International Journal of Medicine and Pharmaceutical Research

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

A Stability Indicating RP-HPLC Method Development and Validation for Simultaneous 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 and Montelukast by RP-HPLC method. The chromatographic conditionsweresuccessfullydevelopedfortheseparationofFaxofenadine and Montelukast by using XterraC185µm(4.6*250mm)column, flow rate was1ml/min, mobile phase ratio was Phosphate buffer (0.05M)pH4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wavelength was 255nm. The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.399mins and 3.907mins. The %purity of Faxofenadine and Montelukast was found to be100.7% and 101.4%respectively.The system suitability parameters for Faxofenadine and Montelukast such as theoretical plates and tailing factor were found to be 1.3, 5117.5and1.4,3877.3the resolution was foundtobe8.0.Theanalyticalmethodwas validated according to ICH guidelines (ICH,Q2(R1)).The linearity study for Faxofenadine and Montelukast was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999and 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 0.5and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87and10 respectively.

Keyword: Faxofenadine and Montelukast, RP-HPLC method

A R T I C L E I N F O

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PAPER-QRCODE

A R T I C L E H I S T O R Y: Received 12 September 2018, **Accepted** 28 Oct 2018, **Available Online**18 January 2019

Citation: *Dr. Gampa Vijay Kumar, et al. A Stability Indicating RP-HPLC Method Development and Validation for Simultaenous Estimation of Montelukast and Fexofenadine Hydrochloride in Bulk and Pharmaceutical Dosage Form. J. Pharm, Biomed. A. Lett., 2019, 7(1): 07-12.*

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.

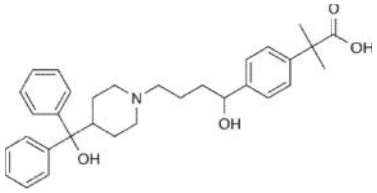

Fig 1: Structure of Fexofenadine

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 broncho constriction otherwise caused by the leukotriene and results in less inflammation.

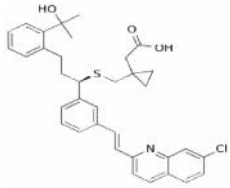

Fig 2: Structure of Montelukast

2. Materials and Methods

Chemicals

Faxofenadine and Montelukast, Potassium dihydrogen, Acetonitrile, Methanol, Water.

Instrumentation

HPLC Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution UV double beam UV 3000 UV Win 5 Lab India Digital weighing pH meter Ultra sonicator Suction pump.

Chromatographic conditions

Column	:XterraC18(4.6*250mm) 5µm
Mobile phase ratio	:Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v)
Detection wavelength	: 260 nm
Flow rate	: 1ml/min
Injection volume	:20µl
Column Temperature	:Ambient

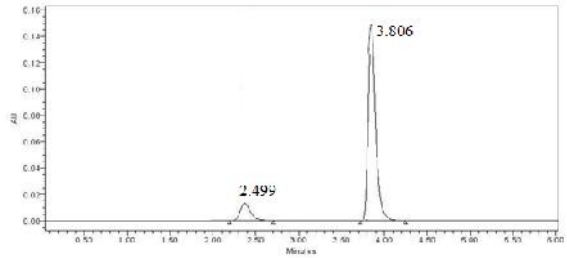

Fig 3: Optimized Chromatogram

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.

Preparation of Sample solutions:

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

Accurately 5mg of Montelukast and 5mg of Faxofenadine 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 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 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 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 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 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 and Montelukast):

Accurately weighed 10 mg of Montelukast and 10mg of Faxofenadine 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:

Repeatability:

Preparation of standard stock solution: Accurately 10 mg of Montelukast and 10mg of Faxofenadine 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 (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 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 Fexofenadine and 500 mg of Montelukast working standard was accurately 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:

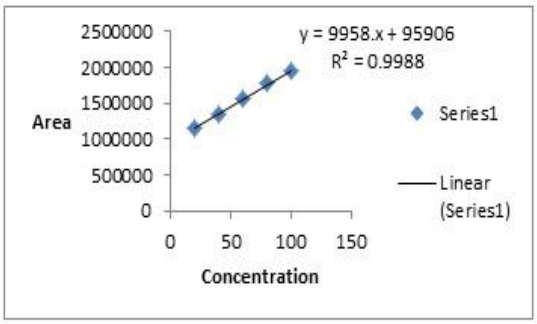

Fig 4: Calibration curve of Montelukast

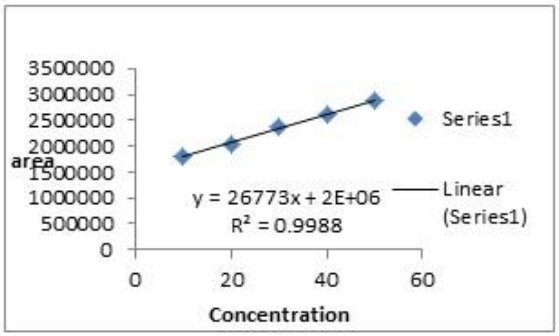

Fig 5: Calibration curve of Faxofenadine

Robustness:

Variation in Flow: Results for actual flow (1.0 ml/min) have been considered from Assay standard.

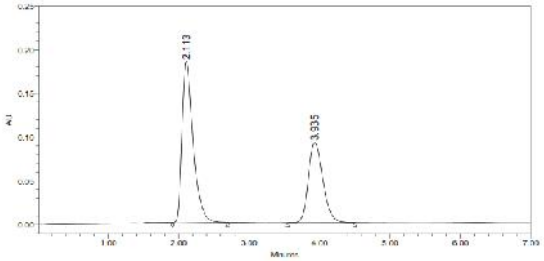

Fig 6: Chromatogram for Robustness more flow

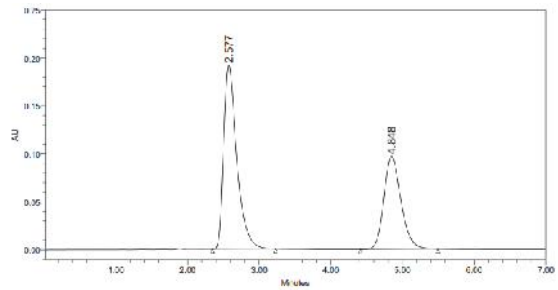

Fig 7: Chromatogram for Robustness less flow

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 Fexofenadine was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

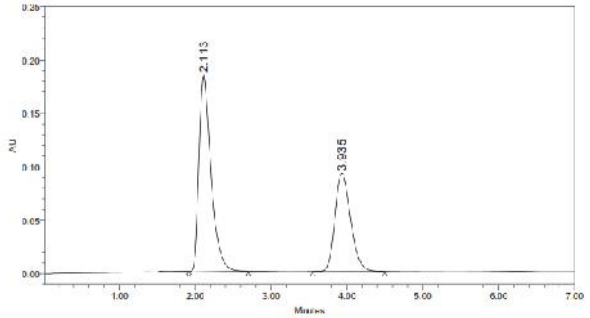

Fig 8: Chromatogram for Robustness more organic

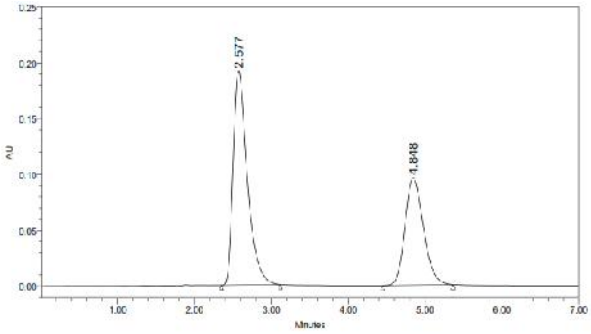

Fig 9: Chromatogram for Robustness less organic

4. Conclusion

A new method was established for simultaneous estimation of Faxofenadine and Montelukast by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Faxofenadine and Montelukast by using Xterra C185µm (4.6*250mm) column, flow rate was1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wavelength was 255nm. The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.399mins and 3.907mins. The % purity of Faxofenadine and Montelukast was found to be 100.7%and 101.4% respectively. The system suitability parameters for Faxofenadine and Montelukast such as theoretical plates and tailing factor were found to be 1.3, 5117.5 and 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 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 0.5and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04 and LOQ value was 9.87and10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Faxofenadine and Montelukast in API and Pharmaceutical dosage form.

Table 1: Results for system suitability of Faxofenadine

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 2: 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	-	-

Table 3: 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 4: Accuracy results of Faxofenadine

%Concentration(at specification level)	Area	Amount Added(mg)	Amount Found(mg)	%Recovery	Mean Recovery
50%	35386	5	5.0	101.3%	100.0%
100%	4735088	10	9.94	99.4%	
150%	5911798	15	14.8	99.2%	

Table 5: Repeatability results of Faxofenadine and Montelukast

Injection	Area	
	Fexofenadine	Montelukast
Injection-1	1501417	2235319
Injection-2	1486940	2240678
Injection-3	1490656	2249490
Injection-4	1487329	2245822
Injection-5	1490384	2251694
Average	1491345	2244601
Standard Deviation	5881.4	6656.8
%RSD	0.39	0.32

Table 6: Intermediate precision results of Faxofenadine and Montelukast

Injection	Area	
	Fexofenadine	Montelukast
Injection-1	2194758	1456296
Injection-2	2195700	1457422
Injection-3	2196191	1456513
Injection-4	2195326	1454579
Injection-5	2200951	1451483
Average	2196585	1455259
Standard Deviation	2496.0	2347.6
%RSD	0.11	0.16

Table 7: System suitability results For Montelukast (Flow rate)

S.No	FlowRate(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

Table 8: System suitability results for Faxofenadine(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

Table 9: System suitability results for Montelukast (Mobile phase)

S.No	Changein Organic Compositionin the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10%Less	1748.5	1.22
2	Actual	1548.2	1.2
3	10%More	1948.0	1.2

Table 10: System suitability results for Faxofenadine (Mobile phase)

S.No	Changein Organic Compositionin the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10%Less	883.3	1.56
2	Actual	1234.0	1.1
3	10%More	969.2	1.6

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