IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 4 Ver. VII (Jul – Aug 2017), PP 01-08
www.iosrjournals.org

Development and validation of simple and rapid LC-MS/MS method for ezetimibe in human plasma and its application to bioequivalence study

Yong Sun Park[a,*], Dong Pil Kim[b], Suk Hong Kang[b], Ki Tak Kim[b], Yong Hwan Kim[b], Jong Hoon Kim[b], Ye Na Kim[a], Jin Young Kim[a], Soo Ji Sim[a], Bo Ra Jin[a], Heon Soo Lee[a]

[a]*Drug Development Supporting Service Division, Bio-Medieng Co. Ltd., Seongnam-si, Gyeonggi-do, Korea*
[b]*Nexpharm Korea. Co. Ltd., Seoul, Korea*
Corresponding Author: YongSun Park

Abstract: *A sensitive and specific liquid chromatography combined with electrospray ionization (ESI) tandem mass spectrometry (LC-MS/MS) method, operating in the negativeionization mode, for quantifying of ezetimibe in human plasma using ezetimibe-d_4 as internal standard (IS) was developed and validated. The analyte and IS were extracted by simple one step liquid to liquid extraction (LLE). The chromatographic separation was performed on a Gemini C_{18} column (50 X 2.0 mm, 5 um) under isocratic conditions using a mixture of acetonitrile/0.1% formic acid (70:30, v/v) as mobile phase at a flow rate of 0.20 ml/min. Quantitation was performed using multiple reaction monitoring (MRM) mode to study parent → product ion transitions of m/z 408.0→270.8 for ezetimibe and m/z412.1→270.8 for IS, respectively. Linearity in plasma was obtained over the concentration range 0.1 ~ 20 ng/ml, with a coefficient of determination (r^2) of 0.9999. The method was has been successfully applied for routine assay to support pharmacokinetic study of ezetimibe in human plasma after an oral administration of 10 mg ezetimibe.*

Keywords: *Ezetimibe, Human plasma, LC–MS/MS, Pharmacokinetics*

Date of Submission: 31-07-2017 Date of acceptance: 17-08-2017

I. Introduction

The chemical name for ezetimibe is (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one, empirical formula is $C_{24}H_{21}F_2NO_3$ and its molecular weight is 409.4 (Fig. 1). The drug is a white, crystalline powder that is freely very soluble in ethanol, methanol, acetone and practically insoluble in water. Ezetimibe is the first of a new class of compounds, the 2-azetidinones, that effectively blocks intestinal absorption of dietary and biliary cholesterol and significantly increases total neutral sterol output and the efficiency of plasma cholesterol excretion into fecal neutral sterols [1-3]. Clinical trials showed that administration of ezetimibe at a dose of 10 mg once daily inhibits cholesterol absorption on average by 54-65% and results in approximately 18% reduction of plasma low-density lipoprotein cholesterol [4-5]. Thus ezetimibe is widely used in treatment of hypercholesterolemia and sitosterolemia [6-7].

Ezetimibe is primarily metabolized in the liver and the small intestine via glucuronide conjugation by UGT1A1 (with some involvement of UGT1A3 and UGT2B15), with subsequent renal and biliary excretion [8]. Both the parent compound and its active metabolite (ezetimibe glucuronide) are eliminated form plasma with a half-life of ezetimibe of ~22 hr, allowing once-daily dosing [9]. Importantly, ezetimibe has no effect on cytochrome P450 enzyme activity, suggesting a low potential for drug-drug interaction [9]. However, whether drug-drug interactions occur when ezetimibe is co-administrated with other glucuronidated drugs remains unclear.

(A) (B)

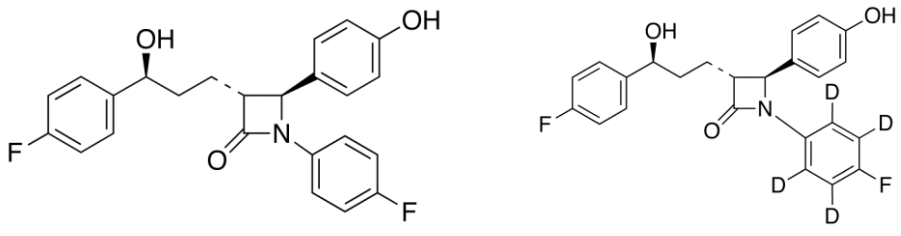


Fig.1. Structural representation of ezetimibe (A) and deuterated ezetimibe-d_4 (B).

Low blood concentrations are achieved following single oral administration of ezetimibe in normal subjects thus, to describe pharmacokinetics of the drug properly; its quantification method in single dose studies should be accurate and sensitive. Several methods including HPLC-UV have been reported for the determination of ezetimibe [10-12]. However, lack of sensitivity hindered the application of HPLC-UV on the pharmacokinetic study of ezetimibe. Furthermore, laborious and time-consuming procedures of sample preparation and long analysis time also restricted its application. Therefore, a highly sensitive, advanced high throughput LC-MS/MS method was developed and validatedfor the determination of ezetimibe in human plasma. This developed method offered higher sensitivity, simpler sample treatment procedure, smaller sample volume requirement and shorter run time of 2.5 min

II. Experimental

2.1. Materials and Instrument

Ezetimibe(100.3%) and ezetimibe-d_4(IS, 98.3%) were purchased from Nexpharm Korea(Korea) and CDN Isotopes Inc. (Canada), respectively.Acetonitrile (J.T. Baker, USA) was HPLC grade, and other chemicals were of analytical grade. All aqueous solutions including the bufferfor the mobile phase were prepared with Milli Q (Millipore, Milford, MA, USA) grade water. Drug-free plasma for the preparation of calibration standard was obtained from H-plus Yangjihospitalblood donor service (Seoul, Korea). Before analysis, the blank samples were analyzed by the present LC-MS/MS method. No significant peaks were observed at the retention times of the analyte and IS.

An Agilent 1200 system consisting of G1312A quaternary pump, G1379B degasser, G1367B auto-sampler, G1330B thermostat,G1316A column oven (TTC) compartment (Agilent, Waldbronn, Germany) was used for solvent and sample delivery. An API 4000 triple-quadrupole mass spectrometer equipped with a TurboIonSpray (ESI) source was used for mass analysis and detection (Applied Biosystems, Foster City, CA, USA). Data processing was performed on Analyst 1.6 software package.

2.2. Chromatographic and Mass Spectrometric Conditions

Isocratic chromatographic separation was achieved on a Gemini C_{18} column, 50mm X 2.0mm, i.d., 5 um (Phenomenex, USA). The mobile phase consisted of acetonitrile / 0.1% formic acid (70:30, v/v) at a flow rate of 0.20 ml/min. The column and auto-sampler temperature were maintained at 35℃ and 10℃, respectively.

The mass spectrometer was operated in negative ionization mode. The tuning parameters were optimized for ezetimibe and the IS by infusing a solution containing 500 ng/ml of both analytes at a flow rate of 10 ul/min into the mobile phase (0.20 ml/min) using an analyte column 'T' connection. Optimized instrument settings specific ezetimibe and IS were as follows: curtain gas was 20 psi, ion source gas 1 was 50 psi, ion source gas 2 was 50 psi, ionspray voltage was -4500 V, turbo heater temperature was 650℃. Quantitation was performed using multiple reaction monitoring (MRM) of the transitions *m/z*408.0→270.8 for ezetimibe and m/z 412.1→270.8 for the IS, respectively, with a dwell time of 150 ms per transition. The precursor ions of ezetimibe and IS were formed using declustering potentials of -75 and -75 V, respectively, and their precursor ions were fragmented at collision energies of -22 and -22 eV by collision-activated dissociation with nitrogen at a pressure setting of 5 (arbitrary units). Both quadrupoles were maintained at unit resolution.

2.3. Preparation of Calibration Standard and QC Samples

A stock solution of ezetimibe with a concentration of 1.0 mg/ml (calculated from purity) was prepared by dissolving 10.0mg of ezetimibe in 10 ml of methanol. Six standard working solutions of1, 5, 10, 50, 100 and 200ng/mlof ezetimibe were made by further dilution of the stock solution with 50% methanol. The quality control (QC) samples were similarly prepared at concentrations of 3, 40 and 160ng/ml, by a separate weighing of the pure standard. A 45ng/mlworking solution of the IS was also prepared by diluting the 0.5 mg/ml stocksolution of ezetimibe-d_4 with 50% methanol.

Matrix-matched calibration standards and QC samples of ezetimibe were prepared by spiking 20 ul of the working solutions into 180ul of drug-free plasma. The calibration standards were prepared at concentrations of0.1, 0.5, 1, 5, 10 and 20ng/mlof ezetimibe in plasma, while the corresponding QC samples were prepared at 0.3, 4 and 16ng/ml.

The stock solutions and standardworking solutionswere stored at 2 ~ 8℃. For each batch of unknown samples to be analyzed, the appropriate standard and QC working solutions were brought to room temperature, and processed through the plasma sample preparation procedure in parallel with the unknown samples.

2.4. Sample preparation

To 10 mlpolypropylene tube, 20 ul of the IS solution (ezetimibe-d_4, 45ng/ml)was added to 200 ul of plasma sampleandvortexed for 10 sec. Ezetimibe and IS were extracted with 3 ml of methyl tert-butyl ether for

10 min on vortex-mixer. The organic and aqueous layers were separated by centrifugation at 4000 rpm for 5 min. The organic layer was transferred to another clean tube and evaporated under nitrogen gas at 50℃ and the dry contents reconstituted with 200 ul of 50% acetonitrile and vortex mixed for 30 sec. The contents were finally transferred into appropriate auto-sampler vials and an aliquot (5 ul) was injected onto the LC-MS/MS for analysis.

2.5. Method validation

Plasma samples were quantified using the ratio of the peak area of analyte to IS as the assay response.The specificity of the method was determined by analyzing six different batches of human plasma as is, to demonstrate the lack of chromatographic interference from endogenous plasma components. Sets of spiked calibration curve (CC) standards and QC samples (n=4 at eachconcentration) were prepared and analyzed on three different occasionsto evaluate linearity, precision and accuracy. To evaluate linearity, plasma calibration curves were prepared and assayed on three consecutive days over the range of 0.1 ~ 20 ng/ml. Least-squares linear regression was used for curve fitting with $1/x^2$ as the weighting factor. For determining the intra-day precision and accuracy, a replicate analysis of plasma samples of ezetimibe in human plasma was performed on the same day. The run consisted of a CC and five replicates of each the lower limit of quantification (LLOQ), low, mid and high concentration QC samples. The inter-day precision and accuracy were assessed by analysis of three batches on different days. The precision was expressed as the coefficient of variation(CV %) and the accuracy as the relative error (R.E.%).The extraction recovery of the analytes from the plasma was evaluated by comparing the mean detector responses of three replicates of processed QC samples at low and high concentration to the detector responses of standardsolutions of same concentration. Endogenous matrix components may change the efficiency of droplet formation or droplet evaporation, which in turn affects the amount of charged ion in the gas phase that ultimately reaches the detector. Three set of samples were prepared by directly spiking the analytes into reconstitution solution and without the presence of residue extracted from blank plasma. Post-preparative stability, three aliquots each of low and high QC samples were stored at 10℃ in an auto-sampler for 23 hr, analyzed and the concentrations were compared with the actual values. Three aliquots of each low and high QC samples were kept in deep freezer at -70℃for 35 day. The samples were processed and analyzed and the concentrations obtained were compared with the actual value of QC samples to determine the long term stability of analyte in human plasma. Three aliquots each of low and high unprocessed QC samples were kept at ambient temperature (25℃) for 19 hr in order to establish the short term stability of the analytes. The stability of the analytes after three freeze and thaw cycles was determined at low and high QC samples. The samples were stored at -70℃ for 24 hr and thawed unassisted at room temperature. After completely thawing, the samples were refrozen for 12 ~ 24 hr. After three freeze-thaw cycles, the concentration of the samples were analyzed. Separate standard working solutions containing 3 ng/ml, 16ng/ml of ezetimibe and 45ng/ml of IS were prepared and stored at 25℃ and 2 ~ 8℃ for 20 hr and 21 day. The response obtained from the two drugs was calculated and compared with that of the freshly prepared solutions of the same concentration.

2.6. Pharmacokinetic study

The validated method was used to determine the plasma concentrations of ezetimibe from a clinical trial in which 35 healthy male volunteers received a single oral dosage of 10 mg ezetimibe. Eligible volunteers were Korea men aged 19 to 57 years (27.5 ±8.1) and the average body weight was 67.3 ± 7.0 kg. The study protocol was approved by the Human Investigation Ethics Committee of H-plus Yangji hospital, Seoul, Korea. Blood samples were collected into heparinized glass tubes before and 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 48 and 72 hr post-dosing, and centrifuged at 4000 rpm (4℃) for 10 min to separate the plasma fractions. The collected plasma samples were stored at -70℃until analysis.

Determination of the pharmacokinetic parameters was performed by non-compartmental assessment of data using the computer program K-BE Test 2007 (ver. 1.1.0, KFDA, Korea). Mean and individual concentration-time profiles were generated and used to determine the maximum plasma concentration (C_{max}) and the time to attain these maximum concentrations (T_{max}). The area under the plasma concentration-time curve from time zero to the time of the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The terminal elimination rate constant (ke) was estimated by log-linear regression of concentrations observed during the terminal phase of elimination.

III. Results and Discussion

3.1. Optimization of the mass spectrometric condition

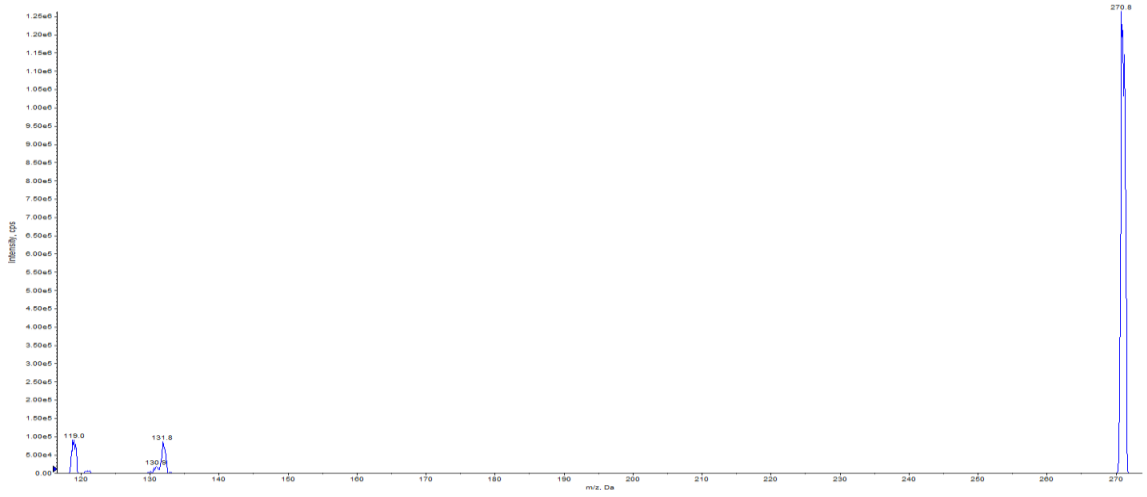
Mass parameters were tuned in both positive and negative ionization modes for theanalytes. Good response was achieved in negative ionization mode. By negative electrospray ionization (ESI) mode, the detector was operated at unit resolution in the multiple-reaction monitoring (MRM) mode using the transitions

of the protonated molecular ions of ezetimibe at *m/z* 408.0→270.8 and IS at *m/z* 412.1→270.8. Optimized parameters were as follows: curtain gas, gas 1 and gas 2 (nitrogen)20, 50 and 50 units, respectively; dwell time 150 ms; source temperature 650℃; ion spray voltage -4500 V. Declustering potential and collision energy were -75V and -22 eV for ezetimibeand -75V and -22 eV for IS, respectively.

3.2. Optimization of the chromatographic condition

In pursuit of symmetric peak shape and retention time of ~0.98 min, feasibility of various mixture(s) of solvents such as acetonitrile and methanol using different buffers such as ammonium acetate, ammonium formate and formic acid with variable pH range of 3.0 ~ 7.0, along with altered flow rates (in the range of 0.2 ~ 0.5 ml/min) were tested for complete chromatographic resolution ofezetimibe and IS (data not shown). The resolution of peaks was achieved with 0.1% formic acid and acetonitrile mixture (30:70, v/v) with a flow rate of 0.2 ml/min, on a Gemini C_{18} column and was found to be suitable for the determination of electrospray response for ezetimibe and IS.

(A) –MS2 (408.0) CE (-22)



(B) –MS2 (412.1) CE (-22)

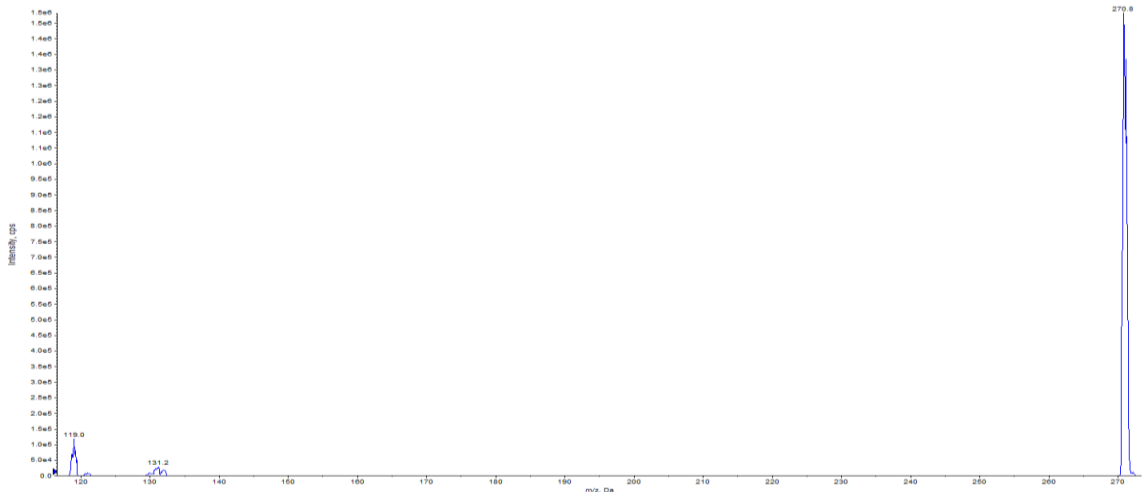


Fig. 2. Product ion spectra of $[M\text{-}M]^-$ of ezetimibe (A) and ezetimibe-d_4 (B).

3.3. Sample pre-treatment

In order to achieve cleanliness in extract,liquid to liquid extract (LLE)was optimized for extraction of analytes from plasma. The extraction solvent (methyl tert-butyl ether) gave consistent results in terms of recovery of ezetimibe and IS and also gave cleaner plasma blank samples.

3.4. Assay specificity

A typical chromatogram for the control human plasma (free of analyte and IS) and human plasma

spiked with ezetimibe at LLOQ are shown in Fig. 3, respectively. No interfering peaks from endogenous compounds are observed at the retention times of analytes and IS. The retention time of ezetimibe and IS was 0.98and 0.98min. The total chromatographic run time was 2.5 min.

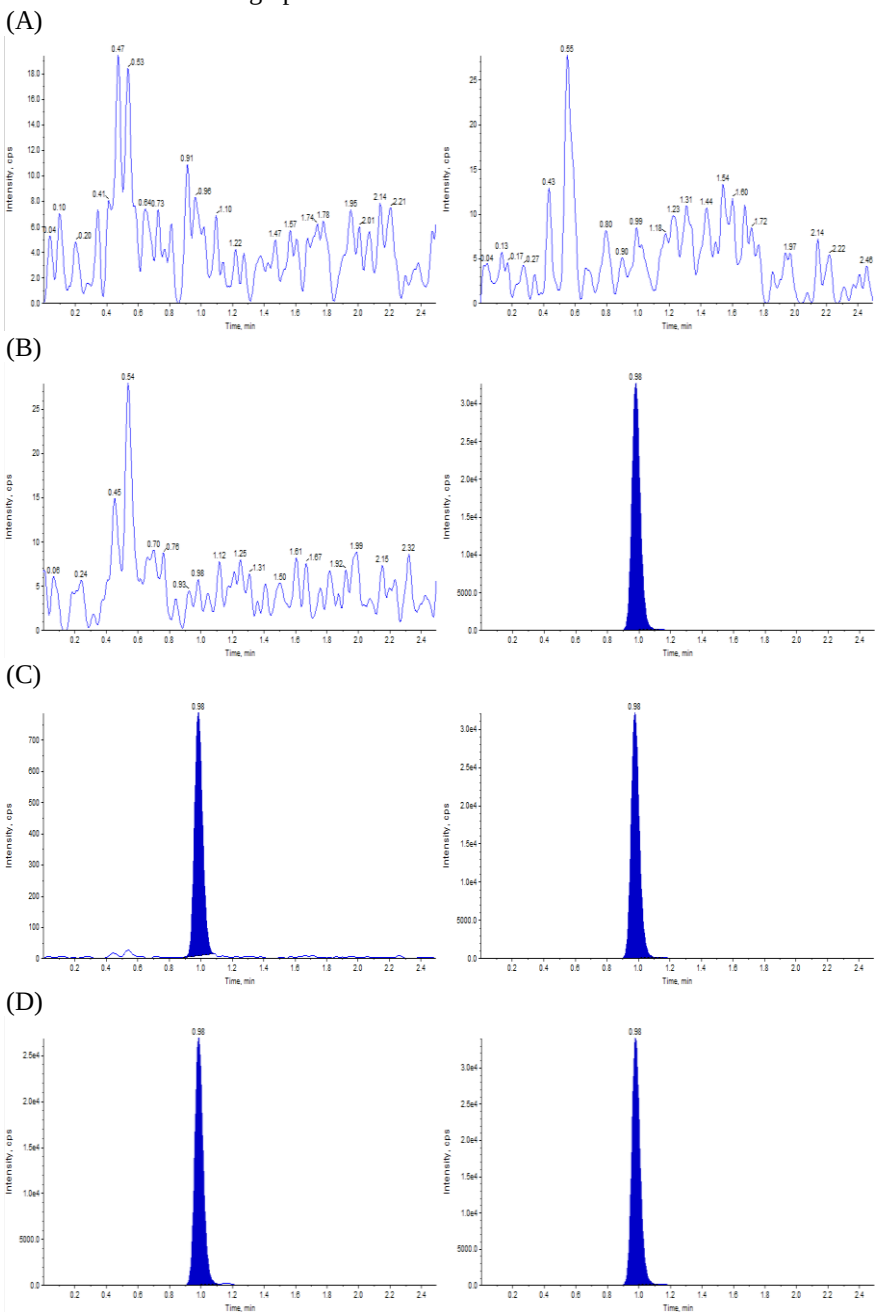


Fig.3. Typical MRM chromatograms of ezetimibe (left panel) and IS (right panel) in (A) human blank plasma, (B) human blank plasma spiked with IS, (C) human plasma spiked with ezetimibeat LLOQ (0.1ng/ml) and IS, (D) a representative chromatogram(5 hr)of extracted a volunteer.

3.5. Linearity and Lower limit of Quantification

The linear regression of the peak-area ratios versus concentrations was fitted over the concentration range of 0.1 ~ 20ng/ml in human plasma. A typical equation of the calibration curves was as follows: y=0.2351x +0.0109(r^2=0.9999), where y represents the peak-area ratio of analyte to IS and x represents the plasma concentration of ezetimibe. Good linearity was seen in this concentration range.

The lower limit of quantification was 0.1ng/ml for determination of ezetimibe in plasma. The precision and accuracy at the concentration of LLOQ are shown in Table 1.

3.6.Precision and Accuracy

The method showed good precision and accuracy. Table 1 summarizes the intra- and inter-day precision and accuracy for ezetimibe from QC samples. The intra-day precision (CV %) for QC samples (0.1, 0.3, 4, 16ng/ml) were 4.38%, 1.50%, 4.27% and 1.08%, respectively and that of inter-day analysis were4.06%, 3.32%, 2.76%, 1.69% with a relative errors (R.E. %) within 0.44% to 2.00%.

3.7. Recovery and Matrix Effect

The extraction recoveries of ezetimibe from human plasma were 96.32% (CV=4.01%),91.83% (CV=2.08%) and 85.23 (CV=1.04%) at concentration levels of 0.3, 4 and 16ng/ml, respectively, and the mean extraction recovery of IS was 91.57% (CV=6.34%).

The endogenous components are mainly the cause of ion suppression effects during electrospray ionization. The extent of this effect is mainly dependent on sample extraction procedure and is also compound dependent. The result indicated that the matrix components did not alter or deteriorate the performance of the proposed method as the % coefficient of variation (CV%) of two QC samples was less than 92.26% and 96.87% for ezetimibe and IS respectively indicates the reproducibility of peak area as well as the extracts were 'clean' and no unseen component interfere with the ionization of the analytes. The extraction recoveries and matrix effect on the estimation of the analytes was shown in Table 2.

3.8. Stability

The result of stability experiments showed that no significant degradation occurred during the chromatography, extraction and sample storage of ezetimibe plasma samples. Stability data are shown in Table 3.

Table 1. Precision and accuracy data for the analysis of ezetimibe in human plasma (n=5).

Added (ng/ml)	Intra-day			Inter-day		
	Found (ng/ml)	CV (%)	R.E. (%)	Found (ng/ml)	CV (%)	R.E. (%)
0.1	0.10	4.38	2.00	0.10	3.13	1.00
0.3	0.30	1.50	0.67	0.29	2.38	2.00
4	4.08	4.27	1.90	4.07	3.16	1.80
16	15.75	1.08	1.55	15.84	1.57	1.01

Table 2. Extraction recovery and matrix effect of ezetimibe and ezetimibe-d_4(IS).

Analyte	Concentration (ng/ml)	Recovery (%)	Matrix effect (%)
Ezetimibe	0.3	96.32 (CV=4.01%)	92.26 (CV=3.35%)
	4	91.83 (CV=2.08%)	-
	16	85.23 (CV=1.04%)	102.44 (CV=12.73%)
Ezetimibe-d_4 (IS)	45	91.57 (CV=6.34%)	96.87 (CV=7.68%)

Table 3. Summary of stability of ezetimibe in human plasma under various storage conditions (n=3).

Storage conditions	Concentration (ng/ml)			CV (%)	Variation (%)
	Added	Initial	After		
Post preparative (23 hr at 10℃)	0.3	0.30	0.30	0.00	1.12
	16	15.91	15.98	1.84	0.48
Short-term (19 hr at 25℃)	0.3	0.30	0.30	0.00	1.12
	16	15.91	16.43	1.27	1.82
Long-term (35 day at-70℃)	0.3	0.30	0.31	1.88	3.37
	16	15.91	16.59	1.15	4.30
Three freeze/thaw (3 cycles)	0.3	0.30	0.31	1.88	3.37
	16	15.91	16.40	1.67	3.08
Stock solution (20 hr at 25℃)*	3	9701	10720	6.57	10.50
	160	433130	449490	7.25	3.78
Stock solution (21 day at 2 ~ 8℃)*	3	9701	9394	2.17	-3.17
	160	433130	432527	3.18	-0.14

* Stability of stock solution was evaluated peak area.

3.9. Application in Pharmacokinetic Study

This validated analytical method was applied to investigate the pharmacokinetic profiles of ezetimibe

in human plasma after an oral administration of 10 mg ezetimibe. Profile of the mean plasma concentration of ezetimibe versus time is shown in Fig. 4. The main pharmacokinetic parameters of ezetimibe in 35healthy male volunteers were calculated.

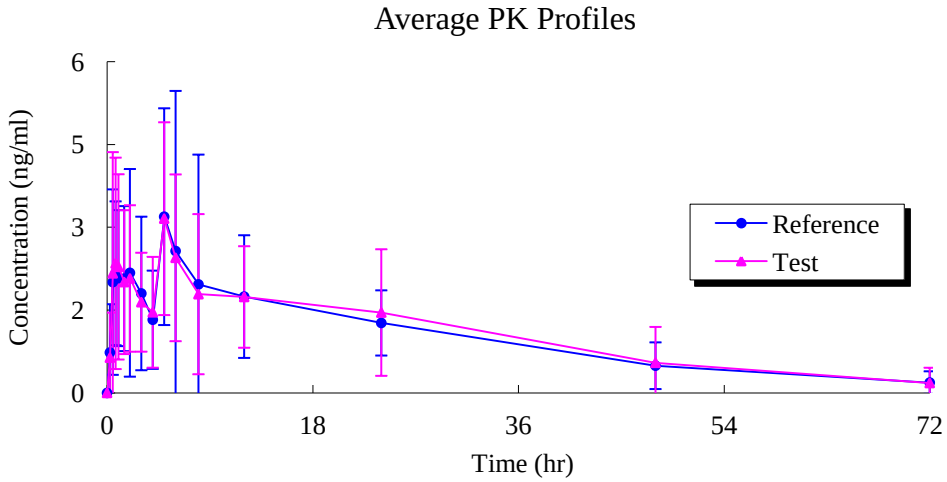


Fig. 4. Mean plasma concentration–time curve of ezetimibe after a single oral dose of 10 mg ezetimibe to 35 healthy malevolunteers. Each point represents the mean±SD.

For the pharmacokinetic analysis of plasma, the mean (SD) values obtained for the test and reference products were as follows: C_{max}, 4.05 (1.96) and 4.32 (2.84) ng/ml; T_{max}, 3.79 (2.76) and 3.79 (3.10) hr; $T_{1/2}$, 19.75 (10.26) and 20.68 (11.70) hr; AUC_{0-t}, 75.81 (46.46) and 71.51 (39.66) ng·hr/ml; and $AUC_{0-\infty}$, 87.09 (54.97) and 81.72 (41.84)ng·hr/ml, respectively (Table 4).The parametric 90% confidence intervals for AUC_{0-t}, and C_{max} values were 95.64 ~ 111.63% and 86.04 ~ 106.94%, respectively, and were entirely within the bioequivalence acceptance limits.

Table 4. Pharmacokinetic parameters of ezetimibe after a single oral dose 10 mg ezetimibe tablets of the test and reference.

Parameters	Test*	Reference**
C_{max}, ng/ml	4.05±1.96	4.32±2.84
T_{max}, hr	3.79±2.76	3.79±3.10
AUC_{0-t}, ng·hr/ml	75.81±46.46	71.51±39.66
$AUC_{0-\infty}$, ng·hr/ml	87.09±54.97	81.72±41.84
$t_{1/2}$, hr	19.75±10.26	20.68±11.70

* Ezerol Tab. (Ezetimibe 10 mg, Nexpharm Korea Co., Ltd. Korea)
** EzetrolTab. (Ezetimibe 10 mg, MSD Korea Ltd. Korea)

IV. Discussion

To the best of our knowledge, we have developed for the first time fully validated LC-MS/MS method for the determination of ezetimibe, which provides the highest sensitivity (0.1ng/ml) using a simple LLE procedure. A good internal standard should track the analyte during extraction and any inconsistent response due to matrix effect. This is also established with almost the same recovery of IS compared to the analyte. The most appropriate IS for typical anions are none other than deuterated compounds and hence ezetimibe-d_4 was used as IS. Results obtained by usage of d_4 internal standard were consistent and reproducible which was evident by incurred sample analysis conducted on this study.

V. Conclusions

An LC-MS/MS assay for ezetimibe in human plasmawas developed and validated with respect to linearity, precisionand accuracy, and analysis of real samples was demonstrated.It was proved to be superior in sensitivity, sample pretreatmentand speed of analysis in comparison to the previously reportedanalytical methods. This method was successfullyapplied to pharmacokinetic studies for ezetimibe andwas found to be sensitive and reliable.

Reference

[1] Altmann S.W., Davis H.R. Jr, Zhu L.J. et. al. Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption.

Science, Feb 20;303(5661), 1201-4, 2004
[2] Davis H.R. Jr, Tershakovec A.M., Tomassinin J.E. et. al. Intestinal sterol transporters and cholesterol absorption inhibition. *Curr Opin Lipidol*, 22, 467-478, 2011
[3] M.H. Davidson, J. Voogt, J. Luchoomun et. al. Inhibition of intestinal cholesterol absorption with ezetimibe increases components of reverse cholesterol transport in human. *Atherosclerosis*, 230(2), 322-329, 2013
[4] Sudhop T., Lütjohann D., Kodal A. et. al. Inhibition of intestinal cholesterol absorption by ezetimibe in humans. *Circulation*, 106(15), 1943-1948, 2002Wang J., Wang J. L., Bao H., RP-HPLC determination of ezetimibe. *Chin. J. New Drugs*, 12, 932-937, 2003
[5] Sudhop T., Reber M., Tribble D. et. al. Changes in cholesterol absorption and cholesterol synthesis caused by ezetimibe and/or simvastatin in men. *J. Lipid Res*, 50, 2117-2123, 2009
[6] R.H. Knopp, H. Gitter, T. Truitt et. al. Effects of ezetimibe, a new cholesterol absorption inhibitor, on plasma lipids in patients with primary hypercholesterolemia. *Eur. Heart. J*. 24(8), 729-741, 2003
[7] Salen G., von Bergmann K., Lütjohann D. et. al. Ezetimibe effectively reduces plasma plant sterols in patients with sitosterolemia. *Circulation*, 109(8), 966-971, 2004
[8] Chosal A., Hapangama N., Yuan Y. et. al. Identification of human UDP-glucuronosyltransferase enzyme(s) responsible for the glucuronidation of ezetimibe (zetia). *Drug Metab Dispos*, 32, 314-320, 2004
[9] Jeu L., Cheng J.W. Pharmacology and therapeutics of ezetimibe (SCH 58235), a cholesterol-absorption inhibitor. *Clin Ther*, 25, 2352-2387, 2003
[10] Basha B.J., Naveed S.A., Tiwari N.K et. al. Concurrent determination of ezetimibe and its phase-I and II metabolites by HPLC with UV detection: quantitative application to various in vitro metabolic stability studies and for qualitative estimation in bile. *J. Chromatogr. B*. 853(1-2), 88-96, 2007
[11] Sistla R., Tata V.S., Kashyap Y.V. et. al. Development and validation of a reversed-phase HPLC method for the determination of ezetimibe in pharmaceutical dosage forms. *J. Pharm Biomed Anal*, 39(3-4), 517-522, 2005
[12] Z. Luo, Z. Deng, Y. Liu et. al. Development and validation of a novel stability-indicating HPLC method for the quantitative determination of eleven related substances in ezetimibe drug substance and drug product. *Talanta*, 139, 67-74, 2015

Yong Sun Park. "Development and validation of simple and rapid LC-MS/MS method for ezetimibe in human plasma and its application to bioequivalence study." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS), vol. 12, no. 4, 2017, pp. 01–08.