

DEVELOPMENT OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD FOR THE DETERMINATION OF MONTELUKAST IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Background: A High-performance liquid chromatography (HPLC) method was designed for the analysis to determine Montelukast in pharmaceutical dosage form. The column chromatographic configurations comprised of a 250 X 4.6 mm column containing 5- μ m; packing L1 (C18), along with mobile phase (Dissolve 0.68 gm of Potassium Dihydrogenphosphate (KH₂PO₄) with 250 ml of HPLC grade water, mix with 750 ml of HPLC grade Acetonitrile. Add 2 ml of Triethylamine with the above mixture and adjust pH to 6.0 with Phosphoric acid) and Pass the solution through 0.45 μ m membrane filter and degas) with flow rate of 2.0 ml/min. Montelukast sodium detection was performed at 220 nm. After analysis of three samples average content of Montelukast is 4.02 mg/tablet and Relative Standard Deviation (RSD) is 0.90%. As average assay result is satisfactory and RSD of average content of three samples is less than 2%, so results are very consistent and can be inferred that the analytical determination procedure is appropriate for routine analysis of this product after validation of this method.

Materials and Methods:

Materials: This HPLC Chromatographic analytical method development of Montelukast sodium uses materials of - 4.6 x 250 mm column containing 5 μ m packing L1(C18), HPLC grade Acetonitrile, HPLC grade water, Potassium Dihydrogenphosphate (KH₂PO₄), Triethylamine, Phosphoric acid.

Methods: The analytical method development has been established through five (5) different experimental procedures which is briefly summarized below. In first step researchers tried to develop the method of analysis for Montelukast Tablet 4 mg as like as Montelukast sodium raw material/drug substance according to the literature review. In second step, the interference of impurities with active drug substance has been examined. In third step, the resolution between two (2) peaks of Montelukast sodium and Montelukast sodium impurity - A has been determined. In fourth step interference of formulated placebo with active drug substance has been examined. In step five researchers evaluated the analytical procedure

through analyzing the finished pharmaceutical dosage form of Montelukast Sodium 4 mg tablet.

Result:

Step-1: In this step, the Relative Standard Deviation (RSD) of conjugated five replicate injections was found 1.2% which is less than 2 and the average area of standard and sample were found very near about. Final computation shown satisfactory assay result of this product.

Step-2: In this step, the study results found that the retention time (RT) of Montelukast sodium and Montelukast Sodium impurity-A are not same. So, Montelukast sodium has no interference with Montelukast sodium impurity-A.

Step-3: In this step, it was observed that resolution of Montelukast sodium and Montelukast Sodium impurity-A above 2. So, this method of analysis ensure that it has a suitable resolution between two peaks.

Step-4: In this step, no remarkable peaks were found for placebo sample solution at the retention time (RT) of Montelukast Sodium and Montelukast Sodium impurity-A. So it is clear that there is no interference of placebo with Montelukast Sodium.

Step-5: In this step, three samples of Montelukast Sodium tablet has been examined and the average content of Montelukast is found 4.02 mg/tablet. The Relative Standard Deviation (RSD) is found 0.90% which is less than 2%.

Conclusion: Based on the laboratory experiments in different ways it is observed that there is no interference of impurities and placebo with Montelukast sodium. After analysis of three samples average content of Montelukast is found 4.02 mg/tablet and Relative Standard Deviation (RSD) is 0.90%. As average assay result is satisfactory and RSD of average content of three samples is less than 2%, so results are very consistent and can be inferred that the quantitative analytical process is suitable for routine analysis of this product after validation of this method.

Keywords: Montelukast Sodium, High-Performance Liquid Chromatography, Analytical Method Development.

1.0 Introduction

Montelukast is prescribed in the management, prevention of asthma symptoms and to improve the symptoms of seasonal allergic rhinitis or hay fever, which belongs to a drug group known as leukotriene receptor antagonists. Montelukast is administered as montelukast sodium is chemically known as (R-(E))-1-(((1-(3-(2-(7-chloro-2-quinolinyl) ethenyl)phenyl)-3(2-(1-hydroxy-1-methylethyl) phenyl) pro- pyl) thio) methyl) cyclopropaneacetic acid, monosodium salt (Ethiraj et al., 2011). It is practically insoluble in acetonitrile and freely soluble in ethanol, methanol, and water. Chemical structure of Montelukast sodium is given in figure 1. Montelukast sodium generally used for the treatment of asthma in adults and children. Montelukast sodium is a selective inhibitor of leukotriene D₄ (LTD₄) at the cysteinyl leukotriene receptor cysLT₁ (Lai, Y., 2018).

The purpose of this research work was to develop a reliable, simple, and fast HPLC analytical method for the quantification analysis of Montelukast in pharmaceutical dosage forms. Literature review of Montelukast Sodium exposed few methods based on HPLC Chromatography for determination of drugs in single dosage forms (Rathore et al., 2010). This research work demonstrates the development of high performance liquid chromatographic (HPLC) method that quantify the drug components simultaneously from the dosage form. Obtained experimental results shown average content of Montelukast is 4.02 mg/tablet and Relative Standard Deviation (RSD) is 0.90% which is less than 2%.

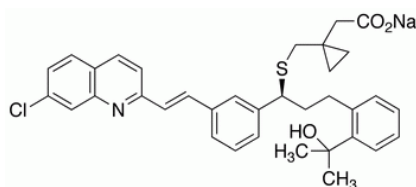


Fig.1: Chemical structure of Montelukast Sodium

2.0 Materials and Methods

2.1 STEP-1:

Literature review of Montelukast Sodium exposed some methods based on HPLC Chromatography have been reported for quantification of drugs in single dosage forms. So, researchers tried to develop the method of analysis for Montelukast Tablet 4 mg as like as Montelukast sodium raw material / drug substance in HPLC method by following ways:

2.1.1 Preparation of diluent

HPLC grade Acetonitrile: HPLC grade water = 50: 50

2.1.2 Standard solution preparation

Weigh about 41.50 mg of Montelukast Sodium (Equivalent to 40 mg Montelukast) into ambered volumetric flask of 100 ml. Dissolve and volume upto 100 ml with diluent. Dilute 5 ml of the above solution up to 50 ml with prepared mobile phase into an amber colored volumetric flask.

2.1.3 Sample solution preparation

Take 1300 mg powder from 20 tablets equivalent to 40 mg of Montelukast into 100 ml ambered volumetric flask. Add sufficient diluent, shake and volume upto 100 ml. Filter the solution with whatman filter paper. Dilute 5 ml of the above solution to 50 ml with prepared mobile phase into an amber colored volumetric flask.

Typical HPLC conditions:

Stationary Phase (Column) : 4.6 x 250 mm column containing 5 µm packing L1(C18)

Mobile phase : Dissolve 0.68 gm of Potassium Dihydrogenphosphate (KH₂PO₄) with 250 ml of HPLC grade water, mix with 750 ml of HPLC grade Acetonitrile. Add 2 ml of Triethylamine with the above mixture and adjust pH to 6.0 with Phosphoric acid. Pass the solution through 0.45µ and degas.

Flow rate : 2.0 ml/min

Wave length : 220 nm

Injected Volume : 20 µl

2.1.4 Procedure:

Inject 20 µl of standard solution one after another until the RSD for replicate injections can not be more than 2%. Inject the sample solution and obtain the chromatograms for the standard and the sample solution.

2.1.5 Calculation:

Content of Montelukast / tablet

$$\frac{AS \times WStd \times 5 \times 100 \times 50 \times PStd \times AWT}{Astd \times 100 \times 50 \times WS \times 5 \times 100 \times 1.0375} \text{ mg}$$

Where,

AS = Peak area of sample

WStd = Weight of standard

AStd = Average peak area of standard

Pstd = Potency of standard in %

WS = Weight of sample

AWT = Average weight of tablets

2.2 STEP-2:

From step-1 got workable method of analysis. Then tried to examine any interference of impurities with active or not. To do this experiment we prepared the impurity solution by following:

2.2.1 Impurity solution preparation

Weigh about 5 mg of Montelukast sodium impurity-A in a volumetric flask of 100 ml. Dissolve it and dilute upto 100 ml with methanol.

Typical HPLC conditions:

Stationary Phase (Column): 4.6 x 250 mm column containing 5 μ m packing L1(C18)

Mobile phase : Dissolve 0.68 gm of Potassium Dihydrogenphosphate (KH_2PO_4) with 250 ml of HPLC grade water, mix with 750 ml of HPLC grade Acetonitrile. Add 2 ml of Triethylamine with the above mixture and adjust pH to 6.0 with Phosphoric acid. Pass the solution through 0.45 μ and degas.

Flow rate : 2.0 ml/min

Wave length : 220 nm

Injected Volume : 20 μ l

2.2.2 Procedure:

Inject 20 μ l of impurity solution and obtain the chromatograms for the Montelukast sodium impurity-A.

2.3 STEP-3:

From step-2, it was clear that the retention time (RT) of Montelukast sodium and Montelukast sodium impurity-A are not same. But the resolution between two (2) peaks was unknown. Resolution between two peaks were recorded by following way:

2.3.1 Standard solution preparation

Take Montelukast Sodium 20 mg working standard and 5 mg Montelukast Sodium impurity-A in a volumetric flask of 100 ml. Dissolve and dilute upto 100 ml with methanol.

Typical HPLC conditions:

Stationary Phase (Column): 4.6 x 250 mm column containing 5 μ m packing L1(C18)

Mobile phase : Dissolve 0.68 gm of Potassium Dihydrogenphosphate (KH_2PO_4) with 250 ml of HPLC grade water, mix with 750 ml of HPLC grade Acetonitrile. Add 2 ml of Triethylamine with the above mixture and adjust pH to 6.0 with Phosphoric acid. Pass the solution through 0.45 μ and degas.

Flow rate : 2.0 ml/min

Wave length : 220 nm

Injected Volume : 20 μ l

2.3.2 Procedure:

Inject 20 µl of standard solution and obtain the chromatograms for the Montelukast Sodium and Montelukast sodium impurity-A.

2.4 STEP-4:

From step-3 we got some clear concept about the method of analysis. Then tried to examine any interference of formulated placebo with active or not. To do this experiment placebo solution has been prepared by following:

2.4.1 Placebo solution-

Take 1258.50 mg formulated placebo in volumetric flask of 100 ml. Add sufficient diluent, sonicate 15 minutes with gentle shaking and volume upto 100 ml with diluent into an amber coloured volumetric flask. Filter the solution. Dilute 5 ml of the above solution upto 50 ml with prepared mobile phase into an amber coloured volumetric flask.

Typical HPLC conditions:

Stationary Phase (Column) : 4.6 x 250 mm column containing 5 µm packing L1(C18)

Mobile phase : Dissolve 0.68 gm of Potassium Dihydrogenphosphate (KH₂PO₄) with 250 ml of HPLC grade water, mix with 750 ml of HPLC grade Acetonitrile. Add 2 ml of Triethylamine with the above mixture and adjust pH to 6.0 with Phosphoric acid. Pass the solution through 0.45µ and degas.

Flow rate : 2.0 ml/min

Wave length : 220 nm

Injected Volume : 20 µl

2.4.2 Procedure:

Inject 20 µl of standard solution and obtain the chromatograms for the placebo solution.

2.5 STEP-5:

From previous experiments it was cleared that Montelukast Sodium has no interference with the formulated placebo. Then we tried to analyze the product by following the method:

2.5.1 Preparation of diluent

HPLC grade Acetonitrile: HPLC grade water = 50: 50

2.5.2 Standard solution preparation

Weigh about 41.50 mg of Montelukast Sodium (Equivalent to 40 mg Montelukast) into ambered volumetric flask 100 ml. Dissolve and volume upto 100 ml with diluent. Dilute 5 ml of the above solution upto 50 ml with prepared mobile phase into an amber colored volumetric flask.

2.5.3 Sample solution preparation

Take 1300 mg powder from 20 tablets equivalent to 40 mg of Montelukast into an ambered volumetric flask 100 ml. Add sufficient diluent, shake and volume upto 100 ml. Filter the solution with whatman filter paper. Dilute 5 ml of the above solution to 50 ml with prepared mobile phase into an amber colored volumetric flask.

Typical HPLC conditions:

Stationary Phase (Column): 4.6 x 250 mm column containing 5 µm packing L1(C18)

Mobile phase : Dissolve 0.68 gm of Potassium Dihydrogenphosphate (KH₂PO₄) with 250 ml of HPLC grade water, mix with 750 ml of HPLC grade Acetonitrile. Add 2 ml of Triethylamine with the above mixture and adjust pH to 6.0 with Phosphoric acid. Pass the solution through 0.45µ and degas.

Flow rate : 2.0 ml/min

Wave length : 220 nm

Injected Volume : 20 µl

2.5.4 Procedure:

Inject 20 µl of standard solution one after another until the relative standard deviation (RSD) for replicate injections can not be more than 2%. Inject the sample solution and obtain the chromatograms for the standard and the sample solution.

2.5.5 Calculation:

Content of Montelukast / tablet

$$\frac{AS \times W_{Std} \times 5 \times 100 \times 50 \times P_{Std} \times AWT}{A_{Std} \times 100 \times 50 \times WS \times 5 \times 100 \times 1.0375} \text{---mg}$$

Where,

AS = Peak area of sample

WStd = Weight of standard

AStd = Average peak area of standard

Pstd = Potency of standard in %

WS = Weight of sample

AWT = Average weight of tablets

3.0 Result

3.1 Step-1

From the above method, the Relative Standard Deviation (RSD) of conjugated five replicate injections was found 1.2% which is less than 2 and the average area of standard and sample were found very near about. Final computation shown satisfactory assay result of this product.

3.2 Step-2

The study results found that the retention time (RT) of Montelukast sodium and Montelukast Sodium impurity-A are not same. So, Montelukast sodium has no interference with Montelukast sodium impurity-A.

3.3 Step-3

It was observed that resolution of Montelukast sodium and Montelukast Sodium impurity-A above 2. So, this method of analysis ensure that it has a suitable resolution between two peaks.

3.4 Step-4

No remarkable peaks were found for placebo sample solution at the retention time (RT) of Montelukast Sodium and Montelukast Sodium impurity-A . So it is clear that there is no interference of placebo with Montelukast Sodium.

3.5 Step-5

After calculating three samples we found following results:

No. of test	Test result (mg/tablet)		Average (mg/tablet)	RSD (%)
1	Montelukast	3.99	4.02 mg	0.90
2		4.01		
3		4.06		

Three samples of Montelukast Sodium tablet has been examined and the average content of Montelukast is found 4.02 mg/tablet. The Relative Standard Deviation (RSD) is found 0.90% which is less than 2%.

4.0 Discussion

For pharmaceutical quantitative analysis, researchers need to develop a suitable analytical procedure. Analytical method development is an essential part to established the routine analytical procedure of pharmaceutical dosage form (Hasin, F., et al 2017). Method development usually based on several experimental considerations. It is require to have maximum sample information to perform an effectual development projected for intentional analytical method application and also on available resources for chromatography. An effective method development assures that laboratory resources are optimized, while methods meet the objectives necessary at each stage of drug development.

Researchers uses literature reviews to build the analytical structure for this experiment. In the first phase of this analysis the interference between active montelucast and impurities has been examined to determine montelucast pick, that helps researchers to compute the

quantity of montelukast in the formulation. The second phase of the analysis researchers examined the resolution between two pick of montelukast and its impurities. This experiment helps to identify the both individual pick of montelukast sodium and montelukast impurity. In the third phase of analysis researchers examined the placebo interference with montelukast pick. In the final phase researchers analysed the three sample of montelukast tablet with the developed method for investigate the performance of analytical activities.

Conclusion

Based on the laboratory experiments in different ways it is observed that there is no interference of impurities and placebo with Montelukast sodium. After analysis of three samples average content of Montelukast is found 4.02 mg/tablet and Relative Standard Deviation (RSD) is 0.90%. As average assay result is satisfactory and RSD of average content of three samples is less than 2%, so results are very consistent and can be inferred that the quantitative analytical process is suitable for routine analysis of this product after validation of this method.

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Declaration

I, Farzana Hasin, as a corresponding author, submitting the manuscript for publication in International Journal of Public Health and Clinical Sciences (IJPCHS). I believe the manuscript represents valid work. I have reviewed the final version of manuscript and approve it for publication. All Authors agree that the contents of the manuscript are confidential and will not be copyrighted, submitted, or published elsewhere (including the Internet), in any language, while acceptance by the Journal is under consideration.

Kindly consider the manuscript for publication in your journal. I declare no conflict of interest. I accept all the terms and conditions displayed in <http://publichealthmy.org> to the best of my knowledge.

Authors contribution

Author 1:..Farzana Hasin.., Author 2:..Md. Mofazzal Hossain.., Author 3:..Md. Jasim Uddin.., Author 4:..AFM Rafiu Ul Islam...and Author 5:..Aditi Bhowmick..

References

- Bodavari, S. (2006). The merck index (No. 3565). Monograph.
- Hasin, F., Noon, S., Hasan, HA., & Jahan, T. (2017). Validation of High Performance Liquid Chromatography (HPLC) Method for Determination of Trimetazidine in Pharmaceutical Dosage Forms, *European Journal of Biomedical and Pharmaceutical Sciences (EJBPS)*. Volume 4, Issue 7, 66-73.
- Hoang, T. H., Farkas, R., Wells, C., McClintock, S., & Di Maso, M. (2002). Application of pressurized liquid extraction technology to pharmaceutical solid dosage form analysis. *Journal of Chromatography A*, 968(1-2), 257-261.
- Hasin, F., Islam, MT., Ahmad, MF., & Rakib, MM. H. (2017). Validation of Assay Method for The Estimation of Imatinib Mesylate in Tablet Dosage Form by HPLC. *European Journal of Biomedical and Pharmaceutical Sciences (EJBPS)*, Volume 4, Issue 7, 74-81.
- Kelly, T. (2012). *Clarke's analysis of drugs and poisons*.
- Hasin, F., Amin, A., Hussain, MI., Jahan, T., & Hama, NA. (2017). Validation of High Performance Liquid Chromatography (HPLC) Method for Determination of Erlotinib Related Substance in Pharmaceutical Dosage Form. *World Journal of Pharmaceutical Research (WJPR)*, Volume 6, Issue 8, 275-288, ISSN 2277 – 7105.
- Liu, L., Cheng, H., Zhao, J. J., & Rogers, J. D. (1997). Determination of montelukast (MK-0476) and its S-enantiomer in human plasma by stereoselective high-performance liquid chromatography with column-switching. *Journal of pharmaceutical and biomedical analysis*, 15(5), 631-638.
- Lai, Y. (2018). U.S. Patent Application No. 15/505,040.
- Riccioni, G., Della Vecchia, R., D'Orazio, N., Sensi, S., & Guagnano, M. T. (2003). Comparison of montelukast and budesonide on bronchial reactivity in subjects with mild-moderate persistent asthma. *Pulmonary pharmacology & therapeutics*, 16(2), 111-114.
- Rathore, A. S., Sathiyarayanan, L., & Mahadik, K. R. (2010). Development of Validated HPLC and HPTLC Methods for Simultaneous Determination of Levocetirizine Dihydrochloride and Montelukast Sodium in Bulk Drug and Pharmaceutical Dosage Form. *Pharm Anal Acta* 1: 106. doi: 10.4172/2153-2435.1000106.

Simons, F. E. R., Villa, J. R., Lee, B. W., Teper, A. M., Lyttle, B., Aristizabal, G., & Menten, J. (2001). Montelukast added to budesonide in children with persistent asthma: a randomized, double-blind, crossover study. *The Journal of pediatrics*, 138(5), 694-698.

Singh, R. M., Saini, P. K., Mathur, S. C., Singh, G. N., & Lal, B. (2010). Development and validation of a RP-HPLC method for estimation of montelukast sodium in bulk and in tablet dosage form. *Indian journal of pharmaceutical sciences*, 72(2), 235.

Sweetman, S. C. (2009). *Martindale: the complete drug reference*.

Tripathi, K. D. (2013). *Essentials of medical pharmacology*. JP Medical Ltd.