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R E S E A R C H A R T I C L E

RP-HPLC Method Development and Validation for Simultaenous Estimation of Montelukast and Fexofenadine Hydrochloride in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

A new method was established for simultaneous estimation of Faxofenadine Hydrochloride Hydrochloride and Montelukast by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Faxofenadine Hydrochloride and Montelukast by using Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was Waters HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.The retention times were found to be 2.399mins and 3.907mins. The % purity of Faxofenadine Hydrochloride and Montelukast was found to be 100.7% and 101.4% respectively. The system suitability parameters for Faxofenadine Hydrochloride and Montelukast such as theoretical plates and tailing factor were found to be 1.3, 5117.5and 1.4, 3877.3 the resolution was found to be 8.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Faxofenadine Hydrochloride and Montelukast was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust and repeatable. LOD value was 2.95 and 3.04 and LOQ value was 9.87 and 10 respectively.

Keywords: Faxofenadine Hydrochloride and Montelukast, RP-HPLC method

A R T I C L E I N F O

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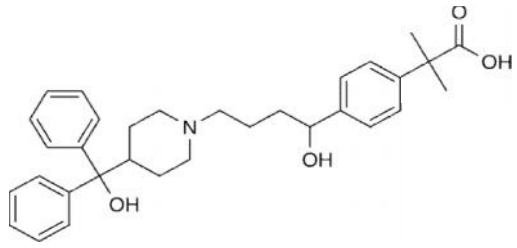
C O N T E N T S

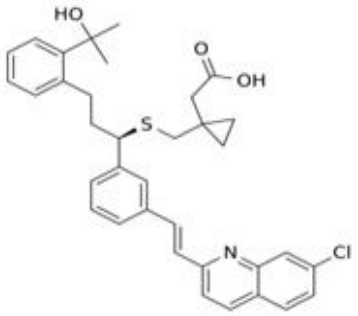
1. Introduction

Fexofenadine, sold under the trade name Allegra among others is an antihistamine pharmaceutical drug used in the treatment of allergy symptoms, such as hay fever and urticaria. Therapeutically, fexofenadine is a selective peripheral H1-blocker.Fexofenadine is classified as a second-generation antihistamine because it is less able to pass the blood-brain barrier and cause sedation, compared to first-generation antihistamines. It has also been called a third-generation antihistamine, although there is some controversy associated with the use of the term.



Fexofenadine

Montelukast (trade name Singulair) is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. Montelukast comes as a tablet, a chewable tablet, and granules to take by mouth. Montelukast is usually taken once a day with or without food.[4] Montelukast is a CysLT1 antagonist; it blocks the action of leukotrieneD4 (and secondary ligands LTC4 and LTE4) on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation.



Montelukast

2. Materials and Methods

HPLC Alliance Waters 2695 Waters Empower UV double beam UV 3000 UV Win 5 Lab India Digital weighing pH meter Ultra sonicator Suction pump. Faxofenadine Hydrochloride and Montelukast, Potassium dihydrogen, Acetonitrile, Methanol, Water.

Chromatographic conditions

Trial-5:

Chromatographic conditions:

Column : Xterra C18 5µm **(4.6*250mm)**

Mobile phase ratio: **P**hosphate buffer (0.05M)

pH 4.6: ACN (55:45%v/v)

Detection wavelength: 255nm

Flow rate : 1ml/min

Injection volume : 20µl Column

Temperature : Ambient

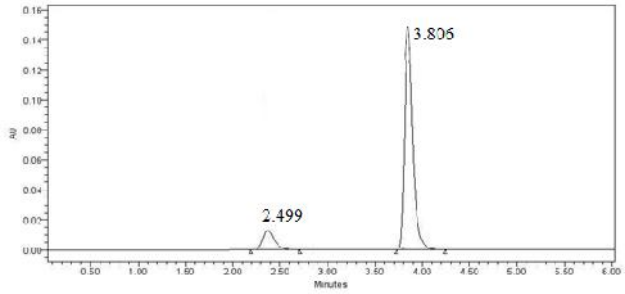


Fig. 1: Chromatogram of Trail-5

Preparation of Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately 5mg of Montelukast and 5mg of Faxofenadine Hydrochloride working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock Solution). Further 3ml and 0.3ml of the above Montelukast and Faxofenadine Hydrochloride stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately 10mg of Montelukast and 10mg of Faxofenadine Hydrochloride working standard were weighed and transferred into a 10mL, 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock Solution). Further 3ml and 0.3ml of the above Montelukast and Faxofenadine Hydrochloride stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately 15mg of Montelukast and 15mg of Faxofenadine Hydrochloride working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock Solution). Further 3ml and 0.3ml of the above Montelukast and Faxofenadine Hydrochloride stock solution were pipetted into a 10ml volumetric flask and diluted up to the mark with diluent.

Method Validation

Accuracy:

Preparation of standard solution (Faxofenadine Hydrochloride and Montelukast): Accurately weighed 10 mg of Montelukast and 10mg of Faxofenadine Hydrochloride working standard were transferred into a 10mL and 100ml of clean dry volumetric flasks. About 7mL and 70ml of Diluents are added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Precision

A) Repeatability:

Preparation of standard stock solution:

Accurately 10 mg of Montelukast and 10mg of Faxofenadine Hydrochloride working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Intermediate Precision (Ruggedness):

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

LOD:

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

LOQ:

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y- intercepts of regression lines.

Linearity

Preparation of stock solution:

Accurately 10 tablets were weighed & crushed in mortar and pestle and weight equivalent to 10 mg of Montelukast and Faxofenadine Hydrochloride (marketed formulation) sample were transferred into a 10mL clean dry volumetric flask and about 7mL of Diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Range:

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 1µg-5µg and 100µg- 500µg of Faxofenadine Hydrochloride and Montelukast respectively.

Robustness:

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

System suitability:

5 mg of Faxofenadine Hydrochloride and 500 mg of Montelukast working standard was accuratel y weighed and transferred into a 100ml clean dry volumetric flask and add about 20ml of diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent.

3. Results and Discussion

Linearity:

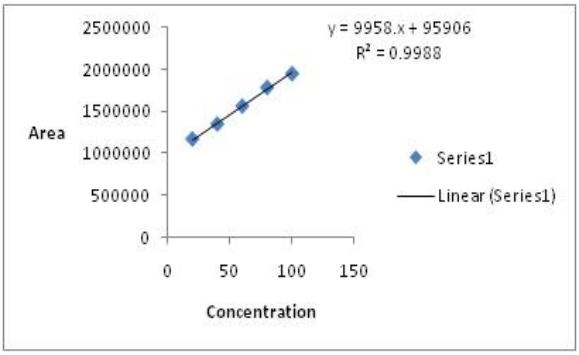


Fig. 2: Calibration curve of Montelukast

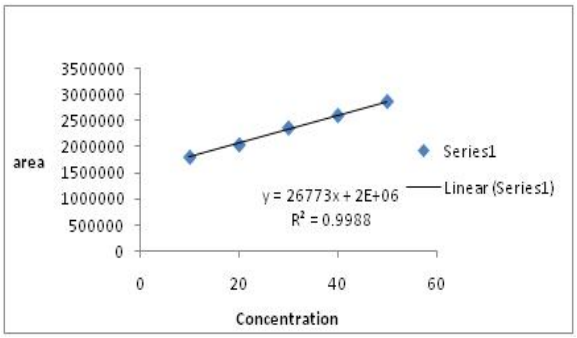


Fig.3: Calibration curve of Faxofenadine Hydrochloride

Robustness

Flow Rate:

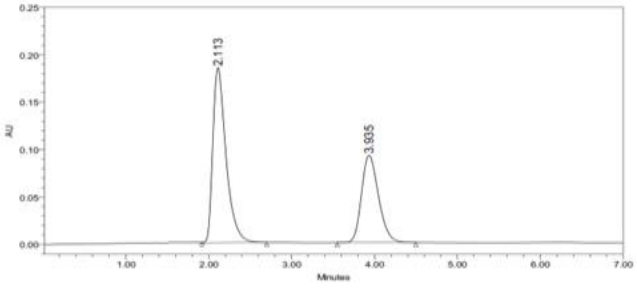


Fig.4: Chromatogram for Robustness more flow

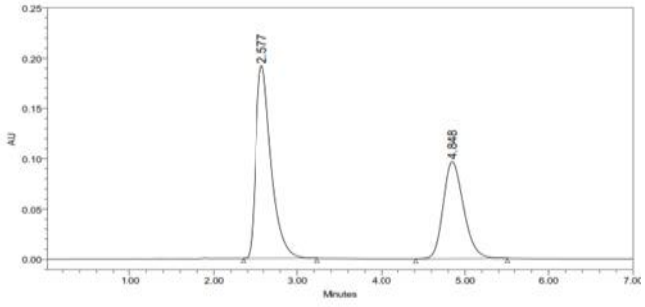


Fig. 5: Chromatogram for Robustness less flow

B) Mobile Phase:

The Organic composition in the Mobile phase was varied from 70% to 60%. Standard solution 300 µg/ml of Montelukast & 3µg/ml of Faxofenadine Hydrochloride was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

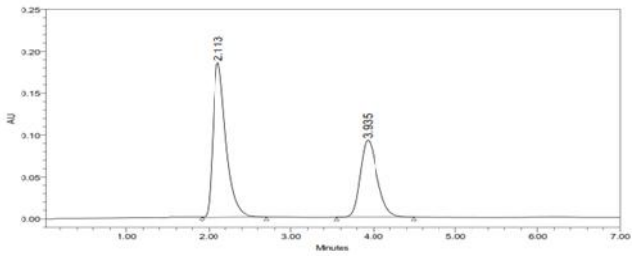


Fig. 7: Chromatogram for Robustness more organic

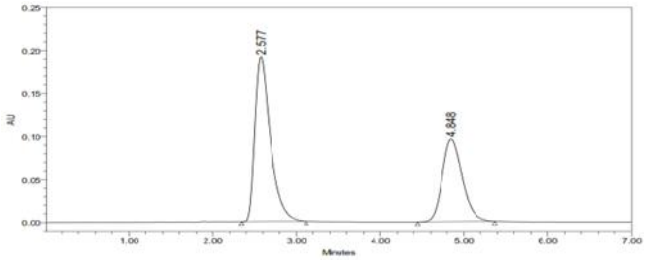


Fig 8: Chromatogram for Robustness less organic

Table 1: Details of Trail-5

S.No	Peak name	R_t	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Faxofenadine Hydrochloride	2.499	945134	156429	5104	1.2	7.1
2	Montelukast	3.806	112541	13839	3787	1.3	

Observation: The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one

Table.2: Results for system suitability of Faxofenadin

Injection	RT(min)	Peak area	TP	TF
1	2.5263	124652	1554.31	1.28
2	2.767	127376	1634.55	1.31
3	2.764	122803	1623.37	1.31
4	2.808	125382	1622.73	1.23
5	2.789	122153	1460.39	1.32
6	2.799	126345	1634.88	1.27
Mean		123634	-	-
SD		631.0	-	-
%RSD		0.6	-	-

Table.3 Results for system suitability of Montelukast

Injection	RT(min)	Peak area	TP	TF
1	3.901	434308	4315.31	1.17
2	4.016	436736	4232.73	1.17
3	4.012	436821	4372.54	1.17
4	4.140	435350	4354.17	1.17
5	4.077	425462	4322.22	1.17
6	4.056	438085	4328.19	1.18
Mean		44531.3	-	-
SD		1257.3	-	-
%RSD		0.3	-	-

Accuracy:

Table 7: Accuracy results of Montelukast

%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
50%	2332744	5	5.10	101.8%	100.5%
100%	3132697	10	9.99	99.9%	
150%	3918997	15	14.9	99.1%	

Table 8: Accuracy results of Faxofenadine Hydrochloride

%Concentration (at specification level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	353867	5	5.0	101.3%	100.0%
100%	4735088	10	9.94	99.4%	
150%	5911798	15	14.8	99.2%	

Precision

i) Repeatability

ii) Intermediate precision (Ruggedness)

Table 9: Accuracy results of Faxofenadine Hydrochloride

Injection	Area
Injection-1	1501417
Injection-2	1486940
Injection-3	1490656
Injection-4	1487329
Injection-5	1490384
Average	1491345
Standard Deviation	5881.4
%RSD	0.39

Injection	Area
Injection-1	2235319
Injection-2	2240678
Injection-3	2249490
Injection-4	2245822
Injection-5	2251694
Average	2244601
Standard Deviation	6656.8
%RSD	0.32

Intermediate precision/Ruggedness

Table 10: Ruggedness results of Montelukast& Faxofenadine Hydrochloride

Injection	Area
Injection-1	2194758
Injection-2	2195700
Injection-3	2196191
Injection-4	2195326
Injection-5	2200951
Average	2196585
Standard Deviation	2496.0
%RSD	0.11

Injection	Area
Injection-1	1456296
Injection-2	1457422
Injection-3	1456513
Injection-4	1454579
Injection-5	1451483
Average	1455259
Standard Deviation	2347.6
%RSD	0.16

Table 11: System suitability results For Montelukast (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	1748.5	1.22
2	1.0	1548.2	1.2
3	1.2	1948.0	1.2

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

System suitability results for Faxofenadine Hydrochloride:

Table 12: System suitability results for Faxofenadine Hydrochloride (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	883.3	1.56
2	1.0	1234.0	1.1
3	1.2	969.2	1.6

4. Conclusion

A new method was established for simultaneous estimation of Faxofenadine Hydrochloride and Montelukast by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Faxofenadine Hydrochloride and Montelukast by using Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.The retention times were found to be 2.399mins and 3.907mins. The % purity of Faxofenadine Hydrochloride and Montelukast was found to be 100.7% and 101.4% respectively. The system suitability parameters for Faxofenadine Hydrochloride and Montelukast such as theoretical plates and tailing factor were found to be 1.3, 5117.5and 1.4, 3877.3 the resolution was found to be 8.0.The analytical method was validated according to ICH guidelines (ICH, Q2(R1)). The linearity study for Faxofenadine Hydrochloride and Montelukast was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Faxofenadine Hydrochloride and Montelukast in API, Pharmaceutical dosage form.

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