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Research Article

Simultaneous Estimation of Montelukast and Bilastine in Bulk and Pharmaceutical Dosage Form by RP-HPLC

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ABSTRACT

A simple, accurate, precise method was developed to estimate Bilastine and Montelukast in bulk and tablet form. Chromatogram was run on Std Inertsil C18 150 x 4.6 mm, 5m. Mobile phase with Buffer 0.01N Na2HPO4: Acetonitrile 70:30 was pumped through column at 1.0ml/min. Method buffer was 0.01N Na2hpo4. The temperature was 30°C. The optimal wavelength was 218nm. Bilastine and Montelukast had 2.524 and 3.153min retention times. Bilastine and Montelukast had 0.9 and 1.2 %RSD. Recovery was 99.98% for Bilastine and 99.48% for Montelukast. Bilastine and Montelukast regression equations yielded LOD, LOQ values of 0.32, 0.97, and 0.24, 0.72. Montelukast regression equation is y = 36464x + 8987, while Bilastine is y = 31133x + 12857. The method developed was simple and economical for regular quality control tests in industries because retention and run times were reduced.

Keywords: Bilastine, Montelukast, RP-HPLC

INTRODUCTION

The frequency and impact of allergic diseases are often underestimated.[1] A key facilitator of the allergic response is immunoglobulin E (IgE) that is present on the surface of mast cells and basophils.[2] Interaction of the allergen with IgE and its receptor complex leads to activation of these cells and release of the substances, including histamine, that cause allergic symptoms. Because of the central role of histamine in allergic responses, many allergic conditions are treated with antihistamines, including allergic rhinitis and urticaria.[3,4] Antihistamines have been in clinical use for 70 years, and the pharmacological characteristics

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of these agents have been evolving over that time. Here we have used Bilastine and Montelukast.

Montelukast: Montelukast was first approved for clinical use by the US FDA in 1998 as Merck's brand name Singulair.[7] The medication is a member of the leukotriene receptor antagonist (LTRA) category of drugs.[7-13] Although capable of demonstrating effectiveness, the use of such LTRAs like montelukast is typically in addition to or complementary with the use of inhaled corticosteroids or other agents in asthma step therapy.[5] Regardless, in 2008-2009, there were FDA-led investigations into the possibility of montelukast to elicit neuropsychiatric effects like agitation, hallucinations, suicidal behaviour, and others in individuals who used the medication.[6] And although these kinds of effects are currently included in the official prescribing information for montelukast, [7-13] the drug still sees extensive use worldwide via millions of prescriptions annually and has since become available as a generic and as a brand name product.

Bilastine: Bilastine is a novel new-generation antihistamine that is highly selective for the H1 histamine receptor, has a rapid onset and prolonged duration of action.

The combination of Bilastine+Montelukast combines two drugs: Montelukast (leukotriene receptor antagonist) and Bilastine (antihistamine). Bilastine+Montelukast belongs to a class of medication called 'anti-allergic medication, primarily used to treat allergic conditions like sneezing, runny nose, congestion, stuffy nose or watery eyes. An allergy is a condition that occurs when the immune system reacts to a foreign substance that is typically not harmful to your body. These foreign substances are known as 'allergens' Some might be allergic to certain foods and seasonal allergies like hay fever, pollen, or pet dander. One of the main symptoms of allergy is cough, which acts as a reflex action in the throat when mucus or foreign irritant enters the respiratory system.

They might be side effects with Bilastine + Montelukast like Rhinitis, Headache, Eczema, Urticaria, Abdominal pain/Upper abdominal pain[14]

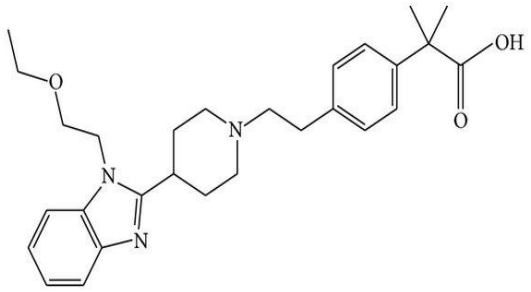

Figure 1 Structure of Montelukast

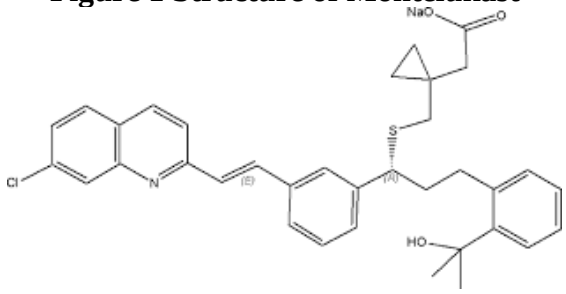

Figure 2. Structure of Bilastine

According to a literature review, there are some techniques for the simultaneous estimate of these medicines as well as others for assessment of the drugs alone or in combination with other drugs. Utilizing UV-Spectrophotometry RP-HPLC. There is no established technique

for the stability-indicating simultaneous measurement of Bilastine and Montelukast by RP-HPLC in pharmaceutical dosage form, according to a survey of the literature. The primary goal of this work is to provide an efficient, quick, and accurate RP-HPLC approach for estimating of Bilastine and Montelukast in medicinal dose and tablet form. According to ICH recommendations, a proven approach was also used to estimate the amounts of Bilastine and Montelukast. [15-20]

MATERIALS AND REAGENTS

Bilastine and Montelukast pure drugs were received from Spectrum Pharma research solutions, Hyderabad. The combination tablet Bilastine and Montelukast (Billargic M) was purchased from a local pharmacy store. Rankem in India provided all of the chemicals and buffers utilised in this method like Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen Ortho phosphate buffer, Ortho-phosphoric acid, Distilled water.

Instruments:

For the development and validation method, an automated sample injector was employed with a WATERS HPLC; model 2695 SYSTEM with Photo diode array detector. For the separation, a Discovery 150 (C18 250 mm x 4.6 mm, 5µm) column was employed. Acetonitrile is employed as mobile phase B, while 0.1% ortho phosphoric acid is used as mobile phase A. (35:65 Ratio). The analysis was done in isocratic mode with an injection volume of 10 mL and a flow rate of 1 mL/min. The duration was six minutes. The measurements were made at 254 nm.

PREPARATION OF SOLUTIONS

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of buffer:

Buffer: (0.1% OPA)

Accurately 1ml of OPA 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.

Buffer: 0.01N Potassium dihyrogen ortho phosphate

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.

Buffer:0.01N Sodium dihydrogen phosphate

Accurately weighed 1.42gm 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 PH adjusted to 3.5 with dil. Orthophosphoric acid solution.

Preparation of Standard stock solutions: Accurately weighed 20mg of Bilastine, 10mg of Montelukast and transferred to 50ml volumetric flask. 3/4th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (400µg/ml of Bilastine and 200µg/ml Montelukast)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (40µg/ml of Bilastine and 20µg/ml of Montelukast).

Preparation of Sample stock solutions: weighed 20mg of Bilastine, 10mg of Montelukast and transferred to 50ml volumetric flask. 3/4th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (400µg/ml of Bilastine and 200µg/ml Montelukast)

Preparation of Sample working solutio
ns (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (40µg/ml of Bilastine and 20µg/ml of Montelukast).

METHOD VALIDATION
To prove that the technique is suggested for routine analysis, the HPLC method's validation was done for the simultaneous estimation of Bilastine and Montelukast drug material in accordance with the ICH criteria.

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.

Linearity: stock solutions of Bilastine and Montelukast is taken in to 6 different volumetric flasks and diluted to 10ml with diluents. Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml.

Accuracy:
Preparation of Standard stock solutions: Accurately weighed 20mg of Bilastine, 10mg of Montelukast and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (400µg/ml of Bilastine and 200µg/ml Montelukast)

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.

Acceptance Criteria:
The % Recovery for each level should be between 98.0 to 102%.

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 (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, 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 0.1ml each of Bilastine and Montelukast, 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 Bilastine and Montelukast, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Bilastine (40ppm) and Montelukast (20ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Degradation studies:

Oxidation: To 1 ml of stock solution of Bilastine 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 20 µg/ml & 10 µg/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 Bilastine and Montelukast, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60 0C. The resultant solution was diluted to obtain 40µg/ml & 20µg/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 Bilastine and Montelukast, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 40µg/ml & 20µg/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 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 40µg/ml & 20µg/ml solution and 10µl 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 200µg/ml& &100µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 40µg/ml &20µg/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 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 40µg/ml&20µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSIONS:

Table 1. System suitability table

S.No.	Bilastine			Montelukast			
Inj	**RT (min)**	**USP Plate Count**	**Tailing**	**RT(min)**	**USP Plate Count**	**Tailing**	**RS**
1	2.533	7830	1.34	3.152	9876	1.34	4.9
2	2.541	7910	1.34	3.159	10276	1.33	4.9
3	2.544	8097	1.34	3.161	10085	1.35	5.1
4	2.549	7916	1.33	3.166	10250	1.35	5.1
5	2.551	7905	1.34	3.172	9916	1.33	4.9
6	2.560	7965	1.35	3.184	9959	1.34	4.9

Table 2. Specificity data

Sample name	Retention time (Mins)	Area
Bilastine	2.543	2396385
Montelukast	3.153	1199241

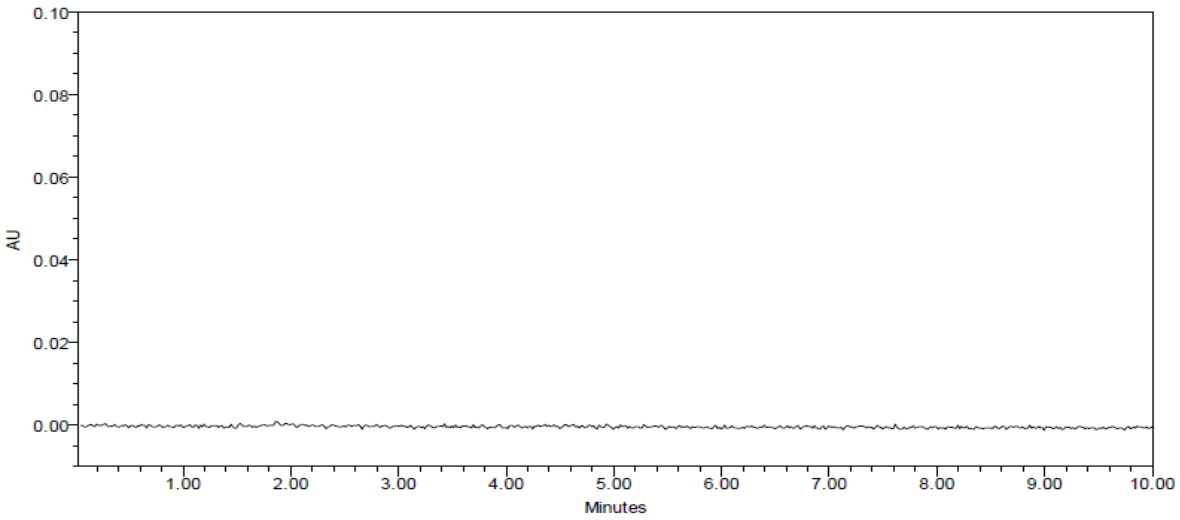

Figure 3. Blank Chromatogram

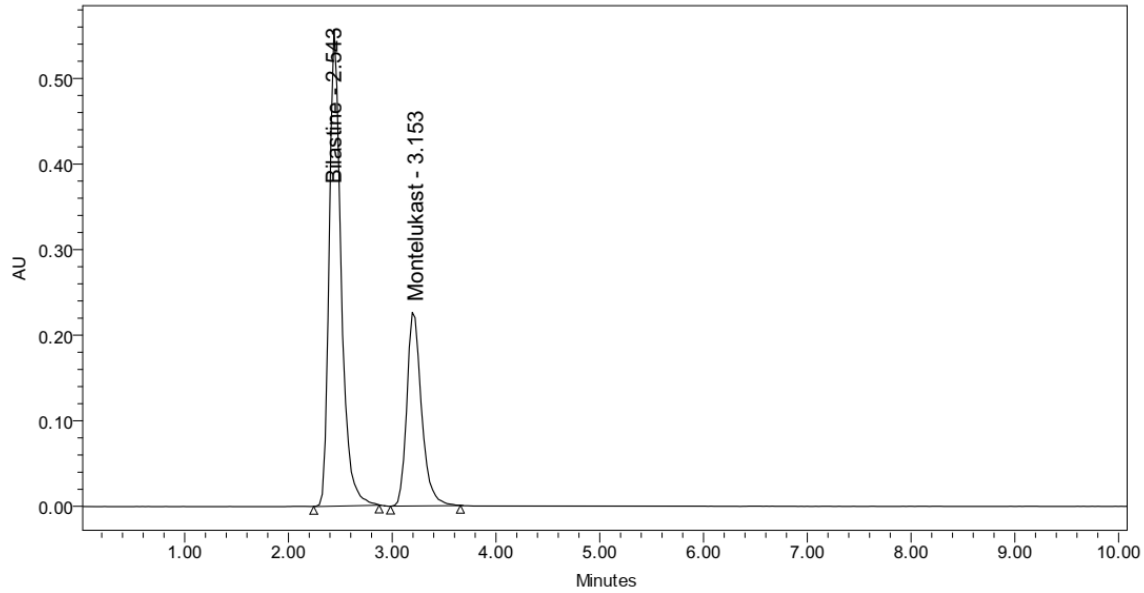

Figure 4. Specificity Chromatograms of Bilastine and Montelukast

Linearity

Table 3. Linearity table for Bilastine and Montelukast:

Bilastine		Montelukast	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	**0**	0
10	328333	**5**	190499
20	645236	**10**	376491
30	946151	**15**	567143
40	1274829	**20**	748145
50	1555762	**25**	913713
60	1877580	**30**	1095611

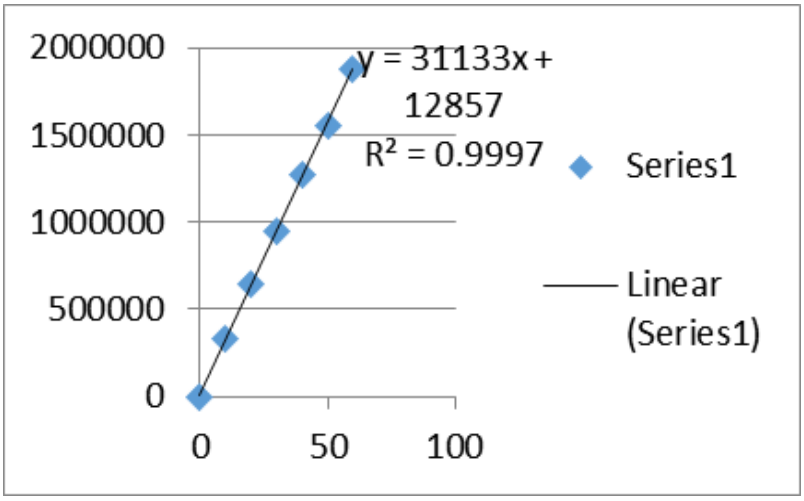

Figure 5. Calibration curve of Bilastine

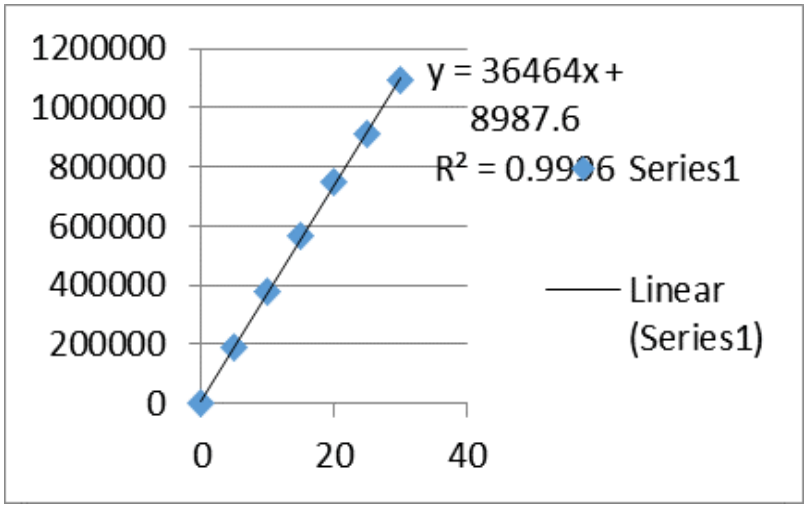

Figure 6. Calibration curve of Montelukast

Accuracy:

Table 4. Accuracy table of Bilastine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	20.0	100.0	**99.98%**
	10	20.1	100.3	
	10	19.8	99.0	
100%	20	39.8	99.6	
	20	39.9	99.7	
	20	39.8	99.6	
150%	30	60.1	100.2	
	30	60.0	100.0	
	30	60.2	100.4	

Table 5. Accuracy table of Montelukast

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	9.96	99.57	**99.48%**
	5	10.02	100.15	
	5	10.02	100.19	
100%	10	19.78	98.91	
	10	20.07	100.37	
	10	19.73	98.64	
150%	15	29.86	99.54	
	15	29.61	98.70	
	15	29.78	99.27	

System Precision: With regard to the working strength of Bilastine and Montelukast, six duplicate injections of the standard solution at 100% of the prescribed limit were analysed to determine the system accuracy. In Table 5, the results of the peak area are compiled.

Table 6. System precision

S. No	Area of Bilastine	Area of Montelukast
1.	1254124	722409
2.	1240740	732487
3.	1235312	718458
4.	1258060	739251
5.	1263108	729060
6.	1241623	739892
Mean	**1248828**	**730260**
S.D	**11110.4**	**8728.6**
%RSD	**0.9**	**1.2**

The % RSD for the peak areas of Bilastine and Montelukast obtained from six replicate injections of standard solution was within the limit of (<2%).

Method precision: Analyzing a sample of Bilastine and Montelukast allowed researchers to gauge the method's accuracy (Six individual sample preparations). Table 6 provides a summary of the data.

Method precision chromatogram

Table 7. Method precision

S. No	Area of Bilastine	Area of Montelukast
1.	527361	772730
2.	526312	773313
3.	518623	777163
4.	523660	779519
5.	522394	774714
6.	523536	774803
Mean	**523648**	**775374**
S.D	**3089.3**	**2544.9**
%RSD	**0.6**	**0.3**

Results shows, the % RSD of Repeatability study was within the range for Bilastine and Montelukast is (<2%)

Table 8. Robustness

S.No.	Condition	%RSD of Bilastine	%RSD of Montelukast
1	**Flow rate (-) 0.9ml/min**	0.9	0.4
2	**Flow rate (+) 1.1ml/min**	0.9	0.5
3	**Mobile phase (-) 60B:40A**	0.9	0.4
4	**Mobile phase (+) 70B:30A**	0.9	0.9
5	**Temperature (-) 25°C**	1.3	0.3
6	**Temperature (+) 35°C**	0.4	1.1

Table 9. Forced degradation for Bilastine and Montelukast

Stress condition	Solvent	Temp (^{0}C)	Exposed time
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxidation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	

DEGRADATION

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 10. Degradation results of Bilastine and Montelukast

Type of degradation	Bilastine		Montelukast	
	%Recovered	% Degraded	%Recovered	% Degraded
Acid	94.71	5.29	93.93	6.07
Base	95.92	4.08	94.72	5.28
Peroxide	93.72	6.28	93.40	6.60
Thermal	98.21	1.79	97.82	2.18
Uv	98.59	1.41	98.57	1.43
Water	99.57	0.43	98.97	1.03

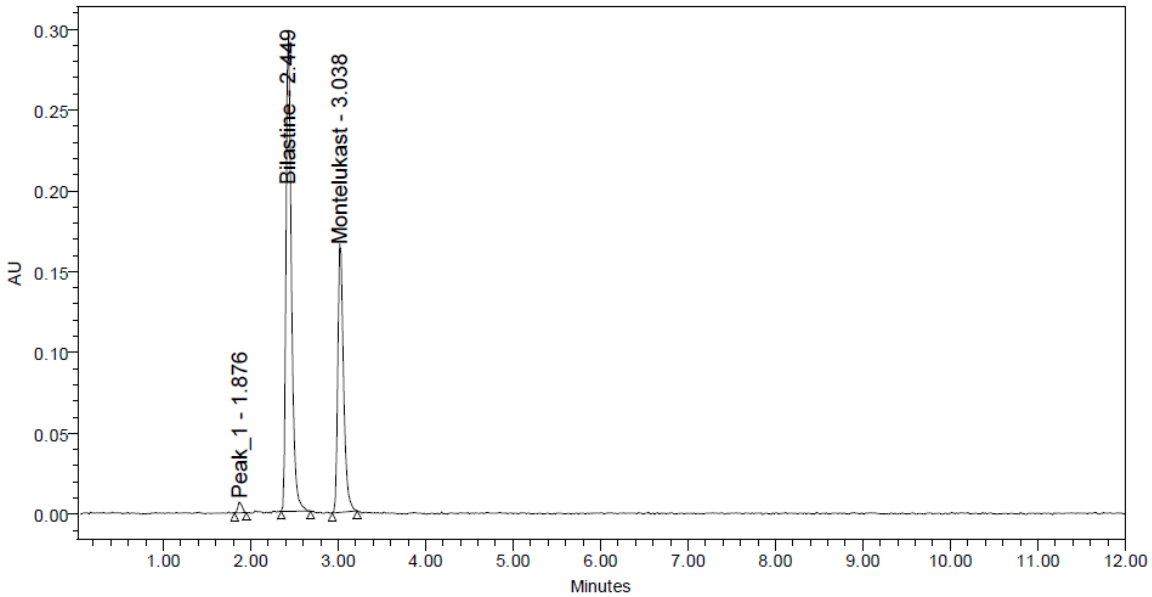

Figure 7. Acid chromatogram of Bilastine and Montelukast

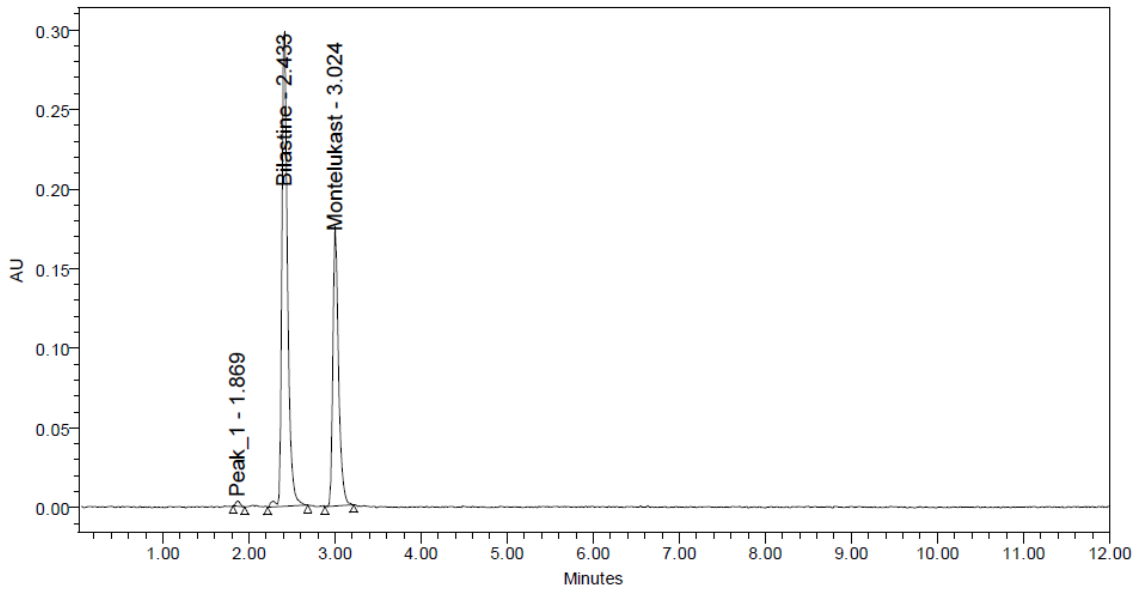

Figure 8. Base chromatogram of Bilastine and Montelukast

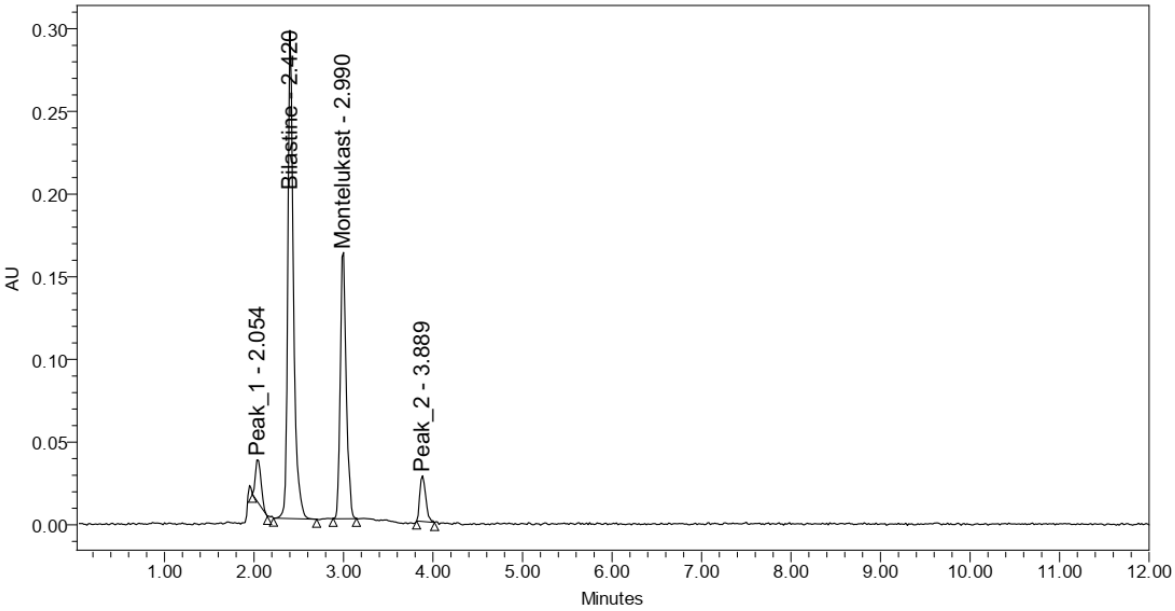

Figure 9. Peroxide chromatogram of Bilastine and Montelukast

According to the results, samples were degraded when they were subjected to an acid, base, and oxidation interaction. Hydrolysis reaction, heat reaction, and light reaction all showed no deterioration. According to the stress research, none of the degradants co-eluted with the maxima of the active medication.

Assay: **(Billargic M)** bearing label claim, **Monteluskast 20mg, Bilastine 10mg**, assay was carried out by injecting sample into HPLC System.

Table 11. Assay Data of Bilastine:

S.no	Standard Area	Sample area	% Assay
1	1254124	1258447	100.67
2	1240740	1261052	100.88
3	1235312	1258024	100.64
4	1258060	1246925	99.75
5	1263108	1252575	100.20
6	1241623	1257703	100.61
Avg	**1248828**	**1255788**	**100.46**
Stdev	**11110.4**	**5144.4**	**0.41**
%RSD	**0.9**	**0.4**	**0.4**

Table 12. Assay Data of Montelukast

S.no	Standard Area	Sample area	% Assay
1	722409	724582	99.12
2	732487	729652	99.82
3	718458	728652	99.68
4	739251	727286	99.49
5	729060	725863	99.30
6	739892	726595	99.40
Avg	**730260**	**727105**	**99.47**
Stdev	**8728.6**	**1848.3**	**0.25**
%RSD	**1.2**	**0.3**	**0.25**

Table 13. Assay outcome for Bilastine and Montelukast

Drug Name	Label claim dose	%Assay	Brand Name
Bilastine	20mg	100.46	**Billargic M**
Montelukast	10mg	99.47	

CONCLUSION

The proposed HPLC method was validated as per ICH guidelines and applied for the determination of Bilastine and Montelukast in tablet dosage form. The method was found to be accurate, precise, robust and specific. Retention time of Bilastine and Montelukast were found to be 2.524 min and 3.153 min. %RSD of the Bilastine and Montelukast were and found to be 0.9 and 1.2 respectively. %Recovery was obtained as 99.98% and 99.48%for Bilastine and Montelukast respectively. LOD, LOQ values obtained from regression equations of Bilastine and Montelukast were 0.32, 0.97 and 0.24, 0.72 respectively. Regression equation of Bilastine is y = 31133x + 12857, and y = 36434x + 8987. of Montelukast. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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