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Original Research Article

Pharmacokinetics of a telmisartan, amlodipine and hydrochlorothiazide fixed-dose combination: A replicate crossover study in healthy Korean male subjects

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Abstract

Purpose: *To compare the tolerability and pharmacokinetic profiles of telmisartan, amlodipine, and hydrochlorothiazide (HCTZ) in a fixed-dose combination (FDC, test product) with a co-administered telmisartan/amlodipine FDC and HCTZ in a single-entity tablet (reference product)*
Methods: *This was a single-dose, randomized, open-label, replicate crossover study conducted in healthy male Korean volunteers aged 19 – 50 years. Fasting randomized subjects received a newly developed test product (telmisartan/amlodipine/HCTZ, 80/10/25 mg) or two tablets of Twynsta® (40/5 mg) and one tablet of HCTZ (25 mg) as reference products. After a washout period, each group replicated the exposure of the other group.*
Results: *The AUC_{last} (h•ng/mL) geometric mean was 3,194.87 and 3,273.77 for the telmisartan test and reference products, respectively; 329.92 and 315.13 for the amlodipine test and reference products; 1,203.98 and 1,150.86 for the HCTZ test and reference products, respectively. The geometric mean of C_{max} (ng/mL) was 543.04 and 497.81 for the telmisartan test and reference products, respectively; 7.74 and 7.34 for the amlodipine test and reference products; 218.71 and 184.39 for the HCTZ test and reference products, respectively. For telmisartan, the 90 % CI of GMRs of AUC_{last} (h•ng/mL) and C_{max} (ng/mL) were 0.9414 – 1.0496 and 1.0246 – 1.2792, respectively; the coefficient of variation (CV) of telmisartan C_{max} was 41.96 %.*
Conclusion: *A formulated FDC tablet containing a telmisartan/amlodipine/HCTZ combination (80/10/25 mg) was bioequivalent to a co-administrated commercially available telmisartan/amlodipine combination and HCTZ tablets at equivalent concentrations.*

Keywords: *Fixed-dose combination, Hypertension, Telmisartan, Amlodipine besylate, Hydrochlorothiazide, Pharmacokinetics*

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INTRODUCTION

Multiple guidelines exist for the treatment of hypertension. The goal of hypertension treatment is usually to reduce blood pressure to the recommended range. A drug of a different class can be added to antihypertensive for patients who fail to achieve control in blood pressure [1].

Angiotensin II receptor blockers (ARBs) are currently the most popular class of drugs used in the treatment of hypertension. ARBs are considered good agents in terms of tolerability,

the convenience of a once-daily dose, and efficiency in lowering blood pressure. Telmisartan is an ARB that has high affinity to the AT1 receptor subtype and exhibits a long half-life [2-4]. Fixed-dose combinations (FDCs) containing telmisartan have been developed with the diuretic hydrochlorothiazide (HCTZ) or calcium channel blocker amlodipine as two active pharmaceutical ingredients [5-8].

A telmisartan-amlodipine combination treatment resulted in clinically relevant blood pressure reduction, and was well tolerated with good compliance [8]. Furthermore, compared with monotherapy in patients with previously uncontrolled blood pressure, the addition of HCTZ to telmisartan has been associated with effective blood pressure reduction, as well as with improved hypertension goal-attainment rates [9].

Amlodipine is an orally active, long-lasting dihydropyridine calcium channel blocker (CCB) used to treat hypertension; the drug works by dilating blood vessels and is available in doses of 5 and 10 mg. Amlodipine is also used to treat certain types of chest pain [10].

HCTZ is a diuretic medication used to reduce the reabsorption of electrolytes from renal tubules, and is often prescribed to treat high blood pressure. It works by increasing urinary output and reducing the amount of fluid in the blood [7-9].

Combination therapy for hypertension can be effective by lowering blood pressure and reducing cardiovascular disease, such as stroke [11,12].

FDCs of antihypertensive agents with different modes of action provide many advantages in treatment while maintaining lower doses of each component drug. Other benefits of FDCs include improvement of compliance, as patients are only required to take one dosage form, and a lower cost of therapy [12-14].

To develop new dosage forms of FDCs, it is necessary to show that the FDC administered is therapeutically equivalent to the combined single-drug doses.

The comparison of therapeutic equivalence can be conducted by evaluating the pharmacokinetic parameters to investigate whether the rate and extent of absorption of individual FDC components are similar to those of the co-administered reference products.

The purpose of this clinical trial was to develop FDCs containing the three drugs, and evaluate the pharmacokinetic profiles after the single-dose and tolerability. The results of this study were used to demonstrate the bioequivalence between test and reference products.

METHODS

Materials

Telmisartan and hydrochlorothiazide were obtained from Ildong Pharmaceutical Co., Ltd (Seoul, Korea). Amlodipine besylate was purchased from HanseoChem Co., Ltd. (Pyeongtaek, Korea). HPLC grade acetonitrile, methanol, water were obtained from Merck Co. (Darmstadt, Germany). The test FDC tablets were supplied from Ildong Pharmaceutical Co., Ltd (Seoul, Korea). The reference tablets telmisartan/amlodipine (Twynsta® 40/5 mg) and hydrochlorothiazide (Dichlozid® 25 mg) were supplied from Boehringer Ingelheim (Ingelheim, Germany) and Yuhan Co. (Seoul, Korea), respectively.

Study subjects

This human pharmacokinetic study was conducted after ethical approval (ref no. CUH-2015-09-019-011, ChonBuk National University Hospital, Jeonju, Korea); all volunteers gave written informed consent to participate in this clinical trial, which was conducted in compliance with Korean GCP [15] and the Declaration of Helsinki [16].

Healthy male subjects aged between 19 to 50 years with > 55 kg body weight and BMI (Body mass index) >17.5 (30.5 kg/m^2) were eligible for participation in this clinical trial. Volunteers with the following history were excluded; cardiovascular, pulmonary, renal, gastrointestinal, endogeneous, or hematologic disease; clinical issues during the laboratory tests or ECG; a history of hypersensitive response to telmisartan, amlodipine, or HCTZ, or the experience of taking one of these ingredients within 10 days of beginning the trial; blood pressure lower than 100/60 or higher than 150/100.

Study design

This trial was a randomized, open-label, single-dose, replicate crossover study in healthy male volunteers. Forty subjects were randomized to two groups. For a pharmacokinetic study of an orally administered single dose, subjects received a newly developed FDC tablet

containing a telmisartan/amlodipine/HCTZ combination (80/10/25 mg, test products) or two tablets of Twynsta® (a telmisartan/amlodipine combination, 40/5 mg) and one tablet of HCTZ (25 mg) (reference products) over the first period. Over the second period, each group received the opposite regimen; the periods were separated by a 21-day washout period. After the second period, the test was replicated with the same washout period.

FDC tablets of telmisartan and amlodipine were used instead of single-entity products as the reference items. Bioequivalence has been reported between a telmisartan/amlodipine 40/5 mg combination (lowest strength) and a telmisartan/amlodipine 80/10 mg combination (highest strength) [17]. Because 80/10 mg Twynsta® tablets are not marketed in Korea, two tablets of 40/5 mg Twynsta® were substituted as the reference items.

After fasting for 10 h, the subjects took the test or reference items with 150 mL of water. Standard meals were provided at 4^{th} and 10^{th} h after administration.

Subjects were forbidden from eating grape fruits 7 days before the first period through the last sampling in the fourth period because a number of calcium channel blockers can interact with the CYP3A4 metabolic enzyme [18].

Blood sampling

For pharmacokinetic analysis, blood were collected before administration and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, 96, and 144 h. Seven milliliters of blood were collected into EDTA tubes. Only 5 mL was collected at 72 h and 3 mL at 96 and 144 h. Plasma was prepared by centrifugation of the blood samples at 3,000 rpm for 10 min at 4 °C. Samples were stored at −70 °C in polypropylene tubes until analysis.

Pharmacokinetic analysis

Analysis of each drug was performed at Biosuntek Laboratories Co. (Seongnam, Korea), which is certified by the MFDS as employing GLP. The methods used have been validated as standard operating procedures and followed MFDS guidelines on bioanalytical method validation [19].

The concentration of telmisartan, amlodipine, and HCTZ in plasma were analyzed by validated LC-MS/MS methods; the pharmacokinetic parameters of each drug were assessed by WinNonlin software (Pharsight, Sunnyvale, CA, USA), including AUC_{last} (h•ng/mL), C_{max} (ng/mL), AUC_{inf} (h•ng/mL), T_{max} (h), and $t_{1/2}$ (h).

Samples were stored at −70 °C until assay and mixed with an internal standard after thawing. Each component was extracted from the plasma using protein precipitation. The liquid chromatographic method used for separation was isocratic.

In the telmisartan assay, the mobile phase consisted of a mixture of ACN (Acetonitrile, A) with 5 mM ammonium formate(B) (A:B=7:3) with a flow rate of 0.25 mL/min and column temperature of 40 °C. Detection was conducted with a positive electrospray ionization multiple reaction monitoring mode set to transmit at *m/z* 515.3 → 276.2 and 441.0 → 263.2 for telmisartan and the internal standard, respectively [20,21].

In the amlodipine assay, the mobile phase consisted of a mixture of ACN(A) with 0.1 % formic acid in water(B) (1:1) with a flow rate of 0.3 mL/min and a column temperature of 40 °C. Detection was conducted with a positive electrospray ionization multiple reaction monitoring mode set to transmit at *m/z* 409.4 → 238.1 and 413.4 → 238.1 for amlodipine and the internal standard, respectively [22,23].

In the HCTZ assay, the mobile phase consisted of a mixture of water and methanol (15:85), with a flow rate of 0.3 mL/min and a column temperature of 40 °C. Detection was conducted with a negative electrospray ionization multiple reaction monitoring mode set to transmit at *m/z* 296.0 → 269.0 and 338.2 → 78.1 for HCTZ and the internal standard, respectively [21,24].

As the primary parameters for establishing bioequivalence, AUC_{last} and C_{max} were evaluated for all components, and AUC_{inf}, T_{max}, $t_{1/2}$, CL/F, and Vd/F as secondary parameters.

Tolerability assessment

Tolerability was assessed from a medical review of adverse events (AEs), clinical laboratory evaluation, vital sign measurements, physical examinations, and electrocardiograms. The subjects who had an experience of taking investigational products at least once were targets of the evaluation.

Statistical analysis

All participants who had an experience of taking

the investigational products were used in statistical analysis of tolerability and only those who finished the study were used in the analysis of pharmacokinetic parameters. The parameters were calculated as the geometric mean.

Establishing bioequivalence according to MFDS regulations was conducted as an estimate of the 90 % CIs of primary parameters (AUC_{last} and C_{max}) for all drugs.

RESULTS

Demographic profile of study subjects

Table 1 displays the demographic characteristics of the 40 male subjects in this study (mean age, 24.25 years; BMI, 23.71 kg/m^2; weight, 72.94 kg).

Six subjects were excluded when they withdrew consent, one was excluded due to an AE (epigastric pain, diarrhea), and one due to concomitant medication. Thus, 32 subjects completed the study (Figure 1).

Pharmacokinetics

The pharmacokinetic parameters of telmisartan, amlodipine, and HCTZ were derived by non-compartmental methods from plasma concentration time curves. The mean plasma concentrations of each drug were similar between the test and reference items; pharmacokinetic parameters are summarized in Table 2.

For telmisartan, the 90 % CIs of the GMRs of AUC_{last} (h•ng/mL), C_{max} (ng/mL) were 0.9414 - 1.0496, 1.0246 – 1.2792 and CV (Coefficient of variation) of C_{max} for telmisartan was 41.96 %. The MFDS has been defined as a highly variable drug (HVD) that has a 30 % or greater intra-subject variability of the C_{max}. The bioequivalent acceptance range of 90% CIs of C_{max} for telmisartan was 0.7363 – 1.3580.

The 90 % CIs of the GMRs for all components were summarized in Table 3 satisfying the MFDS criteria for evaluation of bioequivalence. In previous pharmacokinetic studies, the variation of intra-subjects for telmisartan, amlodipine besylate and HCTZ were similar to this study [25,26].

Figure 2 shows the mean plasma concentration-time profiles for telmisartan, amlodipine and HCTZ and the test and reference items did not show noticeable difference in T_{max} or $t_{1/2}$.

Tolerability

All subjects did not show any serious or unexpected AEs and were considered to be well tolerated. Overall, 40 subjects received FDCs or reference products and 8 subjects experienced 11 AEs. Six AEs were recorded in four subjects who were administered FDCs, and five AEs in four subjects who were administered reference products.

Two AEs were moderate (upper abdominal pain, diarrhea), and the other cases were mild and considered related to the investigational items.

DISCUSSION

In this clinical study, pharmacokinetics profiles and tolerability of newly developed FDCs were investigated in Korean male subjects and compared with co-administration of each reference item.

The pharmacokinetic parameters of telmisartan, amlodipine, and HCTZ were similar profiles between the test and reference product groups in a replicate crossover study design.

Table 1: Demographic characteristics of healthy male subjects

Variable	Statistics	Sequence 1 (N=20)	Sequence 2 (N=20)	All subjects (N=40)
Sex (male)	N	20	20	40
Age (years)	Mean	24.10	24.40	24.25
BMI (kg/m^2)	Mean	24.21	23.20	23.71
Height (cm)	Mean	176.40	173.87	175.14
Weight (kg)	Mean	75.55	70.34	72.94
Alcohol (yes)	N (%)	14 (70.0)	17 (85.0)	31 (77.5)
Smoker (yes)	N (%)	10 (50.0)	6 (30.0)	16 (40)
Caffeine (yes)	N (%)	12 (60.0)	12 (60.0)	24 (60)

BMI = body mass index

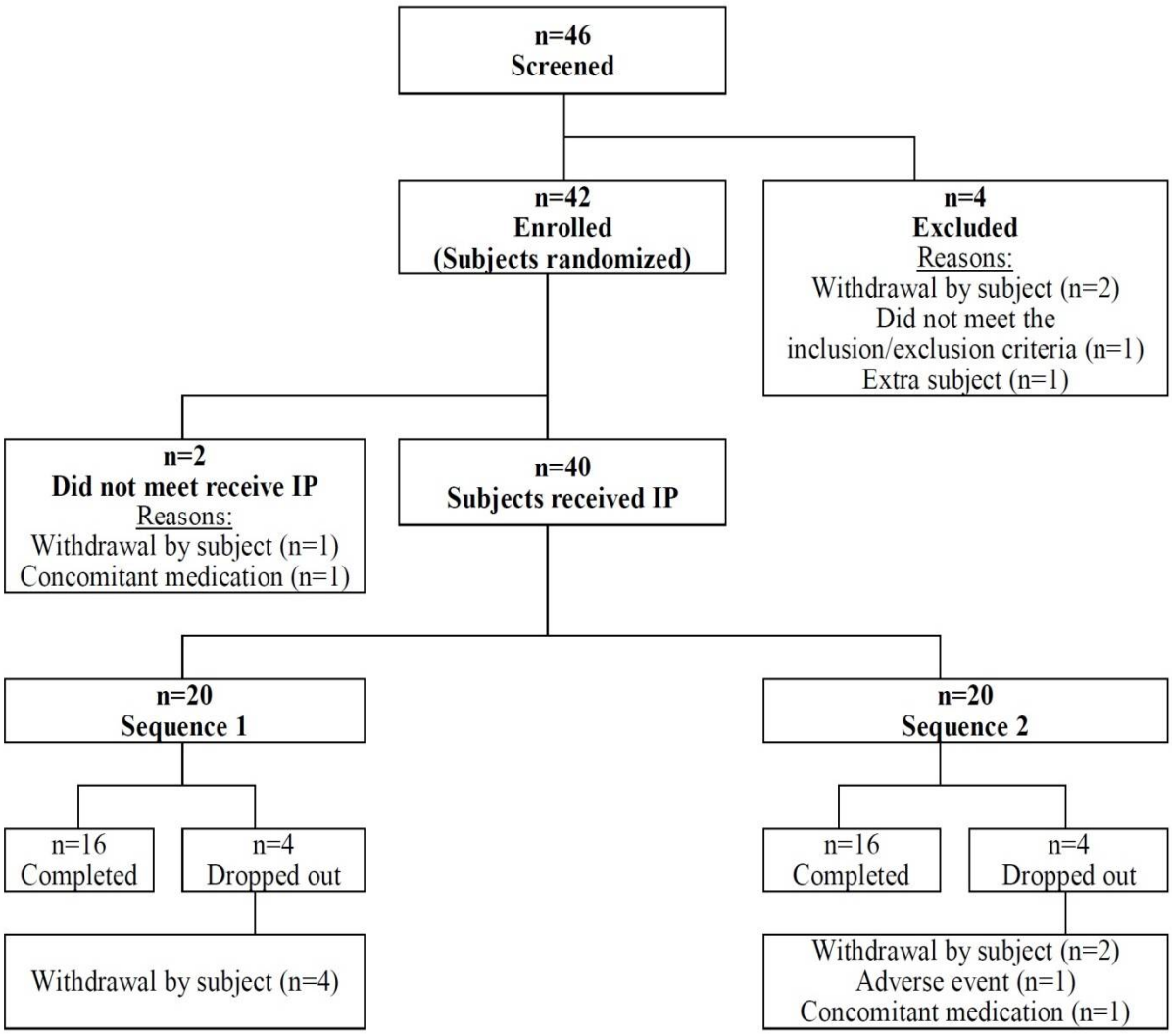

Figure 1: Summary of enrolled subjects (IP, investigational products)

Table 2: Comparison of the single-dose pharmacokinetic parameters of telmisartan, amlodipine, and hydrochlorothiazide (HCTZ) after administration of a fixed-dose combination (FDC, Test) vs. co-administration of reference products (reference)

Component	Parameter	Geometric mean		Geometric mean ratio
		Test	*Reference*	
Telmisartan	AUC_{last} (h•ng/mL)	3,194.87	3,273.77	0.98
	C_{max} (ng/mL)	543.04	497.81	1.09
	AUC_{inf} (h•ng/mL)	3,596.92	3,668.19	0.98
	T_{max} (h)	1.17	1.55	0.75
	$t_{1/2}$ (h)	23.56	20.93	1.13
Amlodipine	AUC_{last} (h•ng/mL)	329.92	315.13	1.05
	C_{max} (ng/mL)	7.74	7.34	1.05
	AUC_{inf} (h•ng/mL)	362.22	347.04	1.04
	T_{max} (h)	6.06	5.66	1.07
	$t_{1/2}$ (h)	39.88	40.35	0.99
HCTZ	AUC_{last} (h•ng/mL)	1,203.98	1,150.86	1.05
	C_{max} (ng/mL)	218.71	184.39	1.19
	AUC_{inf} (h•ng/mL)	1,233.15	1,185.05	1.04
	T_{max} (h)	1.45	2.12	0.68
	$t_{1/2}$ (h)	10.22	10.01	1.02

Table 3: Bioequivalence of fixed-dose combination (FDC) and reference products

Component	Parameter	Geometric least-squares (LS) mean		90% confidence interval of geometric LS mean ratio	Coefficient of variation
		Test	***Reference***		
Telmisartan	AUC_{last} (h•ng/mL)	2,641.68	2,657.76	0.9414–1.0496	-
	C_{max} (ng/mL)	469.56	410.14	1.0246–1.2792	41.96
Amlodipine	AUC_{last} (h•ng/mL)	320.03	303.30	1.0185–1.0930	-
	C_{max} (ng/mL)	7.52	7.11	1.0170–1.1014	13.56
HCTZ	AUC_{last} (h•ng/mL)	1,178.30	1,118.56	1.0213–1.0864	-
	C_{max} (ng/mL)	210.59	178.35	1.1217–1.2428	15.73

However the T_{max} of telmisartan and HCTZ of test was slightly faster than co-administration of reference items, but this difference was not expected to have a significantly influence on overall absorption. Also, since the C_{max} and AUC are major evaluation criteria of bioequivalence by the MFDS, we did not consider parameter of T_{max} further.

The 90 % CIs of the GMRs for AUC_{last} and C_{max} of all drugs were satisfactory with regard to bioequivalence range 0.8 to 1.25 or 0.7363 – 1.3580. This finding from the bioavailability suggested that a replicate crossover study was well designed for newly developed FDCs especially with telmisartan.

In the tolerability, noticeable interaction between telmisartan, amlodipine and HCTZ has not been reported in previous research. Despite co-administration of the highest dose on the markets, serious and unexpected AEs were not different between the FDC groups and the reference product co-administration groups.

The findings from pharmacokinetic profiles and tolerability indicate that these FDCs can be expected to exhibit similar safety and efficacy as commercially marketed products. The newly developed FDCs can replace the co-administration of reference products expected improvement of compliance for patients.

To clear the result of safety and efficacy clinically, other studies will be needed in large scale hypertensive patients.

CONCLUSION

A developed FDC tablet containing telmisartan/amlodipine/HCTZ combination (80/10/25 mg) is bioequivalent to a co-administered commercially available telmisartan/ amlodipine combination and HCTZ tablets at equivalent concentrations. In this clinical study there were no serious or unexpected AEs and noticeable difference in the FDC groups and the reference product co-administration groups.

DECLARATIONS

Acknowledgement

This study was supported and monitored by Ildong Pharmaceutical Co, Ltd, which manufactures the FDCs, and was conducted by a qualified investigator. All coauthors approve of the contents of this manuscript and participated in reviewing it. The corresponding author contributed to the reference search, figure creation, and manuscript writing, and made the final decision on this submission.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

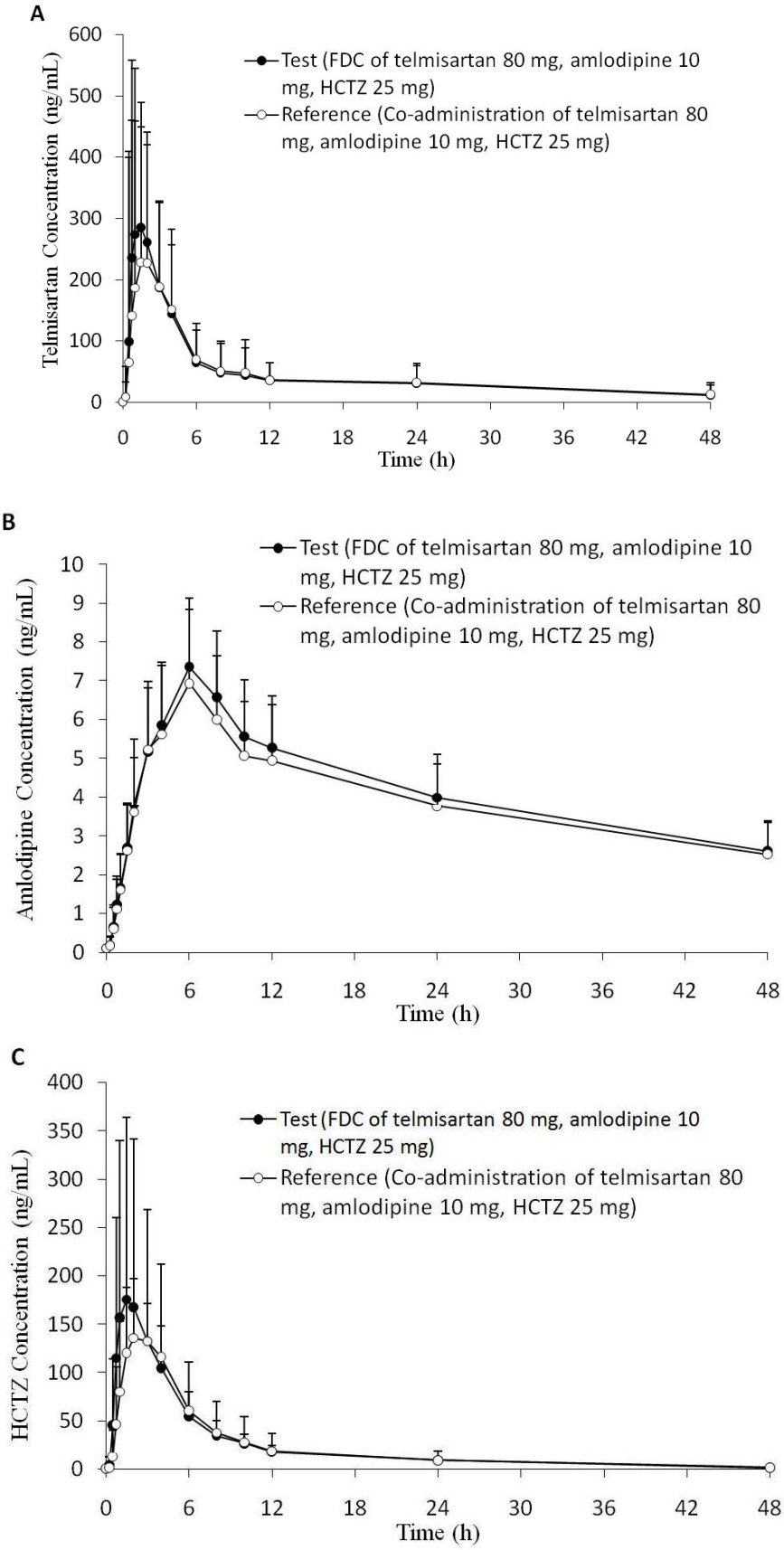

Figure 2: Mean (SD) plasma concentration-time profiles of (A) telmisartan, (B) amlodipine besylate, and (C) hydrochlorothiazide (HCTZ) after a single-dose administration of a fixed- dose combination (FDC) of telmisartan/amlodipine/HCTZ (80/10/25 mg) (Test) vs. co-administration of two tablets of telmisartan/amlodipine (40/5 mg) FDC and HCTZ (25 mg)

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