QbD-Guided Comparative Evaluation of RP-HPLC and UV Methods for Montelukast Sodium Quantification

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ABSTRACT: This study focuses on establishing a fast and novel RP-HPLC method along with its comparable UV spectroscopic approach for the routine evaluation of montelukast sodium. Using Design Expert® software, a 3^2 full-factorial design was deployed to optimize the RP-HPLC method. Retention time, tailing factor, and theoretical plate count were considered as the dependent response parameters in this design, and the mobile phase composition and its flow rate were chosen as the independent variables. A mobile phase composed of methanol and water (40:60, %v/v) was eluted through a C18 column (250 × 4.6 mm, 5 µm) at a flow rate of 1.0 ml/min for the chromatographic separation. The wavelength used for detection was 344.4 nm to ensure optimal sensitivity for the analyte. The RP-HPLC method development yielded statistically significant models (p<0.05). The proposed method was validated in accordance with ICH standards. Retention time of the drug was found to be 5.009 minutes. A linear calibration curve was obtained over the concentration range of 40-60 µg/ml. The method demonstrated a limit of detection of 0.15 µg/ml and a limit of quantification of 0.50 µg/ml. Additionally, a UV spectroscopic method was also developed and validated, showing comparable results to the RP-HPLC method (p>0.05). Overall, the developed RP-HPLC method along with its comparable UV spectroscopic method demonstrated a comprehensive approach for the routine analysis of montelukast sodium.

Key words: RP-HPLC, montelukast sodium, Design Expert®, full factorial design, QbD

INTRODUCTION

Asthma is a chronic condition characterised by a range of symptoms and recurrent airway inflammation, which may lead to a progressive loss in lung function.[1] Typical signs of asthma consist of wheezing, coughing, chest discomfort and difficulty breathing, which may become more or less severe over time.[2] According to a recent World Health Organization research, asthma affects over 339 million individuals globally and by 2025, there will be 400 million people with asthma.[3] Asthma management involves quick-relief drugs like anticholinergics and short-acting beta-agonists, while long-term control relies on biologics, leukotriene modifiers, long-acting beta-agonists (LABAs) and corticosteroids.[4] A newer group of drugs known as leukotriene receptor antagonists works in conjunction with steroids, and bronchodilators seem to lessen the need for steroids.[5]

Montelukast sodium is an orally administered drug with high specificity and potent binding activity toward the cysteinyl leukotriene receptor 1 (CysLT1) and effectively hinders the physiological effects of leukotriene D4 (LTD4) by targeting the CysLT1 receptor.[6] Montelukast is prescribed to prevent and manage asthma in both adult individuals and

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pediatric patients.[7] It is an optically active, hygroscopic powder ranging from white to off-white in appearance.[8] The compound exhibits a molecular weight of 608.18 g/mol and possesses an empirical formula of $C_{35}H_{35}ClNaO_3S$ (Figure 1).[9] The compound is practically insoluble in acetonitrile but shows good solubility in ethanol, methanol, and water.[10]

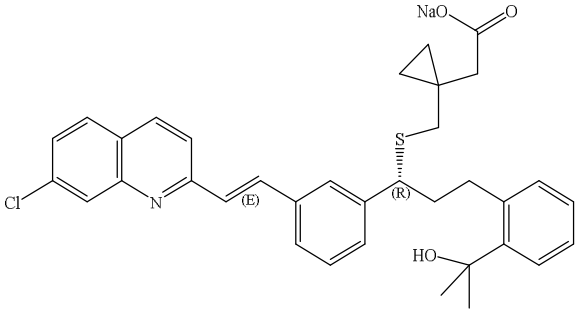

Figure 1. Montelukast sodium.[9]

In analytical method development, quality by design (QbD) adopts a structured and science-based strategy in controlling and understanding the performance characteristics of the method. It renders analytical scientists an extensive understanding of critical process parameters and their underlying principles.[11] The use of QbD tools transformed the development process from traditional trial-and-error methods to a systematic, science-driven approach, leading to the development of robust and dependable analytical methods.[12]

Figure 2 presents the QbD approach applied to develop the RP-HPLC method. The process starts by defining the quality target product profile (QTTP) and identifying the critical quality attributes (CQAs) necessary for a reliable method.[13] Key process parameters, such as flow rate, mobile phase pH, detector wavelength, and solvent ratio are then screened and optimized through design of experiments (DoE).[14] Risk assessment, including ANOVA analysis, evaluates how these parameters impact method performance. The resulting design space ensures robust and reproducible conditions, followed by risk management and continuous improvement to maintain a systematic, science-driven development process.[15]

Product development and analytical method development are closely interconnected throughout the lifecycle of any pharmaceutical product. A thorough literature review showed that the majority of the HPLC methods for determining montelukast sodium involve costly, time-consuming, and intricate processes and also lack a QbD approach in the method development.[16-19]

Patnaik *et al.* (2012) demonstrated an RP-HPLC method for the stability studies of montelukast in formulations. The method exhibits a longer elution time due to broader peaks.[16] Samuel *et al.* (2021) established a new RP-HPLC method using acetonitrile and triethylamine as mobile phase for identifying related compounds of montelukast sodium in a pharmaceutical formulation.[17] In that study, the retention time was 24.2 minutes, suggesting that the method is relatively time intensive and demands a greater volume of solvent compared to standard approaches. Jina *et al.* (2020) utilized a QbD strategy to create an HPTLC technique for the concurrent analysis of montelukast sodium and levocetirizine HCl in a combined tablet formulation. Their approach, however, relied on a complex mobile phase mixture consisting of methanol, ethyl acetate, and triethylamine in a 5:5:0.04 (v/v) ratio together with an elaborate and sequential multi-stage analytical process.[18]

A new UV spectroscopic technique for the quantitative measurement of montelukast sodium was presented by Pallavi *et al.* (2012).[20] Their research showed that the method is accurate and appropriate for routine screening of tablet and bulk formulations. However, this method lacks robustness and ruggedness studies.[20] Consequently, this study focused on developing an improved analytical workflow for montelukast sodium quality control by applying a systematic QbD approach to develop a robust and rapid RP-HPLC method. This strategy addresses common limitations of existing methods, including long run times and inadequate robustness assessment. A complementary UV spectroscopic method was also developed to provide a practical and cost-effective analytical option.

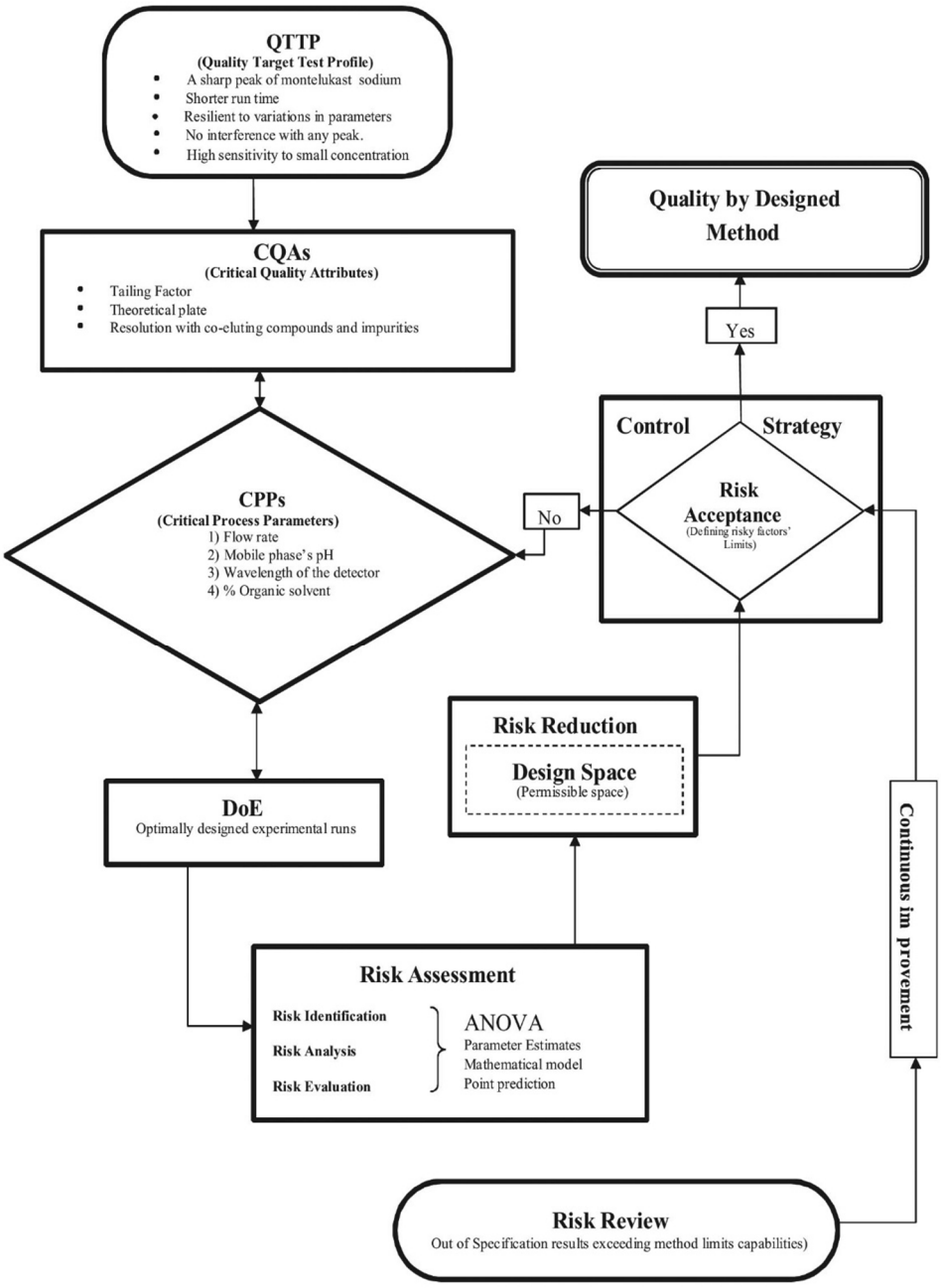

Figure 2. Flowchart for RP-HPLC method development using the QbD approach (adapted[14]).

MATERIALS AND METHODS

Chemicals and reagents. High-purity montelukast sodium reference standards (>99%) were supplied by Incepta Pharmaceuticals Ltd. HPLC-grade methanol, ethanol, and acetonitrile procured from Merck Limited, Mumbai were used. Purified water was obtained through filtration using a Millipore Milli-Q water purification system with 0.22 µm pore size.

Protocol for RP-HPLC method development

Apparatus and instrumentation. The study utilized a Shimadzu SIL-20AHT reversed-phase HPLC instrument equipped with quaternary pump and PDA detector for analysis. LabSolutions CS software was used for data acquisition and integration. Instruments used in the study included a Mettler Toledo ME204 analytical balance, Kemi hot air oven and Remi ultrasonicator.

Chromatographic conditions. The separation was carried out on a Prontosil C18 column (250 × 4.6 mm, 5 µm) with a methanol-water mixture (40:60, %v/v) as the mobile phase. The analysis utilized a 10 µl injection volume at a flow rate of 1.0 ml/min and the total run time was maintained at 10 minutes. Detection of montelukast sodium was achieved at a wavelength of 344.4 nm.

Preparation of mobile phase. The mobile phase was prepared by mixing methanol and HPLC-grade water in a defined ratio, sonicated for 15 minutes, and then filtered under vacuum using a 0.22 µm Restek membrane filtration system. This filtered mobile phase also served as the diluent for preparing analytical samples.

Preparation of standard solution. To prepare the 100 µg/ml working standard solution, 10 mg of montelukast sodium was dissolved in sufficient diluent in a 100 ml volumetric flask. After 15 minutes of sonication, the volume was adjusted to the mark with diluent, homogenized and filtered using a 0.45 µm nylon syringe filter.

Protocol for the development of the UV spectroscopic method. A double beam Shimadzu UV 1700 Pharmaspec spectrophotometer was used for the UV spectroscopic method of montelukast sodium. Different solvent systems (ethanol, methanol, water and acetonitrile) were used to conduct several trials. Ethanol was chosen as a solvent to develop the method because it showed good specificity and linearity for montelukast sodium.

a. Stock solution preparation and determination of absorption maxima (λ_{max}). Accurately measured 10 mg of montelukast sodium was dissolved in ethanol to prepare a stock solution in a 100 ml volumetric flask. The solution was then subjected to spectral scanning, ranging from 400 nm to 200 nm and the chosen λ_{max} of montelukast sodium was found to be 344.40 nm.

b. Standard curve preparation. From stock solution dilutions were performed to obtain concentrations spanning a range of 2-25 µg/ml. UV-spectrophotometric analysis was conducted at 344.4 nm using ethanol as the blank reference. The calibration curve was constructed by correlating measured absorbance values with corresponding concentrations, with data processing performed in Microsoft Excel.

Method validation. Following ICH Q2(R1) validation protocols, both the developed RP-HPLC and UV spectroscopic methods were rigorously assessed for key performance characteristics, including system suitability, precision, specificity, accuracy, linearity range, robustness, ruggedness and sensitivity.[21,22]

System suitability. Six repeated injections of montelukast sodium standards (50 µg/ml for RP-HPLC; 16 µg/ml for UV spectroscopy) were performed to verify system suitability parameters.

Specificity. The specificity of both developed methods was verified through a comparative analysis of working standard solutions against blank solutions. Assessing the chromatograms of the standard and blank solutions for any interfering peaks corresponding to the analyte peaks was the goal of this test.

Linearity. For RP-HPLC method, the standard stock solution of montelukast sodium was diluted to five different drug concentrations ranging from 40 µg/ml to 60 µg/ml and analyzed for linearity. For UV spectroscopic method, six different drug concentrations from 2 to 25 µg/ml were studied for linearity.

Precision. Intra-day (six replicates in one day) and inter-day (six replicates over three days) analyses of standard solutions (50 µg/ml for HPLC and 16 µg/ml for UV) were used to evaluate method precision. The findings were presented as % RSD.

Accuracy. Recovery experiments were used to assess method accuracy which shows percentage recoveries within the analyte's concentration ranges of 40-60 µg/ml (RP-HPLC) and 16-24 µg/ml (UV).

Robustness. For RP-HPLC method, a 50 µg/ml of montelukast sodium reference solution was evaluated for robustness. Key parameters such as flow rate (±0.1 ml/min), methanol composition in mobile phase (±5%) and detection wavelength (±5 nm) were adjusted to observe their impact on the overall chromatographic response. For the UV method, robustness testing focused specifically on wavelength stability (±5 nm) with the analysis of 16 µg/ml of montelukast sodium reference solution.

Ruggedness. Six replicates of the standard solution were analyzed independently by two analysts to assess ruggedness. The method's consistency across these settings was then determined using the mean % recovery and %RSD values.

Sensitivity. For the sensitivity analysis, the baseline parameter was examined. Sensitivity parameters were determined by analyzing drug solutions that were progressively diluted. LOD and LOQ were determined as concentrations that produced signal-to-noise ratios of 3:1 and 10:1, correspondingly, in RP-HPLC analysis. Similarly, the UV method's sensitivity examined the absorbance of the very diluted drug solutions.

Comparison of the RP-HPLC and UV methods. Statistical tests (t-test) of the parameters, such as linearity, accuracy, precision, robustness, and ruggedness studies, were employed to do the comparative study of the developed methods. The outcomes of the RP-HPLC and UV methods were compared using a t-test to see if there were any significant differences (α=0.05). If the *p*-value was greater than 0.05, meaning that there was no significant difference between the two approaches. In this case, the null hypothesis (H_0: $\mu_{HPLC} = \mu_{uv}$) would not be rejected. Results with $p<0.05$ suggested a substantial difference between the analytical processes and provided enough evidence to reject the null hypothesis in favor of the alternative hypothesis (H_a: $\mu_{HPLC} \neq \mu_{uv}$).

RESULTS AND DISCUSSION

RP-HPLC method development and optimization

Method development. A rapid, sensitive, and novel HPLC method was successfully developed for the quantification of montelukast sodium. The chromatographic parameters were systematically optimized to achieve sharp, symmetrical peaks with minimal tailing and excellent resolution. Several reversed-phase columns were evaluated, including Eclipse XDP-C18 (250 mm × 4.6 mm, 5 µm), Zodiac C18 (150 mm × 4.6 mm, 5 µm) and Prontosil C18 column (250 × 4.6 mm, 5 µm), with the last one being found to provide superior analyte separation. Various mobile phase compositions were investigated, such as acetonitrile/water, acetonitrile/phosphate buffer and methanol/water, with optimal separation being achieved using the methanol-water system.

Analysis of Responses. Using Design Expert® software, a 3^2 factorial design was used to optimize the method. To assess their influence on the response parameters, nine experimental runs were carried out. Important chromatographic parameters, such as retention time (RT), tailing factor (TF) and theoretical plate count (TP), were examined in relation to methanol concentration (%) and flow rate (ml/min), as chosen independent factors (Table 1 & 2). As suggested by the DoE, responses from each run were generated. Evaluation was carried out by using the obtained data. Linear mathematical relationships were proposed for each response parameter with the help of statistical analysis.

According to the information presented in table 3, the ANOVA findings revealed F-values of 34.67, 18.57, and 96.57 for R1, R2 and R3 responses, respectively. This suggests that the models are prominent. Every model term (A and B) demonstrated statistically significant, as the p value is less than 0.05. The predicted R^2 values (R1: 0.7908, R2: 0.6934, R3: 0.9335) demonstrated reasonable agreement with their corresponding adjusted R^2 values (0.8938, 0.8236, and 0.9598). Based on these R^2 metrics, Design Expert® suggested linear models for every response. The minimum criterion of 4.0

was surpassed by the signal-to-noise ratios as measured by the adequate precisions (R1: 15.8482, R2: 12.457, and R3: 27.7150). These phenomena showed sufficient model precision. The results demonstrate that the models are appropriate for exploring the design space.

As shown by 3D plots and mathematical models, response surface methodology (RSM) analysis verified that factors A and B both had a positive impact on response 3, whereas both variables had a negative impact on responses 1 and 2 (Figure 3). Because the quantitative analysis of montelukast sodium was unaffected by the experimental settings, the statistical results for these responses showed the method's robustness.

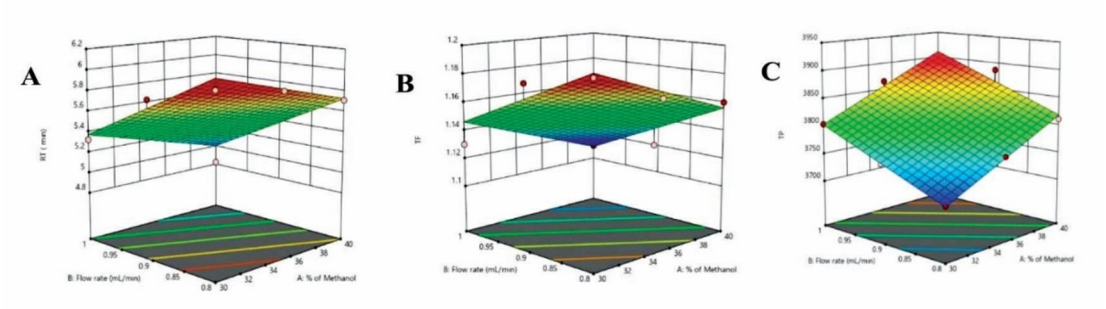

Figure 3. 3D surface plot illustrating the effects of independent factors on the responses RT (A), TF (B) and TP (C).

Table 1. Critical process parameters evaluated across defined ranges in the design space.

Variables	Name	Unit	Category	Coded value			Actual value		
				Low	Mid	High	Low	Mid	High
A	Methanol	%	Numeric	-1	0	1	30	35	40
B	Flow rate	ml/min	Numeric	-1	0	1	0.8	0.9	1.0

Table 2. A 9-run full factorial design (3^2) for method development.

Runs	Variable A: % of Methanol	Variable B: Flow rate (ml/min)	Response 1 RT (min)	Response 2 TF	Response 3 TP
1	30	1.0	5.324	1.13	3804
2	40	0.8	5.709	1.16	3811
3	40	0.9	5.497	1.12	3887
4	35	0.9	5.611	1.16	3800
5	40	1.0	4.82	1.11	3897
6	30	0.8	5.994	1.19	3711
7	30	0.9	5.814	1.18	3755
8	35	0.8	5.892	1.17	3766
9	35	1.0	5.29	1.14	3866

Table 3. ANOVA and regression modeling.

ANOVA for the responses									
	R1			R2			R3		
Source	SS*	F	P	SS	F	P	SS	F	P
Model	0.9822	34.67	0.0005	0.0053	18.57	0.0023	30577.67	96.57	< 0.0001
A-% of Methanol	0.2039	14.39	0.0090	0.0020	15.02	0.0082	17604.17	111.2	< 0.0001
B-Flow rate	0.7783	54.95	0.0003	0.0033	24.33	0.0026	12973.50	81.95	0.0001
Residual	0.0850			0.0008			949.89		
Cor Total	1.07			0.0061			31527.56		
Fit statistics				**Regression equation**					
Source	R1	R2	R3	R1 = +5.55-0.1843A-0.3602B					
Std. Dev.	0.1190	0.0116	12.58						
Mean	5.55	1.15	3810.78	R2 = +1.15-0.0183A-0.0233B					
C.V. %	2.14	1.01	0.3302						
R^2	0.9204	0.8677	0.9699	R3 = +3810.78+54.17A+46.50B					
Adjusted R^2	0.8938	0.8236	0.9598						
Predicted R^2	0.7908	0.6934	0.9335						
Adeq Precision	15.8482	12.4568	27.7150						

SS* = Sum of squares

Optimization of the method. Among the seven candidate solutions generated by Design Expert® software, the optimal chromatographic conditions were selected due to their maximal desirability score (0.944). The DoE optimization criteria are summarized in table 4. An isocratic methanol:water (40:60) system was delivered at 1.0 ml/min through a C18 column (250 × 4.6 mm, 5 µm) maintained at 25°C, with 10 µl injections monitored at 344.4 nm. The experimental data and the deviations from predicted response values are presented in table 5. All the responses are found to be within the acceptable limit (not more than 2.0%). Under these conditions, montelukast sodium had retention times of 5.009 minutes (Table 5). Because of the prolonged retention of montelukast sodium peaks, the total run time of a previously published method was 24.2 minutes.[17] By contrast, the new approach elutes the analyte more quickly while maintaining adequate resolution, resulting in a 10-minute run time. This demonstrates the speed and cost-effectiveness of the new approach.

Table 4. Optimization criteria.

Responses	Criteria
Response 1 (RT)	Minimize
Response 2 (TF)	Minimize
Response 3 (TP)	Maximize

UV method development and optimization. The UV spectrophotometric scanning (200-400 nm) of standard solution of montelukast sodium were performed with different diluting solvents: ethanol, methanol, water and acetonitrile. Ethanol was selected as the solvent to develop the method, as it provides a good specificity for montelukast sodium with the absorption maximum at 344.40 nm (Figure 4).

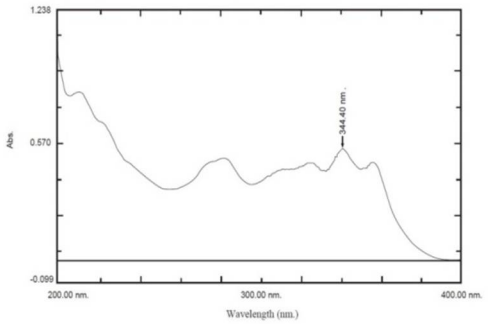

Figure 4. UV- spectrum for the standard solution of montelukast sodium.

Table 5. Predicted error of responses.

Value	Percentage of methanol	Flow rate (ml/min)	Response 1 (RT)	Response 2 (TF)	Response 3 (TP)
Predicted values	40	1.0	5.006	1.109	3911.444
Experimental values	40	1.0	5.009	1.115	3978.247
Predicted errors* (%)			0.0599	0.541	1.708

* Predicted errors (%) = [(Experimental value - Predicted value)/Predicted value] × 100%

Method validation

RP-HPLC method validation. Every system suitability parameter met the requirements set forth by the ICH guidelines, proving that they were all in compliance. The developed method exhibited excellent chromatographic performance with theoretical plate counts exceeding 2,000, tailing factors maintained below 2 and peak response %RSD values consistently less than 2% (Table 6). The chromatogram exhibited sharp resolution with no blank interference, confirming specificity (Figure 5).

Linearity was excellent (R^2 = 0.99, 40-60 µg/ml), with the regression equation y = 84051x – 187581 (Figure 6). Accuracy was demonstrated by recoveries of 99.568-100.713% (%RSD ≤ 1.03). Precision studies (intra- and inter-day) showed high reproducibility (Table 6). Sensitivity was confirmed with LOD (0.15 µg/ml) and LOQ (0.50 µg/ml) as shown in figure 7. As a result of its low LOD and

LOQ value, the current RP-HPLC method can assess minute amounts of drug concentration. Robustness tests (flow rate, mobile phase and wavelength variations) caused minimal peak shifts but maintained recoveries within 0.392-1.420% deviation (Table 6 and Figure 8). These changes were shown to have no discernible effect on the analytical responses for the target analyte. The %RSD for the ruggedness study ranged from 0.847% to 1.039%, respectively, which confirms that the method is rugged.

Table 6. RP-HPLC method validation.

Test	(Mean±%RSD)	Limits	Test	Amount (µg/ml)	Mean % Recovery	RSD (%)
System suitability			Accuracy	40	99.568	1.03
Peak area	4075779.53 ± 1.48	%RSD ≤ 2		50	100.713	0.977
Tailing factor	1.11 ± 1.8%	≤ 1.5		60	99.56	0.684
Retention time	5.0236 ± 0.428%	%RSD ≤ 0.5				
Theoretical plate	3859.833 ± 0.876%	≥ 2000				

Test	Spiked level (%)	Type		Mean % Recovery	RSD (%)
Precision	100	Intra-day		101.083	0.902
		Inter-day	Day-1	101.192	0.853
			Day-2	101.500	1.290
			Day-3	102.145	1.665

Test	**Type**	**Mean % Recovery**	**RSD (%)**
Ruggedness	Analyst-1	101.727	0.847
	Analyst-2	101.663	1.039

Test	**Parameter**	**Variations**	**Amount (µg/ml)**	**Mean % Recovery**	**RSD (%)**
Robustness	Flow rate (ml/min)	0.9	50	100.976	1.024
		1.0	50	100.559	1.338
		1.1	50	102.538	1.081
	Mobile phase (Methanol: water)	45:55	50	100.559	1.336
		40:60	50	101.786	0.392
		35:65	50	101.346	0.605
	Wavelength	349.4 nm	50	100.790	1.420
		344.4 nm	50	101.01	0.650
		339.4 nm	50	100.520	0.590

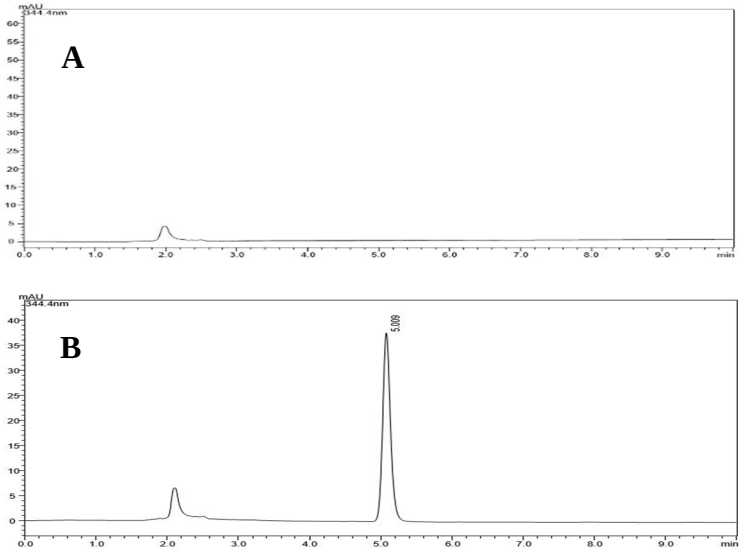

Figure 5. Chromatograms for (A) blank and (B) montelukast sodium standard solution (50 µg/ml).

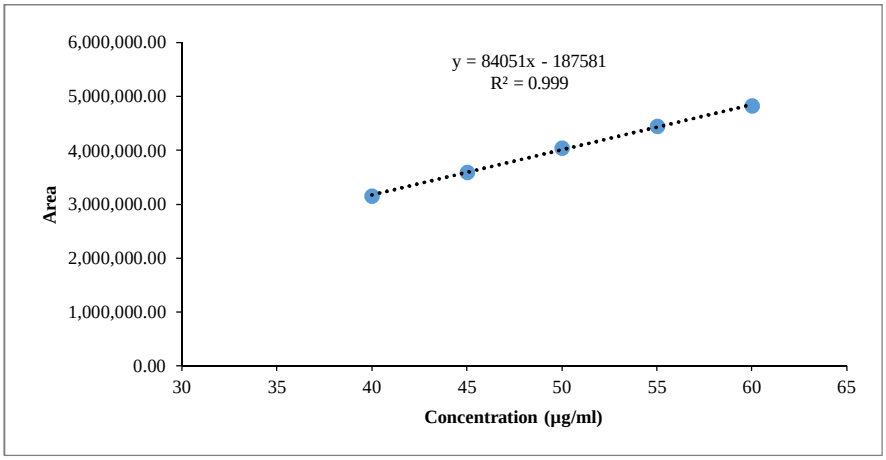

Figure 6. Calibration curve of montelukast sodium constructed by the RP-HPLC method.

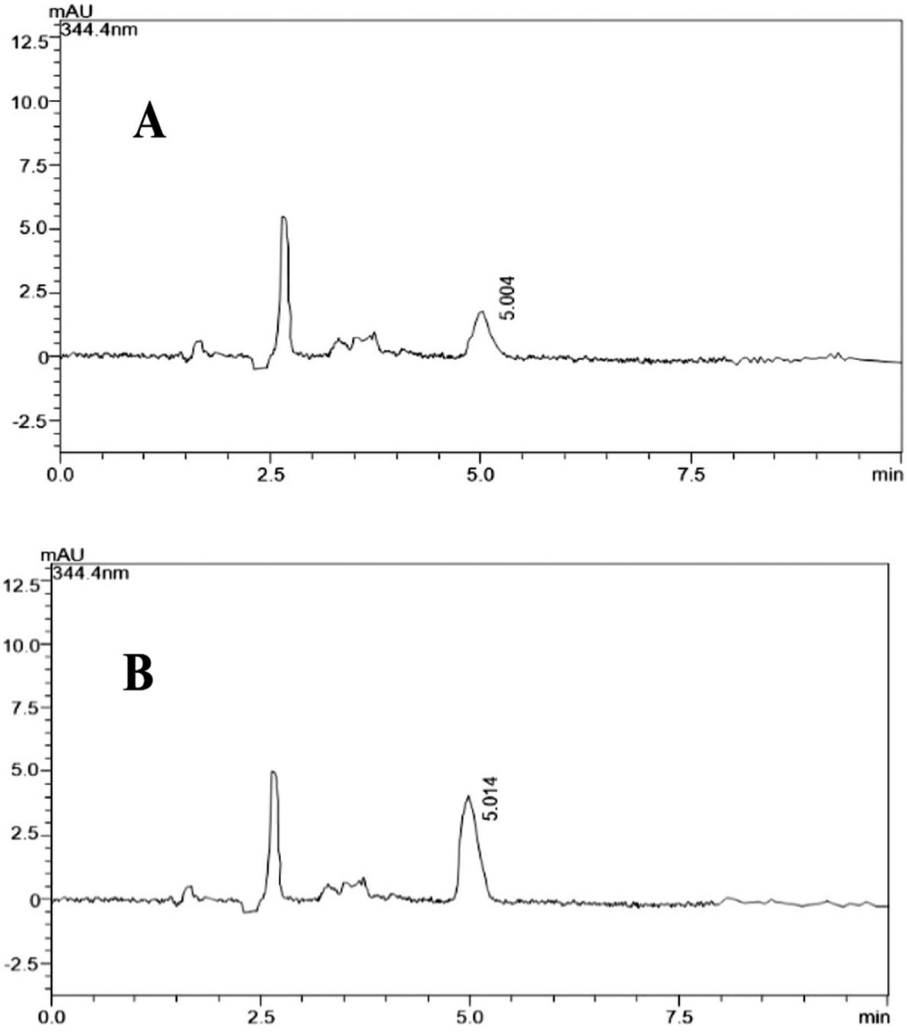

Figure 7. Chromatograms for sensitivity studies. (A) LOD (0.15 µg/ml) and (B) LOQ (0.50 µg/ml).

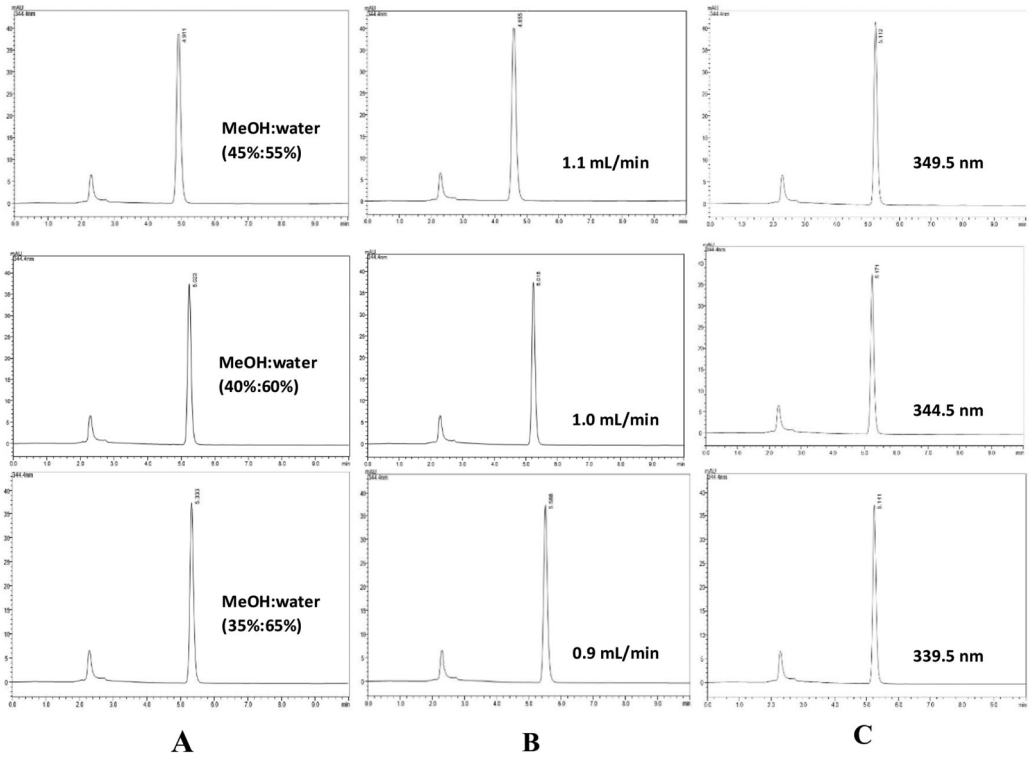

Figure 8. Chromatograms for robustness studies: (A) chromatograms for different % of methanol, (B) chromatograms for different flow rates and (C) chromatograms for different wavelengths.

Validation of the UV method. The system suitability test, evaluated using six replicate measurements of a 16 µg/ml montelukast sodium working standard solution, yielded a mean absorbance of 0.406 ± 1.71 (%RSD). The absence of interfering absorbance in the blank spectrum confirmed the method's specificity within the UV region. The method demonstrated linearity from 2-25 µg/ml, with a calibration curve y = 0.0353x + 0.0318 (R^2 = 0.997), demonstrating acceptable linearity (Figure 9). Recovery studies were used to confirm the accuracy of the method. The results ranged from 98.64 ± 0.225% to 101.834 ± 0.078%. Inter-day and intra-day analyses were carried out to assess precision, demonstrating consistent reproducibility (Table 7). The method's detection limits were established via a LOD (0.556 µg/ml) and LOQ (1.685 µg/ml), which confirmed method's sensitivity. Ruggedness studies between two analysts revealed %RSD values ranging from 0.261% to 0.728%, indicating that the method is rugged. Robustness was investigated by altering the detection wavelength (±5 nm). The method is found to be highly robust even with the changes in method's parameter, with the % RSD of the recovery analysis ranging from 0.078 to 0.876.

Comparative analysis between the developed HPLC and UV methods. The statistical analyses (*t*-test) of validation parameters (linearity, accuracy, precision, robustness, and ruggedness studies) produced *p*-values higher than the significance level ($p>0.05$) for each of the parameters. This suggests that there were no significant differences between the validation parameters of the two methods (Table 8). Thus, the findings prove that both methods are appropriate for regular analysis of montelukast sodium. RP-HPLC would be the chosen method if great sensitivity, versatility, and the capacity to manage a broad concentration range are needed. A simple and affordable drug analysis solution can be obtained using a UV spectrophotometric method that is comparable to the RP-HPLC approach.

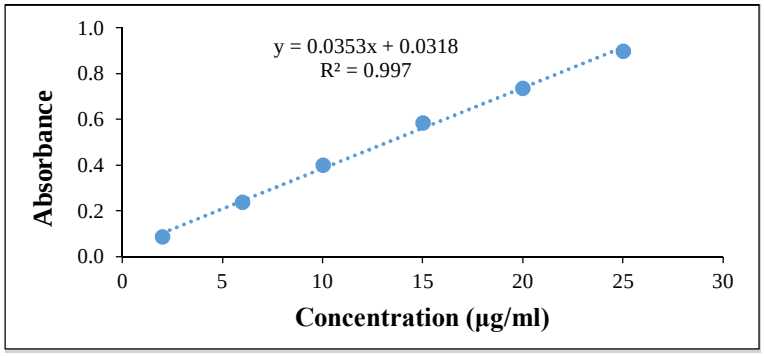

Figure 9. Calibration curve for montelukast sodium constructed by UV spectroscopic method.

Table 7. Validation of the UV method.

Test	Amount (µg/ml)			Mean % Recovery	RSD (%)
Accuracy	16			101.834	0.078
	20			98.64	0.225
	24			101.663	0.171
Test	**Spiked level (%)**	**Type**		**Mean % Recovery**	**RSD (%)**
Precision	100	Intra-day		101.285	1.010
		Inter-day	Day-1	100.258	0.262
			Day-2	99.154	0.294
			Day-3	101.909	0.675
Test	**Type**			**Mean % Recovery**	**RSD (%)**
Ruggedness	Analyst-1			101.258	0.261
	Analyst-2			99.243	0.728
Test	**Parameters**	**Variations**	**Amount (µg/ml)**	**Mean % Recovery**	**RSD (%)**
Robustness	Wavelength	349.4 nm	16	100.263	0.876
		344.4 nm	16	100.278	0.078
		339.4 nm	16	101.154	0.225

Table 8. Comparative analysis between the developed HPLC and UV methods.

Parameters	RP-HPLC	UV	t-test	Remarks
Linearity and range	R^2 values			Both RP-HPLC and UV spectrometric methods had acceptable R^2 values.
	0.999	0.997		
	Range (µg/ml)			
	40-60	2-25		
Accuracy	% Recovery (Mean±%RSD)		**P-value**	Both methods are accurate.
	99.568 ± 1.03	101.834 ± 0.078		
	100.713 ± 0.977	98.64 ± 0.225	0.53	
	99.56 ± 0.684	101.663 ± 0.171		
Precision	Intra-day % Recovery (Mean±%RSD)		**P-value**	Both methods are precise.
	101.083 ± 0.902	101.285 ± 1.01		
	Inter-day % Recovery (Mean±%RSD)			
	101.192 ± 0.853	100.258 ± 0.262	0.249	
	101.500 ± 1.290	99.154 ± 0.294		
	102.145 ± 1.665	101.909 ± 0.675		
Sensitivity	LOD (µg/ml)			The developed methods can be used to analyze minute levels of drug concentration.
	0.15	0.556		
	LOQ (µg/ml)			
	0.50	1.685		
Ruggedness	% Recovery by analyst-1 (Mean±%RSD)		**P-value**	Both methods are rugged.
	101.727 ± 0.847	101.258 ± 0.262		
	% Recovery by analyst-2 (Mean±%RSD)		0.288	
	101.663 ± 1.039	99.2433 ± 0.728		
Robustness	% Recovery (Mean±%RSD)		**P-value**	Both methods are robust.
	100.72 ± 0.65	99.866 ± 0.322	0.187	

CONCLUSION

An RP-HPLC method for accurate montelukast sodium quantitation was successfully developed and validated using a QbD approach. A validated UV spectroscopic method has also been developed for the assay of the drug. This study found that the RP-HPLC and its analogous UV methods were reliable, precise, accurate, and specific. Both analytical techniques are suitable and reliable for quality control applications involving montelukast sodium.

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Conflict of Interests

The authors declare no conflict of interest.

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