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DEVELOPMENT OF EXPEDITIOUS RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF BILASTINE AND MONTELUKAST IN FIXED DOSAGE COMBINATION

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ABSTRACT

Background: Bilastine and montelukast are used to treat allergic rhinitis and chronic urticaria. Due to the recent increase in highly allergic disorders, demand for this combination has risen. Therefore, for regular quality control analysis, a new, rapid, and cost-effective method has become crucial. Compared with the previously reported methods, the present method is ultra-fast, with well-separated peaks within~3.0min and a total run time of 8 min. Because it uses a methanol-buffer system, it is less expensive, more environmentally friendly, and safer than an acetonitrile-based method. This study presents a rapid, specific, and economical "reversed-phase high-performance liquid chromatography (RP-HPLC)" method for the simultaneous estimation of Bilastine and Montelukast. **Methodology:** The chromatographic conditions for the separation of drugs are achieved by leveraging a tailored composition of mobile phase with methanol and acidic phosphate buffer with a volume ratio of 55:45v/v, the "Zorbax C_{18} column (4.6×150mm, 5µ)" maintained at 35ºC with an optimized flow rate of 1.0 mL/min and detected at a wavelength of 260 nm. The injection volume is 10 µL, and the run time is 8 min. **Results and Discussion:** The retention times for the drugs bilastine and montelukast are 2.061 and 2.462 min, respectively. Linearity is observed with correlation coefficients of 0.9993 and 0.9994, respectively, for montelukast and bilastine at 1-5 µg/mL and 100-500 µg/mL. Efficient separation and quantification of these two therapeutically important compounds were achieved in this method. **Conclusion:** The developed analytical method has been validated in accordance with International Conference on Harmonisation (ICH) guidelines, demonstrating satisfactory performance with respect to specificity, accuracy, precision, linearity, and robustness. This novel RP-HPLC approach provides a valuable tool for pharmaceutical analysis and quality control.

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INTRODUCTION

Montelukast (MTK) and bilastine (Figures 1 and 2) are the most commonly prescribed new-generation antihistamines for seasonal allergy symptoms. Among the two, Montelukast has gained popularity since the COVID-19 pandemic, capturing the largest market share due to its rapid onset of action for breathlessness in asthma [1-3].

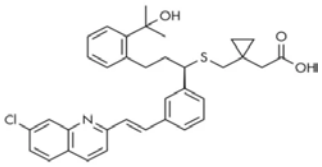

Figure 1: Structure of montelukast

A comprehensive review of the literature reveals that various analytical methods, including RP-HPLC, UPLC, UV Spectroscopy, and Quality by Design (QbD), have been widely used to estimate Montelukast sodium and Bilastine. While several methods are available for the individual estimation of Bilastine and its combination with other drugs, till date a proper quality control method with fastest run time and reduced solvent cost to simultaneously analyze bilastine and montelukast sodium is not reported hence, an attempt is done to estimate the both drugs following RP-HPLC approach as an alternative way to other reported literature [4-7].

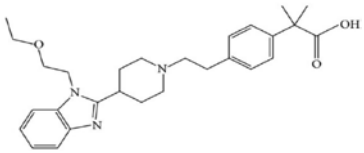

Figure 2: Structure of bilastine

Table 1: Comparative study of developed method vs reported RP-HPLC Methods for bilastine and montelukast

Study	Mobile phase	λ_{max} (nm)	RT (min)		Total run time	Comparison result
			Bilastine	MTK		
Sameera et al. (2023) [1]	Acetonitrile: Phosphate buffer (75:25)	272	2.746	3.668	~8 min	Simple, but longer MTK RT
Sunkara & Ajitha (2022) [2]	Ammonium acetate buffer: acetonitrile (70:30)	220	2.645	3.797	~8 min	Moderate RT, acetonitrile is expensive than methanol
Vijayalakshmi et al. (2021) [3]	Phosphate buffer: acetonitrile (30:70)	260	9.50	7.03	~12 min	Very high RT; not ideal for routine use
Vyas et al. (2022) [4]	Acetonitrile: Water (60:40)	254	2.319	4.299	~9 min	Moderate RT; validated
Present Developed Method	Methanol: Phosphate buffer (55:45)	260	2.061	2.462	~8 min	Fastest RTs; simpler mobile phase; More Affordable, user-friendly, less toxic, and readily available.

MATERIALS AND METHODS

Equipment used

HPLC of Shimadzu equipped with a PDA detector for drug detection, an automated LC solution chromatographic program for recording and data analysis, along with a "Zorbax" C_{18} column for separation.

Chemicals and reagents

Merck provides analytical-grade methanol, orthophosphoric acid, and HPLC-grade water. Montelukast and Bilastine API were procured from Sura Labs Pvt. Ltd., Hyderabad, Telangana, India. The marketed medication was purchased from a local community pharmacy in Hyderabad, Telangana. All the reagents used were of analytical grade.

Preparation of standard stock and working stock solution

Accurately weighed and transferred 10 mg of Montelukast and Bilastine standard drug into a 10 ml of clean, dried volumetric flask, added about 7 ml of diluents, and sonicated to dissolve them completely, and further marked up with the mobile phase. Then a microvolume of 0.03 ml of Montelukast, along with 3 ml of Bilastine from the stock and working solutions, was pipetted into a 10 ml volumetric flask and diluted to the graduated mark with diluent [8 – 9].

Mobile phase preparation

The degassed and sonicated mixtures were prepared for 15 min in an ultrasonicator, consisting of exactly 55 volumes of methanol (55%) and 45 volumes of phosphate buffer (45%), with pH maintained at 3.9 using orthophosphoric acid, and filtered through a 0.45 µ filter under vacuum [9 – 10].

Preparation of the sample solution

An accurate quantity equivalent to 10mg of solution was prepared, and dilutions were performed in the same manner as for the preparation of the Standard and working stock solutions [11,12].

HPLC method development: Optimized chromatographic conditions

The chromatographic separation was carried out under these optimized conditions using Methanol plus Acidic Phosphate buffer as mobile phase in the ratio of 55:45v/v on an "Zorbax C_{18} column (4.6 × 150 mm, 5 µm)" maintained at 35°C ± 1°C, 1.0 mL/min as flow rate for an 8 min run time at a detector wavelength of 260 nm.

Method validation

The developed method was validated, adhering to the guidelines given by the "International Conference on Harmonization (ICH) guidelines". The validation evaluates the method's accuracy, precision, linearity, robustness, system suitability, Ruggedness, Limit of quantification, and limit of detection.

i] System suitability: To ascertain the system's suitability, a series of five replicate injections of the pharmaceutical formulation was performed. The resultant chromatograms were examined for peak characteristics, including peak shape, tailing factors, theoretical plate count, & relative retention time, and the "% RSD" was calculated for the area of five replicate injections.

ii] Specificity study of drug: The specificity parameter for this analysis is assessed by recording the chromatograms of blank, placebo, standard, and sample solutions, and analyzing the chromatograms.

iii] Linearity: The linearity parameter for this analysis is assessed by plotting calibration curves, by taking peak responses with their respective concentrations, with a line of best fit, and regression analysis was done to determine the correlation coefficient. The range of concentration is set to be 100 – 500 ppm for Bilastine and 1-5 ppm for Montelukast.

iv] Precision: To check the extent of agreement among each test result, "intra-day and inter-day" precision was performed.

Intra-day precision: It is achieved by injecting the mixed working stock solution into the HPLC system five times on the same day, without changing the chromatographic operating conditions for a short interval, and subsequently calculating the % RSD of the peak areas from the recorded chromatograms.

Inter-day precision: This was achieved by injecting the mixed working stock solution into the HPLC system five times on different days without changing the chromatographic conditions, and subsequently calculating the % RSD of the peak areas from the recorded chromatograms.

v] Accuracy: This was confirmed by determining the recovery of the spiked samples into the standard working stock solution at the concentration levels of 50%, 100%, and 150% in three repetitive injections. Calculated the amount found, amount added, the individual recovery, and mean recovery values from the chromatogram peak responses.

vi] Robustness: It is assessed by intentionally varying the flow rate to 1.1 and 0.9 ml/min and mobile phase composition (±5%).

vii] Assay determination of Montelukast and Bilastine: The prepared sample solution and mixed standard working solution of Montelukast and Bilastine were injected into the HPLC system in three repetitive injections, and the % Assay is calculated by analyzing the chromatogram [13 – 15].

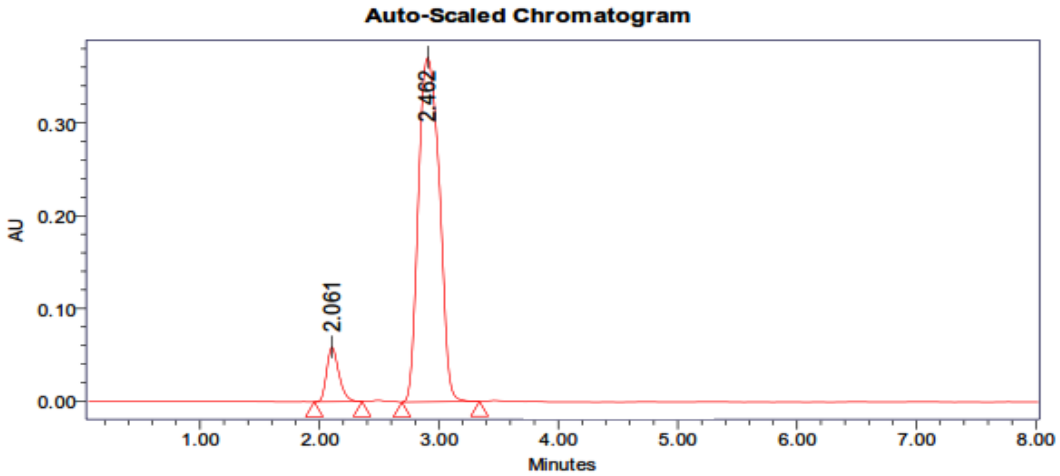

Figure 3: Optimized chromatogram (standard)

RESULTS AND DISCUSSION

Method development

Following an exhaustive examination of various mobile phase combinations, a phosphate buffer: Methanol mixture (55:45, v/v) was found to produce optimum chromatographic results. This carefully calibrated mobile phase enabled the attainment of sharp, well-defined peaks for both Bilastine at 2.061 min and Montelukast at 2.462 min retention time. The optimized RP-HPLC method was performed using a "Zorbax C_{18} column (4.6 × 150 mm, 5 µm)" maintained at a temperature of 35°C, a 1 mL/min flow rate, and 260 nm as wavelength detection. The optimized chromatogram is presented in Figure 3.

Method validation

i] System suitability: The resultant chromatograms evaluation ensured that the proposed method was capable of generating reliable results. The calculated % RSD was within the acceptance criteria. This proved the system performance. The data is provided in Table 2.

Table 2: System suitability tests results (n = 5)

Parameters	Montelukast	Bilastine
Mean	244887.4	3345600
*SD	2202.312	13195.86
*% RSD	0.89	0.39
Retention time	2.030	2.479
Tailing factor	1.2	1.1

**SD- Standard Deviation, RSD – Relative Standard Deviation.*

ii] Specificity: The analysis revealed that the blank and placebo formulations did not exhibit any co-eluting or interfering peaks at the retention times corresponding to Bilastine and Montelukast, thereby confirming the selectivity and specificity of the method.

iii] Linearity: The plotted calibration curve for working stock solutions ranging from 1 to 5ppm for Montelukast and 100 to 500ppm for Bilastine showed that the developed method obeys Beer-Lambert's law with a 0.999 good correlation coefficient. The line graph and the data were reported separately in Figures 4 and 5, respectively, and in Table 3.

iv] Precision: The results of intra-day precision and inter-day precision for Bilastine and Montelukast, depicted in Table 4, demonstrated that the "percentage relative standard deviation (% RSD)" values are in the prescribed limits, confirming the method's precision.

v] Accuracy: The mean recovery for the spiked samples from "50%, 100% and 150%" levels was found to be within the said limits. Thus, proving the Accuracy of the developed method. The data is reported in Table 5.

vi] Assay: The % purity of both drugs in the dosage form is calculated to be 100.2% and 99.89% respectively.

vii] LOD and LOQ: The calculated values by adopting standard error and deviation from intercept data are displayed in Table 6.

Table 3: Linearity and statistical data

Montelukast		Bilastine	
Conc. in ppm	Ave. area of peak	Conc. in ppm	Ave. area of peak
1	88442	100	1131032
2	165724	200	2345302
3	242754	300	3355282
4	315906	400	4429382
5	396371	500	5623754

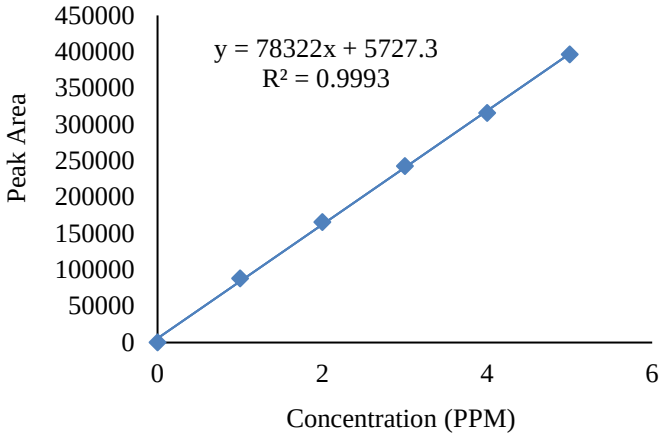

Figure 4: Linearity graph of Montelukast

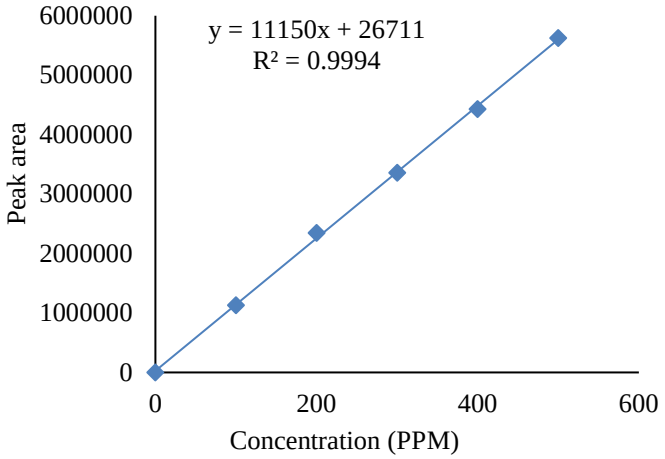

Figure 5: Linearity graph of Bilastine

Table 4: Intra-day and Inter-day precision

Intra-day Precision (n=5)			Inter-day Precision (n=6)			
Drug	Montelukast	Bilastine	Montelukast		Bilastine	
			Day-1	Day-2	Day-1	Day-2
Mean Area	249082.6	3237814	242825.2	242206.7	3326668	3432674
±SD	1380.964	8998.489	3659.692	3277.238	1812.634	6427.897
% RSD	0.55	0.278	1.5	1.35	0.054	0.19

Table 5: The accuracy data

Drugs	Spiking level	Area of Peak	Added amount(µg/ml)	Found amount(µg/ml)	Recovery%	Mean Recovery
Montelukast	50%	124675.7	15	15.1	101%	**100.4%**
	100%	242006.3	30	30.1	100.5%	
	150%	357449	45	44.9	99.7%	
Bilastine	50%	1696259	18.75	18.71	99.8%	**99.2%**
	100%	3351661	37.5	37.2	99.4%	
	150%	4975094	56.25	55.47	98.6%	

Table 6: LOD and LOQ Data

Drugs	Detection limit (µg/ml)	Quantitation limit (µg/ml)
Bilastine	29.58	89.65
Montelukast	0.3	1.0

viii] Robustness: Despite making deliberate changes in the flow rate and change in mobile phase composition, the variations were nil, proving the method to be robust. The data is provided in Table 7.

Table 7: Robustness

Parameters of sample analysis	Drugs	Peak Area	Retention Time	Theoretical plates	Asymmetry factor
Optimized Flow rate of 1.0 mL/min	Montelukast	247392	2.061	7243	1.2
	Bilastine	3530866	2.462	3389	1.1
Less Flow rate of 0.9 mL/min	Montelukast	69214	2.267	4713	1.3
	Bilastine	527373	2.690	5275	1.0
More Flow rate of 1.1 mL/min	Montelukast	388838	1.864	4740	1.2
	Bilastine	4363129	2.284	5611	1.0
Less organic phase	Montelukast	445628	2.165	4709	1.2
	Bilastine	3965572	2.590	5550	1.0
More Organic phase	Montelukast	69404	1.967	5590	1.4
	Bilastine	527708	2.390	6273	1.0

CONCLUSION

This developed method is a unique, rapid, specific, economic, and cost-effective HPLC technique for the simultaneous estimation of Bilastine and Montelukast. All parameters, including system suitability, method specificity, correlation coefficient, accuracy, robustness, and system precision, were The developed method offers advantages in sensitivity, ruggedness, & reproducibility, making it an ideal choice for routine QC analysis. Selecting a 260nm wavelength enhances sensitivity compared to techniques that use higher wavelengths. The chosen mobile phase is less susceptible to fluctuations than complex buffers & highly volatile solvents, demonstrating the method's robustness. The simpler mobile phase & faster RT make it easier to achieve reproducible results across different Laboratories, compared with acetonitrile [2, 3], which can exhibit batch-to-batch variability.

validated using documented evidence, and the results met acceptable criteria. Despite its advantages and proven efficacy, this method can serve as an alternative in quality control laboratories for detecting pharmaceutical dosage forms of Bilastine and Montelukast without pretreatment. Furthermore, the ruggedness, reproducibility, and shorter analysis time ensure that the method has great potential for stability testing, and it could also be adapted for Bioanalytical studies, facilitating the monitoring of drug levels in biological matrices.

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Revathi Boddu contributed to the development of the technique and the execution of the experimental data. Rajeshwar Vodeti contributed to the original draft and data analysis. Suchitra Duddagi supervised the research and provided critical insights. Narender Boggula contributed to manuscript writing and revisions. Jithendar Reddy Mandhadi contributed to experimental work, data interpretation, manuscript preparation, and revisions. All the authors have read and approved the final manuscript.

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