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Development and validation of RP-HPLC method for simultaneous determination of montelukast sodium and levocetirizine dihydrochloride tablets

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ABSTRACT

A simple, sensitive and accurate isocratic reverse phase high performance liquid chromatography method was developed for simultaneous determination of montelukast sodium and levocetirizine dihydrochloride in tablets. The effective separation was achieved on Hypersil C18, 100 x 4.6 mm, 3 µm. The mixture of buffer and acetonitrie in the ratio 90: 10v/v used as a mobile phase. The buffer was prepared as 2.8g of disodium hydrogen orthophosphate in 1000 ml of purified water and adjusts the pH 7.0 with diluted orthophosphoric acid. The flow rate of the mobile phase was 1.0mL/min and the total elution time was 15 minutes. The UV detection wavelength was carried at 230 nm and experiments were conducted at 25°C. The developed method was validated in terms of system suitability, selectivity, linearity, precision, accuracy, limits of detection and quantification for the impurities following the ICH guidelines.

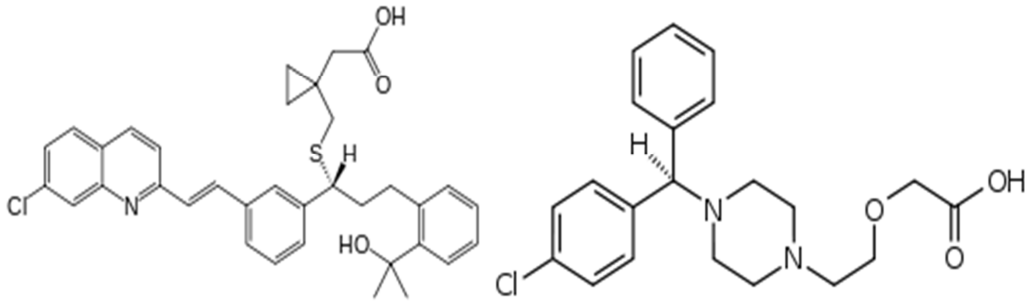
Key Words: Montelukast sodium and levocetirizine dihydrochloride, Method development, Validation and RP-HPLC

INTRODUCTION

Montelukast is chemically belongs to leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies [1,2]. It is usually administered orally in the form of tablets and oral granules etc. Montelukast is a CysLT1 antagonist; it blocks the action of leukotriene D4 (and secondary ligands LTC4 and LTE4) on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. Montelukast is a once-daily leukotriene receptor antagonist, in asthma and allergic rhinitis in both adults and children [3].

Cetrizine is chemically (±)-[2-[4-[(4-chlorophenyl)phenylmethyl]-1- piperazinyl]ethoxy] acetic acid. It is a second-generation4 antihistamine, is a major metabolite of hydroxyzine, and a racemic selective H1 receptor inverse agonist used in the treatment of allergies, hay fever, angioedema, and urticaria. The most commonly it is used in reducing the severity of common cold. Levocetirizine (as levocetirizine dihydrochloride) is a third-generation non-sedative antihistamine, developed from Cetirizine. Chemically, levocetirizine is the active enantiomer of cetirizine5. It is the R-enantiomer of the Cetirizine which is a racemate. Levocetirizine works by blocking histamine receptors. It does not prevent the actual release of histamine from mast cells, but prevents it binding to its receptors. This in turn prevents the release of other allergy chemicals and increased blood supply to the area, and provides relief from the typical symptoms of hay fever.

Allergic rhinitis is the most common allergic disease worldwide and affects about 18% to 40% of the general population. Combination therapy (Montelukast plus levocetirizine) is a more effective strategy than monotherapy in the treatment of persistent allergic rhinitis [6]. Montelukast sodium is a selective and orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene (CysLT 1), receptor. Levocetirizine is the R-enantiomer of Cetirizine. Levocetirizine is an orally active, potent, selective and long acting H 1 -histamine receptor antagonist with no anticholinergic activity. Literature reveals that various spectrophotometric[7,8], HPTLC [9,10], HPLC methods have been reported for analysis of Levocetirizine dihydrochloride and montelukast sodium in single component formulations but a less number of methods are available for the simultaneous estimation of these two drugs in multicomponant dosage forms [11-19]. The quantitative analysis of such multicomponent formulations is very important. HPLC has become a useful instrument for drug analysis since it is the instrument of choice in conducting quantitative estimation. The instrument computes accurate results within minimal time. The method validation which ensures that the selective method will give reproducible and reliable results adequate for intended purpose. Thus, the objective of this work was to develop an accurate, specific, repeatable and validated HPLC method for simultaneous determination of Levocetirizine dihydrochloride and Montelukast Sodium in tablet dosage form as per ICH guidelines [20-22].



a) Chemical structure of montelukast b) Chemical structure of levocetirizine

Fig: 1 Chemical structure of montelukast and levocetirizine

MATERIALS AND METHODS

2.1 Instrumentation and software:

A high performance liquid chromatography system manufactured by Agilent which consist of VWD detector, Quaternary solvent manager, Sample manager, column heating compartment was used for assay determination of montelukast sodium and levocetirizine dihydrochloride. HPLC instrument was controlled by EZChrom Elite software. A Hypersil BDS C_8, 250 x 4.6 mm, column with particle size of 5µm was used as stationary phase for chromatographic separation. Sartorius semi micro analytical balance was used for all weighing, Thermo pH meter was used for buffer pH adjustment, and Bandelin sonicator used to dissolve the standard, sample and were centrifuged by using Hermle centrifuge machine.

2.2 Chemicals and reagents:

All the reagents were of analytical reagent grade unless stated otherwise. Distilled and de-ionized HPLC-grade water, HPLC grade methanol, Disodium Hydrogen Orthophosphate and orthophosphoric acid was purchased from Merck, Mumbai.

2.3 Preparation of mobile phase:

Mixture of buffer solution and methanol in the ratio 250: 750 (v/v) was used as mobile phase. The buffer was prepared as 2.8 g of Disodium Hydrogen Orthophosphate in 1000 mL of water and adjusts the pH of the solution to 7.00 with orthophosphoric acid.

2.4 Preparation of montelukast standard solution:

Weighed accurately and transferred 25mg of montelukast sodium working standard into a 50 mL volumetric flask add 30ml of methanol, sonicate to dissolve and make up with methanol.

2.5 Preparation of levocetirizine standard solution:
Weighed accurately and transferred 31mg of montelukast sodium working standard into a 50 mL volumetric flask add 30ml of methanol, sonicate to dissolve and make up with methanol.

2.5 Preparation of final standard solution:
Transferred 5 mL of montelukast standard solution and 2 mL of levocetirizine standard solution into a 50 mL volumetric flask and make up to the volume with mobile phase as diluent.

2.6 Preparation of sample solutions:
Transferred 5 tablets into a 100 mL volumetric flask, add 5 mL of water, sonicate to dissolve for 5 min then add 60 mL of methanol, sonicate to dissolve for 5 min and dilute to the volume with methanol. Filter the solution through 0.45 µm Nylon filter. Further diluted 5 mL of this solution to 50 mL with diluent.

3. Method validation parameters
The system suitability was conducted using standard preparation and evaluated by injecting five replicate injections. Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present. Performed the specificity parameter of the method by injecting diluent, placebo into the chromatographic system and evaluated by show any peak at the retention time of analyte. Performed the linearity with montelukast sodium and levocetirizine dihydrochloride in the range of 25 to 150% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient.

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation of series of measurements. The system precision was conducted using montelukast sodium and levocetirizine dihydrochloride and evaluated by making six replicate injections. The accuracy of the method by recoveries of montelukast sodium and levocetirizine dihydrochloride sample solutions at different concentration levels ranging from 25 to 150%.The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

RESULTS AND DISCUSSION

4.1 Optimization of chromatographic conditions:
Method development includes selection of appropriate chromatographic conditions/factors like detection wave length, selection and optimization of stationary and mobile phases. The wavelength of 230 nm was selected due to it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify montelukast sodium and levocetirizine dihydrochloride. Preliminary development trials were performed with various columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to Hypersil BDS C_8, 250 x 4.6 mm, 5µm column there a significant improvement in the peak shapes with 1.1 tailing factor.

5. Method validation:
5.1 System suitability:
The RSD from five replicate injections of diluted standard preparation, theoretical plates and asymmetry of main peaks measured. System suitability data is given in Table-1

Table-1: System suitability results of montelukast and levocetirizine

System suitability Parameters & Acceptance criteria	RSD for standard replicates (NMT 2.0%)	Theoretical plates (NLT 2000)	Asymmetry (NMT 2.0)
Montelukast	0.04	7255	1.14
Levocetirizine	0.1	7455	1.17

5.2 Selectivity:
Performed the specificity parameter of the method by injecting diluent, standard preparation sample preparation and placebo preparation into the chromatographic system and recorded the retention times. Specificity study of the

method proved no peak observed at retention time of montelukast sodium and levocetirizine dihydrochloride. The typical selectivity chromatograms are shown in Figure-2.

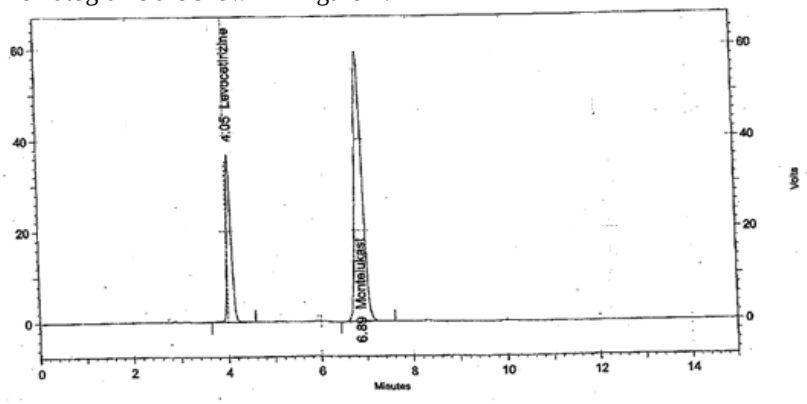


Fig-2: Selectivity chromatogram of montelukast and levocetirizine

5.3 Linearity:

To demonstrate the linearity with montelukast sodium and levocetirizine dihydrochloride in the range of 25 to 150% of specification limit**.** Correlation coefficient of montelukast sodium and levocetirizine dihydrochloride was 0.9999. The linearity results shown in the below Table -2. Linearity curve of montelukast sodium and levocetirizine dihydrochloride shown in the Figure-3.

Table-2: Linearity results of montelukast and levocetirizine

Linearity Level (%)	Concentration of montelukast (µg/mL)	Average area of montelukast	Concentration of levocetirizine(µg/mL)	Average area of levocetirizine
25%	12.42	11566529	6.22	4102897
50%	24.84	22523551	12.45	8133747
75%	37.26	33895077	18.67	11932228
100%	49.68	45584839	24.90	15685745
125%	62.10	56937912	31.12	19789039
150%	74.52	67246758	37.34	23463503
Correlation coefficient :		**0.9999**	**0.9999**	

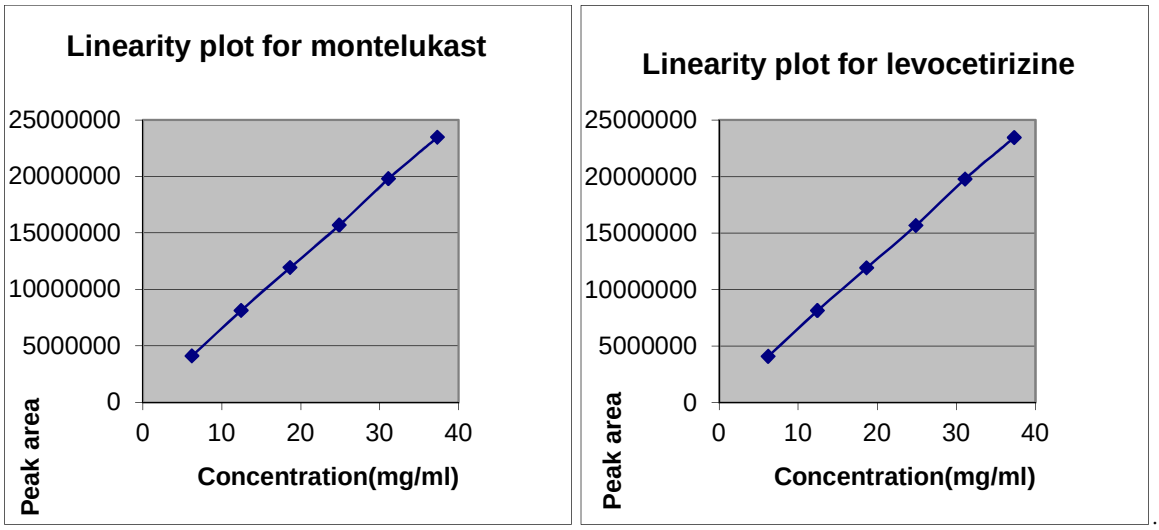


Figure-3 Linearity curve of montelukast sodium and levocetirizine dihydrochloride

5.4 Precision:

The precision of test method was validated by assaying six samples prepared on montelukast sodium and levocetirizine dihydrochloride and calculate relative standard deviation of area results. The precision results are given Table-3.

Table-3: Precision results of montelukast and levocetirizine

Injection No	Area of montelukast	Area of levocetirizine
1	45637547	16044769
2	45640895	16045937
3	45641207	16052102
4	45671551	16066508
5	45667904	16063007
6	45570683	16097593
Average	45638298	16061653
SD	36248.7	19699.6
% RSD	0.1	0.1

5.5 Accuracy:

Accuracy study found that the mean % of recovery was more than 98.0% and less than 102.0% at each level 25 to 150% of concentration levels, hence method is accurate. The accuracy results are given Table-4, 5.

Table-4: Accuracy results of montelukast

Level	Amount Added in (mg)	Amount found in (mg)	% Recovery	Average	% RSD
25%	12.52	12.35	98.7	99.0	0.4
	12.52	12.38	98.9		
	12.51	12.46	99.6		
50%	25.04	25.28	101.0	101.0	0.2
	25.00	25.18	100.7		
	25.02	25.32	101.2		
100%	49.99	49.66	99.3	99.4	0.3
	50.05	49.65	99.2		
	50.00	49.90	99.8		
150%	75.04	74.44	99.2	99.3	0.1
	75.02	74.61	99.5		
	75.04	74.57	99.4		
% RSD					**0.8**

Table-5: Accuracy results of levocetirizine

Level	Amount added in (mg)	Amount found in (mg)	% Recovery	Average	% RSD
25%	6.25	6.21	99.3	99.3	0.1
	6.26	6.23	99.5		
	6.27	6.22	99.2		
50%	12.52	12.56	100.4	100.4	0.2
	12.47	12.56	100.7		
	12.54	12.56	100.2		
100%	25.03	25.01	99.9	99.5	0.3
	25.01	24.81	99.2		
	25.00	24.85	99.4		
150%	37.51	37.40	99.7	100.0	0.2
	37.47	37.47	100.0		
	37.44	37.55	100.3		
% RSD					**0.5**

5.6 Robustness

The method robustness was studied by injecting the system suitability solution at change in the pH of buffer solution, change in minor component, flow rate, and column temperature. The results were obtained as shown in the below Table-6, 7

Table-6: Robustness results of montelukast

Condition	% RSD	Theoretical Plates	Asymmetry
Normal Condition (as such condition)	0.04	7255	1.14
Buffer pH 7.2	0.2	6965	1.10
Buffer PH 6.8	0.5	6616	1.14
Column temperature 30°C	0.1	7934	1.15
Column temperature 20°C	0.1	6996	1.13
Flow 1.1mL/min	0.1	7006	1.12
Flow 0.9 mL/min	0.1	7996	1.17
Change in minor component + 5%	0.03	7565	1.16
Change in minor component - 5%	0.04	7514	1.19

Table-7: Robustness results of levocetirizine

Condition	% RSD	Theoretical Plates	Asymmetry
Normal Condition (as such condition)	0.1	7455	1.17
Buffer pH 7.2	0.2	7969	1.20
Buffer PH 6.8	0.3	7654	1.17
Column temperature 30°C	0.1	8315	1.25
Column temperature 20°C	0.1	7627	1.20
Flow 1.1mL/min	0.1	7761	1.15
Flow 0.9 mL/min	0.02	8738	1.14
Change in minor component + 5%	0.04	7958	1.16
Change in minor component - 5%	0.03	7856	1.21

CONCLUSION

A simple isocratic HPLC method has been developed and validated for the simultaneous determination of montelukast sodium and levocetirizine dihydrochloride. The developed method has been found to selective, sensitive, precise, robust and stability indicating. The method can be directly adopted in quality control laboratories for routine analysis with respect to simultaneous determination and quantification of montelukast sodium and levocetirizine dihydrochloride and also for the analysis of stability samples.

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