Rapid and Simple Method for the Determination of Pregabalin in Human Plasma using Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS): Application to a Bioequivalence Study of Daewoong Pregabalin Capsule To Lyrica® Capsule (Pregabalin 150 mg)

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ABSTRACT − Method using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was developed and validated for the determination of pregabalin in plasma samples. Acquisition was performed by monitoring the transitions: m/z 160.1→142.2 for pregabalin and m/z 423.2→207.1 for losartan (as an internal standard). After cold acetonitrile-induced protein precipitation of the plasma samples, separation was performed with C_{18} column by isocratic mobile phase consisted of 10 mM ammonium acetate and acetonitrile (15:85, v/v). Results were linear over the concentration ranged from 0.1 to 10 µg/mL and the correlation coefficients (r) were ≥0.99. Intra- and inter-day precisions were ≤6.02 and ≤11.04%, respectively, and intra- and inter-day accuracies were 96.60-101.09 and 98.10-102.60%, respectively. This validated method was successfully applied to a bioequivalence study of two formulations of pregabalin, Daewoong pregabalin capsule (Daewoong Pharm. Co., Ltd.) and Lyrica® capsule (Pfizer Korea Ltd.) in twenty eight healthy Korean volunteers. The subjects received a single oral dose of each formulation (150 mg as pregabalin) in a randomized 2 × 2 crossover study and plasma samples were obtained from each subject at predetermined time intervals. Then, the pharmacokinetic parameters (AUC_{0-t}, C_{max} and T_{max}) were calculated and statistically analyzed to assess the differences between two formulations. The 90% confidence intervals for the log-transformed data were acceptable range of log 0.8-log 1.25 (e.g., log 1.0048-log 1.0692 for AUC_{0-t}, log 0.9142-log 1.0421 for C_{max}). Thus, AUC_{0-t} and C_{max} met the criteria of the Korea Food and Drug Administration (KFDA) for bioequivalence test indicating that Daewoong pregabalin capsule was bioequivalent to Lyrica® capsule.

Key words − Pregabalin, Daewoong pregabalin capsule, Lyrica® capsule, Bioequivalence study, Liquid chromatography-tandem mass spectrometry

Pregabalin, (S)-3-aminomethyl-5-methyl hexanoic acid, a member of γ-aminobutyric acid (GABA) analogues, crosses the blood brain barrier and potentially binds to the α_2-δ subunit of the voltage-sensitive calcium channel in CNS (Zareba, 2008). Potent binding at this site reduces calcium influx at hyperexcited nerve terminals, and reduces the release of several neurotransmitters, including glutamate, noradrenaline, and substance P (Dooley et al., 2002; Maneuf et al., 2001; Gee et al., 1996). Based on these activities, pregabalin was approved for the treatment of: neuropathic pain, partial seizure and generalized anxiety disorder (Zareba, 2008; Gray, 2007; Framton and Foster, 2005).

There are few published papers for pharmacokinetics of pregabalin. After oral administration, pregabalin is rapidly absorbed through the intestinal tract with peak human plasma concentrations (C_{max}) occurring at 1 hr and its bioavailability is approximately ≥90%. Pregabalin does not bind to plasma protein and its apparent volume of distribution (V_d) after oral administration is approximately 0.5 L/kg. Less than 2% of the dose is metabolized and the rest of dose is excreted unchanged in urine. The mean terminal disposition half life ($t_{1/2}$) ranged from 4.8 to 6.3 hr in healthy human volunteers with normal renal function and renal clearance ranged from 67 to 81 mL/min (Guay, 2005). The C_{max} has been reported to be 4.04-4.11 µg/mL and the time values achieved (T_{max}) were 1.17-1.25 hr after the oral administration of the 150 mg of pregabalin capsule in twelve healthy human volunteers (Vaidya et al., 2007). In other study, after the oral administration of a single dose 300 mg capsule of pragabalin in eighteen healthy human volunteers, the mean value of area under the plasma concentration-time curve (AUC_{0-t}) has been reported to be 43.08-48.77 µg·hr/mL. The C_{max} ranged from 6.93-7.48 µg/mL at 1.86-2.12 hr (T_{max}) (Mandal et al., 2008). However, there had been no reported studies of pharmacokinetic analysis of pregabalin in Korean.

Several analytical methods have been reported for the deter-

mination of pregabalin in pharmaceutical preparations and biological fluids by LC-UV (Kasawar and Farooqui, 2010; Gujral et al., 2009) or LC-fluorescence (Dousa et al., 2010; Vermeij and Edelbroek, 2004). These methods require pre-column derivatization of analyte with *o*-phthaldialdehyde (OPA) and 3-mercaptopropionic acid or picrylsulfonic acid. In application, derivatization may be difficult and gives inaccurate estimation of analyte due to incomplete derivatization if reaction conditions are not strictly maintained. Therefore, we developed a rapid and simple method for the determination of pregabalin in human plasma using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). This method was fully validated and successfully used to assess the bioequivalence of two formulations of pregabalin after the oral administration of a single dose 150 mg capsule in twenty eight healthy Korean volunteers, where these pharmacokinetic parameters were not yet reported in Korean population.

Materials and Methods

Materials and instruments

The test formulation (Daewoong pregabalin capsule, 150 mg as pregabalin, Daewoong Pharm. Co., Ltd., Korea, lot no. 960524; expiration date, November 7th 2013), which was manufactured in accordance with the Korean Good Clinical Practice (KGCP) guidelines (KFDA, 2009), and the reference formulation (Lyrica® capsule, 150 mg as pregabalin, Pfizer Korea Ltd., Korea, lot no. 0516119; expiration date, November 5th 2012) were supplied in the form of capsules.

Pregabalin (99.2% purity) was supplied from Daewoong Pharm. Co., Ltd., (Gyunggi-Do, Korea) and losartan potassium was supplied from Daewoo Pharm. Co., Ltd., (Busan, Korea), respectively. Chemical structures of pregabalin and losartan (internal standard, IS) are presented in Figure 1. HPLC-grade acetonitrile and methanol were purchased from J.T. Baker (Phillipsburg, NJ, USA). Ammonium acetate was purchased from Sigma-Aldrich (St. Louis, MO, USA) and *o*-phosphoric acid was purchased from Wako (Osaka, Japan). Water was obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA). All other chemicals were of analytical grade.

The Agilent 1100 series HPLC (Agilent technologies, Santa Clara, CA, USA) is used. The chromatography was performed on Shiseido (Tokyo, Japan) Capcell Pak MG C_{18} column (50 × 2.0 mm i.d., 3 μm, 10 nm). The mass spectrometry was performed on API 2000 mass spectrometer (AB Sciex, Toronto, Canada) equipped with Turboionspray ionization source. Data acquisition was performed with Analyst™ 1.5 software (AB Sciex, Toronto, Canada).
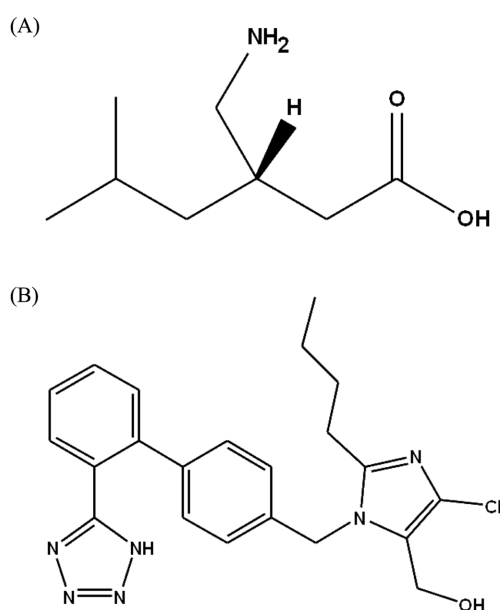

Figure 1. Chemical Structures for (A) pregabalin and (B) losartan (IS).

Determination methods of pregabalin in human plasma
Analytical conditions

The mobile phase, which consisted of 10 mM ammonium acetate buffer and acetonitrile (15:85, v/v), was delivered at a flow rate of 0.2 mL/min as an isocratic mode. The mobile phase was filtered through 0.22 μm membrane and degassed prior to use. The stationary phase, Shiseido Capcell Pak MG C_{18} column, maintained at 45ºC.

The mass spectrometer was operated by electrospray ionization (ESI) at a positive ion mode. Typical source conditions were as follows: the ion spray voltage was set at 5.5 kV, and the source temperature was 500ºC. The curtain gas and collision-activated dissociation were 10 (arbitrary units), respectively. Ion source gas 1 was set at 45 (arbitrary units), ion source gas 2 was 65 (arbitrary units). Quantification was performed using the multiple reaction monitoring (MRM), with a dwell time of 200 ms, transitions were m/z 160.1→142.2 for pregabalin, m/z 423.2→207.1 for the IS, respectively. The optimized collision energy of 14 eV was used for pregabalin and 32 eV was used for the IS. The declustering potential (DP) was adjusted at 20 and 16 (arbitrary units) for pregabalin and IS, respectively. The ion mass spectra and the patterns of fragmentation of pregabalin and IS are presented in Figure 2.

Preparation of the stock solution

Standard stock solution of pregabalin (1 mg/mL) was prepared in methanol and IS (1 mg/mL) was prepared in acetonitrile. For calibration curve and quality control (QC)
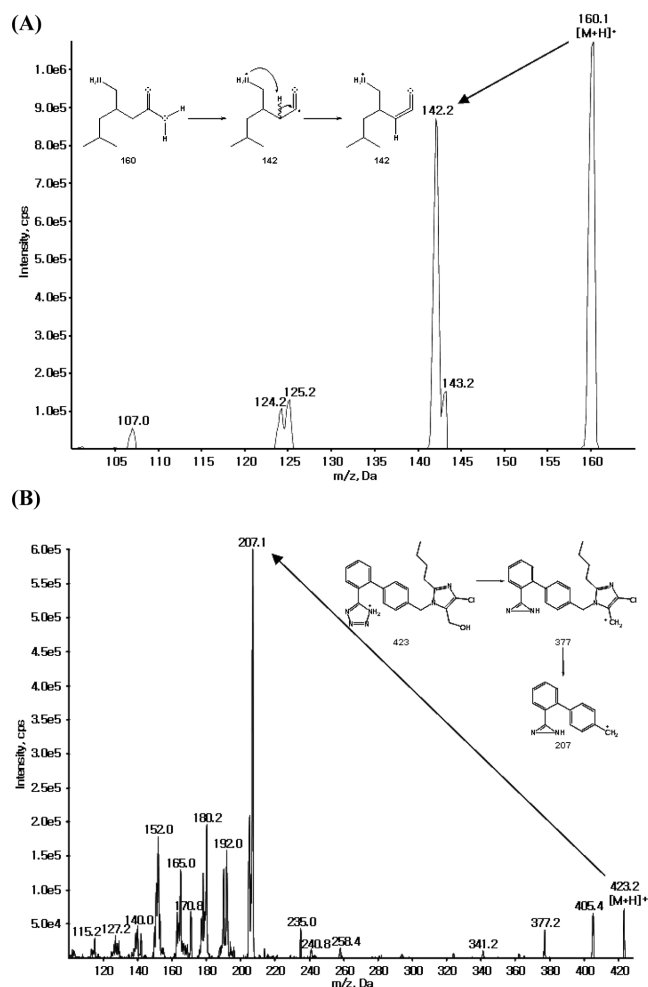
(A)

(B)

Figure 2. Product ion mass spectra and the patterns of fragmentation of (A) pregabalin and (B) losartan (IS).

sample, series of working standard solutions at concentrations of 1, 2, 3, 5, 10, 20, 50, 80 and 100 µg/mL were prepared by appropriate dilution of its stock with 50% methanol. Low-, medium-, high QC working standard solutions were 3, 20, 80 µg/mL, respectively. An IS working solution (10 µg/mL) for routine use was freshly prepared by diluting stock solution with acetonitrile. A seven-point calibration curve was prepared by spiking the blank plasma with appropriate amounts of working standard solution to obtain final concentrations of 0.1, 0.2, 0.5, 1, 2, 5 and 10 µg/mL and quality control samples 0.3, 2 and 8 µg/mL for analyte. All the solutions were stored at -20ºC freezer when they were not in use.

Sample preparation

To 1.75 mL polypropylene tube, 80 µL of human plasma sample was spiked with 20 µL of IS working solution (10 µg/mL) and 15 µL of 10% *o*-phosphoric acid. Then 1.2 mL of cold acetonitrile (stored in -20ºC) was added and mixture was

vortex-mixed for 15 min. All the samples were centrifuged at 4ºC, 14000 rpm for 15 min. The supernatant was filtered through a 0.20 µm syringe filter. The resulting samples were loaded into the autosampler and 2 µL aliquot of each sample was injected into the LC-MS/MS system for analysis.

Method validation

In accordance with Korea Food and Drug Administration (KFDA) guideline for the validation of bioanalytical method (KFDA, 2010b), the method was validated for specificity, sensitivity, linearity, precision, accuracy, recovery and stability.

Specificity and sensitivity

The specificity of the method was evaluated by analyzing drug-free blank plasma from six different subjects. There should not be any interference at the retention times of the analyte and IS. Specificity was assessed by comparing the chromatograms obtained from the blank plasma samples spiked with or without a pregabalin stock solution (1 µg/mL) and IS solution (10 µg/mL). The lower limit of quantification (LLOQ) was used to determine sensitivity and was defined as the concentration of pregabalin at which signal-to-noise ratio (*S/N* ratio) was greater than 10, with a precision less than 20% and accuracy between 80 and 120% of theoretical value. The LLOQ was set as the lowest concentration of calibration curve.

Linearity

The linearity of the calibration curves, ranging from 0.1 to 10 µg/mL, was evaluated using a *$1/x^2$* weighed least-squares regression method of peak area ratios of pregabalin to IS versus actual concentrations. The acceptance criterion for a calibration curve was a correlation coefficient (*r*) of 0.99 or better, and that each back-calculated standard concentration must be within 15% deviation from the theoretical value except for the LLOQ.

Precision and accuracy

To assess the intra- and inter-day precision and accuracy, assays were performed with five replicates on the same day and on four consecutive days. Pregabalin plasma samples at four concentration levels (0.1, 0.5, 2, and 10 µg/mL) were analyzed to evaluate precision and accuracy of the method. Precision is expressed as coefficient of variance (C.V.) at each concentration. The accuracy of the assay was defined as a percentage of the experimental concentration over the theoretical concentration. The acceptance criterion recommended by KFDA for each back-calculated concentration was 15% deviation from the theoretical value except for LLOQ, which was set at 20%.

Recovery and stability

The extraction recovery of pregabalin (low-, medium- and high QC) and IS in human plasma were determined by comparing the peak area of precipitated analyte and IS with that of reference solutions at the same concentration level and reconstituted into blank plasma extracts. The number of replicates for each concentration was three.

The stability of the analyte (QC levels) in human plasma under different temperatures and time conditions was evaluated. For freeze-thaw stability, QC plasma samples were subjected to three cycles from -70℃ to room temperature. An autosampler stability was assessed by placing prepared QC samples in autosampler at 10℃ for 27 hr, and long-term stability was evaluated by freezing QC plasma samples at -70℃ for ten days. Short-term stability was performed by placing QC plasma samples at different temperatures (-70℃, 4℃ and room temperature) for 24 hr. Then the stability was assessed by comparing the area response of prepared stability samples with the area response of fresh QC plasma samples prepared without a storage period.

Selection of volunteers

The bioequivalence study population consisted of twenty eight healthy Korean volunteers with an average age of 24.53 ± 4.47 years and an average body weight of 73.97 ± 5.82 kg. All of the participants were enrolled in the study after performing a medical history assessment, a physical examination and standard laboratory tests (blood analysis: WBC, RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, MPV, platelet, differential counting of WBC, total protein, albumin, AST, ALT, ALP, total bilirubin, direct bilirubin, glucose, BUN, creatinine and total cholesterol, urine analysis: occult blood, bilirubin, urobilinogen, ketone, protein, nitrite, glucose, pH, specific gravity, leucocytes, WBC and RBC). The subjects were excluded if they had possible sensitivity to pregabalin; had a history of gastrointestinal, hepatic, renal, cardiovascular, central nervous system, or endocrinal illness; or had ingested alcohol or any medications within 4 weeks before the study. Written informed consent was obtained from all subjects after the nature and details of the study had been explained, in accordance with the KFDA guideline for bioequivalence test (KFDA, 2010a).

Blood sampling from volunteers

The study protocol was approved by the Institutional Review Board of the Kyung Hee University. The study was performed in accordance with the revised Declaration of Helsinki and the Korean Good Clinical Practice (KGCP) guide-lines (KFDA, 2009).

The study had single-dose, randomized and 2×2 (two-treatment, two-period) crossover designs. The subjects were hospitalized (Kyung Hee University Medical Center, Seoul, Korea) at 6:30 p.m. on the eve of the study. And all of the subjects refrained from smoking, exercising, consuming xanthine-containing foods, drinking alcoholic beverage overnight until 4 hr after each drug (test formulation: Daewoong pregabalin capsule, reference formulation: Lyrica® capsule) administration. The subjects received standardized meals at 4 hr after drug administration. On the day of the study, at 7:00 a.m., a cannula was inserted into the subjects' median cubital vein and was flushed with heparinized normal saline to prevent clotting. At 8:00 a.m., each subject was given an oral dose (two pregabalin capsules; pregabalin 150 mg) with 240 mL of water. There was a one-week washout period between the different formulation doses. After 1.0 mL of blood was discarded, about 7.0 mL of blood samples were collected into heparinized tubes before (0 hr) and at 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 hr after dosing. After sampling, the cannula was flushed with heparinized normal saline. Plasma was separated by centrifugation at 3,000 rpm for 10 min and kept frozen at -70℃ until analysis.

Pharmacokinetics and statistical analysis

After the single oral administration of 150 mg of pregabalin capsules to subjects, pharmacokinetic parameters, including AUC_{0-t}, C_{max} and T_{max} were evaluated for the period of 0-24 hr by the measure of plasma concentration. The parameters were calculated by using BA calc 2007® program (Lee et al., 2007a) applying a non-compartmental method. C_{max} and T_{max} were recorded as actual measurement values and AUC_{0-t} was calculated by linear-trapezoidal method from 0 to 24 hr. In addition, the ratios (test/reference) of AUC_{0-t} and C_{max} using log-transformed data, together with their means and 90% confidence intervals, were analyzed with the analysis of variance (ANOVA) that performed with the K-BE Test 2007® program (Lee et al., 2007b) at a significant level of 0.05. Bioequivalence of two formulations of pregabalin was assessed by AUC_{0-t} and C_{max} criteria of KFDA guideline for bioequivalence test (KFDA, 2010a).

Results and Discussion

Validation results
Specificity and sensitivity
Among the different possible detection techniques that can be coupled with LC, mass spectrometry has been unparalleled for performing bioanalytical determinations with maximum

specificity and sensitivity. LC-MS/MS has high specificity because only ions derived from analytes of interest are monitored. Using drug-free plasma from six different subjects, blank plasma, plasma spiked with pregabalin stock solution (1 µg/mL), plasma spiked with IS solution (10 µg/mL) and pregabalin 0.1 µg/mL (LLOQ) plasma spiked with the IS were analyzed. The chromatograms for pregabalin and IS are presented in Figure 3. The peak retention time of pregabalin and IS were at 0.65 and 0.59 min, respectively. No significant interference in the chromatograms was observed from endogenous substances in human plasma at the retention time of the analyte or IS. Also, there was no interference from the IS to the MRM channel of the analyte. The signal-to-noise ratio of LLOQ (0.1 µg/mL) was greater than 10. The intra- and inter-day precisions for LLOQ were 6.02 and 11.04%, respectively. The intra- and inter-day accuracies for LLOQ were 96.60 and 102.60%, respectively.

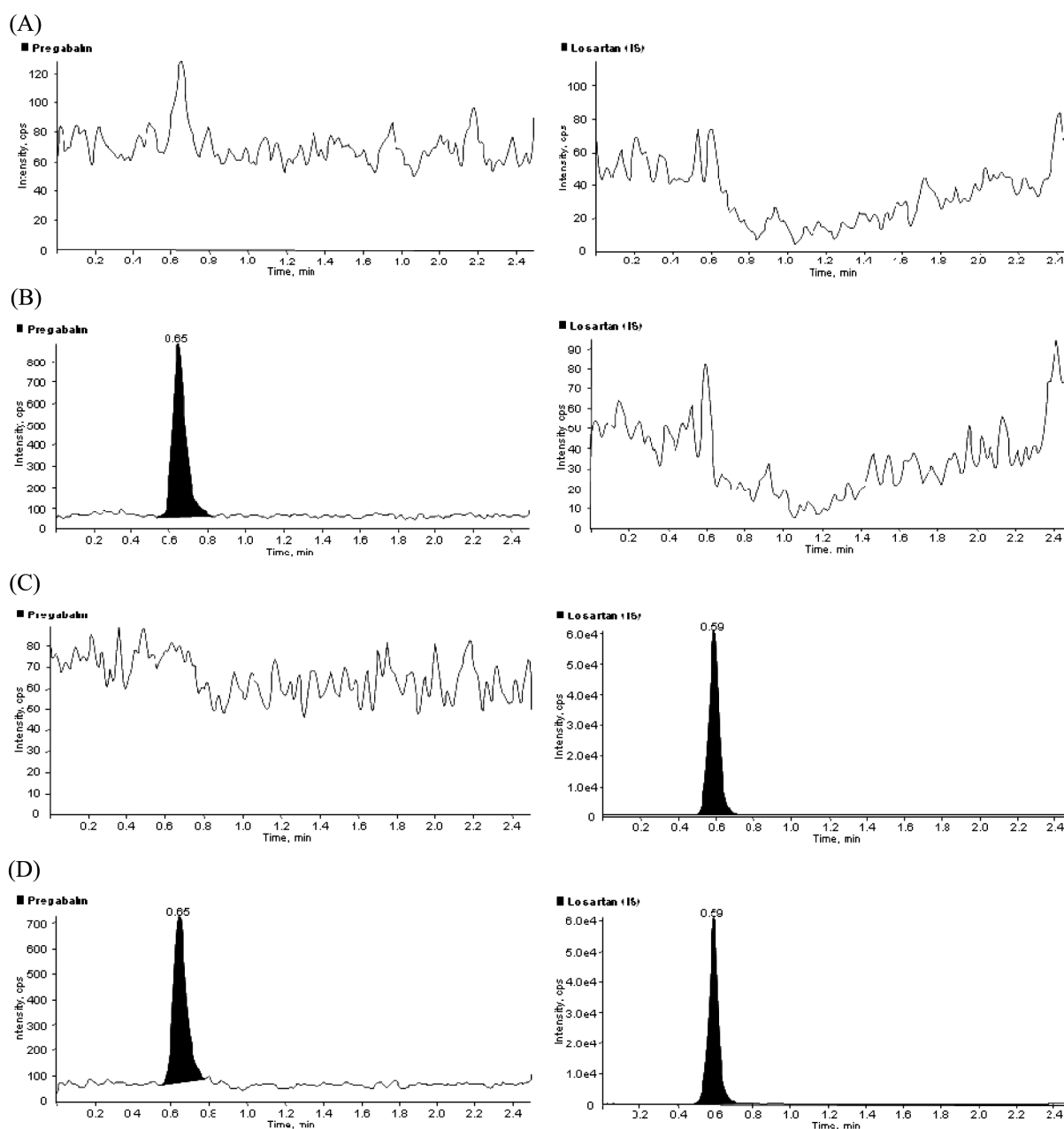
Figure 3. Multiple reaction monitoring chromatograms for pregabalin and IS of (A) blank human plasma, (B) plasma spiked with pregabalin (1 µg/mL), (C) plasma spiked with IS (10 µg/mL) and (D) 0.1 µg/mL (LLOQ) of pregabalin spiked with the IS.

Linearity

To evaluate the linearity of the method, the seven-point calibration curve samples were analyzed on five independent runs. The equation of the calibration curve was obtained by a $1/x^2$ weighed least-squares regression method of the peak area ratios of pregabalin to IS versus actual concentrations. The mean equation of all calibration curves (n=5, intra- and inter-day batches) obtained was y = (0.1352 ± 0.008289) + (0.0008858 ± 0.0008493), and the correlation coefficients (*r*) were ≥0.9987. And the each back-calculated standard concentration of all curves was within 15% deviation from the theoretical value. Thus, the calibration curves showed good linearity within the range of 0.1-10 μg/mL. Results indicated the linearity and repeatability of the method.

Precision and accuracy

The intra- and inter-day precision and accuracy of the method used for pregabalin determination are presented in Table I. Intra-day C.V.s were ≤6.02%, and inter-day C.V.s were ≤11.04%. Intra- and inter-day accuracies ranged from 96.60 to 101.09% and from 98.10 to 102.60%, respectively. All results were within the acceptance criteria of the KFDA guideline for the validation of bioanalytical method (KFDA, 2010b).

Recovery and stability

The mean extraction recoveries for pregabalin at QC concentrations after cold acetonitrile protein precipitation from human plasma were 81.58%, 81.66% and 80.29%, respec-

tively, and the mean extraction recovery of IS (10 μg/mL) was found to be 94.28%.

The evaluated values of stability of the pregabalin in human plasma under different conditions are presented in Table II. Pregabalin stability after three freeze and thaw cycles was performed at QC levels and the percent bias was evaluated to be 6.30, 1.49 and 2.79%, respectively. Autosampler stability was done for 27 hr at the low-, medium-, high QC, respectively. The percent bias was evaluated to be -1.57, -3.61 and -3.23%, respectively. Long-term stability was done for ten days at -70ºC and evaluated at a percent bias of -5.51, -3.24 and -6.80% for QC levels, respectively. Short-term stability (24 hr) was performed at QC levels under different temperatures (-70ºC and 4ºC). Pregabalin QC plasma samples that stored at -70ºC were evaluated at a percent bias of 5.51, 7.22 and 5.27%, respectively. And short-term stability at 4ºC was performed and the percent bias was evaluated to be 11.81, 15.94 and 11.26% for QC levels, respectively. No significant deterioration was observed under any of the examined conditions and then all the plasma samples of the bioequivalence study were stored at -70ºC and thawed at 4ºC.

Pharmacokinetic analysis

The devised method was successfully applied to analyze human plasma samples for a bioequivalence study of pregabalin 150 mg capsules (test formulation: Daewoong pregabalin capsule, reference formulation: Lyrica® capsule) in twenty eight healthy Korean volunteers. Figure 4 presents the

Table I. *Precision and accuracy of the method for determining pregabalin concentration in human plasma (n=5)*

Theoretical concentration (μg/mL)	Intra-day			Inter-day		
	Experimental concentration (mean ± S.D.) (μg/mL)	Precision (%)	Accuracy (%)	Experimental concentration (mean ± S.D.) (μg/mL)	Precision (%)	Accuracy (%)
0.1	0.097 ± 0.006	6.02	96.60	0.103 ± 0.011	11.04	102.60
0.5	0.504 ± 0.023	4.49	100.84	0.502 ± 0.012	2.42	100.48
2	1.983 ± 0.042	2.10	99.17	1.962 ± 0.035	1.79	98.10
10	10.109 ± 0.179	1.77	101.09	9.837 ± 0.335	3.41	98.37

Table II. *Stability of pregabalin in human plasma under different temperatures and time conditions (n=3), (C.V., %)*

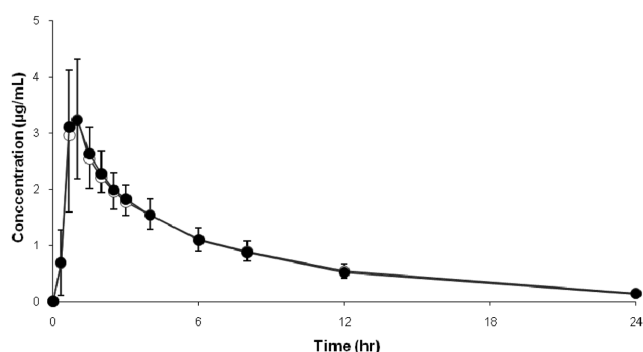| Storage conditions	Low-QC (0.3 μg/mL)	Medium-QC (2.0 μg/mL)	Medium-QC (8.0 μg/mL)
3 freeze/thaw cycles	106.30% (2.22)	101.49% (2.16)	102.79% (2.28)
27 hr at autosampler (10ºC)	98.43% (3.67)	96.39% (0.67)	96.77% (0.74)
10 days frozen (-70ºC)	94.49% (5.00)	96.76% (1.39)	93.20% (3.76)
24 hr at -70ºC	105.51% (1.29)	107.22% (0.35)	105.27% (0.51)
24 hr at 4ºC	111.81% (3.23)	115.94% (3.62)	111.26% (4.37)

Figure 4. Mean plasma concentration-time profile of pregabalin from 28 healthy Korean volunteers after oral administration of a single 150 mg of pregabalin capsule (●, reference formulation; ○, test formulation).

mean (±S.D.) plasma concentration-time curve of pregabalin for test and reference formulations after the oral administration of a single dose of 150 mg capsule, and the pharmacokinetic

parameters derived from these profiles are presented in Table III. The pharmacokinetic parameters were calculated by linear-trapezoidal method in 0-24 hr. In this study, the mean (±S.D.) AUC_{0-t} was 18.70 ± 4.25 μg·hr/mL for the reference formulation and 19.30 ± 4.14 μg·hr/mL for the test formulation. The mean (±S.D.) C_{max} was 3.70 ± 1.13 μg/mL for the reference formulation and the mean T_{max} was 1.05 ± 0.41 hr. The mean (±S.D.) C_{max} was 3.58 ± 0.92 μg/mL for the test formulation and the mean T_{max} was 1.19 ± 0.72 hr.

Bioequivalence study

Bioequivalence analysis result is presented in Table IV. The 90% confidence intervals for the ratios of AUC_{0-t} and C_{max} were log 1.0048-log 1.0692 and log 0.9142-log 1.0421, respectively. The results met the bioequivalence criteria of KFDA (log 0.80-log 1.25) (KFDA, 2010a), therefore, a test formulation (Daewoong pregabalin capsule; pregabalin 150 mg) is

Table III. *Pharmacokinetic parameters obtained after oral administration of Lyrica® capsule and Daewoong pregabalin capsule at the dose of 150 mg of pregabalin*

Subjects	Lyrica® capsule					Daewoong.pregabalin capsule				
	AUC_{0-t} (μg·hr/mL)	Log AUC_{0-t}	C_{max} (μg/mL)	Log C_{max}	T_{max} (hr)	AUC_{0-t} (μg·hr/mL)	Log AUC_{0-t}	C_{max} (μg/mL)	Log C_{max}	T_{max} (hr)
A01	17.34	1.24	3.00	0.48	1.50	19.14	1.28	3.02	0.48	1.00
A02	28.80	1.46	6.27	0.80	0.67	30.21	1.48	5.71	0.76	1.00
A03	22.19	1.35	5.52	0.74	0.67	20.64	1.31	4.24	0.63	0.67
A04	20.30	1.31	2.87	0.46	1.00	19.14	1.28	4.28	0.63	0.67
A05	24.61	1.39	4.08	0.61	1.50	26.78	1.43	4.78	0.68	1.00
A06	12.61	1.10	2.55	0.41	1.00	16.08	1.21	2.56	0.41	1.50
A07	20.59	1.31	4.87	0.69	1.00	18.64	1.27	4.16	0.62	1.00
A08	11.89	1.08	2.36	0.37	0.67	12.35	1.09	3.01	0.48	1.00
A09	11.75	1.07	2.90	0.46	1.00	14.48	1.16	2.29	0.36	1.00
A10	17.81	1.25	3.15	0.50	1.00	17.83	1.25	2.17	0.34	2.50
A11	19.15	1.28	2.64	0.42	1.50	20.31	1.31	2.99	0.48	1.00
A12	16.05	1.21	2.58	0.41	1.50	16.06	1.21	2.95	0.47	1.00
A13	12.91	1.11	3.38	0.53	1.00	16.27	1.21	3.88	0.59	0.67
A14	19.51	1.29	3.75	0.57	1.00	19.86	1.30	3.88	0.59	1.00
B01	13.64	1.13	4.56	0.66	0.67	12.75	1.11	4.10	0.61	1.00
B02	17.84	1.25	3.20	0.51	1.00	16.39	1.21	4.35	0.64	0.67
B03	22.75	1.36	3.75	0.57	1.00	23.56	1.37	3.31	0.52	1.50
B04	18.61	1.27	2.43	0.39	1.50	17.99	1.26	2.70	0.43	2.00
B05	17.08	1.23	3.70	0.57	1.00	16.43	1.22	3.12	0.49	0.67
B06	22.69	1.36	6.13	0.79	0.67	20.91	1.32	5.31	0.73	1.00
B07	20.53	1.31	3.06	0.49	1.50	21.96	1.34	3.44	0.54	0.67
B08	23.16	1.36	3.20	0.51	0.67	22.60	1.35	2.79	0.45	0.67
B09	22.49	1.35	5.51	0.74	0.67	22.79	1.36	4.16	0.62	0.67
B10	21.08	1.32	4.95	0.69	1.00	21.70	1.34	4.86	0.69	1.00
B11	19.48	1.29	3.98	0.60	0.67	20.80	1.32	3.17	0.50	1.50
B12	20.82	1.32	2.97	0.47	1.00	23.81	1.38	3.40	0.53	1.00
B13	13.19	1.12	2.92	0.47	2.50	13.05	1.12	3.31	0.52	2.00
B14	14.71	1.17	3.33	0.52	0.67	17.99	1.26	2.27	0.36	4.00
Mean	18.70	1.26	3.70	0.55	1.05	19.30	1.28	3.58	0.54	1.19
S.D.	4.25	0.10	1.13	0.12	0.41	4.14	0.09	0.92	0.11	0.72

Table IV. *Statistical results of bioequivalence evaluation between two pregabalin capsules*

	Parameters	
	AUC$_{0-t}$	C$_{max}$
Difference (%)	3.21	-3.24
Test/Reference estimate	1.036	0.976
90% C.I. (δ)[1]	log 1.0048 ≤ δ ≤ log 1.0692	log 0.9142 ≤ δ ≤ log1.0421

[1] α = 0.05.

bioequivalent to a reference formulation (Lyrica® capsule; pregabalin 150 mg).

Conclusion

The described LC-MS/MS method for the determination of pregabalin in human plasma is successfully developed and validated. The method has proved to be rapid with each sample requiring a run time of 2.5 min only. And the sample preparation was simple one step protein precipitation. The method has shown acceptable specificity, sensitivity, linearity, precision and accuracy. In addition, this validated method was successfully applied to the bioequivalence study. And the statistical analysis results demonstrated that two different formulations of pregabalin were bioequivalent without significant differences in AUC$_{0-t}$ or C$_{max}$.

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