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Research Paper

A STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF ACEBROPHVLLLN MONTALUKAST IN BULK DOSAGE FORMS

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ABSTRACT

The goal of the current work is to create an RP-HPLC method for the quantitative analysis of Acebrophylline, Montelukast, in Pharmaceutical dosage form.

Chromatographic separation of Acebrophylline, Montelukast was executed on Waters Alliance-e2695, by using Hyper clone 5µ BDS C18 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.

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.

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1. INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure[1].

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action[2]. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation.

The present work is undertaken with an objective to develop economical, simple, precise, accurate and reproducible assay method for the estimation of Acebrophylline, Montelukast in bulk and their single and marketed tablet dosage form by RP-HPLC[3].

Drug profie

Acebrophylline belongs to the class of drugs called bronchodilators primarily used to prevent and treat symptoms of asthma and chronic obstructive pulmonary disease (COPD)[4]. Asthma is a chronic (long-term) respiratory condition in which airways narrow, swell, and produce extra mucus, leading to difficulty in breathing. COPD is a group of lung diseases with emphysema (shortness of breath) and chronic bronchitis (inflammation of the lining of bronchial tubes)[5].

Montelukast was first approved for clinical use by the US FDA in 1998 as Merck's brand name Singulair. The medication is a member of the leukotriene receptor antagonist (LTRA) category of drugs[6]. Although capable of demonstrating effectiveness, the use of such LTRAs like montelukast is typically in addition to or complementary with the use of inhaled corticosteroids or other agents in asthma step therapy. Regardless, in 2008-2009, there were FDA-led investigations into the possibility of montelukast to elicit neuropsychiatric effects like agitation, hallucinations, suicidal behaviour, and others in individuals who used the medication[7].

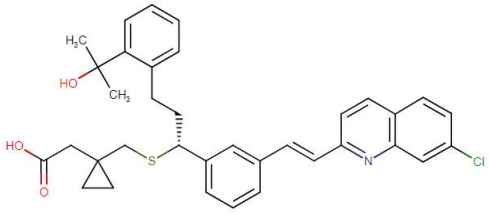


Figure no. 1 Structure of Montelukast

2.MATERIALS AND METHOD

Instrumentation

HPLC instrument used was alliance model, manufactured by Waters, 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[8].

Determination of wave length (λmax)

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[9].

Chromatographic conditions

Column C18 (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 268 nm was employed.

Preparation of Standard Stock Solution

To make the standard stock solution, 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[10].

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)[11].

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)[12].

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.

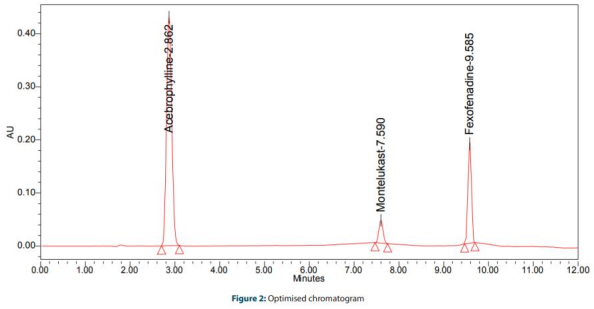


Figure no. 2 Chromatogram

3. RESULT AND DISCUSSION

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.

Specificity

Retention times of Acebrophylline, Montelukast, were 2.862, 7.590 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.

Accuracy

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

Precision

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

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. The correlation coefficient marked down as not more than 0.9999% (acceptance criteria) and hence the method is linear .

Robustness

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

LOD and LOQ

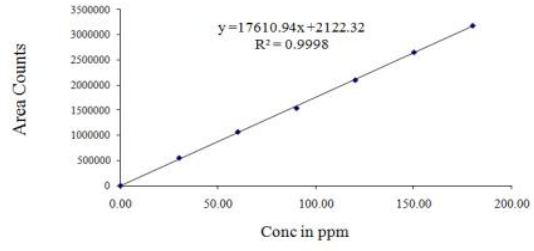
The calculated LOD was 0.60, 0.30 and the LOQ was 1.980, 0.990 for the Acebrophylline, Montelukast respectively.

System suitability

According to ICH criteria, all the system suitability metrics were adequate and within the acceptable range.

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.



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 no.1 Optimised chromatographic condition

DRUG Name	% Concentration (At specificationLevel)	% 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	

Table 2: Recovery results of Acebrophylline, Montelukast by RP-HPLC.

System precision			Method precision	
Sl. No	Area of Acebrophylline Concentration 200(µg/mL)	Area of Montelukast Concentration 10(µg/mL)	Area for Acebrophylline Concentration 200(µg/mL)	Area for Montelukast Concentration 10(µg/mL)
1.	3544621	175138	3569274	175679
2.	3552861	177048	3599553	177432
3.	3543349	176124	3594189	173987
4.	3561143	176612	3585510	177485
5.	3574786	175321	3564115	176066
6.	3569302	175952	3523067	175679
Mean	3557677	176033	3572618	176055
S.D	12952.35	733.25	27913.405	1303.881
%RSD	0.36	0.42	0.78	0.74

Table 3: System precision and method precision table of Acebrophylline, Montelukast by RP-HPLC method

Drug name	Parameter	Condition	Retention time	Peak area	Resolution	Tailing	
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
	Oranic 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
	Oranic 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

Table 4: Robustness results of Acebrophylline, Montelukast

% Degradation results	Acebrophylline		Montelukast	
	Area	% Degradation	Area	% Degradation
Control	3555730	0	176106	0
Acid	3054763	14.1	158044	10.2
Alkali	3072274	13.6	153263	12.9
Peroxide	2981715	16.1	150113	14.7
Reduction	3132892	11.9	175184	0.5
Hydrolysis	3546377	0.3	173412	1.5

Table 5: Forced Degradation results for Acebrophylline, Montelukast

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

5. 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 uncomplicatedto 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[15].

6.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

7. ACKNOWLEDGEMENT

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