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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF MONTELUKAST SODIUM IN BULK DRUG AND DOSAGE FORM

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ABSTRACT

A reversed–phase high-performance liquid chromatography method is developed and validated for the determination of Montelukast Sodium (Montelukast) in bulk drug and pharmaceutical dosage form. The chromatographic determination was performed on Agilent 1220 Infinity LC with *EZ* chrome elite software with variable wavelength detector. The separation was conducted by using Zobrax Eclipse XDB-C18 column (4.6×150×5µm) with mobile phase consisting Methanol:Acetonitrile:Water (60:30:10). The mobile phase was delivered at the flow rate of 1.0 ml/min. The eluent was monitored at wavelength 344 nm and found a sharp and symmetrical peak with retention time 3.582. The method was validated for linearity, accuracy, precision, system suitability, and stability. The method was found to be linear over the concentration range 5-30µg/ml with coefficient R^2 - 0.999. The developed HPLC technique is precise, specific, accurate and stable. Statistical analysis proves that the method is reproducible, selective and suitable to be applied for analysis of montelukast sodium in commercial pharmaceutical dosage form for routine quality control application.

KEYWORDS: Montelukast Sodium (Montelukast), RP- HPLC, Validation.

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INTRODUCTION

Montelukast sodium (1-[[[(1R)-1-[3-[(1E)-2-(7-chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl] -propyl] thio] methyl] cyclopropaneacetic acid, monosodium salt) is a prophylaxis and used for long-term treatment of asthma; relief of symptoms of seasonal allergic rhinitis in patients; relief of symptoms of perennial allergic rhinitis; prevention of exercise-induced bronchoconstriction (EIB)[1]. Its molecular formula and molecular weight are $C_{35}H_{35}ClNNaO_3S$ and Mol. Wt. 608.2g/mol.[1] It is a potent selective inhibitor of leukotriene D4 (LTD4).[2] Montelukast sodium, the active ingredient in montelukast sodium tablets, is a selective and orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene CysLT1 receptor. The most of the studies with these LTRAs were conducted in Asthmatic and then in pediatric patient. Secondly they represent their class effects mostly.[3] In this study, efforts were made to develop a simple, easy and economic RP-HPLC method using mobile phase consisting Methanol:Acetonitrile:Water (60:30:10) for the determination of montelukast in the raw materials as well as in the marketed dosage formulations. The developed method was optimized and validated as per the guidelines of International Conference on Harmonization (ICH) and demonstrated excellent specificity, linearity, precision and accuracy for montelukast. The chemical structure of montelukast is shown in Figure 1. Literature survey reveals that, some study about UV and HPLC determination of montelukast has published. The aim of this study is to develop a fast, simple, reliable, selective, sensitive and inexpensive RP-HPLC method for the determination of montelukast in bulk drug and commercial pharmaceutical formulations as tablet and its validation.

MATERIALS AND METHODS

Montelukast Sodium drug gift samples obtained from Cipla labs, Mumbai. All chemicals and reagents were of analytical grade. Pharmaceutical grade excipients were obtained from Pharmaceutical Technology Lab. of Maharashtra.

Preparation Mobile Phase

To optimize the HPLC parameters several mobile phase compositions were tried. Satisfactory peak symmetry was obtained with mobile phase consisting of Methanol, Acetonitrile and Water in proportion of 60:30:10. The mobile phase was filtered through 0.45 cellulose nitrate filter paper and degassed by ultrasonication for 10 min.

Preparation of standard solution

Weigh accurately 100 mg of Montelukast sodium and transfer in 100 ml of volumetric flask and dissolve in mobile phase and make up the volume with mobile phase. The concentration of stock solution is 1000 µg/ml. This solution is used for further dilutions.

Calibration Curve

Appropriate aliquots of standard stock solution (1000 µg/ml) was diluted to 100 mg/ml in 10ml volumetric flask and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 5, 10, 15, 20, 25, and 30 µg/ml. These solutions were injected into chromatographic system and chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of montelukast were constructed by plotting peak area ratio vs applied concentration of montelukast and regression equation was computed. Similarly the sample solution was chromatographed and concentration of montelukast sodium in tablet samples was found out using regression equation.

Preparation of sample solution

20 Tablets (each tablet contains 10 mg of Montelukast sodium) were weighed and taken into a mortar uniformly mixed. Test stock solutions of Montelukast sodium (10µg/ml) and was prepared by dissolving weight equivalent to 10 mg of Montelukast sodium and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 10 µg/ml of Montelukast sodium was made by adding 1 ml of stock solution to 10 ml of mobile phase.

METHOD VALIDATION [5]

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Montelukast sodium and the solutions were injected six times and the parameters like % RSD, peak tailing, resolution and USP plate count were determined. The results are mentioned in Table 1. The standard chromatogram is shown in Fig. 3.

Specificity

The specificity of the HPLC method was determined by the complete separation of montelukast sodium in presence of its degradation products along with other parameters like retention time (t_r), capacity factor (k), tailing or asymmetrical factor (T). Specificity was also performed by running triplicate analysis of degradants sample under different conditions with mobile phase of different selectivity. The results are mentioned in Table 1.

Linearity

Linearity the method was tested from 90-210 % of the targeted level of the assay concentration for analyte. Standard solutions contained 5-30 µg/mL of Montelukast sodium. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area against the concentration of the drugs. The equations of the calibration curves for Montelukast sodium obtained was y = 03E+06X In the Montelukast sodium determination, the calibration graphs were found to be linear in the aforementioned concentrations with correlation coefficients 0.999. The results are mentioned in Table 2 & calibration curve Fig 2.

Accuracy

20 blank tablets were powdered and mixed. This powder was then spiked with a quantity of montelukast sodium corresponding to 80%, 100% and 120% of the labeled claim. Each of these powder mixtures was analyzed in triplicate and the quantity of montelukast sodium was determined using calibration equation. Accuracy was reported as 97.66% of Montelukast sodium recovered. The results are mentioned in Table 4.

Precision

Prepared sample preparations of Montelukast Sodium as per test method and injected 6 times in to the column. The repeatability of the method was studied by determining the concentrations of Montelukast Sodium six times. The results of the precision study indicate that the method is reliable (%RSD < 2). Intermediate precision of the method was determined by analyzing the samples six times on different days by different chemists using different analytical columns of the same make and different HPLC systems. The results are mentioned in Table 5.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variation in the analytical procedure parameters. To evaluate HPLC method robustness a few parameters were deliberately varied. The parameters included variation of columns C8 (old & new), percentage of acetonitrile in the mobile phase and acetonitrile of different lots. Change in wavelength ± 2nm Change in flow rate ±0.2 ml/min. The results are mentioned in Table 3 & 6.

Limit of Detection (LOD)

The limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa), which may be related to LOD and the slope of the calibration curve, b.

Limit of Quantification (LOQ)

The LOQ is the concentration that can be quantitate reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.

Preparation of samples for Assay

Standard sample

Take weight of 20 tablet of any brand of Montelukast sodium tablets. Crush the tablet in the motor pestle. Accurately weigh the quantity of powder equivalent to 10mg of drug

in 100 ml volumetric flask and add methanol to adjust the volume up to 100 ml. Pipette out the 1 ml in to 10 ml volumetric flask make the volume with mobile phase to get conc. 10µg/ml and analyze the reading on HPLC. Calculate the percentage purity of tablet. The results are mentioned in Table 7.

RESULTS AND DISCUSSION

Chromatographic separation was achieved on a C18 column, mobile phase consisting of a mixture of Methanol: Acetonitrile: Water (60:30:10) with detection of 344 nm. The retention time was found to be 3.582 min and linearity was observed in the range 5-30µg/ml for Montelukast sodium. The method was found to be precise as indicated by the repeatability analysis, showing % RSD < 2. Accuracy, limit of detection, limit of quantification, robustness and ruggudness values were within the limits.

Table 1
System suitability parameters

Injection	Retention time (min)	Peak area	Tailing factor (TF)
1	3.547	2158244	1.345
2	3.540	2160321	1.286
3	3.544	2173645	1.332
4	3.537	2182546	1.203
5	3.542	2212945	1.345
6	3.530	2199364	1.102
Mean	3.540	2181178	1.392
SD	0.0059	21723.14	
%RSD	0.16854	0.9959	

Table 2
Linearity of Montelukast sodium

Sr No	Concentration (µg/ml)	Area
1	5	1006952
2	10	2158244
3	15	3298969
4	20	4432958
5	25	5397699
6	30	6456634
SD	9.3541	2037553
Slope	21771	

Table 3
Results for Ruggedness

Montelukast sodium	%Assay
Analyst 1	98.65
Analyst 2	99.61
%RSD	0.68

Table 4
Recovery results for Montelukast sodium

Recovery level	Accuracy Montelukast sodium					Average % Recovery
	Amount taken(mcg/ml	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	5	3842165	3844741	4.9	98	97.66
	5	3826325				
	5	3865734				
100%	15	4273758	4241898	14.7	98	
	15	4234517				
	15	4217420				
120%	30	4723770	4722146	29.1	97	
	30	4707322				
	30	4735348				

Table 5
Method Precision

Montelukast sodium		
Sr.No.	**Rt**	**Area**
1	3.547	2158244
2	3.540	2160321
3	3.544	2173645
4	3.537	2182546
5	3.542	2212945
6	3.530	2199364
Avg	3.540	2181178
SD	0.0059	21723.14
%RSD	0.16854	0.9959

Table 6
Results for Robustness

Parameter	**Montelukast sodium**	
	Retention time (min)	**Tailing factor**
Flow		
0.8ml/min	3.547	1.384
1.2ml/min	3.510	1.206
Wavelength		
344nm	3.510	1.339
342nm	3.542	1.104

Table 7
Results for Assay

	Montelukast sodium	
	Standard Area	**Sample Area**
Injection 1	2158244	2098876
Injection 2	2160321	2056034
Injection 3	2173645	2043098
Injection 4	2182546	2028431
Injection 5	2177580	2030012
Average Area	2170467	2051290
% Assay	100	94.51
%RSD	0.4935	1.4064

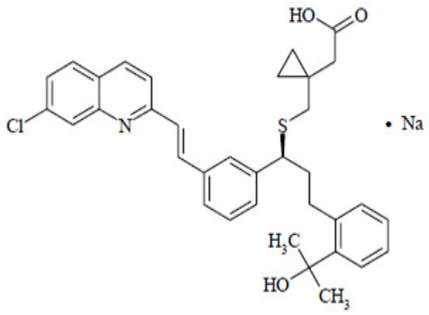

Figure 1
Structure of Montelukast sodium

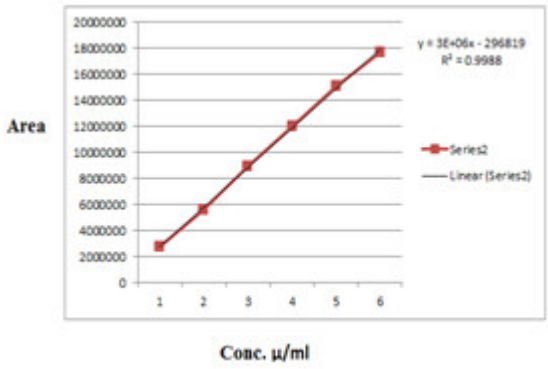

Figure 2
Calibration Curve

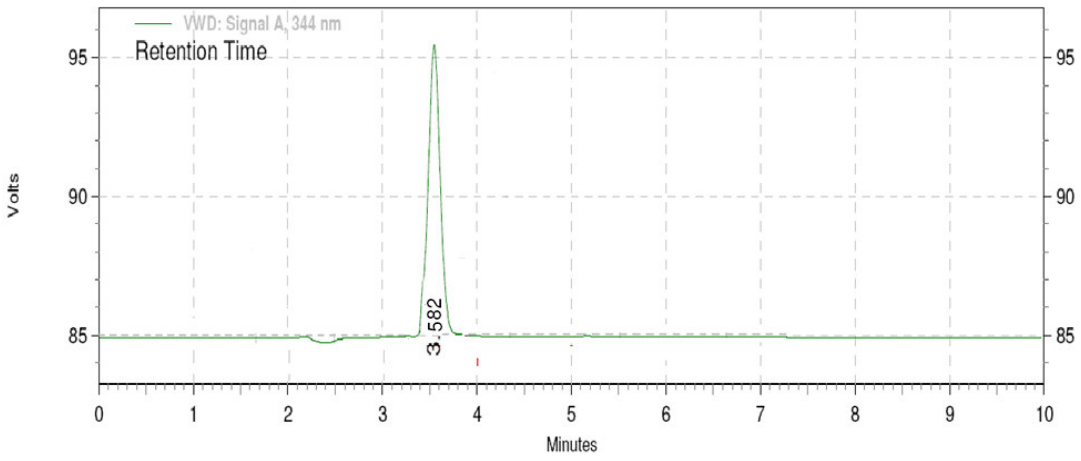

Figure 3
Standard chromatogram

CONCLUSION

The proposed RP-HPLC method was validated as per ICH guidelines and can be applied for the determination of Montelukast sodium in bulk and pharmaceutical dosage forms. The method was found to be system suitability, specificity, accuracy, recovery, robustness, linearity, ruggedness, and limit of detection and limit of quantification. The recovery studies of Montelukast sodium was found to be 97.66 % and get a retention time 3.582 min. the linearity method was investigated and observed in the range of 5-30 µg/ml for Montelukast sodium so and the method was found to be precise as indicated by the repeatability analysis showing % RSD < 2.

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