



# SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF LEUKOTRIENE RECEPTOR ANTAGONIST IN BULK DOSAGE FORMS

J.V. Shanmukha kumar<sup>1\*</sup>, D. Ramachandran<sup>2</sup>, V.S. Settaluri<sup>3</sup> and C. Shechinah Felice<sup>3</sup>

<sup>1</sup>Department of Chemistry, College of Engineering, K L E F University, Vaddeswaram-522502, Guntur, A.P, India.

<sup>2</sup>Department of Chemistry, Acharya Nagarjuna University, Nuzvid Campus, Nuzvid, Krishna District, A.P, India.

<sup>3</sup>Department of Biotechnology, College of Engineering, K L E F University, Vaddeswaram – 522 502, Guntur, A.P, India.

\*E-mail: shanmuk\_fed@klce.ac.in

## ABSTRACT

Three simple, accurate and economical spectroscopic methods were developed for the determination of Montelukast Sodium in bulk and dosage forms. Method A is based on the reduction of ferric ion into ferrous ion by the drug (MTK) in the presence of a ligand 1, 10-phenanthroline to form a highly stable orange red colored complex having  $\lambda_{\max}$  at 510 nm with a molar absorptivity of  $1.652 \times 10^4$ . Method B for montelukast sodium is based on the oxidation of Fe (III) followed by coupling with MBTH, to form a highly stable green colored chromogen having  $\lambda_{\max}$  at 610 nm with a molar absorptivity of  $0.7176 \times 10^4$ . Method C is based on reaction of the drug (MTK) in the presence of the ligand 2, 2'-Bipyridyl to form a highly stable orange colored chromogen having  $\lambda_{\max}$  at 430 nm with a molar absorptivity of  $1.672 \times 10^4$ . Common excipients involved in routine pharmaceutical preparations do not interfere in any of the proposed methods. The results of analysis have been validated statistically and recovery studies confirm the accuracy of the proposed methods.

**Key Words:** Motelukast Sodium (MTK), Spectroscopy, Molar absorptivity, Beer's Law, Analysis validation.

## INTRODUCTION

Montelukast sodium<sup>1, 2</sup> is [R-(E)]-1-[[[1-[3-[2-(7-chloro-2quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]cyclopropaneacetic acid, sodium salt (mono), which is a leukotriene receptor antagonist used as an alternative to anti-inflammatory medications in the management and chronic treatment of asthma and exercise-induced bronchospasm (EIB) drug that is marketed under trade names such as Singulair<sup>®</sup> and Montair. It is usually administered orally. Montelukast blocks the action of leukotriene D<sub>4</sub> on the cysteinyl leukotriene receptor Cys LT<sub>1</sub> in the lungs and bronchial tubes by binding to it. This reduces the bronchi constriction otherwise caused by the leukotriene, receptor and results in less inflammation. Based on its mechanism of operation, it is not useful for the treatment of acute asthma attacks, as also because of its very specific locus of operation; it does not interact with other allergy medications such as theophylline. It is an oral Leucotriene receptor having wide biological and chemical functions. Acute asthma attack, hepatic impairment, phenylketonuria could be reduced by a steady dose of the drug.

It was however considered based on the structure the drug (Fig 1.1) that the analytically important functional groups were not fully exploited for designing suitable analytical methods as was quite evident from the literature, and, only a few methods viz, HPLC<sup>3, 4, 6, 9, 11</sup>, Spectrofluorimetry<sup>5</sup> electrophoresis<sup>10</sup> UV-visible spectrophotometry<sup>7, 8</sup> and LC-ESI-MS<sup>12</sup> appeared in the literature for the determination of

MTK in bulk and pharmaceutical formulations. As the drug has recently come into existence, the number of available procedures that could be of utility to a small-scale industry is less and hence the author has proposed these methods described below for the routine quality control analysis of Montelukast sodium in dosage forms.

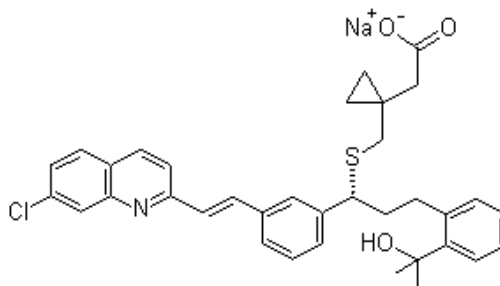


Fig.-1: Structure of Montelukast Sodium

## EXPERIMENTAL

### Instrumentation

After due calibration of the instrument, spectral and absorbance measurements are made using Genesys 10 UV Spectrophotometer procured from Thermo Scientific company marketed by Merck. All the chemicals used were of analytical grade. All the solutions were freshly prepared with double distilled water. Reagents were prepared a fresh for every method.

### Reagents

#### Method A:

Aqueous solution MBTH (0.2%w/v) and ferric chloride (0.7% w/v in 0.5 N HCl) were prepared..

#### Method B:

Aqueous solutions of reagents viz. 1, 10-PTL (0.01 M), Ferric chloride (0.003 M) and ortho phosphoric acid (0.2 M) were prepared.

#### Method C:

Aqueous solutions of reagents such as 2,2'-BPL (0.01 M), Ferric chloride (0.003 M) and ortho phosphoric acid (0.2 M) were prepared.

### Standard and Sample solution of Montelukast Sodium:

About 100 mg of Montelukast Sodium (formulation or pure) was accurately weighed on a digital single pan balance and dissolved in 100 ml of water in a volumetric flask to prepare a solution that has a concentration equal to 1 mg/ml standard solution and further dilutions are made with the same solvent (100 µg/ml) for **Methods A, B and C.**

### Recommended Procedure for the determination of MTK:

**Method A:** Aliquots (0.4-2.0 ml, 100 µg/ml) of standard MTK were transferred into a series of 10 ml calibrated tubes and then solutions of FeCl<sub>3</sub> (1.0 ml) and o-phenanthroline (1.0 ml) was added successively. The total volume in each test tube was brought up to 4.0 ml with distilled water and heated for 10 minutes in a boiling water bath at 90°C. After cooling to the room temperature, 2.0 ml of o-phosphoric acid was added in each test tube. The absorbance of the colored complex solution was measured after 5 minutes at 510 nm against reagent blank prepared similarly.

**Method B:** Aliquots (0.2-1.0 ml, 100 µg/ml) of standard MTK solution (100 µg/ml) was transferred into a series of 10 ml graduated tubes. To each tube 1.5 ml of MBTH (0.2%) solution and 2 ml of FeCl<sub>3</sub> (0.7% in 5N HCl) were added successively and kept aside for 10 minutes. The absorbance of the resulting green colored chromogen was measured at 610 nm against reagent blank prepared similarly.

#### Method C

Aliquots (0.4-2.0 ml, 100 µg/ml) of standard MTK were transferred into a series of 10 ml calibrated tubes and then solutions of FeCl<sub>3</sub> (1.0 ml) and 2, 2 Bipyridyl (1.0 ml) was added successively. The total volume in each test tube was brought up to 4.0 ml with distilled water and heated for 10 minutes in a boiling

water bath at 90°C. After cooling to the room temperature, 2.0 ml of o-phosphoric acid was added in each test tube. The absorbance of the orange colored complex was measured after 5 minutes at 430 nm against reagent blank prepared similarly. In all the above methods, a calibration curve was prepared by plotting the absorbance versus the concentration and the unknown was read from the calibration curve, or deduced using a regression equation, calculated from Beers law data.

### RESULTS AND DISCUSSION

The results obtained in method A were based on oxidation followed by complex formation reaction of Motelukast sodium with 1,10-phenanthroline, ferric chloride and ortho phosphoric acid to form an orange colored chromogen that exhibited maximum absorption at 510 nm against the corresponding reagent blank. The mechanism of reaction is represented in Scheme -1

The results obtained in method B were due to oxidative coupling between MBTH – Fe (III) and Motelukast sodium to form a green colored solution that exhibited maximum absorption at a wavelength of 610 nm against the corresponding reagent blank. The mechanism of reaction is represented in Scheme -2.

The results obtained in method C were based on oxidation followed by complex formation that involved the reaction of Motelukast sodium with 2, 2'-bipyridine, ferric chloride and ortho phosphoric acid to form an orange colored chromogen that exhibited maximum absorption at 430 nm against the corresponding reagent blank. The mechanism of reaction is represented in Scheme -3

Beer's law was obeyed over the concentration range of 2-10  $\mu\text{g.mL}^{-1}$  for method B, 4-20  $\mu\text{g.mL}^{-1}$  for method A and C respectively. The proposed procedures are validated by determining various optical parameters, which are listed in Table-1. The linearity, intercepts and the slope have been calculated using regression equation  $Y = a + bc$ , where Y represents optical density, 'C', the concentration of the drug in  $\mu\text{g.mL}^{-1}$  and 'a' and 'b' represents intercepts and slope respectively. Precision and accuracy of the proposed methods were tested by carrying out the determination of six replicates of pure and dosage samples of the drug, whose concentration lie within Beer's law range.

The values of standard deviation (% R.S.D) and percent range of error (0.05 level and 0.01 level confidence limits) were calculated for the above three methods and are presented in Table 1.1. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical preparation and the mixtures were analyzed by the proposed methods. The percent recoveries are given in Table-2. For method A and C, the reaction of colored species formation was slow at room temperature 25 °c and requires longer time for completion. Hence, efforts were made to accelerate by carrying out the reaction at higher temperatures. It was observed that the maximum color intensity was obtained by heating the reaction mixture at 90 °c on a boiling water bath for 10 minutes, for method A and at 100 °c on boiling water bath for 8 minutes, for method C. For method B, room temperature 25 °c is considered suitable for the development of colored chromogens. The absorbencies remained constant at room temperature for more than 10 and 6 hours for method A and C respectively. In method B, the color was found to be stable for more than 4 hours at room temperature.

Table -1: Optical characteristics, precision and accuracy of the proposed method

Parameter	Method A	Method B	Method C
$\lambda_{\text{max}}$ (nm)	510	610	430
Beer's law limit ( $\mu\text{g/ml}$ )	4-20	2-10	4-20
Sandell's Sensitivity ( $\mu\text{g/cm}^2/0.001 \text{ abs. unit}$ )	0.0368	0.0847	0.0363
Molar absorptivity ( $\text{litre.mole}^{-1}.\text{cm}^{-1}$ )	$1.652 \times 10^4$	$0.7176 \times 10^4$	$1.672 \times 10^4$

Correlation coefficient (r)	0.9999	0.9998	0.9998
Regression Equation (Y)*	0.0534	-0.0043	-0.0919
Slope			
a			
Intercept b	0.00223	0.001195	0.003157
% RSD	0.60	1.62	1.15
%Range of errors (95%Confidence limit)			
0.05 level of Significance	± 0.5016	± 1.3545	±0.9615
0.01 level of Significance	± 0.7422	± 2.004	±1.4226

\*  $Y = a + bx$ , where 'Y' is the absorbance and x is the concentration of Montelukast Sodium in  $\mu\text{g/ml}$ .

\*\* For six replicates

Table-2: Percent Recovery of Motelukast Sodium (MTK) in Pharmaceutical formulations

Formulations	Labeled amount Bulk /Tablet	% Recovery by proposed methods		
		Method A	Method B	Method C
Tablet 1	100 mg	99.4	98.38	98.85
Tablet 2	100 mg	99.7	98.85	99.1
Tablet 3	100 mg	99.8	99.15	99.4
Tablet 4	100 mg	100.2	100.1	99.75

### CONCLUSIONS

The methods reported here are found to be simple, sensitive, accurate and precise. Further, spectrophotometric methods involve simple instrumentation which is cost effective compared with other instrumental techniques, which ordinary laboratories cannot afford to have. The present species which makes it easier for the determination of MTK from pharmaceutical dosage forms in a routine manner. Further statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the methods.

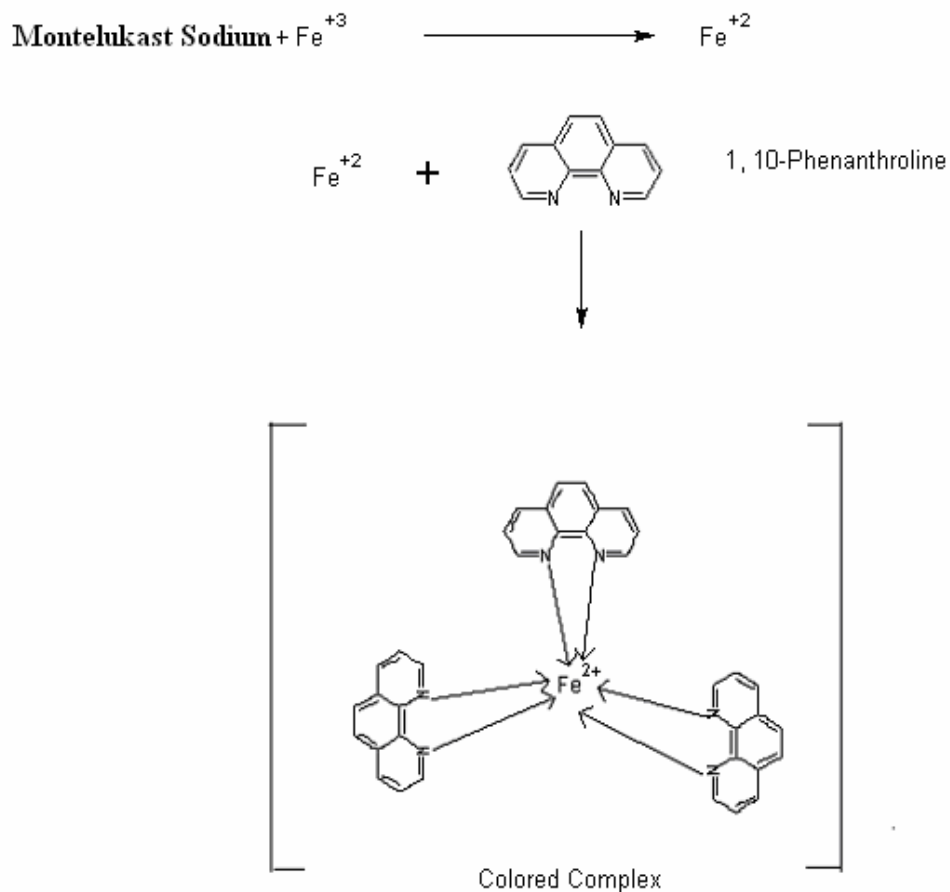
### ACKNOWLEDGEMENTS

The authors are grateful to M/s Vijayasri Chemicals, Hyderabad, for providing pure drug samples, and the management of KLEF University, Vaddeswaram, Guntur Dist. for their continuous support and encouragement and for providing the necessary infrastructure facilities for executing this work.

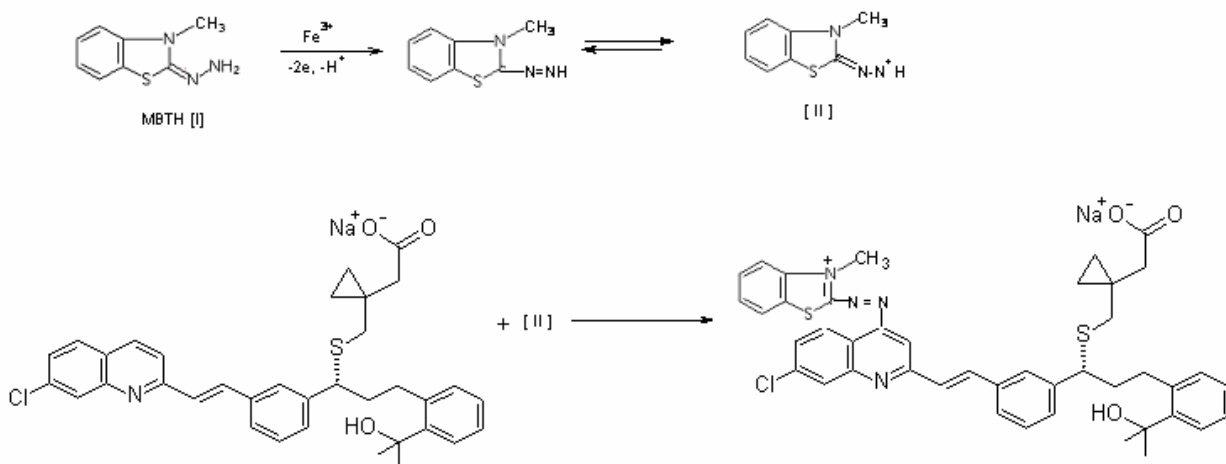
### REFERENCES

1. <http://www.merckfrosst.ca/mfcl/en/corporate/research/accomplishments/singulair.htm>
2. Schering-Plough press release - Schering-Plough/MERCK Pharmaceuticals Receives Not-Approvable Letter from FDA for Loratadine/Montelukast.
3. Smita Patil, Y. V. Pore, B. S. Kuchekar, Aruna Mane And V. G. Khire, *Indian Journal of Pharmaceutical Sciences*, **71(1)**, 58- 61,(2009).

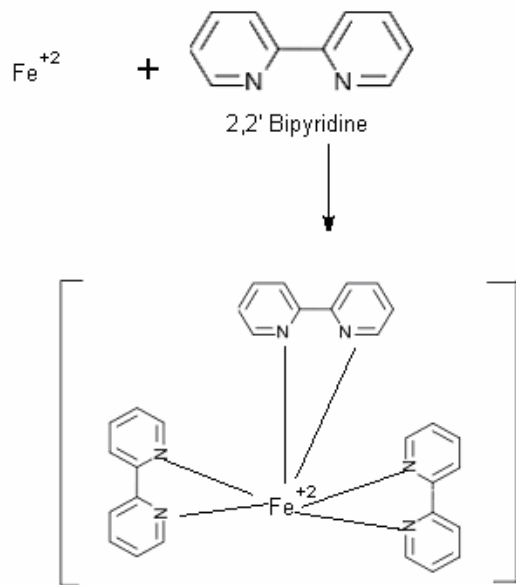
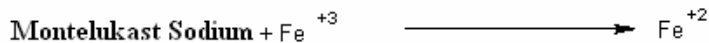
4. H. Ochiai, N. Uchiyama, T. Takano, K. Hara, T. Kamei, *J Chromatogr B Biomed Sci Appl*, **713 (2)**,409-14, (1998).
5. I. Alsarra, N. Y. Khalil, M. Sultan, R. Al-Ashban, F. Belal, *Pharmazie*, **60(11)**3-826,(2005).
6. T. Radhakrishna, A. Narasaraju, M. Ramakrishna and A. Satyanarayana, *Journal of Pharmaceutical and Biomedical Analysis*, **31(2)**, 359-368 (2003).
7. Varun Pawar, N. Lalitha , S.B. Puranik, P.N. Sanjay Pai , G.K. Rao, Development And Validation of Spectrophotometric Method for Determination of Montelukast Sodium in Bulk and Tablet Formulation; KONGPOSH publications; October (2008)
8. M. Saeed Arayne , Najma Sultana and Fida Hussain , *Journal of Analytical Chemistry*, **64(7)** , 690-695, (2009).
9. K. Vanitha Prakash, J. Venkateswara Rao and N. Appala Raju, *Oriental Journal of Chemistry*, **23 ( 3 )**, (2007).
10. Y. Shakalisava, F. Regan, *J. Sep. Sci.*, **31(6-7)**, 1137-43 (2008).
11. R.D. Amin, H. Cheng., J.D. Rogers, *Pharm. Biomed. Anal.*, **13**, 155–158(1995)
12. Yao Huang, Li Ding, Yuan-Yuan Liu, He-Ying Liu, Ai-Dong Wen, Lin Yang, *Journal of Chinese Pharmaceutical Sciences*, **18 (3)**, 261-266.



Scheme-1: Reaction of 1, 10 Phenothroline with Montelukast Sodium



Scheme-2: Reaction of MBTH with Montelukast Sodium



Colored Complex

Scheme-3: Reaction of 2, 2'-Bipyridine with Montelukast Sodium

(Received: 4 March 2010

Accepted: 13 March 2010

RJC-529)