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High-Performance Liquid Chromatographic Determination of Montelukast Sodium in Human Plasma: Application to Bioequivalence Study

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Summary. A simple, sensitive, and precise high-performance liquid chromatographic (HPLC) method for quantitation of montelukast in human plasma has been developed and validated. Commercially available candesartan cilexetil was used as an internal standard. After protein precipitation, montelukast and candesartan cilexetil (I.S.) in human plasma were analyzed using mobile phase containing 62% *v*/*v* acetonitrile and 38% *v*/*v* buffer (containing 1 mL L^{-1} triethylamine as peak modifier, final pH adjusted to 2.5 with orthophosphoric acid). Chromatographic separation was achieved on a BDS Hypersil-C18 column (50 × 4.6 mm i.d., particle size 5 μm; Thermo Electron Corporation, USA) using isocratic elution at a flow rate of 1.5 mL min^{-1}. The signals were monitored using a fluorescence detector set at 350 nm for excitation and 400 nm for emission. The total time for a chromatographic separation was ~3 min. The validated quantitation ranges of this method were 5–300 ng mL^{-1} with coefficients of variation between 1.75% and 9.38%. Mean recoveries were 91.8 ± 3.8%. The within- and between-batch precisions were 0.74–2.46% and 1.64–7.87%, respectively. The within- and between-batch relative errors (bias) were 0.14–3.3% and 0.08–4.6%, respectively. Stability of montelukast in plasma was >94.7%, with no evidence of degradation during sample processing and 30 days storage in a deep freezer at –70°C. This validated method is sensitive and simple with between-batch precision of <8% and successfully applied for the bioequivalence studies. The formulations were compared using the following pharmacokinetic parameters: AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}. No statistically significant difference ($p > 0.05$) was observed between the logarithmically transformed AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} values.

Key Words: montelukast, assay, HPLC, plasma, fluorescence, bioequivalence application

Introduction

Montelukast sodium (2-[1-[1(*R*)-[3-[2(*E*)-(7-chloroquinolin-2yl)vinyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl] propylsulfanylmethyl] cyclopropyl] acetic acid monosodium salt; *Fig. 1a*), is a potent and specific cys-

teinyl leukotriene D_4 (LTD_4) receptor antagonist [1, 2], which is being used in the treatment of asthma [3–8]. The oral absorption of montelukast is rapid and complete with an average bioavailability of 63% to 73%. Following an oral administration of 10-mg film-coated tablet, the mean peak plasma concentration (C_{max}) is achieved in 3–4 h (T_{max}) after administration in adult in fasted state. The mean oral bioavailability is 64% [8]. On the basis of reported literature, the C_{max} of montelukast ranges from 247 to 283 ng mL^{-1}, after oral administration of a 5-mg tablet [9]. Montelukast is more than 99% bound to plasma proteins. The steady-state volume of distribution of montelukast averages 8–11 L. Montelukast is extensively metabolized. In studies with therapeutic doses, plasma concentrations of metabolites of montelukast are undetectable at steady state in adults and pediatric patients. The pharmacokinetics of montelukast is nearly linear for oral doses up to 50 mg. In several studies, the mean plasma half-life of montelukast ranged from 2.7 to 5.5 h in healthy young adults. No difference in pharmacokinetics was noted between dosing in the morning or in the evening [8, 10].

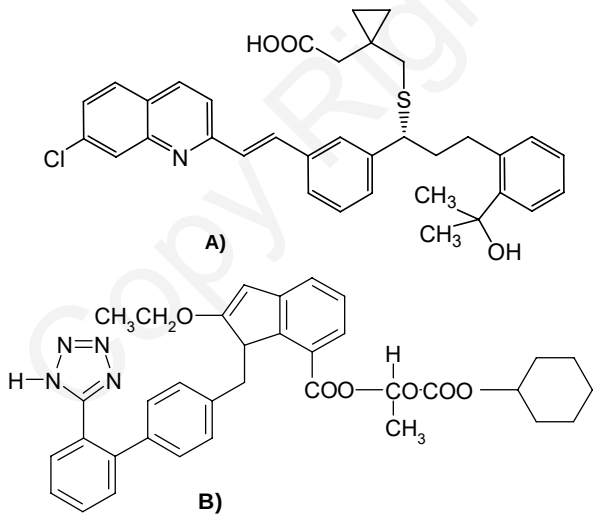


Fig. 1. Chemical structure of (a) montelukast, and (b) candesartan cilexetil

Various analytical methods have been developed to determine montelukast in human plasma samples, such as LC-MS-MS [9, 11], radiochromatographic [12], HPTLC [13], voltammetry [14], and chromatographic assays [15–23]. The use of columns switching [16–17], microtiter plate [19], and dual-column [20] has also been incorporated with the process of high-performance liquid chromatographic (HPLC) analysis. Some methods do

not use internal standards [17, 20], whereas other methods use synthetic derivatives [15–16, 19], quinine bisulfate [18], mefenamic acid [21], or ethoxyquin [23] as internal standards. Literature survey revealed that there is high variation in the limit of quantitation as reported by different authors (0.25 ng mL^{-1} [11]; 1–50 ng mL^{-1} [15–23]). All these methods utilize the 100–500 μL of plasma for sample processing.

For routine clinical analysis, a high throughput analysis is required. A sensitive, rapid, and economical analytical method is necessary for quantitation of the concentrations of montelukast in human plasma in order to support pharmacokinetic, bioavailability, and bioequivalence studies. It was essential to establish an assay capable of quantifying montelukast at concentrations down to ~6.5 ng mL^{-1} (i.e., 2.5% of C_{max}) following oral administration of 5 mg tablet. Thus, the objective of the present investigation was to develop a simple, rapid, sensitive, and economical HPLC-RF method that can estimate montelukast in human plasma samples.

Experimental

Ethics

The bioequivalence study was conducted in accordance with the Declaration of Helsinki and ICH guidelines on good clinical practice. The report was written in accordance with ICH guidelines. The protocol was approved by the IEC of Al-Mowasah Hospital, Amman, Jordan. All subjects were screened within 14 days before entering the study. Before screening, a specific screening-informed consent form was signed. The non-clinical part of the study was conducted at JCPR, Amman, Jordan (data on file).

Chemicals and Reagents

Montelukast sodium (99.5%) and candesartan cilexetil *(Fig. 1b)* (99.6%), were obtained from Tabuk Pharmaceutical Manufacturing Company, Tabuk (KSA). Chemical structures are presented in *Fig. 1*. HPLC grade Lichrosolv acetonitrile, methanol, and water were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade.

Drug Solutions

The entire prepared samples were stored in amber-colored glassware due to the photosensitivity of the drugs. The stock solutions of montelukast and candesartan cilexetil (1 mg mL^{-1}) for generating standard curves were pre-

pared by dissolving an appropriate amount of each compound in acetonitrile–water (70:30 v/v) and acetonitrile to yield concentration of 1.0 mg mL^{-1}, respectively. Working solution of montelukast was obtained by further diluting the stock standard solution with acetonitrile–water (70:30 v/v).

Chromatographic Conditions

The HPLC system (Agilent Technologies, Inc., Santa Clara, CA) consisted of a constant solvent delivery system (G1311A), a spectrofluorometric detector (G1321A) equipped with an auto-sample injector (ALS-G1329) fitted with a 50-µL sample loop was used. The analytical column employed was BDS Hypersil C18 (50 × 4.6 mm i.d., 5 µm particle size, Thermo Electron Corporation, USA). The data were captured using Microsoft Windows-XP based Chem-Station for LC system Rev. B.03.01[317] 2001–2007 chromatographic software. The mobile phase was comprised of phosphate buffer 38% (containing triethylamine 1 mL L^{-1}) and acetonitrile 62% v/v. The pH of the mobile phase was adjusted using concentrated orthophosphoric acid to 2.70 ± 0.05 (using Orion Research Model 611 pH meter). The mobile phase was filtered through 0.45 µm Millipore filter before used and degassed in an ultrasonic bath. All separations were carried out isocratically at a flow rate of 1.5 mL min^{-1}. The detector was operated at an excitation wavelength of 350 nm and an emission wavelength of 400 nm.

Standard and Quality Control (QC) Samples

Working solution of montelukast for the calibration (0.1, 0.2, 0.4, 1.0, 2.0, 4.0, and 6.0 µg mL^{-1}) and QC sample (0.1, 0.3, 3.0, and 5.0 µg mL^{-1}) was prepared separately. Working solution for calibration and QC was prepared by diluting the suitable concentration of stock solution to 20 mL in volumetric flask using acetonitrile–water (70:30 v/v). The internal standard solution (0.6 µg mL^{-1}) was prepared by diluting the stock solution with acetonitrile.

Fifty microliters of working standard solution was added to 950 µL of drug-free plasma to obtain montelukast concentrations of 5, 10, 20, 50, 100, 200, and 300 ng mL^{-1}. Similarly, the QC sample of montelukast as a single batch of concentrations 5 (lower limit of quantitation, LLOQ), 15 (low), 150 (medium), and 250 ng mL^{-1} (high) was prepared by spiking the 5% appropriate working solution to 95% of pooled blank drug-free plasma. The QC samples were divided into aliquot in test tube and were stored at −70±5°C until analysis. All stock solutions were stored between 4 and 8°C.

Sample Preparation

All plasma samples were thawed at room temperature. Plasma (200 μL) was transferred to a 2–mL Eppendorf tube, and 300 μL of acetonitrile containing internal standard (0.6 μg mL^{-1}) was added while vortexing. The mixture was vortex-mixed for 60 s and centrifuged at 16000 rpm for 10 min. The supernatant was transferred to an auto-sampler amber-colored vial and 35 μL was injected into the HPLC system. The blank samples were processed using 300 μL of acetonitrile.

Bioanalytical Method Validation

Calibration curves were made from blank (a plasma sample processed without IS), a zero sample (a plasma processed with IS), and seven non-zero samples covering the total range (5–300 ng mL^{-1}), including lower LLOQ. Each validation run consisted of system suitability sample, blank sample, a zero sample, seven non-zero samples (n = 5) covering the total range (5–300 ng mL^{-1}), and QC sample at three concentrations (n = 10 at each concentration). Samples were analyzed from lower to high concentration at the beginning of each validation run. Other samples were distributed randomly through the run.

Such calibration curves were generated on 3 consecutive days. Linearity was assessed by weighted (x) least square regression analysis. The acceptance criterion for each back-calculated standard concentration was 15% deviation from the nominal value except LLOQ, which was set at 20%.

Specificity and Selectivity

To evaluate the specificity of the method, drug-free plasma sample was carried through the assay procedure, and the retention times of the endogenous compounds in the plasma were compared with those of montelukast or candesartan cilexetil (internal standard). Specificity of the method was assessed to test the matrix influence between different plasma samples. Interference from other or over-the-counter (OTC) medication was also investigated. The tested compounds were aspirin, paracetamol, caffeine, mefenamic acid, ibuprofen, and nicotinamide.

Sensitivity

The limit of detection was defined, as analyte responses are at least five times the response compared to blank response. The lowest standard on the calibration curve was defined as the limit of quantification as an analyte

peak was identifiable, discrete, and reproducible with a precision of less than or equal to 20% and accuracy of 80–120%.

Accuracy and Precision

Within-batch accuracy and precision evaluation were performed by repeated analysis of montelukast in human plasma. The batch consists of calibration standards (n = 5), ten replicates of LLOQ, low, medium, and high QC samples, while between-batch accuracy and precision were assessed by analysis of similar sequence of samples on three separate occasions. The overall precision of the method was expressed as relative standard deviation, and accuracy of the method was expressed in terms of relative error (bias). The QC samples were randomized daily, processed, and analyzed in the following positions: (a) immediately after the standard curve, (b) in the middle of batch, or (c) at the end of the batch. The acceptance criteria for within- and between-batch precisions were 20% or better for the LLOQ and 15% or better for the other concentrations; that for accuracy was 100 ± 20% or better for the LLOQ and 100 ± 15% or better for the other concentrations [24].

Recovery

The efficiency of montelukast extraction from human plasma was measured, analyzing three levels of QC samples. The drug recovery was determined by comparing peak area obtained from the spiked QC plasma samples after extraction and reconstitution to the standard solution at the same concentration of the spiked QC plasma samples.

Stability

The bench top stability was examined by keeping replicates of the low, medium, and high plasma QC samples at room temperature for approximately 24 h. Freeze–thaw stability of the samples was obtained over three freeze–thaw cycles, by thawing at room temperature for 2–3 h, refrozen for 12–24 h. Auto-sampler stability of montelukast was tested by analysis of processed and reconstituted low, medium, and high plasma QC samples, which are stored in the auto-sampler tray for 24 h at 5 ± 1°C. Stability of montelukast in human plasma was tested after storage at approximately −70°C for 30 days. For each concentration and each storage condition, three replicates were analyzed in one analytical batch. The concentration of montelukast af-

ter each storage period was related to the initial concentration as determined for the samples.

Stock Solution Stability

The working solution (150 ng mL^{-1}) of montelukast was repeatedly (n = 3) injected into the chromatograph immediately after preparation (time 0) and at 3, 6, and 9 h after bench top storage at room temperature and 4°C. This injection protocol was repeated after 1, 3, 8, 11, 15, 22, 25, and 30 days of storage of this solution between 4 and 8°C.

Bioequivalence Study

The method was applied to evaluate the pharmacokinetic of montelukast in 31 healthy volunteers (mean age, 29.2 ± 7.3 years; weight, 76.2 ± 8.7 kg; height, 174 ± 7 cm; and body mass index, 25.2 ± 2.1) [25]. Each volunteer was orally administered 5 mg montelukast (Singulair®; Merck Sharp & Dohme Ltd., Northumberland, England or the test product) with 240 mL of drinking water. Blood samples were collected in heparinized tubes before 0.50 h dosing and at 0.33, 0.66, 1.00, 1.33, 1.66, 2.00, 2.33, 2.66, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, and 24.00 h post-dosing and centrifuged to obtain the plasma fraction. The plasma samples were kept in cryogenic vial stored at −70°C until analysis. Test and reference formulations were administered to the same human volunteers under fasting conditions separately with proper washing period as per protocol. The pharmacokinetic parameters for each volunteer were evaluated from the plasma concentration–time profile calculated by non-compartment analysis using Kinetica™ 2000 software (version 4.1; InnaPhase Corporation, Philadelphia, USA). This study was approved by the Ethics Committee before obtaining informed written consent from all volunteers (JCPR, Amman, data on file).

Results and Discussion

Separation and Specificity

Figure 2 shows the representative chromatograms of (a) blank plasma, (b) blank plasma with internal standard, (c) plasma spiked with montelukast at 300 ng mL^{-1}, (d) at 5 ng mL^{-1} (LLOQ), and (e) plasma sample from volunteer 3 h after oral administration of 5 mg dose of montelukast. The analytes were well separated from co-extracted material under the present chromatographic conditions at retention times of ~1.5 and ~2.3 min for montelukast

and candesartan cilexetil, respectively. The total run time was 3 min. The peaks were of good shape and completely resolved one from another. The chromatogram of extracted plasma samples did not show any co-eluting interference peak with the analyte or IS. There were no interfering peaks present in six different randomly selected samples of drug-free human plasma used for analysis at the retention time of either analyte or IS. Several compounds (aspirin, paracetamol, caffeine, mefenamic acid, ibuprofen, and

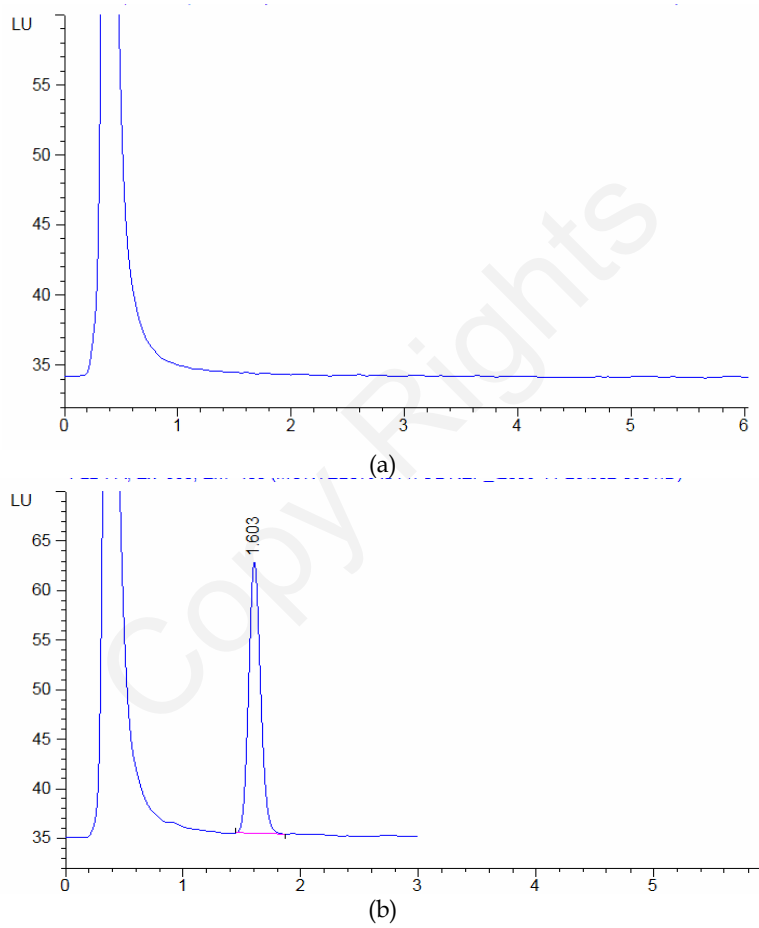
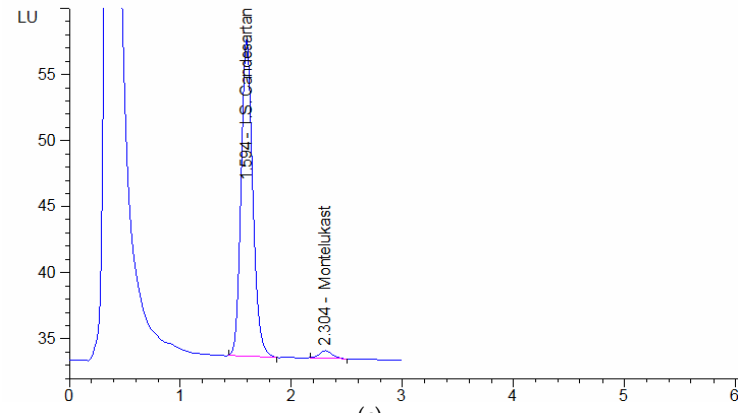


Fig. 2. Chromatograph resulting from (a) analysis of blank human plasma, (b) human plasma spiked with 0.6 μg mL^{-1} candesartan cilexetil (IS), (c) human plasma spiked with 5 ng mL^{-1} of montelukast and 0.6 μg mL^{-1} IS, (d) human plasma spiked with 300 ng mL^{-1} of montelukast and 0.6 μg mL^{-1} IS, and (e) plasma sample from a volunteer 3 h after oral administration of 5 mg dose of montelukast



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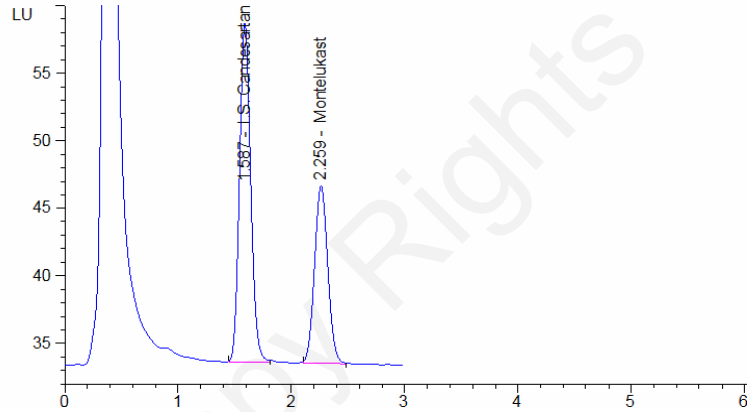

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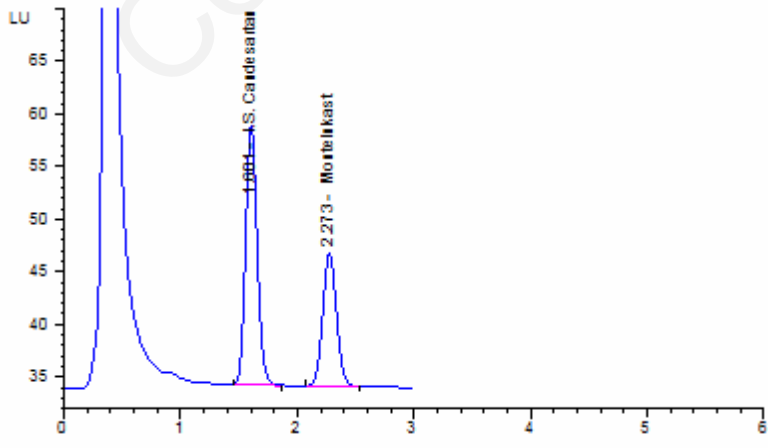

(e)

Fig. 2. (continued)

nicotinamide.) did not produce any interference with the drug or the internal standard during analysis.

Sensitivity

The limits of detection and quantification were 1 and 5 ng mL^{-1}, respectively. The precision and relative errors for LLOQ were 9.4% and 2.4%, respectively.

Linearity

Calibration curves were linear over the concentrations range from 5 to 300 ng mL^{-1} for montelukast (r = 0.999 or better; *Table I*) with coefficients of variation between 1.75% and 9.38%. The best-fit calibration curve were achieved with linear equation $y = mx + c$ with x as the weighting factor. The mean linear equation of calibration curve for the analyte was y = 0.00569(±0.0025)x + 0.00300 (±0.00508), where y was the peak area ratio of the analyte to the IS, and x was the concentration of the analyte. Precision and relative error of back calculated concentrations of standard solutions for montelukast are mentioned in *Table II*.

Table I. Representative calibration curve for HPLC assay of montelukast in plasma[a]

Calibration curve	Slope	y Intercept	r^2
Day 1 (n = 5)	0.005858 ± 0.00012	0.00491 ± 0.00680	0.99963
Day 2 (n = 5)	0.005845 ± 0.00008	−0.00014 ± 0.00399	0.99970
Day 3 (n = 5)	0.005373 ± 0.00007	0.00424 ± 0.00301	0.99954

[a]Seven non-zero calibration standards were included in each calibration curve.

Table II. Statistical evaluation of the analysis results for montelukast in standard curves

Concentration added (ng mL^{-1})	Concentration found (mean ± SD, n = 15) (ng mL^{-1})	Precision (%)	Bias (%)
5.0	5.12 ± 0.48	9.38	2.40
10.0	9.60 ± 0.76	7.91	−3.95
20.0	19.16 ± 1.17	6.09	−4.21
50.0	48.61 ± 2.59	5.33	−2.79
100.0	103.00 ± 3.88	3.76	3.00
200.0	200.67 ± 5.16	2.57	0.33
300.0	298.96 ± 5.24	1.75	−0.35

Recovery

Recovery from plasma was calculated by comparing the peak areas of pure standards prepared in acetonitrile–water (70:30 *v*/*v*) and injected directly into the analytical column with those of precipitated plasma samples containing the same amount of the test compound (n = 6 each). Mean recoveries of montelukast ranged from 87.3% to 95.3% with coefficients of variation 0.20–2.82% at three different concentration ranges for montelukast (15, 150, and 250 ng mL^{-1}). The mean recovery of IS was 87.7% *(Table III)*.

Table III. Extraction recovery of montelukast and candesartan cilexetil from plasma

Analyte	Concentration (ng mL^{-1})	Concentration found (mean ± SD) (ng mL^{-1})	% recovery (mean ± SD)	Mean recovery
Montelukast (n =5)	15.0	13.09 ± 0.27	87.3 ± 1.8	91.8 ± 3.8
	150.0	139.47 ± 3.94	92.9 ± 2.6	
	250.0	238.32 ± 0.49	95.3 ± 0.2	
Candesartan cilexetil (n = 5)	360.0	315.66 ± 7.52	–	87.7 ± 2.1

Accuracy and Precision

Within- and between-day precisions and accuracy were evaluated with different concentrations of montelukast. Within- and between-day precisions (% CV) were less than 2.5% and 7.9%, respectively. Within- and between-day relative errors (bias, %) were less than 3.4% and 4.6%, respectively *(Table IV)*. Accuracy was expressed as percent error (relative error) [(measured

Table IV. Accuracy and precision of the HPLC method for determining montelukast concentrations in plasma samples

Concentration added (ng mL^{-1})	Within-batch precision (n = 10)		
	Concentration found (mean ± SD) (ng mL^{-1})	Precision (%)	Bias (%)
5.0 (LLOQ)	5.17 ± 0.08	1.48	3.33
15.0 (Low)	15.02 ± 0.37	2.46	0.14
150.0 (Med)	152.09 ± 1.12	0.74	1.36
250.0 (High)	250.72 ± 4.05	1.61	0.29
	Between-batch precision (n = 30)		
Concentration added (ng mL^{-1})	Concentration found (mean ± SD) (ng mL^{-1})	Precision (%)	Bias (%)
5.0 (LLOQ)	5.23 ± 0.41	7.87	4.60
15.0 (Low)	15.01 ± 0.69	4.62	0.08
150.0 (Med)	153.17 ± 2.52	1.64	2.11
250.0 (High)	252.88 ± 5.29	2.09	1.15

concentration − spiked concentration) / spiked concentration] × 100 (%), whereas precision was quantified by calculating within- and between-day precisions (% CV).

Stability

Stability results are given in *Table V*. Twenty-four-hour room temperature storage and freeze–thaw cycles for low, mid, and high QC samples indicated that montelukast was stable in human plasma under experimental conditions. QC samples were stable for at least 30 days, which are frozen at approximately −70°C. Results from auto-injector stability indicate that the montelukast is stable when kept in the auto-sampler for up to 24 h at 5 ± 1°C.

Table V. Stability of the samples

Sample concentration (ng mL^{-1})	Concentration found (mean ± SD) (ng mL^{-1})	Precision (%)	Bias (%)
Short-term stability for 12 h (n = 3) in plasma			
15	15.97 ± 0.69	4.31	6.50
150	153.83 ± 0.65	0.43	2.55
250	258.98 ± 2.58	1.01	3.60
Three freeze and thaw cycles (n = 3)			
15	14.28 ± 0.76	5.30	−4.75
150	151.84 ± 7.68	5.05	1.22
250	248.97 ± 9.99	4.01	−0.41
Auto-sampler stability for 24 h (n = 3), at 5 ± 1 °C			
15	14.44 ± 0.18	1.30	−3.73
150	153.48 ± 5.87	3.82	2.32
250	253.85± 7.17	2.82	1.54
30 Days stability at −70 °C (n = 3)			
15	14.21 ± 0.50	3.52	–5.23
150	152.80 ± 5.13	3.36	1.87
250	245.44 ± 3.18	1.29	−1.83

Dilution Integrity

The dilution integrity was also conducted to assess whether upper concentration limit (300 ng mL^{-1}) can be extended or not. QC sample (in six repli-

cates) at a concentration of 500 ng mL^{-1} was diluted two times with blank plasma, and the assay, precision, and accuracy were determined as described earlier. For montelukast, the concentration found was 505.8 ± 4.4 (ng mL^{-1}), and the bias was 1.2%. The result indicated that samples whose concentrations were greater than the upper limit of the standard curve could be re-analyzed by appropriate dilution.

Assay Application

The described assay has been successfully employed without any interference to quantitate montelukast in human plasma samples obtained from the volunteers (n = 31), following the administration of single 5-mg dose of the drug (complete data on file, JCPR, Amman). The analyses were accomplished in accordance with the bio-analytical method validation guidance [24]. The representative chromatogram of plasma sample is shown in *Fig. 2e*. Montelukast concentration appeared to be comparable between montelukast test and reference formulation over the 24-h period (*Fig. 3*). The pharmacokinetic parameters were calculated using Kinetica™ 2000 software (version 4.1; InnaPhase Corporation, Philadelphia, USA) and are summarized in *Table VI*. The C_{max} (mean ± SD) for test and reference formulations are 232.54 ± 51.80 ng mL^{-1} and 268.20 ± 48.52 ng mL^{-1}, respectively, and

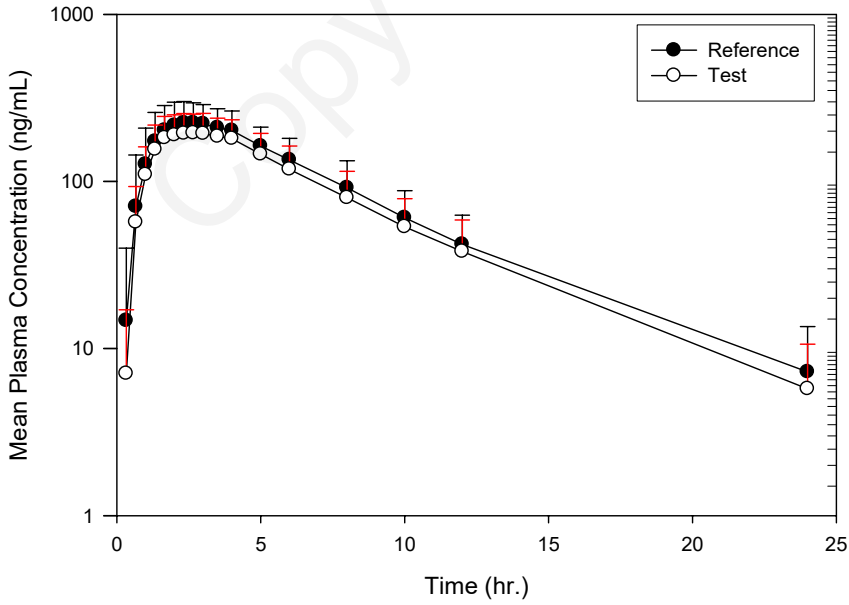

Fig. 3. Mean montelukast plasma concentration–time profile after administration of single oral dose of 5 mg tablet of montelukast of test and reference formulations in human volunteers (n = 31)

Table VI. Mean pharmacokinetic parameters of montelukast in healthy human volunteers (n = 31) after oral administration of 5 mg of test and reference products

Pharmacokinetic parameters	Reference	Test	90% CI	T/R (%)
	Mean ± SD	Mean ± SD		
C_{max} (ng mL^{-1})	268.20 ± 48.52	232.54 ± 51.80	81.0 – 91.2	86.0
AUC_{0-t} (ng h mL^{-1})	1706.13 ± 513.79	1480.47 ± 446.20	81.3 – 93.1	87.0
$AUC_{0-\infty}$ (ng h mL^{-1})	1804.12 ± 539.85	1560.16 ± 444.36	81.4 – 92.9	87.0
T_{max} (h)	2.72 ± 1.23	2.83 ± 1.13	–	–
$T_{½}$ (h)	4.13 ± 1.08	4.07 ± 0.90	–	–
K_{el} (h^{-1})	0.18 ± 0.06	0.18 ± 0.05	–	–

were attained at mean T_{max} of 2.83 and 2.72 h, respectively. There were no significant differences (p > 0.05) between formulations in any of the pharmacokinetic parameters measured, including those used for the evaluation of bioequivalence (AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}). The 90% CI of log transformed AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} of these two formulations were 81.3–93.1%, 81.4–92.9%, and 81.0–91.2%, respectively, which was accepted by the equivalent interval 80–125% [25].

Conclusion

A simple, sensitive, fast, accurate, precise, and economical HPLC method for the quantitation of montelukast in human plasma with dilution integrity has been developed and validated as per guideline [24]. The previously reported methods required large volume of sample for processing and large volume of organic phase. With the use of candesartan cilexetil as appropriate internal standard and BDS Hypersil C-18 column, the total chromatographic run time was reduced to 3 min, which makes it an attractive procedure in high-throughput bioanalysis of montelukast. Plasma sample used is less (0.2 mL instead of 0.5 mL), so the volume of sample per time point from an individual is reduced substantially. Organic waste is lowered by 50% due to short run time and flow rate, this makes it an economical procedure for pharmacokinetic and bioequivalence studies [23]. Tailing factor (1.08) and baseline noise are reduced without affecting the baseline separation of internal standard and the drug. The validated HPLC method described for the estimation of montelukast is successfully applied to bioequivalence studies. The results indicated that it has acceptable precision and adequate sensitivity for the determination of drug in bioequivalence or

pharmacokinetic study. The 90% CI of AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} of these formulations fell within the acceptable bioequivalence range. No statistically significant ($p > 0.05$) differences in pharmacokinetic parameters were found between formulations, treatments, and periods.

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