

Validated HPLC Method for the Pharmacokinetic Study of Atenolol and Chlorthalidone Combination Therapy in Korean Subjects

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ABSTRACT – A rapid, selective and sensitive reverse-phase HPLC methods for the determination of atenolol and chlorthalidone in human serum and whole blood were validated, and applied to the pharmacokinetic study of atenolol and chlorthalidone combination therapy. Atenolol and an internal standard, pindolol, were extracted from human serum by liquid-liquid extraction, and analyzed on a μ -Bondapak C18 10- μ m column in a mobile phase of methanol-0.01 M potassium dihydrogenphosphate (30:70, v/v, adjusted to pH 3.5) and fluorescence detection (emission: 300 nm, excitation: 224 nm). Chlorthalidone and an internal standard, probenecid, were extracted from human whole blood by liquid-liquid extraction, and analyzed on a Luna C18 5- μ m column in a mobile phase of acetonitrile containing 77% 0.01 M sodium acetate and UV detection at 214 nm. These analysis were performed at three different laboratories using the same quality control (QC) samples. The chromatograms showed good resolution, sensitivity, and no interference by human serum and whole blood, respectively. The methods showed linear responses over a concentration range of 10-1,000 ng/mL for atenolol and 0.05-20 μ g/mL for chlorthalidone, with correlation coefficients of greater than 0.999 at all the three laboratories. Intra- and inter-day assay precision and accuracy fulfilled international requirements. Stability studies (freeze-thaw, short-, long-term, extracted sample and stock solution) showed that atenolol and chlorthalidone were stable. The lower limit of quantitation of atenolol and chlorthalidone were 10 ng/mL and 0.05 μ g/mL, respectively, which was sensitive enough for pharmacokinetic studies. These methods were applied to the pharmacokinetic study of atenolol and chlorthalidone in human volunteers following a single oral administration of Hyundai Tenoretic[®] tablet (atenolol 50 mg and chlorthalidone 12.5 mg) at three different laboratories.

Key words – Atenolol, Chlorthalidone, Validation, Pharmacokinetics, HPLC

Atenolol, 4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]benzeneacetamide (Figure 1), is a cardioselective beta-adrenergic blocking agent used in the treatment of hypertension and ischemic heart disease. The drug has no intrinsic sympathomimetic activity or membrane stabilizing properties.¹⁾ The time of peak concentration was reached at 2-4 h, the elimination half-life varied from 6 to 7 h. The AUC_{0-t} and C_{max} (mean \pm S.D.) were 3387.36 \pm 886.77 ng·h/mL and 393.14 \pm 133.54 ng/mL, respectively.^{2,3)}

Chlorthalidone, 2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl)benzenesulfonamide (Figure 2), is an orally effective diuretic agent used alone and in combination for the treatment of hypertension and edema. Chlorthalidone differs from the thi-

azide diuretics by having an unusually long biological half-life (40-60 h) due largely to its extensive distribution into erythrocytes where it has a strong affinity for carbonic anhydrase.^{4,5)}

Atenolol and chlorthalidone are well-known drugs widely used in the treatment of hypertension, the first exerting selective action on β_1 receptors and the second diuretic activity. Pharmaceutical preparations containing both drugs have also been marketed, their combination having the advantage of providing greater therapeutic effects than with either drug alone and permitting once a day administration only, the two drugs not interacting pharmacokinetically with each other or presenting synergic toxic effects.⁶⁾ The bioavailability of atenolol and chlorthalidone from the fixed combination are equivalent to that from the free combination.^{7,8)}

Previous analysis of atenolol was based on the high-performance thin-layer chromatography (HPTLC),⁹⁾ LC/MS/MS¹⁰⁾

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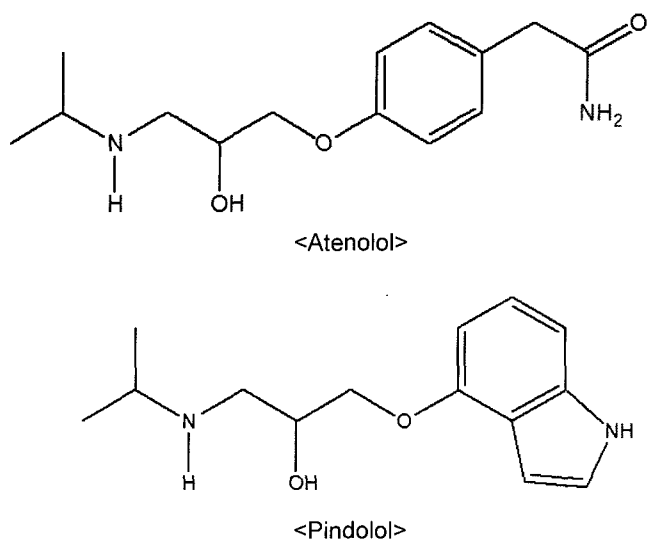


Figure 1—Chemical structures of atenolol and pindolol (I.S.).

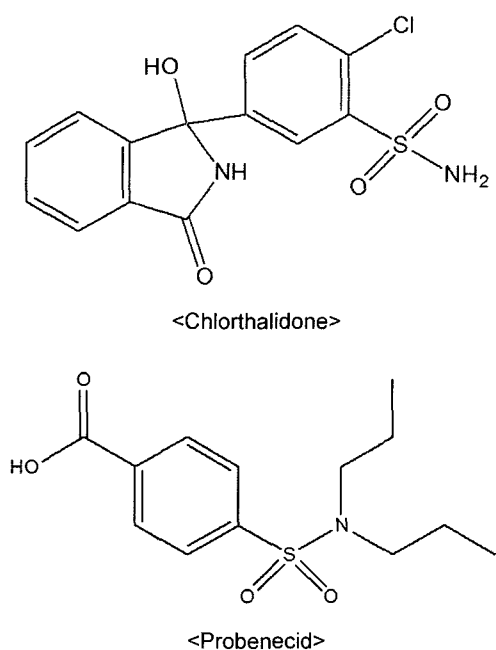


Figure 2—Chemical structures of chlorthalidone and probenecid (I.S.).

and high-performance liquid chromatography (HPLC) with UV^{11,12)} or fluorescence detection.¹³⁾ Those methods used a relatively complicated extraction procedure,¹⁰⁾ had a low sensitivity¹²⁾ and relatively longer analytical time (more than 10 min).¹³⁾ Previous analysis methods of chlorthalidone had a low sensitivity and used a relatively large injection volume.⁴⁾ Therefore, this study was to improve and validate a simple and rapid HPLC method with fluorescence/UV detection for assessing the pharmacokinetics of atenolol and chlorthalidone in Korean subjects in a routine setting.

Experimental Methods

HPLC analysis

Chemicals and reagents—Atenolol (> 99.5% purity) and pindolol (internal standard, 99.8% purity, Figure 1) were purchased from Sigma-aldrich (St. Louis, MO, U.S.A.). Chlorthalidone (> 99.5% purity) and probenecid (internal standard, 99.8% purity, Figure 2) were also purchased from Sigma-aldrich (St. Louis, MO, U.S.A.). Methanol and acetonitrile (HPLC grade) were purchased from Fischer Scientific (Fair Lawn, NJ, USA), and other chemicals were of HPLC grade or the highest quality available. HPLC grade water was obtained from a Milli-Q water purification system (Millipore Co., Milford, MA, USA), and used throughout the study. The mobile phase components such as acetonitrile and methanol were filtered through a 0.2- μ m pore size membrane filter and deionized water was filtered through a 0.45- μ m pore size membrane filter prior to mixing, respectively. Phosphate buffer was prepared by mixing 39 mL of 0.2 M sodium dihydrogen orthophosphate, 61 mL of 0.2 M disodium hydrogen orthophosphate, 33.6 mL of 1 M sodium hydroxide and 66.4 mL of water.

Instruments and chromatographic condition—The HPLC system was from Shimadzu Corporation (Kyoto, Japan). For atenolol analysis, it consisted of two pumps (model LC-10AD), a degasser (model DGU-12A), and a fluorescence detector (model RF-10A_{XL}, emission: 300 nm, excitation: 224 nm). The separation was performed on a μ -Bondapak C18 column (10- μ m particle size, 3.9 \times 300 mm i.d.) at ambient temperature. The mobile phase was a mixture (30:70, v/v) of methanol and 0.01 M potassium dihydrogenphosphate adjusted to pH 3.5, and was prepared daily. The flow rate was 1.0 mL/min for a total running time of 10 min. Detector output was quantified on a model Class LC-10 integrator (Shimadzu, Kyoto, Japan).

For chlorthalidone analysis, it consisted of two pumps (model LC-10AD), a degasser (model DGU-12A), and a UV detector (model SPD-10A_{vp}) set at 214 nm. The separation was performed on a Luna C18 column (5- μ m particle size, 4.6 \times 250 mm i.d.) at ambient temperature. The mobile phase was a mixture of acetonitrile containing 77% 0.01 M sodium acetate, and was prepared daily. The flow rate was 1.0 mL/min for a total running time of 25 min. Detector output was quantified on a model Class LC-10 integrator (Shimadzu, Kyoto, Japan).

Stock solutions and standards—For atenolol analysis, stock solutions of atenolol (1000 μ g/mL) was prepared in distilled water, and stock solution of pindolol (5 μ g/mL) was pre-

pared in sulfuric acid (0.05 M). These solution stored in light-protected glass bottle at 4°C. Serum calibration standards of atenolol were prepared at concentrations of 10, 20, 50, 100, 200, 500 and 1000 ng/mL in drug-free pooled serum obtained from twelve different volunteers. In the same manner, quality control (QC) serum samples at low (20 ng/mL), medium (100 ng/mL), and high (500 ng/mL), were prepared to evaluate accuracy and precision. All solutions and samples of atenolol and pindolol were protected against light during manipulations.

For chlorthalidone analysis, stock solutions of chlorthalidone (1000 µg/mL) and probenecid (10 µg/mL) were prepared in methanol, and stored in light-protected glass bottle at 4°C. Whole blood calibration standards of chlorthalidone were prepared at concentrations of 0.05, 0.1, 0.2, 0.5, 2, 5, 10 and 20 µg/mL in drug-free pooled whole blood obtained from twelve different volunteers. In the same manner, quality control (QC) whole blood samples at low (0.1 µg/mL), medium (0.5 µg/mL), and high (5 µg/mL), were prepared to evaluate accuracy and precision. All solutions and samples of chlorthalidone and probenecid were protected against light during manipulations.

Extraction procedures – To 0.5 mL of atenolol calibration standard sample, 100 µL of pindolol solution (5 µg/mL) was added and vortex-mixed. After vortexing, 4 mL of ethyl acetate was added. The mixture was centrifuged at 3,000 rpm for 10 min. The supernatant was separated and evaporated to dryness under a nitrogen stream in a centrifugal evaporator. The residue was reconstituted with 100 µL of methanol and then 20 µL of solution was injected into the HPLC system and the peak area and retention time were recorded.

To 200 µL of chlorthalidone calibration standard sample, 200 µL of perchloric acid (0.33 M) was added and vortex-mixed for 10 sec. After sonication for 5 min, 500 µL of phosphate buffer with sodium hydroxide was added and vortex-mixed for 10 sec. 100 µL of probenecid solution (10 µg/mL) was added and vortex-mixed for 10 sec. To this solution, 6 mL of *tert*-butyl methyl ether was added and vortex-mixed for 5 min. After vortexing, the mixture was centrifuged at 3,000 rpm for 10 min. The supernatant was separated and evaporated to dryness. The residue was reconstituted with 200 µL of mobile phase and vortexed for 1 min. The mixture was transferred into an eppendorf tube and centrifuged at 12,000 g for 2 min. Then 20 µL of supernatant was injected into the HPLC system.

HPLC method validation

Specificity – The interference of endogenous compounds in blood sample was assessed by analyzing standard atenolol and chlorthalidone, drug-free blood sample, blood sample spiked with atenolol and chlorthalidone, and blood samples obtained

from subjects given atenolol and chlorthalidone combination tablet.

Sensitivity – The lower limit of quantitation (LLOQ) was defined as the lowest concentration yielding a precision with less than 20% CV, and accuracy between 80 and 120% of the theoretical value. The LLOQ was 10 ng/mL for atenolol and 0.05 µg/mL for chlorthalidone in five replicates.

Linearity – The linearity of the calibration curve for atenolol and chlorthalidone was assessed in the range of 10-1000 ng/mL in serum and in the range of 0.05-20 µg/mL in whole blood, respectively. Samples were quantified using the ratio of peak area of analyte to that of I.S.. Peak area ratios were plotted against serum or blood concentrations, and the straight-line regression equation was presented with its correlation coefficient (*r*).

Precision and accuracy – In order to assess the intra- and inter-day precision and accuracy of the assay, QC samples were prepared as described above. The intra-day precision of the assay was assessed by calculating the coefficients of variation (CV) for the analysis of QC samples in five replicates, and inter-day precision was determined by the analysis of QC samples on five consecutive days. Accuracy was determined by comparing the calculated and known concentrations. The evaluation of precision was based on the criteria that relative standard deviation for each concentration level should not be more than ±15% except for the LLOQ, for which it should not exceed ±20%. Similarly, for accuracy, the mean value should not deviate by ±15% of the nominal concentration except for the LLOQ, where the limit was ±20%.

Pharmacokinetic study of atenolol and chlorthalidone combination therapy

Subjects – Twenty-four healthy Korean volunteers, 8 subjects per each institute, participated in the pharmacokinetic study of atenolol and chlorthalidone combination therapy. Their average age was 22.75 ± 2.29 years (mean ± S.D., range: 19-28 years), body weight was 67.61 ± 11.53 kg (range: 45.6-93.0 kg), and mean height was 173.93 ± 7.36 cm (range: 153.2-184.0 cm). All subjects were selected after passing a clinical screening procedure including a physical examination and laboratory tests (blood analysis; hemoglobin, hematocrit, RBC, WBC, platelet, differential counting of WBC, total proteins, albumin, sGOT, sGPT, alkaline phosphatase, total bilirubin, cholesterol, creatinine, blood urea nitrogen, and glucose fasting and urine analysis; specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC and cast). These measures were necessary to ensure that the existing degree of variation would not be due to an influence of disease or other medications. All sub-

jects were excluded if they were possibly sensitive to this type of preparations, had a history of any illness of hepatic, renal or cardiovascular systems, or had taken alcohol or other preparations within 4 weeks prior to the study. Informed consent was obtained from the subjects after explaining the nature and purpose details of the study in accordance with Korean Guideline for Bioequivalence Test.¹⁴⁾ The study protocol was approved by each Institutional Review Board (IRB). This study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of Good Clinical Practice.

Study design—After overnight fasting, each subject received a single oral dose of fixed combination of atenolol and chlorthalidone (one Hyundai Tenoretic[®] tablet, atenolol 50 mg, chlorthalidone 12.5 mg) with 240 mL of water. Food was taken after 4 h, and consumption of alcohol or xanthine containing foods and beverages were restricted during the study.

Sampling was identical for all the subjects. Blood samples were collected in two vacutainer tubes before dosing and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96 and 120 h after drug administration. Following centrifugation (3,000 rpm, 20 min, 4°C), serum samples for atenolol analysis were transferred to polyethylene tubes and immediately stored at -80°C until analysis. The whole blood samples for chlorthalidone analysis were stored at -80°C until analysis. Care was taken against too much exposure to light. This study was sequentially performed at three institutes with an identical protocol. The serum and whole blood samples were analyzed for atenolol and chlorthalidone content by the proposed HPLC methods respectively.

Pharmacokinetic analysis—Pharmacokinetic parameters were calculated by non-compartmental analysis of serum con-

centration-time curve data using WinNonlin software (Pharsight Corporation, CA, USA).¹⁵⁾ The peak concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined from individual serum and whole blood concentration-time profiles for atenolol and chlorthalidone respectively. The area under the serum or whole blood concentration-time curve (AUC_{0-t}) was calculated by the linear trapezoidal rule from 0 to 120 h. The area under the serum or whole blood concentration-time curve from zero to time infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t/\lambda_z$, where C_t is the last measurable concentration. The terminal half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$, where λ_z is terminal rate constant.

Results and Discussion

Chromatography

Serum chromatograms of atenolol are shown in Figure 3C. The retention times for atenolol and I.S. were 3.8 and 6.5 min, respectively. The μ -Bondapak C18 column with the mobile phase of methanol and 0.01 M potassium dihydrogenphosphate (pH 3.5) resulted in the short chromatographic run time (10 min), which was more shorter than that (15 min) reported by Leite *et al.*¹³⁾ The mobile phase used for the assay was of very simple composition, was inexpensive, and achieved optimal resolution of atenolol and the I.S. with no interference from other components in serum.

Whole blood chromatograms of chlorthalidone are shown in Figure 4C. The retention times for chlorthalidone and I.S. were 13.6 and 17.3 min, respectively. The Luna C18 column with the mobile phase of acetonitrile containing 77% 0.01 M sodium acetate resulted 25 min in the chromatographic run

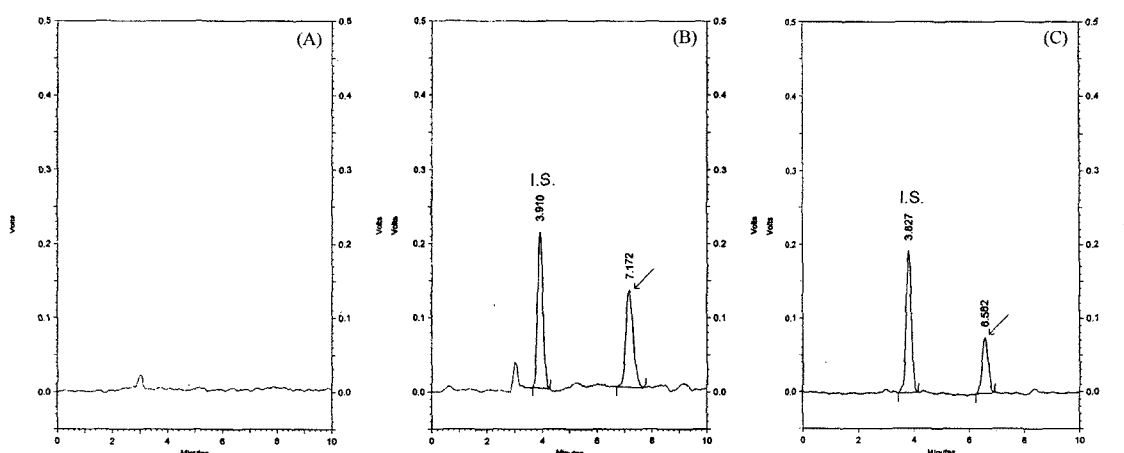


Figure 3—Chromatograms of (A) blank human serum, (B) blank human serum spiked with atenolol (200 ng/mL) and internal standard (I.S., pindolol 5 µg/mL) and (C) serum sample (384.39 ng/mL) at 2 h after oral administration of Hyundai Tenoretic[®] tablet (atenolol 50 mg and chlorthalidone 12.5 mg). \sphericalangle =atenolol peak.

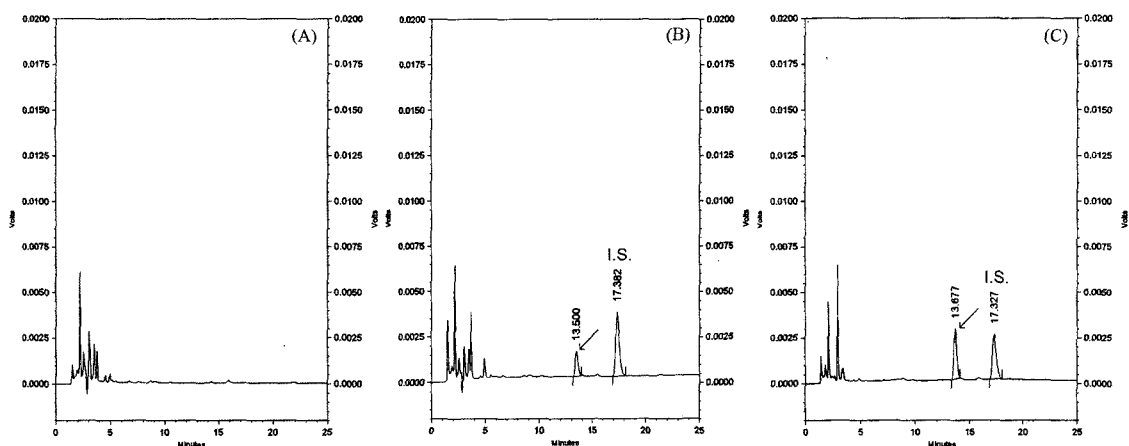


Figure 4—Chromatograms of (A) blank human whole blood, (B) blank human whole blood spiked with chlorthalidone (0.2 µg/mL) and internal standard (I.S., probenecid 10 µg/mL) and (C) whole blood sample (0.56 µg/mL) at 2 h after oral administration of Hyundai Tenoretic[®] tablet (atenolol 50 mg and chlorthalidone 12.5 mg). ✓=chlorthalidone peak.

time. The mobile phase used for the assay was of very simple composition, was inexpensive, and achieved optimal resolution of chlorthalidone and the I.S. with no interference from other components in whole blood.

HPLC method validation

Specificity—Figure 3 shows representative chromatograms of the human serum with extraction using the ethyl acetate. No peaks interfered with atenolol and I.S. at their retention times in blank human serum (Figure 3).

Figure 4 shows representative chromatograms of the human whole blood with extraction using the *tert*-butyl methyl ether. No peaks interfered with chlorthalidone and I.S. at their retention times in blank human whole blood (Figure 4).

Sensitivity—The lower limit of quantitation (LLOQ) was defined as those quantities that were 10-fold of the background noise, with precision errors of less than 20% (CV), and inaccuracy between ±20% (bias).

The LLOQ for 500 µL of human serum was found to be 10

ng/mL for atenolol injected on-column with a 20 µL loop. The mean percent accuracy value for serum samples was 105.99% and precision coefficient of variation (CV) was below 10.62% at the LLOQ (Table I). The LLOQ value for serum was lower than that reported by Wolf-Coporda *et al.*¹²⁾

The LLOQ for 200 µL of human whole blood was found to be 0.05 µg/mL for chlorthalidone injected on-column with a 20 µL loop. The mean percent accuracy value for whole blood samples was 117.43% and precision coefficient of variation (CV) was below 14.63% at the LLOQ (Table II). The LLOQ value for whole blood was lower than that reported by Rosenberg *et al.*⁴⁾ Furthermore, that method used a relatively large injection volume.⁴⁾

Linearity—The calibration curves for atenolol were linear over the concentration range of 10–1000 ng/mL in human serum. The mean regression equations from nine replicate calibration curves on different days for human serum at three different laboratories: $y=0.00557x-0.00818$ at 1st institute; $y=0.00988x+0.00658$ at 2nd institute; $y=0.00693x-0.00075$ at

Table I—Precision and Accuracy for the Determination of Atenolol in Human Serum at Each Institute

Concentration (ng/mL)	Precision CV (%)						Accuracy (%)					
	Intra-day (n=5)			Inter-day (n=5)			Intra-day (n=5)			Inter-day (n=5)		
	Institutes											
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
10 (LLOQ)	12.25	13.88	4.43	10.76	10.68	6.89	100.86	86.46	90.55	102.66	95.57	91.99
20 (low)	7.44	11.03	4.89	12.71	10.09	4.60	90.83	94.85	92.17	103.39	93.84	97.94
100 (medium)	5.63	8.73	3.21	7.17	13.90	2.35	91.41	97.92	101.70	101.11	115.12	100.11
500 (high)	0.88	7.97	4.24	8.56	13.40	3.33	91.82	100.19	98.64	108.12	89.50	101.29

Table II—Precision and Accuracy for the Determination of Chlorthalidone in Human Blood at Each Institute

Concentration ($\mu\text{g/mL}$)	Precision CV (%)						Accuracy (%)					
	Intra-day (n=5)			Inter-day (n=5)			Intra-day (n=5)			Inter-day (n=5)		
	Institutes											
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.05 (LLOQ)	8.78	15.92	13.89	11.90	4.28	7.10	119.77	89.75	102.78	113.98	91.86	116.69
0.1 (low)	7.77	13.92	9.70	9.25	5.99	12.43	112.67	80.82	105.48	110.50	88.17	108.19
0.5 (medium)	4.11	11.40	7.89	10.26	11.50	12.36	103.81	80.76	100.46	94.70	95.86	103.62
5 (high)	11.17	14.84	6.26	7.86	8.88	8.71	101.63	102.45	99.00	97.93	106.80	97.79

3rd institute (where, y =peak area ratio, x =concentration), showed significant linearities ($r=0.9999$, $r=0.9986$, $r=0.9999$, respectively), with statistically insignificant ($P>0.01$) nonlinear elements in the residual sum of squares, as determined by analysis of variance.

The calibration curves for chlorthalidone were linear over the concentration range of 0.05–20 $\mu\text{g/mL}$ in human whole blood. The mean regression equations from nine replicate calibration curves on different days for human whole blood at three different laboratories: $y=1.383x-0.02282$ at 1st institute; $y=4.75797x-0.02948$ at 2nd institute; $y=2.5882x-0.00101$ at 3rd institute (where, y =peak area ratio, x =concentration), showed significant linearities ($r=0.9999$, $r=0.9996$, $r=0.9999$, respectively), with statistically insignificant ($P>0.01$) nonlinear elements in the residual sum of squares, as determined by analysis of variance.

Precision and accuracy—Table I shows a summary of intra- and inter-day precision and accuracy for atenolol. In the range of 10–1,000 ng/mL, intra- and inter-day accuracy ranged from 90.50 to 100.86%, and 101.11 to 113.69%, respectively. The intra- and inter-day assay precision (CV) ranged from 0.88 to 12.25%, and 5.26 to 12.71%, respectively.

Table II shows a summary of intra- and inter-day precision and accuracy for chlorthalidone. In the range of 0.05–20 $\mu\text{g/mL}$, intra- and inter-day accuracy ranged from 98.67 to 119.77%, and 94.70 to 113.98%, respectively. The intra- and inter-day assay precision (CV) ranged from 4.11 to 11.17%, and 4.52 to

12.14%, respectively.

These results indicate that the present method has a satisfactory accuracy, precision, and reproducibility.

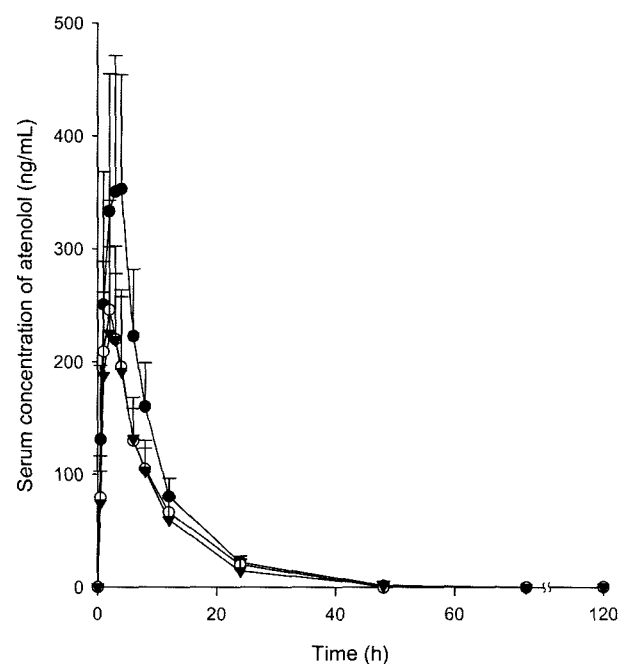


Figure 5—Mean (\pm S.D.) serum concentration-time curves of atenolol for each institute following oral administration of Hyundai Tenoretic® tablet (atenolol 50 mg and chlorthalidone 12.5 mg). Keys: ●; 1st institute (n=8), ○; 2nd institute (n=8), ▼; 3rd institute (n=8).

Table III—Pharmacokinetic Parameter Values of Atenolol for Each Institute Obtained after Oral Administration of Fixed Combination of Atenolol (50 mg) and Chlorthalidone (12.5 mg) (Mean \pm S.D.)

Parameters	1st Institute (n=8)	2nd Institute (n=8)	3rd Institute (n=8)	Total (n=24)
AUC_{0-t} (ng·h/mL)	3231.56 \pm 905.26	2185.16 \pm 521.45	1988.30 \pm 584.37	2468.34 \pm 864.15
$AUC_{0-\infty}$ (ng·h/mL)	3386.16 \pm 872.62	2382.51 \pm 553.30	2190.82 \pm 596.02	2653.16 \pm 848.38
C_{max} (ng/mL)	389.95 \pm 120.67	266.67 \pm 96.55	271.20 \pm 63.00	309.27 \pm 108.98
T_{max} (h)	2.88 \pm 0.99	2.00 \pm 0.76	2.63 \pm 1.06	2.50 \pm 0.98
$t_{1/2}$ (h)	5.91 \pm 1.21	6.77 \pm 0.80	5.94 \pm 1.10	6.21 \pm 1.09

Pharmacokinetics of atenolol and chlorthalidone combination therapy

Pharmacokinetic results—All subjects completed the study and well tolerated the protocol.

Atenolol pharmacokinetic parameters for each institute are provided in Table III. Figure 5 shows the mean (\pm S.D.) serum concentration-time curves of atenolol in twenty-four subjects. Atenolol was not determined after 72 h. Mean values (\pm S.D.) of C_{max} , T_{max} , $AUC_{0-\infty}$ and $t_{1/2}$ were 309.27 ± 108.98 ng/mL, 2.50 ± 0.98 h, 2653.16 ± 848.38 ng·h/mL and 6.21 ± 1.09 h, respectively, and were similar to reported values, T_{max} (2-4 h) and $t_{1/2}$ (6-7 h).³⁾

Chlorthalidone pharmacokinetic parameters for each institute are provided in Table IV. Figure 6 shows the mean (\pm S.D.)

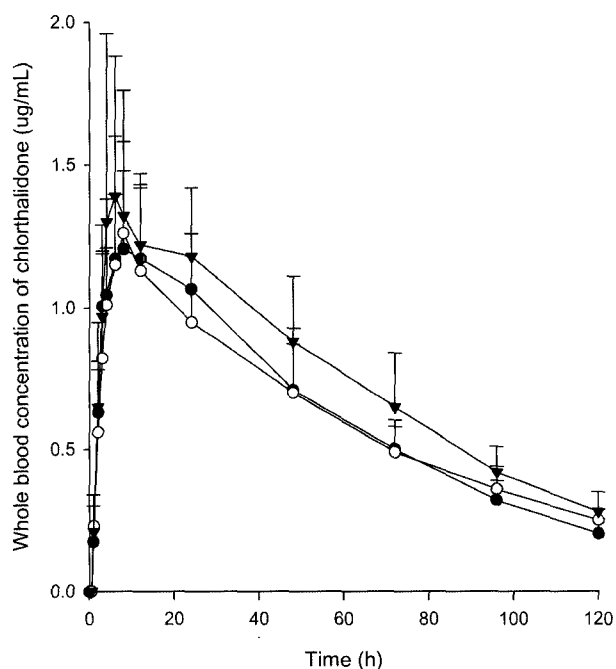


Figure 6—Mean (\pm S.D.) whole blood concentration-time curves of chlorthalidone for each institute following oral administration of Hyundai Tenoretic® tablet (atenolol 50 mg and chlorthalidone 12.5 mg). Keys: ●; 1st institute (n=8), ○; 2nd institute (n=8), ▼; 3rd institute (n=8).

Table IV—Pharmacokinetic Parameter Values of Chlorthalidone for Each Institute Obtained after Oral Administration of Fixed Combination of Atenolol (50 mg) and Chlorthalidone (12.5 mg) (Mean \pm S.D.)

Parameters	1st Institute (n=8)	2nd Institute (n=8)	3rd Institute (n=8)	Total (n=24)
AUC_{0-t} ($\mu\text{g} \cdot \text{h/mL}$)	77.10 ± 13.36	75.52 ± 20.82	91.56 ± 14.53	81.39 ± 17.46
$AUC_{0-\infty}$ ($\mu\text{g} \cdot \text{h/mL}$)	88.91 ± 14.69	92.99 ± 21.07	114.48 ± 27.63	98.79 ± 23.76
C_{max} ($\mu\text{g/mL}$)	1.26 ± 0.27	1.27 ± 0.49	1.71 ± 0.56	1.41 ± 0.49
T_{max} (h)	10.50 ± 5.93	9.00 ± 1.85	9.13 ± 6.62	9.54 ± 5.06
$t_{1/2}$ (h)	38.50 ± 7.90	49.07 ± 10.13	51.23 ± 32.10	46.27 ± 19.90

whole blood concentration-time curves of chlorthalidone in twenty-four subjects. Mean values (\pm S.D.) of C_{max} , T_{max} , $AUC_{0-\infty}$ and $t_{1/2}$ were 1.41 ± 0.49 $\mu\text{g/mL}$, 9.54 ± 5.06 h, 98.79 ± 23.76 $\mu\text{g} \cdot \text{h/mL}$ and 46.27 ± 19.90 h, respectively, and were similar to reported values, T_{max} (about 10 h) and $t_{1/2}$ (40-60 h).¹⁶⁾

Conclusion

A rapid, selective and sensitive HPLC method was validated for the determination of atenolol in human serum and chlorthalidone in human whole blood. It used a simple solvent extraction and chromatography on reverse phase column. The present methods were successfully applied to a pharmacokinetic study of atenolol and chlorthalidone combination in human volunteers.

Acknowledgement

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