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# RP-HPLC Method Development and Validation for the Determination and Stability Indicative Studies of Montelukast in Bulk and its Pharmaceutical Formulations

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**Abstract:** A simple, precise, accurate, economical and reproducible HPLC method for estimation of montelukast in tablet dosage form has been developed. Quantitative HPLC was performed with Shimadzu LC2010c HT with Winchrom Software with UV-Visible detector (SPD-IOA), PUMP (LC-IOAT) and (LC-IOATvp). Phenomenex C<sub>8</sub>, 5 μm, 25 cm x 4.6 mm i.d. column was used in the study. The mobile phase of ACN: Acetate buffer= 6.5:3.5 of pH 3 were used in this study. The conditions optimized were: flow rate (1 mL/minute), wavelength (222 nm) and run time was 20 min. Retention time was found to be 3.08 min. The linearity was found to be in the concentration range of 10-100 μg/mL. The developed method was evaluated in the assay of commercially available tablet moni containing 10 mg of montelukast. The amount of drug in tablet was found to be 10.34 mg/tab for the brand. Results of analysis were validated statistically and by recovery studies. The recovery studies 99.67% was indicative of the accuracy of proposed method. The precision was calculated as repeatability, inter and intraday variation (%RSD) for the drug. By using the method, stability of the drug has been studied.

**Keywords:** Montelukast, RP-HPLC, Degradation studies, Pharmaceutical formulations

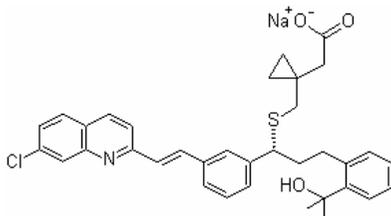
## Introduction

It is necessary to find the content of each drug either in bulk or single or combined dosage forms for purity testing. It is also essential to know the concentration of the drug and its metabolites in biological fluids after taking the dosage form for treatment.

The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise<sup>1</sup>. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation.

Montelukast sodium (MTKT), 1-[(*(R)*-*m*-[(*E*)-2-(7-chloro-2-quinoly) vinyl]- $\alpha$ -[*o*-(1-hydroxymethylethyl)phenethyl]benzyl}thio)methyl] cyclopropaneacetate sodium is a leukotriene receptor antagonist, used in the treatment of asthma<sup>2,3</sup>. It is not official in IP, BP and USP. Various analytical methods, such as liquid chromatography with fluorescence detection<sup>4-6</sup> stereoselective HPLC for MTKT and its *S*-enantiomer<sup>7</sup>, simultaneous HPLC and derivative spectroscopic method with loratadine<sup>8</sup>, stability indicating HPLC method<sup>9</sup> for Montelukast sodium in tablets and human plasma have been reported.

The present study illustrates development and validation of a simple, accurate and precise procedure for determination of montelukast sodium by RP-HPLC in bulk and in tablet dosage form.



**Figure 1.** Montelukast sodium

## Experimental

The chromatographic separation was performed on Shimadzu LC2010c HT (Autosampler) with Winchrom Software with Isocratic--Gradient with UV-Visible Detector (SPD-IOA), PUMP (LC-IOAT). Phenomenex (C<sub>8</sub>) RP Column, 250 mm x 4.6 mm has been used as a stationary phase. pH Analyzer (ELICO), Electronic Balance (AFCOSET), Ultra Sonicator (ENERTECH) has been used in the work. Montelukast sodium working standard was obtained from Wexford Laboratories Pvt. Ltd. in Peenya 2<sup>nd</sup> Stage, Bangalore. Moni Tablets (Montelukast sodium tablets, 10 mg) was purchased from a local pharmacy store. Acetonitrile, acetic acid & water of HPLC grade were taken from standard reagents, Hyderabad.

## Method development

### *Optimised chromatographic conditions*

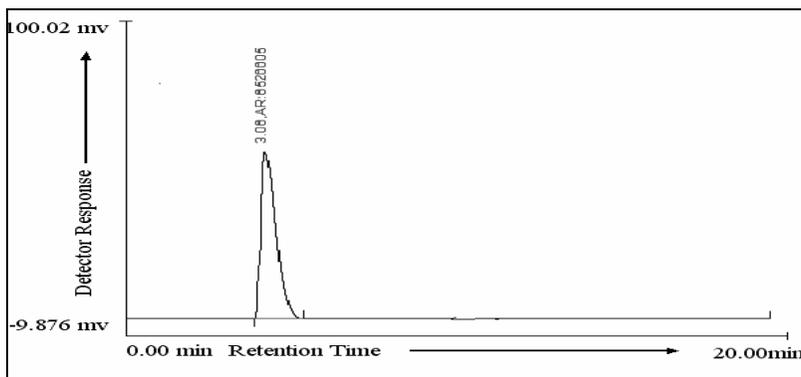
The mobile phase of ACN: Acetate buffer (pH 3) = 6.5:3.5 were used in this study. The conditions optimized were: flow rate (1 mL/minute), wavelength (222 nm) and run time was 20 min, injection volume 20  $\mu$ L. Retention time was found to be 3.08 min. Phenomenex C<sub>8</sub>, 5  $\mu$ m, 25 cm x 4.6 mm i.d. column was used in the study.

### *Preparation of standard drug solutions*

Standard stock solution of a concentration of 100  $\mu$ g/mL of montelukast was prepared by using mobile phase.

### *Preparation of mobile phase*

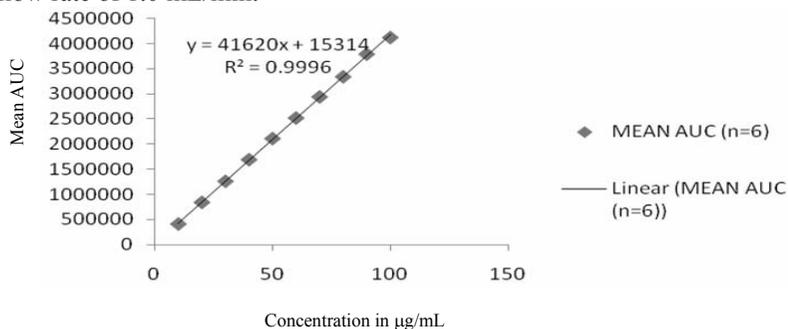
The mobile phase used in this analysis consists of a mixture of glacial acetic acid and acetonitrile in a ratio of 35:65 pH of the solution adjusted to 3.0.



**Figure 2.** HPLC spectrum of montelukast (100 ppm) in optimized conditions (RT 3.08 min)

*Preparation of calibration curve*

Calibration curve was prepared by taking appropriate aliquots of standard montelukast stock solution in different 10 mL volumetric flask and diluted up to the mark with mobile phase to obtain the final concentrations of 10-100 µg/mL of montelukast. The calibration curve has been shown below. Standard solutions (n=6) were injected, the sample volume was 20 µL with a flow rate of 1.0 mL/min.



**Figure 3.** Calibration curve of .montelukast

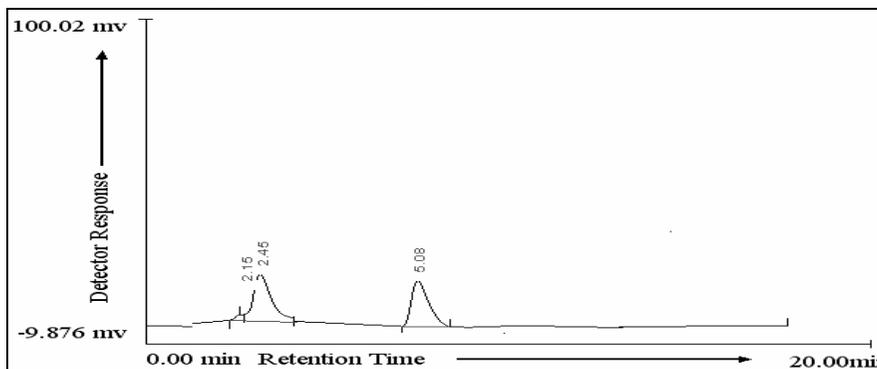
**Table 1.** Results for construct the calibration curve

Conc, µg/mL	Mean AUC (n=6)
10	410341
20	841309
30	1261332
40	1691734
50	2112310
60	2521649
70	2943094
80	3343921
90	3793994
100	4124588

### Forced degradation studies

#### *Acid hydrolysis (0.1 M HCl)*

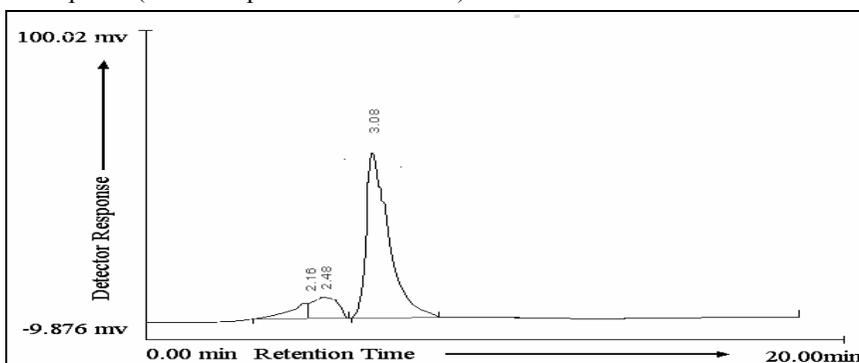
An accurately weighed 10 mg of pure drug was transferred to a clean and dried 10 mL volumetric flask. To which 0.1 M Hydrochloric acid was added and made up to the mark and kept for 24 h. From that 1 mL was taken in to a 10 mL volumetric flask and make up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl and mobile phase (after all optimized conditions).



**Figure 4.** Chromatogram showing degradation in 0.1 M HCl

#### *Basic hydrolysis (0.1 M NaOH)*

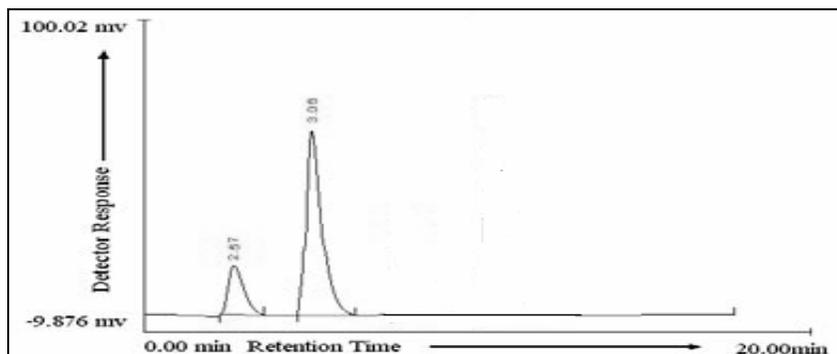
An accurately weighed 10 mg. of pure drug was transferred to a clean and dried 10 mL volumetric flask. To which 0.1 M sodium hydroxide was added and made up to the mark and kept for 24 h. From that 1 mL was taken in to a 10 mL volumetric flask and made up to the mark with mobile phase, then injected into the HPLC system against a blank of NaOH and mobile phase (after all optimized conditions).



**Figure 5.** Chromatogram showing degradation in 0.1 M NaOH

#### *Oxidation with (3%) H<sub>2</sub>O<sub>2</sub>*

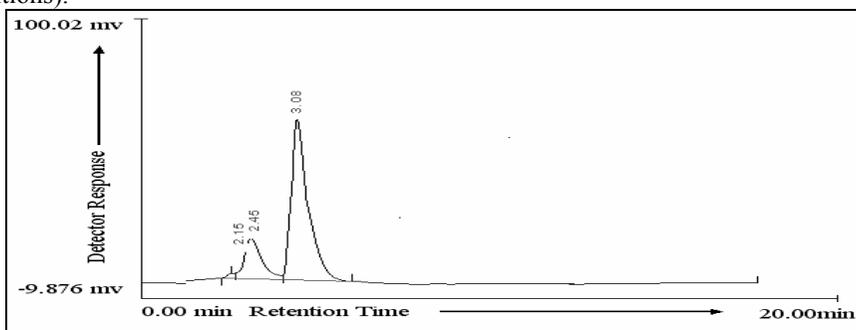
An accurately weighed 10 mg. of pure drug was transferred to a clean and dried 10 mL volumetric flask. To which 3% hydrogen peroxide was added, made up to the mark and kept for 24 h. From that 1 mL was taken in to a 10 mL volumetric flask and made up to the mark with mobile phase, then injected into the HPLC system against a blank of H<sub>2</sub>O<sub>2</sub> and mobile phase (after all optimized conditions).



**Figure 6.** Chromatogram showing degradation in 3% H<sub>2</sub>O<sub>2</sub>

#### *Thermal degradation*

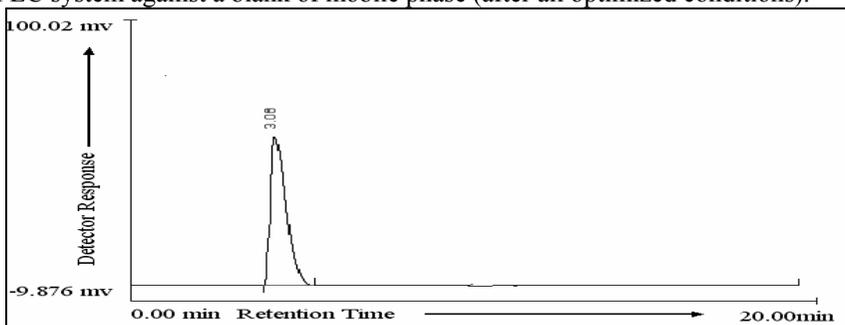
An accurately weighed 10 mg of pure drug was transferred to a clean and dry 100 mL volumetric flask, made up to the mark with methanol and was maintained at 50 °C for 24 h then injected into the HPLC system against a blank of mobile phase (after all optimized conditions).



**Figure 7.** Chromatogram showing thermal degradation

#### *Photolytic degradation*

Approximately 10 mg. of pure drug was taken in a clean and dry Petridis. It was kept in a UV cabinet at 254 nm wavelength for 24 h without interruption. Accurately weighed 1 mg. of the UV exposed drug was transferred to a clean and dried 10 mL volumetric flask. First the UV exposed drug was dissolved in methanol and make up to the mark than injected into the HPLC system against a blank of mobile phase (after all optimized conditions).



**Figure 8.** Chromatogram showing photolytic degradation

## Results and Discussion

### *Results of degradation studies*

The results of the stress studies indicated the specificity of the method that has been developed. The result of forced degradation studies are given in the following table.

**Table 2.** Results of force degradation studies of montelukast

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass balance, %
Acid Hydrolysis (0.1 M HCl)	24 H	-	97.56	97.56
Basic Hydrolysis (0.1 M NaOH)	24 H	80.27	19.06	99.33
Oxidation(3% H <sub>2</sub> O <sub>2</sub> )	24 H	78.73	19.86	98.61
Thermal Degradation (50 °C)	24 H	89.47	10.11	99.58
UV (254 nm)	24 H	98.41	-	98.41

### Method validation

#### *Accuracy recovery study*

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100% and 120%) of pure drug of montelukast were taken and added to the pre-analyzed formulation of concentration 10 µg/mL. From that percentage recovery values were calculated. (Procedure repeated for six times).

### Precision

#### *Repeatability*

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug, montelukast (API) The percent relative standard deviation were calculated for montelukast.

#### *Intra-assay and inter-assay*

The intra and inter day variation of the method was carried out and the high values of mean assay and low values of standard deviation and % RSD (% RSD < 2%) within a day and day to day variations for montelukast revealed that the proposed method is precise. (Procedure repeated for six times).

#### *Linearity and range*

The calibration curve showed good linearity in the range of 10 – 100 µg/mL, for montelukast (API) with correlation coefficient ( $r^2$ ) of 0.999 (Figure 3). A typical calibration curve has the regression equation of  $y = 41620x + 15314$  for montelukast.

#### *Method robustness*

Influence of small changes in chromatographic conditions such as change in flow rate ( $\pm 0.1$  mL/min), temperature ( $\pm 2$  °C), wavelength of detection ( $\pm 2$  nm) and methanol content in mobile phase ( $\pm 2\%$ ) studied to determine the robustness of the method are also in favour of (% RSD < 2%) the developed RP-HPLC method for the analysis of montelukast (API).

*LOD and LOQ*

The minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) were found to be 0.03 and 0.09 µg/mL respectively.

*Specificity and stability in analytical solution*

The results of specificity indicated that the peak was pure in presence of degraded sample. It is important to mention here that the montelukast (API) was stable in solution form up to 24 h at 25 °C.

The results of linearity, precision, inter and intraday assays, method robustness, LOD, LOQ, specificity and stability in analytical solution established the validation of the developed RP-HPLC method for analysis of montelukast.

**Table 3.** Summary of validation parameters By RP-HPLC method

Validation parameters		Montelukast
Specificity		% interference <0.5 %
Range, µg/mL	Linear range	10-100 µg/mL
	Working range	0.03-100 µg/mL
	Target range	44,55,60.5 µg/mL
	Target concentration	55 µg/mL
Accuracy (% Recovery)	80, 100, 120	99.67, 99.19, 99.49
Precision (% RSD)	Repeatability	0.865
	Intra day, 10,30,100 µg/mL	1.05, 0.55, 0.18
	Inter day, 10,30,100 µg/mL	0.24, 0.41, 0.18
LOD, µg/mL		0.03
LOQ, µg/mL		0.09

*Assay of montelukast in dosage form*

Assay was performed as described in previous chapter. Results obtained are tabulated below:

**Table 4.** Assay of montelukast tablet

Brand name of tablets	Labeled amount of Drug, mg	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean Assay (± SD) (n = 6)
Moni	10	10.34 (±0.06)	100.88 (±0.48)

The assay of Moni tablet containing montelukast was found to be 100.88% as per the method.

**Conclusion**

The proposed method is simple, sensitive and reproducible and hence can be used in routine for determination of montelukast in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision.

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## References

1. Rockville M D, United States Pharmacopoeia; United States Pharmacopoeial Convention Inc, 25<sup>th</sup> Edn., 2002, 1, 2150.
2. Budavari S, Editor. The Merck Index. 12<sup>th</sup> Ed., Whitehouse Station, N J: Merck & Co Inc.; 1996, 1070.
3. Morrow J D and Roberts L J, In: Goodman and Gilman's, The Pharmacological Basis of Therapeutics. 10<sup>th</sup> Ed., Hardman JG, Limbird L E and Gilman A G, Editors. New York: McGraw-Hill; 2001, 669.
4. Al-Rawithi S, Al-Gazlan S, Al-Ahmadi W, Alshowaier I, Yusuf A and Raines D, *J Chromatogr B Biomed Sci Appl.*, 2001;754:527–31.
5. Ochiai H, Uchiyama N, Takano T, Hara K and Kamei T, *J Chromatogr B Biomed Appl.*, 1998, **713**, 409–14.
6. Chauhan B, Shubha Rani, Nivsarkar M and Padh H, *Indian J Pharm Sci.*, 2006, **68**, 517–20.
7. Radhakrishna T, Narasaraju A, Ramakrishna M and Satyanarayana A, *J Pharm Biomed Anal.*, 2003, **31**, 359–68.
8. Alsarra I, *Saudi Pharm J.*, 2004, **12**, 136–43.
9. Sweetman S C, Editor. Martindale: The Complete Drug Reference. 33<sup>rd</sup> Ed., London: Pharmaceutical Press, 2002, p 761.



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