



A Validated RP-HPLC Method for the Determination of Impurities in Montelukast Sodium

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Abstract: The present paper describes the development of a reverse phase chromatographic (RPLC) method for montelukast sodium in the presence of its impurities and degradation products generated from forced degradation studies. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The degradation of montelukast sodium was observed under acid and oxidative environment. The drug was found to be stable in other stress conditions studied. Successful separation of the drug from the process impurities and degradation products formed under stress conditions were achieved on an Atlantis dC18 (250 x 4.6 mm) 5 µm column. The gradient LC method employs solution A and solution B as mobile phase. The solution A contains aqueous 0.1% OPA and solution B contains a mixture of water, acetonitrile (5:95 v/v). The HPLC method was developed and validated with respect to linearity, accuracy, precision, specificity and ruggedness.

Keywords: RP-HPLC, Montelukast sodium, Impurities, Degradation products, Validation.

Introduction

Montelukast sodium, the active ingredient in Singulair, is a selective and orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene cys LT₁ receptor¹. 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 salt. The different analytical techniques reported so far for the determination of this drug and its metabolites in biological samples include capillary electrophoresis² and spectrophotometry³. The determination of Montelukast sodium in plasma by RP-LC⁴⁻⁶ and in solid dosage forms by RP-LC⁷⁻⁸ was also reported.

Organic impurities can arise during the manufacturing process and storage of the drug substances and the criteria for their acceptance up to certain limits are based on pharmaceutical studies or known safety data⁹. As per regulatory guidelines, the pharmaceutical studies using a sample of the isolated impurities can be considered for safety assessment. It is, therefore, essential to isolate and characterize unidentified impurities present in the drug sample. During the development of an analytical procedure, the LC method was developed for the determination of montelukast and the impurities arising during its manufacturing. In the present study, we describe a reverse phase column liquid chromatography method for the separation and quantification of process and degradation impurities of montelukast sodium. The accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness of the method were determined in accordance with ICH guidelines¹⁰. This paper reports, a rapid, efficient, simple and validated LC method for separation of potential impurities and degradation products.

Experimental

Reference standard of montelukast and seven impurities namely, Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G (Figure 1) were synthesized and characterized by use of LC-MS, NMR and IR in Aurobindo Pharma Ltd., Hyderabad, India. Montelukast sodium was provided by Chemical Research Division of Aurobindo Pharma Ltd. All reagents used were of analytical reagent grade unless stated otherwise. Milli Q water, HPLC-grade acetonitrile, HPLC-grade orthophosphoric acid (OPA) was purchased from Merck (Darmstadt, Germany).

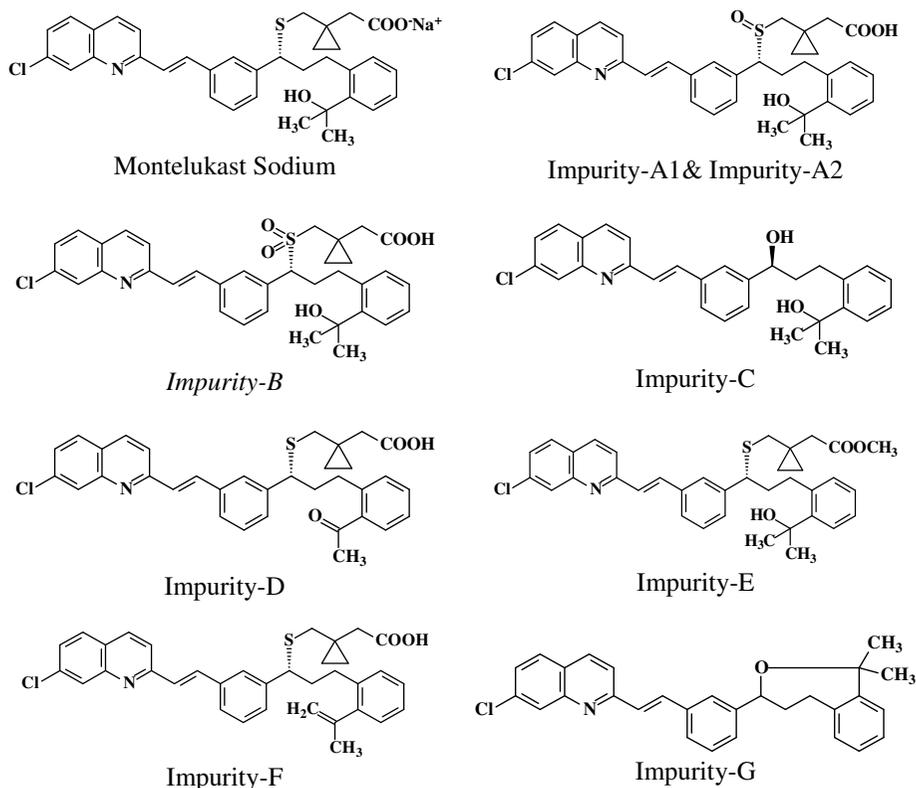


Figure 1. Chemical structures of montelukast sodium and its impurities.

The LC system was equipped with quaternary gradient pumps with auto sampler and auto injector (Alliance 2695, Waters, Milliford, MA, USA) controlled with Empower software (Waters).

Preparation of standard solution

Accurately weighed 40 mg of montelukast was transferred to a 100 mL volumetric flask, added about 50 mL of diluent and sonicated to dissolve, diluted up to the mark with diluent and mixed. Further 5 mL of the resulting solution was diluted to 100 mL with diluent and further 5 mL of the solution was diluted to 50 mL with diluent. This solution contains 0.002 mg/mL.

Preparation of sample solution

Accurately weighed 52 mg of montelukast sample was transferred to 50 mL of volumetric flask and added about 25 mL of diluent and sonicated to dissolve, diluted up to the mark with diluent and mixed.

Chromatographic conditions

The chromatographic separation was achieved on a Waters 250 x 4.6 mm, Atlantis dC18, 5 μ m particles. The gradient LC method employs solution A and B as mobile phase. The solution A contains aqueous 0.1% orthophosphoric acid and solution B contains a mixture of water: acetonitrile (5:95 v/v). The flow rate of the mobile phase was 1.5 mL/min and the peak shape of the montelukast sodium was found to be symmetrical. The HPLC gradient program was set as: time % solution B: 0.01/60, 10/70, 15/90, 20/100, 30/100, 32/60, 40/60 with a post run time of 10 min. The column temperature was maintained at 20 °C and the detection was monitored at a wavelength of 225 nm. The injection volume was 20 μ L. A mixture of water: methanol (30:70, v/v) was used as a diluent.

Validation of the method

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities⁹. The specificity of the developed LC method for montelukast was carried out in the presence of its impurities namely, Imp-A (mixture of two isomers Imp-A1 and Imp-A2) Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G. Stress studies were performed for montelukast drug substance to provide an indication of the stability indicating property and specificity of the proposed method. International degradation was attempted to stress condition of heat (80 °C, 120 h), acid (1.0 M HCl, 85 °C, 15 min), base (5 M NaOH, 85 °C, 120 min) oxidation (3.0% H₂O₂ RT, 10 min) and photolytic degradation (10 K Lux, 48 h) to evaluate the ability of the proposed method to separate montelukast sodium from its degradation product. For heat study period was 120 h, for acid 15 min, for base 120 min, for oxidation 10 min, for photolytic degradation 48 h. Peak purity test was carried out for the montelukast peak by using a PDA detector in stress samples. The purity factor is within the threshold limit obtained in all stressed samples demonstrates the analyte peak homogeneity.

Precision

The precision of the related substance method was checked by injecting six individual preparations of montelukast (1.04 mg/mL) spiked with 0.50% of Imp-A and 0.15% of Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G with respect to the montelukast analyte concentration. The % RSD of the area for each impurity (impurities-A, -B, -C, -D, -E, -F and -G) was calculated. The intermediate precision of the method was also evaluated using different analyst and different instrument in the same laboratory.

Limit of detection and limit of quantification

The LOD and LOQ were determined by measuring the magnitude of analytical background. The LOD and LOQ were determined from slopes of linear regression curves. The LOD and LOQ for Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G were determined by injecting a series of dilute solutions with known concentrations¹⁰.

Linearity

The Linearity of peak areas verses different concentrations was evaluated for montelukast and all the related substances using 10 levels ranging from 0.10 µg/mL to 8.24 µg/mL 0.01 (% w/w) to 0.824 (% w/w) with respect to sample concentration. The above tests were carried out for three consecutive days in the same concentration range for related substance method. The % RSD value for the slope and y-intercept of the calibration curve was calculated.

Accuracy

The accuracy of the method for all the related substances was determined by analyzing montelukast sample solutions spiked with all the related substances at three different concentration levels of 50, 100 and 150% of each in triplicate at the specified limit. The percentage of recoveries for the impurities was calculated from the slope and y-intercept of the calibration curve obtained from linearity studies.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between montelukast, Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G was recorded. The parameters selected were mobile phase composition ($\pm 2\%$ of gradient composition), pH of the mobile phase (± 0.2 units), flow rate ($\pm 10\%$), wavelength (± 5 nm) and column temperature ($\pm 5^\circ\text{C}$). The effect of the percent organic strength on the resolution was studied by varying acetonitrile by -5 to $+5\%$ while other mobile phase components were held constant.

Solution stability and mobile stability

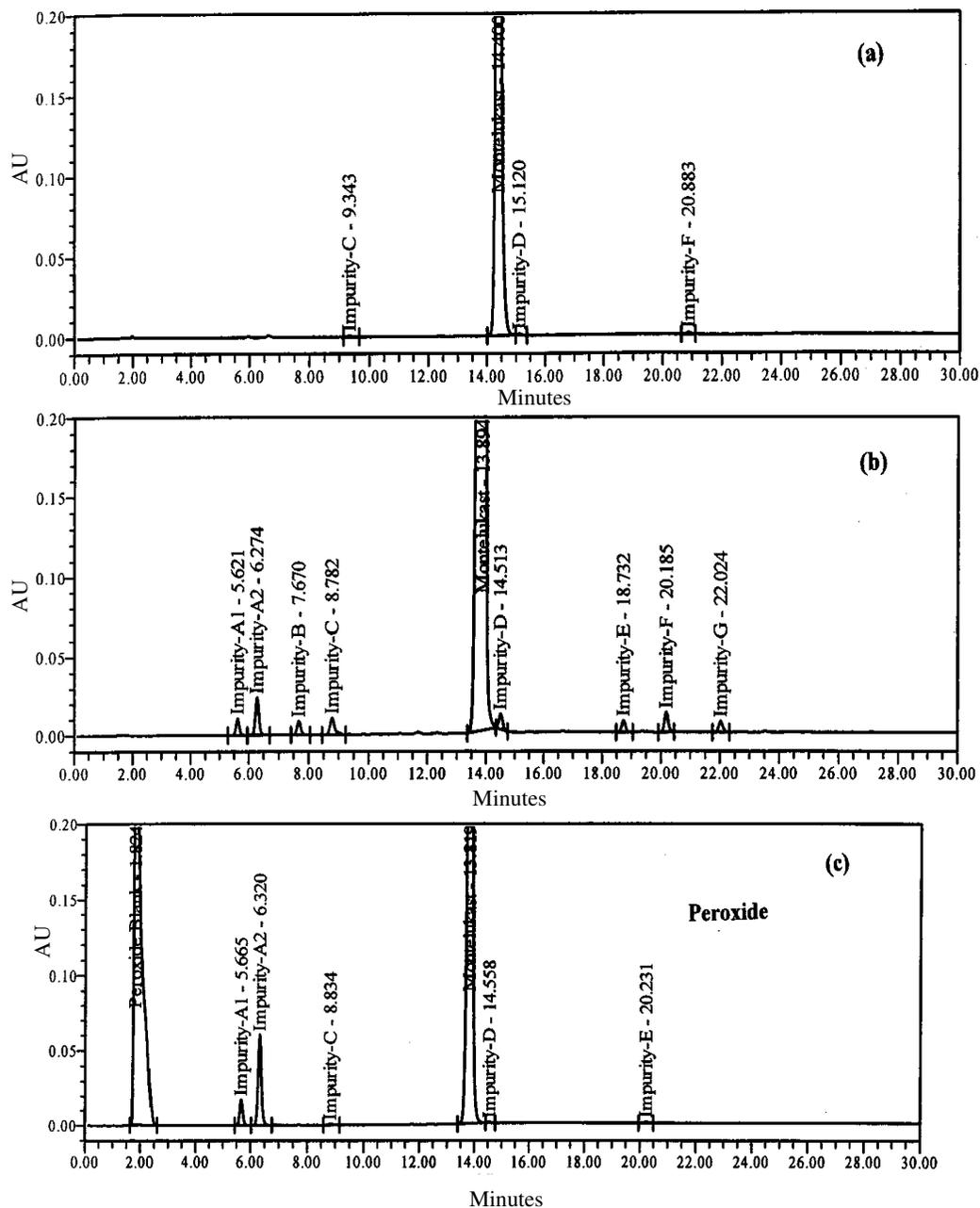
To determine the stability of sample solution, the sample solutions of montelukast spiked with related substances at specified level were prepared and analyzed immediately after preparation and after different time intervals up to 15 h, while maintaining the sample cooler temperature at about 25°C . The results from these studies indicated, the sample solution was stable at room temperature for at least 15 h.

Results and Discussion

Optimization of chromatographic conditions

The main objective of the chromatographic method was to separate montelukast from Imp-A (A1 and A2), Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G. Impurities were coeluted using different stationary phases such as C18, C8 and cyano as well as different mobile phases. During the evaluation of pH study, no effect was observed in elution order and retention times towards acidic side. Elution of impurities required higher ratios of organic modifier, hence 0.1% OPA was chosen as buffer solution to rule out precipitation of aqueous salt buffers with combination of higher organic modifier ratios. During the evaluation of various column chemistries, C18 was observed to give better resolution. Resolution between montelukast and Impurity-D was critical and conditions were optimized as mentioned under section "Chromatographic Conditions". In optimized chromatographic conditions montelukast,

Imp-A (A1 and A2), Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G were separated with a resolution greater than 2, typical relative retention times were approximately 0.40, 0.45, 0.55, 0.63, 1.04, 1.35, 1.45, 1.59 with respect to Montelukast eluted at 13.894 (Figure 2). The system suitability results are given in Table 1 and the developed LC method was found to be specific for Montelukast and all of its impurities namely, Imp-A (A1 and A2), Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G (Figure 2).



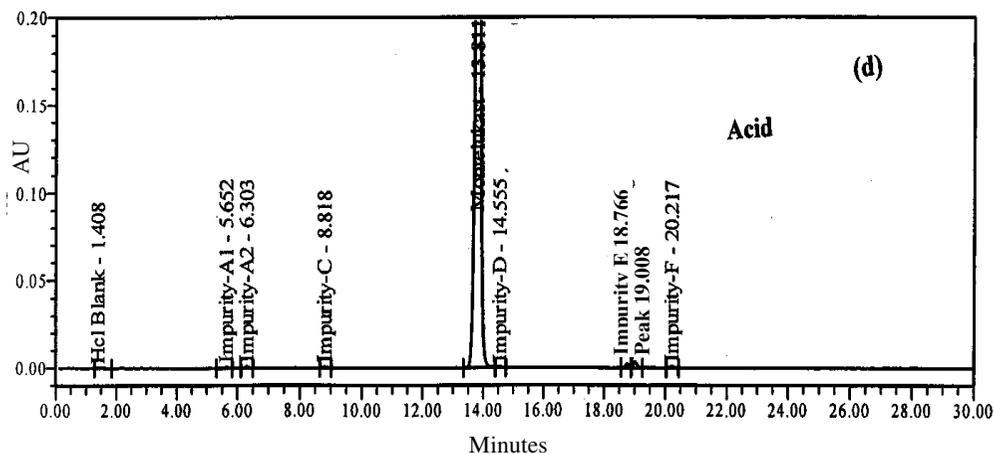


Figure 2. LC-Chromatograms of montelukast sodium and its impurities.

Table 1. System suitability.

Parameter	Impurity-A1	Impurity-A2	Impurity-B	Impurity-C	Montelukast	Impurity-D	Impurity-E	Impurity-F	Impurity-G
RT	5.621	6.274	7.67	8.782	13.894	14.513	18.732	20.185	22.024
RRT	0.40	0.45	0.55	0.63	1.00	1.04	1.35	1.45	1.59
RS	-	3.02	6.02	4.36	2.86	4.05	4.91	2.81	23.35
N	12,326	13,668	216,870	17,630	26,040	60,019	108,491	122,525	120,805
Asymmetry factor	1.07	1.11	1.15	1.39	1.07	1.10	0.97	1.17	1.10

RT = Retention time, RRT = Relative retention time, RS = Response Factor, N = Theoretical plates

Validation of the Method

Forced degradation

Degradation was not observed in montelukast sodium samples when subjected to stress conditions like thermal, photolytic and base hydrolysis (Figure 2). Montelukast sodium was degraded to Imp-A under oxidation and Imp-F under acid conditions. Peak purity test results obtained by using a PDA detector confirmed that the montelukast peak is homogenous and pure in all the analyzed stress samples. The summary of the forced degradation studies was given in Table 3.

Precision

The Precision was determined at the LOQ concentrations for Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G and the %RSD was found to be below 1.3 for all the impurities.

Limit of detection and limit of quantification

The values of LOD and LOQ for montelukast were 0.034 $\mu\text{g/mL}$, 0.103 $\mu\text{g/mL}$ and they were for related substances, in the ranges; 0.033-0.150 $\mu\text{g/mL}$ respectively. The calculated LOD and LOQ concentrations were verified for precision. RSD was in the range of 11.9-16.0 for LOD and 2.7-4.3 % for LOQ respectively. The results were depicted in Table 2.

Table 2. Linearity, LOD, LOQ, precision and accuracy data for montelukast sodium and its impurities.

Parameter	Sum of Impurity-A1 & Impurity-A2	Impurity-B	Impurity-C	Montelukast	Impurity-D	Impurity-E	Impurity-F	Impurity-G
r	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
Slope	38088	45376	40076	39974	38873	43454	43665	38872
Intercept	103	-549	265	149	68	-941	-309	-305
RF	1.01	0.88	1.00	1.00	1.03	0.92	0.92	1.04
Limit of Detection								
Con, µg/mL	0.049	0.033	0.033	0.034	0.033	0.033	0.034	0.034
%RSD	12.3	11.9	14.8	11.3	12.4	12.7	12.2	16.0
Limit of Quantification								
Con, µg/mL	0.150	0.101	0.101	0.103	0.100	0.100	0.102	0.104
%RSD	2.7	4.3	2.9	3.6	2.7	2.2	4.0	2.7
Precision								
%RSD(n=6)	0.8	0.6	1.2	0.9	0.7	1.3	1.3	0.9
Accuracy	98.4-	101.3-	97.3-	98.5-	98.7-	94.7-	95.3-	101.3-
%Recovery(n=3)	101.1	103.4	100.0	101.2	101.3	101.3	101.3	103.8

r = Correlation coefficient. RF = Response Factor

Linearity

Linear calibration plot for the related substance method was obtained over the calibration ranges tested, *i.e.*, LOQ (0.010 %) to 0.014% for impurities, Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G. The correlation co-efficient obtained was greater than 0.999. Linearity was checked for related substance method over the same concentration range for three consecutive days. The %RSD values of the slope and Y-intercept of the calibration curves were 0.03 and 1.83 respectively. The above result show that an excellent correlation existed between the peak area and the concentration of all seven impurities.

Accuracy

The Accuracy of all these related substances was found to be in between the predefined acceptance criterion of 95.0-102% and the data is given Table 2.

Robustness

When the chromatographic conditions flow rate, column temperature amount of organic solvent in the mobile phase, pH were deliberately varied and resolution between the critical pair, *i.e.* montelukast sodium and Imp-D was greater than 2.4, illustrating the robustness of the method.

Solution stability

There were no significant changes in the amounts of the impurities during solution stability experiment performed using the related substances method. The results from the studies indicated, the sample solution was stable at room temperature for at least 15 h.

Table 3. Forced degradation studies data.

Stress condition	Degradation %	Observation	Peak Purity	
			PA	PT
Undegraded	-	-	0.041	0.267
Acid (1M HCl at Rt)	20	Increase in levels of Impurity-F(0.20%) and additional peaks.	0.045	0.252
Base(5M NaOH/85°C/ 120 min)	0.5	No increase in levels of Known impurities.	0.067	0.272
Oxidation (3% H ₂ O ₂ /RT/ 10 min)	12.5	Increase in levels of Impurity-A1(2.8%), Impurity-A2 (8.8%) and additional peaks.	0.034	0.257
Thermal (80°C/120 h)	2.3	No increase in levels of Known impurities.	0.026	0.311
Photolytic (10K lux/48 h)	10	No increase in levels of Known impurities.	0.048	0.307
Humidity (90% RH /25°C /120 h)	1.8	No increase in levels of Known impurities.	0.053	0.266

PA = Purity angle, PT = Purity threshold.

Application of the method

Three batches of montelukast drug substance, each in triplicate are analyzed using the proposed method. The results showed the presence of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G in the range of %RSD (n=3) for the related substances present in the samples were below 10.0%.

Conclusion

A new, accurate and selective gradient RP-HPLC method was proposed for the determination of montelukast related substances in montelukast drug substance and validated as per the ICH guidelines. The method was found to be simple, selective, precise, accurate and robust. Therefore, this method can be used for routine testing as well as stability analysis of montelukast drug substance. All statistical results (Percentage, Mean, RSD, Percentage difference and recovery %) were within the acceptance criteria.

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