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Original Article

A Stability Indicating RP-HPLC Method for Simultaneous Estimation of Acebrophylline, Montelukast, and Fexofenadine in Bulk and Pharmaceutical Dosage Forms

Sreedevi Adikay*, Mounika Bhavanasi, Sai Sruthi Kaveripakam

Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, INDIA.

ABSTRACT

Introduction: The goal of the current work is to create an RP-HPLC method for the quantitative analysis of Acebrophylline, Montelukast, and Fexofenadine in Pharmaceutical dosage form. **Materials and Methods:** Chromatographic separation of Acebrophylline, Montelukast and Fexofenadine was executed on Waters Alliance-e2695 by using Hyper clone 5µ BDS C_{18} 130A (250 x 4.6mm) column and the mobile phase consisting of Methanol: Ammonium formate adjusted to pH-6 and ortho phosphoric acid (70:30). The flow rate: 1.0 mL/min, Column temperature: 25°C and detection wavelength 268nm utilizing a photodiode array detector. **Results and Discussion:** According to ICH criteria, the new approach was validated, and forced degradation tests are also carried out. The procedure is effective for the precise identification and quantification of the three medicines in relation to all of the revealed validation factors. The technique also shows to be suitable for identifying chemical degradation. **Conclusion:** Therefore, the approach created can be utilized for quality control and routine laboratory analysis of these particular medications.

Keywords: RP-HPLC, Acebrophylline, Montelukast, Fexofenadine, Forced degradation.

Correspondence:
Prof. Sreedevi Adikay
Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati-517502, Andhra Pradesh, INDIA.
Email id: sreedeviadikay8@gmail.com

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INTRODUCTION

Allergic rhinitis is an allergic infection and inflammation of nasal airways. It occurs when immune system over react to allergens such as dust, insects, chemical particles, pollens, etc. which response body causing itchy, watery eyes, sneezing, rhinorrhoea, nasal congestion, and other similar symptoms.[1] Many drugs and their combinations are being used to treat allergic infections.

Acebrophylline is chemically 4-[(2-amino-3, 5-dibromophenyl) methyl amino] cyclohexan-1-ol; 2-(1, 3-dimethyl-2, 6-dioxopurin-7-yl) acetic acid.[2] Acebrophylline hinders intracellular phosphodiesterase along with ease bronchial muscles relaxation by elevating cAMP levels. Acebrophylline is a mucolytic and bronchodilator and applied in the treatment and prevention of pulmonary diseases.[3] The chemical structure of Acebrophylline is shown in (Figure 1a).

Montelukast (2-[1-[[(1*R*)-1-[3-[(*E*)-2-(7- chloroquinolin-2-yl) ethenyl] phenyl]-3- [2-(2-hydroxy propan-2-yl) phenyl] propyl] sulfanyl methyl] cyclo propyl] acetic acid) is employed in the treatment for asthma and seasonal allergies.[4] The chemical structure of montelukast is shown in (Figure 1b).

Fexofenadine (2-[4-[1-hydroxy [hydroxyl (diphenyl) methyl] piperidin-1-yl] butyl] phenyl]-2-methyl propanoic acid) that treats allergic reactions such conjunctivitis, hay fever, eczema, and reactions to insect bites and stings as well as food allergies.[5] The chemical structure of fexofenadine as (Figure 1c).

Among various therapeutic approaches in the impairment of allergic disorders, Acebrophylline, Montelukast, Fexofenadine is a combination drug therapy used in the treatment of inflammation in the airways in nose, and reduces symptoms of allergies. Upon wide literature survey it revealed that the Acebrophylline, Montelukast and Fexofenadine is calculated either alone or when used with additional medications by UV-spectrophotometry,[3] in biological fluid by RP-HPLC,[6] LC/MS, LC/MS/MS,[7] RP-UPLC methods.[8] According to a literature review, there hasn't been any method described for a stability-indicating RP-HPLC method enabling simultaneous analysis of Acebrophilline, Montelukast, and Fexofenadine.

Hence in this research, the study is designed for the Method development and validation of a stability indicating RP-HPLC method for simultaneous estimation of Acebrophylline, Montelukast and Fexofenadine bulk and pharmaceutical dosage

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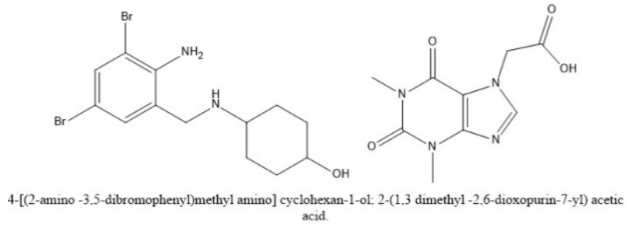

Figure 1a: Molecular structure of Acebrophylline.

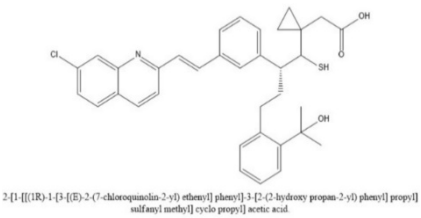

Figure 1b: Molecular structure of Montelukast.

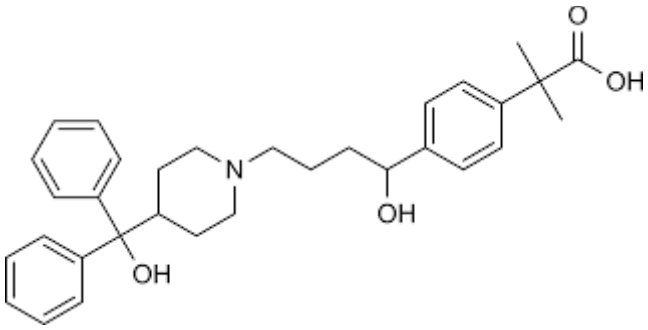

Figure 1c: Molecular structure of Fexofenadine

Figure 1: Molecular structure of Acebrophylline, montelukast and fexofenadine.

form. According to ICH recommendations, the suggested approach has been validated.

MATERIALS AND METHODS

Instrumentation

HPLC instrument used was alliance model, manufactured by Waters, e 2695-Empower software 2.0 versions, pH meter (Eutech), weighing balance (Sartouris), UV-vis-spectrophotometer (UV-1700), Ultra sonicator of model UCA 701 (Unichrome).

Chemicals and reagents

Acetonitrile (Manufactured by Rankem), water (Milli Q) (In house production), methanol (Manufactured by rankem), Ortho Phosphoric Acid (OPA) (Analytical reagents), Ammonium formate (Manufactured by Rankem).

Preparation of buffer solution

0.315 g of Ammonium format was dissolved in 1 Litre HPLC water, pH-6 adjusted with Ortho Phosphoric Acid (OPA) and passed using 0.45µ nylon filter.

Determination of wave length (λ_{max})

The wavelength of maximum absorption of the solution of the drugs in mixture of Methanol: Ammonium formate adjusted to pH-6 and Ortho phosphoric acid (70:30) were scanned utilizing photo diode detector within the wavelength region of 200-400 nm.

Chromatographic conditions

Column C_{18} (250 x 4.6mm, 5µ) was employed as stationary phase. An isocratic operating mode for the chromatographic apparatus was chosen, and the mobile phase was Methanol: Ammonium formate adjusted to pH-6 and ortho phosphoric acid (70:30). Flow rate was maintained at 1mL/min at injection volume of 10µL. Run time: 12 min. Temperature: 25°C the mode of separation is isocratic mode. Detection wavelength of 268nm was employed.

Preparation of Standard Stock Solution

To make the standard stock solution, fexofenadine (12 mg) and Acebrophylline (20 mg) were precisely weighed before being placed to a 10 mL clean, dry volumetric flask. The diluent (50:50 acetonitrile and water) was introduced, sonicated, and the volume was adjusted with the same solvent (Solution 1). An even further 10 mg of montelukast is added to a volumetric flask-10 mL, along with a diluent and sonication to completely dissolve it. The volume was then topped off with the diluent. (Solution 2.) 1mL of the Montelukast solution was pipetted out into above solution 1 then diluted to the appropriate amount (Stock solution). Additional 1 mL of the aforementioned stock solutions were pipetted into a volumetric flask of 10 mL and diluted with diluent until it meets the required level (200ppm of Acebrophylline, 10ppm of Montelukast and 120ppm of Fexofenadine).

Preparation of Sample solution

7.23 mg sample was transferred into a 10 mL volumetric flask which is clean and dry, Diluent was added plus sonicated up till 30 min plus centrifuged for 30 min to dissolve solute completely Using the same solvent, and volume should equal the desired amount. Then it is passed via an injection filter (0.45 microns pore size).

Method validation

Following ICH requirements, the evolved method was validated (Q2) and the parameters "specificity, accuracy, precision, linearity, robustness, Limit of Detection (LOD) and Limit of Quantification (LOQ)" were evaluated.[9,10]

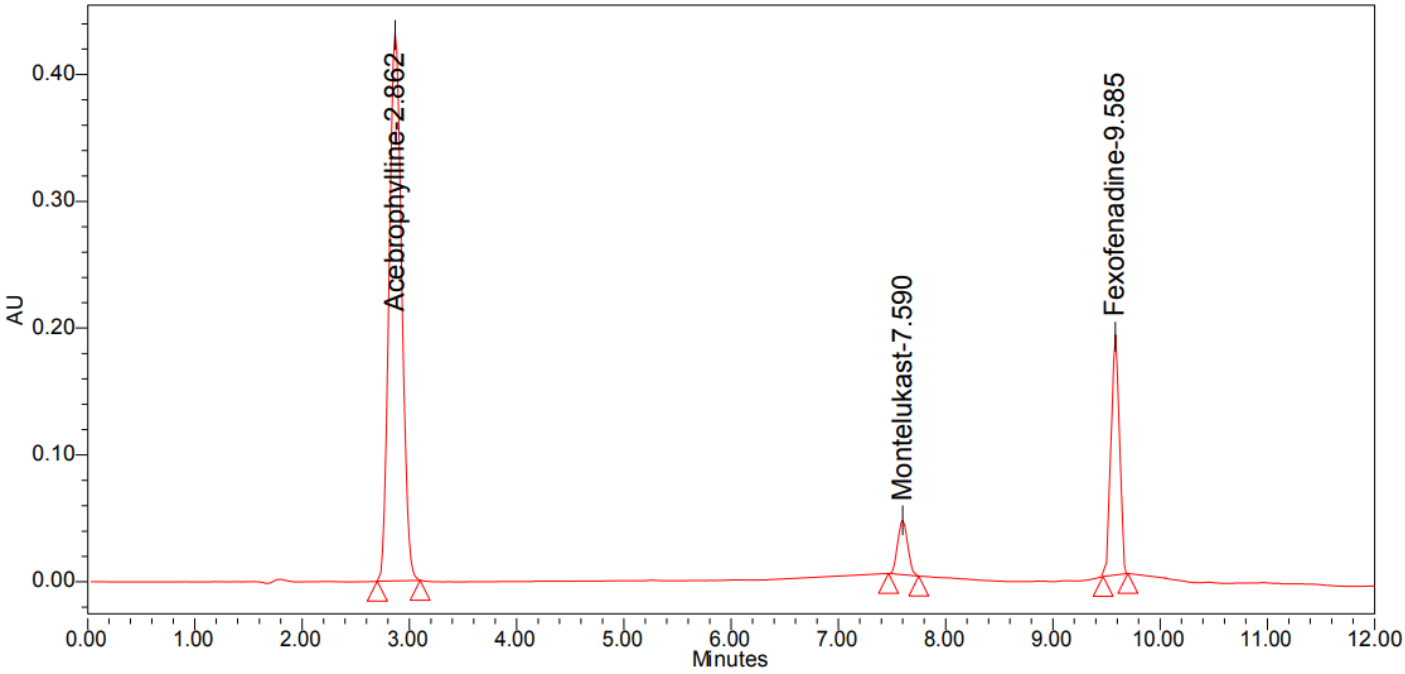

Figure 2: Optimised chromatogram

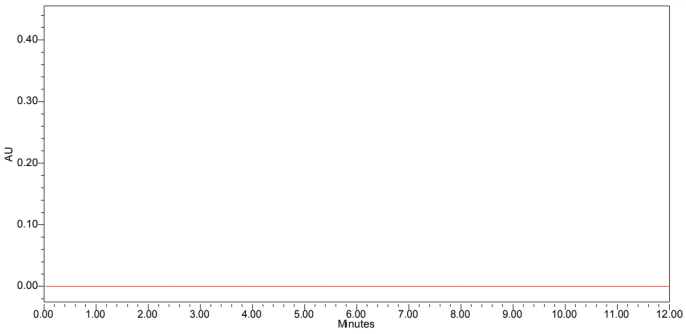

Figure 3a: Chromatogram of blank.

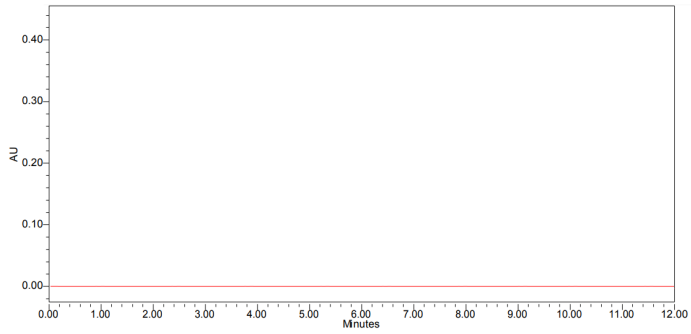

Figure 3b: Chromatogram of placebo.

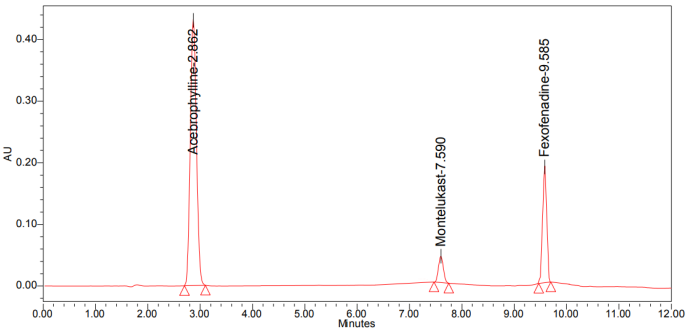

Figure 3c: Chromatogram of standard.

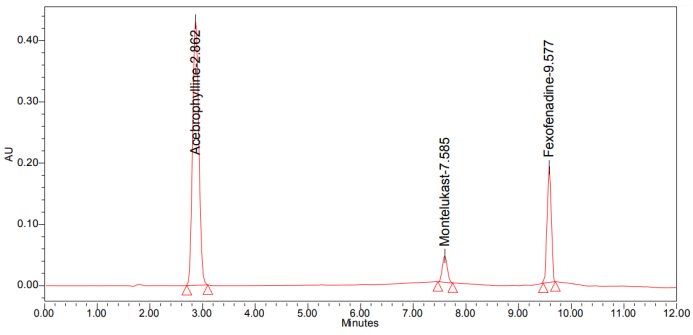

Figure 3d: Chromatogram of sample.

Figure 3: Chromatogram of blank, placebo, standard, and sample.

Evaluation of System suitability

The tailing factor, plate count, and column efficiency were all recorded as system suitability metrics.[11]

Forced degradation studies

Induced degradation studies like acid, hydrolysis (1N HCl), alkali (1N NaOH), peroxide hydrolysis (3% H_2O_2) and reduction ($NaHSO_4$) were carried out as per ICH guidelines.[12]

RESULTS

Method Development and Optimisation

To obtain adequate resolution peaks, a tolerable plate count, and an acceptable tailing factor, numerous trails were created throughout the development of analytical methods. The ideal chromatographic conditions were determined to be the mobile phase with the following parameters: Methanol: Acetonitrile (70:30), flow rate-1 mL/min, injection volume-10 l, run time-12 min, column temperature of 25°C at wavelength (λ), 268 nm. No asymmetric peaks were observed. The method was deemed to be optimized because all of the outcomes were found to be within the acceptable ranges shown here in (Figure 2 and Table 1).

Method Validation

Specificity

Retention times of Acebrophylline, Montelukast, and Fexofenadine were 2.862, 7.590 and 9.585 min respectively. In specificity no interfering peaks were examined at the retention time of the analytes in the blank, placebo, standard and sample. Hence the method was found to be specific. (Figure 3).

Accuracy

The % mean Recovery were obtained as 100.0%, 99.3% and 100.7% for Acebrophylline, Montelukast and Fexofenadine respectively. All these within the acceptable limits and manifesting the accuracy of the method (Table 2).

Precision

Repeatability was deliberated (System, method and intermediate) with six replicate sets and the % RSD was found with in the range (Table 3,4).

Linearity and Range

A regression plot of the peak response area against the level of each drug's concentration demonstrated the method's linearity. It was determined upon the concentration scale of 50-300 µg/mL of Acebrophylline, 2-15 µg/mL of Montelukast and 30-180 µg/mL of Fexofenadine. The correlation coefficient marked down as not more than 0.9999% (acceptance criteria) and hence the method is linear (Figure 4).

Robustness

It was evaluated by changing the flow rate plus organic phase at same wavelength. The results prevailed were summarized in (Table 5) and are indoors the acceptable limits evidencing the method is robust.

LOD and LOQ

The calculated LOD was 0.60, 0.30 and 0.36 and the LOQ was 1.980, 0.990 and 1.188 for the Acebrophylline, Montelukast and Fexofenadine respectively. (Figure 5)

System suitability

According to ICH criteria, all the system suitability metrics were adequate and within the acceptable range and are mentioned in (Table 6).

Degradation Studies

Under various stress situations, forced degradation investigations were carried out. It was observed that the among all findings major degradation was found in peroxide and minimum degradation was on hydrolysis (Table 7).

DISCUSSION

According to ICH (Q2B) requirements, the developed RP-HPLC technique for the estimation of the chosen dosage form was verified.[10] To make sure the performance characteristics of the approach fit the needs of the planned analytical application, the method was validated. To elute the medication, various mobile phase compositions were initially used. On the basis of peak parameters, the mobile phase ratio and flow rate were chosen.

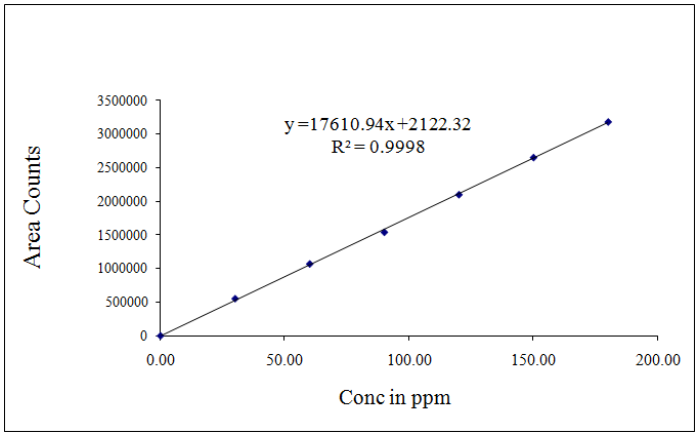

Figure 4: Calibration curve for Acebrophylline, Montelukast.

Table 1: Optimised chromatographic condition

Sl. No	Name	Retention Time	Area	USP Resolution	USP Tailing	USP Plate Count
1	Acebrophylline	2.862	3544621	-	1.09	2733
2	Montelukast	7.590	175138	23.50	1.08	29007
3	Fexofenadine	9.585	2125354	11.38	0.99	52190

Table 2: Accuracy results of Acebrophylline, Montelukast and Fexofenadine by RP-HPLC.

DRUG Name	% Concentration (At specification Level)	% Recovery	Mean Recovery
Acebrophylline	50%	99.0	100.0
	100%	101.0	
	150%	100.0	
Montelukast	50%	100.0	99.3
	100%	100.0	
	150%	98.0	
Fexofenadine	50%	101.2	100.7
	100%	100.9	
	150%	100.0	

Table 3: System precision and method precision table of Acebrophylline, Montelukast and Fexofenadine.

System precision				Method precision		
Sl. No	Area of Acebrophylline Concentration 200(µg/mL)	Area of Montelukast Concentration 10(µg/mL)	Area of Fexofenadine Concentration 120(µg/mL)	Area for Acebrophylline Concentration 200(µg/mL)	Area for Montelukast Concentration 10(µg/mL)	Area for Fexofenadine Concentration 120(µg/mL)
1.	3544621	175138	2125354	3569274	175679	2123194
2.	3552861	177048	2112874	3599553	177432	2132471
3.	3543349	176124	2136034	3594189	173987	2130549
4.	3561143	176612	2135341	3585510	177485	2151383
5.	3574786	175321	2142218	3564115	176066	2162055
6.	3569302	175952	2113021	3523067	175679	2113592
Mean	3557677	176033	2127474	3572618	176055	2135541
S.D	12952.35	733.25	12482.73	27913.405	1303.881	18013.956
%RSD	0.36	0.42	0.59	0.78	0.74	0.84

Table 4: Intermediate precision (Day variation) for Acebrophylline, Montelukast and fexofenadine by RP-HPLC method.

Sl. No.	Area for Acebrophylline		Area for Montelukast		Area for Fexofenadine	
	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
1	3591234	3586217	174786	173548	2134307	2115463
2	3550327	3579354	176367	171963	2153204	2149215
3	3581383	3590365	178542	172241	2134619	2130358
4	3523782	3542871	175143	174629	2143124	2151472
5	3573128	3565423	176357	175702	2165761	2162936
6	3513051	3521668	172987	172196	2111240	2158320
Average	3555484	3564316	175697	173380	2140376	2144627
S.D	31913.328	27071.428	1868.661	1528.854	18630.027	18137.060
%RSD	0.90	0.76	1.06	0.88	0.87	0.85

The development of an HPLC method for the determination of a particular medication combination involved optimizing chromatographic parameters (Acebrophylline, Montelukast, and Fexofenadine). The specificity of the procedure was confirmed by the specificity result, which shows that the analyte peak was pure. Recovery studies determined the method's accuracy. The recovery studies came close to 100%, which is in accordance with the drug's FDA approval.[13] The method and instrument precisions were assessed, and the RSD percentage values were less than 2.0%. The approach that was developed was found to be reliable

Table 5: Robustness results of Acebrophylline, Montelukast and fexofenadine.

Drug name	Parameter	Condition	Retention time	Peak area	Resolution	Tailing	Plate count
Acebrophilline	Flow rate Change(mL/min)	Flow(0.8mL)	3.091	3652641		1.16	2796
		Flow (1mL)	2.862	3544621		1.09	2733
		Flow(1.2mL)	2.548	3356982		1.04	2681
	Organic Phase change	Org (63:37)	3.020	3794138		1.15	2864
		Org (70:30)	2.867	3552861		1.12	2742
		Org (77:23)	2.611	3104138		1.03	2626
Montelukast	Flow rate Change (mL/min)	Flow (0.8m)	8.019	185623	24.01	1.13	29145
		Flow (1mL)	7.591	175138	23.50	1.08	29007
		Flow (1.2m)	7.190	156298	23.95	1.04	28974
	Organic Phase change	Org (63:37)	8.278	197542	26.63	1.09	29536
		Org (70:30)	7.593	177048	23.55	1.06	29015
		Org (77:23)	6.853	138502	21.30	1.01	28760
Fexofenadine	Flow rate Change (mL/min)	Flow (0.8mL)	9.742	2404269	10.00	1.02	52497
		Flow (1ml)	9.582	2125354	11.38	0.99	52190
		Flow (1.2mL)	9.435	1985361	13.07	0.91	52017
	Organic Phase change	Org (63:37)	9.734	2694268	8.66	1.06	52613
		Org (70:30)	9.588	2112874	11.35	0.94	52194
		Org (77:23)	9.371	1748257	14.59	0.90	51956

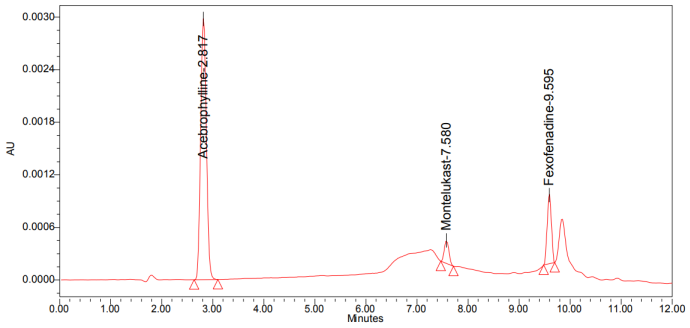

Figure 5a: Chromatogram of LOD.

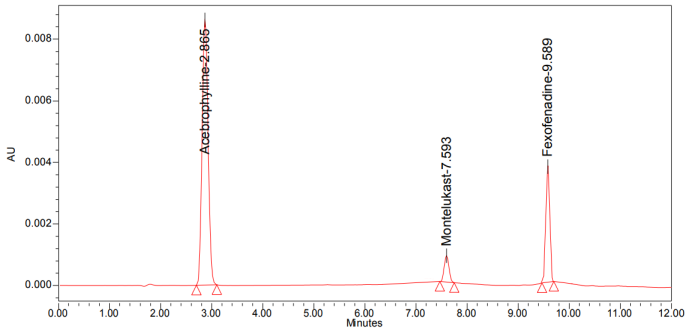

Figure 5b: Chromatogram of LOQ.

Figure 5: Chromatogram of LOD and LOQ.

Table 6: System sutability parameters for Acebrophylline, Montelukast and Fexofenadine.

1	Retention time	2.867	7.593	9.582
2	Plate Count	2751	29154	52180
3	Tailing factor	1.10	1.06	0.94
4	Resolution	--	23.54	11.32
5	%RSD	0.36	0.42	0.59

even when the flow rate, wavelength detection, temperature, and composition of the mobile were changed. The peak regions and retention time did not significantly change. The outcomes of the robustness testing demonstrated that a modest modification of the method's parameters, such as the mobile phase's composition, temperature, flow rate, and wavelength, is reliable within the permitted ranges.[14] The identification and quantification of significantly low drug concentrations were demonstrated by the determination of LOD and LOQ, demonstrating the suitability of this technology for the simultaneous detection and quantification of drug combinations. Additional forced degradation studies indicate that the medication's percentage of degradations was

Table 7: Forced Degradation results for Acebrophylline, Montelukast and Fexofenadine.

% Degradation results	Acebrophylline		Montelukast		Fexofenadine	
	Area	% Degradation	Area	% Degradation	Area	% Degradation
Control	3555730	0	176106	0	2130289	0
Acid	3054763	14.1	158044	10.2	1850970	13.1
Alkali	3072274	13.6	153263	12.9	1861995	12.6
Peroxide	2981715	16.1	150113	14.7	1806507	15.2
Reduction	3132892	11.9	175184	0.5	2090207	1.9
Hydrolysis	3546377	0.3	173412	1.5	2114271	0.8

always determined to be within the allowable range as per ICH guidelines,[15] proving the stability of the established approach. The procedure was discovered to be straightforward, exact, sensitive, quick, reliable, and affordable.

CONCLUSION

Considering the outcomes of precision, linearity, accuracy, recovery, robustness, and specificity the developed stability-indicating RP-HPLC method is ideal for accurate identification and quantitative estimation of selected drugs combination (Acebrophylline, Montelukast, and Fexofenadine). Moreover, the mobile phase and solvents are uncomplicated to prepare, economical and provided good resolution. The investigation also showed that no degradation products or any of the medicinal dosage form's components interfered with the results. Consequently, the established method can be employed for the regular analysis of Acebrophylline, Montelukast, and Fexofenadine in laboratories and quality control purpose.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RP-HPLC: Reverse phase high performance liquid chromatography; **ICH:** International council for harmonisation for technical requirements for pharmaceuticals for human use; **HPTLC:** High performance thin layer chromatography; **RSD:** Relative Standard Deviation; **FDA:** Food and Drug administration.

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