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RESEARCH ARTICLE

Development and Validation of a RP-HPLC Method for Simultaneous Quantification of Montelukast and Fexofenadine in Pharmaceutical Formulations

Syed Ansar Ahmed*[1], Vaishnavi P Shinde[2]

[1] *Associate Professor, Department of Pharmaceutical Chemistry, Indira College of Pharmacy, VishnuPuri, Nanded, Maharashtra, India*
[2] *Research Scholar, Department of Pharmaceutical Chemistry, Indira College of Pharmacy, VishnuPuri, Nanded, Maharashtra, India*

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Abstract: A rapid, sensitive, and precise reversed-phase high-performance liquid chromatographic method was developed for simultaneous estimation of Montelukast and Fexofenadine in pharmaceutical formulations. The chromatographic separation was achieved on a Fortis C18 column (4.6×100µm, 2.5µm) using a mobile phase consisting of methanol and phosphate buffer (75:25 v/v, pH 4.5) at a flow rate of 0.8 mL/min. Detection was carried out at 215 nm using a UV detector. The retention times for Montelukast and Fexofenadine were 3.02 and 6.50 minutes, respectively. The method demonstrated linearity over concentration ranges of 66-396 µg/mL for Montelukast and 10-60 µg/mL for Fexofenadine with correlation coefficients exceeding 0.999. The limits of detection and quantification were 2.80 and 8.485 µg/mL for Montelukast, and 0.55 and 1.674 µg/mL for Fexofenadine, respectively. The method was validated according to ICH guidelines, showing excellent accuracy (99.41-100.81% for Montelukast and 99.59-100.61% for Fexofenadine), precision (RSD < 2%), and robustness. The validated method was successfully applied to the simultaneous determination of Montelukast and Fexofenadine in pharmaceutical formulations, demonstrating its suitability for routine quality control.

Keywords: Montelukast; Fexofenadine; RP-HPLC; Method validation; Pharmaceutical analysis.

1. Introduction

Montelukast and Fexofenadine are widely prescribed medications for managing allergic conditions and asthma. Montelukast (methyl-3-[2-(2-quinolinylmethoxy)phenyl]-2-propenoate), a selective leukotriene receptor antagonist, acts by blocking the action of leukotrienes in airways, thereby reducing inflammation and preventing asthma symptoms [1]. It specifically antagonizes the cysteinyl leukotriene receptor CysLT1, leading to decreased bronchial hyperresponsiveness, inflammatory cell infiltration, and edema of the airways. Montelukast demonstrates particular efficacy in exercise-induced bronchoconstriction and aspirin-sensitive asthma. Fexofenadine (2-[4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]phenyl]-2-methylpropanoic acid), a second-generation H1-antihistamine, provides relief from allergic symptoms without significant central nervous system effects. The active metabolite of terfenadine, it exhibits high selectivity for peripheral H1 receptors and minimal blood-brain barrier penetration. These properties result in effective antiallergic action without the sedation commonly associated with first-generation antihistamines [2].

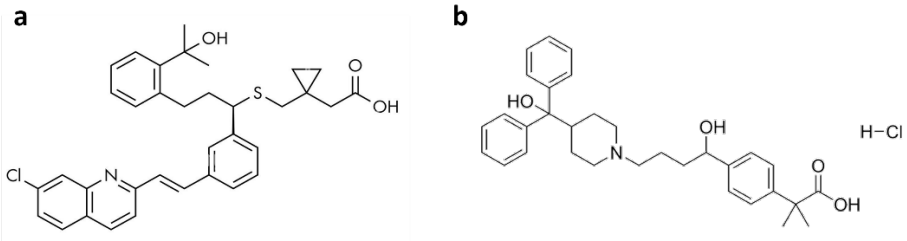

Figure 1. Structure of a. Montelukast and b. Fexofenadine

The simultaneous administration of these drugs has shown enhanced therapeutic benefits in patients with concurrent asthma and allergic conditions [3]. Clinical studies have demonstrated improved symptom control and quality of life metrics when both medications are used concurrently, particularly in patients with allergic rhinitis-associated asthma. The complementary mechanisms

* Corresponding author: Syed Ansar Ahmed

of action - leukotriene antagonism and histamine receptor blockade - provide comprehensive control of the allergic inflammatory cascade. Therefore, developing analytical methods for their concurrent quantification is essential for pharmaceutical quality control and clinical monitoring. Several analytical methods have been reported for the individual estimation of these drugs, including spectrophotometry, HPLC, and LC-MS [4]. Spectrophotometric methods, while simple and cost-effective, often lack the specificity required for complex pharmaceutical matrices. LC-MS methods offer high sensitivity but require sophisticated instrumentation and expertise [5].

High-performance liquid chromatography (HPLC) remains the method of choice for pharmaceutical analysis due to its versatility, precision, and robustness. The technique offers several advantages including high resolution, good reproducibility, and the ability to handle complex sample matrices. Modern HPLC systems, equipped with various detector options and automated sample handling capabilities, provide efficient means for routine pharmaceutical analysis. The development of a simultaneous estimation method using HPLC can significantly reduce analysis time and cost while maintaining accuracy and precision [6]. Reversed-phase HPLC, in particular, offers suitable retention and separation of both Montelukast and Fexofenadine due to their molecular structures and physicochemical properties. The optimization of chromatographic conditions, including mobile phase composition, pH, and stationary phase selection, is crucial for achieving adequate resolution and peak symmetry for both analytes [7]. Current analytical methods for simultaneous determination of these drugs are limited, and many existing procedures involve complex extraction steps or lengthy analysis times [8]. Therefore, there is a need for a simple, rapid, and validated HPLC method suitable for routine quality control analysis of pharmaceutical formulations containing both Montelukast and Fexofenadine. The main aim is this research is to develop and validate a RP-HPLC method for simultaneous estimation of Montelukast and Fexofenadine in pharmaceutical formulations

2. Materials and Methods

2.1. Reagents and Instruments

Reference standards of Montelukast sodium (99.8% purity) and Fexofenadine hydrochloride (99.5% purity) were obtained as gift samples from Cipla Ltd. (Mumbai, India). HPLC-grade methanol was purchased from Rankem (Mumbai, India), while orthophosphoric acid and triethylamine were procured from Finar Chemicals Ltd. (Ahmedabad, India). All other chemicals and reagents used were of analytical grade. Ultrapure water was obtained using a Smart2Pure water purification system (ThermoFisher Scientific India Pvt. Ltd., Mumbai, India). The chromatographic analysis was performed on an Agilent 1260 Infinity II HPLC system (Agilent Technologies, Bangalore, India) equipped with a quaternary pump (G7111B), autosampler (G7129A), column thermostat (G7116A), and diode array detector (G7115A). Data acquisition and processing were performed using Agilent OpenLab CDS ChemStation software version 10.1 (Agilent Technologies, Bangalore, India). Separation was achieved using a Fortis C18 column (4.6 × 100 mm, 2.5 µm particle size) purchased from Phenomenex India (Mumbai, India) maintained at ambient temperature.

2.2. Chromatographic Conditions

The separation was achieved on a Fortis C18 column (4.6×100mm, 2.5µm) maintained at ambient temperature. The mobile phase consisted of methanol and phosphate buffer (75:25 v/v, pH 4.5), delivered at a flow rate of 0.8 mL/min. The injection volume was set at 20 µL, and detection was performed at 215 nm.

2.3. Method Development

2.3.1. Mobile Phase Selection

Initial trials were conducted using various combinations of organic solvents and buffers. The combination of methanol and phosphate buffer (75:25 v/v) was selected based on optimal peak shape, resolution, and system suitability parameters [9]. The pH was adjusted to 4.5 using orthophosphoric acid, as this provided optimal ionization conditions for both analytes [10].

2.3.2. Buffer Preparation

The buffer solution (0.1% OPA) was prepared by diluting 1 mL of HPLC-grade orthophosphoric acid to 1000 mL with ultrapure water. The solution was filtered through a 0.45 µm membrane filter and degassed by sonication for 15 minutes prior to use [11].

2.4. Preparation of Standard Solution

2.4.1. Stock Solutions

Primary stock solutions of Montelukast (660 µg/mL) and Fexofenadine (100 µg/mL) were prepared separately by dissolving accurately weighed amounts in methanol. The solutions were sonicated for 10 minutes to ensure complete dissolution.

2.4.2. Working Standards

Working standard solutions were prepared by appropriate dilution of stock solutions with mobile phase to obtain concentrations within the linear range for both analytes.

2.5. Method Validation

2.5.1. System Suitability

System suitability testing was performed to ensure the analytical system's adequacy before sample analysis. The method's reliability was evaluated through six replicate injections of standard solutions containing both Montelukast and Fexofenadine. Various chromatographic parameters were assessed to confirm the system's performance. The theoretical plates (N) exceeded 8000 for both analytes, demonstrating efficient separation and column performance. Peak symmetry was evaluated through the tailing factor, which remained ≤1.2, indicating minimal peak tailing and good column conditions. The resolution between adjacent peaks was maintained above 2.0, ensuring complete separation of the analytes. Additionally, the relative standard deviation (RSD) of peak areas remained below 2.0%, confirming the injection precision and overall system stability.

2.5.2. Linearity

Linearity was evaluated over the concentration range of 66-396 µg/mL for Montelukast and 10-60 µg/mL for Fexofenadine. Six concentration levels were analyzed in triplicate. The calibration curves were constructed by plotting peak areas against respective concentrations [12].

2.5.3. Precision

Intraday Precision: Six replicate injections of three different concentrations were analyzed on the same day. The %RSD values were calculated for peak areas and retention times.

Interday Precision: The same procedure was repeated on three consecutive days to determine interday precision.

2.5.4. Accuracy

Recovery studies were performed by standard addition method at three concentration levels (80%, 100%, and 120%) in triplicate. Known amounts of standards were added to pre-analyzed samples, and the percentage recoveries were calculated [13].

2.5.5. Sensitivity

Limit of Detection (LOD): The LOD was calculated using the formula: $LOD = 3.3\sigma/S$, where σ represents the standard deviation of the response and S represents the slope of the calibration curve [14].

Limit of Quantification (LOQ): The LOQ was determined using the formula: $LOQ = 10\sigma/S$ [15].

2.5.6. Robustness

The analytical method's robustness was systematically evaluated by introducing deliberate variations in critical chromatographic parameters while maintaining other conditions constant. The flow rate was varied by ±0.1 mL/min from the optimized rate of 0.8 mL/min to assess its impact on retention time and peak characteristics. The mobile phase composition was modified by adjusting the organic phase proportion by ±1% from the established ratio to evaluate its effect on separation efficiency. The detection wavelength was altered by ±1 nm from the selected 215 nm to determine the method's sensitivity to slight changes in detection conditions. Additionally, the column temperature was varied by ±5°C from ambient temperature to assess thermal effects on chromatographic performance. The method's reliability under these varied conditions was determined by monitoring changes in system suitability parameters and analyte quantification.

3. Results and Discussion

3.1. Method development

The optimized chromatographic conditions provided efficient separation of Montelukast and Fexofenadine with retention times of 3.02 and 6.50 minutes, respectively. The selected wavelength of 215 nm provided optimal sensitivity for both analytes [16]. The mobile phase composition of methanol:phosphate buffer (75:25 v/v) resulted in symmetric peak shapes and adequate resolution.

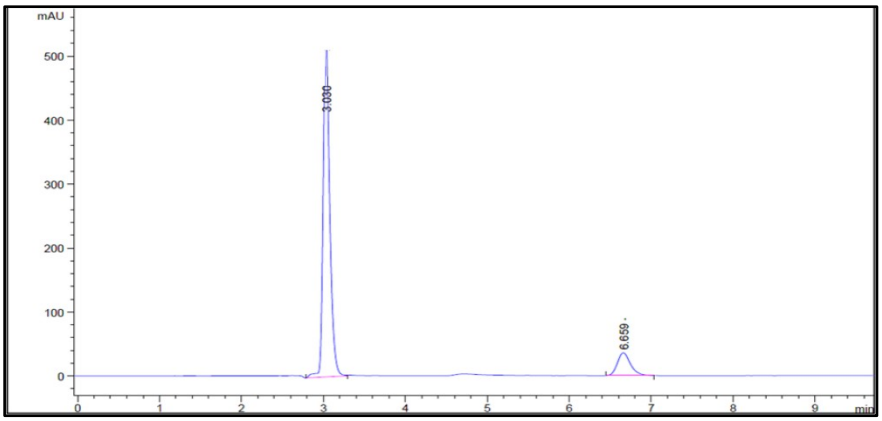

Figure 2. Optimized Chromatogram showing separation of Montelukast and Fexofenadine

3.2. Method Validation

3.2.1. System Suitability

The system suitability parameters met the acceptance criteria, indicating the chromatographic system's adequacy for analysis [17]. The theoretical plates exceeded 8000 for both analytes, demonstrating efficient separation. The tailing factors were within 1.2, indicating good peak symmetry. The results are shown in Table 1.

Table 1. System Suitability Parameters

Parameter	Montelukast	Fexofenadine
Retention time (min)	3.02 ± 0.022	6.50 ± 0.01
Theoretical Plates	8203 ± 0.56	9746 ± 0.11
Tailing factor	1.00 ± 0.02	1.11 ± 0.03
Resolution	2.34	2.15

3.2.2. Linearity and Range

Both analytes showed excellent linearity within their respective concentration ranges as shown in Table 2.

Table 2. Results of Linearity

Parameter	Montelukast	Fexofenadine
Linearity range (µg/mL)	66-396	10-60
Regression equation	y = 39.33x + 758.0	y = 36.54x + 41.32
Correlation coefficient (R^2)	0.9996	0.9995
LOD (µg/mL)	2.80	0.55
LOQ (µg/mL)	8.485	1.674

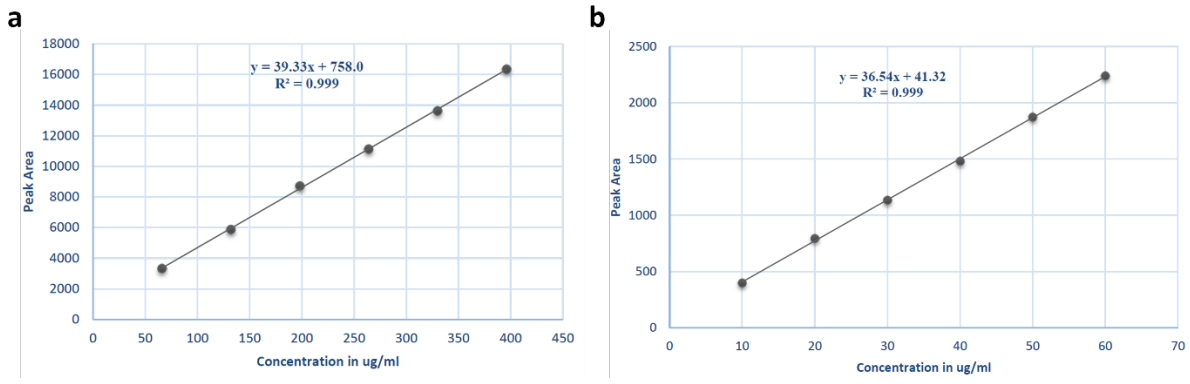

Figure 3. Calibration Curves of a. Montelukast and b. Fexofenadine

3.2.3. Precision

The method showed good precision with %RSD values. The results are shown in Table 3.

Table 3. Results of Precision

Drug	Type	Concentration Range (µg/mL)	%RSD
Montelukast	Intraday	132-264	0.38
	Interday	132-264	0.33
Fexofenadine	Intraday	20-40	0.70
	Interday	20-40	0.51

3.2.4. Recovery

Mean recoveries ranged from 99.41% to 100.81% for Montelukast and 99.59% to 100.61% for Fexofenadine. These values indicate excellent accuracy of the method [18]. The results are shown in Table 4.

Table 4. Results of Recovery Studies

Drug	Level (%)	Amount spiked (µg/mL)	% Recovery	Mean ± SD	%RSD
Montelukast	80	52.8	99.41-99.78	99.46 ± 0.31	0.31
	100	66	100.43-101.07	100.81 ± 0.34	0.33
	120	79.2	100.47-100.81	100.64 ± 0.17	0.17
Fexofenadine	80	8	99.33-99.77	99.59 ± 0.23	0.23
	100	10	100.10-100.70	100.46 ± 0.32	0.31
	120	12	99.51-100.25	99.91 ± 0.37	0.37

3.2.5. Robustness

The robustness of the method was evaluated by deliberately varying the chromatographic conditions. The flow rate was modified by ±0.1 mL/min from the optimal 0.8 mL/min, resulting in %RSD values ranging from 0.05-0.29% for Montelukast and 0.16-0.71% for Fexofenadine. Changes in mobile phase composition (±1% organic phase) showed %RSD values of 0.12-0.16% and 0.07-0.52% for Montelukast and Fexofenadine, respectively. Wavelength variation (±1 nm from 215 nm) demonstrated %RSD values of 0.20-0.38% for Montelukast and 0.70-0.71% for Fexofenadine. Temperature variations (30°C and 40°C) showed %RSD values between 0.28-0.37% for Montelukast and 0.19-0.23% for Fexofenadine. All %RSD values remained well below 2%, indicating that the method is robust and reliable under varying conditions. The results are shown in Table 5.

Table 5. Robustness Parameters (%RSD)

Parameter	Variation	Montelukast	Fexofenadine
Flow rate	0.7 mL/min	0.29	0.71
	0.9 mL/min	0.05	0.16
Mobile phase ratio	74:26	0.16	0.52
	76:24	0.12	0.07
Wavelength	214 nm	0.38	0.71
	216 nm	0.20	0.70
Temperature	30°C	0.28	0.19
	40°C	0.37	0.23

3.3. Assay

The validated method was successfully applied to commercial tablet formulations containing Montelukast and Fexofenadine. The assay results showed good agreement with the label claim, and the excipients did not interfere with the analysis [19]. The results are shown in Table 6

Table 6. Results of Assay of Commercial Formulations

Drug	Amount taken (µg/mL)	Amount found (µg/mL) ± SD	% Amount found	%RSD (n=6)
Montelukast	66	65.97 ± 0.05	99.97 ± 0.57	0.33
Fexofenadine	10	10.26 ± 0.32	100.86 ± 0.16	0.23

4. Discussion

The developed RP-HPLC method shows significant advantages for the simultaneous determination of Montelukast and Fexofenadine. The optimized chromatographic conditions provided excellent separation with retention times of 3.02 and 6.50 minutes for Montelukast and Fexofenadine, respectively, enabling efficient analysis within 7 minutes. This represents a considerable improvement over previously reported methods that typically require longer analysis times. The method's high sensitivity is evidenced by the low LOD (2.80 µg/mL for Montelukast, 0.55 µg/mL for Fexofenadine) and LOQ values (8.485 µg/mL for Montelukast, 1.674 µg/mL for Fexofenadine), making it suitable for routine quality control analysis. The excellent linearity ($R^2 > 0.999$) across wide concentration ranges for both drugs indicates the method's reliability for quantitative analysis. The high recovery rates (99.41-100.81% for Montelukast and 99.59-100.61% for Fexofenadine) and low %RSD values (<2%) in precision studies demonstrate the method's accuracy and reproducibility. The robustness studies confirm that small variations in chromatographic parameters do not significantly affect the method's performance, making it suitable for routine laboratory use.

5. Conclusion

A selective and sensitive RP-HPLC method was developed and validated for simultaneous quantification of Montelukast and Fexofenadine in pharmaceutical formulations. The method demonstrated excellent chromatographic separation with retention times of 3.02 and 6.50 minutes for Montelukast and Fexofenadine, respectively, enabling rapid analysis within 7 minutes. The method exhibited good linearity across concentration ranges of 66-396 µg/mL for Montelukast ($R^2 = 0.9996$) and 10-60 µg/mL for Fexofenadine ($R^2 = 0.9995$). Validation studies revealed high accuracy with mean recoveries ranging from 99.41% to 100.81% for Montelukast and 99.59% to 100.61% for Fexofenadine. The method demonstrated excellent precision with RSD values less than 0.38% and 0.70% for intraday, and 0.33% and 0.51% for interday precision studies for Montelukast and Fexofenadine, respectively. The low LOD (2.80 µg/mL for Montelukast, 0.55 µg/mL for Fexofenadine) and LOQ values (8.485 µg/mL for Montelukast, 1.674 µg/mL for Fexofenadine) indicate adequate sensitivity for routine analysis. System suitability parameters, including theoretical plates (>8000), tailing factor (≤1.2), and resolution (>2.0), met the acceptance criteria. The method's robustness was confirmed through deliberate variations in flow rate (±0.1 mL/min), mobile phase composition (±1%), detection wavelength (±1 nm), and column temperature (±5°C), all results within acceptable limits.

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