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Research Article

ANALYTICAL METHOD DEVELOPMENT AND RAPID ANALYTICAL TECHNIC FOR SIMULTANEOUS ESTIMATION OF MONTELUKAST AND BILASTINE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC METHOD

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ABSTRACT:
A simple, Accurate, precise method was developed for the simultaneous estimation of the Bilastine and Montelukast in bulk and pharmaceutical dosage form. Chromatogram was run through Agilent C18 150 x 4.6 mm, 5mm. Mobile phase containing Buffer :Acetonitrile taken in the ratio 70:30 was pumped through column at a flow rate of 1.0 ml/min. Buffer used in this method was 0.01N Na2Hpo4 buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 265.0 nm. Retention time of Bilastine and Montelukast were found to be 2.141 min and 2.605 min. %RSD of the Bilastine and Montelukast were and found to be 0.4% and 0.2% respectively. %Recovery was obtained as 99.47% and 99.55% for Bilastine and Montelukast respectively. LOD, LOQ values obtained from regression equations of Bilastine and Montelukast were 0.1, 0.03 and 0.03, 0.10 respectively. % Assay was obtained as 99.74% and 99.72% for Bilastine and Montelukast respectively. Regression equation of is Montelukast y = 45726x + 6306.9, y = 43360x + 810 of Bilastine.

Key Words: Bilastine and Montelukast, Rp Hplc, Validation.

INTRODUCTION

Pharmacological medicines like montelukast and bilastine are commonly used to treat allergic diseases like asthma and allergic rhinitis. These medications have complimentary therapeutic effects because they target distinct pathways of the allergic inflammatory cascade. The cysteinyl leukotriene receptor CysLT is inhibited by the leukotriene receptor antagonist (LTRA) montelukast.[1], thus lowering mucus production, bronchoconstriction, and airway inflammation. Although bilastine is more hydrophilic and requires ideal chromatographic conditions to achieve separation and precise detection, it is very helpful in treating asthma and allergic rhinitis, especially in patients who are not susceptible to antihistamines alone[1.2]

Bilastine is a second-generation H1-antihistamine that has a high selectivity for histamine receptors. It relieves histamine-mediated symptoms such sneezing, itching, and congestion quickly and effectively. It is a great option for urticaria and allergic rhinitis because to its good safety profile, which includes low levels of sedation.3

Targeting both leukotriene- and histamine-mediated pathways, montelukast and bilastine provide a dual mode of action. Because of this, the combination works especially well for individuals who have severe or ongoing allergy symptoms, enhancing their quality of life and overall symptom control.[4] Ongoing research explores the broader applications of this combination, including its role in other allergic and inflammatory conditions.5

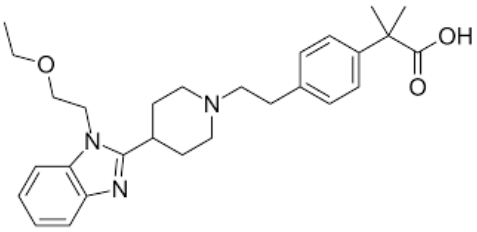

Figure 1: structure of Bilastine

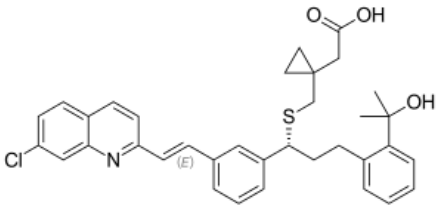

Figure 2: Structure of Montelukast

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Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Bilastine, Montelukast, and their medicinal dose form using RP-HPLC [6- 14] must be validated and developed as per ICH guidelines

MATERIALS AND METHODS: Spectrum pharma Research Solution with Bilastine and Montelukast pure drugs (API) gift samples and Combination Bilastine and Montelukast tablets (Bilzit 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 Montelukast and Bilastine in pharmaceutical formulations at the same time.

Table 1: Chromatographic Conditions

Mobile phase	Acetonitrile and 0.01N Na2Hpo4 (30:70 v/v)
Flow rate	1 ml/min
Column	Agilent C18 (4.6 x 150mm, 5µm)
Detector wave length	265 nm
Column temperature	30°C
Injection volume	10mL
Run time	5.0 min
Buffer	Na2Hpo4

API Preparation:

Preparation of Standard stock solutions: Accurately weighed 10 mg of Montelukast, 5mg of Bilastine and transferred to 50ml volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (200µg/ml of Montelukast and 100µg/ml Bilastine).

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. (20µg/ml of Montelukast and 10µg/ml of Bilastine).

Formulation Preparation:

Preparation of Sample stock solutions: 10 Tablets were accurately weighed and averae 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 (200µg/ml of Montelukast and 100µg/ml of Bilastine).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Montelukast and 10µg/ml of Bilastine).

System suitability parameters: Bilastine (10 ppm) and Montelukast (20 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.

Table 2: System suitability results

S no	**Bilastine**			**Montelukast**			
Inj	**RT(min)**	**USP Plate Count**	**Tailing**	**RT(min)**	**USP Plate Count**	**Tailing**	**RS**
1	2.141	5818	1.25	2.600	8117	1.19	4.7
2	2.142	5542	1.27	2.601	8161	1.19	4.6
3	2.143	5761	1.24	2.602	8250	1.21	4.6
4	2.143	5490	1.28	2.602	8650	1.22	4.6
5	2.149	5457	1.25	2.604	8183	1.21	4.6
6	2.149	5475	1.24	2.606	8347	1.19	4.8

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

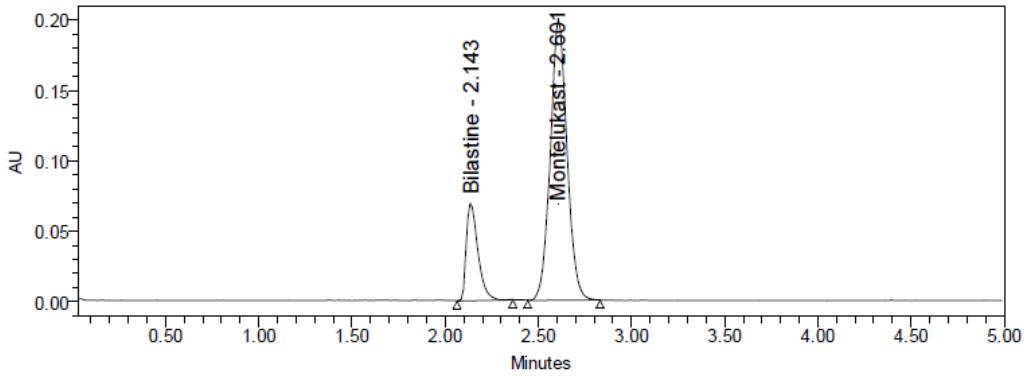

Figure 3: system suitability Chromatogram

Specificity: Checking of the interference in the optimized method. And no interference was observed so, it is specific.

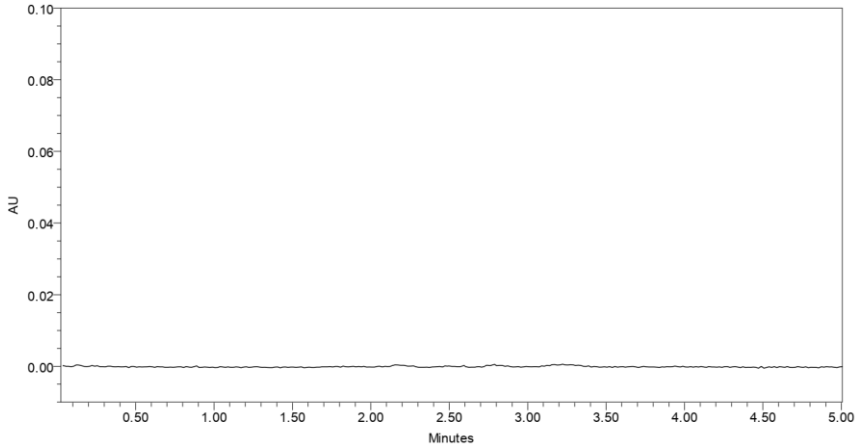

Figure.4 Specificity of Bilastine and Montelukast

Linearity:

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

Table 3: Calibration data of Bilastine and Montelukast

Bilastine		**Montelukast**	
Conc (µg/mL)	**Peak area**	**Conc(µg/mL)**	**Peak area**
0	0	0	0
2.5	108611	5	234274
5	217455	10	467338
7.5	324775	15	696574
10	435673	20	923867
12.5	552432	25	1156275
15	643104	30	1367025

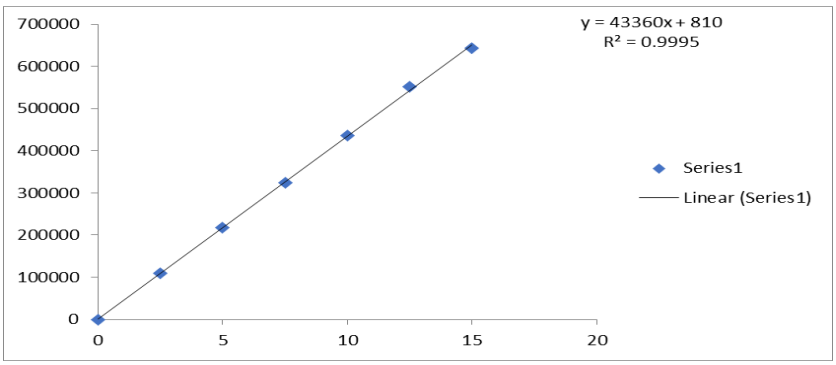

Figure 5 Calibration curve of Bilastine

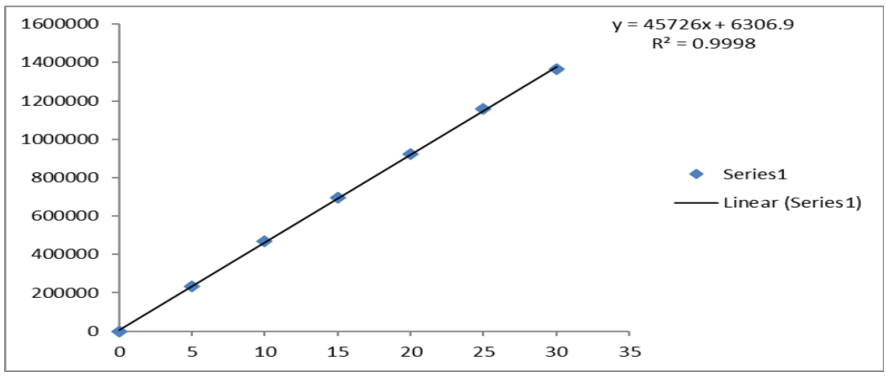

Figure 6 Calibration curve of Montelukast

Table.4: regression data

Parameter	Bilastine	Montelukast
Conc range (µg/mL)	2.5-15	5-30
Regression Equation	y = 43360x + 810	y = 45726x + 6306.9
Co-relation	0.999	0.999

Accuracy:

Recovery data shown in table.

Table.5: recovery data of Bilastine and Montelukast

	Bilastine			Montelukast		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	5	4.96	99.26	10	9.9	99.0
		4.98	99.66		10.0	99.6
		4.98	99.69		9.9	99.0
100%	10	9.99	99.94	20	19.9	99.7
		9.92	99.19		20.0	100.1
		9.95	99.46		19.9	99.5
150%	15	14.96	99.76	30	29.8	99.4
		14.96	99.74		29.9	99.6
		14.89	99.26		29.8	99.3
% recovery	99.74			99.47		

Precision:

System Precision: it is determined and is shown in the table.

Table.6 System Precision

S. No	Area of Bilastine	Area of Montelukast
1.	433817	926254
2.	435922	924285
3.	431622	924711
4.	435987	930375
5.	435811	924960
6.	436487	925298
Mean	434941	925981
S.D	1869.9	2253.0
%RSD	0.4	0.2

Interday and intraday Precision: The precision of the method was determined by analyzing a sample of Bilastine and Montelukast and shown in table.

Table 7: method Precision

	Intraday Precision		Interday Precision	
S. No	**Area of Bilastine**	**Area of Montelukast**	**Area of Bilastine**	**Area of Montelukast**
1.	433788	921122	411527	917413
2.	434112	928726	416607	918988
3.	436227	924227	413573	919965
4.	433812	921067	412329	920492
5.	436747	925480	410629	918111
6.	433471	930891	410851	923533
Mean	**434693**	**925252**	**412586**	**919750**
S.D	**1414.1**	**3988.5**	**2243.7**	**2174.2**
%RSD	**0.3**	**0.4**	**0.5**	**0.2**

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

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.0ml/min), mobile phase minus (40B:60A), mobile phase plus (50B:50A), 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.8: Robustness data for Bilastine and Montelukast.

Parameter	**Optimized condition**	**Used condition**	**Bilastine**	**Montelukast**
			Obtained %RSD	
Flow rate (±0.1ml/min)	1ml/min	0.9ml/min	0.2	0.2
		1.1 ml/min	0.4	0.3
MP (5%v/v)	60:40	65:35	0.6	0.3
		75:25	0.6	0.4
Column temp. (±3^{0}c)	30^{0}c	27 ^{0}C	0.9	0.3
		33 ^{0}C	0.7	0.3

Sensitivity:

Table.9: sensitivity of Bilastine and Montelukast

Molecule	**LOD**	**LOQ**
Bilastine	0.01 µg/ml	0.03 µg/ml
Montelukast	0.03 µg/ml	0.10 µg/ml

Force Degradation Studies: Table shows degradation conditions and table 10 shows the obtained degraded data and chromatogram in figure.

Table.10: 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
Oxidation	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.11: Degradation data

Conc of degradation study	**Bilastine**		**Montelukast**	
	% drug Undegraded	**% drug degraded**	**% drug Undegraded**	**% drug degraded**
2N HCl, 60 min	95.05	4.95	94.02	5.98
2N NaOH, 60min	96.05	3.95	94.13	5.87
Oxidative, 60 min	94.76	5.24	94.75	5.25
Thermal, 1 hr	97.22	2.78	97.78	2.22
Photo, 6 hr	98.05	1.95	98.96	1.04
Neutral, 1 hr	99.10	0.90	99.42	0.58

Acid degradation chromatogram

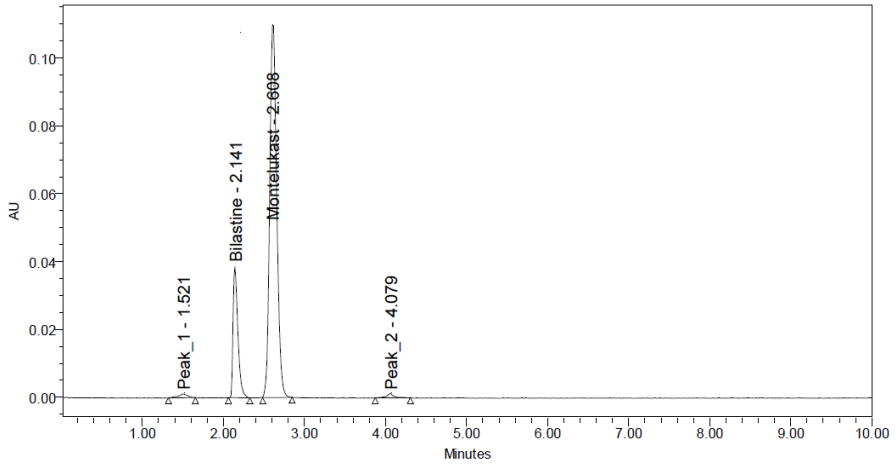

Figure.7 Acid

Base degradation chromatogram

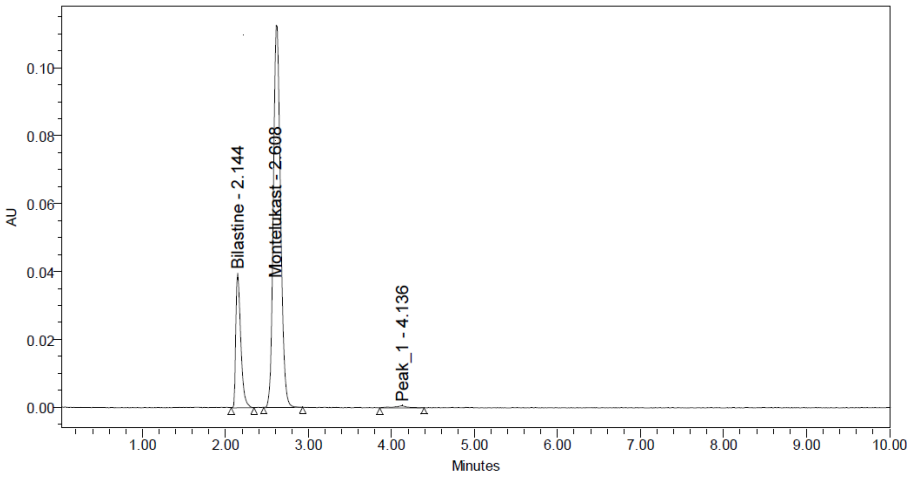

Figure.8 Base

Peroxide degradation chromatogram

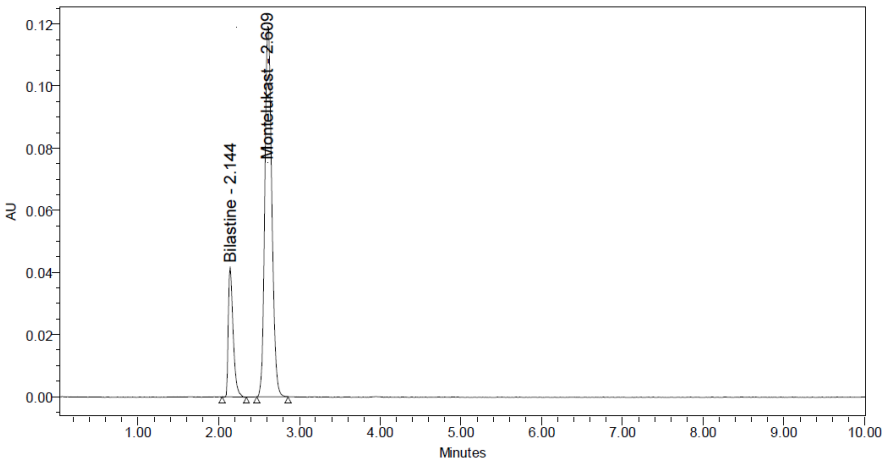

Figure.9 Peroxide

Thermal degradation chromatogram

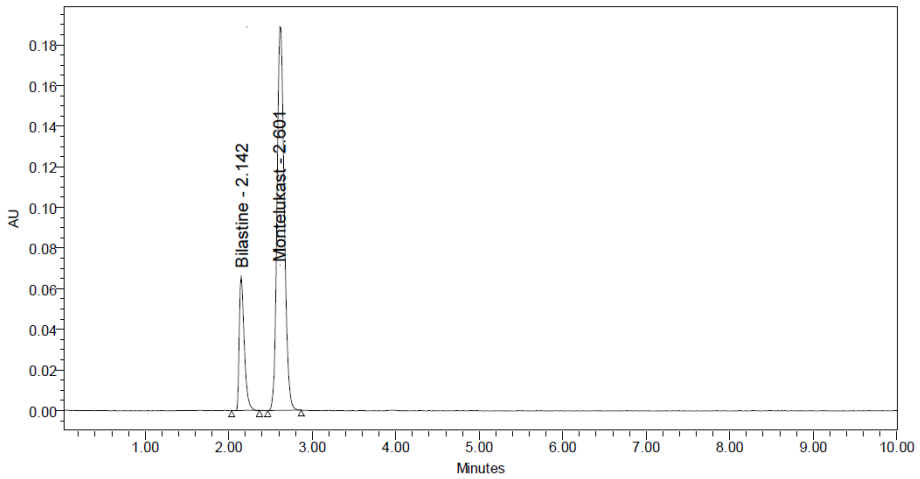

Figure.10 Thermal

UV degradation chromatogram

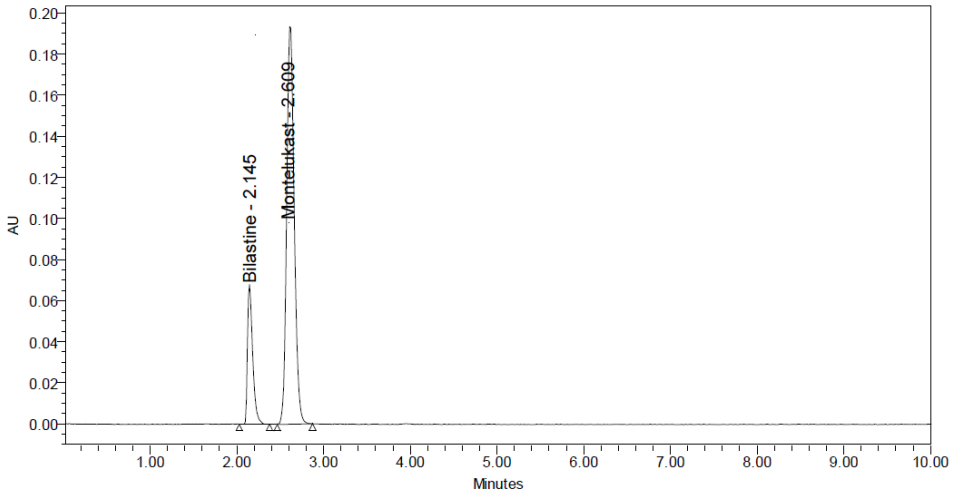

Figure.11 UV

Water degradation chromatogram

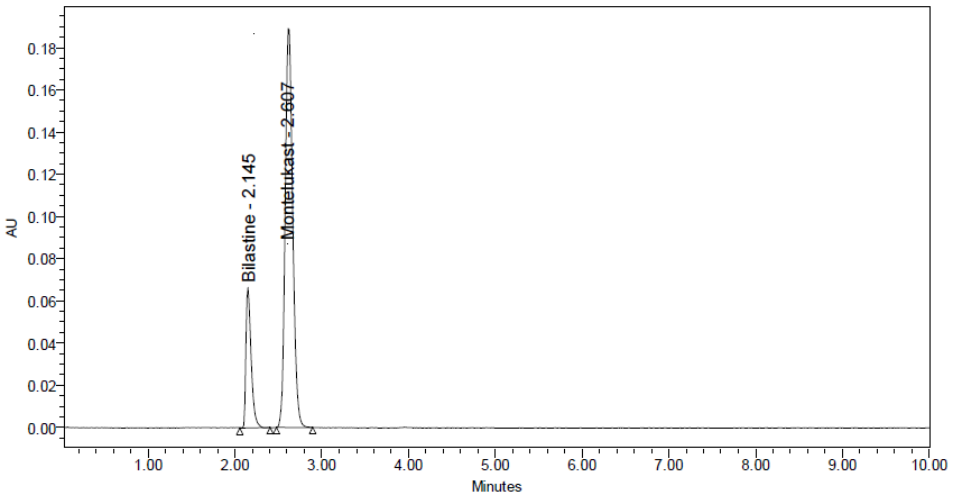

Figure.12 Water

Assay: Average % Assay for Bilastine and Montelukast obtained was 99.58% and 99.65% respectively.

Table 12: Assay data

	Bilastine			Montelukast		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	433817	433788	99.54	926254	921122	99.28
2	435922	434112	99.61	924285	928726	100.10
3	431622	436227	100.10	924711	924227	99.61
4	435987	433812	99.54	930375	921067	99.27
5	435811	436747	100.21	924960	925480	99.75
6	436487	433471	99.46	925298	930891	100.33
Avg	**434941**	**434693**	**99.74**	**925981**	**925252**	**99.72**
Stdev	**1869.9**	**1414.1**	**0.32**	**2253.0**	**3988.5**	**0.43**
%RSD	**0.4**	**0.3**	**0.33**	**0.2**	**0.4**	**0.4**

CONCLUSION:

The study's findings will be very helpful in evaluating the quality of reasonably priced drugs that contain Montelukast and Bilastine. This could be as a result of the study's straightforward sample preparation method, which required little mobile phase and a brief analytical period. The results of evaluating two medications combined in a single dosage demonstrated that the recently created analysis technique was almost entirely successful.

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