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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF MONTELUKAST AND BILASTINE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT:

By using Agilent (250mm 4.6mm, 5µm) simultaneous estimation of the Bilastine and Montelukast in Tablet dosage form was established. Mobile phase containing 0.1% OPA and CH_3CN in 60:40 v/v at a flow of 1.0 ml/min. Temperature maintained at 30°C. Optimized wavelength was 214nm. Retention time of 2.146 min and 3.259 min were found to be Bilastine and Montelukast. %RSD of the Bilastine and Montelukast were and found to be 0.4 and 1.0 respectively. %Recover was 99.85% and 99.98% for Bilastine and Montelukast. LOD, LOQ values were obtained from regression equations of Bilastine and Montelukast were 1.48, 4.47 and 0.30, 0.90 respectively. Regression equation of Bilastine is y = 15205x + 5169.6, and of Montelukast is y = 15205x + 5169.6.

Key Words: Bilastine, Montelukast, RP HPLC, Validation, Method Development.

INTRODUCTION

Montelukast and bilastine are pharmacological agents frequently used in the management of allergic conditions such as allergic rhinitis and asthma. These drugs target different pathways of the allergic inflammatory cascade, making them complementary in their therapeutic effects.

Montelukast, a leukotriene receptor antagonist (LTRA), inhibits the cysteinyl leukotriene receptor CysLT1, thereby reducing airway inflammation, bronchoconstriction, and mucus production. It is particularly effective in managing asthma and allergic rhinitis, especially in patients who are unresponsive to antihistamines alone.[1] while bilastine is more hydrophilic, requiring optimized chromatographic conditions to achieve separation and accurate detection. [2]

Bilastine is a second-generation H1-antihistamine with high selectivity for histamine receptors, providing rapid and long-lasting relief from histamine-mediated symptoms such as nasal congestion, itching, and sneezing. Its favorable safety profile, including minimal sedation, makes it an ideal choice for allergic rhinitis and urticaria.[3]

The combination of montelukast and bilastine offers a dual mechanism of action, targeting both leukotriene- and histamine-mediated pathways. This makes the combination particularly effective in patients with severe or persistent allergic symptoms, improving overall symptom control and quality of life.[4] Ongoing research explores the broader applications of this combination, including its role in other allergic and inflammatory conditions.[5]

Montelukast is A medication used to treat asthma, exercise related breathing problems, and a runny nose due to allergies and it is written as 2-[1-({[(1R)-1-{3-[(1E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl}-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfanyl}methyl)cyclopropyl]acetic acid.[7] Bilastine is a peripheral histamine H1-antagonist used to treat seasonal allergic rhinitis and chronic spontaneous urticaria.[8]

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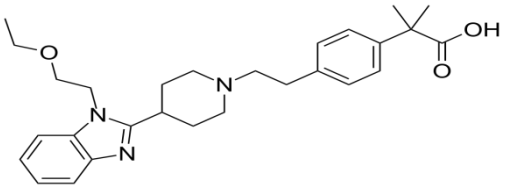
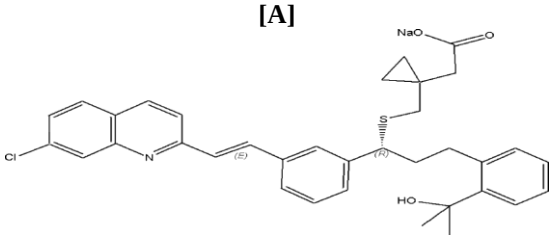


[A]



[B]

Figure 1 and 2 structure of [A] Bilastine and [B]Montelukast

Numerous documented analytical processes, including the identification of more cost-effective methods, have been uncovered by extensive literature research. Therefore, a dependable and economical method for evaluating the stability of Montelukast, Bilastine, and their pharmaceutical dosage form utilising RP-HPLC.[9-13] needs to be established and verified in accordance with ICH criteria.

Materials and Methods: Montelukast and Bilastine pure pharmaceuticals (API) are available as gift samples from Spectrum Pharma Research Solution. And combination of both drug BILLARGIC M was brought from the Pharmacy. The chemicals and buffers used in this estimation were supplied by Rankem, an Indian source.

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 Bilastine and Montelukast in pharmaceutical formulations at the same time.

Table 1: Chromatographic Conditions

Mobile phase	Acetonitrile: OPA (60:40A)
Flow rate	1.0 ml/min
Column	Agilent C18 Column, 5 µm, 4.6 x 250 mm
Detector wave length	214 nm
Column temperature	30°C
Injection volume	10µL
Run time	10.0 min

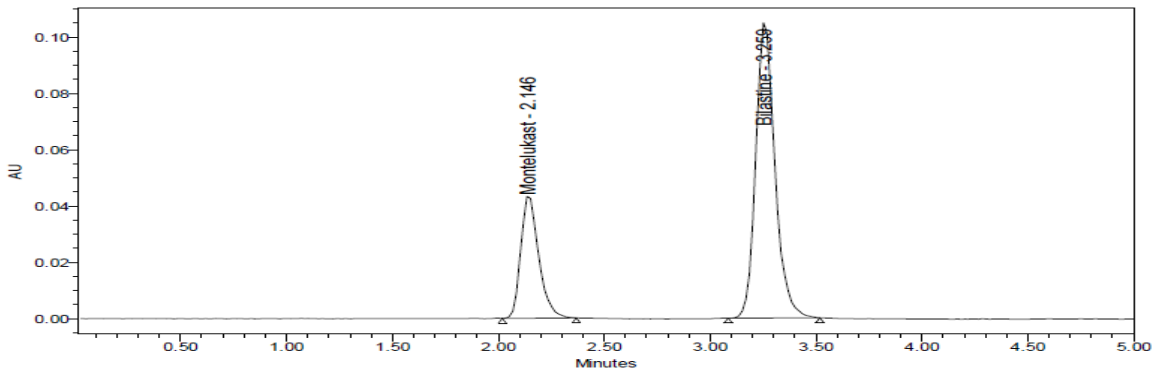


Figure 3: Optimized Chromatogram

Preparation of Standard stock solutions: 10 mg of Montelukast, 20mg of Bilastine added in 50ml VF and filled till 3/4th of diluents and then it was sonicated for 10 minutes. And then it was filled will mark and label as std solution (200µg/ml of Montelukast and 400µg/ml Bilastine). Diluent was added to a 10 ml volumetric flask after 1 ml of each stock solution was pipetted out. (20µg/ml of Montelukast and 40µg/ml of Bilastine)

Preparation of Sample stock solutions: in a 100 ml VF 1 tablet equivalent weight drug was added and 50ml dil was added and sonicated for 20 min and then the solution was filtered with HPLC filter (100µg/ml of Montelukast and 200µg/ml of Bilastine). A 10 ml volumetric flask was then filled with 2 ml of the filtered sample stock solution and diluted. (20µg/ml of Montelukast and 40µg/ml of Bilastine)

System suitability parameters: The system appropriateness characteristics, including peak tailing, resolution, and USP plate count, were assessed after six injections of standard solutions. An RSD of no more than 2% should be present in the region of six standard injection results.

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	Montelukast			Bilastine			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.111	2875	1.29	3.186	5826	1.22	6.6
2	2.112	2891	1.29	3.188	5698	1.19	6.6
3	2.112	2898	1.26	3.188	5946	1.20	6.5
4	2.113	3107	1.28	3.188	6176	1.20	6.6
5	2.113	3131	1.28	3.189	6301	1.20	6.6
6	2.114	2998	1.26	3.189	6101	1.21	6.6

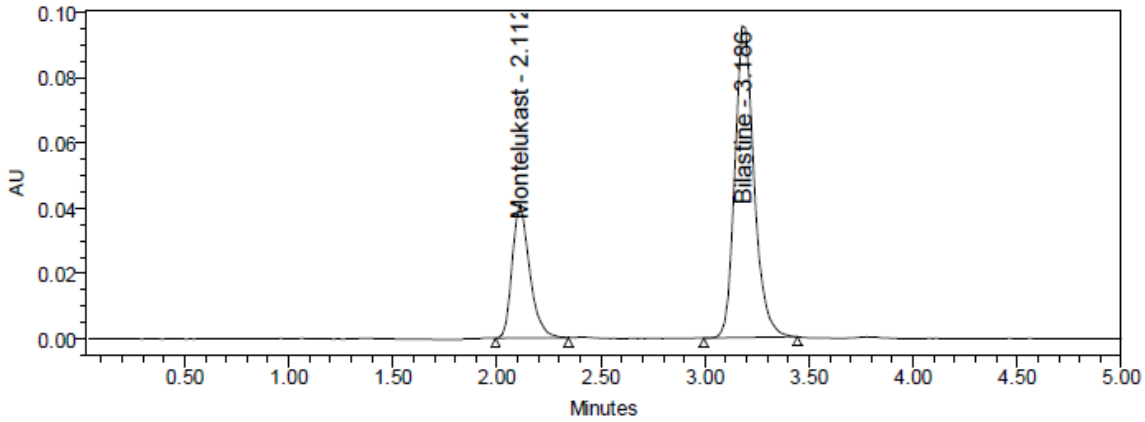
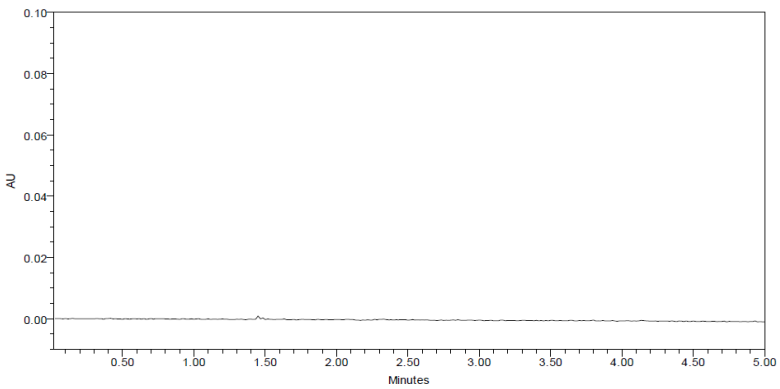


Figure 4: System suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time(mins)	Area
Montelukast	2.146	255518
Bilastine	3.256	660856



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Force Degradation Studies: table 4 shows degradation conditions and table 5 shows the obtained degraded data and purity plot chromatogram in figure 6,7.

Table 4: degradation conditions

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	-

Table 5: degradation data

Type of degradation	Montelukast			Bilastine		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	216729	91.52	8.48	594689	91.18	8.82
Base	231007	97.54	2.46	617833	94.73	5.27
Peroxide	229357	96.85	3.15	628766	96.41	3.59
Thermal	235590	99.48	0.52	635613	97.46	2.54
UV	235338	99.37	0.63	632292	96.95	3.05
Water	230487	97.33	2.67	637844	97.80	2.20

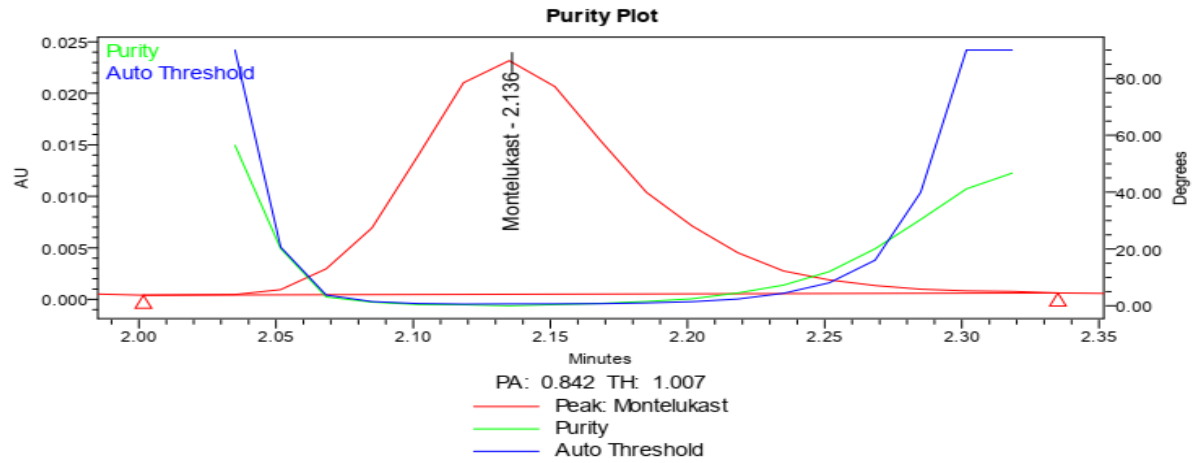

Figure 6: Purity plots for Acid Condition for Montelukast

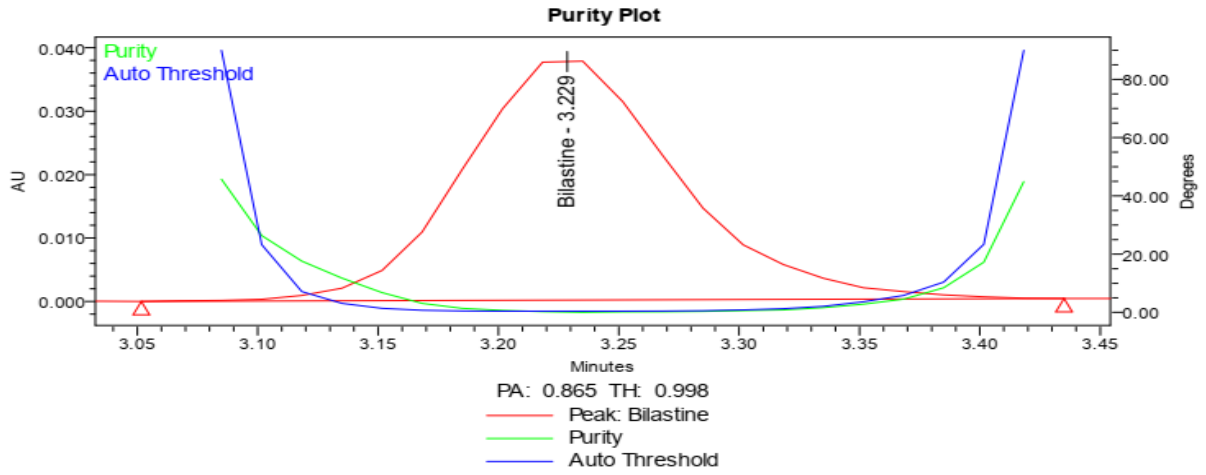

Figure 7: Purity plots for Acid Condition for Bilastine

Linearity:

Calibration data is given in regression data table 6 and calibration curve in figure 8, 9.

Table 6: Calibration data of Montelukast and Bilastine

	Montelukast		Bilastine	
S. no	**Conc (µg/mL)**	**Peak area**	**Conc(µg/mL)**	**Peak area**
1	0	0	0	0
2	5	59644	10	161979
3	10	120168	20	306524
4	15	177128	30	468571
5	20	235126	40	615310
6	25	293199	50	758121
7	30	354785	60	918812
Concentration range	**5-30 µg/mL**		**10-60 µg/mL**	
Regression Equation	**y = 11760x + 747.48**		**y = 15205x + 5169.6**	
Co-relation	**0.9999**		**0.9997**	
LOD	**0.30**		**1.48**	
LOQ	**0.90**		**4.47**	

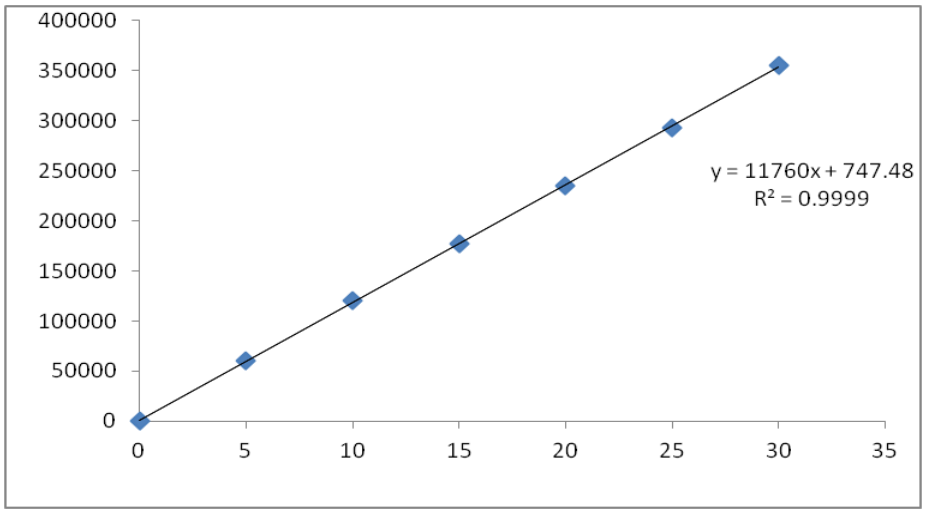

Figure 8 Calibration curve of Montelukast

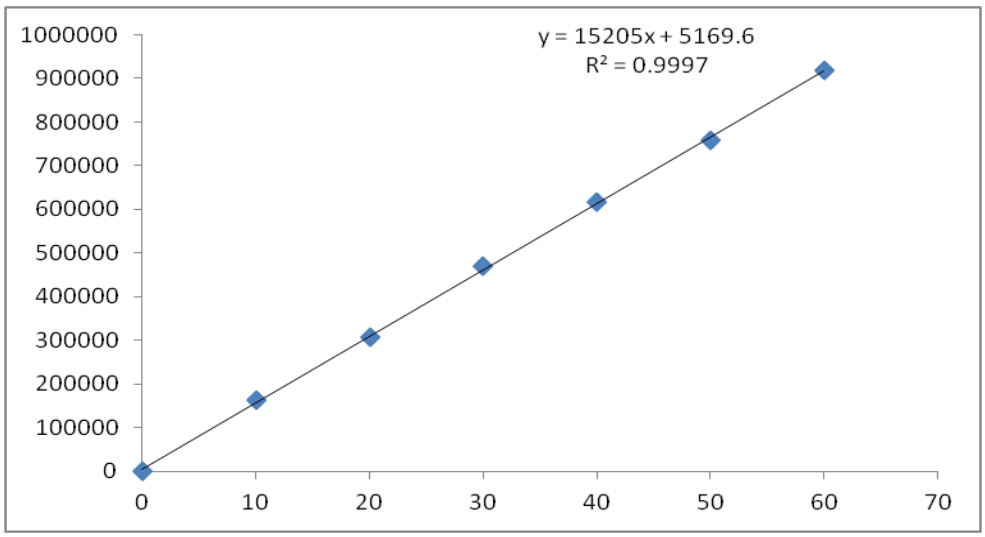

Figure 9 Calibration curve of Bilastine

Accuracy:
Recovery data shown in table 7

Table 7: recovery data of Montelukast and Bilastine

	Montelukast			Bilastine		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	10	9.94	99.37	20	20.09657	100.48
	10	10.01	100.12	20	19.85567	99.28
	10	10.04	100.41	20	19.91729	99.59
100%	20	20.02	100.09	40	39.84139	99.60
	20	20.14	100.68	40	40.01226	100.03
	20	20.06	100.32	40	40.18155	100.45
150%	30	29.79	99.31	60	60.1213	100.20
	30	29.72	99.06	60	59.69519	99.49
	30	30.13	100.42	60	59.68789	99.48
% recovery	**99.98**			**99.85**		

System precision was performed and the data was shown in table 8

Table 8: System precision of Montelukast and Bilastine

S. No	Area of Montelukast	Area of Bilastine
1.	237244	610076
2.	232160	612322
3.	238451	613272
4.	236654	619789
5.	235319	613358
6.	238257	619984
Mean	**236348**	**614800**
S.D	**2347.8**	**4114.2**
%RSD	**1.0**	**0.7**

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

Method Precision: The precision of the method was determined by analyzing a sample of Montelukast and Bilastine and shown in table 9.

Table 9: Method Precision

S. No	Area of Montelukast	Area of Bilastine
1.	237687	615553
2.	236317	617278
3.	235361	618921
4.	238014	616421
5.	239055	611708
6.	236366	619254
Mean	237133	616523
S.D	1354.3	2753.7
%RSD	0.6	0.4

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

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55A:45B), mobile phase plus (65A:35B), 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 10: Robustness data for Montelukast and Bilastine.

Condition	%RSD of Montelukast	%RSD of Bilastine
Flow rate (-) 0.9ml/min	0.4	0.6
Flow rate (+) 1.1ml/min	0.6	0.5
Mobile phase (-) 55A:45B	0.4	0.4
Mobile phase (+) 65A:35B	0.4	0.4
Temperature (-) 27°C	0.3	0.4
Temperature (+) 33°C	0.5	0.2

Assay: Billargic M Tablet label claims Montelukast 10mg, Bilastine 20mg. Assay was performed with the above formulation. The %Assay was found to be 100.08% and 100.13% for Bilastine and Montelukast respectively

Table 11: assay data

	Montelukast			Bilastine		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	237244	237687	100.37	610076	615553	99.92
2	232160	236317	99.79	612322	617278	100.20
3	238451	235361	99.38	613272	618921	100.47
4	236654	238014	100.50	619789	616421	100.06
5	235319	239055	100.94	613358	611708	99.30
6	238257	236366	99.81	619984	619254	100.52
Avg	236348	237133	100.13	614800	616523	100.08
Stdev	2347.8	1354.3	0.572	4114.2	2753.7	0.45
%RSD	1.0	0.6	0.6	0.7	0.4	0.45

Assay was calculated by the formula:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{1} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of drug working standard taken in mg
P	Assay of drug working standard in % on dried basis
L.C	Label Claim

Figure 10. Formula

Summary:

Table 12. Summary of the Method

Parameters		Bilastine	Montelukast
Linearity Concentration		10-60µg/ml	5-30µg/ml
R^2		0.999	0.999
(M)		15205	15205
Intercept		5169.6	5169.6
R^2		y = 15205x + 5169.6	Y= 15205x + 5169.6
Purity Assay		100.08%	100.13%
System Precision		0.7	1.0
Method Precision		0.4	0.6
Accuracy %recovery		99.85%	99.98%
LOD		1.48	0.30
LOQ		4.47	0.90
Robustness	**FM**	0.6	0.4
	FP	0.5	0.6
	MM	0.4	0.4
	MP	0.4	0.4
	TM	0.4	0.3
	TP	0.2	0.5

Conclusion:
The cost-effective approach devised for measuring Montelukast and Bilastine simultaneously utilising high-performance liquid chromatography (HPLC) proven to be accurate, precise, and trustworthy. This approach displayed high linearity, sensitivity, and reproducibility, allowing for exact detection of both medicines in pharmaceutical formulations. Because of its simplicity and low cost, it is suitable for routine quality control and batch release in industrial settings. It was confirmed as suitable for the intended use in accordance with ICH rules.

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