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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF MONTELUKAST AND LEVOCETIRIZINE IN PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Montelukast and Levocetirizine in liquid dosage form. Chromatogram was run through Hypersil BDS 250 x 4.6 mm, 5µ. Mobile phase containing Buffer and Acetonitrie in the ratio of 35:65A was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.02M Potassium di-hydrogen phosphate buffer at pH 3.6. Temperature was maintained at 30°C. Optimized wavelength for Montelukast and Levocetirizine was 231nm. Retention time of Montelukast and Levocetirizine were found to be 2.599min and 3.472min %RSD of the Montelukast and Levocetirizine were and found to be 0.7 and 0.8, respectively. % recovery was obtained as 99.88% and 99.9%for Montelukast and Levocetirizine respectively. Regression equation of Montelukast is y = 35120x + 388.3 and Levocetirizine is y = 29047x + 721.3.

Key Words: Montelukast, Levocetirizine, RP-HPLC.

INTRODUCTION

Montleukast is a leukotriene receptor antagonist used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. [1][2]. Chemically it is known as (R,E)-2-(1-((1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxy propan-2-yl)phenyl)propylthio)methyl)cyclopropyl) acetic acid. Levocetirizine is a third-generation non-sedative antihistamine, developed from the second-generation antihistamine cetirizine. Chemically, levocetirizine is the active enantiomer of cetirizine. Chemically it is known as 2-(2-{4-[(*R*)-(4-chlorophenyl) (phenyl)methyl]piperazin-1-yl}ethoxy) acetic acid.

The literature survey reveals that there is no official analytical method available for estimation of Montelukast and Levocetirizine The reported methods available for the estimation of Montelukast and Levocetirizine individually are spectro-photometric method.

Since the lack of official high performance liquid chromatographic methods for the simultaneous estimation of Montelukast and Levocetirizine, we have planned to develop a simple, precise, economic and accurate Method development and validation for the estimation of montelukast and Levocetirizine in pharmaceutical dosage form by using RP-HPLC.

EXPERIMENTAL

Materials and methods: Active pharmaceutical ingredients Montelukast and Levocetirizine were obtained as a gift sample from Spectrum pharma research solutions, Hyderabad. The pharmaceutical dosage form (Lazine M from Genx (HETERO Healthcare Ltd)) was purchased from local pharmacy. The solvents used in this work were of HPLC grade and obtained from Merck Specialties Private Limited, Mumbai.

Instrumentation and chromatographic conditions: The analysis was performed on a high performance

liquid chromatography system consists of waters 2695 with 2996 module Photo Diode Array detector equipped with a quaternary solvent delivery pump, automatic sample injector and column thermostat. The data acquisition and analysis was performed by using Empower2 software. The chromatographic separation was performed on Hypersil BDS column (250mm x 4.6mm x 5µ).The flow rate was kept at 1ml/min. The column temperature was maintained at 30°C.The mobile phase was made of Potassium di-hydrogen phosphate buffer and Acetonitrile in 35:65 ratio had gave acceptable retention time and good resolution between Montelukast and Levocetirizine. The method was optimized at 231nm. Data acquisition and processing was performed by using empower2 system software. The run time was taken as 7min. All the determinations are carried out at an ambient temperature.

Preparation of Standard stock solutions: Accurately weighed 10mg of Montelukast and 5mg of Levocetirizine and transferred to two 10ml volumetric flasks separately. 5ml of diluent was added to flasks and sonicated for 15mins. Flasks were made up with diluent and labeled as Standard stock solution 1 and 2. 1ml from each stock solution was pipette out and taken into a 10ml volumetric flask and made up with diluent.

Preparation of Sample stock solutions: 5 tablet was weighed, powdered and weigh the powder equivalent to one tablet and it was transferred into a 10mL volumetric flask, 5mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Preparation of buffer: Accurately weighed and transferred 2.72gr of Potassium di-hydrogen phosphate in 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water and PH adjusted to 3.6 with dil. Orthophosphoric acid solution.

Method validation: The method was validated according to ICH guidelines. The different validation characteristics which were performed are following: Linearity, accuracy, Precision, limit of detection, limit of quantification and robustness.

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Montelukast and Levocetirizine and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Linearity: The linearity of the method is determined by preparing three individual series of solutions in the range of Montelukast (25-150µg/ml) and Levocetirizine (12.5-75 µg/ml). The obtained peak areas are plotted against concentration.

Preparation of linearity solutions: Preparation of Standard stock solutions: Accurately weighed 10mg of Montelukast and 5mg of Levocetirizine and transferred to two 10ml volumetric flasks separately. 5ml of diluent was added to flasks and sonicated for 15mins. Flasks were made up with diluent and labeled as Standard stock solution 1and 2.
From two stock solutions pipette out 0.25ml, 0.5ml, 0.75ml, 1.0ml, 1.25ml, 1.50ml into 10ml volumetric flak to get 25%,50%, 75%, 100%, 125%, 150% of standard solutions.

Precision
a) Method precision (repeatability): The method precision/ repeatability can be determined by injecting six working standard solutions and six sample injections. The areas of all the injections were taken and standard deviation, %Relative standard deviation, %assay were calculated.
b) Intermediate precision: The intermediate precision can be determined by injecting six working standard solutions and six sample injections on different days by different operators or by different instruments. The areas of all the injections were taken and standard deviation, %Relative standard deviation, %assay were calculated. The results obtained were within the acceptance criteria.

Accuracy: Accuracy is tested by the standard addition method at three different levels 50, 100 and 150%. The percentage recoveries of Montelukast and Levocetirizine present in the pharmaceutical dosage form were calculated.

Preparation of 50% Spiked Solution: 250 mg tablet powder was taken into a 25ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 50% Sample stock solution. 0.5ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% standard stock solution was spiked and made up with diluents.

Preparation of 100% Spiked Solution: 500 mg of tablet powder was taken into a 25ml volumetric flask and made up with diluents followed by filtration with

HPLC filters and labeled as Accuracy 100% Sample stock solution. 0.5ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Standard stock solution was spiked and made up with diluents.

Preparation of 150% Spiked Solution: 750 mg of tablet powder was taken into a 25ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 150% Sample stock solution. 0.5ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Standard stock solution was spiked and made up with diluents.

Limit of detection and limit of quantification: Limit of detection (LOD) and limit of quantification (LOQ) of Montelukast and Levocetirizine were determined by calibration curve method. Solutions of Montelukast and Levocetirizine were prepared in linearity range and injected in triplicate. Average peak area of two analyses was plotted against concentration

Method robustness: The robustness can be determined by varying the following parameters:
Robustness of the developed method was determined by making small deliberate changes in flow rate (±1ml/min), column temperature (±5%), organic mobile phase ratio (±10%), along with the optimized method.

RESULTS AND DISCUSSIONS

Development and optimization of HPLC method: The present work was focused to develop a RP-HPLC method for the simultaneous estimation of Montelukast and Levocetirizine in pharmaceutical dosage form. The solubility of the active pharmaceutical ingredient was checked in different solvents like methanol, water, acetonitrile and in different ratios but finally the standard is soluble in methanol: buffer (50:50) so it was chosen as a diluent. The different mobile phases like acetonitrile and potassium dihydrogen phosphate buffer and acetonitrile and sodium dihydrogen phosphate buffer were used in compositions with a flow rate of 1ml/min but the peak resolution, retention time and tailing factor were not satisfactory, so at last potassium dihydroger phosphate and acetonitrile was selected as a buffer at flow rate of 1ml/min. Initially kromosil®"(250mm x 4.6mm x 5µ) and "ODS®" (150mm x 4.6mm x 5µ) columns with different temperatures like 30, 35, 40, 45°C were used but the retention time, run time and peak resolution were not exact and the problem was get rid by using BDS C_{18} column (250mm x 4.6mm x 5µ) kept at 30°c with a run time of 7 minutes. Finally the method was optimized by altering the mobile phase composition / ratio and the optimized wavelength of two drugs Montelukast and Levocetirizine was found to be at 231nm.

System suitability parameters: The system suitability tests were conducted before performing the validation and the parameters were within the acceptance criteria like retention times were 2.599min and 3.472min for , Montelukast and Levocetirizine, plate count was >2000, peak tailing was <2 and the %RSD of peak areas of six injections were ≤ 2% (Table 1). Hence the proposed method was successfully applied to routine analysis without any problems.

Linearity range: The linearity range was in the interval of Montelukast (25-150µg/ml) and Levocetirizine (12.5-75 µg/ml), respectively. These were represented by a linear regression equation as follows: y (Montelukast) = 35120x + 388.3 (r^2 =0.999) and y (Levocetirizine) = 29047x + 721.3. Regression line was established by least squares method and correlation coefficient (r^2) for Montelukast and Levocetirizine were found to be greater than 0.999. Hence the curves established were linear. (Table 2).

Precision: Six replicates injections at the same concentration were analyzed on same day and two different days for verifying the variation in the precision and the % RSD for Montelukast and Levocetirizine were within acceptable limit of ≤2. Hence the method is reproducible on different days with different analyst and column. This indicates that the method is precise (Table 3).

Accuracy: The percentage recoveries for Montelukast and Levocetirizine were found to be 99.88% and 99.9% respectively (Table 4, 5). The results of the recovery studies undoubtedly demonstrate accuracy of the proposed method.

Limit of detection (LOD) and limit of quantification (LOQ): The determined values of LOD and LOQ were calculated by using slope and Y-intercept. The LOD and LOQ values of Montelukast were found to be 0.04 and 0.11µg/ml, Levocetirizine were found to be 0.08 and 0.25µg/ml respectively (Table 6).

Robustness: Robustness of the proposed method demonstrated a non-significant alteration through analysis of the sample and standard Montelukast and Levocetirizine solution (Table 5). After this the results obtained were compared with that of optimized method. It was confirmed that by the deliberate changes in the parameters there was no any significant changes in standard deviation, relative standard deviation, theoretical plates, retention time and USP tailing factor.

Assay: The Content of Montelukast and Levocetirizine in the pharmaceutical dosage form was found by using the developed method. The percentage purity of Montelukast and Levocetirizine were found to be 99.88% and 99.9% and %RSD values for Montelukast and Levocetirizine were within limit of ≤2.

CONCLUSION

A new, simple, rapid and precise high performance liquid chromatographic method was developed for the simultaneous estimation of Montelukast and Levocetirizine in pharmaceutical dosage form. Hence this method can be applied for the estimation of Montelukast and Levocetirizine in drug testing laboratories and pharmaceutical industries.

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DISCLOSURE OF INTEREST

The authors declare that they have no conflicts of interest concerning this article.

Table 1: System suitability parameters for Montelukast and Levocetirizine.

S no	Montelukast			Levocetirizine		
Inj	Area	TP	Tailing	Area	TP	Tailing
1	3491334	8205	1.23	1415248	5330	1.14
2	3494399	8634	1.22	1428749	5556	1.15
3	3403896	8357	1.24	1426962	5601	1.13
4	3496761	9109	1.20	1426573	5571	1.13
5	3447969	8326	1.20	1429867	5576	1.14
6	3500058	8186	1.24	1425873	5547	1.13
avrage	3472403			1425545		
Std dev	38705			5256.3		
%RSD	1.1			0.4		

TP: Theoretical plates; Tailing: USP Tailing factor

Table 2 Linearity table for Montelukast and Levocetirizine.

Montelukast		Levocetirizine	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
25	831348	12.5	362056
50	1796570	25	748501
75	2648803	37.5	1088985
100	3531487	50	1414122
125	4386185	62.5	1823895
150	5246585	75	2192428

Table 3 Determination of repeatability and intermediate precision

Drug Name	Repeatability			Intermediate		
	Peak Area	**Std Dev**	**%RSD**	**Peak Area**	**Std Dev**	**%RSD**
Montelukast	3471869	25290.1	0.7	3414298	9335.7	0.3
Levocetirizine	1425607	12045.2	0.8	1416100	5559.1	0.4

Table 4 Determination of Accuracy of Montelukast

% Level	**Amount Spiked (µg/mL)**	**Total amount found (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	50	150.2	50.2	100.42	99.73%
	50	149.6	49.6	99.19	
	50	149.9	49.9	99.74	
100%	100	199.6	99.6	99.61	
	100	200.5	100.5	100.52	
	100	199.5	99.5	99.53	
150%	150	250.1	150.1	100.04	
	150	248.7	148.7	99.12	
	150	249.1	149.1	99.38	

Table 5 Determination of Accuracy of Levocetirizine

% Level	**Amount Spiked (µg/mL)**	**Total amount found (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Mean %Recovery**
50%	25	75.3	25.3	101.35	100.04%
	25	75.1	25.1	100.53	
	25	75.1	25.1	100.23	
100%	50	100.0	50.0	99.95	
	50	100.4	50.4	100.72	
	50	99.6	49.6	99.25	
150%	75	124.4	74.4	99.15	
	75	125.0	75.0	100.03	
	75	124.4	74.4	99.19	

Table 6 Sensitivity table of Montelukast and Levocetirizine

Molecule	**LOD(µg/ml)**	**LOQ(µg/ml)**
Montelukast	0.04 µg/ml	0.11 µg/ml
Levocetirizine	0.08 µg/ml	0.25 µg/ml

Table 7 Robustness data for Montelukast and Levocetirizine.

S.no	Condition	%RSD of Montelukast	%RSD of Levocetirizine
1	Flow rate (-) 0.9ml/min	0.4	0.4
2	Flow rate (+) 1.1ml/min	0.1	0.2
3	Mobile phase (-) 61B:39A	0.3	0.1
4	Mobile phase (+) 68B:32A	0.6	0.9
5	Temperature (-) 25°C	0.1	0.2
6	Temperature (+) 35°C	0.7	0.8

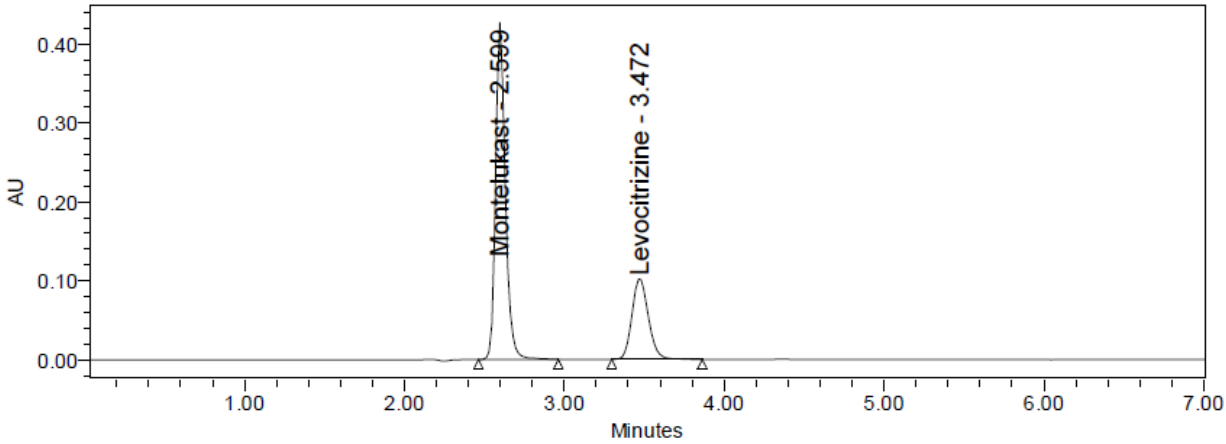

Fig. 1: Optimized chromatogram of Montelukast and Levocetirizine

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