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Eco-Friendly and UV Spectrophotometric HPLC Methods for Multivariate Calibration Based Montelukast Sodium and Fexofenadine Estimation

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Abstract

A new green Reverse Phased-HPLC technique have been developed for the analysis of Montelukast sodium in both bulk and pharmaceutical formulations, using ethanol and sodium dihydrogen orthophosphate buffer as mobile phase in an 80:20 v/v ratio. The above method was developed and validated in accordance with ICH guidelines for linearity and range, sensitivity, recovery, precision, detection limit, quantification limit, and robustness. The results were found to be within the acceptance limits in accordance with ICH guidelines. This eco-friendly method reduces the usage of harmful organic solvents, making it a sustainable alternative in pharmaceutical analysis. Furthermore, by using the multilinear regression technique, for the assessment of Fexofenadine and Montelukast in their bulk pharmaceutical formulation, a novel, precise, and progressive spectrophotometric technique was created. The method was validated using various validation parameters and were found to be within the limits specified. This makes the method suitable for routine quality control analysis. It also demonstrates strong potential for application in stability studies and pharmacokinetic evaluations. Future research may focus on extending this green approach to other antihistaminic and anti-asthmatic drug combinations. This advancement highlights the growing importance of green analytical chemistry in modern pharmaceutical development, promoting safer, cleaner, and more sustainable laboratory practices globally.

Keywords: ICH Guideline, Multilinear Regression, Pharmaceutical Formulations, RP-HPLC, Validation.

Introduction

Some food sources can cause allergies such as hay fever, dust, or pet dander. These unfamiliar substances are known as 'allergens'. Cough is one of the main symptoms of sensitivity, and it persists as a reflex movement in the throat when an unknown growth or bodily fluid enters the respiratory system (1).

Montelukast sodium is 2-[1-(1R)-1-[3-[(E)] [phenyl] -2-(7-chloroquinolin-2yl) ethenylPhenyl-2(2hydroxypropan2yl) -3- sulfonylmethyl] propyl the acid cyclopropyl acetic (2). It is soluble in ethanol and methanol (3). Montelukast belongs to the class of monocarboxylic acids, aliphatic sulphides, and quinolines. It acts as an anti-arrhythmia, anti-asthmatic, and leukotriene antagonist. It is a conjugate acid of a montelukast. In 1998, the US FDA authorised montelukast for clinical use under the Merck brand, singular (4). The drug belongs to the class of medications known as leukotriene receptor antagonists (LTRA). Despite their potential for efficacy, LTRAs like montelukast are usually used in conjunction with or in addition to inhaled corticosteroids or other medications as part of asthma step therapy. Nevertheless, the FDA conducted studies in 2008 and 2009 to determine whether montelukast users would experience neuropsychiatric side effects such as agitation, hallucinations, suicidal thoughts and actions, and more.

Fexofenadine is 2-[4-[1-hydroxy-4-[4hydroxy (diphenyl) methyl] piperidin-1yl] butyl] phenyl]-1-methylpropanoic acid. It is Soluble in ethanol and methanol (5). It is H1 receptor converse agonist, second generation Anti- histamines. Binds with the H1 receptor and surrogates the release of mast cells, and interleukins that are responsible for allergic events. The side-effects are Headache, vomiting, cough, diarrhoea. To treat allergic conditions like running nose and watery eyes and to create a reliable green HPLC technique for assessing montelukast in API using ethanol as a mobile phase solvent (6). To create a green HPLC method to evaluate montelukast sodium in both forms (API and dosage) in accordance with ICH

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guidelines (7). Create a UV spectroscopic technique to quantify montelukast sodium in both formats (pure and dosage), Create a UV spectroscopic strategy to quantify fexofenadine in both existing forms (pure and dosage). Conventional analytical techniques such as HPLC and LC-MS, though precise and sensitive, often involve hazardous solvents and complex procedures, raising concerns about environmental impact and sustainability. Recent advancements in green analytical chemistry have introduced eco-friendly RP-HPLC methods using ethanol as a safer mobile phase alternative, aligning with principles of sustainability while maintaining analytical performance. Additionally, chemometric approaches, such as multilinear regression and partial least squares, have enabled simultaneous, accurate quantification of both drugs without the need for extensive separation, offering cost-effective and rapid alternatives. These developments highlight a growing shift toward greener and computationally driven methodologies in pharmaceutical analysis. A combination of two medications, specifically Fexofenadine and Montelukast functions by blocking the action of synthetic substances known as leukotriene binding sites, which are expelled from the lungs and cause irritation (enlargement), as well as the accumulation and expansion of bodily fluids in the airways. As a result, bodily fluid build-up and restriction are reduced (8). In addition to other allergic symptoms, it is used to treat runny and stuffy noses, airway blockages, sneezing, itching, and watery eyes. It works by preventing the production of the chemicals that trigger allergic reactions. Montelukast + Fexofenadine is available as a pill, syrup, suspension, and chewable tablet (9).

Several analytical methods have been developed and validated for the estimation of Montelukast and Fexofenadine across various matrices. Literature review revealed that one of the method validated Fexofenadine using RP-HPLC with an acetate buffer: ACN (50:50) mobile phase, reporting high linearity (R^2 = 0.999) over 31.5–500 µg/ml. Dipti *et al*., 2016 analyzed Montelukast using HPLC with methanol: OPA (10:90), achieving r^2 values of 0.9982 and 0.9979 in tablets and dosage forms, respectively. Another reported method applied UV spectrophotometry using first-order derivative and AUC methods, obtaining strong correlations (r^2 = 0.9998 and 0.9941), while another quantified Montelukast in rabbit plasma using NaH_2PO_4: ACN (20:80), with recoveries above 66.47%. A method used UV spectrophotometry at 287.3 nm for Montelukast with linearity between 2–100 µg/ml. A stability-indicating RP-HPLC method with acetate buffer:ACN (6.5:3.5), achieving RT of 3.08 min was also reported. Finally one method evaluated Fexofenadine via UV spectrophotometry at 220 nm, with ethanol as solvent and excellent recovery (~99–100%) (10–14).

This paper quantifies multivariate calibration for montelukast sodium in both formulation and pure dose forms (15). The estimation of fexofenadine in formulation and pure dose forms is done using a multivariate calibration. The methodical approach to figuring out the concentration of later models is being verified for usage in the research facility. The validation process must be followed when validating the developed method.

Materials and Methodology

Instruments/ Reagents and Chemicals

The instruments used include a Shimadzu UV-1800 UV-visible double beam spectrophotometer, a sonicator, a pH meter, an analytical balance, and Product No. G4294B: Agilent 1220 Infinity Gradient LC System, which is a high-performance liquid chromatography system that includes a double-channel gradient pump, a desegregated degassing chamber, an auto sampler, a column unit, and a detector (Diode array). By using Agilent Open LAB CDS Chem Station Edition and Agilent Open LAB CDS 3D UV Add-On Software the data was gathered and processed.

Montelukast sodium was purchased as a gift sample from Ephi Drugs, Pondicherry. The Fexofenadine was obtained as a gift sample from Softgel pvt. Ltd, Chennai. Solvents and Chemicals employed are Ethanol, Sodium dihydrogen phosphate, Distilled water, Methanol, Orthophosphoric acid.

HPLC Method Development and Validation for Evaluating Montelukast Sodium in Pure Form

Preparation of Stock Solution: Montelukast sodium was transferred into a 10 mL standard flask at a precise weight of 10 mg. To dissolve the drug, ethanol was used, and a concentration of 1000 µg/ml was obtained.

Preparation of Sodium Dihydrogen Phosphate Buffer 0.025M: Sodium dihydrogen phosphate buffer was prepared by adding 1.499g of drug into 500ml of HPLC grade H_2O. Initially, the pH was 4.7. Later on, it was adjusted to 3.7 by using 5% ortho-phosphoric acid.

Preparation of Working Standard Solution: The concentration of 100 µg/ml was achieved by diluting the stock solution.

Chromatographic Conditions: The chromatogr-aphic condition used for the intended method is summarised in Table 1.

Table 1: Chromatographic Conditions for the Development and Validation of Montelukast Sodium in Pure Form

Column	ZORBAX eclipse C18 by Agilent (4.6x150 mm, 5 µm)
Wavelength	254nm
Flow rate	1mL/min
Run time	6 min
Injection volume	5 µL
Mobile phase	0.025 sodium dihydrogen phosphate: Ethanol (20:80 v/v)

Assay of Active Pharmaceutical Ingredient: Montelukast sodium weighing exactly 100 mg was dispersed with ethanol in a 100 ml flask, and dilutions were made to reach a 100 µg/ml concentration.

Analytical Method Validation

Each validation parameter was performed in compliance with the ICH Q2 (R1) guidelines. The specifications carried out are referenced beneath;

System Suitability: To assess system suitability, a 100 µg/ml standard solution of Montelukast sodium was injected into the system in a volume of 5 µl. Table 2 Underneath results and discussion refers to data obtained for these parameters.

Specificity: It was assessed by comparing blank chromatograms to those of Montelukast sodium at 100 µg/ml.

Linearity: To evaluate the drug's linearity report, a concentration ranging from 50 to 150 µg/ml was chosen. The obtained chromatographic details were subjected to least square regression analysis. Linearity was established by drawing a plot of Montelukast sodium concentration vs peak area.

Limit of Detection (LOD): The LOD was calculated by the formula referenced beneath;

Limit of Detection = $3.3 \times \frac{\sigma}{S}$

Limit of Quantification (LOQ): The LOQ was calculated by the formula referenced beneath;

Limit of Quantification = $10 \times \frac{\sigma}{S}$

Precision: Reproducibility of the samples was calculated three times a day and on three different days using a 100 µg/ml solution of Montelukast sodium. The precision for the observed responses was conveyed by the standard deviation (SD) and percentage relative standard deviation (%RSD).

Accuracy: The method precision was measured in terms of percentage recovery from a standard stock solution of 100 µg/mL when impaled with 80%, 100%, and 120% Montelukast sodium. Furthermore, the SD and RSD were calculated to determine whether the accuracy data falls within the specified limit.

Robustness: The mobile phase's flow rate was changed from 1.0 ± 0.1ml, and mobile phase composition was changed from the organic phase ethanol 80 ± 2ml.

Method A: Quantification of Montelukast Sodium by UV Spectrophotometric Aided Multivariate Calibration Technique

Preparing the Stock Solution (Standard): In a 100ml standard flask, 100 mg of drug (Montelukast sodium) was dispersed with methanol. Take 2.5ml of this and dissolve it in 50ml of methanol. From this standard stock solution, different concentrations of 2 - 10 µg/mL of solution were prepared.

Determination of λ max: The stock solution was diluted with MeOH to obtain a concentration of 6 µg/ml. This was measured in the UV region between 200 and 400 nm. The maximum wavelength was determined to be 344 nm. A linear curve was produced when the absorbance was plotted against the concentration (Table 3). 340, 342, 344, 346, and 348 nm are the range around 344 nm that the solutions were scanned in order to increase correlation and decrease instrumental fluctuations.

Preparation of sample solution: Accurately weigh and grind 20 Montelukast sodium tablets, then dissolve the powder in 10 millilitres of MeOH to obtain a concentration of 1 mg/mL. The solution

was then used for further testing after being purified.

Method Validation

The complete validation process was conducted in compliance with the required conditions of ICH Q2 B. Below is a list of the parameters that were employed:

Linearity: A range of concentrations from 2 to 10 µg/mL was created using the standard stock solution of Montelukast sodium. In order to reduce instrumental variations and enhance correlation, these solutions were scanned across a range of wavelengths from 340 to 342 to 346 to 348 nm, which are the wavelengths surrounding their absorbance maxima. The absorbances were measured, and concentration versus absorbance was plotted to make standardization graphs.

LOD and LOQ: The detection and quantification limits were computed using the linear regression line's slope and intercept values. The sensitivity of the developed approach was established.

Assay: The extracted tablet's solution's λ max was determined to be 344 nm. Table 4 provides the calculation of the total amount present.

Accuracy (Recovery studies): Recovery study resolutions of 80%, 100%, and 120% were achieved by applying the conventional addition approach. The sample stock and the standard were prepared. Pipette 0.5 ml of the standard into a three-standard flask, then add 0.46, 0.7, and 0.94 ml of sample solution to the volumetric flasks above. Finally, add methanol to make up to 10 ml. Using a UV spectrometer, the dilutions were measured in order to calculate the recovery percentage.

Method B: Quantification of Fexofenadine by UV Spectrophotometric Aided Multivariate Calibration Technique

Preparation of Stock Solution: It was made by mixing 100 mg of the medication (Fexofenadine) with methanol in a 100 ml standard flask. Using this standard stock solution, various concentrations from 2 to 10 µg/mL of solution were made.

Determination of λ max: Methanol should be incorporated into the stock solution to achieve a dilution resulting in a concentration of 15 µg/ml. The measurements for this solution were conducted in the UV wavelength ranging from 200 to 400 nm, which reveals the maximum absorbance (λ max) at 217 nm. A linear relationship was observed when absorbance was plotted against concentration. To enhance the relationship and lessen the instrumental variations, analysis was done on the solutions across a range surrounding 217 nm, specifically at 213, 215, 217, 219, and 221 nm.

Preparation of Sample Solution: Accurately weigh and grind 20 Fexofenadine tablets, subsequently dissolving the total weight of 10 mg in 10 ml of methanol to obtain a concentration of 1 mg/mL. Following filtration, the sample solution was utilized for further analysis.

Method Validation

Each validation parameter was performed in accordance with the guideline suggested by ICH Q2 B. The parameters that were used are listed below.

Linearity: Different concentrations between 5 and 25 µg/ml were created using the standard Fexofenadine stock solution. These solutions underwent a wavelength scan around their absorbance maxima of 213, 215, 217, 219, and 221 nm in order to reduce instrumental variations and enhance correlation. The concentration vs. absorbance was plotted to create the standardization graphs after the absorbances were recorded. By implementing the slope and intercept values of the linear regression line, the LOD and LOQ detection limit and quantification limit data were determined. It was determined that the devised approach was sensitive.

Precision: To evaluate this method, a 15 µg/ml solution was analyzed six times within a brief period on same day, as well as on six individual days, to conduct studies on intra- and inter-day precision.

Assay: The maximum wavelength (λ max) for the solution containing the extracted tablet was determined to be 217 nm. The calculation of the total amount present is detailed in Table 12.

Accuracy (Recovery Studies): The recovery study of the proposed technique was conducted using the standard addition method at concentrations of 80%, 100%, and 120%. A stock solution of both the sample and the standard was prepared. A quantity of 0.5 mL of standard solution was pipetted out into a three-necked flask, followed by adding a concentration of 0.3, 0.5, and 0.7 mL of the sample solution into the respective volumetric flasks. The total volume of solution was then adjusted with methanol to 10 mL. The solutions were analyzed

using a UV spectrometer, and the percentage recovery was computed.

Results and Discussion

The outcomes for the estimation of Montelukast sodium by using the HPLC method are as follows:

The chromatogram obtained for blank solution, standard stock and sample solution for Montelukast sodium are shown in Figure 1, 2 and 3 and respectively.

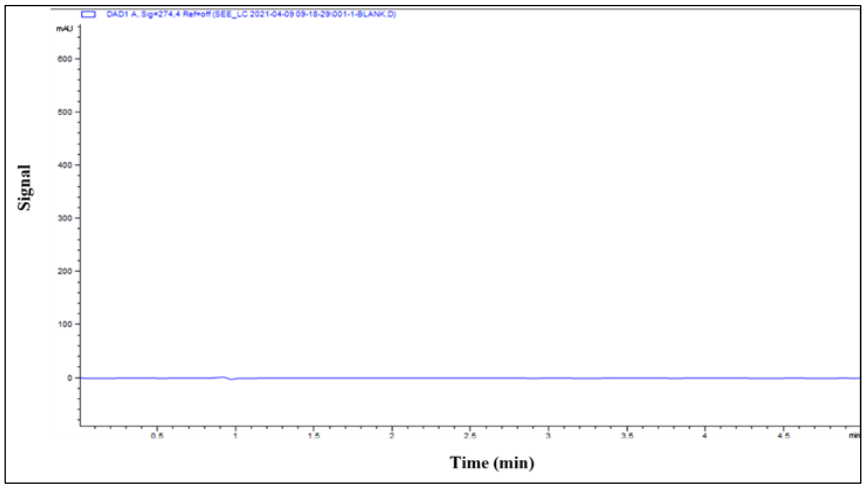

Figure 1: Blank Chromatogram of Montelukast Sodium

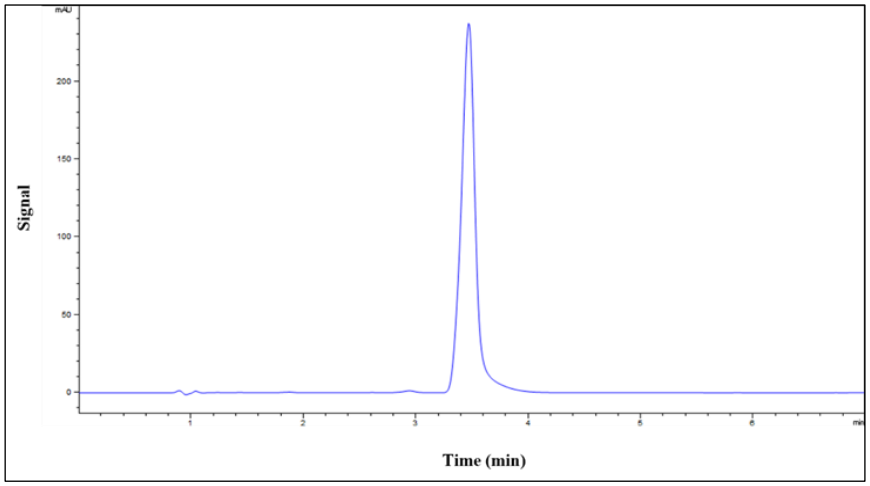

Figure 2: Standard Chromatogram of Montelukast Sodium at 254 nm

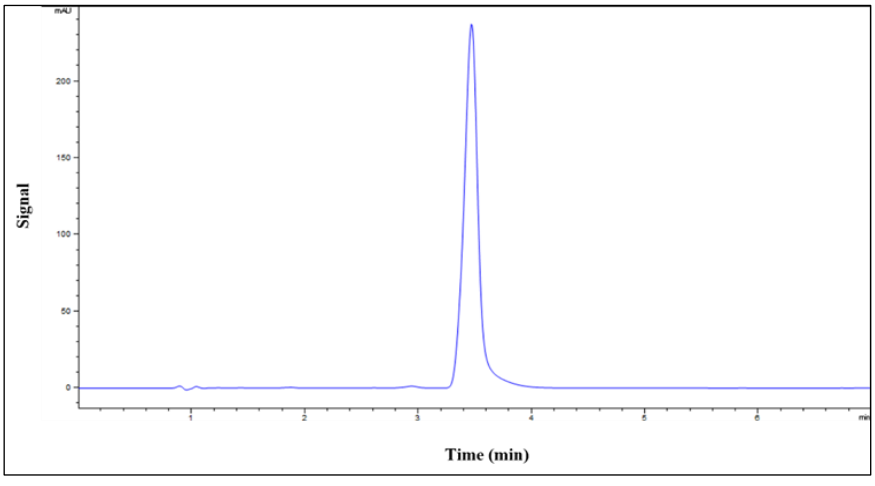

Figure 3: Sample Chromatogram of Montelukast Sodium at 254 nm

Analytical Method Validation

The outcomes of the system suitability assessment are illustrated in both a figure and a table. The percentage RSD values for theoretical plates over 2000, the retention time (RT), peak area, capacity factor (K), and the tailing factor show a high degree of system fit (Table 2).

Specificity: The chromatogram of the blank solution, standard stock and sample solution was shown in the Figure 1-3 and there was no interference found at the retention time (RT) of Montelukast sodium. The system suitability previously determined is depicted in Figure 4.

Linearity: Results of linearity were presented in Table 3 and Figure 5, and the corresponding overlay chromatogram in Figure 6.

LOD and LOQ: Detection and quantification limits were found to be 0.542µg / mL and 1.619µg / mL.

Precision: Chromatograms for system precision, interlay and intraday precision are presented in Table 4, 5 and Figure 7, 8. The low % RSD values obtained shows good precision.

Accuracy: The results for accuracy were presented in Figure 9 and Table 6 and found to be within limits. The % Accuracy was found to be in the range of 99.63-100.18%.

Table 2: Assessment of System Appropriateness for the Suggested HPLC Technique

Parameter	Average ± SD	% RSD	Reference value
Retention time (RT)	3.012±0.05	1.62	-
Peak Area	2588+2.58	0.10	-
Capacity Factor	1.62±0.01	0.64	1-10
Tailing Factor	1.08±0.02	1.40	NMT 2
Theoretical Plates (N)	2405+6.06	0.25	NLT 2000

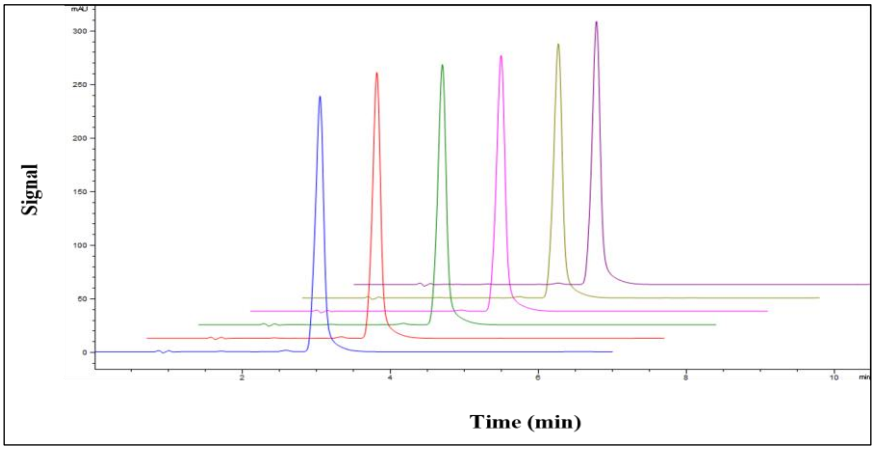

Figure 4: System Suitability

Table 3: Linearity Results of Montelukast Sodium

Conc (µg/ml)	Peak area
50 µg/ml	1294
75 µg/ml	1941
100 µg/ml	2589
125 µg/ml	3236
150 µg/ml	3883

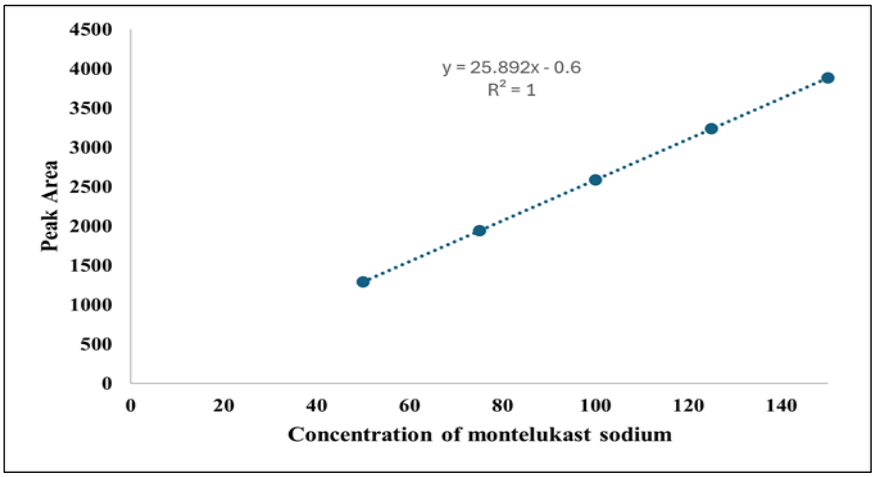

Figure 5: Calibration Curve of Montelukast Sodium at 254 nm

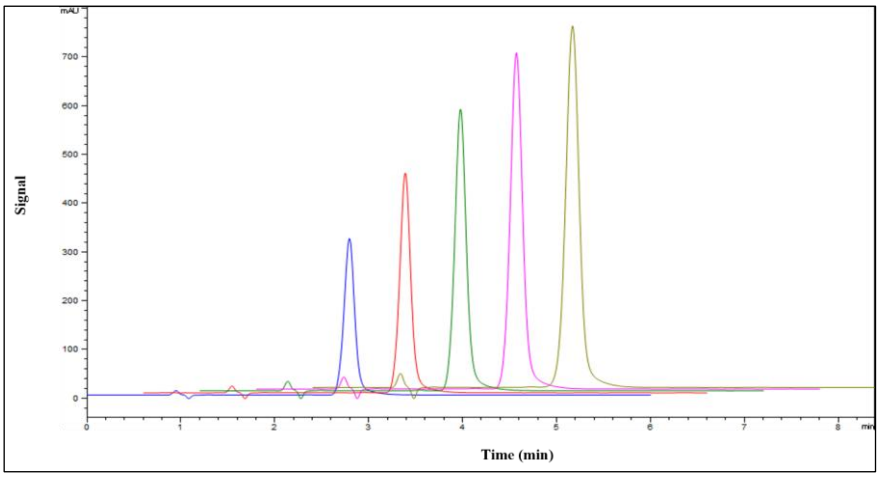

Figure 6: Overlay Chromatogram of Linearity

Table 4: System Precision of Montelukast Sodium

Description	System Precision (Area)	Interday Precision (Assay)		
		Day 1	Day 2	Day 3
1	2589	99.25	99.57	99.54
2	2587	99.22	99.62	99.85
3	2585	99.64	99.54	99.55
4	2582	99.55	99.48	99.47
5	2592	99.22	99.75	99.56
6	2589	99.52	99.62	99.35
Average	2587	99.40	99.60	99.55
Standard Deviation	3.50	0.19	0.09	0.17
% RSD	0.14	0.19	0.09	0.17

Table 5: Method Precision of Montelukast Sodium

Sl. No	Retention Time	Peak Area
1	2.99	2587
2	3.0	2589
3	3.1	2591
4	3.05	2590
5	3.08	2590
SD	1.243	1.516
%RSD	41.07	0.058

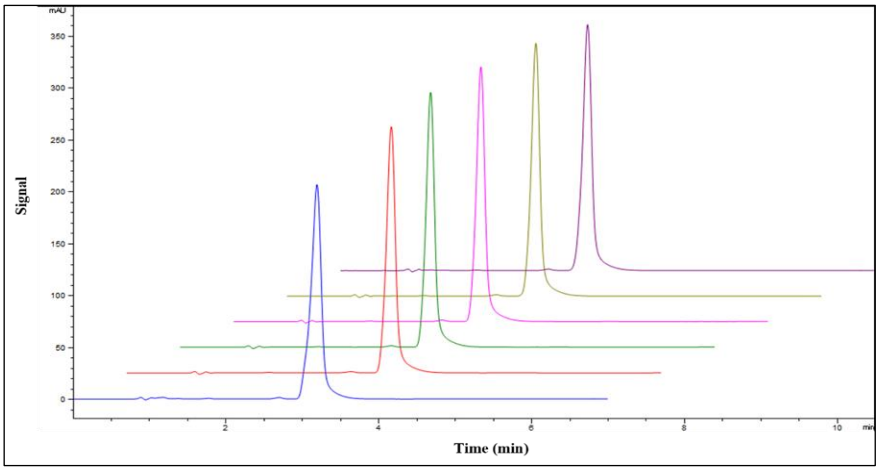

Figure 7: Chromatogram of Method Precision

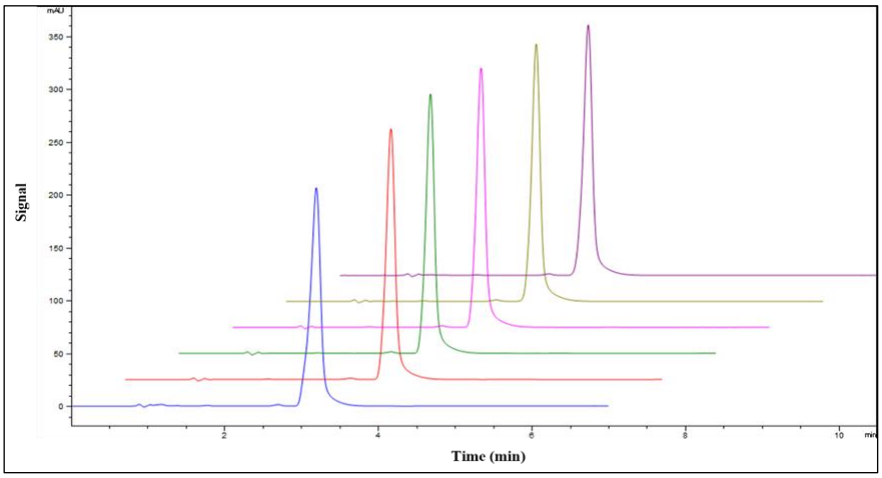

Figure 8: Chromatogram of System Precision

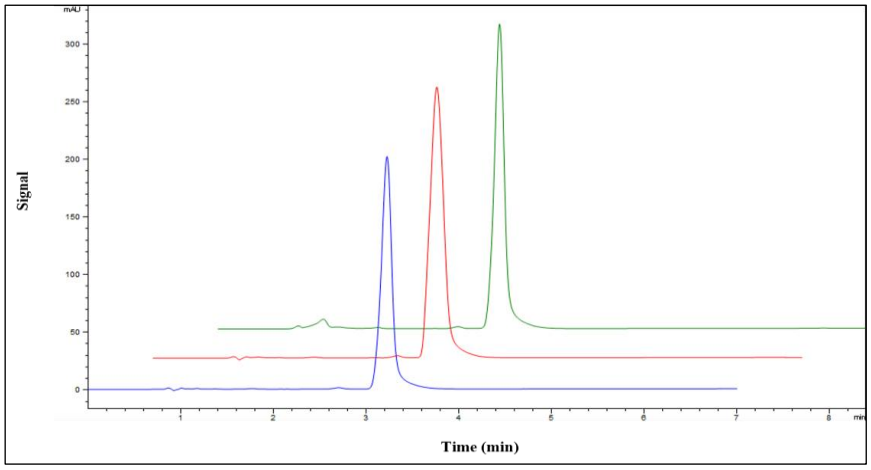

Figure 9: Chromatogram of Accuracy

Table 6: Accuracy Results for Montelukast

Avg Area	80µg/ml	100 µg/ml	120 µg/ml
	2071*	**2589***	**3106***
Mean recovery	79.76	100.18	119.56
% Accuracy	99.70	100.18	99.63

Quantification of Montelukast by UV Spectrophotometric Aided Multivariate Calibration Technique

Montelukast's maximum wavelength (λ max) was determined to be 344 nm when methanol was used as the solvent. The UV spectra is depicted in Figure 10.

Linearity: The linearity was observed at wavelengths of 340, 342, 344, 346, and 348 nm within 2–10 µg/mL concentration range (Table 7). The corresponding calibration curves are presented in Figures 11 -15. At each wavelength, the lower standard deviation values indicate that the technique demonstrated precision. Additionally, the quantification limits (LOQ) and detection limits (LOD) were computed and are described in Table 8.

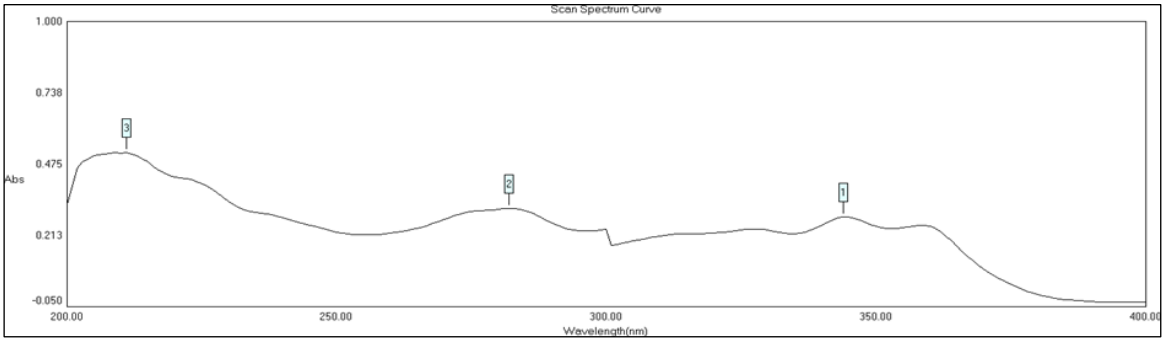

Figure 10: UV spectrum of Montelukast sodium

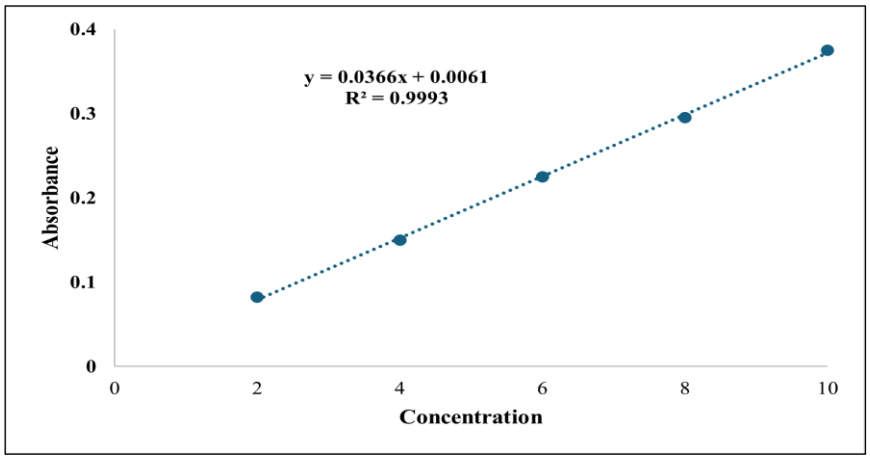

Figure 11: Calibration Curve at 340 nm

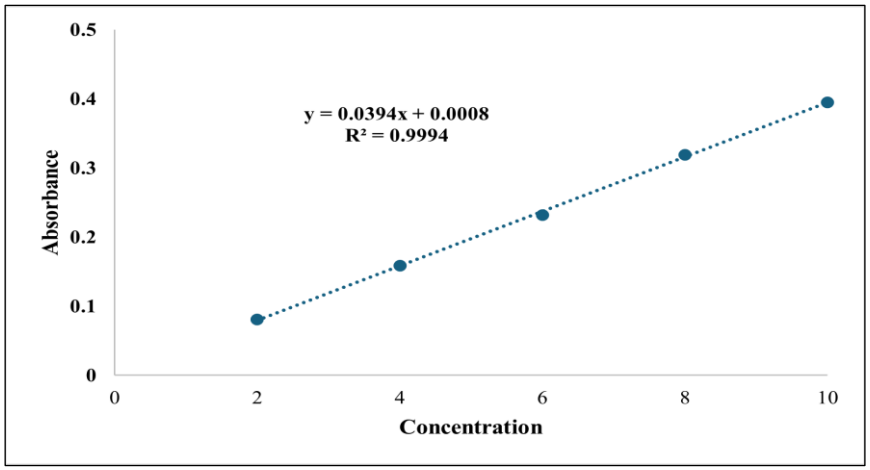

Figure 12: Calibration Curve at 342 nm

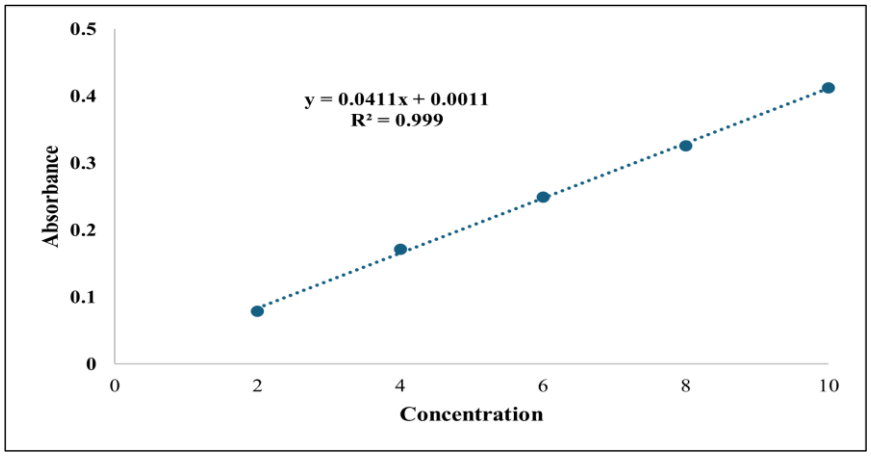

Figure 13: Calibration Curve at 344 nm

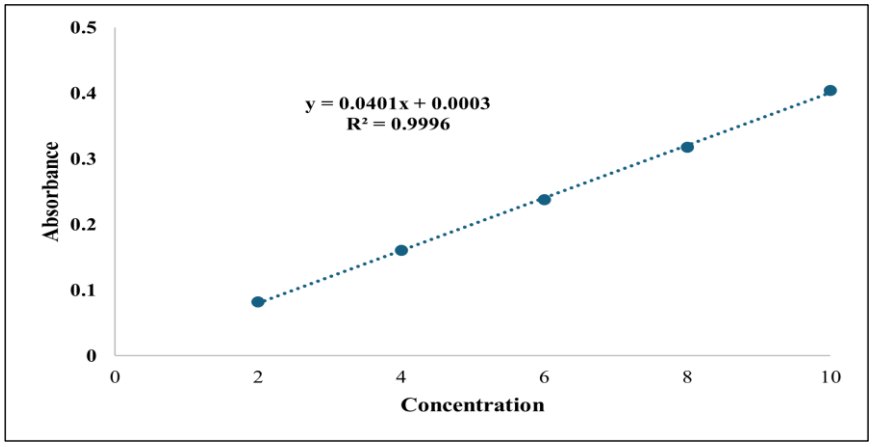

Figure 14: Calibration Curve at 346 nm

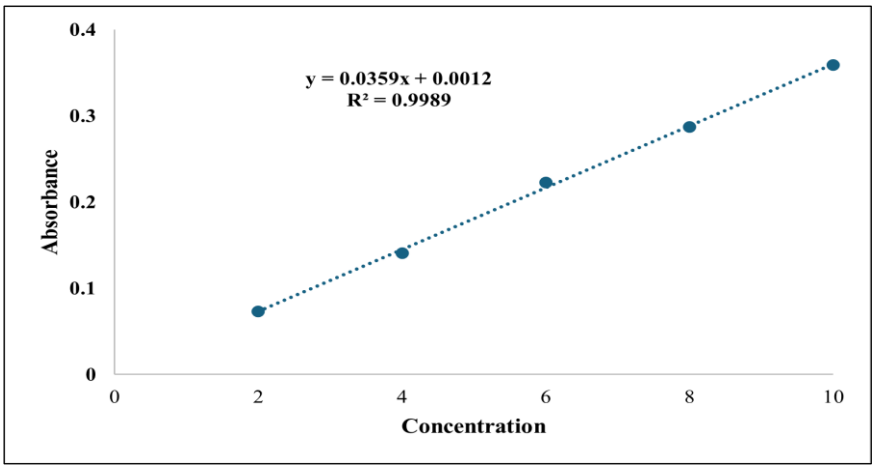

Figure 15: Calibration Curve at 346 nm

Table 7: Multivariate Ultraviolet Calibration Achieved at Five Distinct Wavelengths

conc (µg/ml)	Absorbance				
	340 nm	342 nm	344 nm	346 nm	348 nm
2	0.082	0.081	0.079	0.082	0.073
4	0.150	0.159	0.171	0.161	0.141
6	0.225	0.232	0.249	0.238	0.223
8	0.295	0.319	0.326	0.318	0.287
10	0.375	0.395	0.412	0.404	0.359

Table 8: Linearity Data at Each of the Five Wavelengths Displaying LOD and LOQ

Wavelength (nm)	Regression equation	R^2	LOD (µg/mL)	LOQ (µg/mL)	% RSD
340	y = 0.0366x + 0.0061	0.9993	0.317	0.961	1.5602
342	y = 0.0394x + 0.0008	0.9994	0.300	0.908	1.5083
344	y = 0.0411x + 0.0011	0.999	0.374	0.952	1.8829
346	y = 0.0401x + 0.0003	0.9996	0.247	0.750	1.2492
348	y = 0.0359x + 0.0012	0.9989	0.465	1.410	1..9269

Limits of Quantification (LOQ) and Detection (LOD): The LOD and LOQ were calculated to be 0.374 µg/mL and 0.952 µg/mL, respectively.

Precision: The reduced standard deviation indicates that the technique demonstrated specificity, with the % RSD for interday and intraday precision recorded at 0.0409 and 0.0228, respectively. These values fall within the acceptable threshold less than two percent for every wavelength. The low % RSD further confirms that the proposed technique is both accurate and precise (Figure 16, 17).

Assay: At a wavelength of 344 nm, the tablet formulation's absorbance was measured. The measured quantity and assay percentage were determined to be 9.02 mg and 100.1% w/w, respectively, with a % RSD value as indicated in (Table 9).

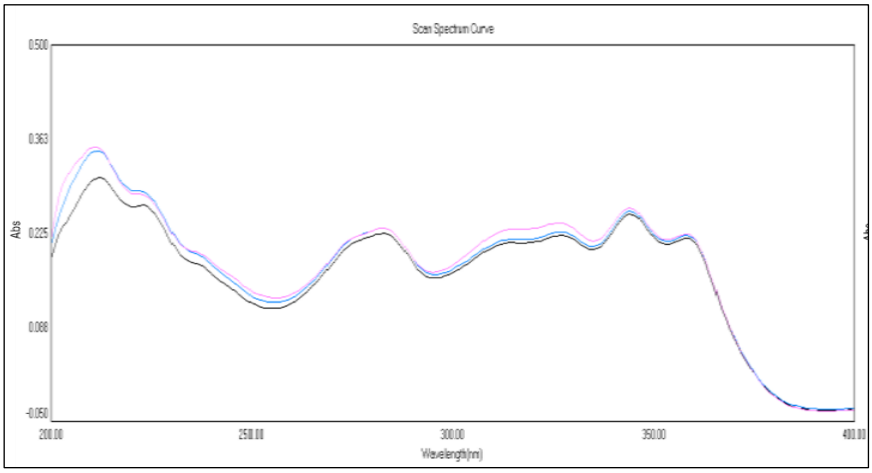

Figure 16: Overlay UV Spectra of Intraday Precision

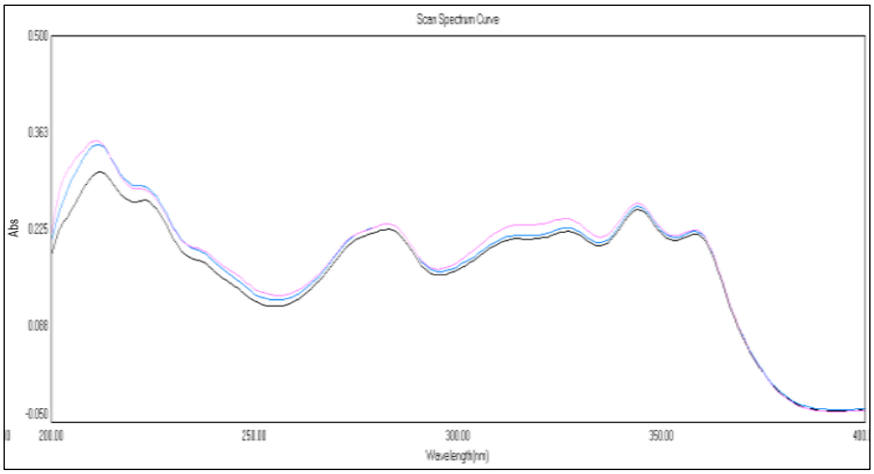

Figure 17: Overlay UV Spectra of Interday Precision

Table 9: Assay of Montelukast Sodium

Label claim (mg)	Amount Estimated (mg)	% Assay
10	9.97	99.7
10	10.09	100.9

10	9.99	99.9
Average	9.02	100.1
SD		1.6073
% RSD		1.6006

Quantification of Fexofenadine by UV Spectrophotometric Aided Multivariate Calibration Technique

With methanol as the solvent, the λ max of fexofenadine was measured at 217 nm and depicted in Figure 18.

Linearity: The linearity was observed at wavelengths of 213, 215, 217, 219, and 321 nm within a 5–25 µg/mL concentration range, as shown in Figure 19 and Table 10. The corresponding calibration curves are presented in Figures 20 - 24. At each wavelength, the lower standard deviation values indicate that the technique demonstrated precision. Additionally, the quantification limits (LOQ) and detection limits (LOD) were computed and are described in (Table 11).

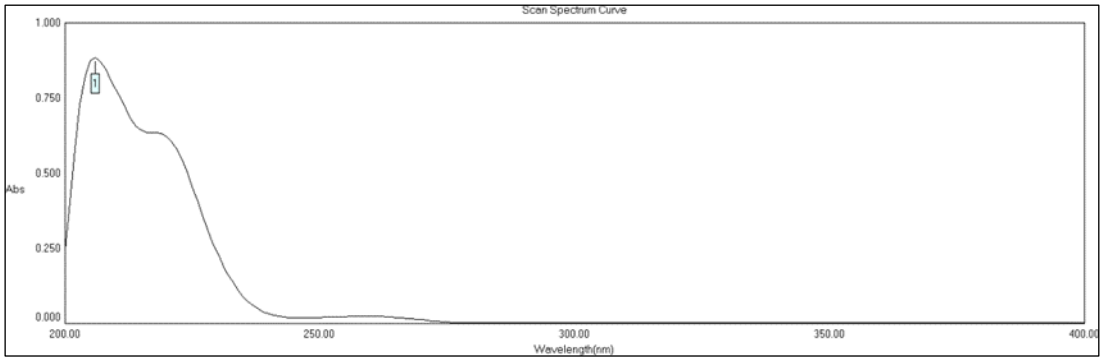

Figure 18: UV Spectrum of Fexofenadine

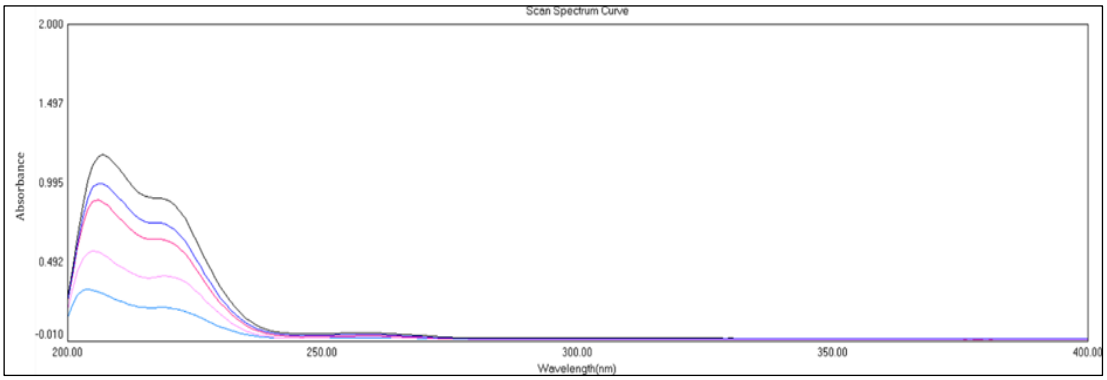

Figure 19: Overlay UV Spectra of Fexofenadine Showing Linearity

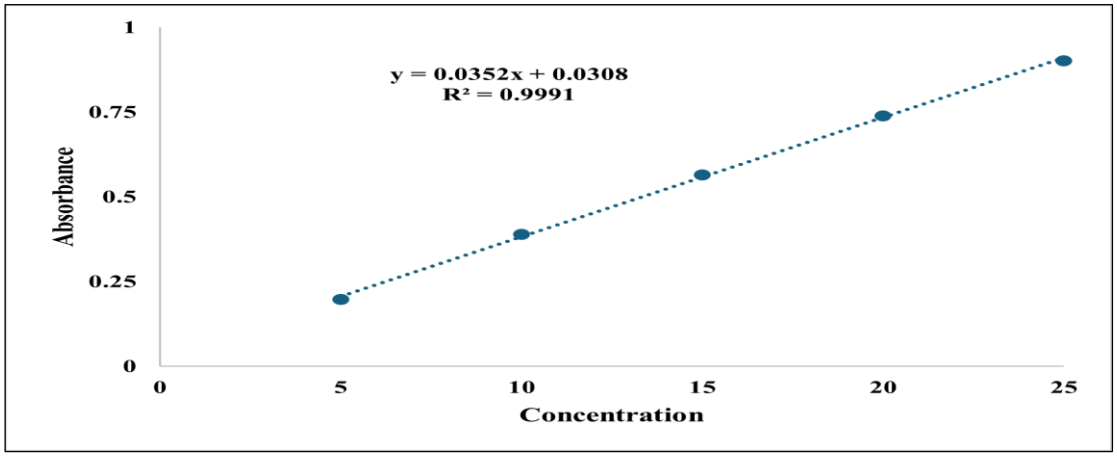

Figure 20: Calibration Curve at 213 nm

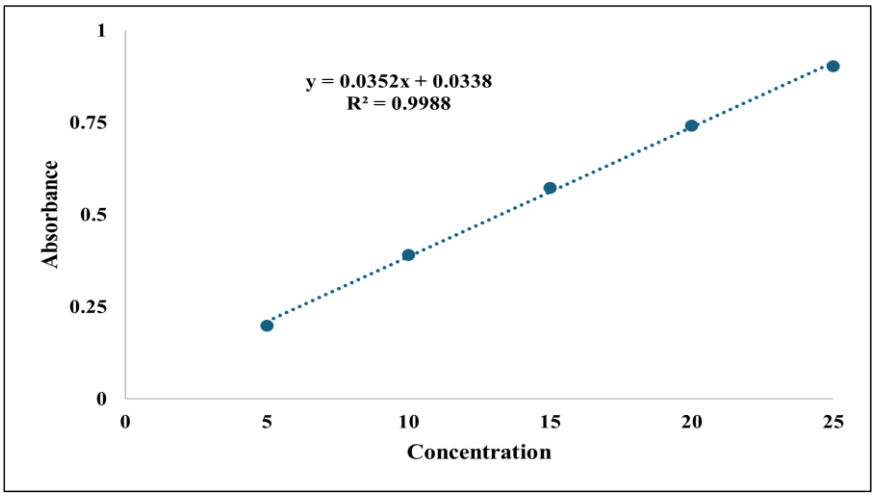

Figure 21: Calibration curve at 215 nm

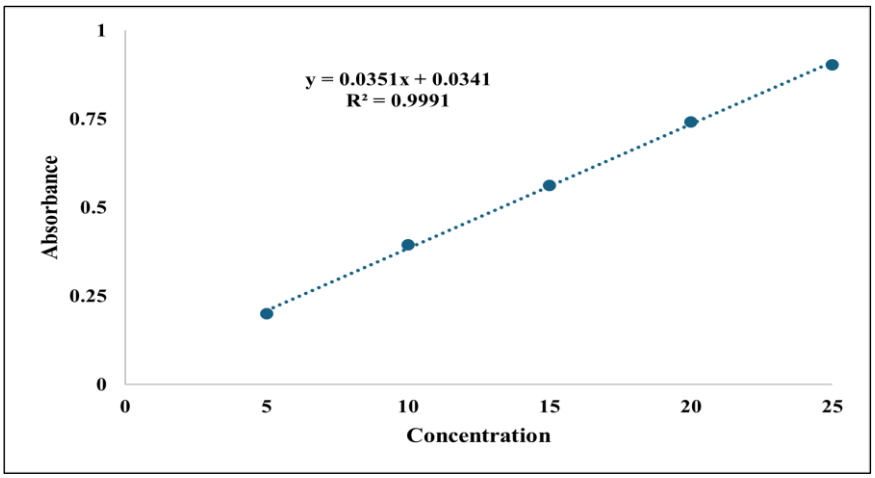

Figure 22: Calibration Curve at 217 nm

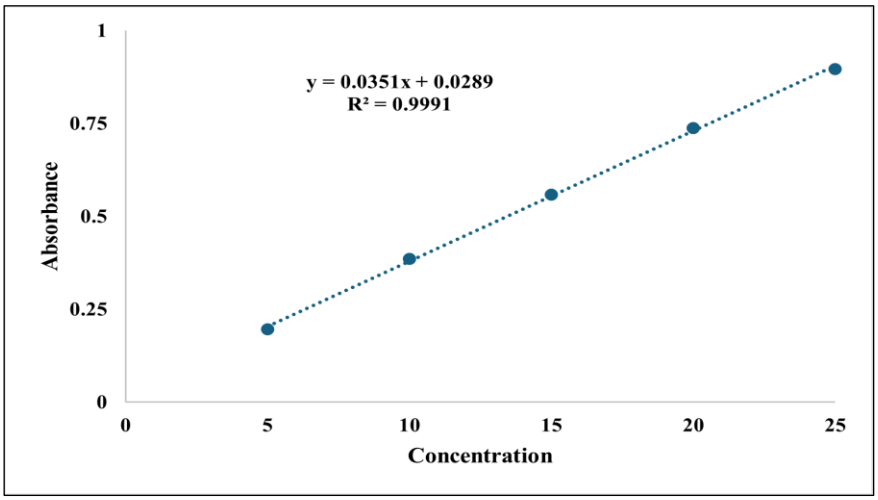

Figure 23: Calibration Curve at 219 nm

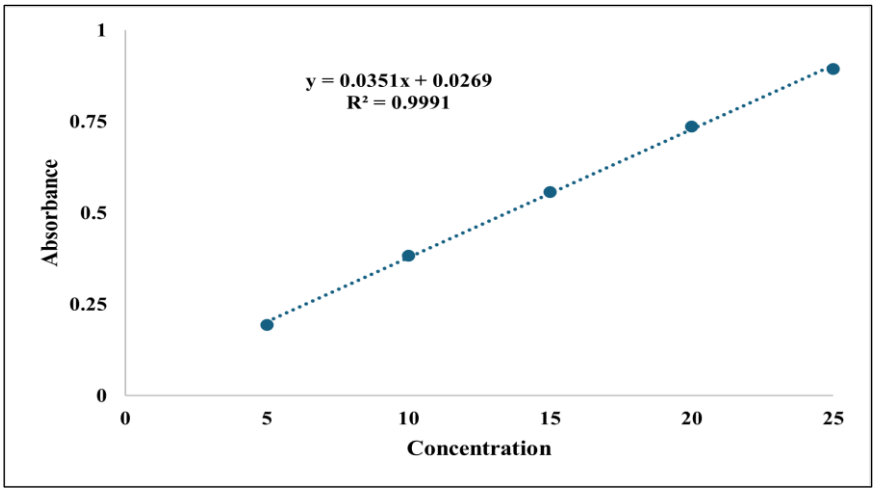

Figure 24: Calibration Curve at 221 nm

Table 10: Multivariate Calibration Achieved at Five Distinct Wavelengths

Conc (µg/mL)	Absorbance (nm)				
	213 nm	215 nm	217 nm	219 nm	221 nm
5	0.197	0.199	0.2	0.196	0.194
10	0.389	0.391	0.394	0.385	0.383
15	0.565	0.572	0.562	0.559	0.557
20	0.739	0.741	0.741	0.738	0.736
25	0.901	0.903	0.903	0.896	0.894

Table 11: Linearity Data at Each of the Five Wavelengths Displaying LOD and LOQ

Wavelength (nm)	Regression equation	R^2	LOD	LOQ	% RSD
			(µg/mL)	(µg/mL)	
213	0.0879x + 0.0308	0.9991	0.366	1.108	1.7443
215	0.0879x + 0.0338	0.9988	0.412	1.248	1.9463
217	0.0872x + 0.0361	0.9991	0.418	1.268	1.9853
219	0.0877x + 0.0289	0.9991	0.354	1.073	1.6969
221	0.0877x + 0.0269	0.9991	0.354	1.073	1.7031

LOD and LOQ: The detection and quantification limits were found to be 0.418 µg/mL and 1.268 µg/mL.

Precision: The reduced standard deviation indicates that the method demonstrated specificity, with the % relative standard deviation (RSD) for the intraday and interday precisions were recorded at 0.0399 and 0.0218, respectively. These values fall within the acceptable threshold of less than 2% for each wavelength. The low % RSD further confirms that the proposed technique is both accurate and precise (Figure 25, 26).

Assay: The wavelength at which the tablet formulation's absorbance was measured was 217 nm. The measured quantity and assay percentage were determined to be 9.02 mg and 100.1% w/w, respectively, with a % RSD value as indicated in (Table 12).

Table 12: Assay of Fexofenadine

Label claim (mg)	Amount Estimated (mg)	% Assay
120	119.2	99.3
120	120.5	100.4
120	119.6	99.6
Average	119.76	99.76
SD		0.5686
% RSD		0.6232

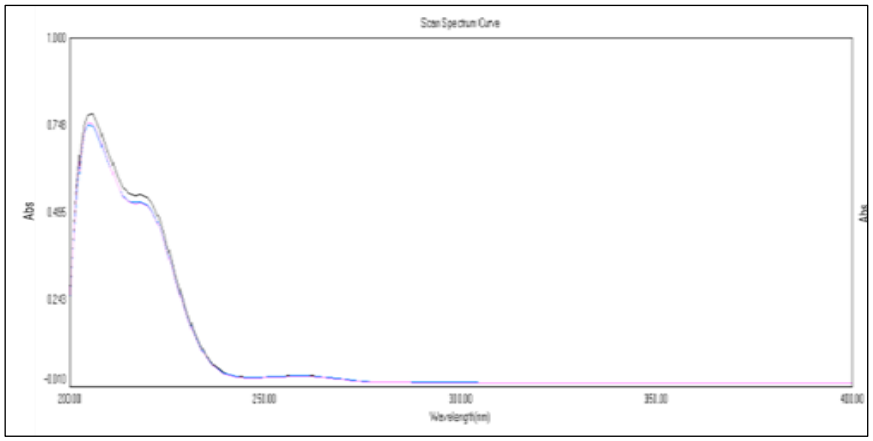

Figure 25: Overlay UV Spectra Intraday Precision

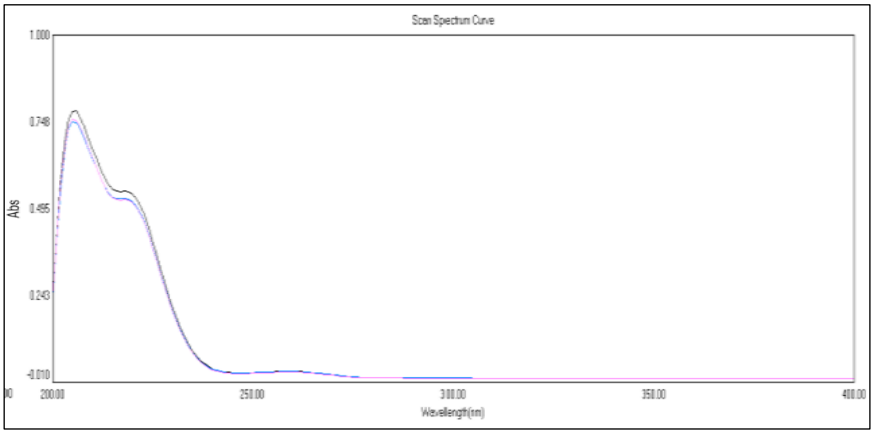

Figure 26: Overlay Spectra of UV Interday Precision

Conclusion

The recently advanced green analytical method for the determination of Montelukast sodium utilizing RP-HPLC with ethanol as the mobile phase has been demonstrated to be environmentally friendly, precise, specific, sensitive, and reproducible. Through the use of multilinear regression, a simple and precise method for evaluating fexofenadine and montelukast in their pharmaceutical forms was developed. The method underwent validation against various parameters and was found to comply with the limits set by ICH guidelines. This method offers a simple operational procedure in comparison to more costly techniques such as HPLC, making it suitable for routine analysis. While the recently advanced green analytical method using RP-HPLC with ethanol as the mobile phase for the determination of Montelukast sodium offers several advantages, it is not without limitations. One key limitation is its applicability primarily to formulations containing Montelukast and Fexofenadine; its effectiveness in analyzing other drug combinations or complex matrices has yet to be extensively explored. Additionally, although ethanol is a greener alternative, its use still requires proper handling and storage, and the method's robustness under varied environmental or instrumental conditions may need further evaluation. The spectrophotometric method based on multilinear regression, though simple and cost-effective, may be limited by its dependency on accurate baseline correction and potential interference from excipients in complex formulations.

Future perspectives involve expanding the scope of this green RP-HPLC method to include a broader range of antihistaminic and anti-asthmatic drug combinations. There is also potential to apply this technique in stability-indicating studies and bioanalytical applications, particularly in pharmacokinetics. Integration with newer green solvents or deep eutectic solvents may further enhance the method's environmental profile. Additionally, miniaturization and automation of this technique could increase throughput and reproducibility in routine quality control laboratories.

Abbreviations

RP-HPLC: Reversed Phase High Pressure Liquid Chromatography, RSD: Relative Standard Deviation, LTRA: Leukotriene Receptor Antagonists.

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Author Contributions

All the authors have contributed equally.

Conflict of Interest

The authors declare no competing interests.

Ethics Approval

Not applicable.

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