Rapid and Sensitive Determination Of Montelukast in Human Plasma by High Performance Liquid Chromatographic Method Using Monolithic Column: Application to Pharmacokinetic Studies

Alireza Shafaati1, Afshin Zarghi2, Seyed Mohsen Foroutan1, Arash Khoddam1 and Babak Madadian2

1School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, P.O.Box: 14155-6153, Iran
2Noor Research and Educational Institute, Tehran, Iran

Abstract

A rapid, simple and sensitive high-performance liquid chromatography (HPLC) method, using a monolithic column and fluorescence detection, has been developed for quantification of montelukast in human plasma. Ethoxyquine, easily available as a pharmaceutical substance, was selected as internal standard. The assay enables the measurement of montelukast for therapeutic drug monitoring with a minimum detectable limit of 5 ng/ml. The method involves simple, one-step extraction procedure and analytical recovery was about 97%. The separation was carried out in reversed-phase conditions using a Chromolith RP® (RP-18e, 100 mm×4.6 mm) column at ambient temperature. The mobile phase was 56% acetonitrile and 50mM sodium dihydrogen phosphate, and distilled water to 100%, adjusted to pH 7.0 at a flow rate of 2 ml/min. The excitation wavelength was set at 350 nm, emission at 450 nm. The calibration curve was linear over the concentration range 20–800 ng/ml. The coefficients of variation for inter-day and intra-day assay were found to be less than 7%. The method was applied to the determination of montelukast in plasma from 12 subjects dosed with montelukast 10 mg tablets and pharmacokinetic parameters were determined.

Keywords: Montelukast; Determination; Human Plasma; HPLC; Monolithic Column

Introduction

Montelukast sodium [1] is described chemically as [R-(E)]-1-[[1-[3-[2-(7-chloro-2quinolinyl)] ethenyl]phenyl]-3-2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio] methyl] cyclopropane acetic acid, monosodium salt (Figure 1). Montelukast is a selective and orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene CysLT1 receptor [2,3]. The drug is indicated for the prophylaxis and chronic treatment of asthma in adults and pediatric patients 12 months of age and older. It is also indicated for prevention of exercise-induced bronchoconstriction in patients 15 years of age and older and for the relief of symptoms of seasonal allergic rhinitis in adults and pediatric patients 2 years of age and older [2-4]. Montelukast is rapidly absorbed following oral administration and is extensively metabolized. Montelukast and its metabolites are excreted almost exclusively via the bile [5]. In several studies, the mean plasma half-life of montelukast ranged from 2.7 to 5.5 hours in healthy young adults [5,6]. The pharmacokinetics of montelukast is nearly linear for oral doses up to 50 mg [6].

In order to study the pharmacokinetics of montelukast in humans, a sensitive and reliable assay of the drug in biological fluids is necessary. Various analytical methods have been reported for the determination of montelukast in bulk or pharmaceutical dosage forms [7-10] and in biological fluids [1,10-13]. Recently, [14] published an excellent paper on development and validation of a bioanalytical method for determination of montelukast in human plasma using HPLC. In their paper, they also reviewed and compared the published HPLC methods for determination of montelukast in human plasma. Compared to other reports, the method reported by Sripalakit and colleagues was simple and the total run time (i.e. 9 minutes) was less than the time previously reported for similar methods. Furthermore, they used mfenamic acid as internal standard, which is available commercially. In our study, we have developed and validated a very simple method based on using a monolithic column, by which the consumption of acetonitrile in the mobile phase has been reduced from 70% to 56%. Moreover, although we have achieved a base line resolution between the internal standard and the major peak, the lag time between the two peaks (which is more than 4 minutes in Sripalakit’s paper) has been reduced to less than 2 minutes. This in turn, led to a shortened analysis time (<5 min.) and further reduction in the acetonitrile consumption. Thus, the present method provides a simple, rapid and sensitive assay for determination of montelukast with a minimum detectable limit of 5 ng/ml.

Figure 1: Chemical structure of montelukast (MO) and ethoxyquine (internal standard, I.S.).

*Corresponding author: Alireza Shafaati, Professor of pharmaceutical chemistry, School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, P.O.Box: 14155-6153, Iran, E-mail: ashafaati@yahoo.com

Received May 24, 2010; Accepted November 22, 2010; Published December 30, 2010


Copyright: © 2010 Shafaati A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
method is less hazardous to human health and to the environment as well as being faster and more economic.

Materials and Methods

Chemicals and reagents

Montelukast sodium and ethoxiquine standard powders were kindly gifted by Sobhan Daru Pharmaceutical Company (Tehran, Iran). HPLC grade acetonitrile, analytical grade disodium hydrogen phosphate, phosphoric acid, and all other chemicals were purchased from Merck (Darmstadt, Germany). High purity water was supplied by Milli-Q Water Purification System (Millipore, Billerica, MA). Human plasma was supplied by Noor Educational and Research Institute (Tehran, Iran).

Chromatographic conditions

The HPLC system (Knauer, Germany) consisted of a K1001 pump and a RX-10AXL spectrofluorometric detector and controlled by Eurochrom 2000 (Integration Package) software. The analytical column employed was Chromolith RP* (100mm x 4.6mm i.d.) purchased from Merck (Germany). The mobile phase was comprised of acetonitrile—50 mM phosphate buffer pH 7.0 (56% - 46% v/v, respectively). The mobile phase was prepared freshly and was filtered before use. All separations were performed isocratically at a flow rate of 2.0ml/min and column condition was maintained at ambient temperature. The detector was operated at an excitation wavelength of 350 nm and an emission wavelength of 450 nm.

Standard solutions

Blank plasma was prepared from heparinized whole-blood samples collected from healthy volunteers and stored at -20°C. Stock solution of montelukast sodium was prepared with concentration of 0.01 mg/ml in water and diluted with drug free human plasma or the mobile phase to the desired concentration. Due to the sensitivity of montelukast to the light [8,9], exposure of the drug solution to the light was carefully avoided. Ethoxiquine was dissolved in acetonitrile at a concentration of 0.4 mg/ml. A series of plasma standard solutions of montelukast sodium were prepared with concentrations of 20, 100, 200, 350, 500, 600, and 800 ng/ml each containing the internal standard at a concentration of 40 ng/ml. The samples were then prepared for analysis as described above. Calibration curves were constructed by plotting peak area ratio (y) of montelukast to the internal standard versus the drug concentration (x). A linear regression was used for quantification.

Pharmacokinetic design and analysis

The proposed method was applied to evaluate the pharmacokinetic of montelukast in 12 healthy male volunteers with a mean age of 25.9 ± 4.2 years and body mean weight of 72.7 ± 2.5 kg. Each volunteer was orally administered 10mg montelukast (Sobhan Daru Pharmaceutical Company, Tehran, Iran) under fasting conditions. Blood samples were collected in heparinized tubes before and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7, 10 and 24 h after dosing and centrifuged to obtain the plasma fraction. The plasma samples were kept in cryogenic vial stored at −20°C until analysis. Montelukast pharmacokinetic parameters were determined by non-compartmental methods. Elimination rate constant (K) was estimated by the least-square regression of plasma concentration—time data points lying in the terminal log-linear region of the curve. Half-life (T½) was calculated as 0.693 divided by K. The area under the plasma concentration–time curve from time zero to the last measurable concentration at time (AUC0–∞) was calculated using the trapezoidal rule (Foroutan et al, 2003). The area was extrapolated to infinity (AUC0–∞) by addition of C/K to AUC0–t, where C, is the last detectable drug concentration. Peak plasma concentration (Cmax) and time to peak concentration (Tmax) were derived from the individual subject concentration time curves.
Results

Chromatography separation

Under the chromatographic conditions described, montelukast and the internal standard peaks were well resolved. No interfering peaks from endogenous plasma components were observed. Typical chromatograms of blank plasma in comparison to spiked samples analyzed for pharmacokinetics study are shown in Figure 2. The monolithic column has lower flow impedance comparing to the particulate packings, and therefore allows applying higher flow rate and easy optimization of chromatographic conditions [16] to obtain desirable resolution in a short time (<5 min). A mobile phase consisted of acetonitrile and phosphate buffer in the ratio of 56: 44% v/v, respectively, was optimum to achieve the best resolution between montelukast and the internal standard. The average retention times of the internal standard I and montelukast were 2.3 and 3.7 min, respectively. Ethoxyquine, easily available as a pharmaceutical substance, was selected among many compounds we tested as internal standard because of its structural similarity to montelukast and appropriate chromatographic behavior.

Method validation

As shown in Table 1, the calibration curve for the determination of montelukast in plasma was linear over the range 20–800 ng/ml. The linearity of this method was statistically confirmed. For each calibration curve, the intercept was not statistically different from zero. The correlation coefficients ($r$) for calibration curves were equal to or better than 0.999. The relative standard deviation (RSD) values of the slope were less than 2%. For each point of calibration standards, the concentrations were recalculated from the equation of the linear regression. The analytical recovery for plasma at three different concentrations of montelukast was determined. Known amounts of the drug were added to drug-free plasma in concentrations ranging from 20-600 ng/ml. The internal standard was added and the absolute recovery of montelukast was calculated by comparing the peak areas for extracted montelukast from spiked plasma and a standard solution of montelukast in acetonitrile containing internal standard with the same initial concentration. The average recovery was 98.2 ± 1.5%. Using fluorescence detection, the limit of quantification (LOQ), as previously described, was 5 ng/ml for montelukast. This is sensitive enough for drug monitoring and for pharmacokinetic studies.

We assessed precision of the method by repeated analysis of plasma specimens containing known concentrations of montelukast. As shown in Table 2, coefficients of variation were less than 7%, which is acceptable for the routine measurement of the drug. Stability was determined for spiked plasma samples under the conditions as described above. The results showed that the samples were stable under the conditions used for storage.

Application to pharmacokinetic study

In this study plasma concentrations were determined in twelve healthy volunteers, who received a single oral dosing of 10-mg tablet formulation. The derived pharmacokinetic parameters of 12 healthy volunteers are summarized in Table 3. The mean plasma concentration–time curve of montelukast is shown in Figure 3. The plasma concentration of montelukast reached to a maximum $2.42 ± 0.19$ h after dosing with a level of $499.34 ± 133.69$ ng/ml. These pharmacokinetic parameters are in good agreement with those found previously and no

Table 1: Assay linearity results for montelukast

<table>
<thead>
<tr>
<th>Concentration added (ng/ml)</th>
<th>Peak area ratio (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-day</td>
</tr>
<tr>
<td>20</td>
<td>0.21 ± 0.01 (4.8)</td>
</tr>
<tr>
<td>300</td>
<td>2.16 ± 0.13 (6.1)</td>
</tr>
<tr>
<td>600</td>
<td>3.61 ± 0.17 (4.4)</td>
</tr>
</tbody>
</table>

Values in parentheses are relative standard deviations (%)
significant difference was observed between our pharmacokinetic data and results reported in the literature [5,6].

Discussion

The aim of our study was to develop a rapid and sensitive method for the routine analysis of biological samples in pharmacokinetic montelukast research. This method is well suited for routine application in the clinical laboratory because of the speed of analysis and simple extraction procedure. Over 300 plasma samples were analyzed by this method without any significant loss of resolution. No change in the column efficiency or increase in backpressure was observed over the entire study time, thus proving its suitability.

In the present method we used a protein precipitation extraction, HPLC on a monolithic column and fluorescence detection to give high sensitivity [16]. The published HPLC methods for determination of montelukast in human plasma were summarized in an excellent paper published by [14]. They presented in their paper, a simple bioanalytical assay for determination of montelukast in human plasma and its application to a pharmacokinetic study and compared to the previously reported methods. Then, they concluded that their method is advantageous over most of the reported methods because of the shorter analysis time and availability of the internal standard. In our method, in this work, total analysis time is even shorter and separation achieved in less than 5 minutes. Moreover, separation was performed on a monolithic column which resulted in lower consumption of the acetonitrile as an organic modifier in the mobile phase. Further reduction in the acetonitrile consumption was resulted due the shorter total analysis time. Therefore, the present method is less hazardous to human health and to the environment as well as being faster and more economic. These results were obtained along with the same sensitivity and selectivity as described by [14].

The present method proved to be reproducible and reliable based on the results of validation assessment. Also, sensitivity and selectivity of the method allowed us to apply it successfully for the routine analysis of biological samples in pharmacokinetic research on montelukast.

Conclusions

We developed a rapid, simple, accurate and reproducible method for determination of montelukast in plasma. The proposed method is less hazardous to human health and to the environment as well as being faster and more economic. This method will permit pharmacokinetic and pharmacodynamic studies of the drug in humans.

Acknowledgements

This work was supported by Noor Research and Educational Institute.

References