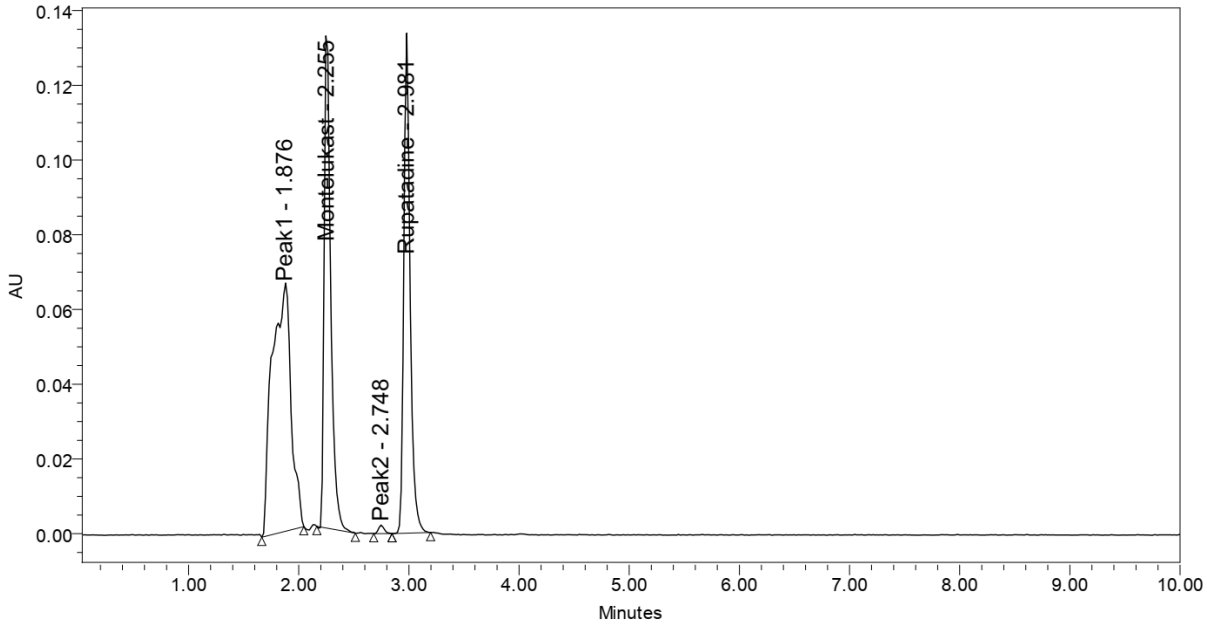

Fig 9 peroxide

Thermal degradation chromatogram

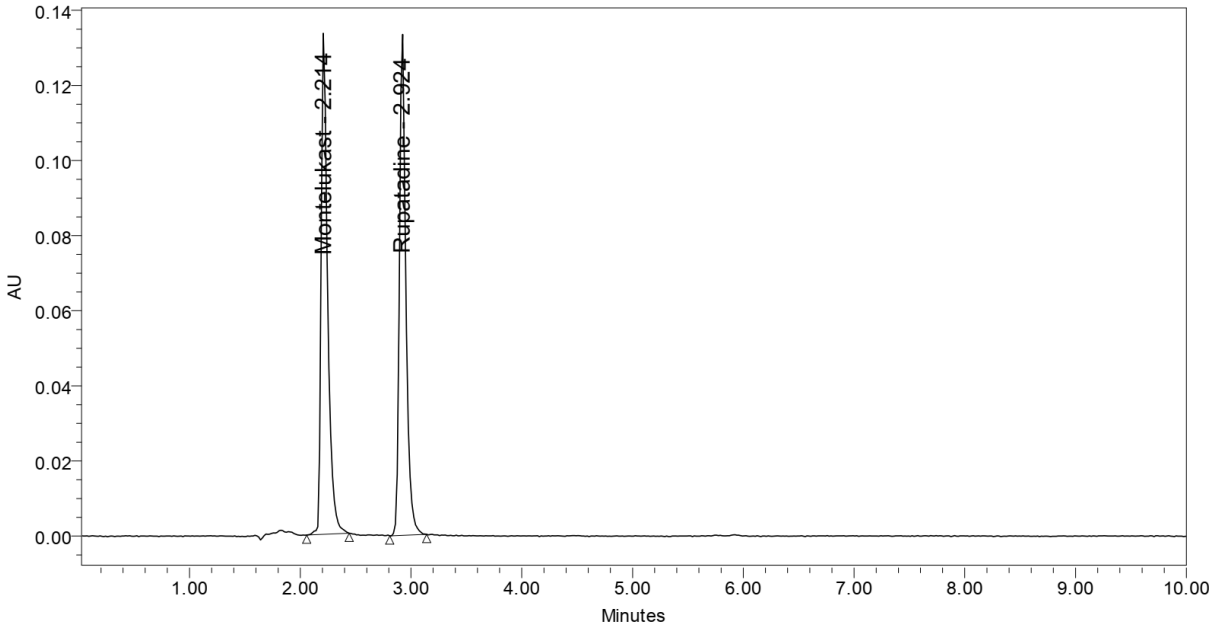

Fig 10 thermal

UV degradation chromatogram

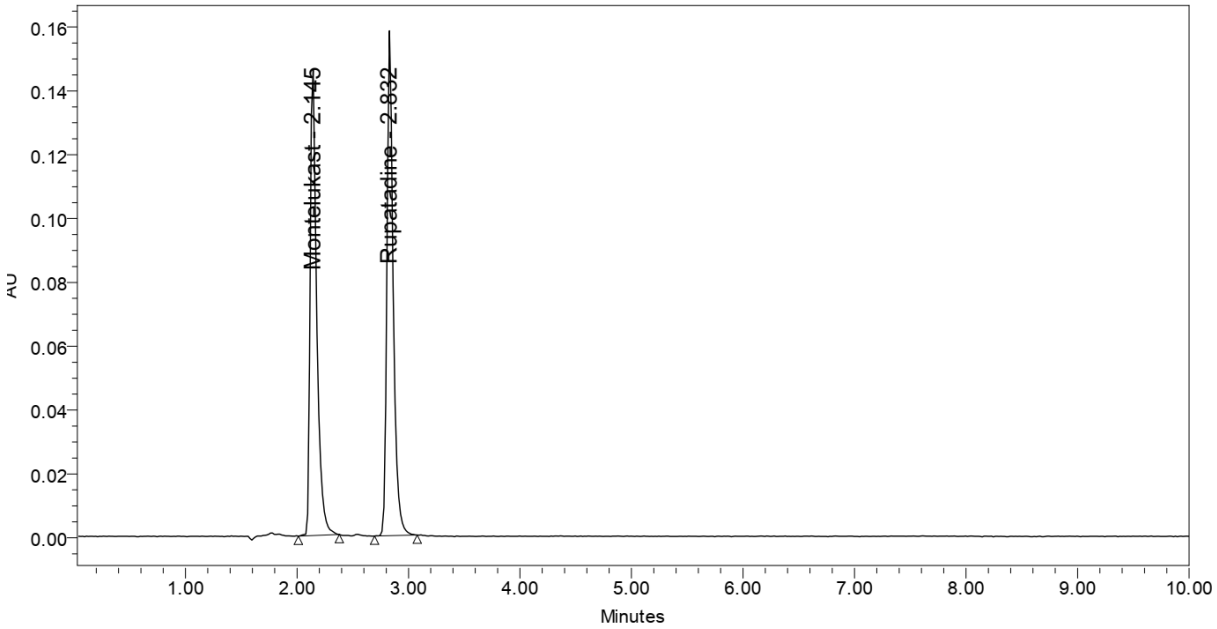

Fig 11 UV

Water degradation chromatogram

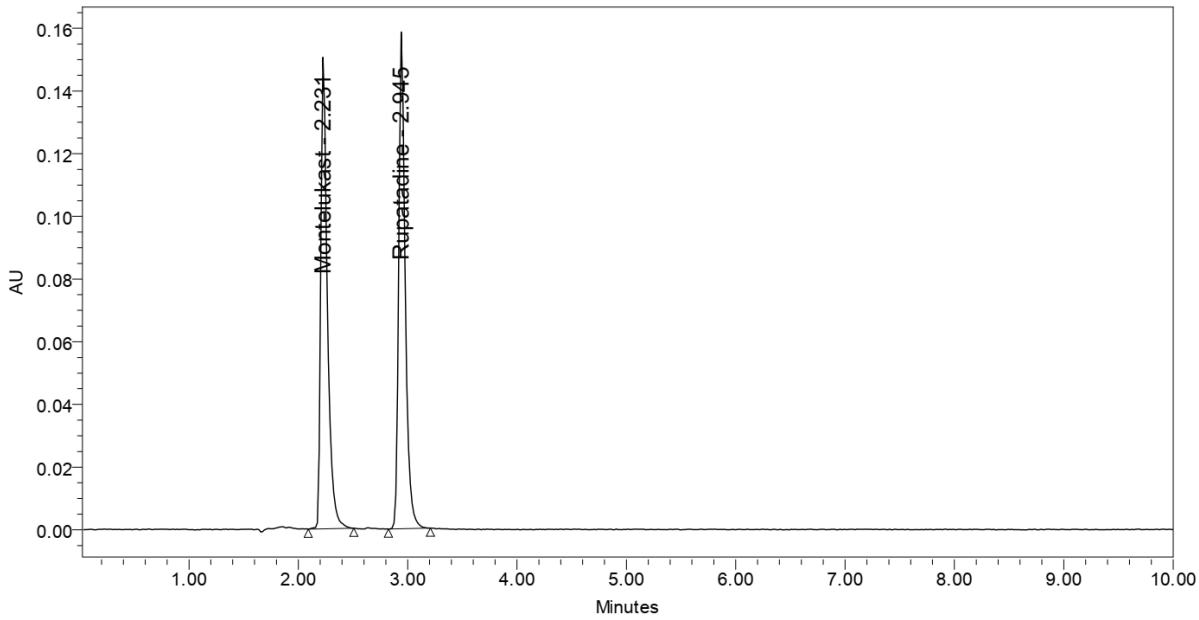

Fig 12 water

Assay: Rupanex M, bearing the label claim Montelukast 10mg, Rupatadine 10mg. Assay was performed with the above formulation. Average % Assay for Montelukast and Rupatadine obtained was 99.28% and 99.37% respectively.

Table 13: assay data

Formulation	Label claim(mg)	% Assay*
Rupanex M	Rupatadine 10mg.	99.37%w/w
	Montelukast 10mg	99.28%w/w

Conclusion:

The developed RP-HPLC method provides a simple, precise, and highly efficient analytical approach for the simultaneous estimation of Montelukast and Rupatadine in pharmaceutical dosage forms. The optimized chromatographic conditions enabled excellent separation of both analytes with well-resolved retention times, satisfying all system suitability requirements. Validation parameters confirmed that the method is linear, accurate, precise, robust, and sensitive within the tested concentration ranges. The low percentage of RSD values and high recovery rates demonstrate the reliability and reproducibility of the method. Therefore, this RP-HPLC method is suitable for routine quality control analysis, stability studies, and formulation development of Montelukast and Rupatadine combinations

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