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Research Article

SIMULTANEOUS AND ESTIMATION OF MONTELUKAST AND BILASTINE BY USING RP-HPLC METHOD WITH STABILITY INDICATING

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ABSTRACT
An easy-to-use, precise method was developed for the simultaneous estimation of bilastine and montelukast in bulk and tablet dose form. Chromatogram was run through Inertsil C18 150 x 4.6 mm, 5µm. mobile phase comprising 0.01N disodium hydrogen phosphate and in the ratio of (70:30) was pumped through column at a flow rate of 1.0ml/min. 30°C was kept as the temperature. The chosen optimized wavelength was 230.0 nm. Retention time of Bilastine & Montelukast were 2.523 min & 3.140 min respectively. Bilastine &Montelukast %RSD were determined to be 1.2 and 0.6% respectively. %Recovery of Bilastine & Montelukast were 99.55% & 99.51 %respectively. Bilastine & Montelukast 's regression equation yielded LOD and LOQ values of 0.16,0.48ppm and 0.12,0.36ppm. Bilastine regression equation is y = 62266x + 12857 and Montelukast regression equation is 72927x + 8987. Stability experiments of Bilastine & Montelukast under distinctive environments of stress were also performed. As a result of shorter retentions durations and shorter run times, the method was created to be straightforward and cost-effective, and it may be used for routine Quality Control Tests in Industries.

Keywords: RP-HPLC, Bilastine ,Montelukast, ICH Guidelines.

INTRODUCTION

La Renon Healthcare Pvt L developed Bilastine and Montelukast belongs to the medication class known as leukotriene receptor antagonists and antihistamines. It is used as an anti-allergic agentt to treat allergic rhinitis and asthma. Mechanism of Action:Bilastine and Montelukast inhibits the high-voltage calcium channels in the central nervous system. It modulates the release of neurotransmitters such as norepinephrine and glutamate, reducing abnormal brain electrical activity and blockage of pain signals transmission. Bilastine + Montelukast treats allergic rhinitis (sneezing, runny nose, congestion, stuffy nose, or watery eyes) and asthma.[1,2,3].

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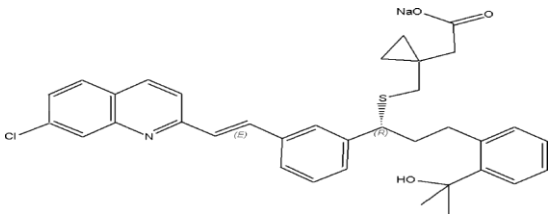

Figure.1 Structure of Montelukast

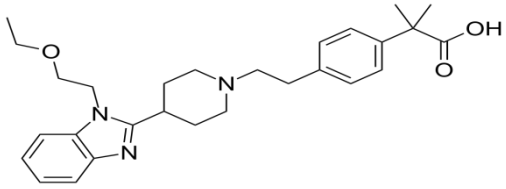

Figure.2 Structure of Bilastine

Adverse Events: The most common side effects of Bilastine + Montelukast are headache, drowsiness, fatigue, upper respiratory infection, fever, pharyngitis, cough, abdominal pain, diarrhea, influenza, rhinorrhea, and sinusitis The analytical methods for identifying both substances were recently disclosed. These approaches include spectrophotometry4 and HPLC 5-11. However, the suggested technique has the benefits of being more sensitive than the existing methods for both pharmaceuticals, greener since a smaller quantity of acetonitrile was used compared to previous methods, and the breakdown route of montelukast was disclosed..

MATERIALS AND METHODS

Chemicals and reagents

Bilastine and Montelukast pure drug (API),Bilastine and Montelukast tablets (Billargic C), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Disodium hydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation:

- Electronics Balance-Denver
- pH meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- 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 Bilastine and Montelukast solution.

Preparation of Standard stock solution:

Accurately weighed 10mg of bilastine and 5mg of montelukast were 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. (200µg/ml bilastine and 100µg/ml montelukast)

Preparation of Standard working solution: 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml bilastine and 10µg/ml montelukast).

Preparation of Sample stock solution: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml 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 (100µg/ml bilastine and 20µg/ml and 10 µg/ml montelukast)

Preparation of Sample working solution: 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml bilastine and 10µg/ml montelukast).

Chromatographic conditions:

- **Mobile phase** **:** 70**%** Na2hpo4: 30% Acetonitrile
- **Flow rate** **:** 1.0ml/min
- **Column** **:** **Inertsil**(4.6x 150mm, 5.0µm).
- **Detector wave length** **:** 230.0nm
- **Column temperature** **:** 30°C
- **Injection volume** **:** 10.0µL
- **Run time** **:** **6** min
- **Diluent** **:** Water and Acetonitrile in the ratio 50:50

Results: Bilastine and Montelukast drug peaks were good resolution, tailing Factor, theoretical plate count and resolution.

Degradation: To conduct the forced degradation experiment, standard stock solutions of Bilastine and Montelukast were exposed to various stress conditions, including 1 mL of 20% H_2O_2 (for oxidative degradation), 1 mL of 2N HCL (for acidic degradation), and 1 mL of 2N NAOH (for acidic degradation) (for basic degradation). The produced solutions were refluxed for 30 minutes at 60^{o}C. To examine the descent, the standard solutions were also subjected to UV radiation and temperature conditions. The resulting solutions were diluted to yield 20µg/ml and 10 µg/ml Bilastine and Montelukast respectively for degradation studies. To examine sample stability, 10µl samples were fed into the system and chromatograms were obtained.

Method Validation: The method was validated in accordance with ICH recommendations Q2R1. System appropriateness, specificity, linearity, accuracy, precision, LOD& LOQ, and robustness are among the validation parameters.

RESULTS AND DISCUSSION

System suitability parameters: The system suitability parameters were assessed by making standard solutions Bilastine and Montelukast (20µg/ml and 10 µg/ml) and injecting them six times. Peak tailing, resolution, and USP plate count were all determined. For three medications in combination, the USP Plate count exceeded 2000 and the tailing factor was less than 2. All of the system's appropriate parameters were passed and remained within the limitations. Table 1 shows the results.

Specificity: In the optimised method, the interference is checked. Bilastine and Montelukast, had retention time of 2.534 min and 3.150 minutes. We did not found any interfering peaks in the chromatograms of blank and placebo samples during the retention periods of the drug in our approach. As a result, this procedure was stated to be particular. Figures 3, 4, and 5 show the chromatograms for specificity.

Linearity: Six linear concentrations Bilastine (5-30µg/ml) and Montelukast (2.5-7.5µg/ml) was injected in triplicate manner. Correlation coefficients obtained was 0.999 for all the three drugs. The results were shown in table 2 and fig 6.

Precision:

Repeatability: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (20µg/ml and 10 µg/ml Bilastine and Montelukast) were created. Each injection was given from each working sample solution, and the results are shown in table 3. The average area, standard deviation, and % RSD for the medication were computed and found to be 1.2% and 0.6% for Bilastine and Montelukast. The system precision was passed for this procedure since the precision limit was less than "2 %." Table 3 shows the information results.

Intermediate Precision: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (20µg/ml and 10 µg/ml Bilastine and Montelukast) was prepared. Each injection from each working sample solution was given on the following day of the sample preparation, and the obtained areas are listed in table 4. The average area, standard deviation, and % RSD for the medicationwas computed and found to be 1.3% and 0.7% for Bilastine and Montelukast. Because the precision limit was less than "2%" the intermediate precision was used for this procedure. Table 4 shows the information results.

Accuracy: The conventional addition procedure was used to create three levels of accuracy samples. Triplicate injections were administered at each degree of accuracy, and the mean % recovery for Bilastine and Montelukast were found to be 99.55% & 99.51%. Tables 5 show the outcomes. Because satisfactory recover values were achieved, the accuracy for this approach was passed.

Robustness: Robustness conditions such as flow minus (0.9ml/min), flow plus (1.1ml/min), mobile phase minus (65:35 v/v), mobile phase plus (55:45 v/v), temperature minus (25°C), and temperature plus (35°C) were maintained, and samples (20µg/ml and 10 µg/ml Bilastine and Montelukast) was injected in duplicate. The % RSD was computed and determined to be within the acceptable range. Table 6 shows the data.

Assay: Billargic C tablets had a label claim Bilastine and Montelukast 20mg and 10mg per unit formulation. The aforementioned formulation was used for the assay. The average % assay achieved for Bilastine and Montelukast were 99.70% and 100.20%.

Degradation Studies: Degradation studies were performed with the stock standard solution and the degraded samples were analysed using proposed method. Assay % Bilastine and Montelukast in the injected samples was calculated and all the samples passed the limits of degradation. The purity plots obtained in degradation studies.

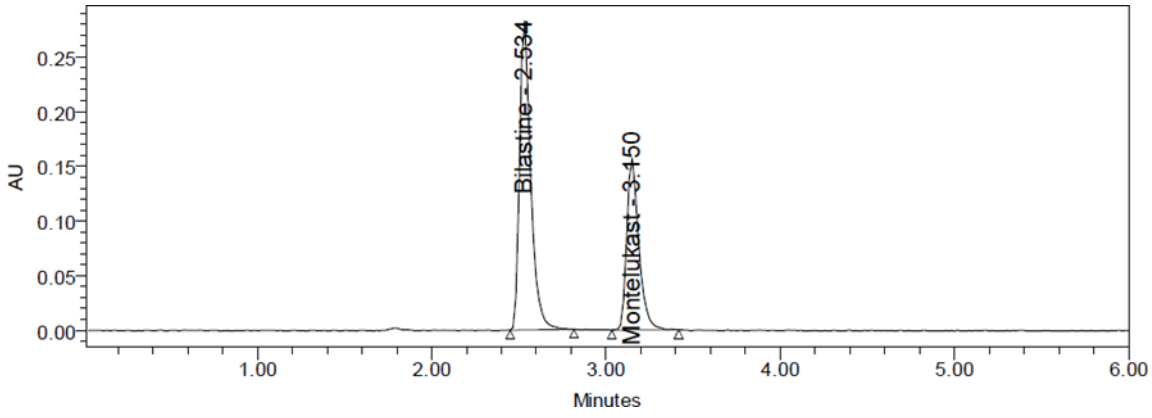

Figure 3. Optimized chromatogram

S:No	Bilastine			Montelukast			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.529	7830	1.34	3.147	9876	1.34	4.9
2	2.534	7910	1.34	3.150	10276	1.33	4.9
3	2.537	8097	1.34	3.156	10085	1.35	5.1
4	2.538	7916	1.33	3.158	10250	1.35	5.1
5	2.540	7905	1.34	3.165	9916	1.33	4.9
6	2.556	7965	1.35	3.181	9959	1.34	4.9

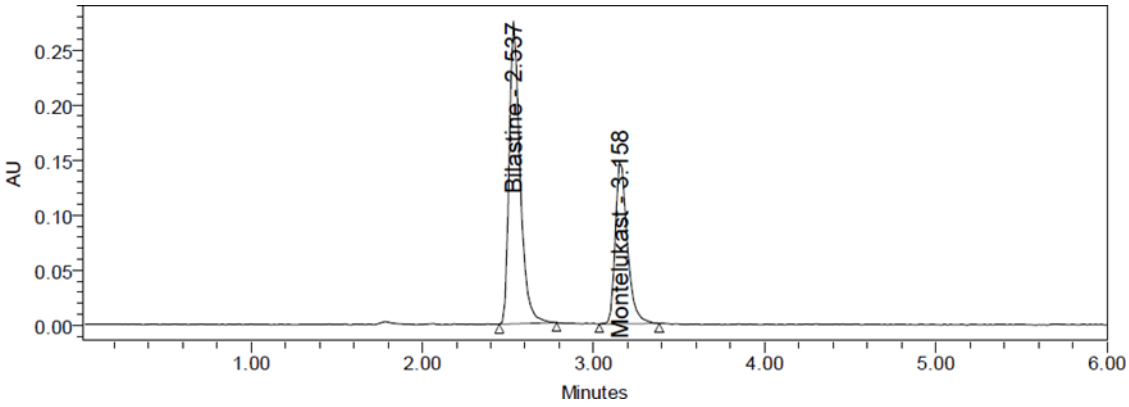

Figure.4: Standard solution chromatogram

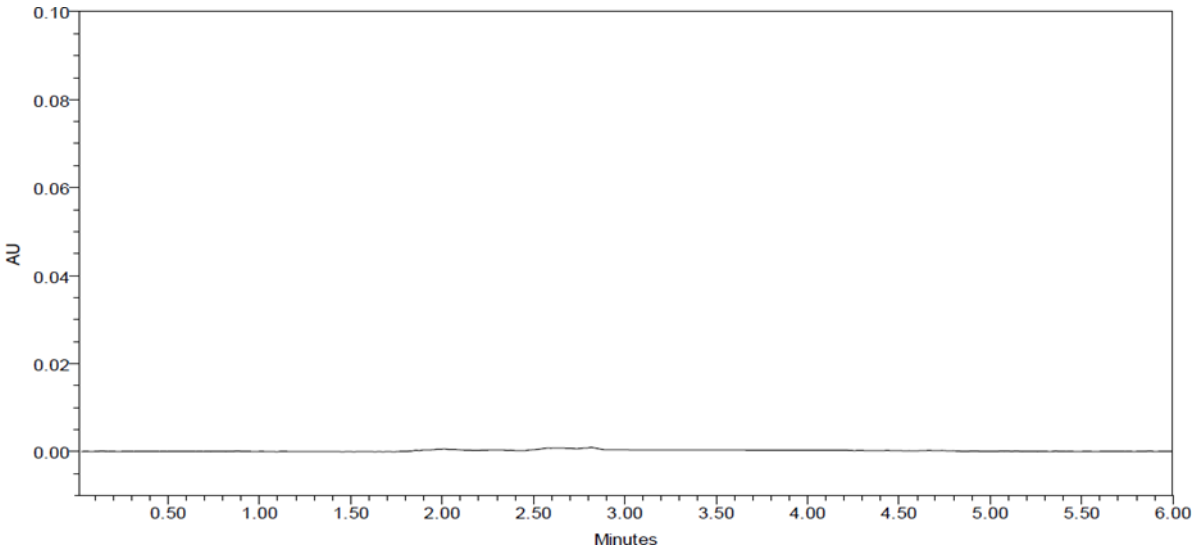

Figure.5: Blank chromatogram

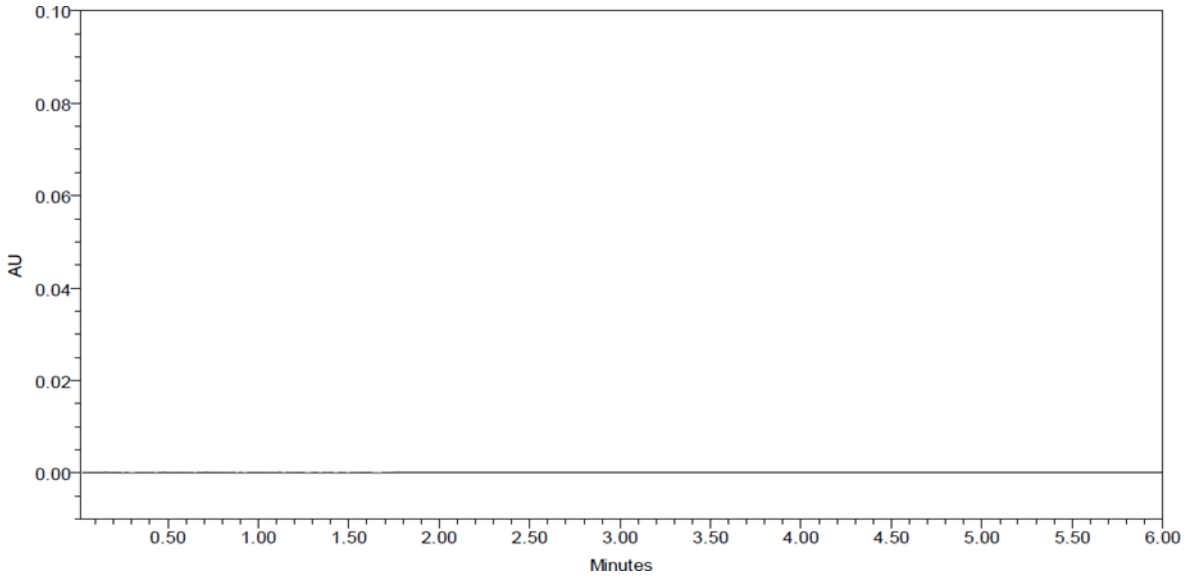

Figure.6: Placebo chromatogram

Table No.1: Linearity table for Bilastine and Montelukast,

Bilastine		Montelukast	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
5	328333	2.5	190499
10	645236	5	376491
15	946151	7.5	567143
20	1274829	10	748145
25	1555762	12.5	913713
30	1877580	15	1095611

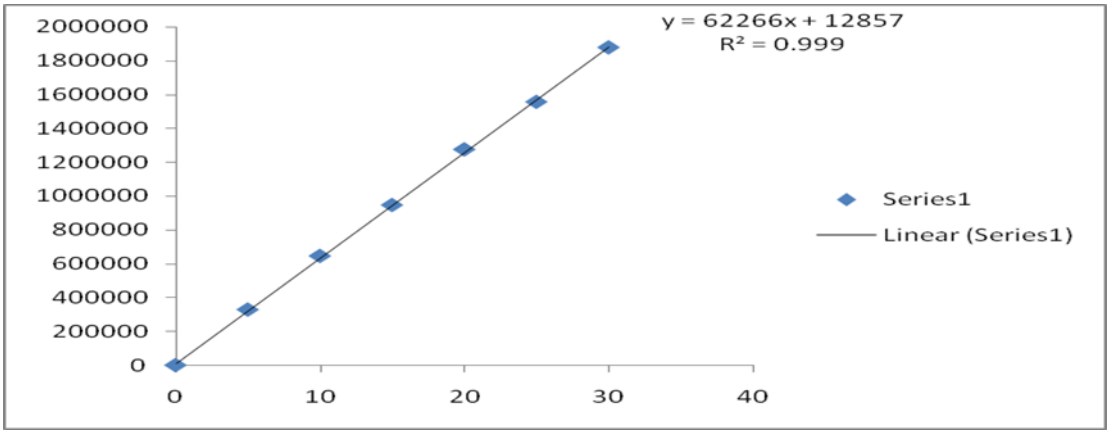

Figure.7: Calibration curve Bilastine

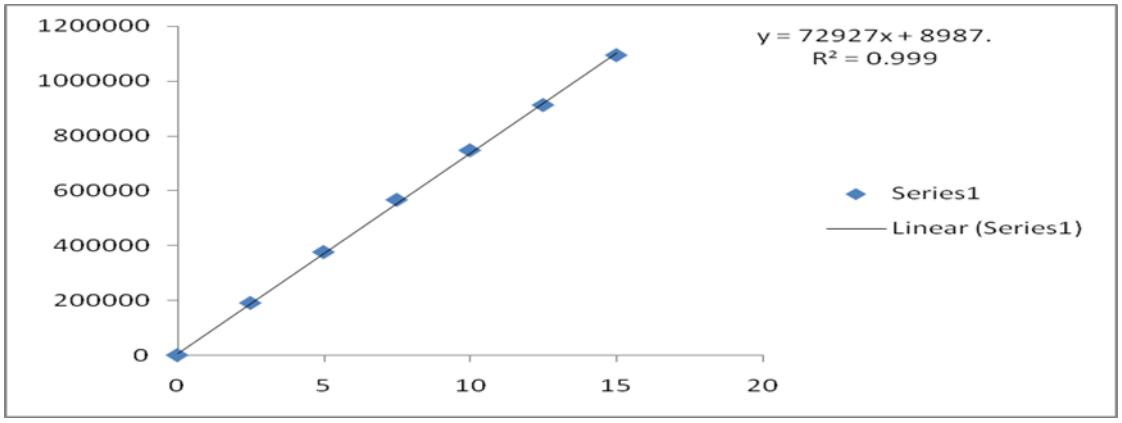

Figure.8: Calibration curve Montelukast

Table No.2: Repeatability for Bilastine and Montelukast

Bilastine	Montelukast
1254124	725909
1240740	732487
1235312	728358
1258060	739293
1263108	729060
1241623	739892
1248828	732500
11110.4	5885.9
0.9	0.8

Table No.3: Intermediate Precision for Bilastine and Montelukast

S. No	Area of Bilastine	Area of Montelukast
1.	1189197	698447
2.	1183347	701052
3.	1158103	698024
4.	1177451	696925
5.	1162793	692575
6.	1197268	707703
Mean	**1178027**	**699121**
S.D	**15183.3**	**5034.1**
%RSD	**1.3**	**0.7**

Table No.3: Accuracy for Bilastine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	10.2	101.6	99.55%
	10	10.0	100.3	
	10	9.9	99.0	
100%	20	19.7	98.7	
	20	19.9	99.7	
	20	19.8	98.8	
150%	30	30.1	100.2	
	30	29.7	98.9	
	30	29.6	98.8	

Table No.4: Accuracy for Montelukast

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	4.98	99.62	99.51%
	5	5.01	100.21	
	5	5.01	100.24	
100%	10	9.89	98.94	
	10	10.04	100.40	
	10	9.87	98.67	
150%	15	14.93	99.56	
	15	14.81	98.71	
	15	14.89	99.29	

Table No.5: Robustness Data

S.no	Condition	%RSD Bilastine	Montelukast
1	Flow rate (-) 0.9ml/min	0.9	0.4
2	Flow rate (+) 1.1ml/min	0.9	0.5
3	Mobile phase (-) 75B:25A	0.9	0.4
4	Mobile phase (+) 65B:35A	0.9	0.9
5	Temperature (-) 27°C	1.3	0.3
6	Temperature (+) 33°C	0.4	1.1

Table No.6 Degradation Data

Type of degradation	Bilastine			Montelukast		
	Area	%Recovered	% Degraded	Area	%Recovered	% Degraded
Acid	1183953	94.52	5.48	688696	94.21	5.79
Base	1199094	95.73	4.27	694547	95.01	4.99
Peroxide	1201577	95.93	4.07	699860	95.73	4.27
Thermal	1227727	98.02	1.98	717255	98.11	1.89
Uv	1232477	98.40	1.60	722736	98.86	1.14
Water	1244641	99.37	0.63	725678	99.26	0.74

Fig No 7: Degradation Chromatogram

Acid degradation chromatogram

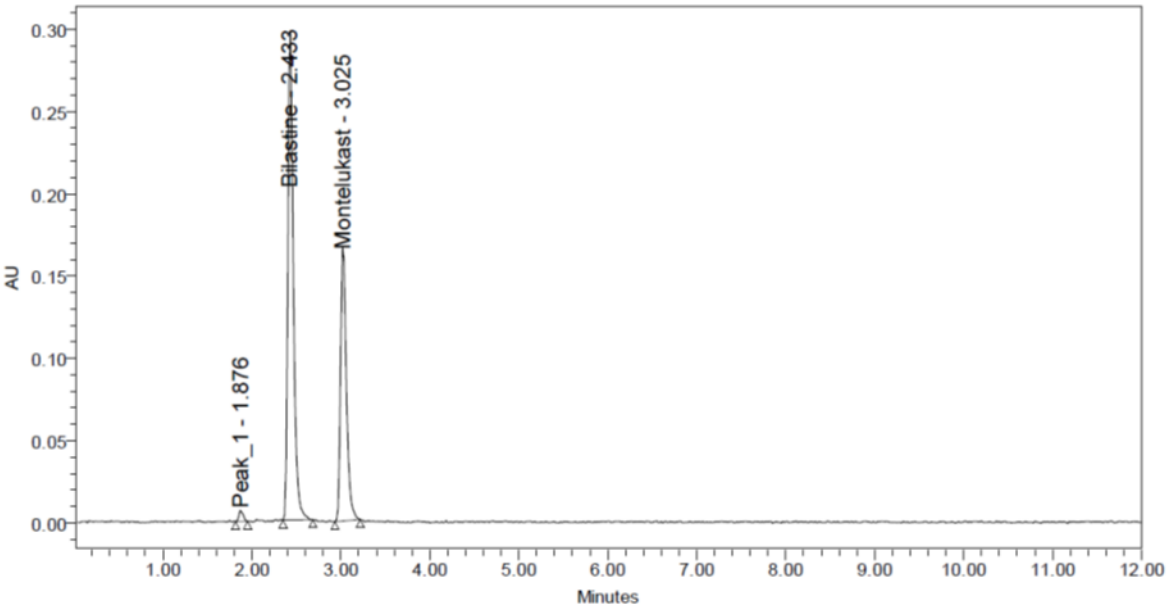

Figure.9 acid

Base degradation chromatogram

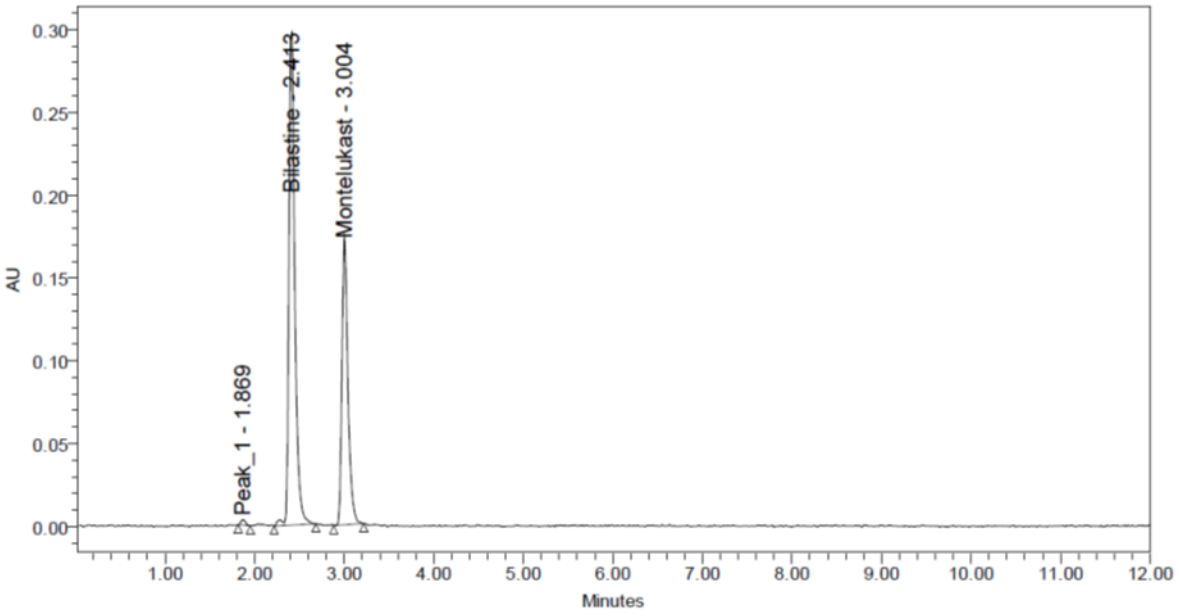

Figure.10 base

Peroxide degradation chromatogram

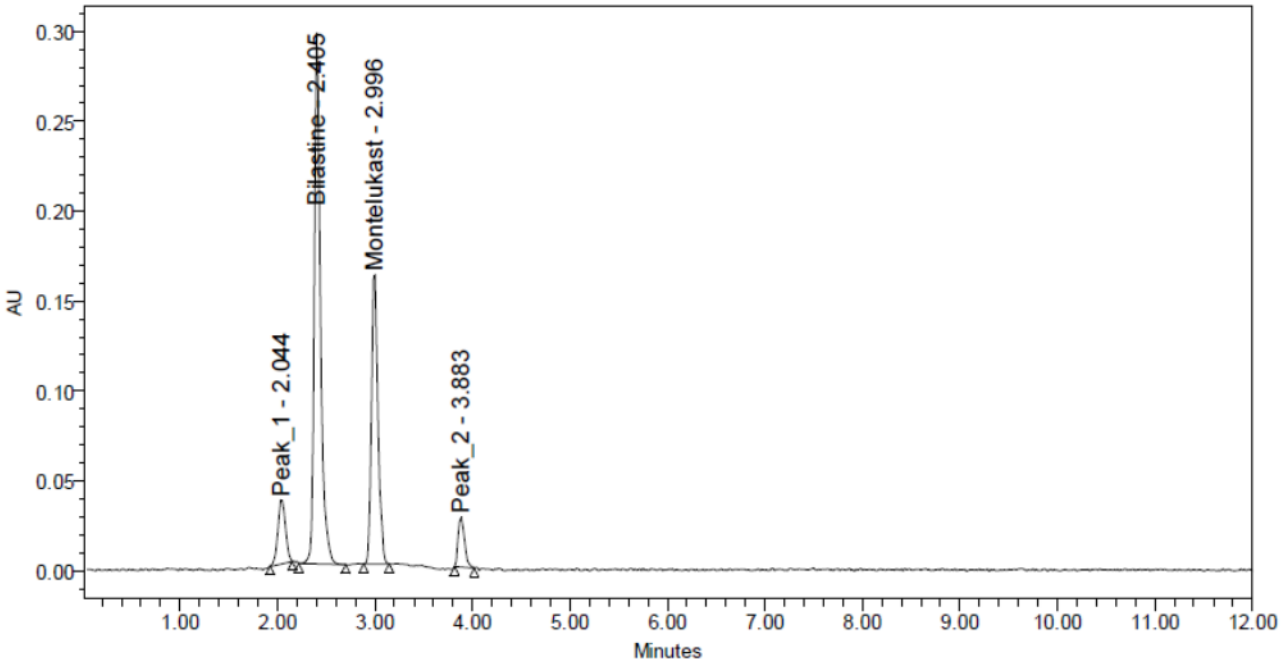

Figure.11 peroxide

Thermal degradation chromatogram

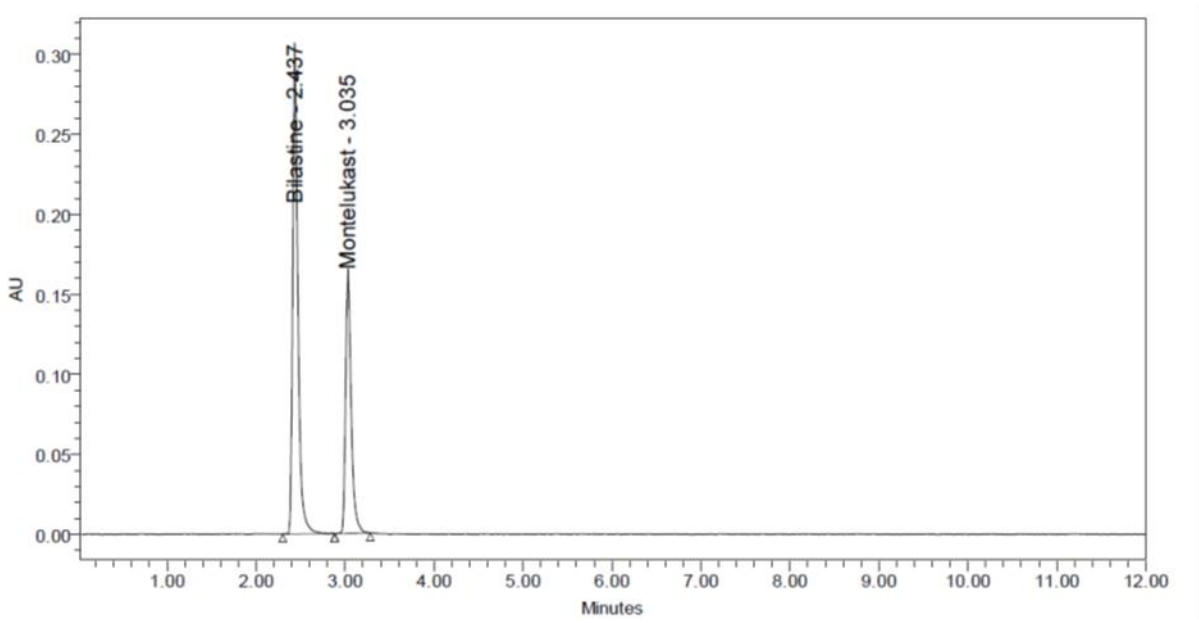

Figure.12 thermal

Uv degradation chromatogram

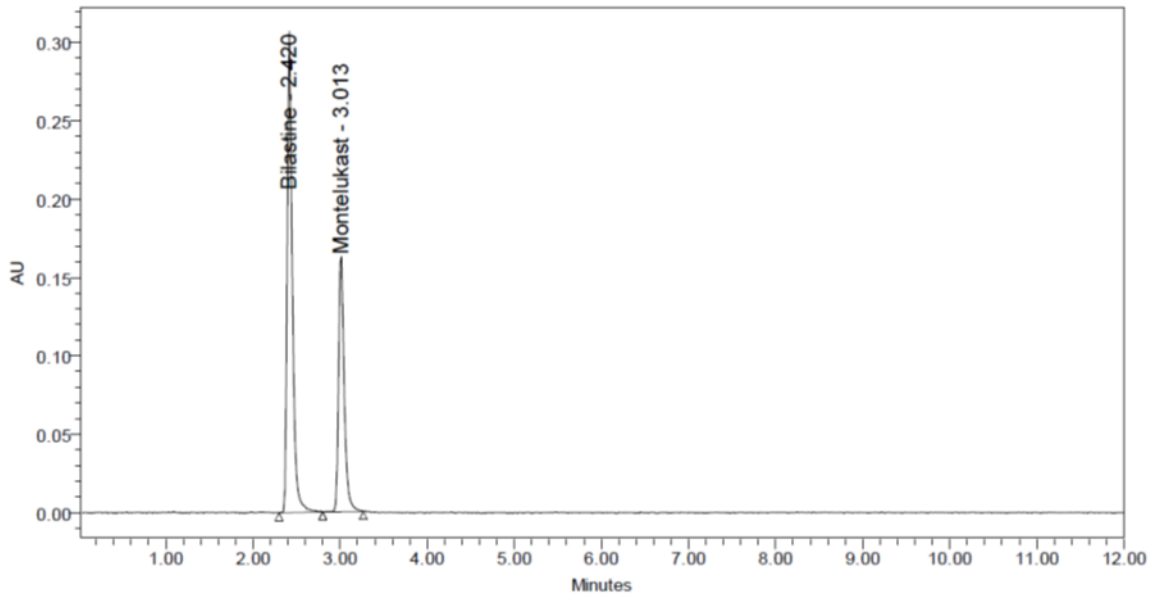

Figure.13 uv

Water degradation chromatogram

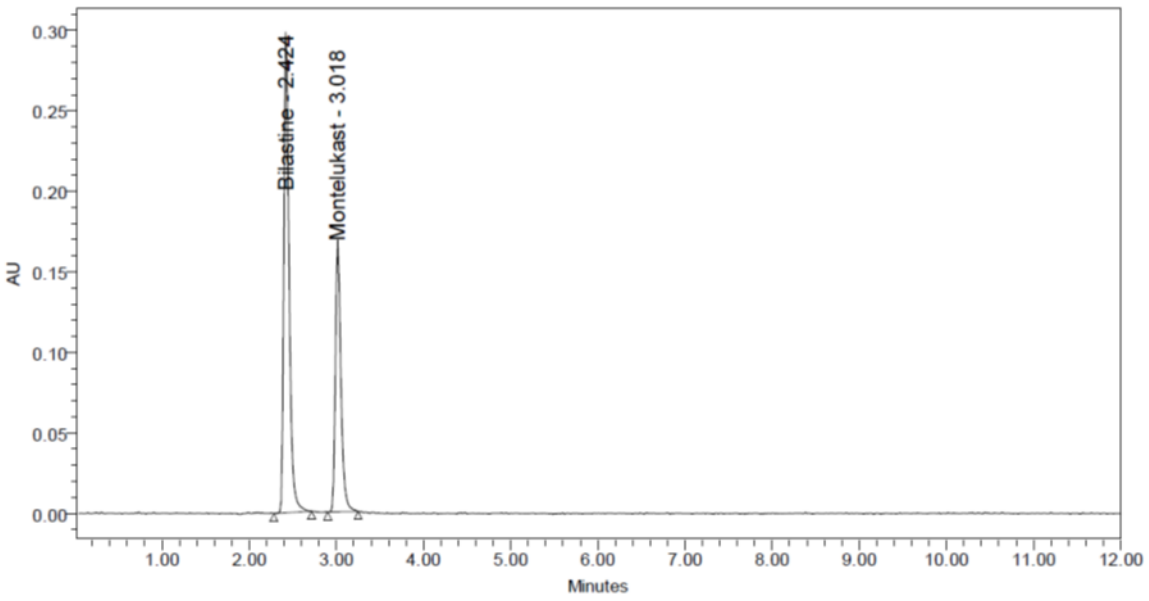

Figure.14 water

Conclusion: The purpose of Bilastine and Montelukast in drug quantity methods is discussed using a straightforward and selective LC approach. On an Inertsil C8 (150 mm x 4.6 mm x 5.0 µm) column, chromatographic departure was skillful by a mobile phase made up of 0.01N di sodium hydrogen phosphate buffer: Acetonitrile (70:30)%v/v prepared with detection at 230 nm. For Bilastine and Montelukast, breadth was understood in the attention range of 5–30 µg/ml and 2.5-7.5 µg/ml (r2 = 0.9995), indicating that the amount of pharmaceuticals calculated by the suggested methods was reasonably consistent with the label claim.

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