

Analytical Method validation and development of Bilastine and Montelukast Sodium Tablets and Simultaneous Quantification by a Validated RP-HPLC Method

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Abstract—The present study was undertaken to develop and validate a simple, accurate, precise and reliable Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for the simultaneous quantification of Bilastine and Montelukast Sodium in fixed-dose combination tablets. Bilastine is a second-generation H1 antihistamine, while Montelukast Sodium is a selective cysteinyl leukotriene receptor antagonist, and their combination is used in the management of allergic rhinitis and associated respiratory conditions. The analytical method was developed for the determination of both drugs in pharmaceutical dosage form and subsequently validated for its intended purpose. The developed method was evaluated for specificity, system precision, method precision, intermediate precision, ruggedness, linearity and range, limit of detection (LOD), limit of quantification (LOQ), accuracy, robustness, stability in analytical solution and forced degradation studies. The method demonstrated clear and sharp chromatographic peaks for Bilastine and Montelukast without significant interference from blank or placebo components. Linearity was evaluated at different concentration levels ranging from 50% to 150% of the sample concentration, showing a good linear relationship between peak area and concentration for both analytes. The accuracy study was performed at 80%, 100% and 120% concentration levels. The overall mean recovery was $99.9 \pm 1.09\%$ for Bilastine and $100.3 \pm 1.06\%$ for Montelukast, with an overall %RSD of 1.1% for both drugs. Intermediate precision showed %RSD of 1.6% for both analytes. The LOD was found to be $4.83 \mu\text{g/mL}$ for Bilastine and $3.53 \mu\text{g/mL}$ for Montelukast, while the LOQ was $14.64 \mu\text{g/mL}$ and $10.70 \mu\text{g/mL}$, respectively. The validated RP-HPLC method was found suitable for the simultaneous assay of Bilastine and Montelukast Sodium in pharmaceutical dosage forms and can be applied for routine quality-control analysis.

Index Terms—Bilastine, Montelukast Sodium, RP-HPLC, Method Development, Method Validation, Simultaneous Quantification, Pharmaceutical Analysis.

I. INTRODUCTION

Allergic rhinitis (AR) and seasonal allergic rhinoconjunctivitis (SARC) are common allergic disorders that may occur independently or in association with asthma. Histamine and cysteinyl leukotrienes (CysLTs) are important inflammatory mediators involved in the development of allergic symptoms and airway inflammation. Therefore, appropriate pharmacological management targeting these mediators is important for effective control of allergic rhinitis and related respiratory symptoms.^[3-5,13,16] Bilastine is a second-generation, selective H1-antihistamine used in the management of allergic rhinoconjunctivitis and urticaria. It exerts its therapeutic effect primarily by selectively antagonizing H1 histamine receptors and thereby reducing histamine-mediated allergic responses.^[3-5] Montelukast Sodium is a selective cysteinyl leukotriene receptor type-1 (CysLT1) antagonist that inhibits leukotriene-mediated effects such as bronchoconstriction, airway edema and inflammatory responses.^[10-13] The complementary mechanisms of action of Bilastine and Montelukast Sodium provide a rationale for their use in combination therapy for allergic conditions.^[16] Fixed-dose combination formulations containing Bilastine and Montelukast Sodium have been developed in tablet dosage forms for the management of allergic rhinitis and related allergic

conditions. Tablets are widely preferred because of their ease of administration, stability and economical manufacturing.^[16,18,19] However, reliable analytical methods are essential for the quality assessment of pharmaceutical formulations and for ensuring the identity, purity, potency and consistency of the active pharmaceutical ingredients.^[1,2] High-performance liquid chromatography (HPLC), particularly reverse-phase HPLC (RP-HPLC), is a widely used analytical technique for the separation, identification and quantitative determination of pharmaceutical compounds.^[20-22] Previous studies have reported RP-HPLC and spectrophotometric methods for the individual or simultaneous determination of Bilastine and Montelukast in pharmaceutical formulations. The available literature includes validated methods for Bilastine as well as methods for Montelukast in combination with other active pharmaceutical ingredients.^[30-52] Analytical method validation is an essential component of pharmaceutical quality control. A validated analytical procedure should demonstrate that it is suitable for its intended purpose by providing reliable and reproducible results. Important validation characteristics include specificity, precision, accuracy and linearity.^[23,24] These parameters provide evidence regarding the capability of an analytical method to consistently quantify the active pharmaceutical ingredients in a pharmaceutical dosage form. In view of the importance of reliable simultaneous estimation of the two active ingredients, the present study was undertaken to develop and validate a simple, accurate, precise and specific RP-HPLC method for the simultaneous quantification of Bilastine and Montelukast Sodium in fixed-dose combination tablets. The developed method was evaluated for specificity, precision, accuracy and linearity and was intended for routine quality-control analysis of the combined dosage form.

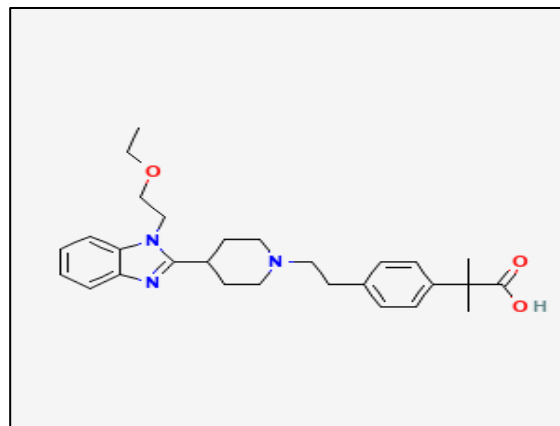


Fig 1: Structure of Bilastine

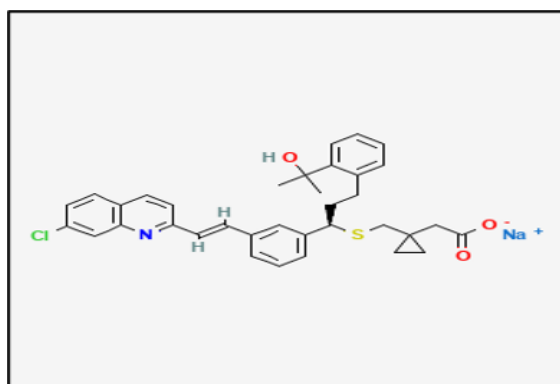


Fig 2: Structure of Montelukast

II. MATERIALS AND INSTRUMENTS

A Shimadzu LC-2010 HPLC with Hypersil BDS C-18 Column (100×4.6 mm, 3 μm) as a stationary phase and 0.1% v/v Triethylamine buffer of pH-3.00: Acetonitrile as a mobile phase in gradient mode. The buffer was filtered through 0.45 μm membrane filter and degassed by sonication. The mobile phase solvent was pumped at 1.0 ml/min, column oven temperature kept at 40°C and λ_{max} was set at 220 nm. Injection volume for all samples was fixed at 10 μl and under these conditions; the run time was 13 min.

III. EXPERIMENTAL CONDITIONS

Chromatographic separation was achieved on a Hypersil BDS C-18 column (100 × 4.6 mm, 3 μm) with the column oven temperature maintained at 40 °C and the sample cooler set to 10 °C. The mobile phase comprised 0.1% v/v triethylamine buffer adjusted to

pH 3.00 (Component A) and acetonitrile (Component B). Elution was performed at a constant flow rate of 1.0 mL/min using a gradient program structured as follows: 0.01–3.00 min, 60% A and 40% B; 5.00–8.00 min, 80% A and 20% B; and 10.00–13.00 min, returning to 60% A and 40% B. The total analytical run time was 13 min. The injection volume for all samples was 10 μ L, and detection was carried out at a wavelength of 220 nm.

IV. PREPARATION OF STANDARD SOLUTIONS

Preparation of Buffer Solution

Accurately added 2 mL of Triethylamine in 2000 mL of water. Mixed well and pH adjusted to 3.00 with Orthophosphoric acid and filtered it through 0.22 μ m membrane filter.

Preparation of Mobile phase

Buffer solution pH 3.00 prepared and acetonitrile in different channels.

Preparation of Diluent and blank solution

Acetonitrile and HPLC grade water in 1:1 ratio,

Preparation of Stock Solution

The stock solution of Bilastine and Montelukast was prepared by transferring the 101.02 mg of Bilastine and 52.67 mg of Montelukast Sodium (equivalent to 50.77 mg of Montelukast) into 100 ml volumetric flask. About 70 ml of acetonitrile was added to the flask and sonicated at temperature below 20°C, with occasional shaking and final volume was made up to 100 ml.

Preparation of Standard Solution

A 5 ml of stock solution was transferred into 25 ml of volumetric flask, and the final volume was made up with diluent to get final concentration of 200.14 μ g/ml of Bilastine and 100.75 μ g/ml of Montelukast (after potency correction, 99.06% for Bilastine and 99.23% for Montelukast) and used as standard solution after filtration through 0.45 μ m nylon syringe filter.

Preparation of Common Placebo Solution

Accurately weighed and transferred the placebo powder equivalent to 200 mg of Bilastine (about 2000 mg placebo, the average weight of 1 tablet is 230 mg) into a 200 ml volumetric flask. About 120

ml of acetonitrile was poured and sonicated for 20 minutes below 20°C, and final volume was made up of diluent. A 5 ml of aliquot was transferred in a 25 ml flask, and the volume was made up with diluent, mixed and filtered through a 0.45 μ m nylon syringe filter and used as a placebo solution.

Preparation of Method Precision sample solution

Ten intact tablets of Bilastine and Montelukast Sodium was put in a 200 ml volumetric flask, about 120 ml of acetonitrile was added and sonicated for 20 minutes below 20°C, with occasional shaking. The final volume was made up after cooling the solution. A 5 ml solution was transferred into a 25 ml volumetric flask and volume made up with diluent and filtered through a 0.45 μ m nylon syringe filter and used as a method precision sample solution.

V. METHOD VALIDATION

The analytical method for assay of Bilastine and Montelukast in Bilastine and Montelukast Sodium tablets was validated as per the following parameters.

Method Validation

The developed RP- HPLC method for the simultaneous estimation of Bilastine and Montelukast was validated according to ICH Q2(R1) guidelines for specificity, precision, ruggedness, linearity, range, sensitivity, accuracy, robustness, and solution stability.

Specificity

Specificity was evaluated to ensure zero matrix interference from blank vehicles or common excipients. Single injections of the blank and common placebo solutions were analyzed alongside six replicate injections of the standard solution. The acceptance criteria required no peak interference at the retention times of Bilastine and Montelukast.

Precision and Ruggedness

Method precision was evaluated across three distinct parameters:

- System Precision: Six replicate injections of the standard solution were tested. The relative standard deviation (RSD) for the peak regions of both analytes must not be greater than 2.0%.

- **Method Precision:** Six independent sample solutions prepared from the same formulation batch were injected in duplicate. The assay values must fall within $100 \pm 2\%$ with an overall mean assay RSD of $\leq 2.0\%$.
- **Intermediate Precision and Ruggedness:** Analysis was repeated by a different analyst on a different day using a separate HPLC instrument. The six independent samples were injected in duplicate, adhering to the $100 \pm 2\%$ assay limit and a maximum RSD of 2.0% . For system ruggedness, the cumulative RSD across all twelve assay values (method and intermediate precision combined) must not exceed 5.0% .

Linearity and Range

Linearity was verified by injecting varying concentration levels spanning the target analytical range. Following blank and system suitability checks, each linearity level was injected in duplicate. Calibration curves were constructed by plotting mean peak areas against respective concentrations. The method was considered linear if the correlation coefficient (r^2) was not less than 0.999 for both Bilastine and Montelukast over the specified analytical range.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The sensitivity thresholds (LOD and LOQ) were determined using multiple approaches. These included visual estimation, the signal-to-noise (S/N) ratio method—targeting a ratio of 3:1 for LOD and 10:1 for LOQ—and statistical evaluation using the STEYX function in MS Excel applied directly to the calibration curve data.

Accuracy (Recovery)

Method accuracy was determined via recovery studies using the standard addition method. Known amounts of pure Bilastine and Montelukast APIs were spiked into the common placebo matrix at low, medium, and high concentration levels. Each sample level was analyzed in duplicate. The individual recovery at all spiked levels must fall within 98.0% to 102.0% , with an RSD of no more than 2.0% at each level.

Robustness

Robustness was evaluated by deliberately introducing small, intentional modifications to critical

chromatographic parameters: flow rate ($\pm 10\%$), column temperature ($\pm 5^\circ\text{C}$), and detection wavelength ($\pm 5\text{ nm}$). For each modified condition, system suitability was verified using six standard replicates and three injections from the first three method precision samples. The method demonstrated adequate robustness by meeting all system suitability requirements, with an overall RSD from the nine combined assay values not exceeding 2.0% .

Stability in Analytical Solution

Solution stability was monitored by storing the first method precision sample solution in the sample cooler (10°C) and re-injecting it at predefined time intervals. The method met the stability criteria since the absolute percentage area difference for both Bilastine and Montelukast peaks between the initial injection and subsequent time intervals did not exceed 5.0% .

VI. RESULTS

Method Optimization and System Suitability

To develop an accurate and precise method for simultaneously estimation of Bilastine and Montelukast % assay from bulk mixture and tablet formulation, chromatographic conditions have been optimized for validation purpose after attempting several hits and trials on different composition of mobile phases, flow rate and column composition. Under these chromatographic conditions the RT of Bilastine and Montelukast was observed at 1.67 and 7.43 min, respectively, with the resolution of 42.3 without any interference in the blank solution.

Preparation of Buffer Solution

Accurately added 2 mL of Triethylamine in 2000 mL of water. Mixed well and pH adjusted to 3.00 with Orthophosphoric acid and filtered it through $0.22\mu\text{m}$ membrane filter.

Preparation of Mobile phase

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Specificity

After reviewing the chromatograms, it can be concluded that no factorial interference was observed at the retention time of Bilastine peak (1.67 min) and Montelukast peak (7.43 min) in blank chromatogram and in placebo sample solution

Precision

The precision of a method was calculated in terms of System Precision, Method Precision and Intermediate precision. The Ruggedness of method was calculated by comparing the data of method precision and intermediate precision statistically.

System Precision

The %RSD of six replicates injection of standard solution was 0.1% for Bilastine and 0.0% for Montelukast and summarized

Method Precision (MP)

Mean area of two injections used for calculation of % assay value. The results obtained in terms of area count, mean and value of % RSD of six method precision samples are presented. The Chromatogram of method precision sample shows the clear and sharp peaks of Bilastine and Montelukast

Intermediate Precision (IP)

Prepared standard solution for Intermediate study was injected into six replicates and system suitability was established. The %RSD of Bilastine and Montelukast in standard solution for IP was found to be 0.3% and 0.0 % respectively and presented the % assay for IP samples was calculated by comparing the mean area count of standard solution for IP with the mean area count of particular IP sample. The %RSD of all six IP samples was calculated and presented. The Chromatogram of Intermediate precision sample shows the clear and sharp peaks of Bilastine and Montelukast

Ruggedness

The overall relative standard deviation of twelve assay values; six of method precision samples and six of intermediate precision samples were calculated and presented.

Linearity and Range

Calibration standards of various concentrations i.e., 50%, 70%, 90%, 100%, 120% and 150% were prepared from standard stock solution and diluted with diluent to get the concentration of 100.07-300.21 µg/ml and 50.38-151.14 µg/ml of Bilastine and Montelukast respectively. The chromatograms at each concentration level were recorded. The linear

regression was calculated from the data of the calibration curve and linearity curves are shown as per Figure 3,4. Linearity curves of Bilastine and

Montelukast shows a good linear relationship between the area vs. concentration, and the obtained results are summarized in Table 1 and 2.

Table 1: Result of Bilastine Linearity

% level of Calibration Standards	Stock vol. taken(ml)	Dilution (mL)	Bilastine	
			Conc. ($\mu\text{g/ml}$)	Mean Area
Linearity 50%	2.0	20	100.07	958365
Linearity 70%	3.5	25	140.10	1320912
Linearity 90%	4.5	25	180.13	1688638
Linearity 100%	4.0	20	200.14	1846238
Linearity 120%	6.0	25	240.17	2215541
Linearity 150%	6.0	20	300.21	2786982
Intercept			43187	
Slope			9094.9	
Correlation coefficient (r^2)			0.99966	

Table 2: Result of Montelukast Linearity

% level of Calibration Standards	Stock vol. taken(ml)	Dilution (mL)	Montelukast	
			Conc. ($\mu\text{g/ml}$)	Mean Area
Linearity 50%	2.0	20	50.38	747861
Linearity 70%	3.5	25	70.53	1051012
Linearity 90%	4.5	25	90.68	1356839
Linearity 100%	4.0	20	100.76	1491488
Linearity 120%	6.0	25	120.91	1818571
Linearity 150%	6.0	20	151.14	2311953
Intercept			-44076	
Slope			15473	
Correlation coefficient (r^2)			0.99929	

LOD and LOQ

The limit of detection and limit of quantitation is calculated using the steypx function of MS Excel on results of linearity standards and the obtained results are presented.

Accuracy (Recovery)

Accuracy of the assay method was calculated at three levels i.e., 80%, 100% and 120% of sample concentration and the obtained result was presented.

Table 3: Result of Bilastine Accuracy (Recovery)

Recovery Sample Name	% Recovery Bilastine			
	Bilastine added (mg)	Bilastine Recovered (mg)	Bilastine % Recovery	Mean $\pm$ SD
Recovery 80% -1	79.496	80.336	101.1	100.6 $\pm$ 1.23
Recovery- 80% -2	80.357	79.720	99.2	
Recovery 80% -3	79.981	81.199	101.5	
Recovery 100% -1	101.101	100.425	99.3	
Recovery 100% -2	102.141	100.709	98.6	98.9 $\pm$ 0.35
Recovery 100% -3	101.596	100.514	98.9	
Recovery 120% -1	120.209	121.809	101.3	
Recovery 120% -2	121.448	121.151	99.8	100.3 $\pm$ 0.87
Recovery 120% -3	120.873	120.658	99.8	
Overall Mean $\pm$ SD			99.9 $\pm$ 1.09	
Overall %RSD			1.1	

Table 4: Result of Montelukast Accuracy (Recovery)

Recovery Sample Name	% Recovery Montelukast			
	Montelukast added (mg)	Montelukast Recovered (mg)	Montelukast % Recovery	Mean $\pm$ SD
Recovery 80% -1	40.168	40.091	99.8	100.6 $\pm$ 1.36
Recovery- 80% -2	39.980	40.859	102.2	
Recovery 80% -3	40.535	40.490	99.9	
Recovery 100% -1	50.726	50.585	99.7	99.6 $\pm$ 0.12
Recovery 100% -2	51.242	50.967	99.5	
Recovery 100% -3	50.855	50.721	99.7	
Recovery 120% -1	60.759	60.464	99.5	100.7 $\pm$ 1.25
Recovery 120% -2	60.133	61.356	102.0	
Recovery 120% -3	60.421	60.845	100.7	
Overall Mean $\pm$ SD			100.3 $\pm$ 1.06	
Overall %RSD			1.1	

Robustness

Robustness of the analytical method was carried out as per methodology and overall mean and overall SD was calculated by from % assay value of six method precision plus three % assay value of each robustness parameters. From the obtained area count of Bilastine and Montelukast, Mean, SD, Overall % RSD and Mean of overall % RSD was calculated.

From the obtained data it was observed that the area count and retention time of Bilastine and Montelukast peaks increased as the flow rate decreased to 0.9 ml/min. The area counts and retention time of Bilastine and Montelukast was

decreased as we increase the flow rate to 1.1 ml/min. From the column oven temperature plus and minus study, no significant effect in area counts of Bilastine and Montelukast was seen. From the wavelength minus (215 nm) study, significant increase was seen in the area count of Bilastine and Montelukast.

Stability In Aqueous Solution (SIAS)

Stability in aqueous solution was carried out by injecting method precision sample at specific time interval and % difference in area count with respect to the area count of first method precision sample. Also, we have observed no significant change in RT, TF, TP and resolution over 27 hours.

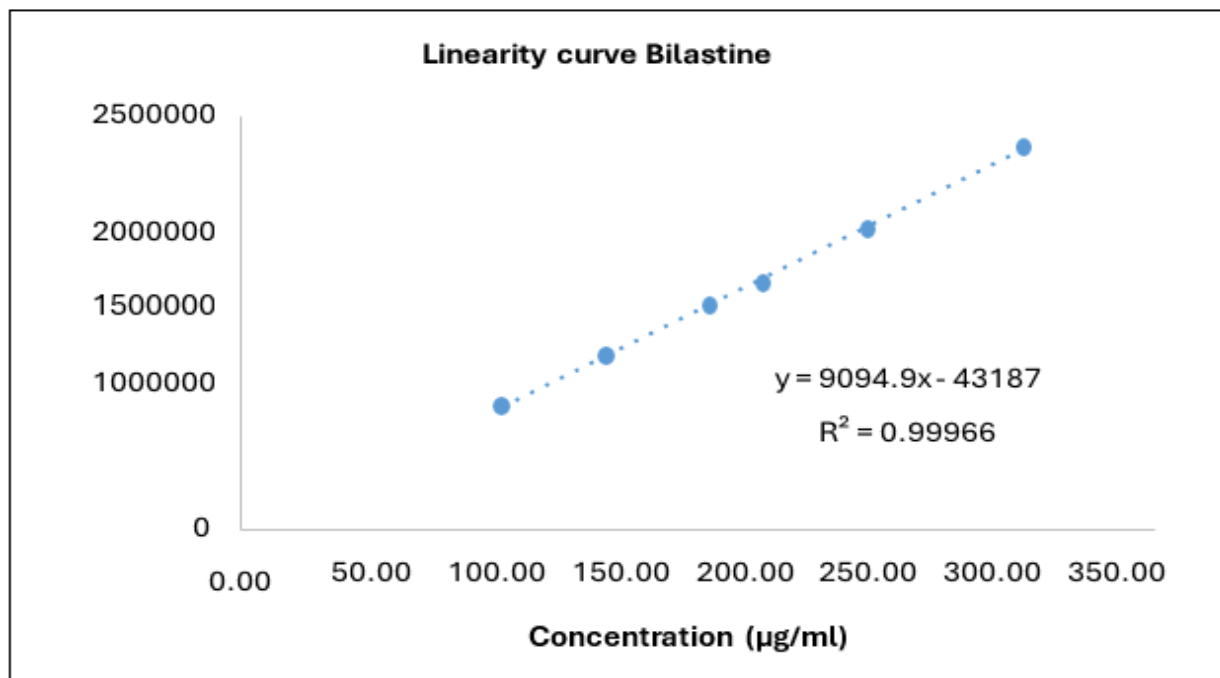


Figure 3: Graphical representation of linearity of Bilastine

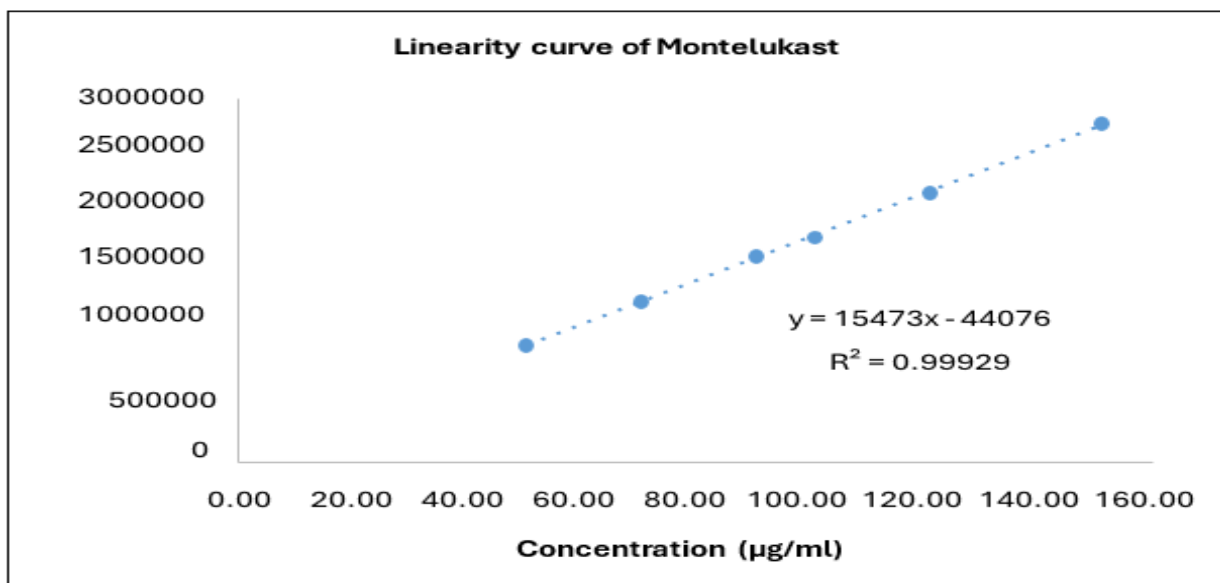


Figure 4: Graphical representation of linearity of Montelukast

VII. DISSCUSSION

The developed RP-HPLC method demonstrated satisfactory performance for the simultaneous assay of Bilastine and Montelukast in bulk and tablet formulations. Both analytes exhibited acceptable chromatographic behaviour, with adequate retention, low tailing factors, and satisfactory theoretical plate

counts. The method showed excellent linearity over the investigated concentration ranges, with correlation coefficients of 0.99966 and 0.99929 for Bilastine and Montelukast, respectively. Precision and intermediate precision results indicated good reproducibility, while accuracy values close to 100% confirmed reliable recovery. The low LOD and LOQ values demonstrated adequate sensitivity.

Robustness studies further indicated that the method remained consistent under deliberate analytical variations.

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