AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Development and Validation of RP HPLC Method For the Simultaneous Estimation of Bilastine and Montelukast Tablet Formulation

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ABSTRACT

A new simple, rapid, precise and accurate assay method was developed for simultaneous estimation of Bilastine and Montelukast in pure form and tablet form. The analytes were separated by RP HPLC on a RP-Purosnosphere C18 column (5 µm, 4.6mm* 250 mm).The mobile phase was Acetonitrile: water: methanol (30:25:45 v/v) at 1.1 ml/min flow rate satisfactorily resolve the tertiary mixture. The UV detector was operated at 214 nm for the determination of all the drugs. Linearity, accuracy and precision were found to be acceptable over the concentration ranges of 10-50 µg/ml for Bilastine and 5-25 µg/ml for Montelukast with a R^2 0.9960 and 0.9974 values respectively. The optimized methods proved to be specific, robust and accurate for the quality control of drugs in bulk drug and pharmaceutical formulations.

Keywords: Bilastine, Montelukast sodium, ICH, Validation etc.

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Received 22 May 2024, Accepted 05 June 2024

Please cite this article as: Itnare DG *et al*., Development and Validation of RP HPLC Method For the Simultaneous Estimation of Bilastine and Montelukast Tablet Formulation. American Journal of PharmTech Research 2024.

INTRODUCTION

Drugs play a vital role in the progress of human civilization by curing diseases. Analytical chemistry is divided into two branches qualitative and quantitative[1.] Today a majority of the drugs used are of synthetic origin. These are produced in bulk and used for their therapeutic effects in pharmaceutical formulations. Pharmaceutical product quality is of vital importance for patient safety. Pharmaceutical analysis is the branch of pharmacy that is responsible for developing sensitive, reliable and accurate methods for the estimation of drugs in pharmaceutical dosage forms and biological fluids.[2] Histamine and cysteinyl leukotrienes (CysLTs) are potent inflammatory mediators involved in both seasonal allergic rhinoconjunctivitis (SARS) and asthma. A combination therapy against both these agents may provide additive benefit.[1]

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.1 It has a chemical formula of C28H37N3O3. Montelukast is a member of the leukotriene receptor antagonist (LTRA) category of drugs with molecular formula C35H36ClNO3S and is indicated for the prophylaxis and chronic treatment of asthma. The Recommended dose of Bilastine and Montelukast sodium is 20 mg and 10 mg respectively.[1]

Bilastine is chemically known as 2-[4-[2-[4-[1- (2-ethoxyethyl) benzimidazol-2-yl] piperidin-1- yl] ethyl] phenyl]-2-methylpropanoic acid.[2]For symptomatic relief of nasal and non-nasal symptoms of seasonal rhinitis in patients 12 years of age and older and for symptomatic relief in chronic spontaneous urticaria in patients 18 years of age and older. 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. Histamine plays a major role in the allergic reaction and is released by mast cell degranulation. This histamine binds with H1 receptors, activates the receptors and causes allergic reactions. Bilastine binds with H1 receptor and prevents the activation of H1 receptor by histamine. Thus, it acts as an antagonist for histamine. Bilastine shows no cardiotoxic, sedative side effects and undergoes minimal or no first pass metabolism. It has less chance to undergo drug-drug interactions. Therefore, it is useful for treating patients suffering with renal/ hepatic dysfunction. Bilastine, a piperidine class antihistamine medication used for the treatment of allergic rhinitis and chronic urticaria.[3]

Montelukast is 1-[[[(1R)-1-[3-[(1E)-2-(7-Chloro-2-Quinolinyl) Ethynyl] Phenyl]-3-[2-(1-hydroxy-1-methyl-ethyl) Phenyl] Propyl] Thio] Methyl] cyclopropane acetic acid.[4] Montelukast Sodium is a selective, Potent and Orally Active Antagonist of the Cysteinyl, CysTL1, Leukotriene receptor used for the treatment of Asthma in children's and adults. Montelukast Sodium is a potent drug,

selectively CystLT1 receptors antagonist. It is indicated for the prophylaxis and chronic treatment of asthma in adults and pediatric patients[5,] The synergistic combination of bilastine and montelukast has a dual action and is an attractive treatment option in allergic rhinitis patients with hyperreactive airway disease such as asthma. Both classes of drugs are required for achieving better results.[7,8]

BIL and MONT is a new drug combination. Therefore, there are very few reports of HPLC and UV method development for this new combination. Therefore, this is a attempt to develop novel, simple, robust, accurate method for the determination of efficacy and safety of BLS and MLT combination. This method was fully validated according to International Conference on Harmonization (ICH) and ready for the application in routine analysis without interference of excipients.

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Published Papers on this drug combination and in combination with other drug by UV[9,10,11], HPLC[12,13,14,15].

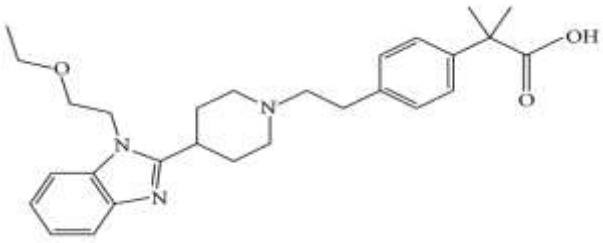

Figure 1: Structural formula of Bilastine

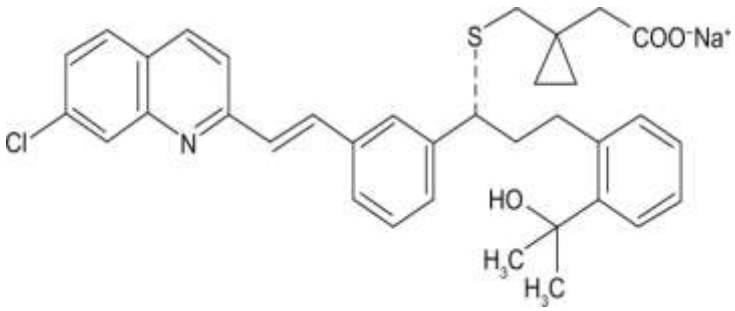

Figure 2: Structural formula of Montelukast

MATERIALS AND METHOD

Chemicals and reagents

Bilastine (20 mg) and Montelukast (10 mg) pure drugs were obtained as a gift sample from Centaur Pharmaceuticals India. The combined formulation B-Latine-MK (20 mg/10 mg) of the

two drugs purchased from Vikram Pharmacy Jalgaon. Analytical grade methanol purchased from Merck Chemicals Pvt. Ltd. Mumbai. Acetonitrile, methanol and water used were of HPLC grade (Qualigens Fine Chemicals, Mumbai, India). Ammonium Acetate Buffer was AR grade (Qualigens Fine Chemicals, Mumbai, India). A 0.2 µm nylon filter (Pall life Sciences, Mumbai, India) was used. All other chemicals and reagents used were analytical grade unless otherwise indicated.

Apparatus

The chromatographic system (Systronics Corporation, India) consisted of LC 8600aprominence solvent delivery module, a manual injector with a 20 µL fixed loop and a UV-visible detector. The separation was performed on a Hibar® (Merk, Germany) RP-Presnosphere Star C18 column (5 µm, 4.6mm* 250 mm) at an ambient temperature. Chromatographic data were recorded and processed using Chemitochrom 2000 software. A Fast clean ultrasonicate cleaner (India) was used for degassing the mobile phase. Shimadzu UV 1800 double beam UV visible spectrophotometer and Sansui-vibra DJ-150S-S electronic balance were used for Spectrophotometric and weighing purposes respectively.

Chromatography Conditions

Chromatographic separations of active (BIL and MONT)substances were obtained by using Systronics LC-138 RP-Presnosphere Star C18 column (5 µm, 4.6mm* 250 mm),.Mobile phase Acetonitrile: water: methanol (25:45:30 v/v)) (pH 4.4 was adjusted with o-phosphoric acid Buffer) was prepared, filtered through a 0.2 µm nylon filter and degassed for 5 min in an ultrasonicator. The mobile phase was pumped through the column at flow rate of 1.1 ml/min^{-1}. Analyses were carried out at ambient temperature with detection at 214 nm. The injection volume was 20 µL and each analysis required 12 min.

Standard Solutions

Stock standard solutions of BIL 1 mg/ml and MONT 1 mg/ml were prepared by dissolving 20 mg BIL standard and 10 mg MONT standard in 25 ml methanol. Working standard solutions of BIL 0.1 mg/ml and MONT 0.1 mg/ml were prepared by diluting suitable aliquots of corresponding stock solutions with mobile phase.

Sample Solution

Twenty B-Latine-MK Tablets containing BIL (20 mg) and MONT (10 mg) were weighed and ground to fine powder. A quantity of sample equivalent to BIL (20 mg)and MONT(10 mg) was transferred into 100 ml volumetric flask containing methanol (60 ml), sonicated for 15 min and the volume was made up to the mark and filtered through 0.45µm nylon membrane filter. This solution was (1 ml) transferred to 10 ml volumetric flaks, dissolved and volume was adjusted to

the mark. The response of solution was measured at 214 nm and quantification of BIL and MONT was done by using present HPLC method. Typical chromatogram of final resultant formulation solution was shown in (Figure 1).

Validation of Proposed Method

Calibration curve (linearity)

Accurately measured aliquots of working standard solutions equivalent to 10-50 µg/ml BIL, and 5-25 µg/ml MONT were transferred to series of 10 ml volumetric flasks and the contents of the flasks were diluted to volume with mobile phase. A 20 µL aliquot of each solution was injected in triplicate into the liquid chromatography. The conditions including the flow rate of mobile phase at 1.1 ml/min, detection at 214 nm and run time program for 12 min, were adjusted. A calibration curve for each drug was obtained by plotting area under the peak versus concentration. The graphs of area vs concentration were recorded for all the drugs and are shown in (Figure 2 and 4).

Accuracy (% recovery)

Recovery studies were carried out by adding a known amount of pure drugs BIL and MONT to a pre analyzed sample solution. These studies were carried out by spiking 80%, 100% and 120% respective drug. The recovery studies showed that the results were within acceptable limits, above 99% and below 101%. The results are given in (Table 2).

Method Precision (repeatability)

The precision of the developed method was assessed in terms of repeatability, intraday and inter-day precision by analyzing six replicate standard samples. The % R.S.D. values of the results corresponding to the peak area and retention time were expressed for intra-day precision and on 3 days for inter-day precision.

Intermediate Precision (reproducibility)

The intraday and interday precisions of the proposed method were determined by estimating the corresponding responses 5 times on the same day and on 5 different days for present method. The results are reported in terms of relative standard deviation (RSD).

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ of the drug were calculated using the equations according to International Conference on Harmonization (ICH) guidelines.

Robustness

Robustness of the method was determined by making slight changes in chromatographic conditions. Effect of % of methanol (59, 60 and 61%) in mobile phase on the retention time and slight changes in flow rate were applied as variable parameters. Flow rate varied at three levels (-1,

0, 1). One factor at the time was changed to estimate the effect. Thus standard solution at varied pH (pH 4.2, 4.3 and 4.4) three pH levels was performed.

Specificity

Specificity is the ability of the analytical method to measure analyzed response in presence of interferences including degradation products and related substances. Specificity was checked by determining BIL and MONT in laboratory prepared binary mixture and in binary mixture containing different degradation products.

System suitability Test

In the system suitability test tertiary solution of 40µg/ml of BIL and 20µg/ml of MONT (n=6) was prepared and injected. Then the system suitability parameters like retention time, theoretical plates, tailing factor and resolution were calculated from the chromatogram.

RESULTS AND DISCUSSION

The absorption spectra of BIL and MONT greatly overlap; so conventional determination of these compounds in mixture is not possible. To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for BIL and MONT were obtained with a mobile phase consisting of Acetonitrile: Water: Methanol (25:30:45v/v), pH 4.4 adjusted using o-phosphoric acid Buffer. Quantification of the drugs was performed at 214 nm. Resolution of the components with clear baseline separation was obtained.

Validation of the Proposed Method

Linearity

Linear correlation was obtained between peak areas and concentrations of BIL and MONT in range of 10-50 and 5–25 µg/ml respectively. The linearity of calibration curves was found to be acceptable over the concentration ranges of 20-120 µg/ml for BIL while 5-30 µg/ml for MONT with a R^2 0.9960 and 0.9974 values respectively.

(Table 1, Figure 2 and 3). The results show that good correlation existed between the peak area and concentration of the analysts.

Table 1: Regression analysis of the calibration curves for Bilastine and Montelukast in the proposed HPLC Method

Parameter	Bilastine	Montelukast
Linearity Range (µg/ml)	10-50	5-25
Detection Wavelength (nm)	214	
Slope ± SD	70.17	55.036
Correlation coefficient	0.9960	0.9974

(n= mean of three determinations)

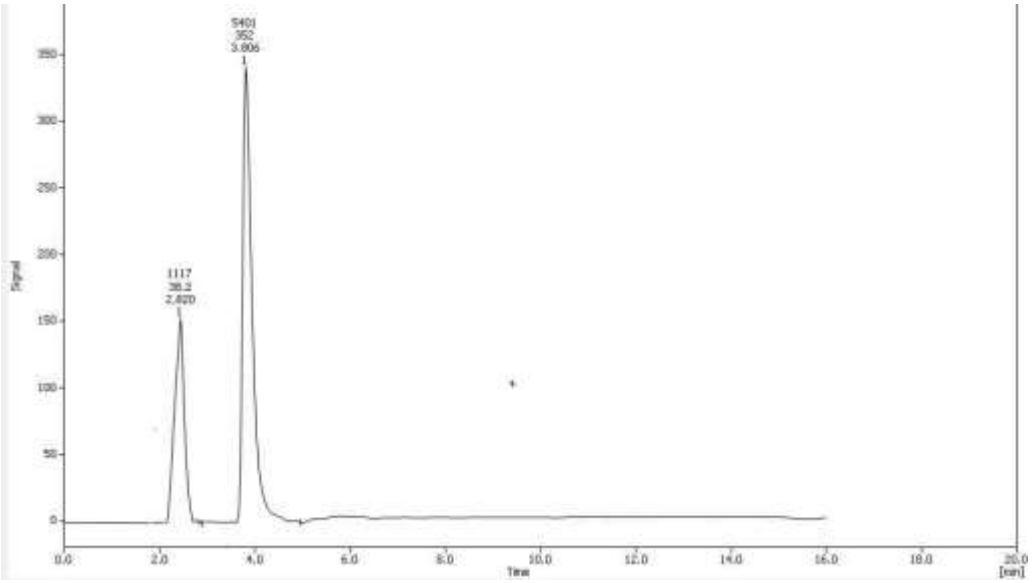

Figure 1: Typical liquid chromatogram obtained for a 20 µL injection of a synthetic mixture of BIL and MONT

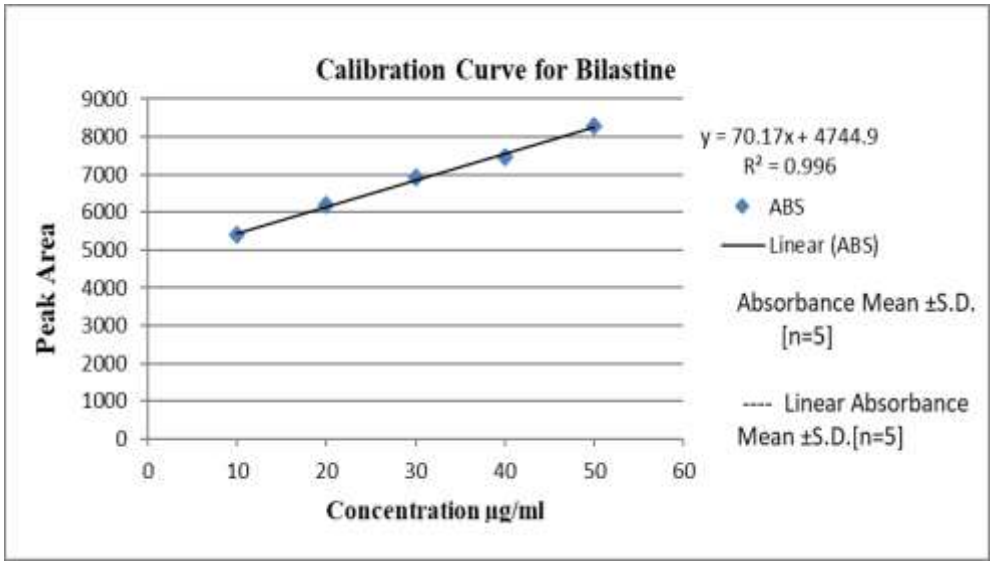

Figure 2: Calibration Curve for Bilastine

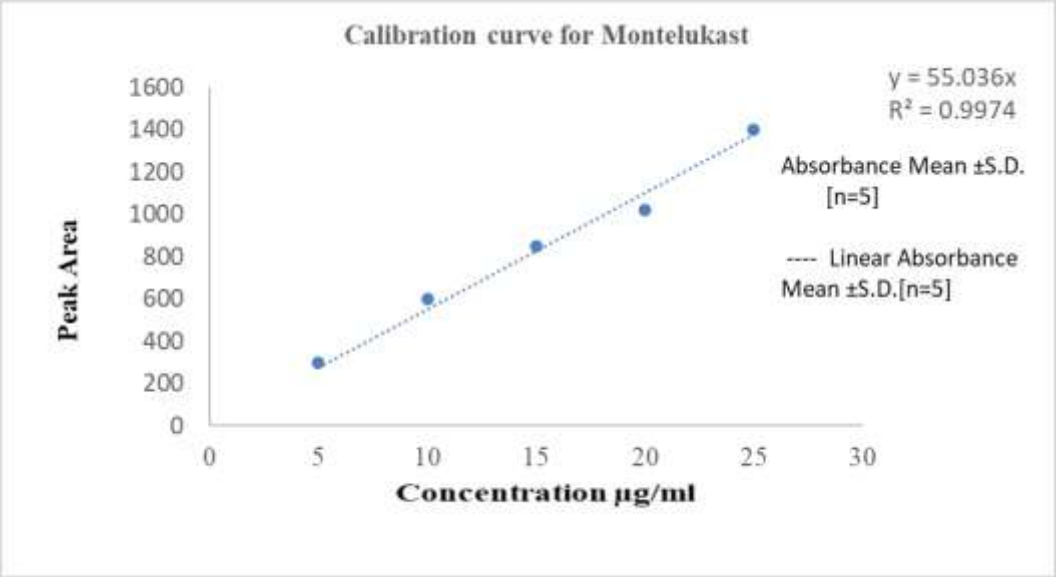

Figure 3: Calibration Curve for Montelukast

Accuracy

The recovery experiments were performed by the standard addition method. The recoveries obtained were 99.88% and 99.85% for BIL and MONT respectively (Table 2). The high values indicate that the method was accurate.

Method precision

Precision study was carried out using parameter like method repeatability study which showed that results were within acceptable limit 0.437 and 1.5530 i.e. % RSD below 2.0 indicating that the method is reproducible. The results are shown in (Table 2)

Intermediate precision

The intraday RSD values for BIL and MONT were 0.5894-0.7344 % and 0.4419-1.1590 %, respectively. The interday RSD values for BIL and MONT were 0.4378-0.8105 %, and 0.8254–0.9479%, respectively. The % RSD (< 2%) values indicate that the method was sufficiently precise (Table 2).

LOD and LOQ

LOD values for BIL and MONT found to be 3.8238µg/ml and 1.2973µg /ml, respectively. LOQ values for BIL and MONT were found to be 8.5873µg /ml and 3.9314µg /ml, respectively (Table 2). These data showed that the method was sensitive enough for the determination of BIL and MONT.

Table 2: Summary of the validation parameters for the proposed HPLC method

Parameter	Bilastine	Montelukast
LOD	3.8238µg /ml	1.2973µg /ml
LOQ	8.5873µg /ml	3.9314µg /ml
Accuracy,%	99.88 ± 0.65	99.85 ± 0.62
Repeatability (%RSD, n = 3)	0.5595	0.6185
Precision (RSD, %)		
Interday, n = 3	0.592	0.836
Intraday, n = 3	0.416	0.537

LOD = Limit of detection.

LOQ = Limit of quantification

RSD = Relative standard deviation.

Robustness

The method was found to be robust with no significant changes on test result upon change of analytical conditions like different flow rate, % methanol in mobile phase and pH of mobile phase with the standard deviation was found to be below 1 and % RSD is less than 2 for all results. It was found that under small deliberate changes of chromatographic factors, there was no considerable change in under study parameters.

System Suitability Test

A tertiary solution of 40 µg/ml of BIL and 20µg/ml of MONT (n=5) was prepared and same was injected, then the system suitability parameters were calculated from the chromatogram. The parameters, retention times, resolution factor, tailing factor and theoretical plates were evaluated. The results (Table) obtained from system suitability tests are in agreement with the official requirements.

Table 3: System suitability test parameters for BIL and MONT for the proposed HPLC method

System Suitability Parameters	Proposed Method	
	BIL	MONT
Retention Time (t_R)	3.80	2.82
Area	5401.63	849.48
Theoretical Plate Number (N)	19267	2315
Asymmetry factor	0.817	1.820
Resolution Factor (R)	2.30	

CONCLUSION

The proposed LC method presented in this paper has advantages of simplicity, accuracy, precision and convenience for separation and quantitation of BIL and MONT in combination and can be used for the assay of their respective dosage form. Moreover, the proposed LC method is a

stability indicating assay method that can determine BIL and MONT in presence of their degradation products. Thus, the proposed LC method can be used for the quality control of BIL and MONT in typical laboratories.

ACKNOWLEDGEMENT

The authors are thankful to Centaur Pharmaceuticals Pvt. Ltd. for supplying generous gift samples of pure drugs. Hereby, the authors declare that there is no conflict of interests regarding the publication of this paper.

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