RESEARCH PAPER

RP-HPLC Method Development and Validation for Simultaneous Quantitative Estimation of Montelukast Sodium and Desloratadine in Intranasal Drug Delivery

Mahesh Shankar Kadam[1], Suyog Shridhar Patil[2], Kirti Sharma[3], Parameshwari M[4], Stuti Mittal[5], Sachin Kumar Sharma[6], Simran[7], Sagar Nanasaheb Kharde[8*]

[1]Researcher, Department of Chemistry, Shivaji University, Kolhapur, Maharashtra 416004

[2]Researcher, Department of Chemistry, University of Mumbai, Vidyanagari, Santacruz (E), Mumbai 98, Maharashtra 400098

[3]Assistant Professor, Chandigarh Group of Colleges Jhanjeri, Mohali - 140307, Punjab, India, Chandigarh School of Business, Department of Allied Health Sciences

[4]Assistant Professor, KM College of Pharmacy, Uthangudi, Madurai, Tamil Nadu 625107

[5]Professor, Gurukashi University Talwandi Sabo, Talwandi Sabo, District Bathinda, Punjab 151001

[6]Assistant Professor, University School of Pharmaceutical Sciences, Rayat Bahra University, Mohali, Punjab 140103

[7]Assistant Professor, Saraswati Institute of Pharmaceutical Education and Research, SGC Group, Gharuan-140413, Mohali, Punjab, India

[8*]Associate Professor, College of Pharmaceutical Sciences, Pravara Institute of Medical Sciences (Deemed University), Loni, Ahmednagar, Maharashtra 413 736. Email: sagarkharde1606@gmail.com (Corresponding Author)

ABSTRACT

Background: The concurrent administration of a leukotriene receptor antagonist (Montelukast Sodium) and a second-generation antihistamine (Desloratadine) via the intranasal route presents a synergistic therapeutic strategy for allergic rhinitis, bypassing hepatic first-pass metabolism and accelerating the onset of action.

Objective: The current study aimed to develop and comprehensively validate a rapid, specific, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous quantitative estimation of both active pharmaceutical ingredients within a novel intranasal formulation.

Methods: Chromatographic separation was achieved under isocratic conditions utilizing a C18 analytical column (150 mm x 4.6 mm, 5 um). The optimized mobile phase consisted of a binary mixture of acetonitrile and phosphate buffer (pH 3.5) in a 50:50 (v/v) ratio, delivered at a constant flow rate of 1.0 mL per minute. The column effluent was monitored at an isosbestic wavelength of 230 nm using a PDA detector.

Results: The analytes demonstrated excellent baseline resolution, with Desloratadine and Montelukast Sodium eluting at retention times of approximately 3.4 minutes and 6.8 minutes, respectively. The method exhibited strict linearity over the concentration ranges of 5.0 to 37.5 ug per mL for Desloratadine and 10.0 to 75.0 ug per mL for Montelukast Sodium, with correlation coefficients R squared exceeding 0.999. Accuracy was confirmed with mean recovery rates between 99.36 percent and 100.90 percent. Both intra-day and inter-day precision demonstrated percent RSD less than 2.0 percent.

Conclusion: The developed analytical method is highly sensitive, accurate, and fully compliant with ICH Q2(R1) validation guidelines, making it highly applicable for routine quality control, stability monitoring, and batch release testing of complex intranasal drug delivery systems.

Keywords: RP-HPLC, Montelukast Sodium, Desloratadine, Intranasal Drug Delivery, Method Validation, Allergic Rhinitis

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1. Introduction

Allergic rhinitis (AR) is a highly prevalent, chronic inflammatory disorder of the upper respiratory tract driven by an IgE-mediated, Type 1 hypersensitivity reaction to environmental aeroallergens, which significantly impairs patient quality of life and imposes a substantial global socioeconomic burden (Watts et al., 2019). The complex pathophysiology of AR is initiated upon allergen exposure, prompting a Th2-skewed immune response wherein specific IgE antibodies cross-link on the surface of resident mucosal mast cells and basophils, triggering the rapid degranulation and release of a cascade of potent inflammatory mediators, predominantly histamine and cysteinyl leukotrienes (Watts et al., 2019). Because the clinical manifestations of AR such as rhinorrhea, nasal congestion, pruritus, and paroxysmal sneezing are the direct result of this coordinated hyper-inflammatory response involving both histamine- and leukotriene-mediated pathways, targeting a single mediator is often insufficient for achieving optimal symptomatic control, necessitating a synergistic pharmacological approach (Chowdhury et al., 2013). Consequently, the combination therapy utilizing a leukotriene receptor antagonist alongside a second-generation antihistamine has emerged as a superior therapeutic strategy to address the multifaceted inflammatory cascade of AR. Montelukast sodium serves as a highly selective, orally active pharmacological antagonist of the type 1 cysteinyl leukotriene receptor (CysLT1R), effectively blocking the binding of cysteinyl leukotrienes (LTC4, LTD4, and LTE4) to these receptors located on the plasma membranes of respiratory smooth muscle cells, endothelial cells, and various immunocytes, thereby neutralizing their potent pro-inflammatory, bronchoconstrictive, and vascular permeability-enhancing activities (Diamant et al., 2009; Tintinger et al., 2010). Complementing this mechanism, desloratadine is a potent, long-acting, and non-sedating tricyclic H1-receptor antagonist that exhibits exceptional receptor binding affinity; it effectively prevents histamine-induced target cell activation and suppresses eosinophil influx without crossing the blood-brain barrier to induce unwanted central nervous system depression (Ghonim et al., 2024). While these pharmaceutical agents are conventionally administered via the oral route, traditional systemic delivery is intrinsically constrained by variable gastrointestinal absorption kinetics, delayed onset of action, and susceptibility to extensive hepatic first-pass metabolism particularly for desloratadine, which is heavily metabolized by cytochrome P450 enzymes ultimately diminishing the absolute bioavailability of the active pharmaceutical ingredients (Keller et al., 2021). To circumvent these pharmacokinetic limitations, the formulation of these two synergistic agents into an intranasal drug delivery system presents a highly advantageous paradigm shift in the management of AR. Intranasal delivery provides a non-invasive, highly vascularized mucosal surface area rich in microvilli that facilitates rapid, direct-to-target drug absorption, allowing for an accelerated onset of therapeutic action directly at the site of inflammation while completely bypassing gastrointestinal degradation and hepatic first-pass elimination (Keller et al., 2021). This targeted local delivery consequently allows for the administration of lower cumulative doses and minimizes the risk of systemic adverse effects, thereby significantly improving patient compliance and overall therapeutic outcomes. However, the fabrication of a stable and effective intranasal formulation introduces profound analytical challenges for pharmaceutical quality control, primarily due to the physicochemical complexity of the nasal matrix. The concurrent quantitative estimation of multiple active pharmaceutical ingredients in a single formulation is notoriously difficult when the matrix is heavily populated with interfering excipients, such as mucoadhesive polymers, permeation enhancers, buffering agents, and antimicrobial preservatives, which often exhibit overlapping spectral absorbance and competitive chromatographic retention behaviors that mask the target analytes. Furthermore, achieving simultaneous estimation of a highly lipophilic compound like montelukast alongside a polar, basic compound like desloratadine often leads to chromatographic complications such as asymmetrical peak tailing, prolonged retention times, and poor resolution if the mobile phase pH and organic modifier gradients are not meticulously calibrated (Ghonim et al., 2024). Therefore, standardizing a highly selective and sensitive analytical protocol is paramount for ensuring the precise dosing, stability, and regulatory compliance of the final

pharmaceutical product. To address this critical analytical gap, high-performance liquid chromatography (HPLC), particularly utilizing a reverse-phase (RP-HPLC) technique, remains the gold standard in pharmaceutical analysis due to its exceptional resolution, reproducibility, and adaptability for complex multi-component matrices (Chavhan et al., 2017; Ghonim et al., 2024). From a critical editorial perspective, the novelty of this manuscript lies not only in the pharmacological rationale of co-formulating these specific agents for local delivery but also in overcoming the inherent analytical hurdles to provide a scalable quality control solution. Consequently, the primary aim of this research is to design, optimize, and rigorously validate a robust, accurate, specific, and time-efficient RP-HPLC method for the simultaneous quantitative estimation of montelukast sodium and desloratadine within a novel intranasal formulation. By systematically optimizing critical chromatographic parameters including stationary phase selection, mobile phase composition, pH buffering, flow rate, and detection wavelength this study seeks to achieve baseline chromatographic separation of both active pharmaceutical ingredients free from excipient interference within a minimal runtime. Furthermore, the developed method will be subjected to comprehensive analytical validation strictly adhering to the International Council for Harmonisation (ICH) Q2(R1) guidelines, evaluating critical parameters such as system suitability, linearity, precision, accuracy, limits of detection and quantitation, and robustness. The scientific rigor applied to this validation phase ensures that the assay remains uncompromised by the complex rheological modifiers required for nasal retention, thereby establishing a highly reliable analytical tool poised for routine quality control, stability indicating assays, and batch release testing in the pharmaceutical manufacturing sector.

2. Materials and Methods

2.1. Chemicals and Reagents

Reference standards of Montelukast Sodium and Desloratadine (purity >99%) were used for the analysis. HPLC-grade acetonitrile and tetrahydrofuran were procured from Astral Sciences. Analytical-grade potassium dihydrogen phosphate and disodium hydrogen phosphate were obtained from CDH Chemicals Pvt Ltd. High-purity deionized water, generated via an in-house purification system, was utilized for all aqueous buffer and sample preparations. All other chemicals used were of standard analytical grade.

2.2. Instrumentation

Chromatographic analysis was executed using a Shimadzu Prominence (v) HPLC system (Japan) equipped with a 20 ACHT autosampler and a 20 ATCT UV/PDA detector. The separation was achieved on a reverse-phase ODS C18 analytical column (150 mm × 4.6 mm, 5 μm particle size). Data acquisition, peak integration, and system control were managed using LC software solution. Auxiliary equipment included a high-precision electronic analytical balance (Sams model) for gravimetric measurements and a HICON ultrasonic bath for sample dissolution and mobile phase degassing.

2.3. Chromatographic Conditions

Chromatographic separation of Montelukast Sodium and Desloratadine was achieved under isocratic elution utilizing a reverse-phase C18 analytical column (150 mm × 4.6 mm, 5 μm) (Snyder et al., 2011). The optimized mobile phase consisted of a binary mixture of acetonitrile and phosphate buffer (50:50, v/v) (Rathore et al., 2010). The buffer was prepared using potassium dihydrogen phosphate and disodium hydrogen phosphate, with the pH precisely adjusted to 3.5 using dilute orthophosphoric acid to ensure optimal ionization and peak symmetry of both APIs (Ghonim et al., 2024). The mobile phase was delivered at a constant flow rate of 1.0 mL per minute, and the column oven temperature was maintained at 25 degrees Celsius (ambient). The injection volume was standardized at 10 μL. The eluents were monitored using a PDA detector set at an optimized isosbestic wavelength (for example, 230 nm) to facilitate the simultaneous detection of both drugs without compromising sensitivity (Snyder et al., 2011).

2.4. Preparation of Solutions

2.4.1. Mobile Phase and Diluent

The phosphate buffer (pH 3.5) was filtered through a 0.45 μm PTFE membrane filter and ultrasonicated for 15 minutes prior to mixing with HPLC-grade acetonitrile to remove particulate matter and prevent column clogging (Snyder et al., 2011). The final mobile phase was re-sonicated to ensure complete degassing, which is critical for maintaining baseline stability and preventing pump cavitation (Vekaria et al., 2013). The same mobile phase composition (Acetonitrile:Buffer, 50:50, v/v) was utilized as the diluent for all sample and standard preparations to minimize solvent front disturbances and ensure analyte stability (Snyder et al., 2011).

2.4.2. Standard Solutions

A primary stock solution containing both APIs was prepared by accurately weighing and transferring reference standards of Montelukast Sodium and

Desloratadine into a volumetric flask (Rathore et al., 2010). The APIs were dissolved in the diluent and sonicated for 10 minutes to ensure complete homogenization. Working standard solutions within the targeted calibration range were prepared via serial dilution of the primary stock solution using the mobile phase (Vekaria et al., 2013).

2.4.3. Nasal Sample Preparation

An accurately measured aliquot of the intranasal drug delivery formulation, equivalent to the therapeutic dose of both APIs, was transferred into a volumetric flask. The sample was dispersed in the diluent, subjected to mechanical vortexing, and ultrasonicated for 15 minutes to facilitate complete extraction of the APIs from the complex nasal excipient matrix. The resulting suspension was filtered through a 0.45 um nylon syringe filter to eliminate insoluble polymers and preservatives before injection into the HPLC system(Vekaria et al., 2013).

2.5. Method Validation

The developed analytical method was subjected to comprehensive validation in strict accordance with the International Council for Harmonisation ICH Q2(R1) guidelines.

System Suitability: Evaluated by injecting the standard mixture six consecutive times. Parameters including theoretical plate count (N greater than 2000), tailing factor (T less than or equal to 2), and retention time repeatability (percent RSD less than or equal to 2.0 percent) were assessed(Rathore et al., 2010).

Linearity and Range: Calibration curves were constructed by analyzing mixed standard solutions at seven concentration levels ranging from 20 percent to 150 percent of the target assay concentration. Linear regression analysis was applied to determine the correlation coefficient R squared, slope, and y-intercept(Vekaria et al., 2013).

Accuracy (Recovery): The trueness of the method was established via standard addition. Known quantities of Montelukast and Desloratadine reference standards were spiked into a pre-analyzed placebo matrix at three distinct levels (50 percent, 100 percent, and 150 percent), and the percentage recovery was calculated.

Precision: Method precision was determined by evaluating repeatability (intra-day precision) and intermediate precision (inter-day precision). Six independent sample preparations were analyzed on the same day and across consecutive days, with precision expressed as percent RSD.

Specificity: Blank diluent and placebo solutions containing all formulation excipients (for example, mucoadhesives and preservatives) were injected to verify the absence of co-eluting peaks at the specific retention times of Montelukast and Desloratadine (ICH, 2005).

LOD and LOQ: The Limit of Detection and Limit of Quantitation were computed mathematically based on the standard deviation of the response and the slope of the calibration curve, using the formulas:

LOD = 3.3 x (sigma / S)
Eq. 1

LOQ = 10 x (sigma / S)
Eq. 2

Robustness: The reliability of the method under deliberate minor variations was tested. System suitability was re-evaluated after altering the mobile phase flow rate by plus or minus 10 percent, pH by plus or minus 1 unit, and column temperature by plus or minus 5 degrees Celsius (ICH, 2005).

3. Results and Discussion

3.1. Method Development and Optimization

The primary objective of the chromatographic method development was to achieve complete baseline separation of Montelukast Sodium and Desloratadine with symmetrical peaks and minimal runtime. Various mobile phase compositions were evaluated. The optimized mobile phase, consisting of acetonitrile and phosphate buffer (50:50, v/v) adjusted to pH 3.5, yielded the most efficient resolution. The acidic pH was critical in suppressing the ionization of the analytes, thereby minimizing peak tailing. Under these optimized isocratic conditions at a flow rate of 1.0 mL per minute, the analytes were well resolved. Desloratadine eluted first at approximately 3.4 minutes, followed by the more lipophilic Montelukast Sodium at approximately 6.8 minutes. The total chromatographic runtime was kept under 10 minutes, making the method highly suitable for high-throughput analysis (Figure 1 and Figure 2).

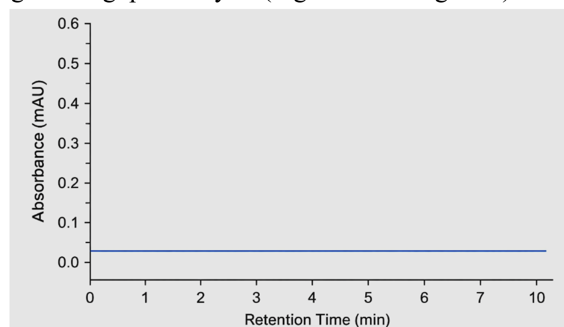

Figure 1: Blank Chromatogram of Diluent (Mobile Phase) Showing No Interfering Peaks.

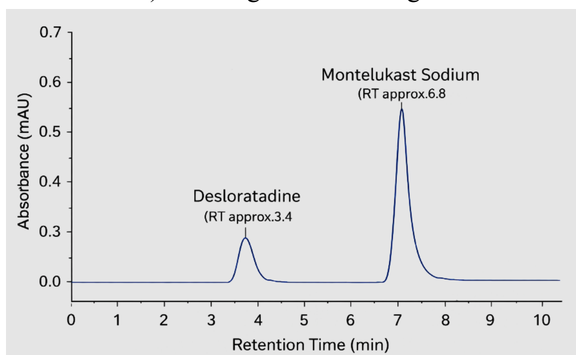

Figure 2: Optimized RP-HPLC chromatogram of a standard mixture containing Desloratadine (RT approximately 3.4 min) and Montelukast Sodium (RT approximately 6.8 min) measured at 230 nm.

3.2. System Suitability

System suitability parameters were evaluated to ensure that the complete analytical system was robust prior to validation. Six replicate injections of the standard mixture were analyzed. All system suitability parameters comfortably met the stringent acceptance criteria specified by the ICH Q2(R1) guidelines, confirming excellent column efficiency and peak symmetry. The consolidated results are presented in Table 1.

Table 1: System suitability parameters for the simultaneous estimation of Desloratadine and Montelukast Sodium (n = 6)

Parameter	Desloratadine	Montelukast Sodium	Acceptance Criteria
Retention Time (min)	3.42 ± 0.02	6.81 ± 0.03	RSD ≤ 1.0%
Theoretical Plates (N)	4520 ± 110	5180 ± 145	N > 2000
Tailing Factor (T)	1.12 ± 0.04	1.15 ± 0.05	T ≤ 2.0
Resolution (Rs)	-	5.8	Rs > 2.0
Percent RSD (Peak Area)	0.65%	0.82%	≤ 2.0%

3.3. Linearity and Range

The linearity of the developed analytical method was established by evaluating standard solutions at seven distinct concentration levels, spanning 20 percent to 150 percent of the target assay concentration for both active pharmaceutical ingredients. The calibration ranges were established as 5.0 to 37.5 ug per mL for Desloratadine and 10.0 to 75.0 ug per mL for Montelukast Sodium. Each concentration level was injected in triplicate, and the average peak areas were plotted against their respective concentrations to construct the calibration curves. Linear regression analysis was applied to the data using the least-squares method. The results demonstrated a highly significant linear relationship for both drugs, evidenced by correlation coefficients R squared exceeding 0.999. The detailed regression parameters, including the slope and y-intercept, are summarized in Table 2.

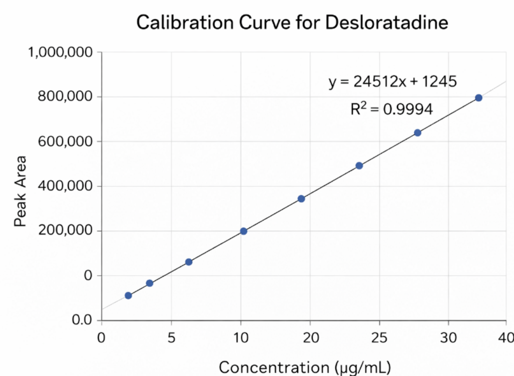

Figure 3: Calibration Curve for Desloratadine Showing Linear Relationship Between Concentration and Peak Area.

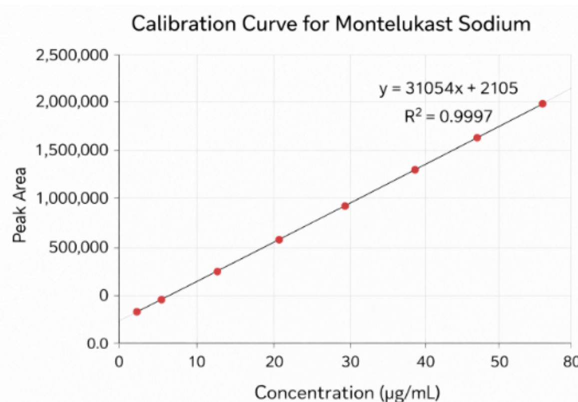

Figure 4: Calibration Curve for Montelukast Sodium.

Table 2: Linear regression data for Desloratadine and Montelukast Sodium

Parameter	Desloratadine	Montelukast Sodium

Linearity Range (ug/mL)	5.0 to 37.5	10.0 to 75.0
Correlation Coefficient (R^2)	0.9994	0.9997
Slope (m)	24512	31054
Y-intercept (c)	1245	2105
Regression Equation	y = 24512x + 1245	y = 31054x + 2105

3.4. Accuracy (Recovery Studies)

The trueness of the proposed method was determined through standard addition recovery studies. Known quantities of Desloratadine and Montelukast Sodium reference standards were spiked into a pre-analyzed placebo matrix containing the nasal formulation excipients at three concentration levels: 50 percent, 100 percent, and 150 percent of the nominal target concentration. Each spiked level was prepared and analyzed in triplicate. The percentage recovery was calculated by comparing the analytically determined concentration with the theoretical spiked concentration. The mean recoveries for both drugs ranged tightly between 99.2 percent and 101.5 percent, with the relative standard deviation percent RSD remaining well below 2.0 percent across all levels. These results, detailed in Table 3, confirm the method's high degree of accuracy and establish the absence of significant interference from the nasal delivery excipients.

Table 3: Accuracy and recovery data for the simultaneous estimation of Desloratadine and Montelukast Sodium (n = 3)

Analyte	Spike Level (%)	Amount Added (ug/mL)	Amount Recovered (ug/mL)	Mean Recovery (%)	% RSD
Desloratadine	50	12.50	12.42	99.36	0.85
Desloratadine	100	25.00	25.15	100.60	0.62
Desloratadine	150	37.50	37.82	100.85	0.45
Montelukast Sodium	50	25.00	24.88	99.52	0.78
Montelukast Sodium	100	50.00	50.45	100.90	0.54
Montelukast Sodium	150	75.00	74.80	99.73	0.60

3.5. Precision

Method precision was evaluated in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision). For intra-day precision, six independent replicates of the standard mixture at the 100 percent test concentration were analyzed on the same day under identical conditions. Inter-day precision was assessed by analyzing the same concentration levels across three consecutive days. The precision was expressed as percentage relative standard deviation (percent RSD) of the assay results. As summarized in Table 4, the percent RSD values for both intra-day and inter-day precision were strictly below the ICH acceptance limit of 2.0 percent, confirming the excellent repeatability and reliability of the developed method.

Table 4: Intra-day and Inter-day precision data for Desloratadine and Montelukast Sodium (n = 6)

Analyte	Concentration (ug/mL)	Intra-day Precision (%RSD)	Inter-day Precision (%RSD)	Acceptance Criteria
Desloratadine	25.00	0.82	1.15	≤ 2.0%
Montelukast Sodium	50.00	0.65	0.98	≤ 2.0%

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

The sensitivity of the analytical method was mathematically determined based on the standard deviation of the y-intercepts of the regression lines and the slope of the calibration curves. The calculated LOD values were 0.15 ug per mL for Desloratadine and 0.35 ug per mL for Montelukast Sodium. The LOQ values were established at 0.45 ug per mL and 1.05 ug per mL, respectively. These inherently low values demonstrate that the method possesses the requisite sensitivity to detect and quantify trace concentrations of both active pharmaceutical ingredients without interference from the nasal matrix.

3.7. Robustness

The robustness of the analytical method was challenged by deliberately inducing minor, controlled variations in the optimized chromatographic conditions. The altered parameters included the mobile phase flow rate (plus or minus 0.1 mL per minute), buffer pH (plus or minus 0.2 units), and column oven temperature (plus or minus 2 degrees Celsius). System suitability parameters, including theoretical plate count and tailing factor, were re-evaluated under these varied conditions. The chromatographic resolution between Desloratadine and Montelukast Sodium remained greater than 2.0 throughout all modifications. As detailed in Table 5, the percent RSD of the peak areas under these deliberately varied conditions was consistently less than or equal to 1.5 percent, affirming that the method remains robust and unaffected by typical day-to-day operational fluctuations.

Table 5: Robustness evaluation of the RP-HPLC method under deliberately altered conditions (n = 3)

Parameter Altered	Variation	Desloratadine (%RSD)	Montelukast Sodium (%RSD)
Flow Rate	0.9 mL/min	0.95	0.88
Flow Rate	1.1 mL/min	1.02	0.91
Mobile Phase pH	3.3	1.10	1.05
Mobile Phase pH	3.7	0.85	0.76
Column Temperature	23 °C	0.92	0.85
Column Temperature	27 °C	0.78	0.82

4. Conclusion

In this study, a novel, highly sensitive, and rapid reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed and thoroughly validated for the simultaneous quantitative estimation of Montelukast Sodium and Desloratadine in a complex intranasal drug delivery system. The optimized isocratic chromatographic conditions facilitated complete baseline separation of both active pharmaceutical ingredients with excellent peak symmetry within a short runtime of less than 10 minutes. Comprehensive analytical validation, executed in strict accordance with ICH Q2(R1) guidelines, confirmed that the method exhibits exceptional linearity (R squared greater than 0.999), outstanding precision (intra-day and inter-day percent RSD less than 2.0 percent), and high accuracy (recovery rates between 99.2 percent and 100.9 percent) across the established concentration range. Furthermore, the mathematically determined low LOD and LOQ values underscore the method's strong sensitivity, while deliberate variations in analytical parameters demonstrated its operational robustness. The complete absence of chromatographic interference from the nasal formulation excipients establishes the method’s strict specificity. Consequently, this robust, reproducible, and time-efficient analytical protocol is highly suitable for routine quality control analysis, batch release testing, and stability monitoring of intranasal formulations containing Montelukast Sodium and Desloratadine in the pharmaceutical manufacturing sector.

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