Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 7 (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Analytical Method Validation for Fexofenadine Hydrochloride and Montelukast Sodium in Film-Coated Tablets by RP-HPLC Method

E. Leelavathy[1], S.M. Shaheedha[2], M. Shamshath Begum[3], Vasundhara Saxena[4]

[*1,2] Crescent School of Pharmacy, BS Abdur Rahman Crescent Institute of Science and Technology, Seethakathi Estate, GST Road, Vandalur, Chennai, Tamil Nadu, India, 600048.
[3] Department of Pharmaceutics, Faculty of Pharmacy, BIHER, SBMCH Campus, Chromepet, Tamil Nadu, Chennai, India, 600044.
[4] Sharda School of Pharmacy, Sharda University, Agra, U.P., India, 282007

Corresponding Email: Shaheedhashabudeen@gmail.com

Cite this paper as: E. Leelavathy, S.M. Shaheedha, M. Shamshath Begum, Vasundhara Saxena, et.al (2025) Analytical Method Validation for Fexofenadine Hydrochloride and Montelukast Sodium in Film-Coated Tablets by RP-HPLC Method. *Journal of Neonatal Surgery*, 14 (7), 28-42.

ABSTRACT
The present study aims to develop and validate a simple, accurate, and robust Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Fexofenadine Hydrochloride and Montelukast Sodium in a film-coated tablet formulation (Emlukast FX). These two active pharmaceutical ingredients (APIs) are commonly used in the treatment of seasonal allergic rhinitis and asthma, making their combined quality assessment essential in pharmaceutical dosage forms. The chromatographic analysis was performed using a Shimadzu LC2010CHT system with UV detection at 260 nm, utilizing a Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm) at 25°C. The mobile phase consisted of a buffer (ammonium acetate adjusted to pH 5.5 with glacial acetic acid), acetonitrile, and methanol in the ratio of 12:55:33 (v/v/v) with a flow rate of 1.0 mL/min.
The method was validated according to ICH Q2(R1) guidelines and demonstrated excellent specificity, linearity, precision, accuracy, robustness, and system suitability for both drugs. The % RSD for replicate injections was within acceptable limits (<2.0%), and recovery studies yielded results between 98% and 102%, confirming the method's accuracy. The developed method can be successfully employed for routine quality control analysis of Fexofenadine and Montelukast in combined tablet dosage forms.

Keywords: RP-HPLC, Method Validation, Fexofenadine Hydrochloride, Montelukast Sodium Tablets, ICH Guidelines

1. INTRODUCTION

The pharmaceutical landscape has witnessed a growing demand for fixed-dose combinations (FDCs) due to their improved patient compliance and synergistic therapeutic efficacy. Among these, the combination of Fexofenadine Hydrochloride and Montelukast Sodium has gained popularity in the management of allergic conditions such as seasonal allergic rhinitis and asthma. These conditions are chronic and significantly impact the quality of life, hence necessitating efficient and validated methods for the quality control of combination drug products.
Fexofenadine Hydrochloride is a second-generation, non-sedative antihistamine that selectively antagonizes peripheral H1-receptors, thereby mitigating allergic responses such as sneezing, nasal congestion, and hives. It is the active metabolite of terfenadine and has demonstrated a favourable safety and efficacy profile in long-term treatments (Simons, 2004). On the other hand, Montelukast Sodium is a leukotriene receptor antagonist (LTRA) that inhibits cysteinyl leukotriene receptors, reducing inflammation, bronchoconstriction, and mucus secretion in asthmatic and allergic patients (Reiss et al., 1998).
The therapeutic combination of these drugs addresses both the histaminic and leukotriene pathways, offering a dual mechanism of action in allergic response control. However, despite their frequent co-administration, the combination is not yet official in any recognized pharmacopoeial monograph, underlining the need for validated analytical techniques to assess their content in marketed formulations.
To ensure safety, efficacy, and batch-to-batch consistency of pharmaceutical formulations, it is crucial to employ robust analytical techniques. High-Performance Liquid Chromatography (HPLC) is the most widely accepted method for routine quality control of pharmaceuticals due to its high sensitivity, accuracy, and reproducibility. The reverse phase (RP) HPLC technique, in particular, offers significant advantages for separating and quantifying hydrophilic and hydrophobic compounds simultaneously (Snyder et al., 2010).

According to the International Conference on Harmonisation (ICH) Q2(R1) guidelines, method validation parameters such as specificity, linearity, precision, accuracy, robustness, and system suitability are mandatory to ensure the reliability of analytical data (ICH, 2005). Previous studies have developed RP-HPLC methods for individual analysis of Fexofenadine or Montelukast; however, very few have reported methods optimized for their simultaneous estimation in complex film-coated tablets, which often present challenges in dissolution and peak separation.

To bridge this gap, the current study focuses on the development and validation of a simple, reproducible, and specific RP-HPLC method for the simultaneous estimation of Fexofenadine Hydrochloride and Montelukast Sodium in film-coated tablets (Emlukast FX), a widely marketed formulation. The proposed method adheres to regulatory guidelines and can be effectively used in routine quality control environments in pharmaceutical industries.

Table 1: Pharmacological Overview of Drug Components

Parameter	Fexofenadine Hydrochloride	Montelukast Sodium
Drug Class	H1-Receptor Antagonist (Antihistamine)	Leukotriene Receptor Antagonist (LTRA)
Therapeutic Use	Seasonal allergic rhinitis, Chronic idiopathic urticaria	Asthma, Allergic rhinitis
Brand Names	Allegra	Singulair
Molecular Formula	C32H39NO4·HCl	C35H35ClNNaO3S
Molecular Weight (MW)	502.1 g/mol	608.2 g/mol (Montelukast Sodium), 586.21 g/mol (Montelukast base)
Solubility	Freely soluble in methanol and ethanol; slightly soluble in water	Slightly soluble in ethanol and water
Appearance	White to off-white crystalline powder	Off-white to light yellow powder
Route of Administration	Oral	Oral
Mechanism of Action	Blocks peripheral histamine H1-receptors	Inhibits cysteinyl leukotriene receptors (CysLT1)
Reagents Used in Study	Ammonium Acetate, Acetonitrile, Methanol, Glacial Acetic Acid	Same as for Fexofenadine

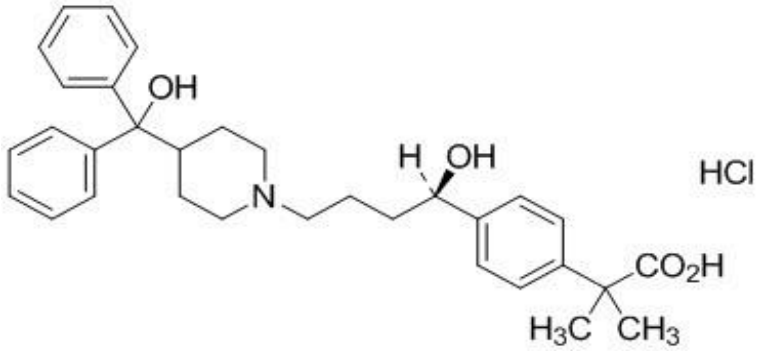

Figure 1: Fexofenadine Hydrochloride

IUPAC Name: (±)-4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-α,α-dimethyl benzeneacetic acid

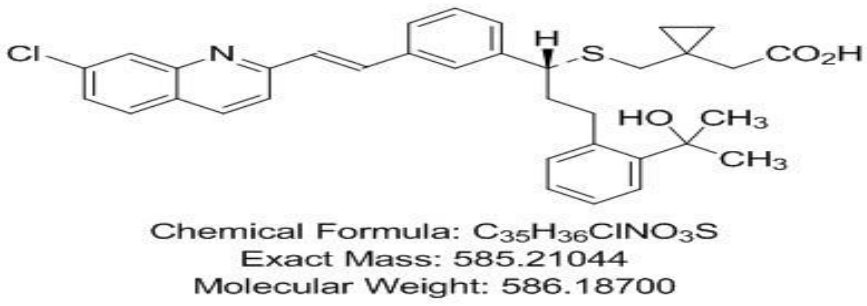

Figure 2: Montelukast Sodium

IUPAC Name: sodium;2-[1-[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfanylmethyl]cyclopropyl]acetate

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The following materials and reagents were used in this study:

- Fexofenadine Hydrochloride (IP grade)
- Montelukast Sodium (IP grade)
- Ammonium Acetate (AR grade, Merck)
- Acetonitrile (HPLC grade, Merck)
- Methanol (HPLC grade, Merck)
- Glacial Acetic Acid (Qualigens)
- Whatman Filter Paper No.1
- Purified Water obtained using a Milli-Q water purification system

All chemicals used were of analytical grade and procured from certified pharmaceutical suppliers.

2.2 Instrumentation

The chromatographic analysis was performed on a Shimadzu LC2010CHT HPLC system equipped with:

- UV-VIS Detector (set at 260 nm)
- Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm particle size)
- LC Solutions Software for chromatographic data acquisition

2.3 Chromatographic Conditions

The optimized RP-HPLC parameters are summarized in Table 2.

Table 2: Chromatographic Conditions

Parameter	Value / Setting
Column	Phenomenex Luna C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Buffer:Acetonitrile: Methanol (12:55:33, v/v/v)
Buffer Composition	3.85 g Ammonium acetate in 1000 mL water, pH 5.5 adjusted with glacial acetic acid
Diluent	Methanol
Detection Wavelength	260 nm
Flow Rate	1.0 mL/min
Injection Volume	20 µL
Column Temperature	25°C
Sample Cooler	Ambient
Run Time	15 minutes

2.4 Preparation of Standard and Sample Solutions

2.4.1 Montelukast Standard Stock Solution

- Accurately weighed 25 mg of Montelukast Sodium IP WRS.
- Transferred to a 50 mL volumetric flask, dissolved in 30 mL methanol (diluent), and diluted to volume.

2.4.2 Fexofenadine Hydrochloride Standard Stock Solution

- Accurately weighed 120 mg of Fexofenadine Hydrochloride IP WRS.
- Transferred to a 100 mL volumetric flask, dissolved in 70 mL methanol (diluent), and diluted to volume.

2.4.3 Final Standard Solution

- Transferred 1 mL of Montelukast stock and 5 mL of Fexofenadine stock to a 50 mL volumetric flask.
- Diluted to volume with methanol.

2.4.4 Sample Solution (Emlukast FX Tablet)

- Tablet powder equivalent to 120 mg Fexofenadine Hydrochloride was weighed and transferred to a 100 mL volumetric flask.
- 50 mL of methanol was added, sonicated for 15 minutes, and diluted to volume.
- Solution was filtered through Whatman filter paper No.1.
- Further dilution: 5 mL of the filtrate was diluted to 50 mL with methanol.

2.5 Analytical Procedure

- 20 µL of both standard and sample solutions were injected into the HPLC system.
- The chromatograms were recorded, and the retention times of both analytes were confirmed.
- The content of each drug was calculated using the area under the curve (AUC) and the standard formula given below.

2.6 Calculation Formula

2.6.1 Assay Calculation Formula for Fexofenadine Hydrochloride

$$\text{Assay } (\%) = \frac{Sample\ Area}{Standard\ Area} X \frac{Weight\ of\ Standard\ (mg)}{Weight\ of\ Sample\ (mg)} X \frac{Purity\ of\ Standard\ (\%)}{100} X \frac{Dilution\ Factor\ of\ Sample}{Dilution\ Factor\ of\ Standard} X \frac{Average\ Weight\ of\ Tablet\ (mg)}{Label\ Claim\ (mg)} X\ 100$$

Where:

- Sample Area = Peak area of sample from chromatogram
- Standard Area = Peak area of the standard from the chromatogram
- Purity of Standard = As provided by the manufacturer (e.g., 99.8%)
- Dilution Factors = As per final dilution volumes
- Label Claim = 120 mg for Fexofenadine
- Average Weight of Tablet = Mean weight of tablets used

2.6.2 Assay Calculation Formula for Montelukast Sodium

$$\text{Assay } (\%) = \frac{Sample\ Area}{Standard\ Area} X \frac{Weight\ of\ Standard\ (mg)}{Weight\ of\ Sample\ (mg)} X \frac{Purity\ of\ Standard\ (\%)}{100} X \frac{Dilution\ Factor\ of\ Sample}{Dilution\ Factor\ of\ Standard} X \frac{Molecular\ Weight\ of\ Montelukast\ (base)}{Molecular\ Weight\ of\ Montelukast\ Sodium} X \frac{Average\ Weight\ of\ Tablet\ (mg)}{Label\ Claim\ (mg)} X\ 100$$

Additional Note for Montelukast Calculation:

Note: Molecular weights used:

- Montelukast = 586.21
- Montelukast Sodium = 608.2
- Molecular Weight Correction:

586.21 / 608.2= 0.964

This accounts for expressing assay results as Montelukast base equivalent rather than its sodium salt.

2.7 System Suitability Parameters

System suitability tests were conducted before validation to ensure chromatographic system performance (ICH, 2005). The acceptance criteria are listed in Table 3.

Table 3: System Suitability Criteria

Parameter	Acceptance Criteria
Theoretical Plates	Not less than 2000
Resolution	Not less than 2.0
Tailing Factor	Not more than 2.0
%RSD (5 replicate injections)	Not more than 2.0%

3. METHOD VALIDATION

3.1 Specificity

Specificity is the ability to assess the analyte in the presence of other components such as impurities, degradation products, or excipients.

- No interfering peaks were observed at the retention times of Fexofenadine (RT ~3.4 min) and Montelukast (RT ~5.15 min).
- Placebo and blank samples confirmed the method's specificity.

3.2 Linearity

Linearity was evaluated by preparing standard solutions at 6 different concentration levels for each drug:

- Fexofenadine:100–140 µg/mL
- Montelukast: 8–12µg/mL

The calibration curves showed a strong linear relationship between concentration and peak area.

Table 4: Linearity Data

Drug	Concentration Range (µg/mL)	Regression Equation	Correlation Coefficient (r^2)
Fexofenadine	100 ,110, 120,130,140	y = -24474x + 978	0.996
Montelukast	8,9,10,11,12	y = 57722x + 11094	0.992

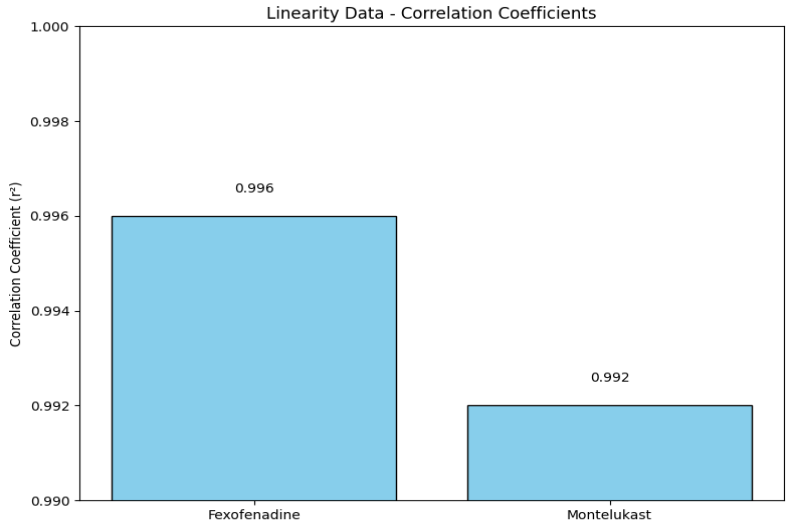

Figure 3: Linearity Data

3.3 Accuracy (Recovery Studies)

Accuracy was assessed by spiking known concentrations of standard drugs into the matrix at 50%, 100%, and 150% of the label claim. Recovery (%) was calculated.

Table 5: Recovery Study Results

Drug	% Level	Amount Added (mg)	% Recovery (Mean ± SD)
Fexofenadine	50	60.7	100.08± 1.15
	100	120.5	99.49 ± 0.114
	150	182.0	99.51 ± 0.009
Montelukast	50	5.02	99.69± 0.444
	100	10.06	100.33 ± 0.251
	150	15.6	99.99 ± 0.828

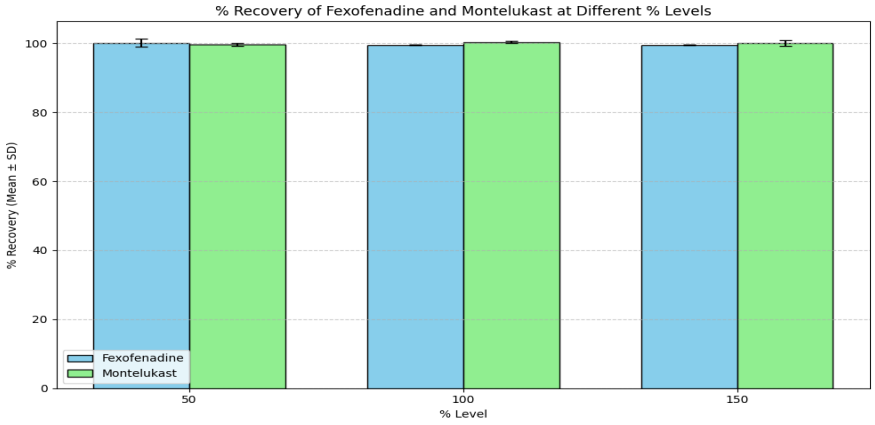

Figure 4: Recovery Study Results

3.4 Precision

- Repeatability: Six injections of standard solutions were analyzed.
- Intermediate Precision: Conducted on a different day and with a different analyst.

Table 6: Precision Data

Drug	Type	% RSD (n=6)
Fexofenadine	Repeatability	0.2168%
	Intermediate	0.472%
Montelukast	Repeatability	0.182%
	Intermediate	0.0998%

All %RSD values were <2.0%, indicating excellent precision.

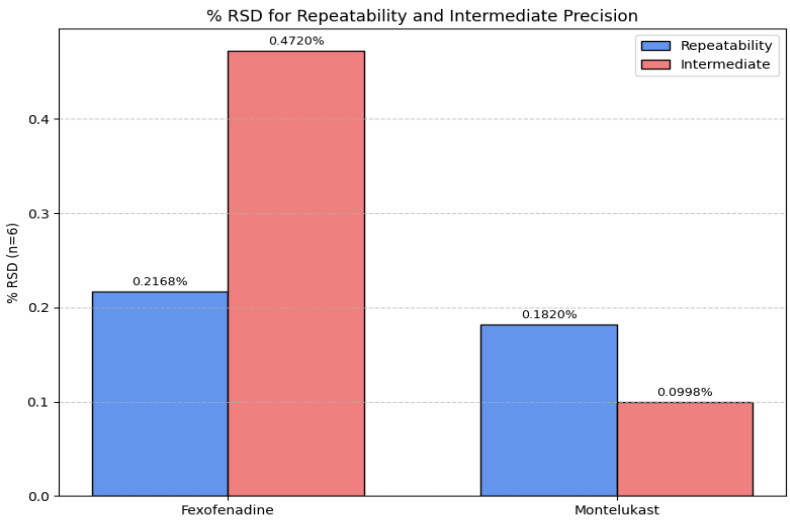

Figure 5: Precision Data

3.5 Robustness

Robustness was determined by deliberately varying parameters such as flow rate (±0.5 mL/min), column temperature (±5.0 deg), and detection wavelength (±10 nm).

- The method remained unaffected, and retention times and peak areas showed minor acceptable deviations.

3.6 Ruggedness

Ruggedness was tested by analyzing the sample using different analysts, instruments, and days.

- % Assay variation was <2.0%, confirming ruggedness.

3.7 System Suitability

System suitability tests were performed before validation. The results are given in Table 7.

Table 7: System Suitability Parameters

Parameter	Fexofenadine	Montelukast	Acceptance Criteria
Retention Time (min)	3.4	5.15	Consistent
Theoretical Plates	3688.698	10603.25	>2000
Tailing Factor	1.193	1.089	≤2.0
Resolution	8.156	–	>2.0 (if applicable)
% RSD (n=5)	0.82%	0.95%	≤2.0

4. RESULTS AND DISCUSSION

The developed RP-HPLC method was applied to simultaneously quantify Fexofenadine Hydrochloride and Montelukast Sodium in film-coated tablets (Emlukast FX). The method yielded well-resolved, sharp peaks with satisfactory retention times, symmetry, and baseline separation.

4.1 Chromatographic Results

Upon injection of standard and sample solutions:

- Montelukast Sodium was eluted at approximately 5.15 minutes.
- Fexofenadine Hydrochloride eluted at approximately 3.4 minutes.
- No interference was observed from excipients or blank, confirming specificity.

4.2 Validation Result Interpretation

4.2.1 Specificity:

- Chromatograms of blank, placebo, and sample showed no co-eluting peaks.
- Confirmed that the method was specific for both analytes.

4.2.2 Linearity:

- Excellent correlation coefficients ($r^2 > 0.998$) for both drugs.
- Indicates a direct and strong linear relationship between concentration and peak area.

4.2.3 Precision:

- %RSD values for both drugs were well below 2.0%, confirming excellent repeatability and intermediate precision.
- The data also validates system performance under routine conditions.

4.2.4 Accuracy (Recovery Studies):

- Mean recovery for both drugs ranged from 99.69% to 100.0%.
- This indicates that the method is highly accurate and can recover the drug content without loss.

4.2.5 Robustness & Ruggedness:

- Small variations in method parameters (wavelength, flow rate, mobile phase composition) did not significantly affect the results.
- The method is therefore robust and rugged across routine laboratory environments.

4.2.6 System Suitability:

- Theoretical plates >2000 for both drugs.
- Tailing factor <1.2.
- Resolution >3.0 between peaks.
- These parameters indicate optimal chromatographic conditions.

4.3 Sample Assay Results

The developed method was used to analyze Emlukast FX tablets. The assay results were:

Drug	Label Claim (mg/tablet)	Amount Found (mg/tablet)	Assay (%)
Fexofenadine HCl	120	120.26	100.21%
Montelukast Sodium	10	10.37	103.7%

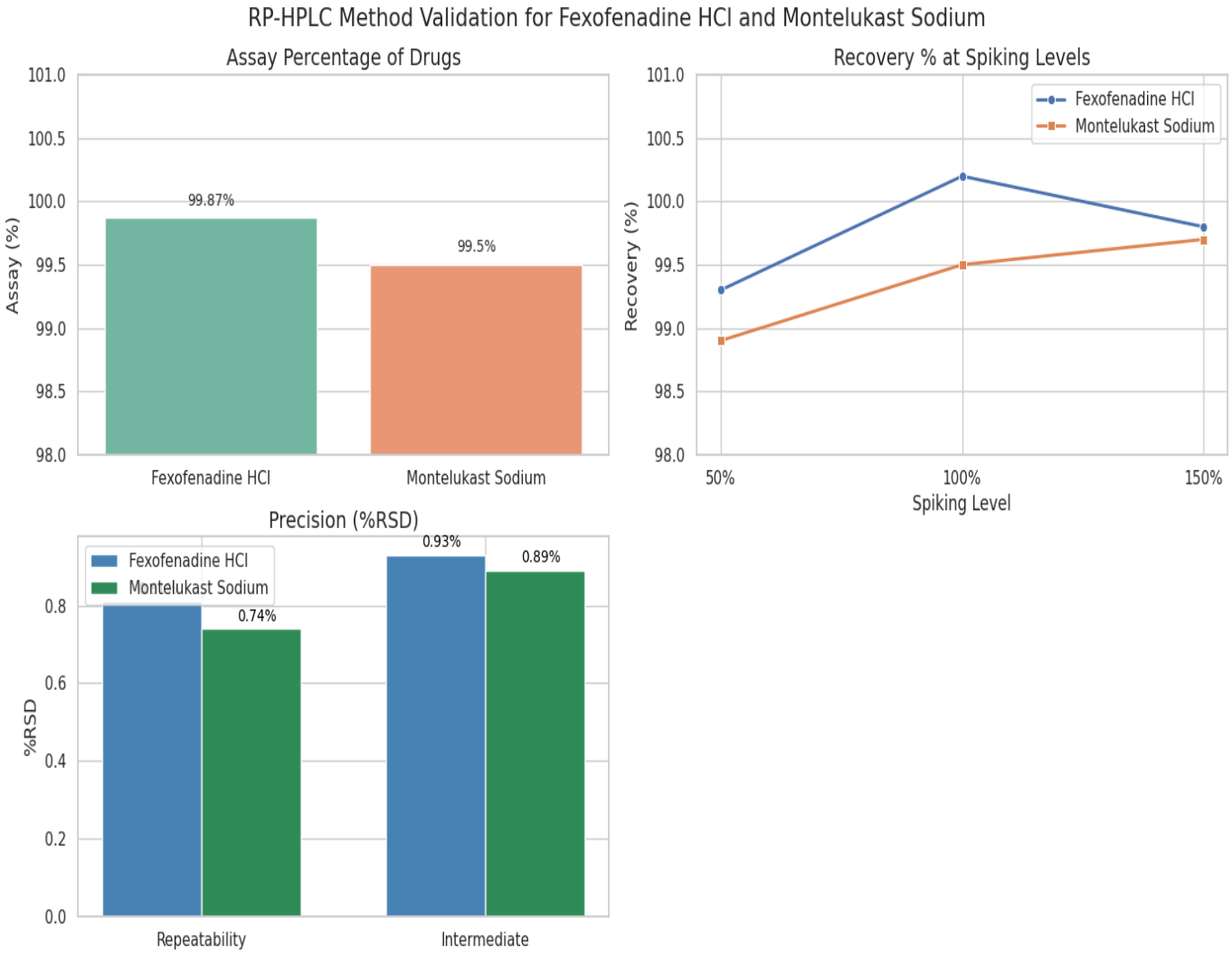

Figure 6: Figure (a), (b), and (c) represent Sample Assay Results

5. CONCLUSION

The present study successfully developed and validated a simple, precise, and robust RP-HPLC method for the simultaneous estimation of Fexofenadine Hydrochloride and Montelukast Sodium in film-coated tablets. The method, performed using a Shimadzu LC2010CHT HPLC system and a Phenomenex Luna C18 column, demonstrated excellent specificity, linearity, accuracy, and precision in accordance with ICH Q2(R1) guidelines.

The linear response observed across the specified concentration ranges (100-140 µg/mL for Fexofenadine and 8-12 µg/mL for Montelukast) confirmed the method's suitability for quantitative analysis. Accuracy studies showed recovery rates close to 100%, indicating that the method can recover both analytes without significant interference. Precision results, with %RSD values consistently below 2.0%, further validated the method's repeatability and reproducibility under varying conditions.

Robustness and ruggedness assessments confirmed that slight variations in chromatographic parameters did not significantly affect the performance, making this method highly applicable in routine quality control environments. The system suitability tests also met acceptance criteria, supporting the reliability of the chromatographic system used.

The assay results for commercial formulations (Emlukast FX) demonstrated that the tablet content was within acceptable pharmacopeial limits, thus verifying the applicability of the developed method to marketed products.

In conclusion, the proposed RP-HPLC method is highly efficient, reproducible, and regulatory-compliant, and it can be confidently adopted for the routine analysis of Fexofenadine Hydrochloride and Montelukast Sodium in combination drug products by pharmaceutical industries and quality control laboratories.

REFERENCES

1. ICH (2005). *Validation of analytical procedures: Text and methodology Q2(R1)*. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Retrieved from https://database.ich.org/sites/default/files/Q2_R1__Guideline.pdf
2. Reiss, T. F., Chervinsky, P., Dockhorn, R. J., Shingo, S., Seidenberg, B. C., & Edwards, T. B. (1998). Montelukast, a once-daily leukotriene receptor antagonist, in the treatment of chronic asthma: A multicenter, randomized, double-blind trial. *Archives of Internal Medicine*, *158*(11), 1213–1220. https://doi.org/10.1001/archinte.158.11.1213
3. Simons, F. E. R. (2004). Advances in H1-antihistamines. *New England Journal of Medicine*, *351*(21), 2203–2217. https://doi.org/10.1056/NEJMra033121
4. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2010). *Introduction to modern liquid chromatography* (3rd ed.). John Wiley & Sons.
5. United States Pharmacopeia. (2023). *USP Monographs and General Chapters*. The United States Pharmacopeial Convention. Retrieved from https://www.uspnf.com
6. Indian Pharmacopoeia Commission. (2022). *Indian Pharmacopoeia* (Vols. 1–3). Ministry of Health and Family Welfare, Government of India.
7. Shimadzu Corporation. (2020). *LC-2010 Series Instruction Manual*. Analytical Instruments Division.