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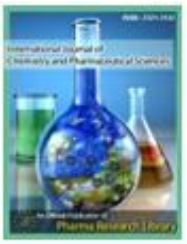

R E S E A E C H A R T I C L E

Analytical Method Development and Validation for the Simultaneous Estimation of Desloratadine and Montelukast by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

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 (P^H: 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 150µg/mL for Montelukast. The LOD and LOQ for Desloratadine were found to be 2.759, 9.195 respectively. 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: Desloratadine, Montelukast, High performance liquid chromatography.

A R T I C L E I N F O

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1. Introduction

Analytical methods

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available [2].

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3,5]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements.

Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6].

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [9].

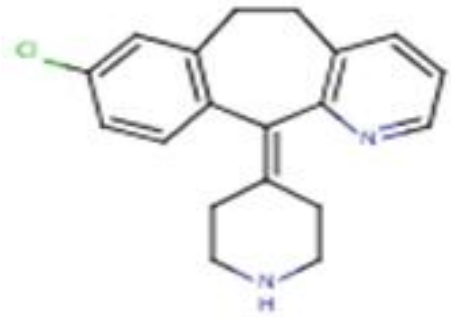

Figure 1: Desloratadine

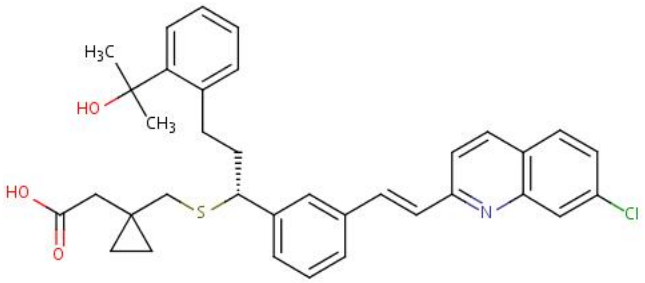

Figure 2: Montelukast

2. Materials and Methods

Apparatus

WATERS HPLC, Model: Aglient 2695, Photo diode array detector (PDA), with an automated sample injector. The output signal was monitored and integrated using Empower 2 software. ELIPSE C8 (150mm*4.6, 5 µm, Make: Waters) column was used for separations [10].

Chemicals: KH_2PO_4, Methanol, Water, Dipotassium hydrogen phosphate, Desloratadine, Montelukast.

Mobile phase:

Transfer 17.41mg of K_2HPO_4 into 1000ml of beaker and adjust pH to 8.6. Transfer the above solution 600ml and 400ml of buffer and methanol is used as mobile phase. They are mixed and sonicated for 20min [11].

Optimization Chromatographic trials for Simultaneous Estimation of Desloratadine and Montelukast by RP-HPLC.

Optimization chromatographic conditions

Mobile Phase: K2HPO4: Methanol (600:400)
Column: Agilent ZorbaxC18, 250x4.6, 5µ
Flow Rate: 1.0ml/min
Injection Volume: 10 µl
Column Temperature: 30˚C
Detector: 237nm

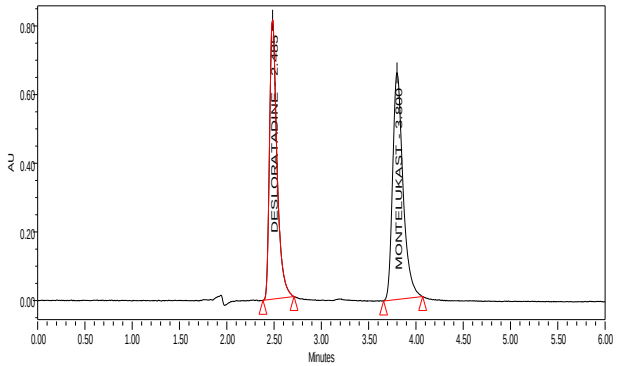

Figure 3: Optimization Chromatogram

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.

3. Results and Discussions

Method Validation Parameters

Specificity: Solution of standard sample and placebo were prepared as per test procedure and injected into the HPLC system.

Blank Interference

A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure [12].

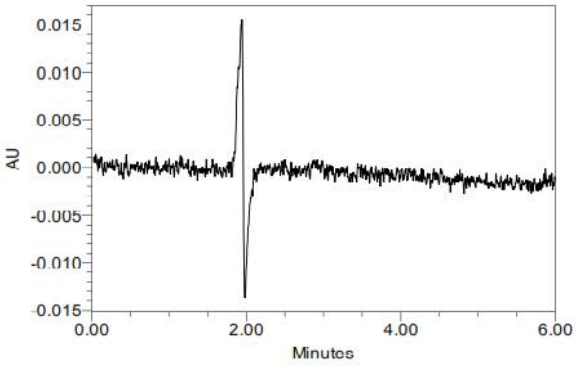

Figure 4: Chromatogram of sample

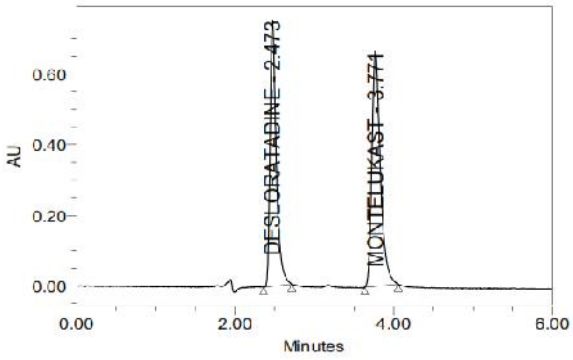

Figure 5: Chromatogram of blank

Linearity

Prepare a series concentrations of 50µg/ml, 75µg/ml, 100µg/ml, 125µg/ml,150µg/ml of standard solutions and inject into HPLC system. Plot the graph of standard versus the actual concentration in µg/ml and determine the coefficient of correlation and basis for 100% response [13,14].

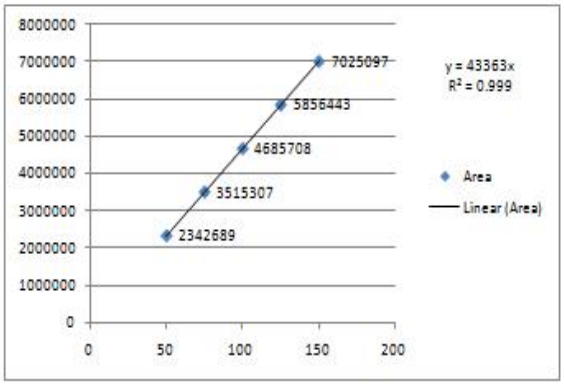

Figure 6: Calibration graph of Desloratadine

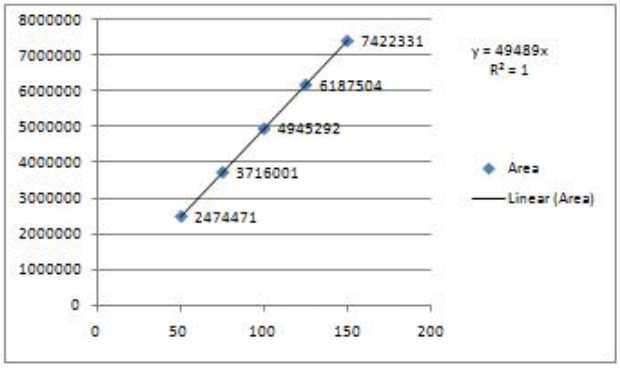

Figure 7: Calibration graph of Montelukast

Standard Stock for Linearity solutions

Accurately weighed 10mg of Doxylamine Succinate and 10mg of Pyridoxine HCl taken into clean and dry 100 ml volumetric flask. To this 70mL of diluent added. Sonicated to dissolve volume made up to the mark with diluents [15]

Acceptance criteria [16,17]

Linearity regression coefficient of average peak area response of replicate injections plotted against respective concentration should not be less than 0.999. The % y-intercept as obtained from the linearity data (without extrapolation through origin 0, 0) should be within ±2.0.

Acuuracy

Recovery study can be performed in the concentration of 50%,100% & 150% of the target concentration of the test and inject 50% and 150% six times and 100%three times into HPLC. Then determine %Recovey and %RSD [18,19].

Precision

Preparation of sample:

Transfer the 797.6mg of sample into a 50ml of volume at flask and add 10ml of diluent and sonicate 20min and makeup with diluent. Transfer the above solution into 5ml into 25ml volumetric flask dilute to the volume with diluent. The method precision parameters were evaluated from sample chromatograms obtained, by calculating the % RSD of peek areas from 6 replicate injection [20,21].

Robustness

Effect of variation in flow rate: Prepare the system suitability solution as per the test method and inject into the HPLC system with ±0.2 ml of the method flow. Evaluate the system suitability values as required by the test method for both flow rates. Actual flow rate was 0.8 ml/min and it was changed to 0.6ml/min and 1.0ml/min and inject into HPLC and system suitability was checked [22].

Effect of variation in Temperature: Prepare the system suitability solution as per the test method and injected into the HPLC with ±5°c of the method temperature [23]. Evaluate the system suitability values as required by the test method for both temperatures [24].

Limit of Detection: The sensitivity of measurement of Desloratadine and Montelukast proposed method was estimated in terms of the limit of detection (LOD). The LOD was calculated by the use of signal to noise ratio. In order to estimate the LOD value, the blank sample was injected six times and peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level [25,26].

$$LOD = 3.3\ \sigma / S$$

Where,

σ = standard deviation of intercepts of calibration curves

S = mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

Limit of Quantification: The sensitivity of measurement of Desloratadine and Montelukast by the use of proposed method was estimated in terms of limit of quantification (LOQ)[27,28]. The LOQ was calculated by the use of signal to noise ratio. In order to estimate the LOQ value, the blank sample was injected six times and the peak area of this blank was calculated at noise level. The LOQ was calculated as ten times the noise value gave the LOQ [29].

$$LOQ = 10\ \sigma / S$$

Where,

σ = standard deviation of intercepts of calibration curves

S = mean of slopes of the calibration curves. The slope S may be estimated from the calibration curve of the analyte.

Limit of Detection for Desloratadine

Minimum concentration of standard component in which the peak of the standard gets merged with noise called the LOD

$$LOD = 3.3^* \sigma/S$$

Where; σ = standard deviation, S = slope

LOD for Desloratadine= 2.759

LOD for Montelukast=2.9091

Limit of Quantification for Montelukast

Minimum concentration of standard component in which the peak of the standard gets detected and quantification

$$LOQ = 10^*\sigma/S$$

Where; σ = standard deviation, S = slope

LOQ for Desloratadine=9.195

LOQ for Montelukast=9.69

Table 1: Linearity Results for Desloratadine

S.NO	Conc (µg/ml)	RT	Area
1	50	2.455	2342689
2	75	2.453	3515307
3	100	2.450	4685708
4	125	2.457	5856443
5	150	2.450	7025097
Std.dev			
Slope			
Intercept			
Correlation coefficient (r^2)			0.999

Table 2: Linearity Results for Montelukast

S.NO	Conc(µg/ml)	RT	Area
1.	50	3.723	2474471
2.	75	3.720	3716001
3.	100	3.718	4945292
4.	125	3.726	6187504
5.	150	3.721	7422331
Std.dev			
Slope			
Intercept			
Correlation coefficient(r^2)			1

Table 3: Accuracy results for Desloratadine

S.NO	Accuracy level	Injection	Sample area	RT
1	50%	1	2348613	2.462
		2	2349714	2.457
		3	2347105	2.458
		4	2348073	2.464
		5	2350074	2.453
		6	2347111	2457
2	100%	1	4686132	2.451
		2	4689860	2.456
		3	4685674	2.459
3	150%	1	7028349	2.460
		2	7020503	2.459
		3	7027333	2.451
		4	7023698	2.455
		5	7025101	2.454
		6	7025097	2.450

Table 4: Accuracy results for Montelukast

S.NO	Accuracy level	Sample Name	Sample area	RT
1	50%	1	2476168	3.744
		2	2474957	3.737
		3	2478210	3.733
		4	2474256	3.743
		5	2476899	3.728
		6	2476516	3.731
2	100%	1	4942582	3.722
		2	4946656	3.725
		3	4949436	3.732
3	150%	1	7422081	3.730
		2	7420920	3.727
		3	7428108	3.721
		4	7424803	3.723
		5	7428503	3.725
		6	7422331	3.721

Table 5: Precision data for Desloratadine

S.No	RT	Area	%Assay
injection1	2.473	4945121	99
injection2	2.473	4941492	99
injection3	2.472	4945109	99
injection4	2.459	4943465	99
injection5	2.461	4942694	99
injection6	2.464	4949517	99
Mean			99
Std. Dev.			0.06
% RSD			0.05

Table 6: Precision data for Montelukast

S.No	RT	Area	%Assay
injection1	3.771	4945121	100
injection 2	3.774	4941492	100

injection 3	3.759	4945109	100
injection 4	3.748	4942695	100
injection 5	3.750	4942694	100
injection 6	3.744	4949517	100
Mean			100
Std. Dev.			0.06
%RSD			0.06

Table 7: Robustness data for Desloratadine

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate(0.6ml/min)	3.267	6117979	1.51
Actual flow ate(0.8ml/min)	2.485	4480500	1.52
Increased flow rate(1.0ml/min)	1.961	3585343	1.48
Decreased temperature(20^0c)	2.459	4511175	1.52
Actual temperature(25^0c)	2.485	4480500	1.52
Increased temperature(30^0c)	2.451	4512813	1.54

Table 8: Robustness data for Montelukast

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate(0.6ml/min)	4.955	4511175	1.32
Actual flow rate(o.8ml/min)	3.800	5041081	1.39
Increased flow rate(1.0ml/min)	2.978	4512813	1.32
Decreased temperature(20^0c)	1.39	4960210	1.39
Actual temperature(25^0c)	3.800	5041081	1.39
Increased temperature(30^0c)	1.35	4927514	1.35

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. A new method was established for simultaneous estimation of Desloratadine and Montelukast by RP-HPLC method. The method was validated and the response was found to be linear in the drug concentration range of 50 µg/mL to 150µg/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 respectively. 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. The study is focused to develop and validate HPLC methods for estimation of Desloratadine and Montelukast in tablet dosage form. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Desloratadine and Montelukast.

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