Original Article

Newly Developed and Validated Method of Montelukast Sodium Estimation in Tablet Dosage Form by Ultraviolet Spectroscopy and Reverse Phase-High Performance Liquid Chromatography

Selvadurai Muralidharan[1*], Low Jia Qi[1], Lai Ting Yi[1], Narvinder Kaur[1], Subramani Parasuraman[2], Jayaraja kumar[3], Vijayan Venugopal[3], Palanimuthu Vasanth Raj[3]

[1]Unit of Pharmaceutical Chemistry, Faculty of Pharmacy, AIMST University, MALAYSIA.
[2]Unit of Pharmacology, Faculty of Pharmacy, AIMST University, MALAYSIA.
[3]Unit of Pharmaceutics, Faculty of Pharmacy, AIMST University, MALAYSIA.

ABSTRACT

Objective: Simple high performance liquid chromatographic (HPLC) and ultraviolet (UV) methods were newly developed and validated for the quantitative estimation of Montelukast (ML) in pharmaceutical dosage form. **Material and Methods:** HPLC was carried out by reverse phase-HPLC technique on a C_{18} column with a good mobile phase consist of 10 mM ammonium acetate and acetonitrile [(pH 5.5 adjusted with orthophosphoric acid (25:75, v/v)]. UV method was developed with the wavelength at 280 nm. Two methods showed good linearity, reproducibility and precision. Both the methods (UV and HPLC) showed no spectral or chromatographic interferences from the tablet excipients. **Results:** The developed methods were successfully applied to dosage forms. Validation parameters were carried out such as linearity, precision, accuracy, and specificity. The HPLC LOD and LOQ for montelukast were found to be 150 ng/ml and 500 ng/ml.

The UV LOD and LOQ for montelukast were found to be 1 μg/ml and 5 μg/ml. Both the results were statistically differentiated using one-way analysis of variance (ANOVA). The developed and validated economical methods could be applicable for analysis of ML and monitoring of the quality of available drugs.

Key words: HPLC, UV, Montelukast, Estimation, Validation, Tablets.

Correspondence:

Dr. Selvadurai Muralidharan, Senior Lecturer, Faculty of Pharmacy, Semeling-08100, Bedong, Kedah, MALAYSIA.
Phone no: +60164953594; Fax no: +6044298009
Email: murali23pharm@hotmail.com
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INTRODUCTION

Montelukast sodium is chemically (R-(E))-1-(((1-(3-(2-(7-chloro-2-quinolinyl) ethenyl)phenyl)-3(2-(1-hydroxy-1- methylethyl) phenyl) propyl) thio) methyl) cyclopro paneacetic acid, monosodium salt.[1,2] Montelukast sodium primarily used for the treatment of asthma in children and adults.[3–5] It is a potent selective inhibitor of leukotriene D4 (LTD4) at the cysteinyl leukotriene receptor cysLT1.[6–7] Only limited analytical methods such as liquid chromatography with fluorescence detector,[8] stereoselective high performance liquid chromatography (HPLC) for montelukast and its S-enantiomer,[9] column switching HPLC with fluorescence detector,[10] semi-automated 96-well protein precipitation,[11] HPLC with derivative spectroscopy,[12] pressurized liquid extraction followed by HPLC[13-14] and LC-MS methods[15–17] The objective of the present work was to develop and validate a simple, fast and reliable isocratic RP-HPLC and UV method for the estimation of ML in pharmaceutical dosage forms. The significant features and innovation of the proposed method included sample treatment with sonication of small amount of powder sample at ambient temperature, centrifugation, dilution; short elution time with ML; good precision (R.S.D. less than 5%) and high recovery (greater than 95%). Confirmation of the applicability of the developed method validated according to the International Conference on Harmonisation (ICH), to determination of ML in Pharmaceutical preparations.

Experimental

Chemicals

HPLC grade acetonitrile (ACN) and Triethyl amine (A.R. grade) was purchased from Merck, Malaysia. Water HPLC grade was obtained from a Milli-QRO water purification system. Montelukast was provided by vitalife, Haryana, India.

Instrumentation and analytical conditions

HPLC separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50μL loop volume. The version was used for the HPLC is 1.25. The HPLC was conceded out at a flow rate of 1.0 ml/min using a mobile phase constituted of 10 mM ammonium acetate–acetonitrile (pH 5.5 adjusted with orthophosphoric acid) (25:75, v/v) and detection was made at 280 nm. Every day prepared the mobile phase and filtered through a 0.45 μm membrane filter (Millipore®) and sonicated before use. A Princeton SPHER C_{18} column (250×4.6 mm i.d., 5μ) was used for the separation. UV method was performed on a UV–VISIBLE spectrophotometer, Beckman Coulter-DU800 with the λ_{max} at 280 nm and using 1.0 cm quartz cell.

Preparation of standard solutions

HPLC method

Accurately weighed 100.0 mg of ML was transferred to a 100 ml standard flask and dissolved in a mixture of water and methanol in the ratio 1:1 v/v. From the stock solution, other solutions with concentrations of 150.0, 200.0, 250.0, 300.0, 350.0, and 500.0 ng/ml were obtained by diluting adequate amounts in triplicate and used for the calibration curve.

UV method

Accurately weighed 100.0 mg of ML was transferred to a 100 ml standard flask and dissolved in a mixture of water and methanol in the ratio 1:1 v/v. From the stock solution, other solutions with concentrations of 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 μg/ml were obtained by diluting adequate amounts in triplicate and used for the calibration curve.
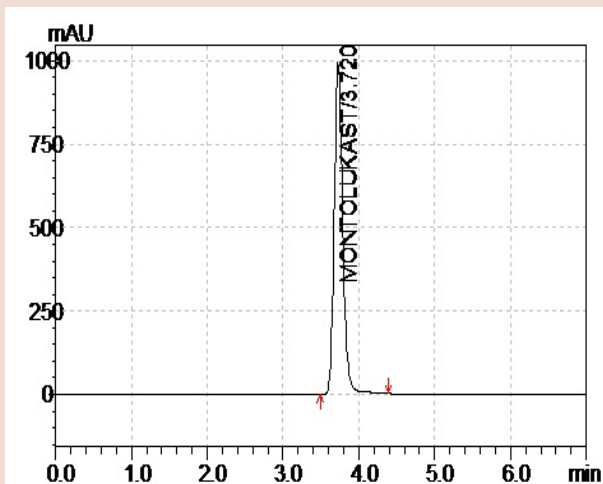
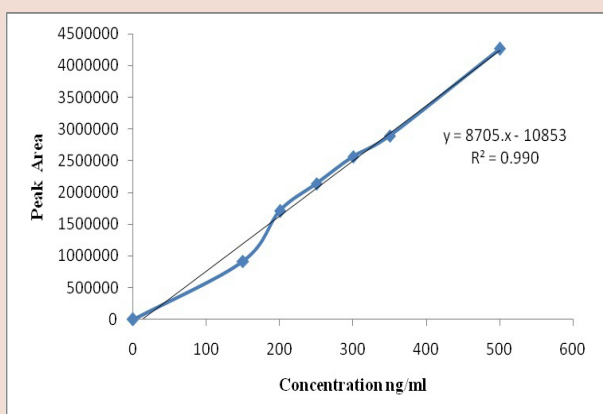**Figure 1:** Typical sample chromatogram of Montelukast
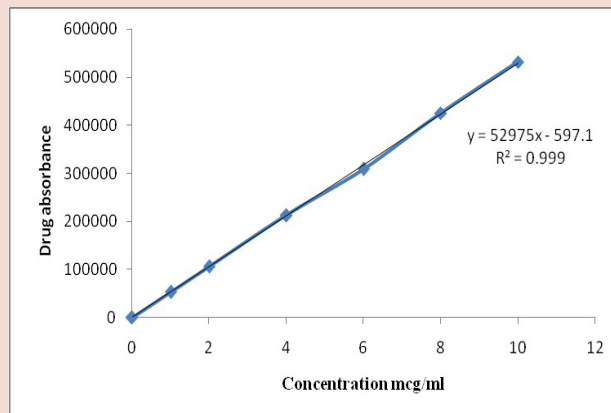

Figure 2: Calibration Curve of Montelukast by HPLC

Preparation of sample solutions

HPLC method

Twenty tablets, 10.0 mg of ML were weighed and finely made in to powder form; a amount of powder equivalent to 10.0 mg of ML was weighed and transferred to a sintered glass crucible. To this 5.0 ml of 1.0 mg/ml solution of montelukast was added and the drug was extracted with methanol and water (1:1 v/v). The extract was made up to 100 ml with mobile phase and further dilutions were made to get a concentration of 10.0 ng/ml of montelukast was used for the estimation. Figure 1 shows an HPLC chromatogram of ML in pharmaceutical tablets.

UV method

Accurately weighed quantity of powder equivalent to 10.0 mg of ML was transferred to 100 ml standard flask and dissolved in the mobile phase to obtain an each concentration of 100.0 mcg/ml. An aliquot of this solution was diluted in mobile phase to obtain a solution with final concentration.

Method validation

The aim of method validation is to reveal that the method is suitable for its intended idea as it is stated in ICH guidelines.[18] The method was validated for linearity, precision (repeatability and intermediate precision), accuracy specificity, short-term stability and system suitability.

Figure 3: Calibration Curve of Montelukast by UV

Calibration plots were constructed with six concentrations in the range of 150.0–500.0 ng/ml prepared in triplicates to test linearity. The ratio of peak area signal of ML was plotted against the corresponding concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared ML solution. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area ratios of ML to that of IS were determined and precision was reported as % R.S.D. Method accuracy was tested (% recovery and % R.S.D. of individual measurements) by analysing samples of ML at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of AMH recovered in the samples. Specificity was assessed by comparing the chromatograms obtained from sample of pharmaceutical preparation and standard solution with those obtained from excipients which take part in the commercial tablets and verifying the absence of interferences. Sample solution short-term stability was tested at ambient temperature (20 ± 1℃) for three days. In order to confirm the stability of both standard solutions at 100% level and tablets sample solutions, both solutions protected from light were reinjected after 24 and 48 h at ambient temperature and compared with freshly prepared solutions. A system suitability test was performed by six replicate injections of the standard solution at a concentration of 150 ng/ml verifying ML resolution >2; %R.S.D. of peak area ratios of ML. %R.S.D. of each peak retention time ± 2%.

RESULTS AND DISCUSSION

Validation of method

Linearity

Six point's calibration graphs were constructed covering a concentration range 150–550 ng/ml. Three independent determinations were performed at each concentration. Linear relationships between the ratio of the peak area signal of ML the corresponding drug concentration were observed, as shown by the results presented in Table 1. The standard deviations of the slope and intercept were low. The determination coefficient (r^2) exceeded 0.99 (Figure 2.). The UV calibration is presented in Figure 3.

Precision

The repeatability study (*n*=6) carried out showed a R.S.D. of 0.81% for the peak area ratios of ML obtained, thus showing that the equipment

Table 1: Results of regression analysis of data for the quantitative determination of Montelukastby the proposed methods

Statistical parameters	HPLC	UV
Concentration range	150.0-500.0 (ng/ml)	1.0-10.0 (µg/ml)
Regression equation	y=8705 X+-10853	y=52975 X-597.1
Correlation coefficient (r)	0.990	0.999

Table 2: Accuracy study for Montelukast (n=5)

Nominal Concentration ng/ml (HPLC)	Nominal Concentration µg/ml (UV)	Mean recovery (%) HPLC UV		R.S.D. (%) HPLC UV	
150.0	1.0	1.92	1.85	0.31	0.13
300.0	6.0	7.85	7.55	0.68	0.55
500.0	10.0	11.99	11.78	0.91	0.97

Table 3: Results obtained for determination of Montelukast in Marketed formulations

	HPLC		UV		HPLC	UV
Drug	Amount mg/tab		Amount mg/tab		% Recovery	
	Labelled	Found	Labelled	Found		
Montelukast	10.0	9.74	10.0	9.52	99.55	98.89

used for the study worked correctly for the developed analytical method and being highly repetitive. For the intermediate precision, a study carried out by the same analyst working on two consecutive days (*n*=3) indicated a R.S.D. of 0.69%. Both values were far below 5%, the limit percentage set for the precision and indicated a good method precision.

Accuracy

The data for accuracy were expressed in terms of percentage recoveries of ML in the real samples. These results are summarized in Table 2. The mean recovery data of ML in real sample were within the range of 99.55 and 98.89%, mean % R.S.D. was 0.71%, satisfying the acceptance criteria for the study.

Specificity

The HPLC chromatogram recorded for the mixture of the drug excipients revealed no peak within a retention time range of 5 min. The results showed that the developed method was specific as none of the excipients interfered with the analytes of interest.

Stability

The stability of ML in standard and sample solution was determined by storing the solutions at ambient temperature (20 ± 1ºC) protected from light. The solutions were checked in triplicate after three successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 72 h, as during this time the results did not decrease below 96%. This denotes that ML is stable in standard and sample solutions for at least 3 days at ambient temperature, protected from light and is compatible with IS.

System suitability

The % R.S.D. of peak area ratio of ML is within 2% representing the suitability of the system. These results point out the applicability of this method to routine with no interferences, its suitability being proved. This method evaluated statistical such good linearity, reproducibility

and its validation for different parameters and led us to the conclusion that it could be used for the rapid and reliable determination of ML in pharmaceutical forms.

Assay of tablets

The developed and validated method was applied for the estimation (assay) of marketed tablets containing 10.0 mg of ML each sample was analysed in triplicate after extracting the drug as mentioned in assay sample preparation of the experimental section and injections were carried out in triplicate. No interference was found in analyte peak. The report presented in Table 3.

CONCLUSION

A validated isocratic HPLC and UV methods have been developed for the determination of ML in dosage forms. The proposed methods are simple, rapid, accurate, precise and specific. Its chromatographic run time of 6.0 min allows the analysis of a large number of samples in a short period of time. Therefore, it is suitable for the routine analysis of ML in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS/MS or GC–MS/MS that are complicated, costly and time consuming rather than a simple HPLC–UV method. Considering the possible worldwide development of counterfeit Mukonil, the proposed method could be useful for the national quality control laboratories in developing countries.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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