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Pharmacokinetic Analysis of Montelukast in Healthy Korean Volunteers by High Performance Liquid Chromatography-Tandem Mass Spectrometry

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ABSTRACT − A rapid and specific high performance liquid chromatography-tandem mass (LC/MS/MS) method for the analysis of montelukast in human plasma has been developed and validated. After cold acetonitrile-induced precipitation of the plasma samples, montelukast and glipizide (internal standard, IS) were eluted on a reverse-phase C_{18} column by isocratic mobile phase consisted of 10 mM ammonium formate buffer (adjusted to pH 3.5 with formic acid) and acetonitrile (3:97, v/v). Acquisition was performed with multiple reaction monitoring (MRM) mode by monitoring the transitions: *m/z* 587.2→ 423.2 for montelukast and *m/z* 446.0→321.2 for IS. Ranges of concentration for calibration curves (10-1000 ng/mL) showed correlation coefficients (r^2) were better than 0.9948. Precision of intra- and inter-day ranged from 3.70 to 11.68% and from 3.04 to 12.95%, accuracy of intra-day and inter-day ranged from 93.34 to 102.75% and from 100.79 to 107.63%, respectively. The described method provides a fast and sensitive analytical tool for determining montelukast levels in plasma, and was successfully applied to a pharmacokinetic study in 16 healthy human subjects after oral administration of 10mg tablet formulation of montelukast sodium under fasting conditions.

Key words − Montelukast, Pharmacokinetic study, Human plasma, LC/MS/MS, Korean

Montelukast, (S, E)-2-(1-((1-(3-(2-(7-chloroquinolin-2-yl) vinyl) phenyl)-3-(2-(2-hydroxy propan-2-yl) phenyl) propylthio) methyl) cyclopropyl) acetic acid, is antihistamic drug as a leukotrien receptor antagonist for using treatment of seasonal allergy and chronic asthma (Mastalerz et al., 2010; Castro-Rodriguez et al., 2010; Yasar et al., 2011; Schäper et al., 2011).

Several groups have reported methods for the determination of montelukast in biological matrices. These methods include chromatographic assay (Amin et al., 1995; Liu et al., 1997; Al-Rawithi 2001), usage of dual column (Smith et al., 2004), column switching (Ochiai et al., 1998), spectrofluorometry (Alsarra et al., 2005) or mass spectrometry (Challa et al., 2010; Sripalakit et al., 2008). However, most of these methods required laborious sample pretreatment or derivatization, and long chromatographic run times, and thus, are not convenient for analyzing the large numbers of samples generated during pharmacokinetic research. Thus, in the present study, our objective was to develop a rapid, sensitive, high throughput method for the routine determination of montelukast in human plasma.

Pharmacokinetic studies of montelukast were performed in various laboratories. In case of oral administration of 10 mg film-coated tablet, it was reported the $T_{max,}$ $AUC_{0\text{-}t}$ and C_{max} was 3.7 ± 0.8 hr, 2441 ± 441 ng·hr/mL and 385 ± 85 ng/mL, respectively (Cheng et al., 1996). Challa BR et al. reported pharmacokinetic research in 31 healthy Indians volunteers after oral administration of 5 mg montelukast; $AUC_{0\text{-}t}$, C_{max} and T_{max} are decided to be 2417.26 ± 63.58 ng·hr/mL, 369.29 ± 137.35 ng/mL and 2.67 hr, respectively. Meanwhile other group (Sripalakit et al. 2008) investigated method for pharmacokinetic study of 10 mg montelukast to 48 healthy Thai male volunteers. $AUC_{0\text{-}t}$, C_{max} and T_{max} are presented each of 3712.41 ± 1020.29 ng·hr/mL, 535.73 ± 156.81 ng/mL and 3.61 ± 1.25 hr, respectively. Pharmacokinetic data for montelukast 10 mg chewable tablet (Bharathi et al., 2009) were also found similar to those of tablet formation in human plasma; C_{max} (325 ± 134.4 ng/mL) was achieved at 3.50 ± 0.00 hr and the $AUC_{0-\infty}$ was observed 3018.83 ± 845.0 ng·hr/mL.

Although pharmacokinetic studies of montelukast have been reported in various races, there has not been reported in the Korean populations. The purpose of the present study was designed to assess a pharmacokinetic study of montelukast in 16 healthy Korean volunteers who were administrated a 10 mg tablet of montelukast sodium.

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Materials and Methods

Materials and reagents

Montelukast sodium (99.8% purity) was supplied from Daewoong Pharm. Co., Ltd. (Kyunggi-Do, Korea) and glipizide (internal standard, IS) was purchased Sigma-Aldrich (St.Louis, MO, USA), respectively. The structural formulas of montelukast (Figure 1A) and IS (Figure 1B) are shown. Ammonium formate and formic acid were also purchased from Sigma-Aldrich. Acetonitrile was obtained from J.T. Baker (Phillipsburg, USA). Water was obtained from aquaMAX™ ultra water purification system (YOUNGLIN, Kyunggi-Do, Korea). All other chemicals and reagents were of the highest analytical grade.

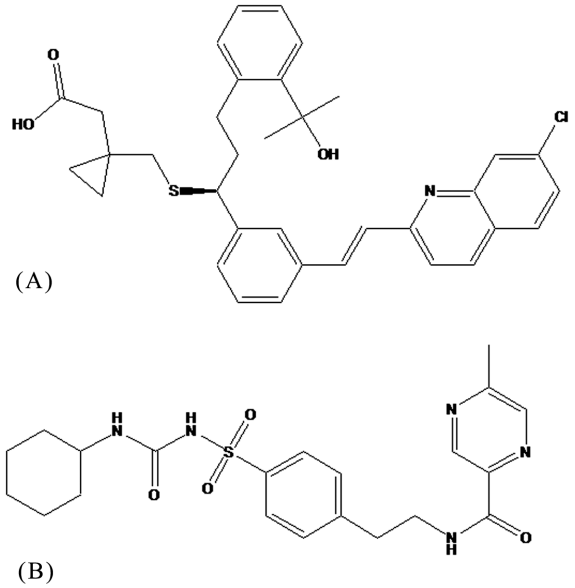

Figure 1. Chemical Structures of montelukast (A) and glipizide (B).

LC/MS/MS condition for Montelukast in plasma samples

Chromatographic conditions

HPLC system was consisted of Agilent 1200 series model (Agilent Technologies, Waldbronn, Germany). LC separation was performed on Phenomenex luna C_{18} column (50×2.0 mm, 3 μm) at 35°C with a mobile phase consisting of acetonitrile : ammonium formate buffer (adjusted to pH 3.5 with formic acid) = 97 : 3 (v/v), at a flow rate of 0.2 mL/min. The solution filtered using 0.22 μm pore membrane filter and ultrasonically degassed prior to use.

Mass spectrometric conditions

Mass spectrometric detection was performed on API 4000 triple quadrupole mass spectrometer (Applied Biosystems MDS Sciex, Toronto, Canada) equipped in electro-spray ionization (ESI) positive ion mode. Injection for mass conditions was carried out using a solution of montelukast and IS, delivered at a constant flow-rate of 0.2 mL/min. The nebulizer and TurboIonSpray gases (nitrogen) were set at a value of 40 and 50 psi, respectively. The optimized TurboIonSpray voltage and temperature were set at 5500 V and 550°C. Nitrogen was also used as curtain gas and collision gas, which were set at 14 and 6 psi. Quantification was performed using the multiple reaction monitoring (MRM) transition *m/z* 587.2 → *m/z* 423.2 for montelukast, *m/z* 446.0 → *m/z* 321.2 for IS, respectively (Figure 2). The optimized collision energy (CE) of 37 V was used for montelukast and 25 V for IS. The declustering potential (DP) was set at 57 V and 70 V for montelukast and IS, respectively. Analyst software (version 1.5) from Applied Biosystems MDS Sciex was used for data acquisition and handling.

Preparation of calibration standard and sample preparation

The primary stock solution of montelukast (1 mg/mL cal-

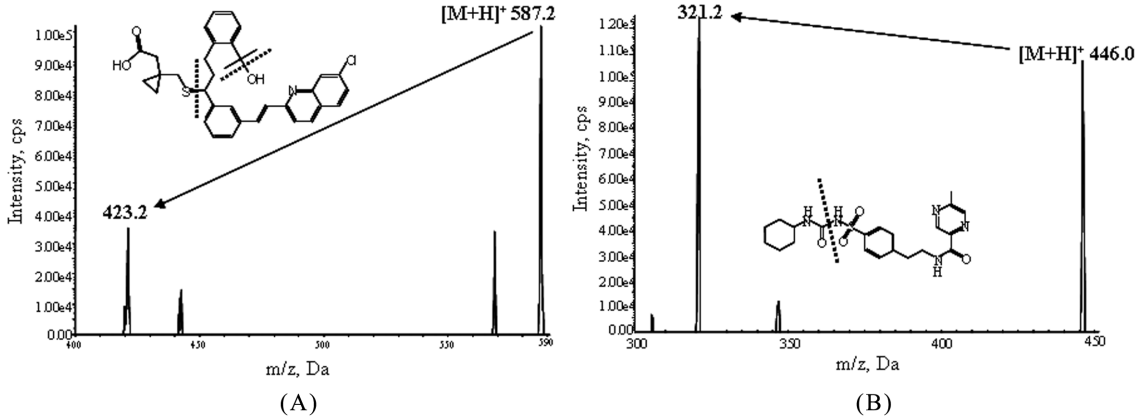

Figure 2. Product ion spectra of $[M+H]^+$ ions of montelukast (A) and IS (B).

culated as free base) was prepared in 100% acetonitrile. Standard stock solutions were diluted with acetonitrile to achieve concentration to 100, 200, 500, 1000, 5000 and 10000 ng/mL. IS primary stock solution was also prepared in dimethyl sulfoxide (1 mg/mL). IS stock solution was prepared to 3 ng/mL of solution diluted with acetonitrile. These were then stored at -20°C.

After thawing at room temperature and vortexing briefly, an aliquot of each sample (100 μL) was added 35 μL of IS (3 μg/mL) and 600 μL of cold acetonitlile. The mixture was vortex-mixed for 15 min and then centrifuged at 14000 rpm for 20 min at 4°C. Each of sample supernatant (150 μL) was filtered in syringe filter and then 5 μL aliquot of the sample was injected onto the analytic column for LC-MS/MS analysis.

Montelukast is generally known as a light-sensitive compound and showed instability when exposed to light leading to the formation of its *cis*-isomer as the major photoproduct; ethylene moiety of montelukast readily changes from *trans*-form to *cis*-form (Al Omari et al., 2007). Therefore, we performed all procedures on sodium lamp based on the reports of advantage of sodium lamp.

Method validation

The method was validated for selectivity, linearity, sensitivity, and precision, accuracy according to the Korean Food and Drug Administration (KFDA) guidelines (KFDA, 2003) for validation of bioanalytical methods.

Selectivity

The selectivity of the method towards endogenous plasma matrix components was assessed in blank human plasma. Blank plasma samples from six different volunteers were tested for the presence of endogenous compounds, which might interfere with analytes, using the proposed extraction procedure and chromatographic/spectroscopic conditions. And then, it is compared with those obtained with a solution of the analyte at a concentration near the LLOQ.

Calibration curve and linearity

A calibration curve was constructed from six calibration samples covering the whole range (10, 20, 50, 100, 500, 1000 ng/mL) by the peak area ratio of montelukast against IS. Concentration of montelukast was calculated from their area ratio with the calibration curve. The linearity of the calibration curve was also calculated. The calibration curve had to have a correlation coefficient (r^2) of 0.99 or better. The limit of detection (LOD) was determined at a signal-to-noise (S/N) ratio of 3, and the lowest standard on the calibration curve was to be accepted as the lower limit of quantitation (LLOQ), if the analyte response was at least 10 times more than that of drug free (blank) extracted plasma. In addition, the analyte peak of LLOQ sample should be identifiable, discrete, and reproducible with a precision (%, C.V.) not greater than 20.0 and accuracy within 80.0-120.0%.

Precision and accuracy

The intra- and inter-day assay precisions were determined as coefficient of variance (%, C.V.), and intra- and inter-day assay accuracies were expressed as percentages of the theoretical concentration, as accuracy (%) = (found concentration/theoretical concentration)×100. Intra-day assay was performed with five replicates on the same day and inter-day assay was accomplished on four separate days. The acceptance criterion recommended by KFDA for each back-calculated standard concentration was 15% deviation from the normal value except LLOQ, which was set at 20%.

Pharmacokinetic study

The proposed analytical method was applied to a pharmacokinetic study. A single oral dose of 10 mg of montelukast was given to 12 healthy male volunteers. Blood samples were collected in tubes containing heparin before and after 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12 and 24 hr of administration of drug. These were centrifuged at 3500rpm for 8 min to obtain plasma, and the supernatant plasma was put into micro-tube. These were then frozen at -70°C.

Pharmacokinetic parameters were calculated from plasma levels applying a non-compartmental statistic using BA calc 2002. Blood samples were drawn up to a period of three to five times the terminal elimination half-life ($t_{1/2}$). The highest concentration (C_{max}) and the time to reach the highest concentration (T_{max}) values were determined by visual inspection of the plasma montelukast concentration-time curve (AUC_{0-t}) obtained by the linear trapezoidal method in 0-24 hr.

Results and Discussion

MS optimization and quantification

MS/MS optimization was performed by directly infusing solutions of montelukast and glipizide (IS) into the electrospray injection (ESI) unit of a mass spectrometer at a constant flow-rate of 0.2 μL/min. Quadrupole full scans (Q1 scans) were carried out in positive ion detection mode in order to optimize ESI conditions. The mass spectra of montelukast and glipizide revealed base peaks at *m*/*z* 587.2 and 446.0, respectively, which corresponded to the protonated molecular ions.

The MS/MS condition parameters including collision gas, collision energy and collision cell entrance/exit potential were set to maximize the amounts of product ions produced. Product ion mass spectra and the fragmentation patterns of montelukast and glipizide are illustrated in Figure 2. The product ions of montelukast and glipizide were both at *m*/*z* 423.2 and *m*/*z* 321.2. The instrumental parameters used are mentioned in the experimental section.

Method validation

Selectivity

No visible interferences were observed in the chromatograms of six blank plasma samples. Figure 3A showed the chromatogram of a blank plasma sample with no endogenous peaks at the retention positions of montelukast or glipizide, which occurred at *ca.* 1.40 and 0.87 min, respectively. The total run times for samples were *ca.*2.5 min (Figure 3).

Calibration curve and linearity

A six-point calibration curve exhibited good linearity for LC/MS/MS in the concentration range 10-1000 ng/mL ng/mL. The linear regression equation of the analysis calibration curve was $y = 0.004393 (\pm 0.000065) x + 0.005013 (\pm 0.006571)$ ($r^2 = 0.9964 \pm 0.00223$). Where y peak area is the area ratio of montelukast to IS, x is the concentration ratio of montelukast to IS. The LLOQ for montelukast was proved to be 10 ng/mL.

Precision and accuracy

The precision calculated as the C.V. and the accuracy was always below 15%, other than near the LLOQ (below 20%) in

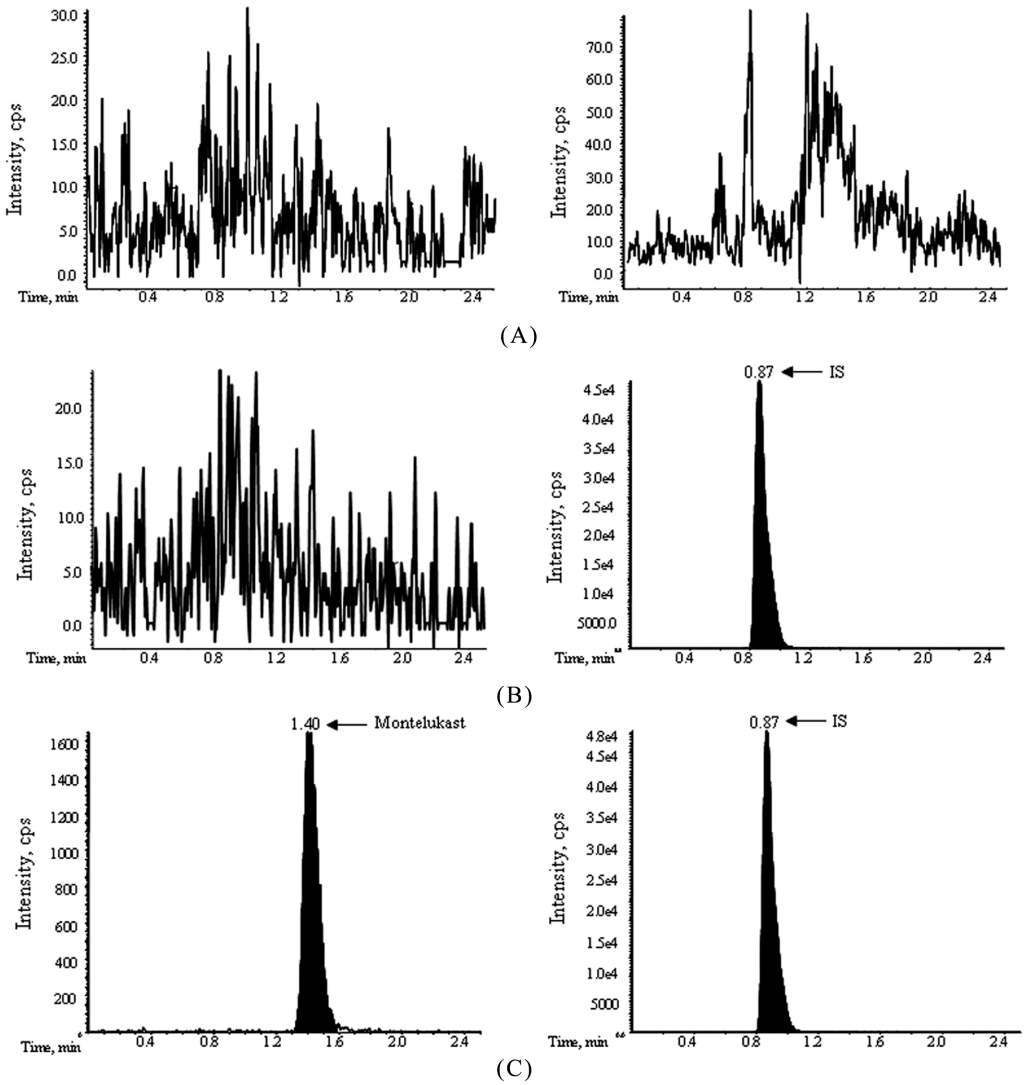

Figure 3. Chromatograms of the plasma blank (A), plasma blank spiked IS (B), plasma the lower limit of quantification (C).

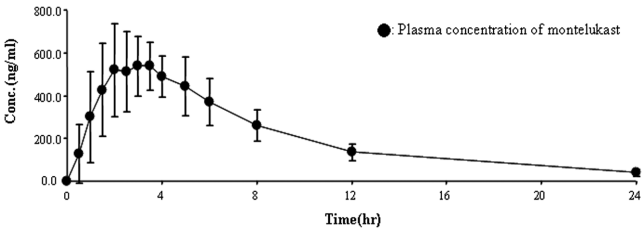

Figure 4. Mean (±S.D, n=16) plasma concentration-time curves of montelukast following oral administration of tablet (montelukast sodium 10 mg).

Table I. *Precision and accuracy for the determination of montelukast in human plasma*

Concentration (ng/mL)	Precision (C.V. %)		Accuracy (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
10.00	12.95	3.70	100.79	96.68
50.00	9.02	11.68	103.64	93.34
500.00	3.04	9.04	107.63	102.75
1000.00	7.38	6.52	104.69	98.26

the concentration range from 10 to 1000 ng/mL. Intra-day C.V.s of the method for montelukast ranged from 3.04% to 12.95%, while its accuracy ranged from 93.34% to 102.75%. Inter-day C.V.s ranged from 3.70% to 11.68%, and accuracies from 100.79% to 107.63% (Table I). These results satisfy KFDA criteria and confirm the reproducibility of this method. These results enable us to conclude that the devise method is well suited for routine high-throughput analyses and pharmacokinetic studies.

Pharmacokinetic study

The validated method was successfully applied for the determination of montelukast in 16 healthy male volunteers that orally administered 10 mg of montelukast in tablet form. Fig. 4 shows a plasma concentration vs. time profile plot for montelukast in human subjects under fasting conditions. The method was sensitive enough to monitor montelukast concentrations for 24 hr. Approximately 350 samples including calibration, QC standards and volunteer samples were run and analyzed over 2 days, and the precisions and accuracies obtained for calibration and QC samples were well within acceptable limits. Percentage differences during sample reanalysis testing for assay reproducibility were less than 15%. Mean area under the plasma-concentration versus time curve from 0 to 24 hr (AUC_{0-24}) after administration was 4910 ± 916.391 ng·hr/mL, and the mean area under the plasma-concentration curve from time 0 to infinity (AUC_{0-inf}) was 5250.316 ± 1012.806 ng·h/mL. The observed mean maximum

Table II. *Pharmacokinetic parameters obtained from the time-plasma concentration of montelukast after oral administration of 10 mg montelukast sodium in 16 Korean male volunteers*

Subjuct	Pharmacokinetics parameters				
	AUC_{0-t}	AUC_{0-inf}	C_{max}	T_{max}	$T_{1/2}$
1	4637.895	5083.587	494.9	2	6.4
2	5478.815	5652.399	980.2	1.5	4.8
3	3658.415	3883.2	688.5	2	5.8
4	6174.715	6832.171	612.9	4	6.3
5	4522.650	4643.801	641.3	2.5	4.1
6	5370.525	5895.324	557.0	1.5	6.8
7	3688.260	4022.515	380.4	4	6.4
8	3570.130	3714.309	496.7	2.5	4.6
9	5780.995	6293.051	778.4	2.5	6.5
10	5775.345	6181.728	841.8	2.5	6.2
11	4383.190	4711.297	569.3	2	5.8
12	4749.570	5050.893	697.8	2.5	5.9
13	5737.960	6255.163	767.0	5	6.5
14	5638.165	5769.146	702.4	3.5	4.1
15	5766.150	6124.913	767.9	3.5	5.1
16	3635.815	3891.557	748.9	1.5	3.0
Mean	4910.537	5250.316	670.3	2.7	5.5
S.D	916.391	1012.806	149.8	1.0	1.1

plasma concentration (C_{max}) was 670.3 ± 149.8 ng/mL, mean T_{max} was 2.7 ± 1.0 hr, and the half-life of montelukast during the terminal phase ($T_{1/2}$) was 5.5 ± 1.1 hr (Table II). Pharmacokinetic studies of montelukast have been reported in healthy human (Challa BR et al., 2010; Sripalakit P et al., 2008; Cheng H et al., 1996; Bharathi DV et al., 2009). Among the data, pharmacokinetic parameters are shown to similar property considering different administration dose. Although our study was determined somewhat higher AUC and C_{max}, a little difference in parameters between research groups may be made from the variation due to the method of determining drug concentrations, and individual variation; race, gastrointestinal motility or difference in metabolism. Therefore we could not find the significant difference in pharmacokinetic parameters of montelukast between races by the comparison of these parameters.

Conclusions

In this study, we have developed and validated more effective, rapid and simple method for determination montelukast in human plasma using LC-MS/MS. The devised method was

also found to be satisfactory in terms of its selectivity, sensitivity, and reproducibility, and was successfully used in a pharmacokinetic study of montelukast administered orally as a single 10 mg tablet in healthy Korean. This method may be useful for the pharmacokinetics and bioequivalence study of montelukast.

References

Challa, B.R., Awen B.Z., Chandu, B.R., Khagga, M., Kotthapalli, CB., 2010. Method development and validation of montelukast in human plasma by HPLC coupled with ESI-MS/MS: Application to a bioequivalence study. Sci Pharm. 78(3), 411-422.

Sripalakit, P., Kongthong, B., Saraphanchotiwitthaya, A., 2008. A simple bioanalytical assay for determination of montelukast in human plasma: application to a pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci. 869(1-2), 38-44.

Mastalerz, L., Kumik, J., 2010. Antileukotriene drugs in the treatment of asthma. Pol Arch Med Wewn. 120(3), 103-108.

Castro-Rodriguez, J.A., Rodrigo, GJ., 2010. The role of inhaled corticosteroids and montelukast in children with mild-moderate asthma: results of a systematic review with meta-analysis. Arch Dis Child. 95(5), 365-370.

Yasar, H., Kiran, B., Cagatay, T., Ozkul, H., Icten, S., 2011. The effect of montelukast sodium on serum arginase levels in patients with seasonal allergic rhinitis. Am J Rhinol Allergy.

Schäper, C., Noga, O., Koch, B., Ewert, R., Felix, S.B., Gläser S., Kunkel, G., Gustavus B., 2011. Anti-inflammatory properties of montelukast, a leukotriene receptor antagonist in patients with asthma and nasal polyposis. J Investig Allergol Clin Immunol. 21(1), 51-58.

Amin, R.D., Cheng, H., Rogers, J.D., 1995. Determination of MK-0476 in human plasma by liquid chromatography. J Pharm Biomed Anal. 13(2), 155-158.

Liu, L., Cheng, H., Zhao, J.J., Rogers, J.D., 1997. Determination of montelukast (MK-0476) and its S-enantiomer in human plasma by stereoselective high-performance liquid chromatography with column-switching. J Pharm Biomed Anal. 15(5), 631-638.

Al-Rawithi, S., Al-Gazlan, S., Al-Ahmadi, W., Alshowaier, IA., Yusuf, A., Raines, D.A., 2011. Expedient liquid chromatographic method with fluorescence detection for montelukast sodium in micro-samples of plasma. J Chromatogr B Biomed Sci. 754(2), 527-531.

Smith, G.A., Rawls, C.M., Kunka, R.L., 2004. An automated method for the determination of montelukast in human plasma using dual-column HPLC analysis and peak height summation of the parent compound and its photodegradation product. Pharm Res. 21(9), 1539-1544.

Bharathi, D.V., Hotha, K.K., Jagadeesh, B., Mullangi, R., Naidu, A., 2009. Quantification of montelukast, a selective cysteinyl leukotriene receptor (CysLT1) antagonist in human plasma by liquid chromatography-mass spectrometry: validation and its application to a human pharmacokinetic study. Biomed Chromatogr. 23(8), 804-810.

Cheng, H., Leff, J.A., Amin, R., Gertz, B.J., De Smet, M., Noonan, N., Rogers, J.D., Malbecq, W., Meisner, D. Somers, G., 1996. Pharmacokinetics, bioavailability, and safety of montelukast sodium (MK-0476) in healthy males and females. Pharmaceutical Research. 13, 445-448.

Ochiai, H., Uchiyama, N., Takano, T., Hara, K., Kamei, T., 1998. Determination of montelukast sodium in human plasma by column-switching high-performance liquid chromatography with fluorescence detection. J Chromatogr B Biomed Sci Appl. 713(2), 409-414.

Alsarra, I., Khalil, NY., Sultan, M., Al-Ashban, R., Belal, F., 2005. Spectrofluorometric determination of montelukast in dosage forms and spiked human plasma. Pharmazie. 60(11), 823-826.

Al Omari, M.M., Zoubi, R.M., Hasan, E.I., Khader, T.Z., Badwan, A.A., 2007. Effect of light and heat on the stability of montelukast in solution and in its solid state. J Pharm Biomed Anal. 45(3), 465-471.

Korea FDA, 2003 Guideline for the Validation of Bioanalytical Method 2003.05.