INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

A RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF MONTELUKAST SODIUM IN BULK AND PHARMACEUTICAL DOSAGE FORMS

B.V.V Ravi kumar[1]*, Patnaik A.K.[2], Raul Saroj Kumar[3], Nagireddy Neelakanta Rao[4]

[1]Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India

[2]Department of Chemistry, Khalikota Autonomous College, Berhampur, Orissa, India

[3]M R College of Pharmacy, Phool-Baugh, Vizianagaram, A.P, India

[4]Suraksha Pharma, Suraksha Towers, Hyderabad, A.P, India

Article Received on: 14/09/12 Revised on: 10/10/12 Approved for publication: 03/11/12

*Email: ravikumar_bvv@yahoo.co.uk

ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Montelukast Sodium in pharmaceutical dosage form. Isocratic elution at a flow rate of 1.2 mL min $^{-1}$ was employed on a symmetry C_{18} column at ambient temperature. The mobile phase consisted of acetonitrile: phosphate buffer 65:35 (v/v). The UV detection wavelength was at 234 nm. Linearity was observed in concentration range of 1-100 µg/mL. The retention time for Montelukast Sodium was 4.2 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Montelukast Sodium in pharmaceutical dosage forms.

Keywords: Montelukast Sodium, RP-HPLC, Method development and Validation.

INTRODUCTION

Montelukast sodium is a selective and orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene CysLT1 receptor used in the treatment of asthma[1]. Montelukast sodium is described chemically as [R-(E)]-1-[[[1-[3-[2-(7-chloro- 2quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy -1-methylethyl) phenyl] propyl] thio] methyl] cyclopropaneacetic acid, monosodium salt1 (Figure 1). Montelukast binds with high affinity and selectivity to the CysLT1 receptor (in preference to other pharmacologically important airway receptors, such as the prostanoid, cholinergic, or β-adrenergic receptor). Montelukast inhibits physiologic actions of LTD4 at the CysLT1 receptor without any agonist activity.

Literature survey reveals that various HPLC method[2-6], methods with other drugs [7-9] and spectrophotometric method[10] have been reported for the estimation of Montelukast sodium. The aim of the study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Montelukast Sodium in pharmaceutical dosage form as per ICH guidelines.

MATERIALS AND METHOD

Instrumental and analytical conditions

The HPLC analysis were carried out on YOUNGLIN ACME 9000 (Korea) with Autochrrome 3000 integrater and UV Visible detector. The column used is Inertsil-Extend C_{18} (250 × 4.6 mm, packed with 5 µm). UV detection was performed at 234 nm. The injection volume of sample was 20 µL and the run time was 8 minutes. An isocratic mobile phase containing acetonitrile and 0.02 M phosphate buffer at 65: 35 (v/v) at the pH 3.5 was carried with the flow rate at 1.2mL min^{-1}. The mobile phase was filtered through 0.4µm membrane filter and degassed before use.

Reagents and chemicals

Montelukast Sodium working standard was kindly gifted by Dr Reddy's laboratories, Hyderabad. Tablets were purchased from local pharmacy manufactured by Piramal health care (Monti). Ultra pure water was obtained from a millipore system. HPLC grade acetonitrile was obtained from Merck (India) limited. All other chemicals used were AR grade. The optimum chromatographic conditions were summarized in table 7.

Preparation of mobile phase

Dissolved 2.7218 g of Potassium Di hydrogen orthophosphate in 1000 mL of water and mixed, pH adjusted to 3.5 using ortho phosphoric acid, sonicated to degas the buffer. Transferred 650 volumes of Acetonitrile and 350 volumes of buffer into a 1000 volumes mobile phase bottle and mixed. Then sonicated up to 15 minutes for degas the mobile phase.

Preparation of Standard Solution

Accurately weighed about 10 mg of Montelukast Sodium and transferred into a 10mL volumetric flask and 5 mL of acetonitrile was added to ensure the complete solubilization and the volume was adjusted with the mobile phase to get stock solution of 1000 µg/mL. Then 0.5 mL of stock solution is transferred into 10 ml volumetric flask and make up to volume with mobile phase which gives a solution of strength 50 µg/mL.

Method Validation

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and system suitability.

Linearity

From the standard stock solution, the various dilutions of Montelukast Sodium in the concentration of 1, 2, 20, 25, 50, 75 and 100 µg/mL were prepared. The solutions were injected using 20 µL injection volume in to the chromatographic system at the flow rate of 1.2 mLmin^{-1} and the effluents were monitored at 234 nm, chromatograms were recorded. Calibration curve of Montelukast Sodium was obtained by plotting the peak area ratio versus the applied concentrations of Montelukast Sodium, given in table 1. The linear correlation coefficient was found to be 0.999, shown in figure 2.

Precision

Repeatability of the method was checked by injecting replicate injections of 50 µg/mL of the solution for six times on the same day as intraday precision study of Montelukast Sodium and the % RSD was found to be 0.07, given in table 2.

Accuracy

Montelukast Sodium reference standards were accurately weighed and added to a mixture of the tablets excipients, at three different concentration levels (50, 100 and 150 % of Montelukast Sodium). At each level, samples were prepared in triplicate and the recovery percentage was determined and presented in table 3.

Specificity

Spectral purities of Montelukast Sodium chromatographic peaks were evaluated for the interference of the tablet excipients as per the methodology. In the work, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure to evaluate possible interfering peaks and no interference peaks were observed.

Robustness

To determine the robustness of the method, two parameters (flow rate, composition of mobile phase) from the optimized chromatographic conditions were varied. Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust which is shown in table 4.

Ruggedness

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst.

Detection and quantitation limits

According to the determined signal-to-noise ratio, Montelukast Sodium presented limits of detection of 0.15 µg/mL and limits of quantitation of 0.70 µg/mL, where the compounds proportion found in the sample solutions injected onto the chromatograph. However, the objective of the method is the quantitation of Montelukast Sodium, so that the values obtained for Montelukast Sodium should be considered as the limit of method sensitivity.

System Suitability Parameter

System suitability tests were carried out on freshly prepared standard stock solutions of Montelukast Sodium and it was calculated by determining the standard deviation by injecting standards in six replicates at 6 minutes interval and the values were recorded and the system suitability parameters are shown in table 5.

Assay of Montelukast Sodium tablet

Three different batches of Monti were analyzed using the validated method. For the analysis, six replicates of each batch were assayed. The tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 50mg of Montelukast Sodium was transferred to a 100 ml volumetric flask followed by the addition of 25 ml of acetonitrile. The solution was sonicated for 3 minutes then filtered through 0.45 µm membrane filter and volume adjusted with the mobile phase. Further dilutions were made to get the final concentration equivalent to 50 µg/mL of Montelukast Sodium. The results were presented in table 6.

All the analyzed batches presented Montelukast Sodium were very close to the labeled amount. The Montelukast Sodium content in the tablets samples varied from 98.98 to 100.24%.

Table 1: Linearity parameter for Montelukast Sodium

Concentration (µg/mL)	Average area
1	53387
2	83479
20	1026327
25	1294197
50	2666178
75	3885428
100	5122408

Table 2: Precision parameter of Montelukast Sodium

Injections	Area
I.P-1	2657614
I.P-2	2658634
I.P-3	2655374
I.P-4	2654362
I.P-5	2653286
I.P-6	2656455
Mean	2655954
SD	2008.885
% RSD	0.075637

Table 3: **Accuracy parameter for Montelukast Sodium**

Concentration (µg/mL)		**inj-1**	**inj-2**	**inj-3**	**Mean**	**% Recovery**	**STD**	**% RSD**
25	50%	1307462	1316543	1308643	1310883	98.334	4937.43	0.37664
50	100%	2663574	2654278	2656174	2658009	99.693	4912.06	0.18480
75	150%	4045734	4025573	4043463	4038257	100.974	11042.9	0.27345

Table 4: Robustness parameter of Montelukast Sodium

Parameters	**Adjusted to**	**Average Area**	**R_t**	**SD**	**% RSD**
Flow rate as per method 1.2mL/min	1.1 mL/min	2657384	4.37	3465.89	0.13
	As it is	2666153	4.21	9983.68	0.48
	1.3ml/min	2947538.8	4.96	15528.59	0.53
Mobile phase composition (Buffer : Acetonitrile, 35:65)	Buffer : Acetonitrile (30:70)	2655152.5	4.38	3672.43	0.14
	As it is	2277340.2	4.27	8126.60	0.36
	Buffer : Acetonitrile (40:60)	3270501.7	4.83	12826.87	0.39

Table 5: System Suitability for Montelukast Sodium

Concentration	Injection	Area	R_t
50 µg/mL	Inj-1	2657926	4.04
	Inj-2	2652798	4.1
	Inj-3	2658245	3.97
	Inj-4	2653846	4.02
	Inj-5	2661947	3.99
	Inj-6	2659542	3.98
Statistical Analysis	Mean	2657384	4.016667
	SD	3465.887	0.048442
	% RSD	0.130425	1.206035
	Tailing Factor	1.0289	
	Plate Count	9765.8	

Table 6: Contents of Montelukast Sodium in tablets (n=6)

Sample tablet	Batch	Content of Montelukast Sodium (%) + S.D.
Monti	1	98.98± 0.25
	2	99.51 ± 0.42
	3	100.24± 0.45

S.D=Standard Deviation

Table 7: Developed Chromatographic Conditions

Parameters	Method
Stationary phase (column)	Inertsil-Extend C_{18} (250 × 4.6 mm, packed with 5 µm)
Mobile Phase	65:35 (Acetonitrile : Phosphate Buffer)
pH	3.5 ± 0.02
Flow rate (ml/min)	1.2
Run time (minutes)	8.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	234
Drugs RT (min)	4.2

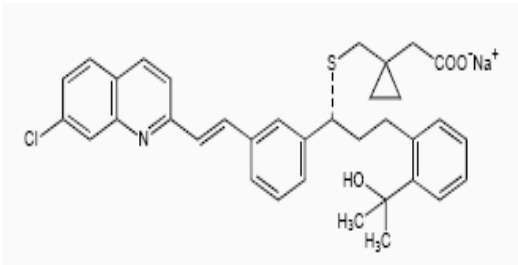

Figure 1: Structure of Montelukast Sodium

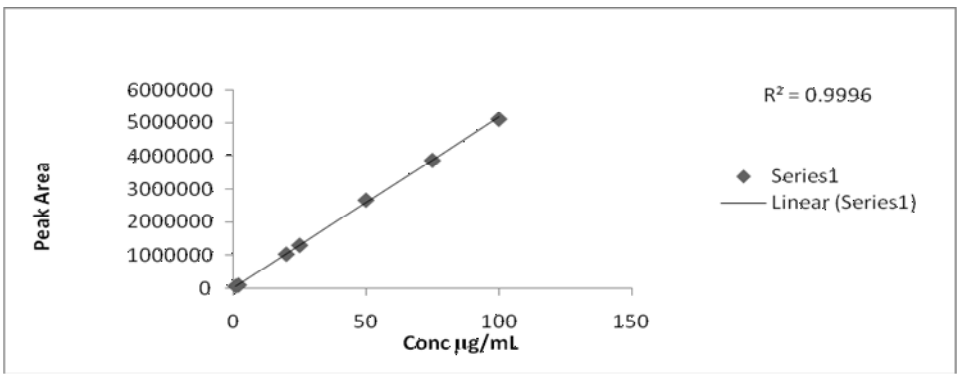

Figure 2: Linearity curve of Montelukast Sodium

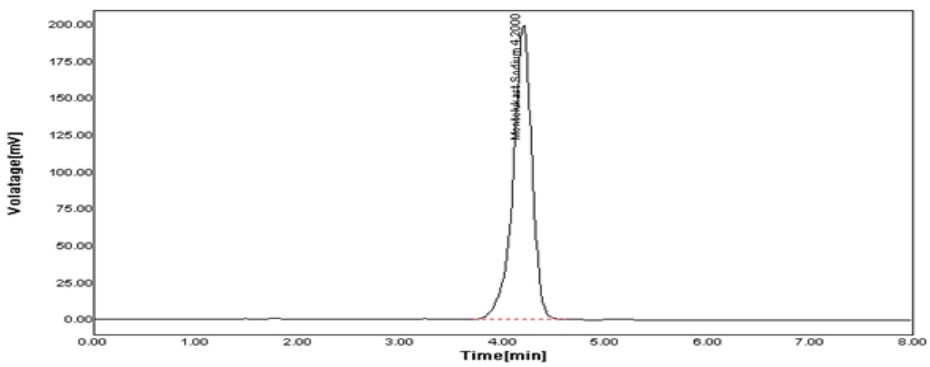

Figure 3: Standard Chromatogram of Montelukast Sodium

RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Montelukast Sodium was preferably analyzed by reverse phase chromatography and accordingly C_{18} column was selected. The elution of the compound from the column was influenced by polar mobile phase. The ratio of the acetonitrile to phosphate buffer was optimized to give symmetric peak with short run time. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase of acetonitrile: phosphate buffer at the ratio of 65:35 (v/v).The retention time of Montelukast Sodium was found to be 4.2 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 5. Developed chromatographic method was applied for the determination of Montelukast Sodium in tablet formulation, table 7. A typical chromatogram showing the separation of Montelukast sodium is shown in figure 3.

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Montelukast Sodium in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Therefore, it is suitable for the routine analysis of Montelukast Sodium in pharmaceutical dosage form.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Reddy's Laboratories, Hyderabad for providing gift sample of Montelukast Sodium and Bright's Lab., Hyderabad for providing necessary facilities to carry out the research work.

REFERENCES

1. Budavari S. The Merck Index.12th.ed.White house station NJ.Merck and Co INC; 1996:1070
2. Sing RM, Saini PK, Mathur SC, Sing GV, Lal B. Development and validation of a RP-HPLC method for estimation of Montelukast sodium in bulk and in tablet dosage form. Current trends in biotechnology and pharmacy 2010; 72(2): 235-237.
3. Raju KN, Swamy TG, Rao AL. Development and validation of RP-HPLC method for the determination of Montelukast sodium in bulk and in pharmaceutical formulations. International journal of pharmaceutical, chemical and biological Sciences 2011; 1(1):12-16.
4. Devi NK, Rani AP, Madhavi BR, Mrudula BS. New RP-HPLC method for the analysis of Monteleukast sodium in pharmaceutical dosage forms. International journal of chemtech research 2010; 2(1): 471-475.
5. Nataraj KS, Babu TV, Duza MB. Method development and validation of Montelukast sodium in bulk and pharmaceutical dosage forms by RP-HPLC .Journal of chemical and pharmaceutical sciences 2011; 4(2): 55-57.
6. Pattnaik A, Panda SS, Sahoo S, Patro VJ. RP-HPLC Method development and validation for the determination and stability indicative studies of Montelukast in bulk and its pharmaceutical formulations. E- Journal of chemistry 2012; 9(1):35-42.
7. Giriraj P, Shajan A. Simultaneous estimation and method validation of Montelukast sodium and Doxofylline in solid dosage form by RP-HPLC. International journal of chemical and pharmaceutical sciences 2011; 2(1):31-36.
8. Ravisankar M, Uthirapathy S, Thangadurai A, Munusamy J, Dhanapal K. Simultaneous estimation of Fexofenadine Hydrochloride and Montelukast sodium in bulk and marketed formulation by RP-HPLC method. International research journal of pharmacy2012; 3(4):256-259.
9. Eswarudu MM, Junapudi S, Chary TN. RP-HPLC method development and validation for simultaneous estimation of Montelukast sodium and Levocetirizine Dihydrochloride in tablet dosage form. International journal of pharma world research 2011; 2(4):1-18.
10. Srihari G, Setty KN, Reddy NR, Chakravarth IE. A simple spectrophotometric assay of Montelukast in pharmaceutical formulation. Journal of chemical and pharmaceutical research 2011; 3(6):23-27.

Source of support: Nil, Conflict of interest: None Declared