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Development and Validation of Method for Simultaneous Quantification of Bilastine, Montelukast by HPLC

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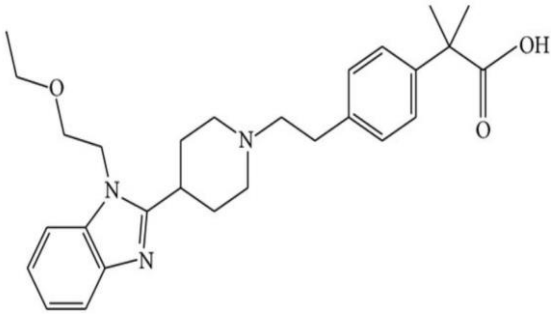
Abstract

A new, simple, rapid, specific, linear, accurate and robust analytical method by HPLC-UV has been developed for simultaneous quantification of Bilastine and Montelukast in tablet formulation. The degradation was done using individual drug substances. The separation was achieved by using Zorbax XDB C-18 column (150*4.6) mm, 5µ, using Water:Acetonitrile:Formic acid (50:50:1) as mobile phase. The flow rate was 1mL/min and injection volume was 20 µl. The retention time of Bilastine and Montelukast was observed at 3.22 minutes and 4.80 minutes respectively. The method was validated as per ICH guidelines. The proposed method was specific, linear, accurate and robust. The method was successfully applied on the marketed formulation, so it could be effectively used for formulation containing either Bilastine or Montelukast or both of the drugs.

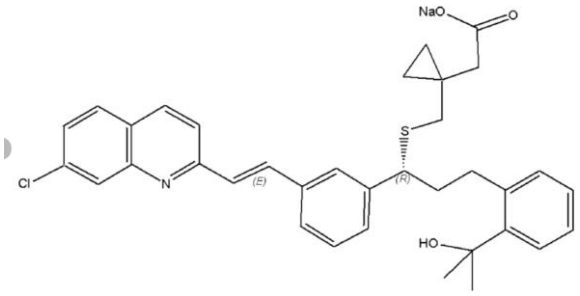
Keywords: Liquid Chromatography, Bilastine, Montelukast, simultaneous estimation

Introduction

Bilastine, or 2-[4-[2-[4-[1-(2-ethoxyethyl) benzimidazol-2-yl] piperidin-1-yl] ethyl] phenyl]-2-methylpropionic acid. Bilastine can be therefore classified into the same chemical group as many of the new antihistamines on the market, although it is not structurally derived, nor is it a metabolite or enantiomer of any of them, but an original molecule designed with the intent of fulfilling all the requirements of a second-generation antihistamine. s selective Histamine H1 receptor antagonist, leading to decreased nasal congestion and urticaria. It reduces the development of allergic symptoms by binding to and preventing activation of the H1 receptor. It is used for management of seasonal rhinitis and spontaneous urticaria [1-3].



Bilastine



Montelukast

Figure 1: Structure of Bilastine and Montelukast

Montelukast sodium [R-(E)}-1-[[[1-[3-[2-(7-Chloro-2 quinolinyl) ethynl] phenyl]-3-[2-(1-hydroxy-1- methylethyl) phenyl] propyl]thio]methyl] cyclopropaneactic acid, monosodium salt is a Cysteinylleukotriene 1(CysLT1) receptor antagonist. It is used for management of asthma, exercise induced bronchoconstrucion and allergic rhinitis. It works by blocking the action of leukotrine D4 in the lungs resulting in decreased inflammation and relaxation of smooth muscle.

There are few methods available for the estimation of Bilastine and Montelukast, but in the current method an effort is made to develop a simple, short, precise method which can estimate simultaneously both the drugs in the tablet formulation [1-4].

Experimental Work

Chemical and Reagents: Samples of Bilastine API was received from Montage Laboratories Pvt Ltd, Montelukast API was received from Mediwin Pharmaceuticals. The marketed sample Bilzest M was used for the study procured from market. The LC-MS grade Methanol and Acetonitrile was purchased from JT-Baker. LC-MS grade water from Aquarch. LC-MS grade formic acid was purchased from Merck.

Instrumentation and Chromatographic conditions: An Agilent Zorbax XDB C-18, (150*4.6) mm, 5µ column was used. The HPLC used was Shimadzu LC-20 AT connected with a UV detector. The analytical balance capable of weighing up to 4 decimal places of Shimadzu ATX-224 make was used. The Sonicator used was Frontline 1870 make and pH meter of Analab Scientific Private ltd. Mobile phase is Binary with Mobile phase A as 0.1% Formic acid in water prepared by added 1mL of formic acid in 1000mL of water and Mobile phase B as Acetonitrile. The composition of mobile phase is 50:50 Mobile phase A and Mobile phase B. The flow rate of the mobile phase is 1.0 mL/min with an isocratic program with a run time of 10 minutes. The column temperature is maintained at 35°C. The injection volume is 20 μL. The diluent consisted of Water and acetonitrile in a ratio of 50: 50 (v/v). Sample compartment temperature kept at Room temperature. The UV detection wavelength selected is 280nm. The linearity of the method was proved from LOQ to 200% of sample concentration, hence the Impurity and main component quantification can be done in the same method [5-16].

Preparation of Standard Stock Solution

Standard Stock Solution of Bilastine: Added 20mg of Bilastine in 100mL of volumetric flask and dissolved in diluent. This is stock solution of Bilastine (200µg/mL).

Standard Stock Solution of Montelukast: Added 10mg of Montelukast in 100mL of volumetric flask and dissolved in diluent. This is stock solution of Montelukast (100µg/mL).

Combined Standard of Montelukast and Bilastine: Further diluted the Stock solution of Bilastine and montelukast by transferring 1mL to 100mL and diluting the solution to 100mL with diluent. This is Combined standard solution of Bilastine and Montelukast (2 µg/mL and 1 µg/mL respectively).

Preparation of Sample Solution: The sample was prepared from the from formulation containing Bilastine (20mg) and Montelukast (10mg). 10 tablet of formulation was powdered and sample equivalent to 20mg and 10mg was transferred to 100mL volumetric flask. Added 50mL of diluent and sonicated to disperse and dissolved. Made up to

volume with diluent. This is the sample solution. 200µg/mL Bilastine and 100µg/mL Montelukast. Further dilution was made to get a sample solution of concentration of 2 µg/mL of Bilastine and 1µg/mL Montelukast.

Various trials were taken for the method development, but the good chromatography was observed in the following trials, with mobile phase as 0.1% of Formic acid in Water and 0.1% Formic acid in Acetonitrile. Column finalized was Zorbax XDB C-18 (150*4.6)mm, 5µ, with column temperature of 30℃ and injection volume of 20µL. The run time finalized was 10 minutes [17-28].

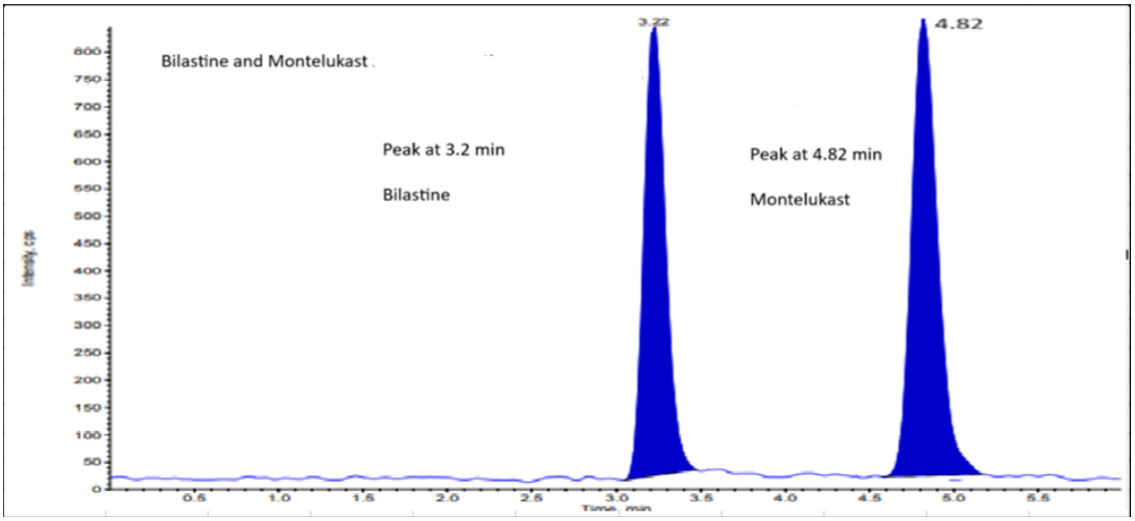


Figure 2: Representative chromatogram with Bilastine and Montelukast Peak

Method Validation

The method was fully validated as per ICH Q1 (R2) guidelines. Specificity was tested by injecting the blank, standard, and marketed sample. Linearity and accuracy was proved from LOQ to 125% level of Sample concentration, Method found robust for flow rate variation (1.2mL/min and 0.8mL/min) and for Mobile phase composition variation (40:60-Mobile Phase A: Mobile Phase B and 60:40- Mobile Phase A: Mobile Phase B). All system suitability parameters were passing for robustness conditions.

System Suitability, Specificity, Linearity, accuracy, Precision and Robustness was planned as per ICH guidelines. The diluent and sample chromatogram was overlaid and no interference was seen at retention time of Bilastine and Montelukast.

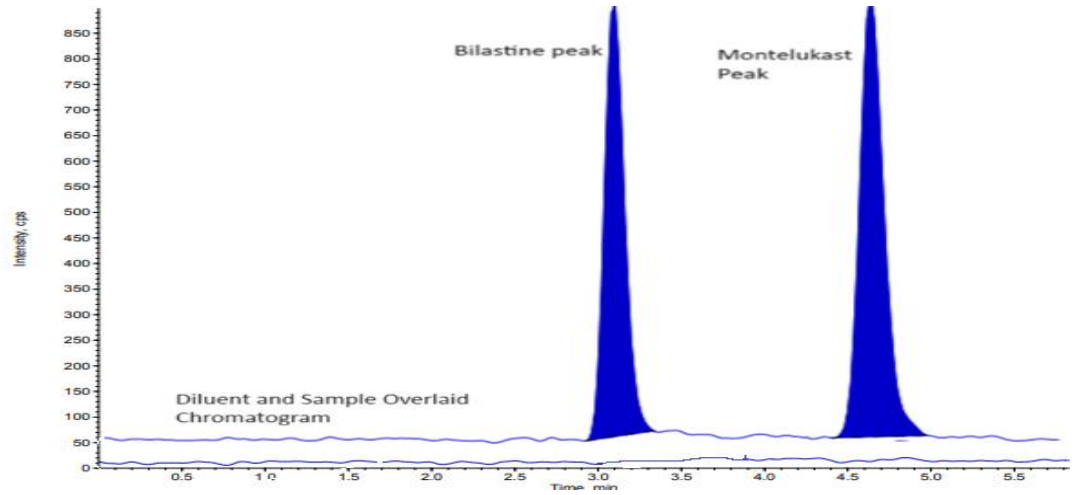


Figure 3: Overlaid Chromatogram of Diluent and Sample solution

System Suitability was established before initiating the Validation by Injection 6 replicate injections of Combined Standard solution.

Table 1: System Suitability of Bilastine

S. No.	Peak Name	Retention Time (Minutes)	Peak Area	USP Plate Count	USP Tailing
01	Bilastine	3.20	7000	4500	1.23
02	Bilastine	3.22	7105	4230	1.30
03	Bilastine	3.17	7011	4290	1.22
04	Bilastine	3.10	6945	4000	1.20
05	Bilastine	3.13	6803	5002	1.22
06	Bilastine	3.11	6500	4200	1.34
Average		3.16	6894	The USP plate count and USP tailing was passing.	
Standard Deviation		0.049	217.05	The %RSD of Retention time and Peak area was also	
%RSD		1.56	3.15	passing.	

Table 2: System Suitability of Montelukast

S. No.	Peak Name	Retention Time (Minutes)	Peak Area	USP Plate Count	USP Tailing
01	Montelukast	4.75	5000	2100	1.00
02	Montelukast	4.85	4850	3000	1.02
03	Montelukast	4.90	4903	3201	1.06
04	Montelukast	4.73	4950	3200	1.05
05	Montelukast	4.70	4801	3000	1.10
06	Montelukast	4.85	4600	3500	1.00
Average		4.79	4851	The USP plate count and USP tailing was passing.	
Standard Deviation		0.08	141.5	The %RSD of Retention time and Peak area was also	
%RSD		1.68	2.92	passing.	

Linearity was planned from LOQ to 125% as per below table.

Table 3: Linearity Concentration of Bilastine and Montelukast

Linearity Dilutions of Bilastine and Montelukast														
Weight of Standard (Mg)		Volume of Stock solution (mL)	Concnetration of Stock solution (ppm)		mL	mL	mL	mL	Concnetration Achieved (ppm)		Target concnetration (ppm)		% Level	
Bilastine	Montelukast		Bilastine	Montelukast	From Common 100mL volumetric				Bilastine	Montelukast	Bilastine	Montelukast	Bilastine	Montelukast
20	10	100	200	100	5	100	1	100	0.1	0.05	0.1	0.05	5	5
							1	50	0.2	0.1	0.2	0.1	10	10
							5	100	0.5	0.25	0.5	0.25	25	25
							5	50	1	0.5	1	0.5	50	50
							5	25	2	1	2	1	100	100
							5	20	2.5	1.25	2.5	1.25	125	125

The correlation coefficient of Bilastine and Montelukast observed was 0.998 and 0.999 respectively. The %Y intercept was less than 3 for both the components.

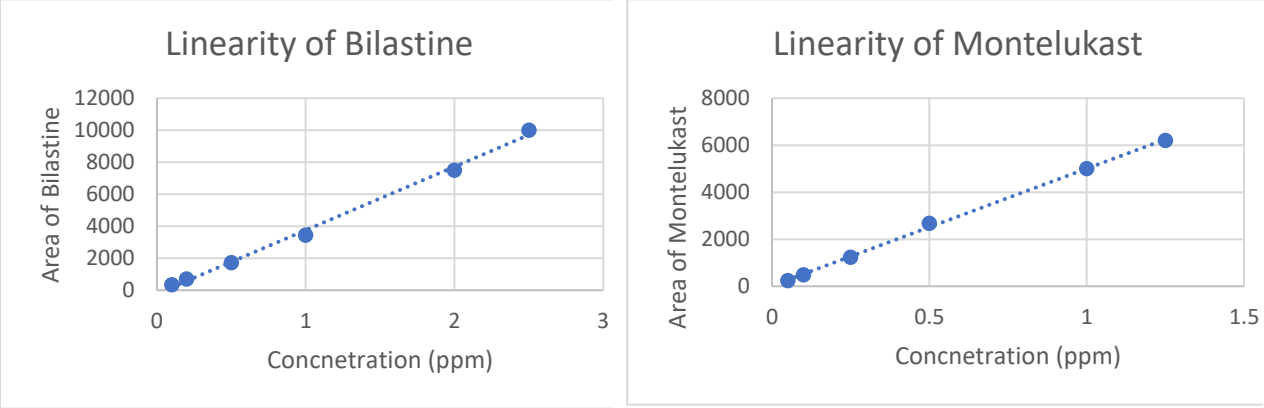


Figure 4: Linearity Plots of Montelukast and Bilastine

Table 5: Accuracy of Bilastine

Accuracy was planned by Spiking the Bilastine and Montelukast solutions in Diluent at level of LOQ to 125%.

Concentration/ Sample ID (Bilastine)	Amount added (Bilastine) (ppm)	Amount recovered (Bilastine) (ppm)	% Recovery (Bilastine)	Average	% RSD
LOQ-Sample 1	0.05	0.045	111.11		
LOQ-Sample 2	0.05	0.044	113.64	109.7	4.5
LOQ-Sample 3	0.05	0.048	104.17		
100%-Sample 1	2.0	1.98	101.01		
100%-Sample 2	2.0	1.90	105.26	104.2	2.7
100%-Sample 3	2.0	1.88	106.38		
125%-Sample 1	2.5	2.45	102.04		
125%-Sample 2	2.5	2.47	101.21	100.4	2.1
125%-Sample 3	2.5	2.55	98.04		
Overall Statistical Analysis					
	% Overall Mean			104.8	
	Overall % RSD			4.79	

Table 6: Accuracy of Montelukast

Concentration/ Sample ID (Montelukast)	Amount added (Montelukast) (ppm)	Amount recovered (Montelukast) (ppm)	% Recovery (Montelukast)	Average	% RSD
LOQ-Sample 1	0.1	0.09	111.11		
LOQ-Sample 2	0.1	0.09	111.11	111.1	0.0
LOQ-Sample 3	0.1	0.09	111.11		
100%-Sample 1	1.0	1.0	100.0.00		
100%-Sample 2	1.0	0.98	102.04	101.4	1.16
100%-Sample 3	1.0	0.98	102.04		
125%-Sample 1	1.25	1.23	101.62		
125%-Sample 2	1.25	1.22	102.46	101.6	0.8
125%-Sample 3	1.25	1.24	100.80		
Overall Statistical Analysis					
	% Overall Mean			104.7	
	Overall % RSD			4.64	

Method Precision was planned by preparing 6 replicated solution of the tablet formulation and injecting in HPLC

Table 7: Precsion on Marketed Formulation sample

Sample	Bilastine		Montelukast	
	20mg Label Claim	% w/w	10mg Label Claim	% w/w
1	19.5	97.5	9.9	99.0
2	19.2	96.0	9.8	98.0
3	19.3	96.5	9.2	92.0
4	19.4	97.0	9.3	93.0
5	20.3	101.5	10.0	100.0
6	20.1	100.5	9.9	99
Mean	98.2		96.8	
% RSD	2.31		3.54	

Results and Discussion

Method Development and Optimization

The main aim of a chromatographic method is to have good peak shape and able to quantify the sample. Various trials were taken to optimize the method using different columns and mobile phase combinations. Method Development was initiated with Mobile phase A as water and Mobile phase B as Acetonitrile in ratio of 70:30, with flow rate of 0.8mL/min, column as Phenomenex Luna C-18 (250*4.6) mm, 5µm. Bilastine was observed at 6 minutes while Montelukast peak was not observed. Further the flow rate was increased to 1.0mL/min and mobile phase composition was changed to 60:40, Montelukast peak was not eluted in this trial. The mobile phase A was replaced by 0.1% Formic acid in water and flow rate was changed to 0.8mL/min and column as Zorbax XDB C-18 (150*4.6)mm, 5 µm and in this trial Bilastine and Montelukast was observed. During forced degradation few changes in the method were made to elute the degradation peaks within 10 minutes of run time. The chromatographic condition were finalized with column Agilent, Zorbax XDB C-18 (150*4.6) mm, 5µm, and mobile phase as 0.1% Formic acid in water and Acetonitrile (50:50) as mobile phase, with mode of elution as isocratic and column temperature of 25℃ with flow rate of 1.0mL/min and run time of 10 minutes.

Method was found specific with no interference at retention time of Bilastine and Montelukast. Linear and accurate in range from LOQ to 1.25%, precise at the sample concentration level. The assay of Marketed formulation was performed and result observed was in range of 98 to 102% for both Bilastine and Montelukast.

Conclusion

The method is validated as per ICH guidelines and is short, simple, precise, linear and accurate and can be implemented for analysis of routine samples without further validation.

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