Stability Indicating Rp-Hplc Method for the Estimation of Montelukast in Pharmaceutical Dosage Form

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Abstract: *A simple, specific, accurate, precise and stability-indicating reverse phase high performance liquid chromatography (RP-HPLC) method is developed for estimation of montelukast in tablet dosage form. The HPLC method, C-8 Column consisting of (25mm x 4.6mm x 5um – BDS) i.d in isocratic mode, with mobile phase containing 0.01M Potassium di-hydrogen orthophosphate buffer and acetonitrile in the ratio of 35:65 v/v was used. The flow rate was 1mL min^{-1} and effluent was monitored at 222 nm. Retention time was found to be 3.08min, respectively. The method was validated in terms of Linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 10 - 100 µg/ml respectively. The LOD and LOQ values for HPLC method were found to be 0.03 and 0.09 µg/ml respectively. No chromatographic interference from tablet excipients was found. The proposed method was successfully used for estimation of montelukast in tablet dosage form.*

Keywords*: Hplc method, Montelukast, Stability studies*

I.   Introduction

Montelukast is an oral leukotriene receptor antagonist for the treatment of asthma and seasonal allergic rhinitis (hay fever). **Montelukast** reduces the production or blocks the action of Leukotrienes – substances released by cells in the lungs during an asthma attack.   Leukotrienes are a group of naturally occurring chemicals in the body that causes the lining of the airways to become inflamed, which in turn leads to wheezing, shortness of breath and mucus production. They are formed by cells, released, and then bound to other cells, and it is the binding to these other cells that stimulates the cells to promote inflammation.

Leukotriene modifiers include motelukast (Singulair) and zafirlukast (Accolate), selective competitive inhibitors of LTD_4 and LTE_4 receptors. Montelukast is used for the treatment of asthma and seasonal allergic rhinitis. Montelukast begins working after 3 to 14 days of therapy. Therefore, it should not be used for the treatment of an acute asthmatic attack.

Leukotriene modifiers decrease the need for oral glucocotricoid rescue therapy and permit the reduction of inhaled glucocorticoid doses, their effects have not been directly compared with those of inhaled glucocorticoids in published reports. The data that are available indicate that the magnitude of the improvement in FEV_1 in response to inhaled glucocorticoids exceeds that in response to antileukotrienes in patients with mild-to-moderate persistent asthma.

II.   Experimentalwork

1.1 Instruments

UV-Vis Spectrophotometer	:	Schimadzu-model-UV-2450
HPLC instrument	:	Schimadzu-model LC-2010 CHT
Colomn type	:	25mm x 4.6mm x 5um – BDS
		C-8 Column

2.2 Materials

Acetic acid, Acetonitrile, Water (HPLC grade), Montelukast tablet, Montelukast standard.

2.3 Method

First, the Montelukast solution in mobile phase. (Buffer : ACN = 35:65) has been prepared and scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of moutelukast, so that the same wave number can be utilized in HPLC UV detector for estimating the montelukast. While scanning the montelukast solution we observed 3 maxima namely 223.20nm, 211.00nm and 206.70nm. The UV spectrum has been recorded on shimadzu make UV – Vis spectophotometer model UV-2450. The scanned UV spectrum is attached in the following page.

After doing all the hit and trials we have arrived to a suitable method of analysis for montelukast and the details of the procedure is as explained below.

HPLC analysis was performed on SCHIMADZU make instrument model LC-2010$_{CHT}$ with auto injector facility. Column used for the analysis was supplied with a dimension of 25mm x 4.6mm, 5 μm and packing of C-8.

III. Hplc Method

3.1 Preparation of standard solution

10mg of montelukast standard was transferred into 10ml volumetric flask, dissolved & make up to volume with mobile phase.Further dilution was done by transferring 1ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

3.2 Preparation of Calibration graph

Stock solution was diluted with mobile phase get a series of concentration ranging from 10-100 μg ml^{-1} each containing 10 μg ml^{-1} of Montelukast and analyzed in triplicate. The peaks obtained were integrated, peak areas were noted and Calibration graph was plotted using peak area ratios to standard peak areas vs. concentration of standard solutions.

3.3 Preparation of sample solution

Twenty tablets, each containing 10 mg of Montelukast were weighed and average weight was calculated. Quantity equivalent to 10 mg Montelukast was weighed, transferred to 100ml standard flask, extracted with acetonitrile and made up with the same solvent. This solution was filtered through Whatman filter paper and suitable aliquots of formulation solutions were prepared to obtain concentration in the linearity range.

IV. Method Validation

The developed method was validated according to ICH guidelines for validation of analytical procedure.

The linearity was evaluated by linear regression analysis. The calibration graph was plotted for the HPLC method (10-100 μg ml^{-1}) in triplicate.

Precision studies were done in terms of repeatability (intraday precision) and intermediate (inter day precision) and was expressed as relative standard deviation (RSD) of a series of measurements. Intraday precision was calculated from six replicate readings at 3 concentration levels with-in the linearity range. Inter day precision was studied by comparing the results on three different days.

To study the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre analyzed sample at 3 different levels i.e., 80, 100 and 120%. The resultant solutions were then reanalyzed by the proposed method.

LOD and LOQ was calculated using single-to-noise (S/N) ratio method. LOD was taken as a concentration of analyte where S/N is 3 and LOQ was taken as a concentration of analyte where S/N is 10.

Robustness was evaluated by studying the influence of small deliberate changes of the analytical parameters on the retention times and peak shapes.

The method should be robust enough with respect to all critical parameters so as to allow routine laboratory use.

Specificity of the method towards the drug was studied by determining the purity of drug peak in a mixture of stress samples using a UV detector.

V. Stability Indicating Studies

PROCEDURE

The stress testing was conducted as per ICH [5] guidelines and USP [6]

Forced degradation for the drug substance was carried out under acid / base / neutral hydrolysis, photolytic and oxidative stress conditions.

Drug at a concentration of 1 mg/ml was used for all degradation studies.

In each study, blanks and controls (zero hour samples) were used to compare and calculate the percent degradation.

There were four samples prepared in each stress test,

1) Blank solution stored under normal condition.
2) Blank solution subjected to stress same as the drug.
3) Zero time sample containing the drug stored under normal condition   (control).
4) Drug solution subjected to stress.

Hydrolytic Studies

5.1 Acid hydrolysis :

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 M Hydrochloric acid was added & make up to the mark & kept for 24 hrs. from that 1 ml was

taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, than injected into the HPLC system against a blank of HCl & mobile phase (after all optimized conditions)

5.2 Basic Hydrolysis:

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 M Sodium hydroxide was added & make up to the mark & kept for 24 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase , than injected into the HPLC system against a blank of . NaOH and mobile phase (after all optimized conditions)

5.3 Oxidation with (3%) H_2O_2:

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 3% Hydrogen Peroxide was added, make up to the mark & kept for 24 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase , than injected into the HPLC system against a blank of . H_2O_2 and mobile phase (after all optimized conditions)

5.4 Thermal degradation:

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 100 ml volumetric flask, make up to the mark with mobile phase & was maintained at 50 ^{0}C. for 24 hrs. than injected into the HPLC system against a blank of mobile phase (after all optimized conditions)

5.5 Photolytic degradation:

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

VI.   Results

6.1 HPLC method

Chromatographic conditions were optimized to get best resolution and peak. The selection of mobile phase was done based on the peak parameters, (symmetry, theoretical plates and capacity factor) ease of preparation and cost. Symmetrical peaks with good separation (retention time montelukast 3.08min) was obtained with C_8 column using mobile phase buffer and acetonitrile in the ratio of 35:65 v/v at a flow rate of 1ml/min. The optimum wave length for montelukast was found at 222 nm.

To check the Linearity, standard calibration curve of the drug was constructed by plotting ratios of peak areas of standard drug montelukast vs. concentration of standard solutions. The curve showed good linearity over a concentration range of 10 – 100 μg ml^{-1} use UV/Vis detector. Linearity range and the linear regression equation for the drug are tabulated in Table 1. The LOD and LOQ values of montelukast was found to be 0.03μg ml^{-1} and 0.09 μg ml^{-1} respectively.

The Precision of the method was determined by repeating the intraday and intermediate (interday) precision. Precision was expressed as the RSD of the results. The values obtained for the precision studies are presented (Table 1), the results were with good repeatability, low interday variability were obtained.

6.2 DISCUSSION:-

The robustness of the method was evaluated by slight modification in the organic composition of mobile phase and flow rate. During the studies it was found that there was no significant change in retention time, area and symmetry of the peak.

The accuracy of the developed method was done by performing recovery studies. The results of the recovery studies range from 50-100%.

The developed method was used for the assay of commercially available tablets and six replicate determinations were performed. Experimental values obtained for the determination of tablets are represented. The interference of excipients was studied by comparing the chromatography of standards and formulations. The same shape and retention times of peaks showed that there was no interference from the excipients.

The system suitability test of the chromatographic system was performed before each run. Five replicate injections of standards were made and peak asymmetry, theoretical plate number, RSD of peak areas determined. For all system suitability injections, asymmetry was < 1.5, theoretical plate number was > 5000 and RSD of peak areas is < 1%.

6.3 Stability Indicating Studies
Result and discussion

HPLC studies of samples obtained on stress testing of montelukast under different conditions using Acetonitrile: buffer (65:35% v/v) as mobile solvent system suggested the following degradation behavior.
Hydrolytic studies
Acidic condition

It was observed that around 20% of the drug degraded on heating it in 0.1 M HCl for 30 min at 100°C forming degradation products at retention time 2.15, 2.45, and 5.08 min. The rate of hydrolysis in acid was faster as compared to that of alkali or water.

Alkaline condition

For alkaline hydrolysis it was found that around 20% of the drug degraded on heating it in 0.1 M NaOH for 3 hrs at 100°C.

Oxidative studies

In oxidative condition, 20% degradation of the drug was obtained after exposure to 3% H_2O_2 for 24hrs.

Photolytic studies

Sufficient degradation was achieved by exposing the drug product to sunlight during the "day time" for 3.5 days. The resultant degradation products have retention times 3.08min. respectively.

In all degradation studies except, acid hydrolysis and photolytic studies there was no significant formation of degradation products when compared to the standard. This indicates that, the drug may be degraded to low molecular weight non-chromophoric compounds. According to Singh and Bakshi the, stress testing suggests a target degradation of 20-80 % for establishing stability indicating nature of the method. The drug shows extensive degradation in acid hydrolytic condition. UV/Vis detection was used as an evidence for specificity of the method and to evaluate the homogeneity of the drug peak. Chromatographic peak purity data was obtained from the spectral analysis report and peak purity index value of 1.0000 indicates a homogeneous peak thus establishing the specificity of the method assayed. The developed RP-HPLC method is simple, specific and precise and can be used for routine analysis and stability indicating studies of montelukast in pharmaceutical dosage forms as well.

VII.   Conclusion

Stability indicating reversed phase high performance liquid chromatography method for montelukast is developed and validated as per ICH guidelines. The analysis of montelukast in the presence of degradants is possible now. This method can also be used for further impurity profiling of the drug. As the retention time of montelukast is 3.08 min, it can be used for the determination of montelukast from the tablets too. Since the developed methods showed no interferences from the diluents like lactose, mannitol etc. and excipients like talc, magnesium stearate etc., and these methods can be used for the routine analysis of montelukast from its dosage forms.

From the purposeful degradation study it is clear that the molecule is quite stable to basic, oxidative, dry heat and it shows degradation in acidic and photolytic conditions.

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**Table I: Summary of validation parameters for proposed methods**

| Parameters	HPLC method
Linearity	10-100 μg ml^{-1}
Linear regression	
Intercept (c)	15314
Slope (m)	41620
Correlation coefficient(r)	0.9996
Limit of detection (LOD)	0.03 μg ml^{-1}
Limit of quantification (LOQ)	0.09 μg ml^{-1}
Precision (% RSD)	
Intraday (n=6)	1.05
Interday (n=6)	0.24
Repeatability of injection (n=5)	0.865

Table II: Degradation studies:

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	----------	97.56	97.56
Basic Hydrolysis (0.I M NaOH)	24Hrs.	80.27	19.06	99.33
Oxidation(3% H_2O_2)	24Hrs.	78.73	19.86	98.61
Thermal Degradation (50 ^{0}C)	24Hrs.	89.47	10.11	99.58
UV (254nm)	24Hrs.	98.41	----------	98.41

Table III: Analysis of formulation

Labeled amount, mg tablet^{-1}	Amount found, mg tablet^{-1}	% Label Claim
	HPLC	HPLC
10	10.34	103.4
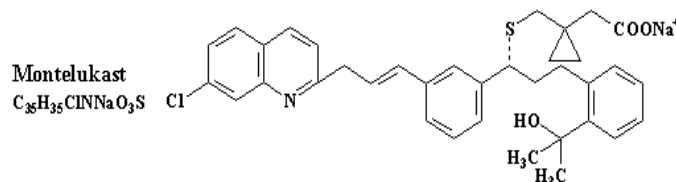
Fig.1, Structure of Montelukast

Montelukast
$C_{35}H_{35}ClNNaO_3S$
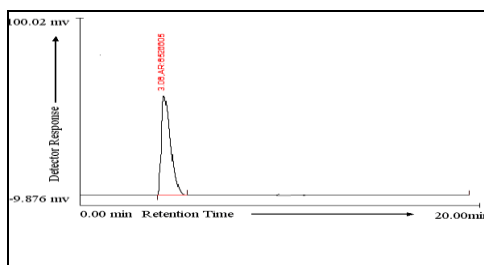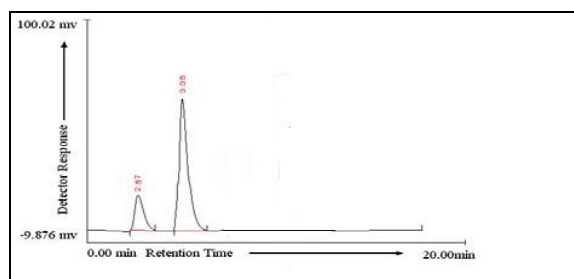

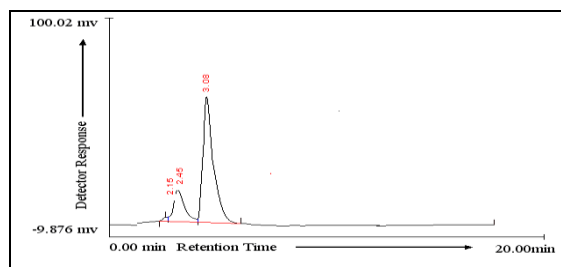**Fig.2: HPLC Chromatogram of Standard Montelukast**
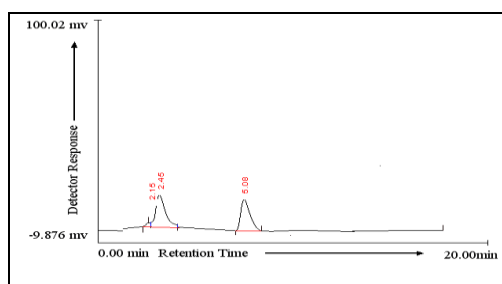
a. *Chromatogram showing degradation in 0.1 M HCl*
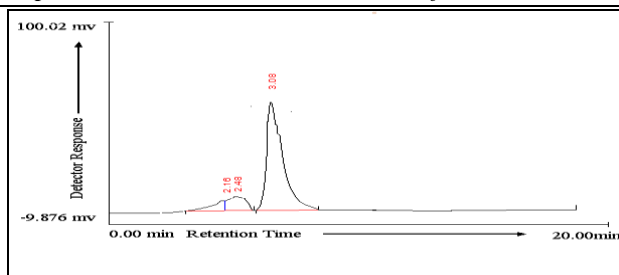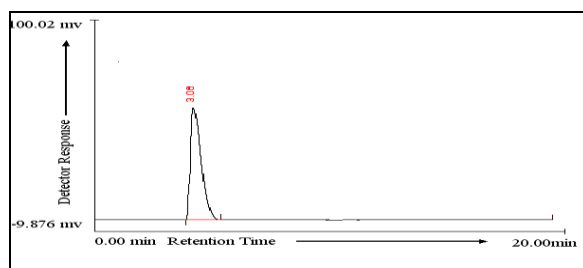
b. *Chromatogram showing degradation in 0.1 M NaOH*

c. *Chromatogram showing degradation in 3% H_2O_2*

d. *Chromatogram showing thermal degradation*

e. *Chromatogram showing photolytic degradation*

Fig.3: HPLC Chromatogram of forced degradation samples of Montelukast