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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DESLORATADINE AND MONTELUKAST SODIUM BY RP-HPLC

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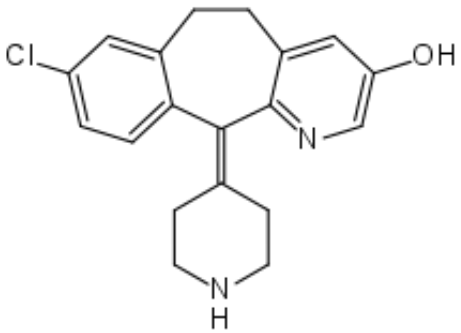
ABSTRACT

A novel, precise, accurate, rapid and cost effective isocratic Reverse-Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed, optimized and validated for the simultaneous estimation of Desloratadine and Montelukast Sodium in pharmaceutical dosage forms. The drugs were estimated using Imp Sil,C_{18}HS(250 mm x 4.6 mm i.d, 5µm)column. The mobile phase composed of Acetonitrile, Methanol, water with ratio of 15:80:05 v/v, at a flow rate of 1.0 ml/min was used for the separation. Detection was carried out at 280 nm. The linearity range obtained was 2-10 µg/ml for Desloratadine and 10 – 50 µg/ml for Montelukast with retention times of 2.46 min and 3.73 min for Desloratadine and Montelukast respectively. The correlation coefficient values were found to be 0.9994 and 0.9998. Precision studies showed % RSD values less than 2% for both the drugs in all the selected concentrations. The percentage recoveries of Desloratadine and Montelukast were in the range of 99.32% - 99.58% and 99.38%- 109% respectively. The limit of detection (LOD) and limit of quantification (LOQ) were 0.522µg/ml, 0.584µg/ml for Desloratadine and 1.384µg/ml 1.268µg/ml for Montelukast respectively. The method was validated as per the International Conference on Harmonization (ICH) guidelines. The proposed validated method was successfully used for the quantitative analysis of commercially available tablet dosage forms.

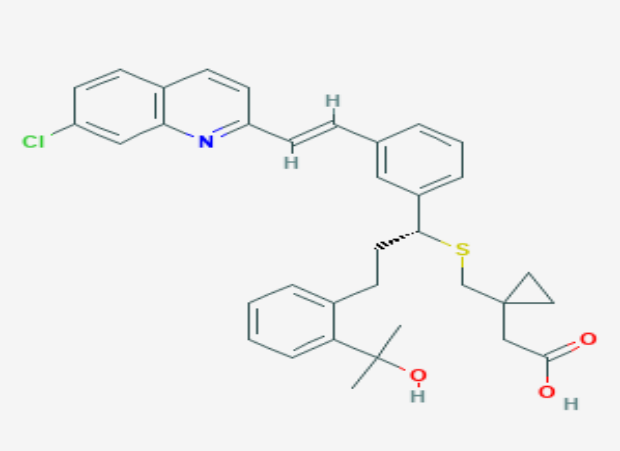
KEYWORDS: Montelukast Sodium, Desloratadine, HPLC, Validation.

INTRODUCTION

Desloratadine, [8-chloro-11-piperidin-4-ylidene-5,6-dihydrobenzo [1,2]cyclohepta [2,4-b] pyridine] is a second generation, tricyclic antihistamine that which has a selective and peripheral H1-antagonist action. It is the active descarboethoxy metabolite of loratidine (a second generation histamine). Desloratidine has a long-lasting effect and does not cause drowsiness because it does not readily enter the central nervous system. Desloratadine is a selective H_1-antihistamine which functions as an inverse agonist at the histamine H_1 receptor. Montelukast sodium, [2-[1-[[(1R)-1-[3-[(E)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(2-hydroxypropan-2yl)phenyl]propyl]sulfanylmethyl]cyclopropyl]acetic acid] is a selective and orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene CysLT1 receptor. The cysteinyl leukotrienes (LTC4, LTD4, LTE4) are products of arachidonic acid metabolism and are released from various cells, including mast cells and eosinophils. These eicosanoids bind to cysteinyl leukotriene receptors (CysLT) found in the human airway. Cysteinyl leukotrienes and leukotriene receptor occupation have been correlated with the pathophysiology of asthma, including airway edema, smooth muscle contraction, and altered cellular activity associated with the inflammatory process, which contribute to the signs and symptoms of asthma. Montelukast is an orally active compound that binds with high affinity and selectivity to the CysLT1 receptor (in preference to other pharmacologically important airway receptors, such as the prostanoid, cholinergic, or β-adrenergic receptor). Montelukast inhibits physiologic actions of LTD4 at the CysLT1 receptor without any agonist activity.



Desloratadine



Montelukast

Fig.1: Desloratadine and Montelukast.

Desloratadine and Montelukast in combination used for the treatment of persistent allergic rhinitis. Literature survey have few analytical methods are available for the simultaneous estimation of Desloratadine and Montelukast (Fig.1) in pharmaceutical formulations by using UV and HPLC. Hence, we made an attempt to develop a simple method for the simultaneous estimation of Desloratadine and Montelukast by RP-HPLC in pharmaceutical dosage forms. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines.[1-3]

Method Development and Optimization

Development of RP-HPLC method for simultaneous estimation of desloratadine and montelukast in combined dosage forms. Shimadzu gradient HPLC system with following configurations was used for the present study such as LC-20AD prominence solvent delivery system (Pump), Rheodyne 7725i injector with 20 µl loop, SPD-20A Prominence UV-VISBLE detector, LC-solution Version 1.25SP4 data station and Analytical column: Imp Sil,C_{18}HS (250 x 4.6 mm i.d., 5µ).

The wavelength of 281 nm was selected for the final method as these drugs has shown good absorbances. The drugs selected in the present study were polar in nature and hence RP-HPLC method was preferred because of its simplicity and suitability.[4-6]

Optimized chromatographic conditions

The following chromatographic conditions were used:

Stationary phase: Imp Sil, C_{18}HS (250 mm x 4.6 mm i.d, 5µm)

Mobile phase : Solvent A: Acetonitrile
Solvent B: Methanol
Solvent C: water
Solvent ratio : 15:80:05
Detection wavelength : 281 nm
Flow rate : 1.0 ml/min
Temperature : Room temperature of 20 ± 2^0 C

Estimation of Desloratadine and Montelukast

Preparation of Standard Solution

10 mg of Desloratadine was taken in a 10 ml standard flask. To this, mobile was added for dissolving the drug. It was shaken for one min. to obtain a clear solution and the volume was made up to 10 ml with mobile phase. 5 mg of Montelukast was taken in a 5 ml standard flask and diluted with few ml of mobile phase until the sample dissolves completely and the volume was made up to 5 ml with mobile phase.

Preparation of Formulation Solutions

Twenty tablets were weighed and finely powdered. Powder equivalent to 5 mg of desloratadine and 10 mg of montelukast was accurately weighed into a 100 ml volumetric flask, 30 ml of diluents was added and sonicated for 15 min, made up to the volume with diluents and mixed. Filter the solution through 0.45 nylon membrane filter. Dilute 10 ml of the above solution to 100 ml with diluents. A representative chromatogram of sample preparation (10ug/ml of desloratadine and 25 ug/ml of montelukast).[7-10]

Method of Recording of chromatogram

With the optimized chromatographic conditions mentioned above, a steady baseline at about 20 min. was recorded. After the stabilization of the baseline at about 30 min., the standard solutions were injected and chromatograms were recorded until the reproducibility of the peak areas was satisfactory. Finally 2 µg/ml of the standard solution of desloratadine and 10 ug/ml of montelukast individually were injected and the chromatograms were recorded *(Fig.2)*. The typical chromatograms of the sample solutions for brand was also recorded and given below.[11-12]

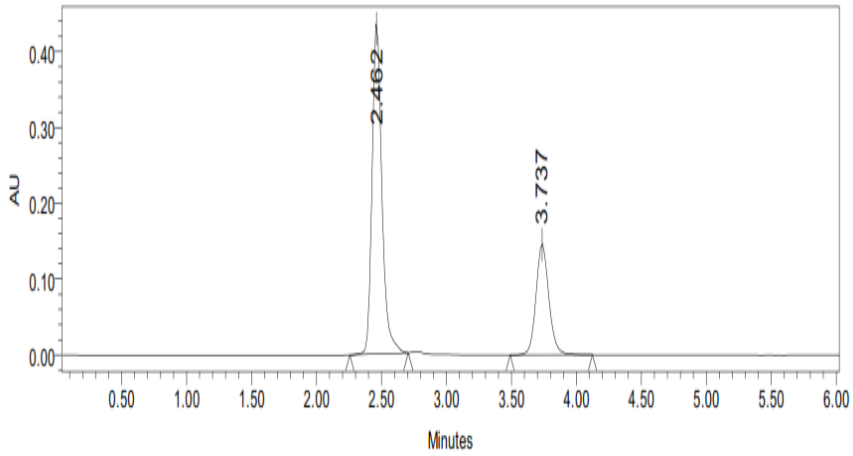


Fig2: Chromatogram of Sample solution of brand (DESTIN-M).

This procedure was repeated for the sample solution. The peak areas were noted and the response factors of the standard and sample solution peaks were calculated. The elution order of mixture was found as desloratadine (retention time 2.46 min), and montelukast (retention time 3.73 min).

Validation of HPLC Method

After the development of HPLC method for the estimation of multicomponent dosage form, validation of the method was performed statistically. This section describes the procedure followed for validation of the method developed.

a) Accuracy

Accuracy of the method was determined by recovery experiments. To the formulation, the reference standards of the respective drugs were added at the level of 50% and100 %. These were further diluted by procedure as followed in the estimation of formulation. The concentrations of the drugs present in the resulting sample solution were determined by using assay method.

b) Precision

Repeatability and reproducibility studies demonstrated the precision of the method. Repeatability studies were done by consequently injecting the standard solution at three different concentrations. These solutions were prepared in duplicate and injected as per assay procedure. For the same, Intraday, Interday and reproducibility of injection were studied.[13-14]

c) Linearity and Range

From the standard stock solutions, a suitably mixed standard solution was prepared to contain 50 to 150% of targeted level of the assay concentration of the standard drugs. The solutions were examined by the assay procedure. The calibration curve (Fig. 3& 4) was plotted using response factor (peak area ratio of the standard peak area Vs concentration of the standard solutions for both the drugs. The values thus obtained were mentioned in Table 7 for desloratadine and table 8 for montelukast from the calibration curve, the slope and intercept are calculated.

d) Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the developed method were determined by analyzing progressively lower concentrations of the standard solution using optimized chromatographic conditions. The minimum concentration of the standard solution, which gave signal to noise ration of 3 and 10 were taken as the LOD and LOQ values respectively. The LOD and LOQ were found by formula given in the ICH guide line for validation.[15-16]

e) System Suitability Studies

The system suitability studies were carried out as specified in USP. These parameters include column efficiency, resolution and capacity factor Table 6.

RESULTS AND DISCUSSION

The peak area ratios of standard and sample solutions were calculated .The assay procedure was repeated for 6 times and mean peak area, mean peak area ratio, mean weight of standard drugs, mean weight of sample taken for assay were calculated. The percentages of individual drugs found in formulations, mean, and standard deviation in formulations were calculated and presented in Table 1. The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulations.[17]

Table 1: Analysis of Formulation.

Formulation	Drug	Label Claim (mg/tablet)	Estimated Amount* (mg/tablet)	% Label claim*	SD*
(DESTIN-M)	Desloratadine	5	4.96	99.88	0.23
	Montelukast	10	10.57	100.31	0.11

*** Average of six Determination**

2. Validation of the method

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and results are percentage relative standard deviation were calculated and presented in Table 2. Recoveries of standard drugs were found to be accurate and were within the specified limits.

Table 2: Accuracy (Recovery Studies).

Drug name	Label Claim (mg/tablet)	% Amount added	Amount found	%Recovery	% RSD
DESLORATADINE	5	50	54.63	99.32%	0.85
		100	104.56	99.58%	
MONTELUKAST	10	50	59.63	99.38%	1.475
		100	109.03	109%	

The precision of the method was determined by studying repeatability and reproducibility. The intraday, Interday studies were carried out and the Peak areas of drug peaks and percentage relative standard deviation were calculated and results are presented in Table. 3 & 4. The results revealed that the method developed is reproducible and its results are shown in Table 5& 6.

Table 3: Intraday Studies.

Level	Concentration (µg/ml)		Peak area		% RSD	
	Desloratadine	Montelukast	Desloratadine	Montelukast	Desloratadine	Montelukast
I	2	10	220812	350714	1.384	2.363
			214781	334524		
			217871	343257		
II	4	20	440450	797603	0.031	0.143
			440179	795324		
			440250	796244		
III	6	30	582190	106132	0.003	0.010
			502145	106111		
			582175	106125		

Table 4: Interday Studies.

Day	Concentration (µg/ml)		Peak area		% RSD	
	Desloratadine	Montelukast	Desloratadine	Montelukast	Desloratadine	Montelukast
1	2	10	220812	350714	0.375	1.460
2			219174	347312		
3			220174	33710		
1	4	20	440450	797603	0.115	0.072
2			441375	796597		
3			4411275	797601		
1	6	30	582190	106132	0.011	0.490
2			582250	105236		
3			582117	106137		

The linearity range for desloratadine was found to be from 2 to 10 µg/ml and the linearity range for montelukast was found to be 10-50µg/ml respectively.

Repeatability of injection

A standard solution of mixture of drugs were injected six times and its % RSD was calculated and results shown in table 5**.**

Table 5: Repeatability of injection.

Concentration (µg/ml)	Injection	Peak area		% RSD	
		Desloratadine	Montelukast	Desloratadine	Montelukast
Desloratadine (2 µg/ml) Montelukast (10 µg/ml)	1	440573	797603	0.0401	0.0230
	2	440273	797907		
	3	440733	797808		
	4	440676	797748		
	5	440567	797562		
	6	440373	797403		

The response factor, slope, intercept and correlation coefficient values were calculated. The correlation coefficient of desloratadine and Montelukast were found to be 0.9994 and 0.99985 respectively. The calibration curves were plotted using response factor Vs concentration of the standard solutions.

LOD of Desloratadine and Montelukast were found to be 0.522 ug /ml, 1.384. The LOQ of Desloratadine and Montelukast were found to be 0.584, 1.268 ug /ml. The resolution, capacity factor, theoretical plates/meter, peak symmetry was finding out for the standard solutions and is presented in Table 6. The values obtained demonstrated the suitability of the system for the analysis of the above drug combination.

Table 6: System suitability studies.

Drug	R_s	N	K'	Tailing factor	HETP	LOD ug/ml	LOQ ug/ml
Desloratadine	2.749	2277.4	0.421	1.106	65.864	0.522	0.584
Montelukast	10.097	8240.2	2.175	1.12	18.203	1.384	1.268

Rs =Resolution, N =Theoretical plate, K' =Capacity factor, LOD = Limit of detection, LOQ = Limit of quantification, HETP = Height equivalent to therapeutic plates

Table 7: Linearity of Desloratadine.

Desloratadine		
S.No	Concentration (µg/ml)	Peak area
1.	2	220819
2.	4	440459
3.	6	582194
4.	8	820879
5.	10	997021

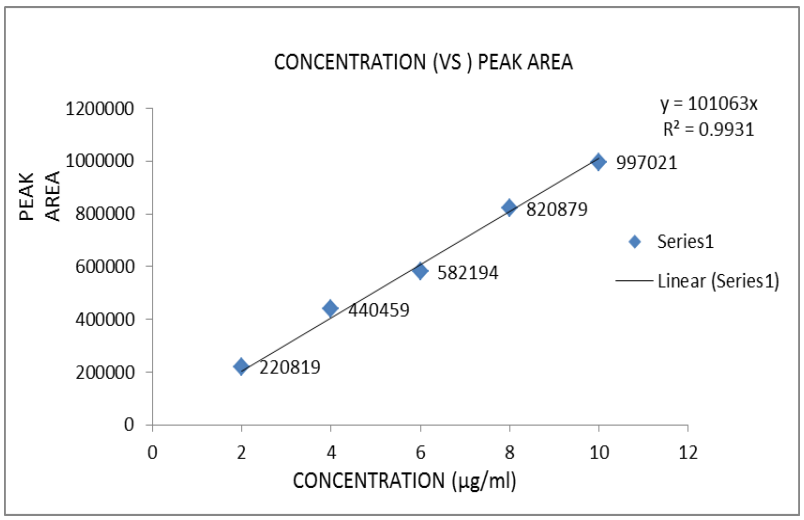


Fig. 3: Calibration curve for Desloratadine.

Table 8: Linearity of Montelukast.

Montelukast		
S.No	Concentration (µg/ml)	Peak area
1.	10	356814
2.	20	797707
3.	30	1062328
4.	40	1551919
5.	50	1954541

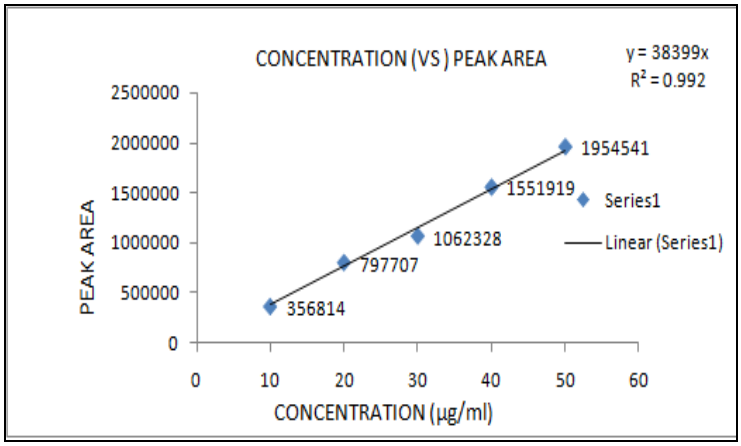


Fig. 4: Calibration curve for Montelukast.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameter and provides an indication of its reliability during normal usage. 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. The typical variations are variation in flow rate by ±0.2 ml/min and variation in temperature varied from ±10%. Mixed samples of both MON and DES were analyzed under these changed experimental conditions. The results of robustness study are shown in Table 8.

Table 8. Robustness for Montelukast and Desloratadine.

S. No.	Parameter	Montelukast		Desloratadine	
		Peak area	Tailing factor	Peak area	Tailing factor
1.	Flow Rate (+0.2ml/min)	356814	1.654	210829	1.254
2.	Flow Rate (-0.2ml/min)	356752	1.234	201802	1.325
3.	Temp. change (10% more)	349581	1.204	210212	1.214
4.	Temp. change (10% less)	356789	1.320	220312	1.242
% RSD		0.16		0.02	

Ruggedness

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay was performed in different condition, different analyst and different dates. The results of ruggedness study are given in Table 9.

Table 9. Ruggedness for Montelukast and Desloratadine.

S. No.	Parameter	Montelukast		Desloratadine	
		Peak area	Tailing factor	Peak area	Tailing factor
1.	Analyst-1	356814	1.654	210829	1.254
2.	Analyst-2	356752	1.234	201802	1.325
3.	Analyst-3	349581	1.204	210212	1.214
4.	Analyst-4	356789	1.320	220312	1.242
% RSD		0.101		0.011	

CONCLUSION

The deliberate changes in the method and operational conditions have not much affected the chromatograms. This indicates that the proposed method was robust and rugged. The proposed RP-HPLC method was simple, specific, sensitive, precise and accurate and can be used for simultaneous estimation of Montelukast and Desloratadine in bulk samples and its tablet dosage forms.

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