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Simultaneous Estimation of Desloratadine and Montelukast in Bulk and Pharmaceutical Formulations by RP-HPLC

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Abstract: **A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Desloratadine and Montelukast in bulk and pharmaceutical formulations. Separation of Desloratadine and Montelukast was successfully achieved on a ECLEPSE XDB C8 (4.6 x 150mm, 5 µm, Make: Waters) or equivalent in an isocratic mode utilizing K_2HPO_4 buffer (pH: 8.6) Methanol (60:40%v/v) at a flow rate of 0.8 mL/min and elute was monitored at 261 nm, with a retention time of 2.485 and 3.800 minutes for Desloratadine and Montelukast. The method was validated and the response was found to be linear in the drug concentration range of 50 µg/mL to 150 µg/mL for Desloratadine and 50 µg/mL to 150 µg/mL for Montelukast. The LOD and LOQ for Desloratadine were found to be 2.759, 9.195 respectivly. The LOD and LOQ for Montelukast were found to be 2.9091, 9.6970 respectively. This method was found to be good percentage recovery for Desloratadine and Montelukast were found to be 100.00% and 100.00% respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuacy, Precesion, Specificity and Robustness.**

Keywords: **RP-HPLC; Desloratadine, Montelukast.**

I. INTRODUCTION

Desloratadine: 13-chloro-2-(piperidin-4-ylidene)-4 azatricyclo [9.4.0.0^{3,8}] pentadeca 1(11),3,5,7,12,14-hexaene. It belongs to the benzocycloheptapyridines. These are aromatic compounds containing a benzene ring and a pyridine ring fused to a seven membered carbocycle. Desloratadine is a second generation, tricyclic antihistamine that which has a selective and peripheral H1-antagonist action.

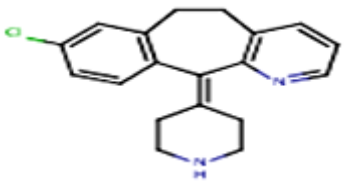

Figure1: Chemical structure Of Desloratadine

Montelukast: 2-[1-({[(1R)-1-{3-[(E)-2-(7-chloroquinolin-2-yl) ethenyl] phenyl}-3-[2-(2-hydroxypropan-2-yl) phenyl] propyl] sulfanyl} methyl) cyclopropyl] acetic acid. Montelukast inhibits the actions of LTD_4 at the $CysLT_1$ receptor, preventing airway edema, smooth muscle contraction, and enhanced secretion of thick, viscous mucus. It is an Antilipidemic Agents

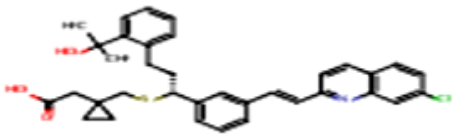

Figure2: Chemical structure of Montelukast

II. MATERIALS AND METHOD

A. *Instrumentation*

The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software with Eclipse XBD-C8, (150mm × 4.6 ; 5µm) column.

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B. *Chemicals and Reagents*

Desloratadine and Montelukast was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. K_2HPO_4, Methanol of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Ortho phosphoric acid of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and mille Q water.

C. *HPLC Conditions*

The mobile phase consisting of K_2HPO_4 buffer (pH: 8.6) Methanol was degassed and were pumped from the solvent reservoir in the ratio of 60:40v/v was pumped into the column at a flow rate of 0.8 ml/min. The column temperature was 40°C. The detection was monitored at 261nm and the run time was 6min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 15 min. with the mobile phase flowing through the system.

D. *Preparation of standard solution*

Accurately weigh and transfer 5mg of Desloratadine and 10mg of Montelukast into 50ml of volumetric flask and add 10ml of methanol to each and sonicate 10min (or) shake 5min and makeup the volume with methanol. Pipette out 5.0ml standard stock standard stock into 25ml volumetric flask dilute to volume with methanol and inject into HPLC.

E. *Preparation Of Sample Solution*

Accurately weighed 797.6mg of sample. Transfer the sample powder into 50ml of volumetric flask add 10ml methanol, sonicate for 20mins. Then make up the volume with methanol and filter through the 0.45µm filter paper. Transfer 5ml of above solution 25 ml volumetric flask and make up the volume with methanol.

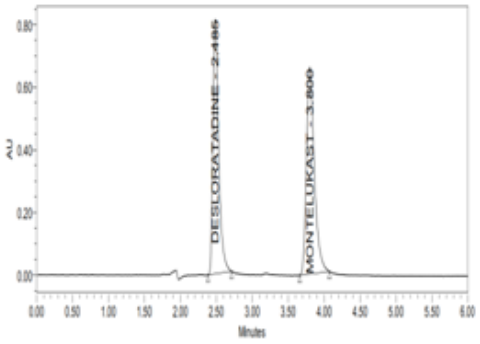

Figure 3: Standard chromatogram for Desloratadine and Montelukast

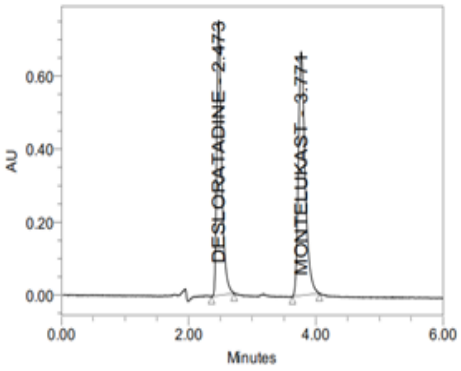

Figure 4: Formulation chromatogram for Desloratadine and Montelukast

F. *Method validation*

1. *System Suitability Studies*

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table I).The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

TABLE I: SYSTEM SUITABILITY PARAMETERS

Parameter	Desloratadine	Montelukast
Retention time	2.485	3.800
Theoretical plates	5123	5986
Tailing factor	1.39	1.52
% RSD	0.4	0.3

2. *Specificity*

Specificity is the ability to assess unequivocally the analyte in the presence of components which may expect to be present. Typically these might include impurities, degradants, matrix, etc.

3. *Accuracy and precision*

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times. The percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate (Table-II&III).

TABLE II: ACCURACY FOR DESLORATADINE

S. No	Accuracy level	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	398.80	39.640	39.78	100	100
		398.80	39.640	39.79	100	
		398.80	39.640	39.76	100	
		398.80	39.640	39.77	100	
		398.80	39.640	39.80	100	
		398.80	39.640	39.75	100	
2	100%	797.60	79.280	79.36	100	100
		797.60	79.280	79.43	100	
		797.60	79.280	79.36	100	
3	150%	1196.40	118.920	119.03	100	100
		1196.40	118.920	118.90	100	
		1196.40	118.920	119.01	100	
		1196.40	118.920	118.95	100	
		1196.40	118.920	118.98	100	
		1196.40	118.920	118.98	100	

TABLE III: **ACCURACY** *FOR MONTELUKAST*

S. No	Accuracy level	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	398.80	79.760	79.83	100	100
		398.80	79.760	79.79	100	
		398.80	79.760	79.89	100	
		398.80	79.760	79.77	100	
		398.80	79.760	79.85	100	
		398.80	79.760	79.84	100	
2	100%	797.60	159.20	159.34	100	100
		797.60	159.20	159.47	100	
		797.60	159.20	159.56	100	
3	150%	1196.40	239.280	239.28	100	100
		1196.40	239.280	239.24	100	
		1196.40	239.280	239.47	100	
		1196.40	239.280	239.49	100	
		1196.40	239.280	239.37	100	
		1196.40	239.280	239.29	100	

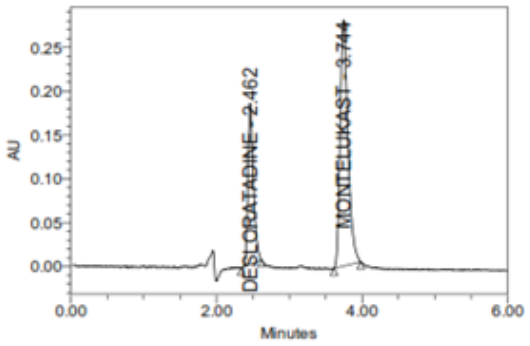

Figure 5: AccuracyChromatograms-50%of Desloratadine and Montelukast

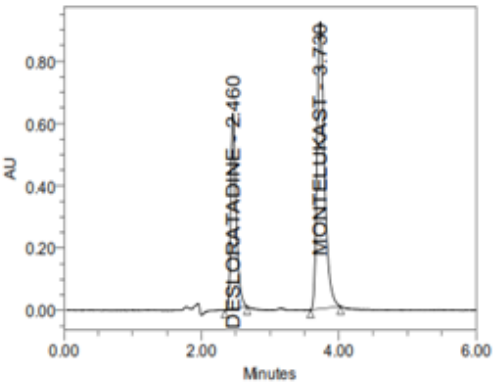

Figure 6: AccuracyChromatograms-100%of Desloratadine and Montelukast

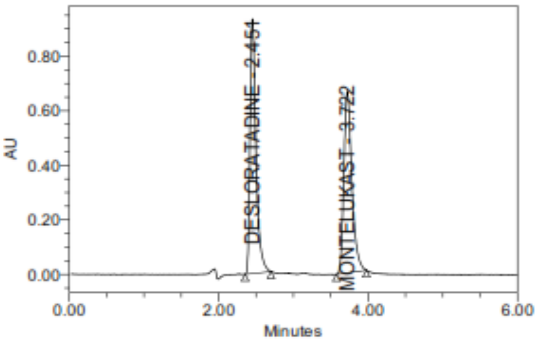

Figure 7: AccuracyChromatograms-150%of Desloratadine and Montelukast

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies. six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. The chromatograms of three different levels shown in Figure 5, 6 &7. From the data obtained, the developed RP-HPLC method was found to be precise. (Table-IV)

TABLE IV: PRECISION STUDIES

S.No	Area (Des)	Area (mon)	%Assay (Des)	%Assay (mon)
1	4678289	4945121	99	100
2	4677549	4941492	99	100
3	4676508	4945109	99	100
4	4675202	4943465	99	100
5	4677862	4942694	99	100
6	4678822	4949517	99	100

4. Linearity and range

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was Y=43363X (R^2=0.999) for desloratidine and Y=49489X (R^2=1) for montelukast. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The overlay chromatograms of Linearity for Desloratadine and Montelukast shows in Figure 10 and the results for calibration curves are given in Figure 8&9.

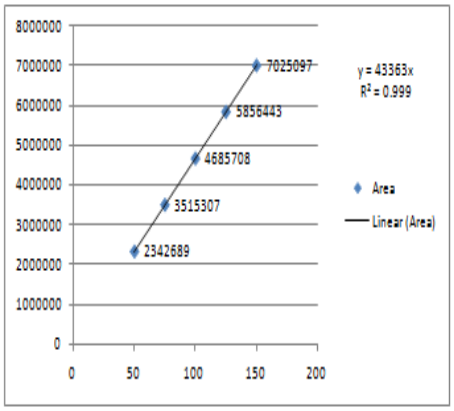

Figure 8: Linearity Curve for Desloratidine

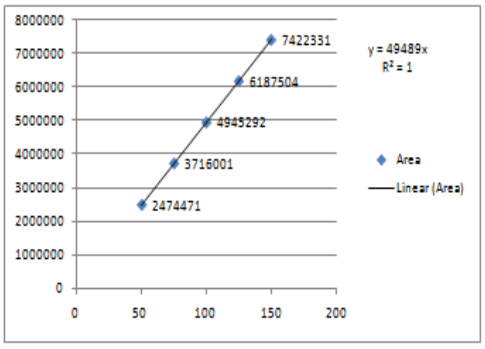

Figure 9: Linearity Curve for Montelukast

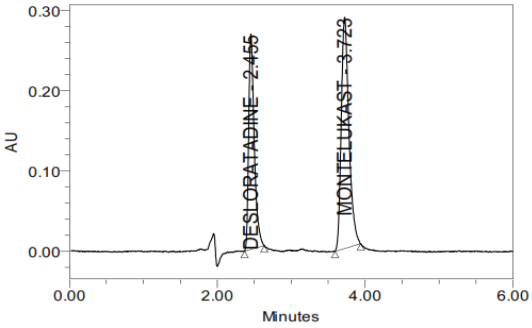

Figure 10: Overlay chromatograms of Linearity for Desloratidine and Montelukast

5. Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms which demonstrated that the RPHPLC method developed, are robust (Table-V&VI).

TABLE V: ROBUSTNESS FOR DESLORATIDINE

S.No	Sample Name	RT	Area	USP Tailing
1	Temp-1	4.955	4960210	1.39
2	Temp-2	2.978	4927514	1.35
3	Flow-1	3.725	4511175	1.32
4	Flow-2	3.722	4512813	1.32

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TABLE VI: ROBUSTNESS FOR MONTELUKAST

S.No	Sample Name	RT	Area	USP Tailing
1	Temp-1	3.267	4511175	1.52
2	Temp-2	1.961	4512813	1.54
3	Flow-1	2.451	6117979	1.51
4	Flow-2	2.459	3585343	1.48

6. LOD&LOQ

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of Desloratadine and Montelukast. RSD (σ) method was applied; the LOQ and LOD values were predicted using following formulas (a) and (b). Precision was established at these predicted level

(a) LOQ = 10 σ / S
(b) LOD = 3.3 σ / S
Where
σ = residual standard deviation of response
S = slope of the calibration curve.

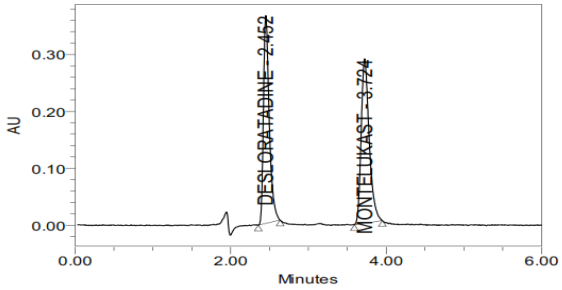

Figure 11: Chromatograms for LOQ

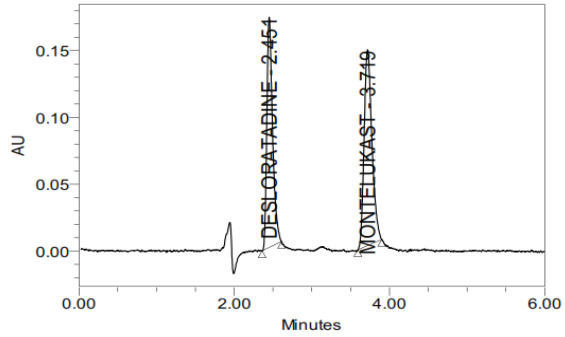

Figure 12: Chromatograms for LOD

TABLE VII: LOD and LOQ for Desloratidine and Montelukast

S.No	Sample Name	Name	RT	Area
1	LOD	Desl	2.451	881757
2	LOQ	Desl	2.452	1898664
1	LOD	Mont	3.719	1025560
2	LOQ	Mont	3.724	2071910

III. RESULTS AND DISCUSSION

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1. So we can say system is suitable for analysis method specificity was concluded by figure:3 and figure:4 those figures are Desloratadine and montelukast standard chromatogram and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table I say that the method precision passed for both Desloratadine and montelukast studies. The method accuracy was evaluated by recovery studies. Desloratadine and montelukast recovery was founded 99%&100% as per ICH 97%- 103% and also percentage RSD was very low so method is accurate shown in table II&III. Linearity calibration curve was given below fig: 8&9 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation co-efficient. Linear correlation was found to be Y=43363X for Desloratidine and y = 49488X for Montelukast Method robustness results were given by table V&VI, LOQ and LOD Results were given by table VII.

IV. CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Desloratidine and Montelukast pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for rountine quality control analysis of Desloratidine and Montelukast pure and its pharmaceutical dosage forms.

V. ACKNOWLEDGEMENT

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