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(RESEARCH ARTICLE)

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Analytical method development and validation for the simultaneous estimation of montelukast and Acebrophylline by RP- HPLC method

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Abstract

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Montelukast and Acebrophylline was done by RP-HPLC. The Phosphate buffer was pH 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Montelukast and Acebrophylline were found to be from 100-500 µg/ml of Montelukast and 1-5µg/ml of Acebrophylline . Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Montelukast and acebrophylline. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: Methanol; Phosphate buffer; Inertsil C_{18} column; Montelukast; Acebrophylline

1. Introduction

Pharmaceutical Analysis is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and quantitative measurements of the substances present in bulk and pharmaceutical preparation. The technique employed in quantitative analysis is based upon the quantitative performance of suitable chemical reactions and either measuring the amount of reagent needed to complete the reaction, or ascertaining the amount of reaction product obtained [1]. Quality is important in every product or service but it is vital in medicine as it involves life. Unlike ordinary consumer goods there can be no "second quality" in drugs. 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. Physico-chemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the Physicochemical methods, the most important are optical (Refractometry, Polarimetry, Emission, Fluorescence methods of analysis, Photometry including Photocolorimetry and Spectrophotometry covering UV-Visible and IR regions and Nephelometry or Turbidimetry) and chromatographic (Column, Paper, TLC, GLC, HPLC) methods. Methods such as Nuclear Magnetic Resonance and Para Magnetic Resonance are becoming more and more popular. The combination of Mass Spectroscopy with Gas Chromatography and Liquid Chromatography are the most

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powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation; acid-base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis [2].

2. Materials and Methods

2.1. . Materials

The gift sample Montelukast is from Mylon, and other polymers such as Acebrophylline, KH_2PO_4, Acetonitrile for HPLC, and Ortho phosphoric acid.

2.2. Methods

2.2.1. HPLC Method Development [3]

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30: 70 v/v respectively.

2.2.2. Wave length selection

UV spectrum of 10 µg / ml Montelukast and Acebrophyllinein diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 260. At this wavelength both the drugs show good absorbance.

2.2.3. Optimization of Column

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS(4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow.

2.3. Preparation of buffer and mobile phase

2.3.1. Preparation of Phosphate buffer

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

2.3.2. Preparation of mobile phase

Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration [4].

2.3.3. Diluent Preparation

The Mobile phase was used as the diluent.

2.4. Preparation of the montelukast & acebrophylline standard & sample solution

2.4.1. Standard Solution Preparation

Accurately weigh and transfer 10 mg of Montelukast and Acebrophylline10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents [5].

2.4.2. Sample Solution Preparation

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to

10 mg of Montelukast and Acebrophylline (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3 ml of Montelukaste and Acebrophyllineof the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

2.4.3. Procedure

Inject 20 μL of the standard, sample into the chromatographic system and measure the areas for Montelukast and Acebrophylline peaks and calculate the %Assay by using the formulae.

2.5. System Suitability

Tailing factor for the peaks due to Montelukast and Acebrophyllinein Standard solution Should not be more than 2.0Theoretical plates for the Montelukast and Acebrophylline peaks in Standard solution should not be less than 2000.

Method validation summary

2.6. Precision

2.6.1. Preparation of stock solution

Accurately weigh and transfer 25 mg of Montelukast and Acebrophylline working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3 ml of Montelukast & Acebrophylline of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

2.6.2. Procedure

The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits [6].

2.6.3. Acceptance Criteria

The % RSD for the area of five standard injections results should not be more than 2%.

2.7. Intermediate precision/ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method,

Precision was performed on different day by using different make column of same dimensions.

2.7.1. Preparation of stock solution

Accurately weigh and transfer 25 mg of Montelukast and 10mg of Acebrophylline working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3ml of Montelukast & Acebrophyllineof the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

2.7.2. Procedure

The standard solution was injected for five times and measured the area for all five

injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

2.7.3. Acceptance Criteria

The % RSD for the area of five standard injections results should not be more than2% [7].

2.8. Accuracy

2.8.1. Preparation of Standard stock solution

Accurately weigh and transfer 10 mg of Montelukast and Acebrophylline10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

2.9. Linearity

2.9.1. Preparation of stock solution

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Montelukast and Acebrophylline (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [8].

(Stock solution)

2.10. Limit of detection

2.10.1. Limit of Detection: (For Montelukast)

Preparation of 300µg/ml solution

Accurately weigh and transfer 10 mg of Montelukast working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [9].

2.10.2. Limit Of Detection: (For Acebrophylline)

Preparation of 3µg/ml solution

Accurately weigh and transfer 10mg of Acebrophylline working standard into a 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and diluteup to the mark with diluents [10].

2.11. Limit of quantification

2.11.1. Limit of Quantification (for Montelukast)

Preparation of 300µg/ml solution

Accurately weigh and transfer 10 mg of Montelukast working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

2.11.2. Limit of Quantification: (for Acebrophylline)

Preparation of 3µg/ml solution

Accurately weigh and transfer 10mg of Acebrophylline working standard into a 100mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [11].

2.12. Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a). The flow rate was varied at 0.8 ml/min to 1.2ml/min. Standard solution 300ppm of Montelukast& 3ppm ofAcebrophylline was preparedand analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10% .

Results for actual flow (1.0ml/min) have been considered from Assay standard [12].

3. Results and discussion

3.1. Optimized chromatogram is obtained by following conditions

Trial 1:

- Mobile phase: Water: Methanol (50:50%v/v)
- Column:Xterra C18 (4.6*250mm) 5µm
- Flow rate :1.0 ml/min
- Wavelength: 260 nm
- Column temp : Ambient
- Sample Temp :Ambient
- Injection Volume :10 µl

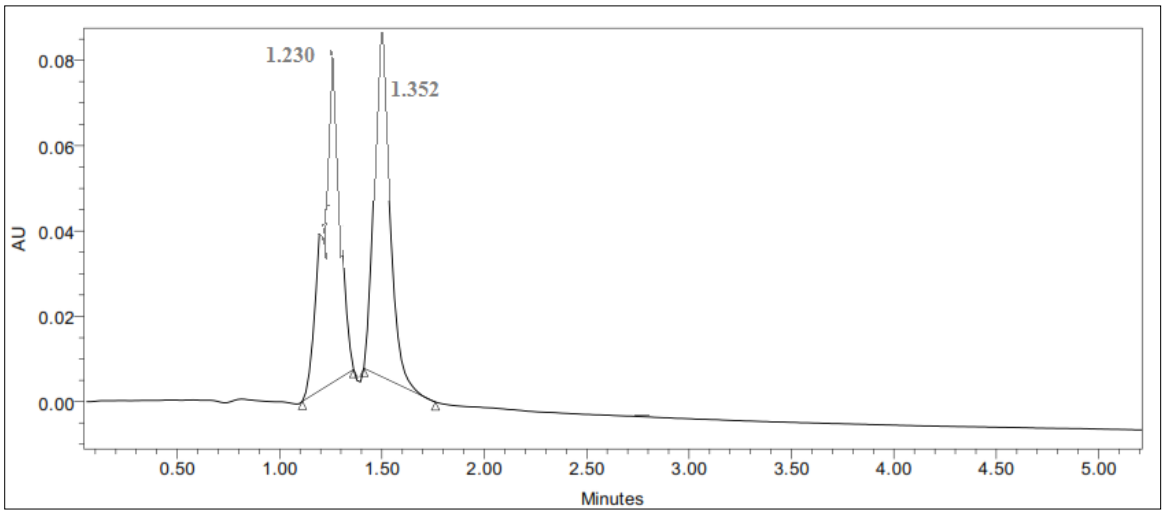

Figure 1 (a) Trial chromatogram for Montelukastand Acebrophylline

3.2. Chromatogram for Montelukastand Acebrophylline

- Column : Inertsil C18 (4.6 x 250mm, 5µm)
- Buffer pH : 3.0.
- Mobile phase :30% buffer 70% Methanol
- Flow rate : 1.0ml per min
- Wavelength : 260 nm
- Temperature : ambient.
- Run time : 10min.

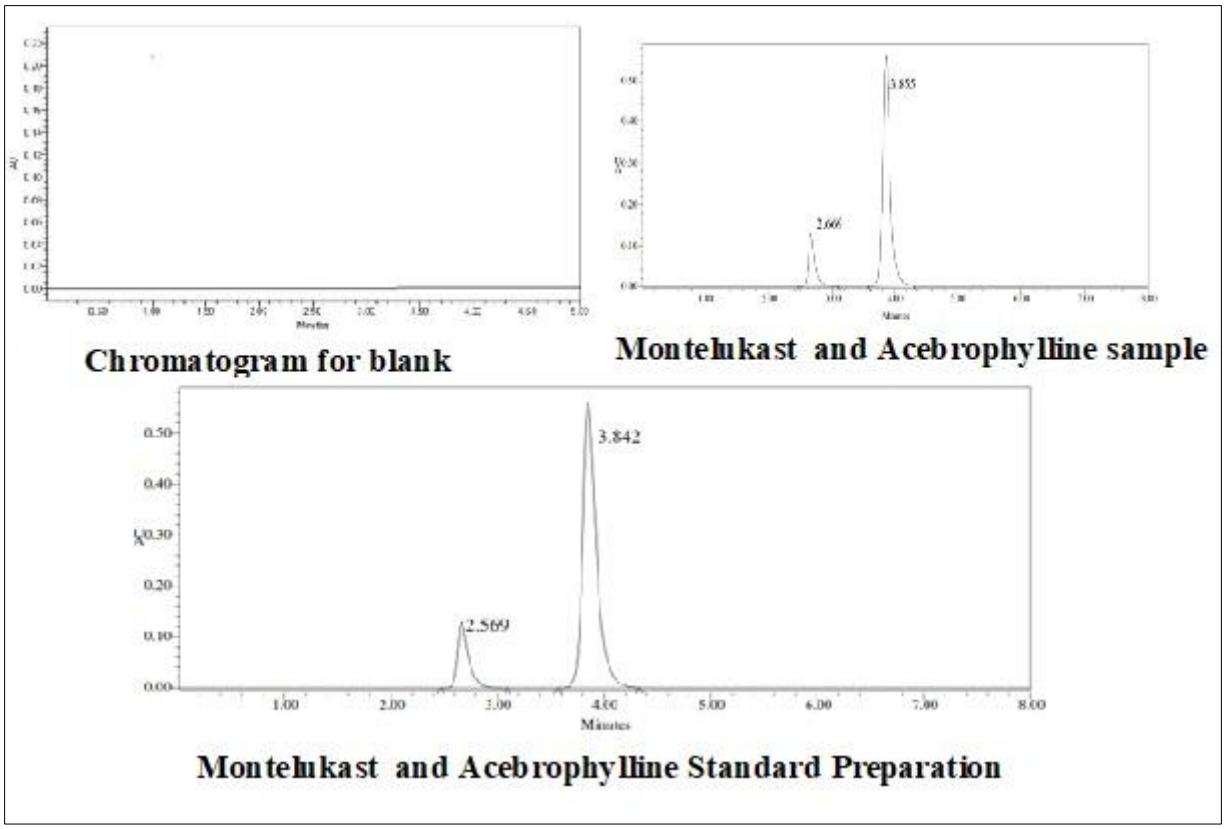

Figure 2 Chromatogram for blank, sample and Standard Preparation

Retention time of Montelukast– 2.569 min

Retention time of Acebrophylline - 3.842 min.

3.3. System suitability

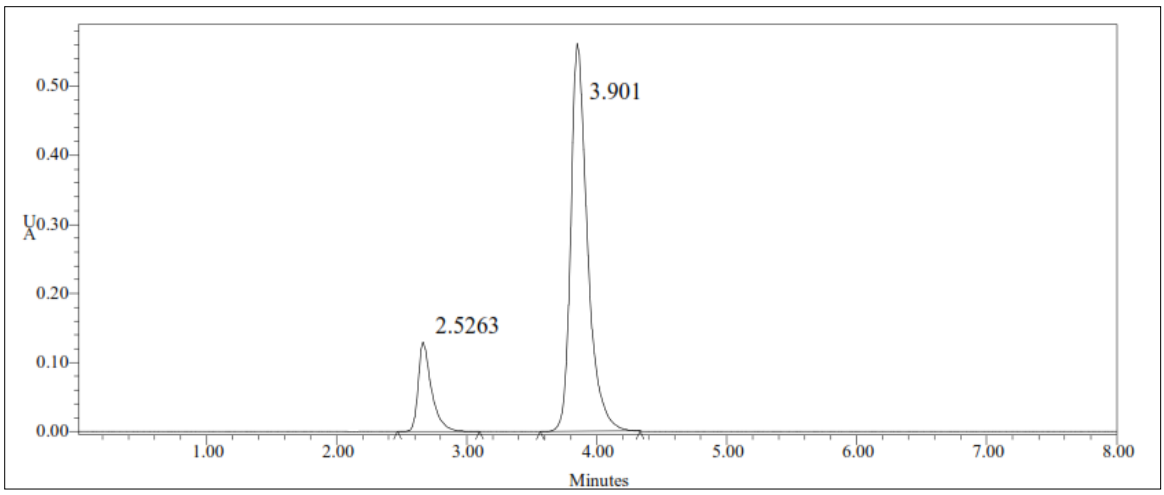

Figure 3 Chromatogram for system suitability

Results

System suitability results

- Tailing factor Obtained from the standard injection is 1.3
- Theoretical Plates Obtained from the standard injection is 4668.7

Results

System suitability results

- Tailing factor Obtained from the standard injection is 1.3
- Theoretical Plates Obtained from the standard injection is 6090.3

Table 1 Results of system suitability parameters for Montelukastand Acebrophylline

Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
Montelukast	2.5	124505	213642		1.2	4673.4
Acebrophylline	3.9	1308495	154566	6 0	1.3	6090.3

- Acceptance criteria
- Resolution between two drugs must be not less than 2
- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.
- Validation parameters

3.4. Precision

Precision of the method was carried out for standard solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Table 2 Results of method precession for Montelukast

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

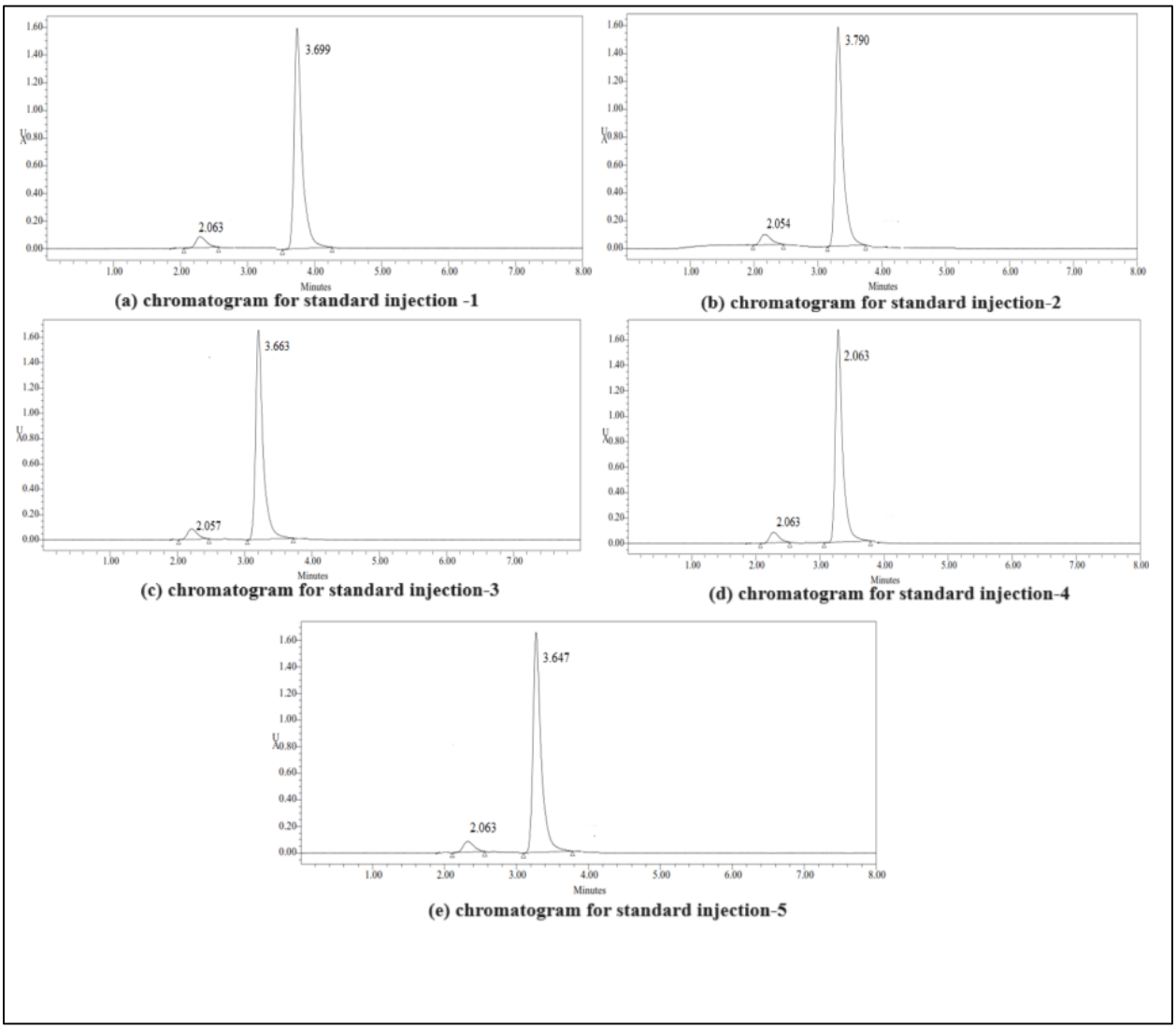

Figure 4 Precision chromatograms for standard injections 1-5

Table 3 Results of method precession for Acebrophylline

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

3.5. Intermediate precession (ruggedness):

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Table 4 Results of Intermediate precision for Montelukast

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 5 Results of Intermediate precision for Acebrophylline

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

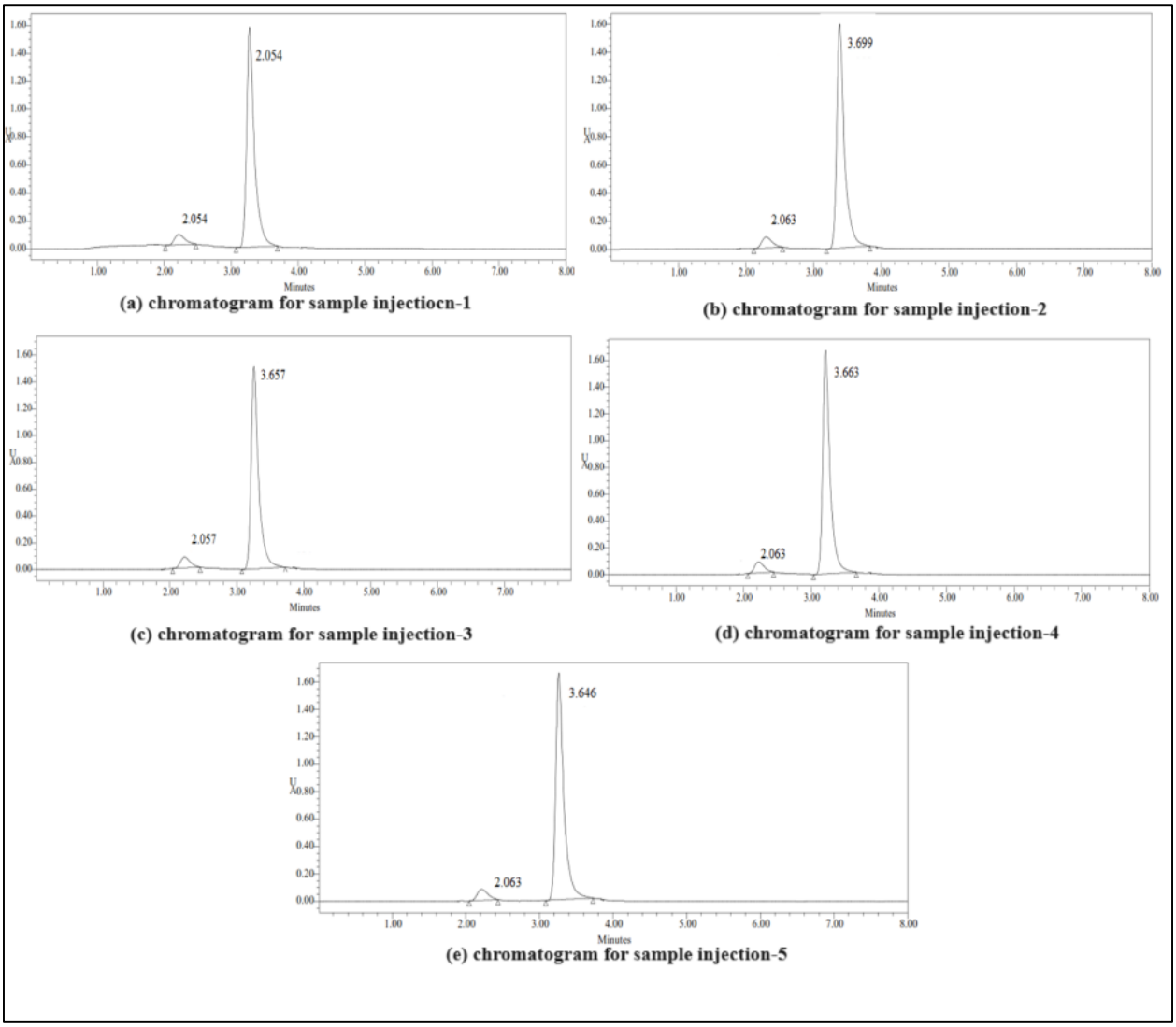

Figure 5 Intermediate precision chromatograms for sample injections 1-5

Acceptance criteria

%RSD of five different sample solutions should not more than 2

The %RSD obtained is within the limit, hence the method is rugged.

3.6. Accuracy

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table 6 Accuracy (recovery) data for Montelukast

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table 7 Accuracy (recovery) data for Acebrophylline

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

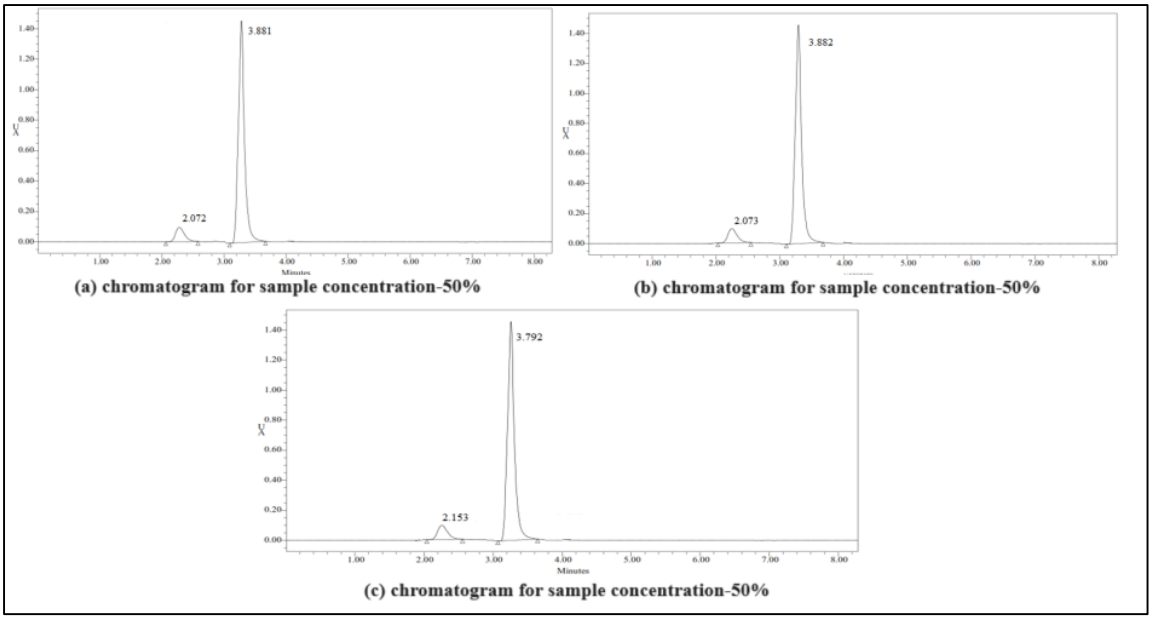

Figure 6 Accuracy chromatograms for sample concentration-50%

Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102.0%.

The percentage recovery was found to be within the limit (97-103%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate

3.7. Linearity

The linearity range was found to lie from 100µg/ml to 500µg/ml of Montelukast, 5µg/ml to 25µg/ml 0f Acebrophylline and chromatograms are shown below.

Table 8 Area of different concentration of Montelukast

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

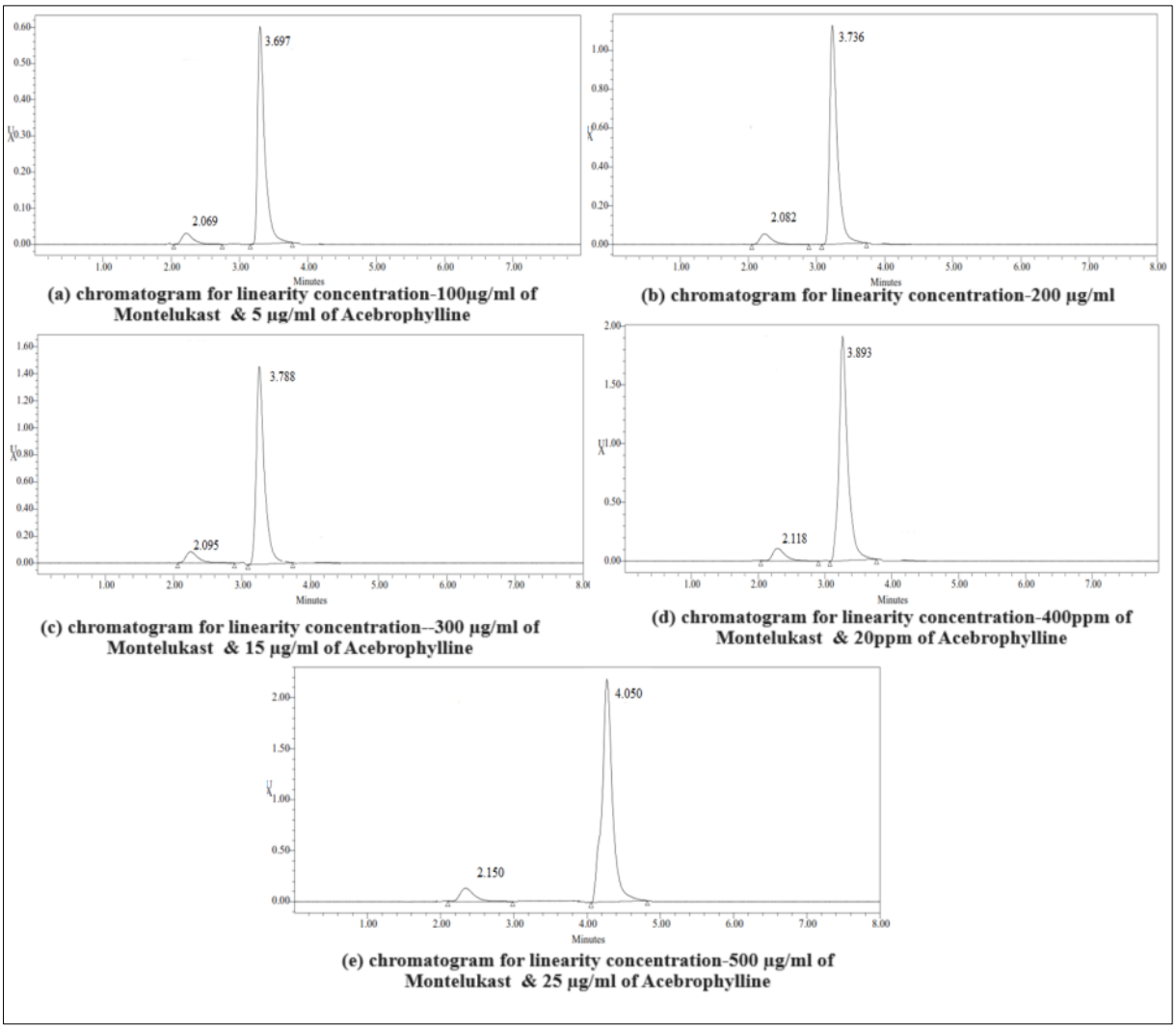

Figure 7 Linearity Chromatograms

3.8. Limit of detection for montelukast and acebrophylline

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio

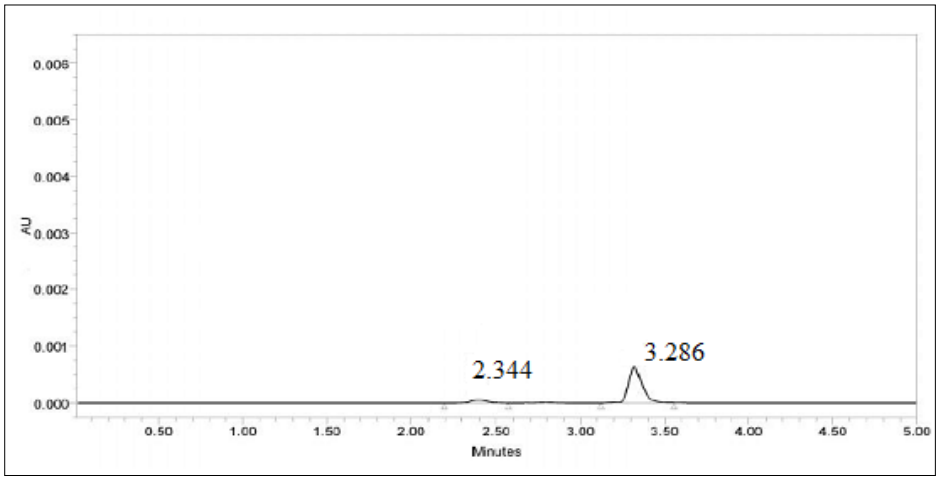

Figure 8 Chromatogram of Montelukast&Acebrophylline showing LOD

Table 9 Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Montelukast	52	152	2.9
Acebrophylline	52	156	3

Signal to noise ratio shall be 3 for LOD solution

The result obtained is within the limit.

3.9. Limit of quantification (LOQ):

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

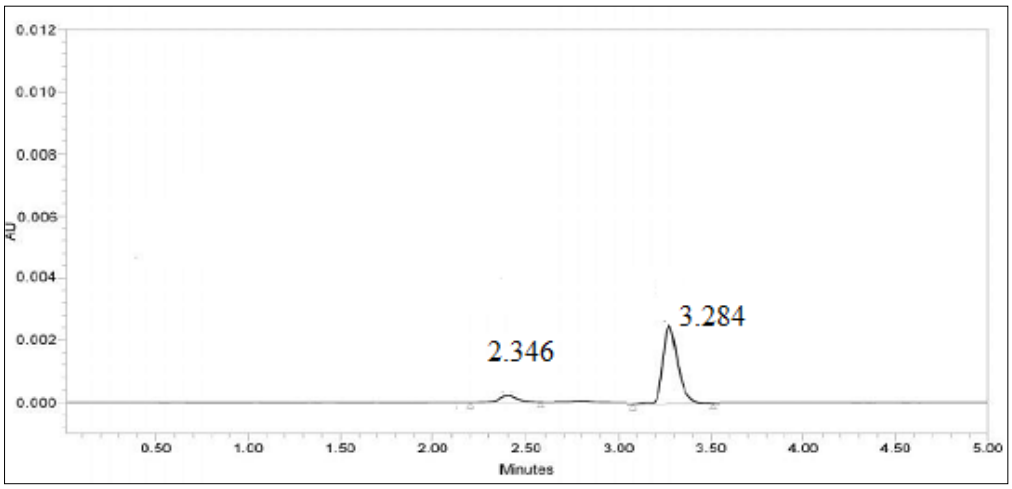

Figure 9 (a) chromatogram of Montelukast&Acebrophylline showing LOQ

Table 10 Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Montelukast	52	522	10.03
Acebrophylline	52	524	10.1

Signal to noise ratio shall be 10 for LOQ solution

The result obtained is within the limit.

3.10. Robustness

The standard and samples of Montelukastand Acebrophylline were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

3.10.1. Variation in flow

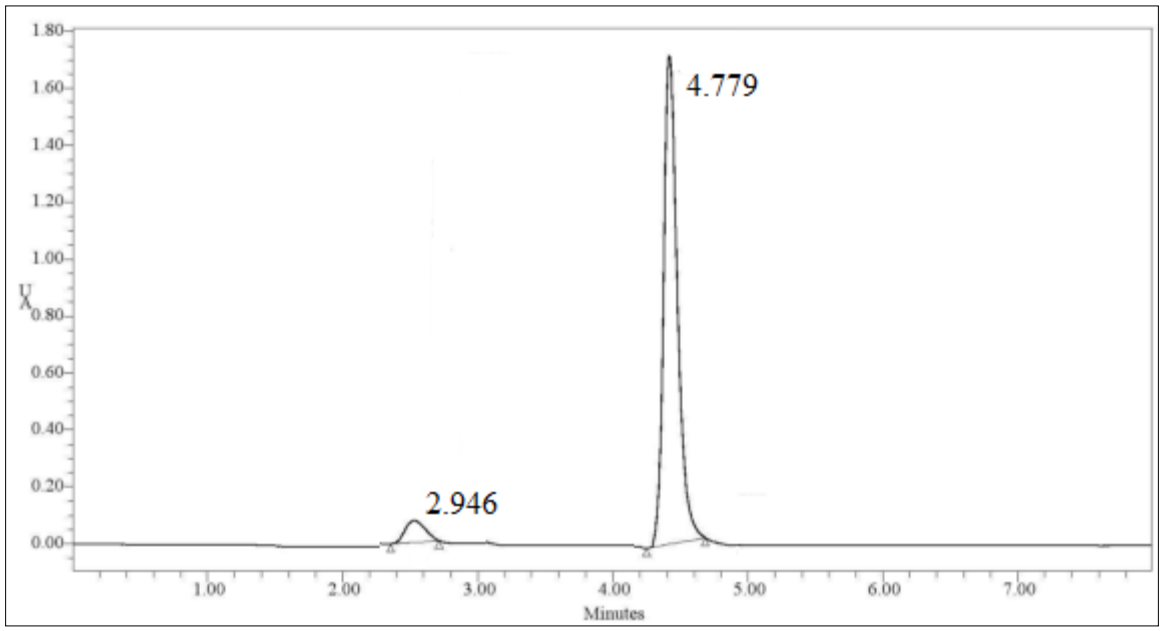

Figure 10 (a) chromatogram showing less flow of 0.6ml/min

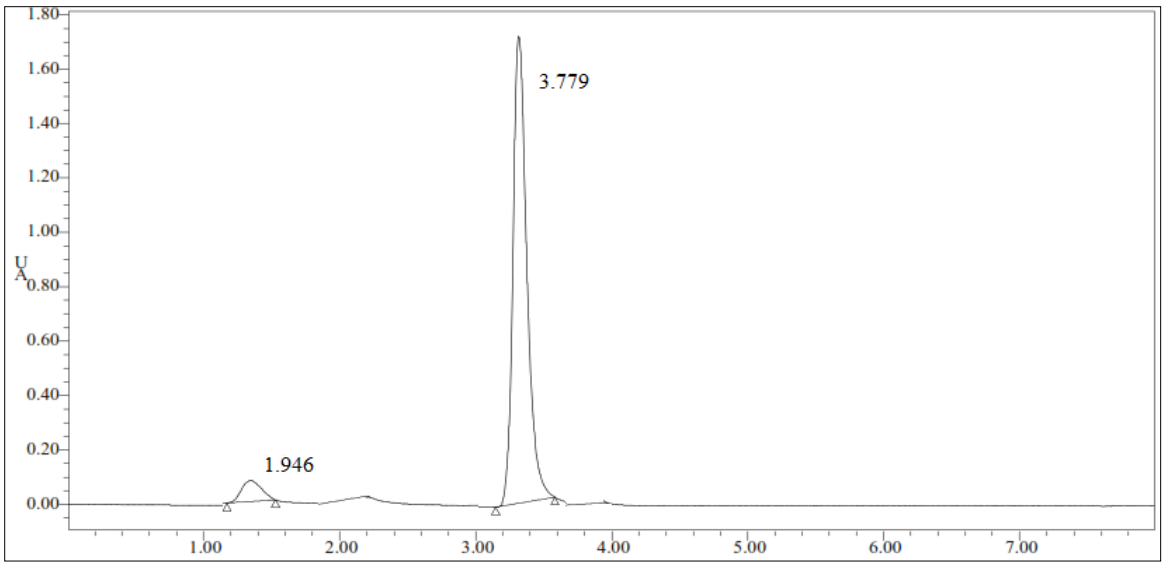

Figure 11 (b) chromatogram showing more flow of 1.0ml/min

Table 11 Flow Rate (ml/min) data for Montelukast

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 12 Flow rate (ml/min) data for Acebrophylline

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table 13 Change in Organic Composition in the Mobile Phase for Montelukast

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 14 Change in Organic Composition in the Mobile Phase for Acebrophylline

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

Conclusion

The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors attest that they have no conflict of interest in this study.

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