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RP-UPLC method development and validation for the simultaneous estimation of montelukast and ebastine in bulk and pharmaceutical dosage form

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ABSTRACT

A simple, accurate, precise and reliable RP-UPLC method was developed for the simultaneous estimation of the Montelukast and Ebastine in pharmaceutical dosage form. Mobile phase with 0.1% Ortho phosphoric acid: Acetonitrile in the ratio of 60:40 run through Waters C18 150 x 3 mm, 2µ.column at a rate of 0.3ml/min. at temperature 30°C and optimized wavelength was set at 244nm. Retention time of Montelukast and Ebastine were found to be 1.298 min and 1.636 min. The method developed was validated in accordance with ICH guidelines with respect to the stability indicating capacity of the method including system suitability, accuracy, precision, linearity, range, limit of detection, limit of quantification and robustness. This method passed all the validation parameters; hence, it can be employed for routine quality control of Montelukast and Ebastine in pharmaceutical industries and drug testing laboratories.

Keywords: Montelukast, Ebastine, UPLC

INTRODUCTION

Montelukast (Fig. 1), (*R*)-1-[(1-(3-(2-(7-chloro-2-quinolinyl)- ethenyl) phenyl)-3-(2-(2-hydroxy-2-propyl)phenyl) propyl) thiomethyl] cyclopropane acetic acid, is a potent and selective antagonist of the cysteinyl leukotriene receptor 1 subtype (CysLT1). Montelukast is a leukotriene receptor antagonist used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. [1] Montelukast comes as a tablet, a chewable tablet, and granules to take by mouth. [2] Montelukast is usually taken once a day with or without food. 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. This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation. Because of its mechanism of action, it is not useful in the treatment of acute asthma attacks. The Mont in Montelukast stands for Montreal, the place where Merck (MSD) developed the drug. Ebastine is1-[4-(1,1-dimethylethyl)phenyl]-4-[4-

(diphenylmethoxy)-1-piperidinyl]-1-butanone. It is a selective, long-acting, nonsedating, second-generation antihistamine. It has been reported to be safe and effective in the treatment of allergic rhinitis and chronic idiopathic urticaria. [3] Ebastine (Fig 2) is a second-generation H_1-receptor antagonist with an oxypiperidine-based structure, whose active form is the metabolite carebastine. Ebastine is administered orally once daily and is indicated for the treatment of the symptoms of allergic rhinitis and chronic idiopathic urticaria. [4] Ebastine is a H_1 antihistamine with low potential for causing drowsiness. It does not penetrate the blood–brain barrier to a significant amount and thus combines an effective block of the H_1 receptor in peripheral tissue with a low incidence of central side effects, i.e. seldom causing sedation or drowsiness.

The literature survey was carried out for the simultaneous estimation of Montelukast and Ebastine, few analyical methods are methods avaliable for Montelukast and Ebastine individually and in combination with other drugs [5-10]. According to literature survey there is no official method for the estimation of Montelukast and Ebastine by ultra performance liquid chromatography (UPLC) in pharmaceutical dosage forms. Hence, an attempt has been made to develop new method for the simultaneous estimation and validation of Montelukast and Ebastine in pharmaceutical formulation in accordance with the ICH guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

Montelukast and Ebastine pure drugs (API) were obtained from spectrum Pharma research solutions and eye drops containing ophthalmic solution equivalent to (Ebast M) 10 mg and 10mg of montelukast and ebastine was purchased from local Pharmacy store. All the chemicals and solvents like Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, Ortho-phosphoric acid are from RANKEM- Mumbai.

Instruments and Chromatographic Conditions

Electronics Balance-Denver, P^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS UPLC Aquity system equipped with quaternary pumps, UV detector and Auto sampler integrated with Empower 2 Software was used for LC peak integration and Data processing. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV-win 6 Software was used for measuring absorbance of Montelukast and Ebastine solutions. The mobile phase used was 0.1% Ortho phosphoric: Acetonitrile in the ratio of 60:40 run through Waters C18 150 x 3 mm, 2µ.column at a rate of 0.3ml/min. for 3 min at Temperature 30°C and Optimized wavelength was 244nm at the injection volume of 3µL.

Preparation of Solvents and Solution

Diluent

Diluent was selected Based on the solubility of the drugs, Water: Acetonitrile (50:50) were taken as diluent.

Preparation of buffer

Preparation of 0.1% Ortho phosphoric acid Buffer

Accurately weighed 1.36gm of Potassium dihyrogen Ortho 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 added 1ml of Triethylamine then PH adjusted to 4.8 with dil. Orthophosphoric acid solution

Preparation of Mobile Phase

Mobile phase was prepared my mixing 0.1% Ortho phosphoric: Acetonitrile in the ratio of 60:40 and sonicated using ultrasonic bath to degas and subjected to vacuum filtration with 0.45µ Millipore Nylon filter.

Preparation of Standard stock solutions

Accurately Weighed and transferred 10mg of Montelukast and 10mg of Ebastine working Standards into a 10 ml&10ml clean dry volumetric flasks, add 7ml of diluent , sonicated for 5 minutes and make up to the final volume with diluents.

Preparation of Standard working solutions (100% solution)

Accurately about1ml of above each stock solution was pipetted out and transfered into a 10ml volumetric flask and the final volume was made up with diluent. (100 µg/ml of Montelukast and 100µg/ml of Ebastine).

Preparation of Sample stock solutions

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 10mL volumetric flask, 5mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluents.

Method Validation

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

Specificity

It is the ability of analytical method to measure the response of the analyte and have no interference from other extraneous components and well resolved peaks are obtained.

LINEARITY

25% Standard solution

0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Montelukast and 25 µg/ml of Ebastine)

50% Standard solution

0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (50µg/ml of Montelukast and 50µg/ml of Ebastine)

75% Standard solution

0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (75 µg/ml of Montelukast and 75 µg/ml of Ebastine)

100% Standard solution

1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (100µg/ml of Montelukast and100 µg/ml of Ebastine)

125% Standard solution

1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (125µg/ml of Montelukast and 125 µg/ml of Ebastine)

150% Standard solution

1.5ml each from two standard stock solutions was pipetted out and made up to 10ml (150µg/ml of Montelukast and 150 µg/ml of Ebastine)

ACCURACY

Preparation of Standard stock solutions

Accurately Weighed and transferred 10mg of Montelukast and 10mg of Ebastine working Standards into a 10 ml&10ml clean dry volumetric flasks, add 7ml of diluent , sonicated for 5 minutes and make up to the final volume with diluents. From the above stock solution (1000µg/ml&1000µg/ml)

Preparation of 50% Spiked Solution

0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution

1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution

1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Robustness

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.27ml/min), Flow plus (0.33ml/min), mobile phase minus (65:35) mobile phase plus (55:45) temperature minus (25°C) and temperature plus (35°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.

LOD sample Preparation

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.3ml each of Montelukast and Ebastine solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Montelukast and Ebastine solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent. Samples were injected in duplicates.

System Suitability

By preparing standard solutions of Montelukast (100ppm) and Ebastine (100ppm) the system suitability parameters were determined the solutions were injected six times and the parameters like peak tailing, resolution and the USP theoretical plate count were assessed to check whether the results complies with Recommended limits.

Assay of Montelukast and Ebastine

An Accurately measured weight equivalent to (Ebast M)) 10 mg and 10 mg of montelukast and ebastine respectively was used to perform assay by utilizing the method developed and under the optimized chromatographic conditions. Sample solutions were injected in to the UPLC system and scanned at 244 nm from which the % of drug was estimated.

RESULTS & DISCUSSIONS

Optimization of Chromatographic Conditions

To develop and establish a suitable RP-UPLC method for simultaneous estimation of Montelukast and Ebastine in bulk and Tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 60% Ortho phosphoric acid:40% Acetonitrile at a flow rate of 1.0 ml/min. samples were analyzed at 244 nm detector wave length and at an injection volume of 3 µL using Waters C18 150 x 3 mm, 2m column with run time of 3 min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for Montelukast and Ebastine , the optimized chromatogram was obtained as shown in (Figure-3).

Chromatographic conditions

Flow rate	: 0.3 ml/min
Column	: Waters C18 150 x 3 mm, 2µ.
Detector wave length	: 244nm
Column temperature	: 30°C
Injection volume	: 3µL
Run time	: 3min
Diluent	:Water:Acetonitrile (50:50)

Validation

Linearity was established for Montelukast (25-150µg/ml) and Ebastine (25-150µg/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained as y =22149x + 8177 for Montelukast and y = 25658x + 4211 for Ebastine, Correlation coefficient (R^2) was determined as 0.999 for the two drugs. The Linearity calibration curves were plotted as shown in (Figure-4&5) for Montelukast and Ebastine, respectively. Retention times of Montelukast and Ebastine were 1.300 min and 1.636min respectively. Where no interfering peaks in blank and placebo at retention times of these drugs were not found in this method. So this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared and Triplicates of injections were given for each level of accuracy and mean %Recovery was obtained as 100.01% and 100.13% for Montelukast and Ebastine respectively were shown in (Table-2).% RSD was calculated from the corresponding peaks obtained by injecting six times a known concentration of Montelukast and Ebastine the repeatability was obtained as 0.5% and 0.6% respectively for Montelukast and Ebastine and the % RSD for intermediate Precision was obtained as 0.3%, 0.3% for Montelukast and Ebastine, Low % RSD values

indicates that the method developed was precise as shown in (Table-3). The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve Montelukast and Ebastine. The detection limit values were obtained as 0.38 and 1.15 and Quantitation limit were fund to be 0.09 and 0.28 for Montelukast and Ebastine Respectively as given in (Table-4).

Robustness conditions like Flow minus (0.27ml/min), Flow plus (0.33ml/min), mobile phase minus (65:35) mobile phase plus (55:45) temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner Table -5).. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table -6). Montelukast and Ebastine pure drugs (API) were obtained from spectrum Pharma research solutions and eye drops containing ophthalmic solution equivalent to (Ebast M)10 mg and 10mg of montelukast and ebastine equivalent to 10 mg and 10 mg of montelukast and ebastine respectively was used to perform assay and the Average % of drug was found to be 100.93 and 101.65% for Montelukast and Ebastine respectively the results were shown in (Table-7) and the chromatograms for Montelukast and Ebastine standard drugs and pharmaceutical dosage forms were shown in (Figure-6, 7) Respectively.

Degradation Studies

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 8&9).

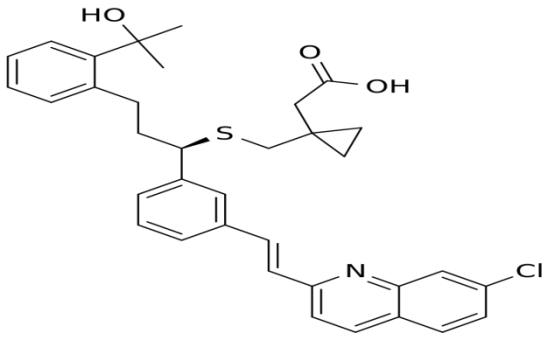

Figure-1: Chemical Structure of Montelukast

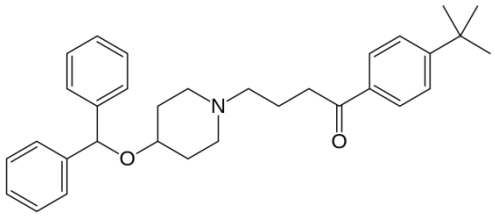

Figure-2: Chemical Structure of Ebastine

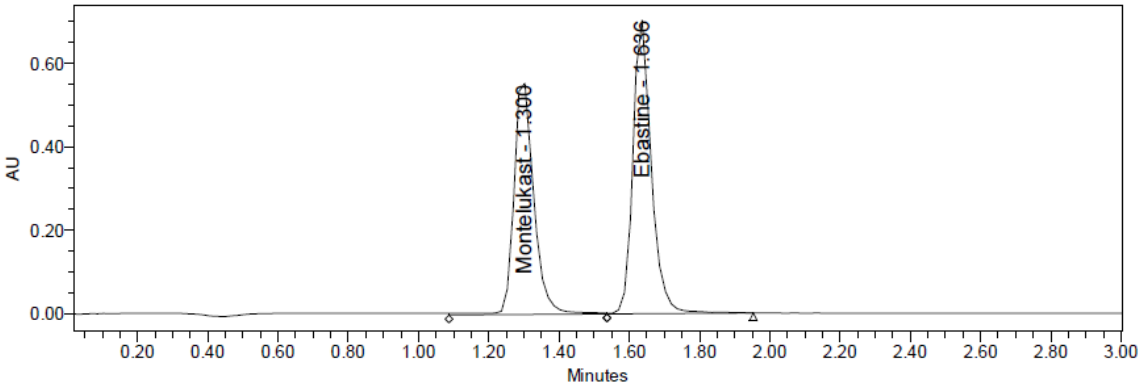

Figure-3: Optimized Chromatogram of Montelukast and Ebastine

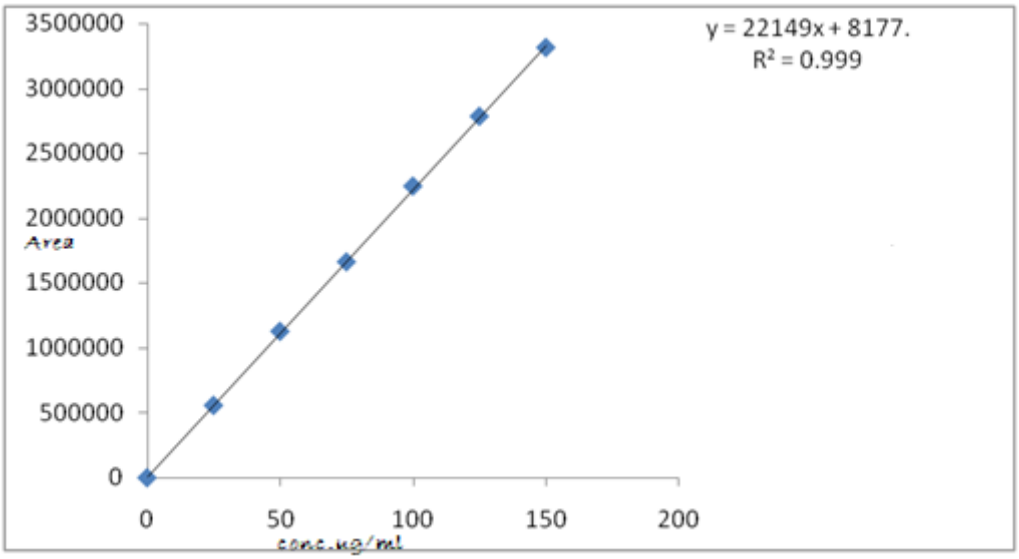

Figure-4: Linearity Curve of Montelukast

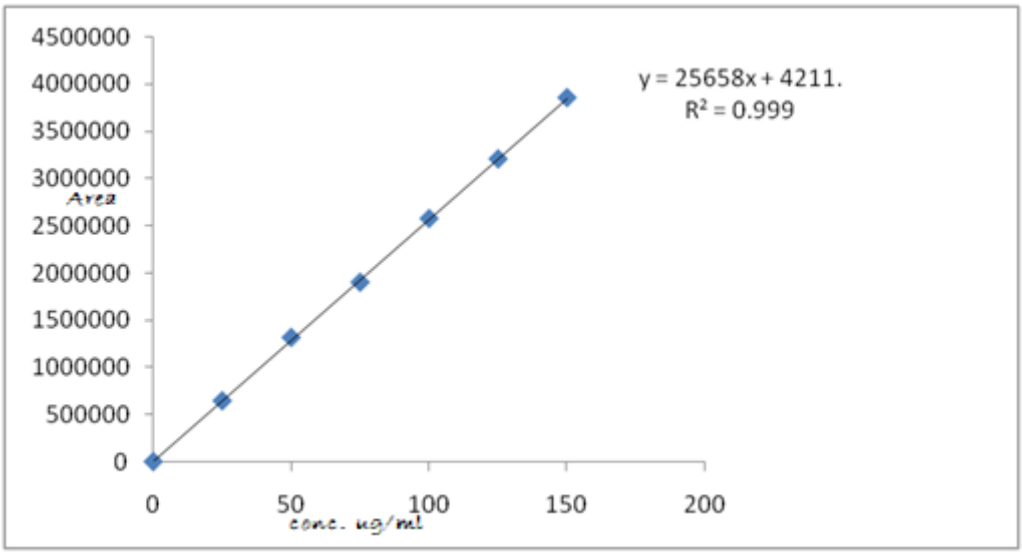

Figure-5: Calibration Curve of Ebastine

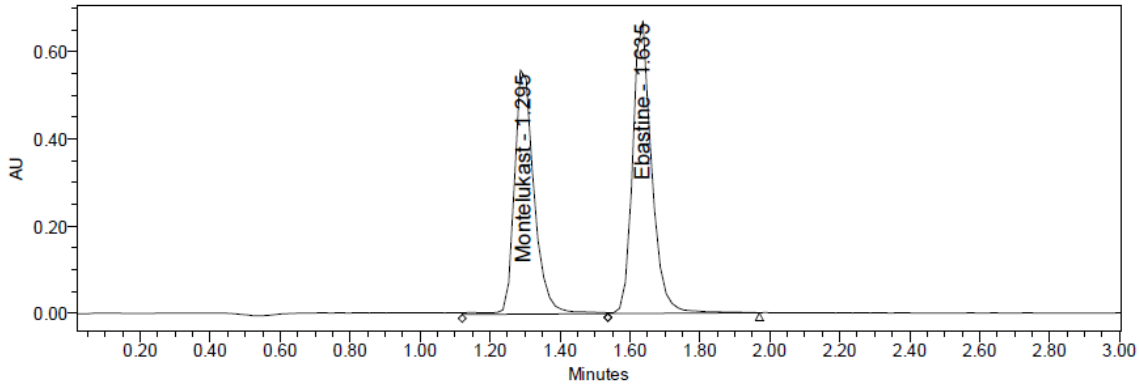

Figure-6: Standard Chromatogram of Montelukast and Ebastine

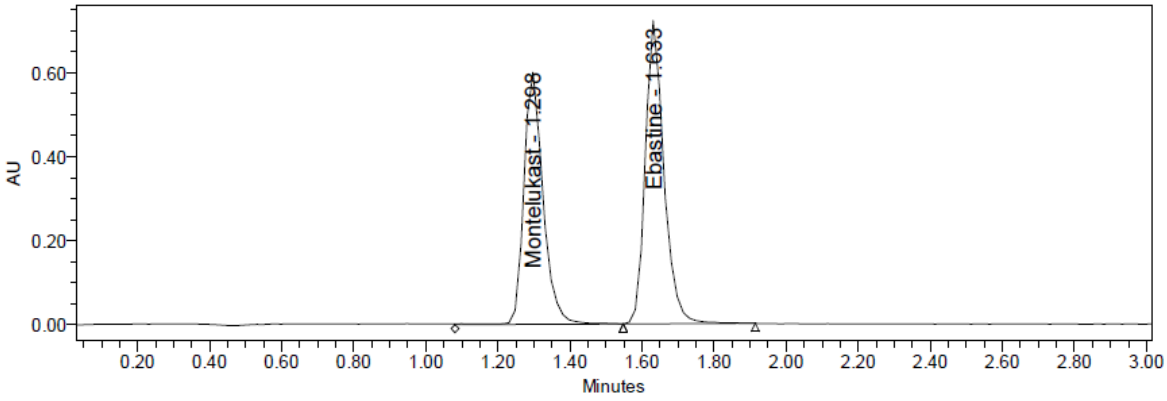

Figure-7: A Sample Chromatogram of Montelukast and Ebastine in Pharmaceutical Dosage Form

Table-1: Optimized Chromatographic Conditions

Parameter	Condition
RP-UPLC	WATERS UPLC SYSTEM equipped with quaternary pumps with TUV detector
Mobile phase	Buffer and ACN: taken in the ratio 60:40.
Flow rate	0.3ml/min
Column	Waters C18 150 x 3 mm, 2μ.
Detector wave leng	244nm
Column temperatu	30°C
Injection volume	3μL
Run time	3 min
Diluent	Water and Acetonitrile in the ratio 50:50
Retention Time	Montelukast 1.300 min and Ebastine 1.636 m
Theoretical Plates	Montelukast 2700 and Ebastine 4854

Table-2: Accuracy results of Montelukast and Ebastine

Conc.	Montelukast			Ebastine		
	Amount added (µg/ml)	**Amount recovered (µg/ml)**	**% Recovery**	**Amount added (µg/ml)**	**Amount recovered (µg/ml)**	**% Recovery**
50%	50	49.64	99.28	50	50.11	100.23
	50	49.83	99.67	50	49.60	99.20
	50	50.27	100.53	50	50.73	101.46
100%	100	99.83	99.83	100	101.21	101.21
	100	101.40	101.40	100	99.03	99.03
	100	99.83	99.83	100	99.72	99.72
150%	150	148.81	99.21	150	150.07	100.05
	150	149.90	99.93	150	151.48	100.99
	150	150.59	100.39	150	148.98	99.32
Mean % Recovery			**100.01%**	**Mean % Recovery**		**100.13%**

Table-3: Precision Results of Montelukast and Ebastine

S.No	Repeatability		Intermediate precision	
	Area of Montelukast	**Area of Ebastine**	**Area of Montelukast**	**Area of Ebastine**
1.	2242085	2593353	2067472	2235855
2.	2244548	2602117	2075676	2232146
3.	2212966	2619485	2058202	2225542
4.	2234176	2614953	2075429	2232241
5.	2238071	2579639	2066073	2247538
6.	2239127	2609946	2076196	2233303
Mean	2235162	2603249	2069841	2234438
S.D	11435.4	14846.5	7224.1	7268.1
%RSD	0.5	0.6	0.3	0.3

Table-4: LOD and LOQ values of Montelukast and Ebastine

Molecule	LO	LO
Monteluka	0.3	1.1
Ebastine	0.0	0.2

Table-5 Robustness Data of Montelukast and Ebastine

S.no.	Condition	%RSD of Montelukast	%RSD of Ebastine
1	Flow rate (-) 0.9ml/min	0.5	1.4
2	Flow rate (+) 1.0.3ml/min	0.8	1.3
3	Mobile phase (-) 35B:65A	1.8	1.9
4	Mobile phase (+) 45B:55A	1.5	1.7
5	Temperature (-) 25°C	0.9	0.5
6	Temperature (+) 35°C	0.4	0.6

Table-6: System Suitability Parameters Results of Montelukast and Ebastine

S no	Montelukast			Ebastine			
Inj	**RT(min)**	**USP Plate Count**	**Tailing**	**RT(min)**	**USP Plate Count**	**Tailing**	**Resolution**
1	1.295	2611	1.30	1.634	4661	1.16	3.4
2	1.295	2609	1.27	1.635	4264	1.15	3.3
3	1.296	2507	1.29	1.636	4369	1.15	3.3
4	1.296	2474	1.27	1.636	4854	1.18	3.4
5	1.299	2470	1.23	1.636	4634	1.15	3.3
6	1.300	2700	1.20	1.637	4460	1.15	3.3

Figure-7: Assay Results of Montelukast and Ebastine

S.No	Montelukast			Ebastine		
	Standard Area	**Sample area**	**% of Drug**	**Standard Area**	**Sample area**	**% of Drug**
1.	2211642	2242085	101.24	2581317	2593353	101.27
2.	2229330	2244548	101.35	2534616	2602117	101.61
3.	2231862	2212966	99.93	2535195	2619485	102.29
4.	2202595	2234176	100.89	2561234	2614953	102.11
5.	2194107	2238071	101.06	2573801	2579639	100.73
6.	2204468	2239127	101.11	2563694	2609946	101.92
Mean	2212334	2235162	100.93	2558310	2603249	101.65
S.D	15229.1	11435.4	0.52	19506.6	14846.5	0.5797
%RSD	0.7	0.5	0.51	0.8	0.6	0.6

Table 8. Degradation Data of Montelukast

S.NO	Degradation Condition	% Drug Degraded
1	Acid	4.29
2	Alkali	5.43
3	Oxidation	2.37
4	Thermal	1.15
5	UV	2.46
6	Water	0.31

Table 9. Degradation Data of Ebastine

S.NO	Degradation Condition	% Drug Degraded
1	Acid	5.66
2	Alkali	5.56
3	Oxidation	4.21
4	Thermal	4.40
5	UV	2.50
6	Water	0.56

CONCLUSION

A new Accurate, Precise, Simple and reliable method for the simultaneous estimation of the Montelukast and Ebastine in Pharmaceutical Dosage Form has been developed. The method developed was validated and was found to be sensitive, accurate, precise and reliable for the analysis of Montelukast and Ebastine in Bulk and Pharmaceutical dosage forms. The Results obtained were within the prescribed limits of ICH Guidelines and shown accuracy and preciseness of the method developed. As the Retention times were decreased and that run time was less the method can be effectively adopted in regular quality control testing in industries which is also economical too. Finally it can be concluded from the results that the method developed was simple and accurate with robust and reliability as added values to the method.

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