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RESEARCH ARTICLE

Validation of Assay for Simultaneous Estimation of Ebastine and Montelukast in Tablet Dosage Forms by RP-HPLC Method

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ABSTRACT

A simple, accurate, precise, economical method was developed for the simultaneous estimation of the Ebastine and Montelukast in tablet dosage form by the RP-HPLC method. The chromatogram was run through Kromosil (250mm x 4.6 mm, 5m.) The mobile phase containing potassium dihydrogen phosphate buffer and Acetonitrile was taken in the ratio 60:40 was pumped through the column at a flow rate of 1ml/min. The pH was adjusted to 4.8 with Orthophosphoric acid. A buffer used in this method was potassium dihydrogen phosphate solution. The temperature was maintained at 30°C. The optimized wavelength for Ebastine and Montelukast was 244nm. The retention time of Ebastine and Montelukast were found to be 2.447 min and 3.436 min respectively. With the optimized chromatographic conditions, the drug was linear in the concentration range of 0 - 150 µg/ ml. The correlation coefficient was found to be 0.999. The average percentage assay in the formulation was found to be 99.05% and 99.20% for Ebastine and Montelukast respectively. % Recovery for Ebastine and Montelukast was found to be 99.93% and 99.69% respectively. %RSD for repeatability was found to be 0.2 respectively. LOD, LOQ values are obtained from regression equations of Ebastine and Montelukast were 0.11ppm, 0.33ppm and 0.14ppm, 0.43ppm respectively. Regression equation of Ebastine is y = 19263x+1149, and y = 19946x+1095 of Montelukast. Hence the suggested RP-HPLC method can be used for routine analysis of Ebastine and Montelukast in API and Pharmaceutical dosage form.

KEYWORDS

Ebastine, Montelukast, RP-HPLC, Simultaneous estimation, Validation

INTRODUCTION

Ebastine is a second-generation H_1 receptor antagonist that is indicated mainly for allergic rhinitis and chronic idiopathic urticaria.

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It is chemically known as 1-(4-tert-butylphenyl)-4-[4-(diphenylmethoxy) piperidin-1-yl] butan-1-one. Figure 1. It is soluble in methanol, chloroform, and dimethyl sulfoxide. Ebastine and its active metabolite is selective peripheral histamine H1 receptor antagonist. Thus it prevents the attachment of histamine on receptors and its activation (Activation of receptors of histamine on various tissues produce various allergic symptoms e.g. a Runny nose). Ebastine also has a specific inhibitory effect on Th2-type cytokine production and inhibit T cell migration and pro-inflammatory

cytokine production by T cells and macrophages.

Montelukast is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. It is chemically known as Sodium; 2-[1-[[(1R)-1-[3-[(E)-2-(7-chloroquinolin-2-yl) ethenyl] phenyl]-3-[2-(2hydroxypropanyl) phenyl] propyl] sulfanylmethyl] cyclopropyl] acetate. Figure 2. It is freely soluble in ethanol, methanol, and water. Montelukast blocks the action of leukotriene D4 on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation.

A detailed survey of the literature for Ebastine and Montelukast reveals that the available analytical methods are costly and with more retention time. Hence we developed a rapid and sensitive RP-HPLC method with UV detection (244 nm) for routine analysis of montelukast sodium and ebastine in a pharmaceutical formulation (Ebast-M). A literature review revealed few methods on method development and validation of Ebastine and Montelukast by RP-HPLC. So now the main aim is to develop a method with less run time and retention time compared to those methods.[1-7]

MATERIAL AND METHOD

Instruments

HPLC from Waters with model No HPLC 2965 system with Empower 2 software.

Materials

Ebastine and Montelukast (API) were received from spectrum lab, Combination Ebastine and Montelukast (EBAST M TABLET) tablets were obtained from Micro Labs, Distilled water (HPLC grade), acetonitrile, ammonium acetate buffer, methanol, Potassium dihydrogen phosphate buffer, Triethylamine, orthophosphoric acid (HPLC grade) were obtained from MERCK.

Methods

Diluent

Based upon the solubility of the drugs, diluent was selected, Methanol and Water were taken in the ratio 50:50.

Preparation of Standard Stock Solutions

Accurately Weighed and transferred 10mg and 10mg of Ebastine and Montelukast working Standards into 10ml and 10ml clean dry volumetric flasks separately, add 3/4th volume of diluent, sonicated for 30 minutes and makeup to the final volume with diluents.

Preparation of Standard Working Solutions (100% solution)

From the above each stock solution, 1 ml was pipetted out into a 10ml volumetric flask and then makeup to the final volume with diluent.

Preparation of Sample Stock Solutions

20 tablets were weighed and calculate the average weight of each tablet then the tablet powder weight equivalent to 10 mg of Ebastine and 7.5 mg of Montelukast was transferred into a 10ml volumetric flask, 7ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered.

Preparation of Sample Working Solutions (100% solution)

From the filtered solution, 1ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent.

Preparation of Buffer

1ml of OPA was taken in 1000 ml volumetric flask and makeup to the mark with milli-Q water.

Preparation of Buffer: 0.01N Potassium dihydrogen orthophosphate (pH 4.8)

Accurately weighed 1.36gm of Potassium dihydrogen orthophosphate 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 the pH was adjusted to 4.8 with Orthophosphoric acid.

RESULTS AND DISCUSSION

Method Development

Table 1: Different trials were performed by changing Mobile phase and buffer

Trials	Column Used	Mobile phase	Buffer	Flow rate	Wave length	Temperature	Injection Volume
Trial: 1	Discovery 250 x 4.6 mm, 5µ.	Water: Methanol (50:50)		1ml/ min	244nm	25°C	10µl
Trial: 2	Discovery 250 x 4.6 mm, 5µ.	Water: Acetonitrile (50:50)	Water	1ml/ min	244nm	30°C	10µl
Trial: 3	Discovery 250 x 4.6 mm, 5µ.	buffer: ACN (60:40)	0.1%OPA	1ml/ min	244nm	30°C	10µl
Trial: 4	Discovery 250 x 4.6 mm, 5µ.	buffer: Acetonitrile (60:40)	0.01N KH_2PO_4 (4.8) solution	1ml/ min	244nm	30°C	10µl
Trial: 5	buffer: Acetonitrile (70:30A)	buffer: Acetonitrile (70:30)	0.01N KH_2PO_4 (4.8) solution	1ml/ min	244nm	30°C	10µl
Optimized Method	Kromosil 250 x 4.6 mm, 5µ.	Buffer: Acetonitrile (60:40)	0.01N KH_2PO_4 (4.8) solution Diluent : Water: ACN: (50:50)	1.0ml/min	244nm	30°C	10µl

Table 2: Optimization of chromatographic conditions

Trials	Observation
Trial: 1	Ebastine peak was eluted but Montelukast peak was not eluted and peak shape also not good so further trial is carried out.
Trial: 2	Peaks were eluted but peak shape was not good and baseline disturbances hump, USP plate count were not good so further trial is carried out.
Trial: 3	Both peaks were eluted but resolution was less so further trial is carried out.
Trial: 4	Retention time is more and ebastin eluted at void range so further trial is carried out.
Trial: 5	Increasing buffer ratio montelukast retention time is more and ebastine eluted at void range so further trial is carried out.
Optimized Method	Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits. Peak shape and retention time is good so, further process is carried out.

Method Validation

The present study was carried method was validated based on ICH (Q2B) parameters.[8]

The following parameters were validated for the proposed method.

System Suitability

All the system suitability parameters are within range and satisfactory as per ICH guidelines. Table 3

Discussion: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were within the limits.

Discussion: Retention times of Ebastine and Montelukast were 2.447 min and 3.436 min respectively. We did not find any interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity

Six Linear concentrations of Ebastine (25-150ppm) and Montelukast (20-120ppm) were prepared and injected. Regression equation of the Ebastine and Montelukast were found to be, y = 19263x +1149, and y = 19946x + 1095 and the regression coefficient was 0.999. Table 4 Figure 4 & 5

Precision

Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Ebastine and Montelukast were found to be 0.2% and 0.2% respectively. Table 5

Inter-day precision: Inter-day precision was performed with 24 hrs time lag and the %RSD Obtained for Ebastine and Montelukast were 0.3% and 0.2%. Table 6

Accuracy

Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery was displayed in Table 7. Figure 6-8

Robustness

Small deliberate changes in a 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 Guidelines. Table 6 Figure 9 & 10

Discussion: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (65B:35A), mobile phase plus (55B:45A), temperature minus (25°C) 3and temperature plus (35°C) was maintained and samples were injected in a duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Assay

Standard preparations are made from the API and Sample Preparations are from Formulation (EBAST M TABLET). Both sample and standards are injected six homogeneous samples. The drug in the formulation was estimated by taking the standard as the reference. The Average % assay was calculated and found to be 99.05% and 99.20% for Ebastine and Montelukast respectively. Table 7

Degradation Studies

Standards and degraded samples are injected and calculated the percentage of drug degraded in solution by applying different conditions like acid, alkali, and oxidative, photolytic, thermal and neutral analysis. Table 8 Figure 11-15

Table 3: System Suitability Studies of Ebastine and Montelukast

Property	Ebastine	Montelukast
Retention time (t_R)	2.447min	3.436min
Theoretical plates (N)	8019 ± 63.48	10040 ± 63.48
Tailing factor (T)	1.37 ± 0.117	1.33 ± 0.117

Table 4: Calibration Data of Ebastine and Montelukast Method

S. No	Concentration Ebastine (µg/ml)	Response	Concentration Montelukast (µg/ml)	Response
1	0	0	0	0
2	25	533631	20	478732
3	50	987156	40	956442
4	75	1467357	60	1501069
5	100	1976938	80	1885033
6	125	2503069	100	2386656
7	150	3011189	120	2913242

Table 5: Repeatability results for Ebastine and Montelukast

Sl. No.	Ebastine	Montelukast
1	1958452	1868712
2	1957170	1870107
3	1952368	1865840
4	1959570	1870800
5	1953581	1865343
6	1952026	1859653
Mean	1955528	1866743
S.D.	3274.9	4115.7
%RSD	0.2	0.2

*Average of six determinations

Table 6: Inter-Day Precision Results for Ebastine and Montelukast

S. No.	Ebastine	Montelukast
1	2159276	1882066
2	2168976	1884258
3	2165538	1892454
4	2158679	1885947
5	2162743	1875128
6	2157355	1882066
Mean	2162095	1883653
S.D	4510.1	5671.0
%RSD	0.2	0.3

Table 7: Table of Accuracy

Sample	Concentration (%) (µg/ml)	Recovery (%)	Mean % Recovery	%RSD
Ebastine	50	101.07	99.93%	0.07
	100	98.93		0.33
	150	99.81		0.30
Montelukast	50	100.8	99.69%	0.08
	100	99.63		0.74
	150	98.64		0.52

Table 8: Robustness Data of Ebastine and Montelukast

S. No	Robustness condition	Ebastine %RSD	Montelukast %RSD
1	Flow minus (0.9ml/min)	0.1	0.2
2	Flow Plus (1.1ml/min)	0.4	0.5
3	Mobile phase minus (65:35)	0.3	0.3
4	Mobile phase Plus (55:45)	0.2	0.1
5	Temperature minus (25^{0c})	0.2	0.2
6	Temperature Plus (30^{0c})	0.3	0.1

Table 9: Assay of Tablet

S. No.	Ebastine %Assay	Montelukast % Assay
1	99.15	98.91
2	99.23	99.19
3	99.00	98.10
4	99.26	99.84
5	98.97	99.46
6	98.67	99.70
AVG	99.05	99.20
S.D	0.2184	0.6339
% RSD	0.2	0.64

Table 10: Different Types of Degradation Studies

Types of Degradation	EBASTINE		
	Area	% Recovered	% Degraded
Acid	1800991	95.56	4.44
Base	1833505	97.28	2.72
Peroxide	1853657	98.35	1.65
Thermal	1871146	99.28	0.72
UV	1868750	99.15	0.85
Water	1867367	99.08	0.92
MONTELUKAST			
Acid	1867882	95.28	4.72
Base	1910567	97.45	2.55
Peroxide	1930866	98.49	1.51
Thermal	1950620	99.50	0.50
UV	1951371	99.54	0.46
Water	1947871	99.36	0.64

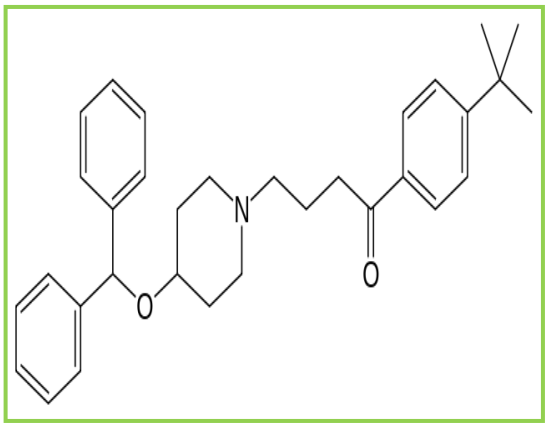

Figure 1: Structure of Ebastine

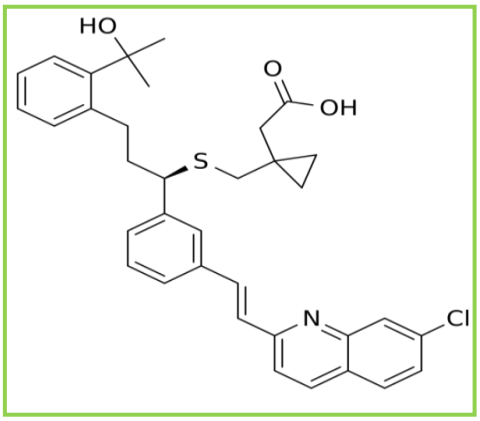

Figure 2: Structure of Montelukast

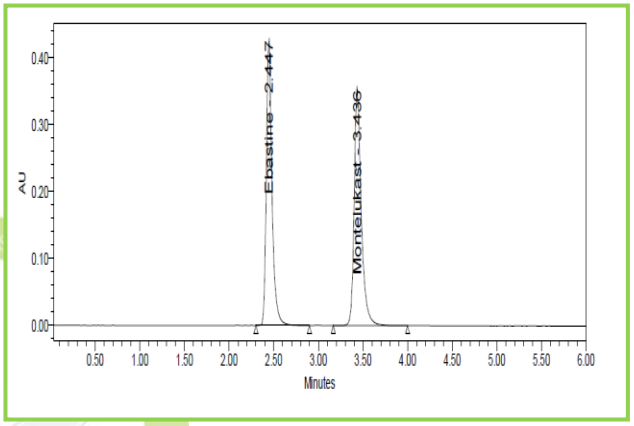

Figure 3: Typical chromatogram of Ebastine and Montelukast

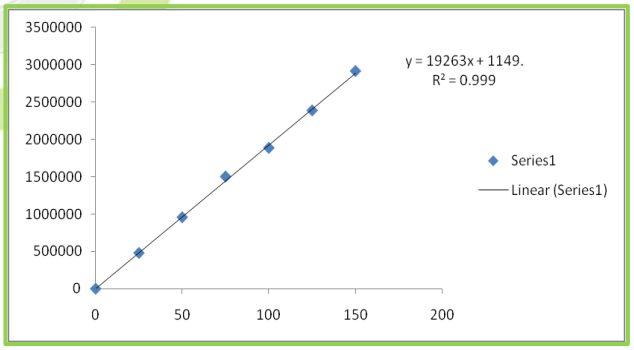

Figure 4: Calibration curve of Ebastine

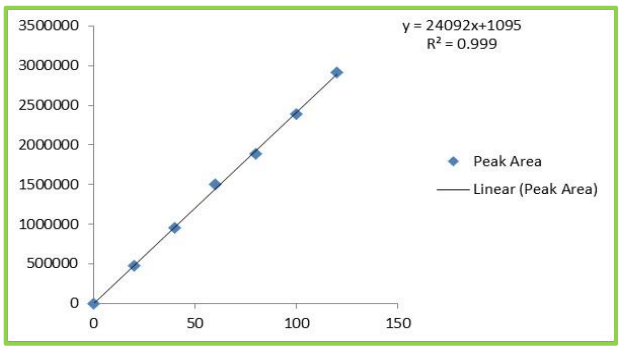

Figure 5: Calibration curve of Montelukast

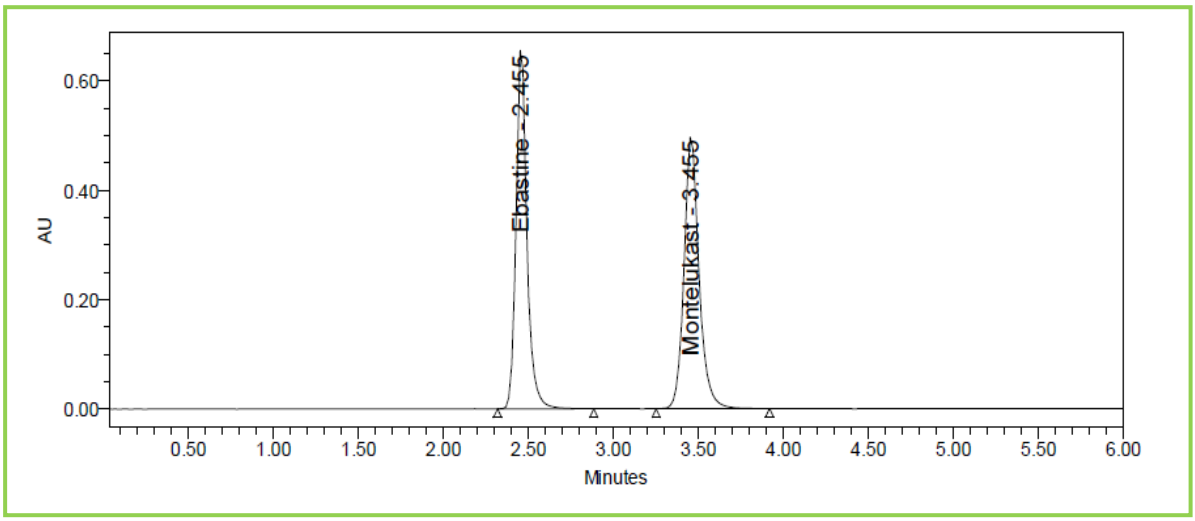

Figure 6: Accuracy 50% Chromatogramof Ebastine and Montelukast

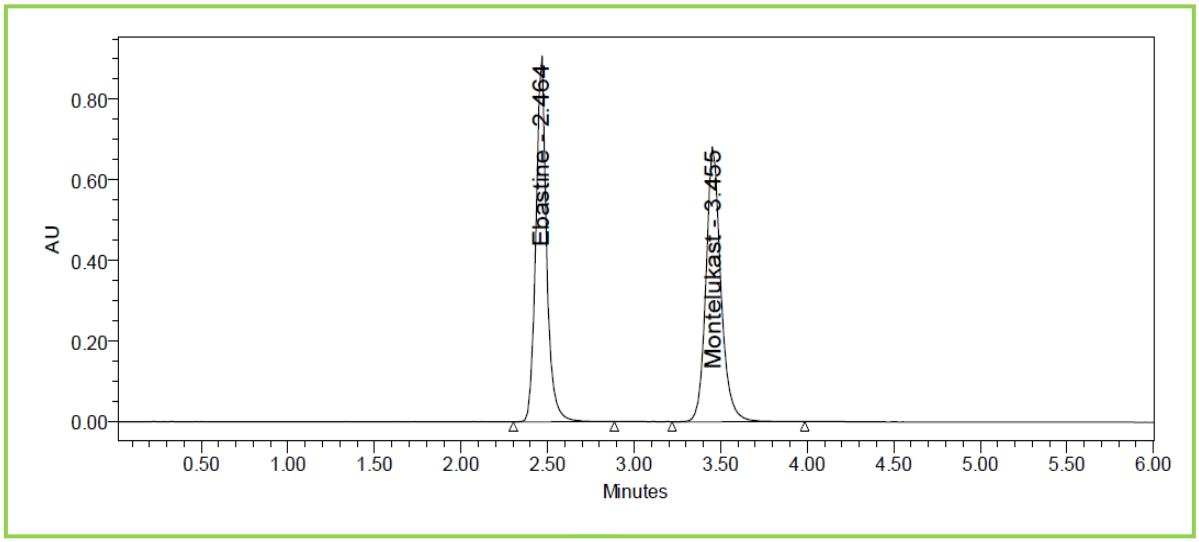

Figure 7: Accuracy 100% Chromatogram of Ebastine and Montelukast

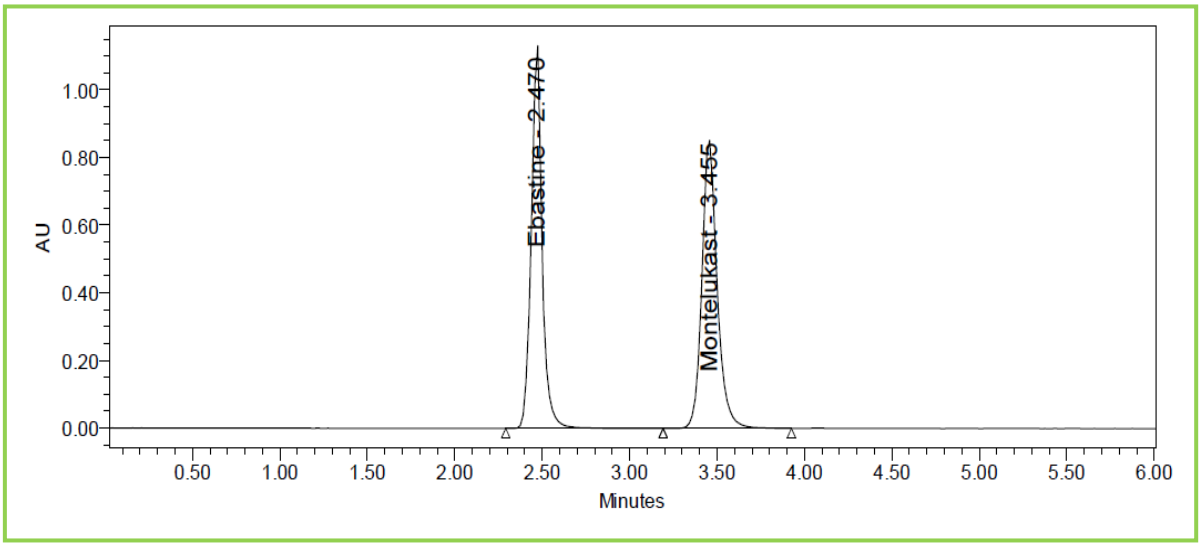

Figure 8: Accuracy 150% Chromatogram of Ebastine and Montelukast

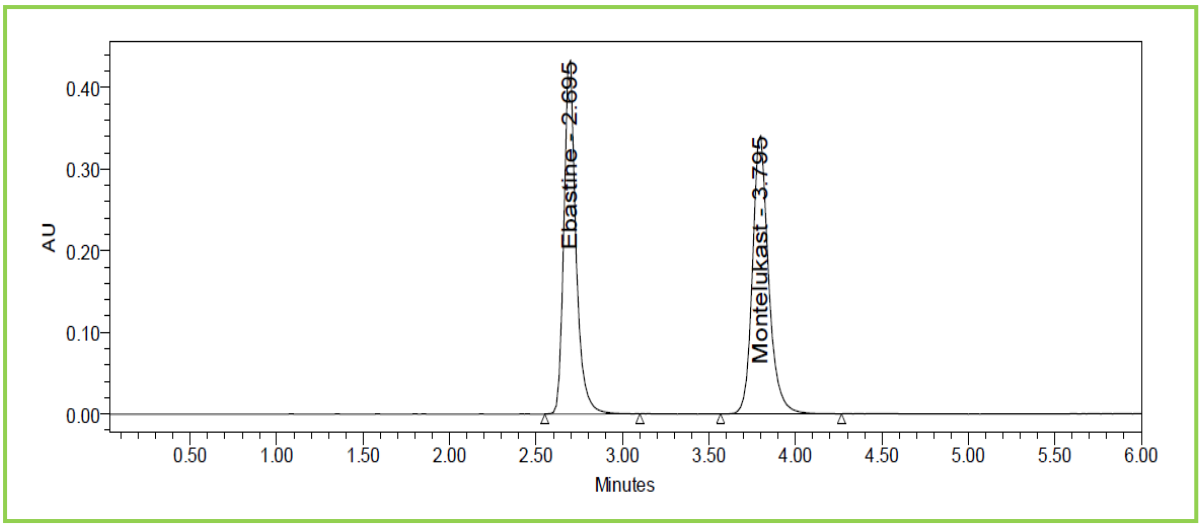

Figure 9: Flow minus Chromatogram of Ebastine and Montelukast

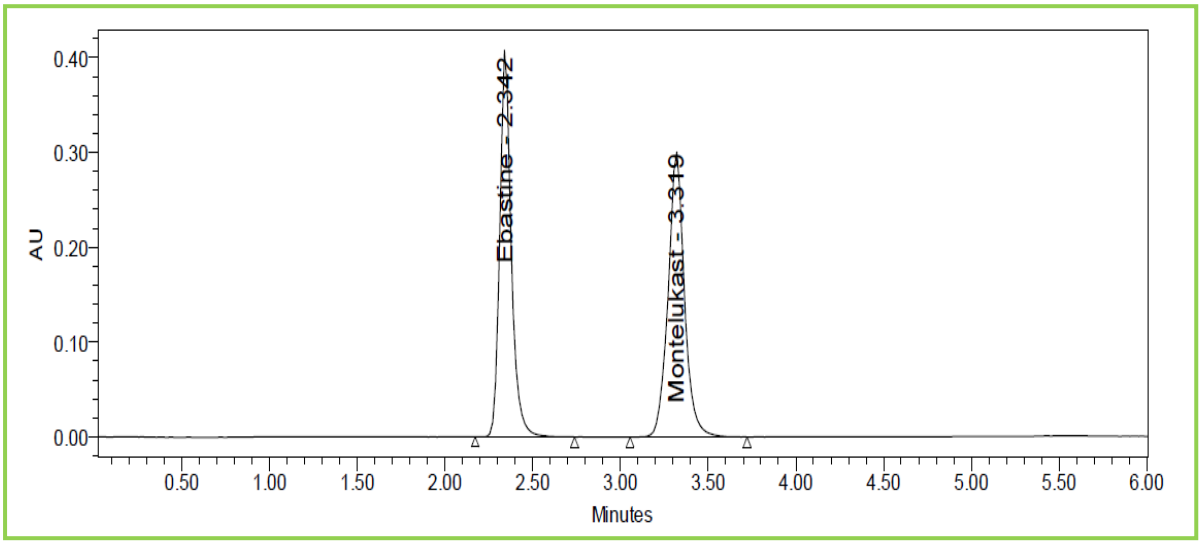

Figure 10: Flow plus Chromatogram of Ebastine and Montelukast

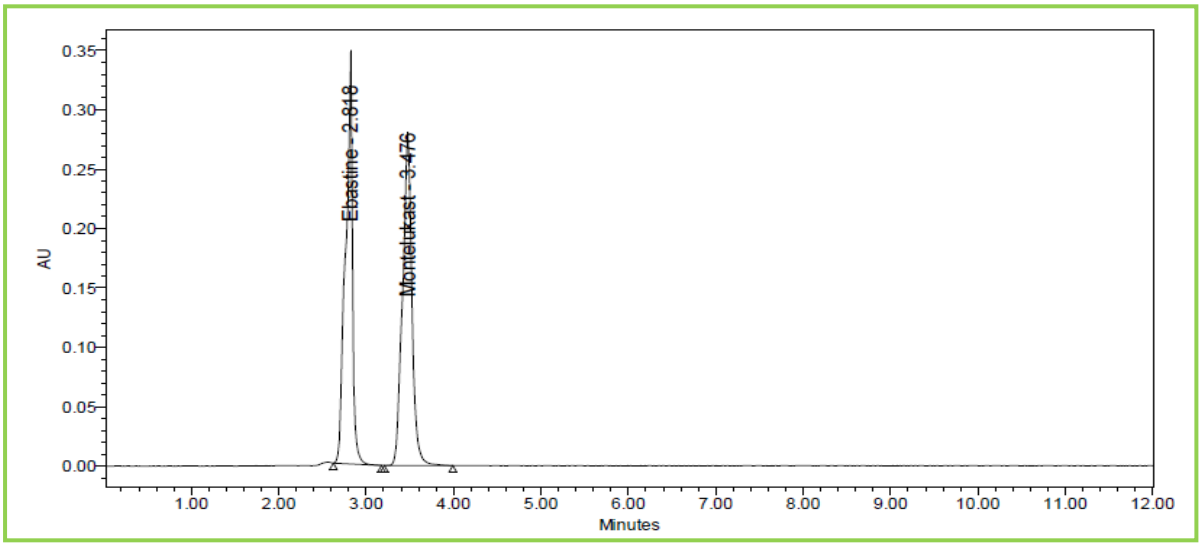

Figure 11: Chromatogram showing Acid degradation

CONCLUSION

A new method was established for simultaneous estimation of Ebastine and Montelukast by RP-HPLC method. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Ebastine and Montelukast in the pharmaceutical dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness, and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. Hence the suggested RP-HPLC method can be used for routine analysis of Ebastine and Montelukast in API and Pharmaceutical dosage form.

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