Bioequivalence of Two Brands of Valsartan 80 mg Coated Breakable Tablets in 15 Healthy Algerian Volunteers: A Pilot Study

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Abstract: A randomized, two-way, crossover study was conducted in 15 fasting, healthy, Algerian volunteers to compare the bioavailability of two brands of Valsartan 80 mg coated breakable tablets. The present study aimed to evaluate the intra-subject variability of this active substance in the Algerian population. The test brand was compared to TAREG (Novartis) as the reference product. The study was performed at the bioequivalence center of the national control laboratory for pharmaceuticals products, in joint venture with University Hospital Center Ibn Badis, Constantine, Algeria. The drug was administered with 200 mL of water after a 10 h overnight fasting on two treatment days separated by one week washout period. After dosing, serial blood samples were collected for a period of 24 h. A reliable, simple, and robust liquid chromatography-tandem mass spectro-metric (LC-MS/MS) method has been developed and validated that employs protein precipitation (or denaturation) for the estimation of valsartan in human plasma using losartan as internal standard. The assay was found to be linear over the range of 50-5,000 ng/mL, with a lower limit of quantitation of 50ng/mL. Various pharmacokinetic parameters including AUC_{0-t} , $AUC_{0-\infty}$, C_{max} , T_{max} , and $T_{1/2}$ were determined from plasma concentrations of both formulations and found to be in good agreement with reported values. The pharmacokinetic and statistical analysis was conducted with Kinetic 4.4.1. AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} were tested for bioequivalence after log-transformation of data. No significant difference was found based on ANOVA; 90% confidence interval ([85.82%, 118.76%] for AUC_{0-t} [86.09%, 118.83%] for $AUC_{0-\infty}$) of test/reference ratio for these parameters were found within bioequivalence acceptance range of 80-125%. But for the C_{max} , the 90% confidence interval of test/reference ratio wasn't in this acceptance range [90.18%, 131.07%]. The results of PK analysis suggested that the reference and test formulations of valsartan 80 mg coated breakable tablets weren't bioequivalent during fasting state in these healthy algerian volunteers.

Key words: Valsartan, bioequivalence, LC-MS/MS, pharmacokinetics.

1. Introduction

Bioequivalence of two formulations of the same drug includes equivalence with respect of the rate and extent of their absorption. The area under concentration time curve (AUC) generally serves as the characteristic of the extent of absorption while the peak concentration (C_{max}) and the time of its occurrence (T_{max}) reflect the rate of absorption [1].

Valsartan, a nonpeptide orally active angiotensin II receptor blocker with selectivity for the type 1 receptor subtype, is indicated for the treatment of hypertension [2]. The oral absorption of valsartan is rapid; T_{max} is 2

to 4 h [3]. The absolute bioavailability of oral valsartan has been reported to be 23%. Valsartan is extensively (94%-97%) bound to plasma proteins (principally albumin) [2, 4], plasma values decrease bi-exponentially, with a $t_{1/2}$ of 6-9 h. Elimination is mostly in the unchanged form, predominantly in bile (> 80%) and urine (< 20%) [4, 5].

Food was reported to decrease the exposure (as measured by AUC) to valsartan by 40% and C_{max} by 50%, although from 8 hours after administration, plasma valsartan concentrations were reported to be similar between the fed and fasted groups. This reduction in AUC was not, however, accompanied by a clinically significant reduction in the therapeutic effect.

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Thus, valsartan is labeled for administration with or without food [6].

Because of the low absorption and the high inter and intra individual variability of valsartan, it is necessary to perform comparative bioavailability studies [5, 7]. The aim of this study was to evaluate, in healthy Algerian volunteers, the bioavailability of a local generic valsartan formulation in order to evaluate the intrasubject variability of valsartan and to validate the application of the developed LC-MS/MS valsartan quantification method.

2. Materiel and Methods

2.1 Study Products

Two formulations of valsartan 80 mg were evaluated:

Reference formulation: TAREG® 80 mg coated breakable tablet (batch number 135,024, expiry date 05/2017 manufactured by Novartis).

Test formulation: valsartan 80 mg coated breakable tablet (batch number 5,063, expiry date 05/2018).

2.2 Study Subjects

16 healthy Algerian subjects (12 male and 04 female), suitable for a pilote study, were enrolled, but only 15 subjects completed the study, one voluntary withdrawal before the study with mean (SD) age, 28.93 (4.13) years (range 23-38); mean (SD) body weight, 80.75 (12.09) kg (range 46.1-90 kg); mean (SD) height, 1,746 (0,000854) m (range 1.55-1.87 m) and mean (SD)

BMI (body mass index), 22.14 (3.50) kg/m² (range 18.47-29.06 kg/m²).

The volunteers were screened by a complete clinical examination and laboratory tests (hematological, biochemical and urinary analysis and serological test) and were requested to be abstained from taking any medication for two weeks before and during the study, from taking vitamins two days prior the study, from taking grapefruit seven days before the study and from smoking, as well as consuming caffeine or drinks or foods containing xanthines related for 48 h prior to the study drug administration.

2.3 Ethical Consideration

This research was carried according to the Declaration of Helsinki (Seoul, 2008) and GCP (good clinical practice) Guidelines.

The study was conducted at National Control Laboratory for Pharmaceuticals Products (Algiers, Algeria) according to a protocol approved by Research Ethics Committee of University Hospital Center Issad Hassani (Beni Messous, Algiers) and by ministry of health. All the subjects provided written informed consent before entering the study.

2.4 Study Design

The study was based on a randomized, single dose, two way crossover designs under fasting condition with a washout period of one week.

In the morning of phase I and II, after an overnight

Fig. 1 Chemical structures of valsartan *N*-pentanoyl-*N*-{[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-*L*-valine and losartan (1-((2'-(1*H*-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-butyl-4-chloro-1*H*-imidazol-5-yl)methanol [8, 9].

fast (10 h) volunteers were given a single dose of either formulation (reference or test) of valsartan 80 mg with 200 mL of water. No food was allowed until 4 h after dose administration, lunch, snack and dinner were given to all volunteers according to a time schedule. The volunteers were continuously monitored by University Hospital Center Ibn Badis staff throughout the confinement period of the study.

2.5 Blood Sampling

Approximately, 04 mL of blood samples for Valsartan assay was obtained through an heparin-locked catheter before (0 h) and at 30', 1h, 1h30', 1h50', 2h10', 2h30', 2h50', 3h10', 3h30', 3h50', 4h10', 4h40', 5h10', 5h40', 6h40, 7h40', 9h40', 11h40', 13h40' et 24h after dosing. The blood samples were collected in glass tubes containing heparin, and centrifuged at 3,600 rpm for 10 min at 20 °C; plasma was separated and kept frozen at -80 °C in properly labeled tubes. After a period of 7 days the study was repeated in the same manner to complete the crossover design.

2.6 Optimization of MS Parameters and Chromatographic Conditions

An LC-MS/MS method was developed and validated for valsartan analysis in plasma samples. All solvents were HPLC grade, other chemicals and reagents were analytical grade. Valsartan (hydrochloride) and losartan (K⁺) (internal standard) are used as reference standards (Fig. 1).

The LC-MS/MS system consisted of: -HPLC Perkin Elmer SER 200 which contain an autosampler SER 200 and a binary pump (LC-200Q).

Masse spectrometer AB Sciex Instruments, 4,000 Q Trap triple quadrupole instrument equipped with an ESI source. Analyste 1.5.1 software was used for data interpretation.

The method was developed in negative mode with turbospray source (ESI) by infusion of 0.1 µg/mL aqueous solutions of valsartan and losartan reference

standards. The ion transitions m/z 434.1→179.2 and 421.1→127.1 were selected for the MRM of valsartan and losartan respectively. The compound parameters were optimized as follows: Declustering potential: -75 V and -85 V, entrance potential: -5 V and -9 V, collision cell exit potential: -1 V and -9 V, and collision energy: -37 V and -35 V for valsartan and losartan respectively. The source/gas parameters were optimized as follows: Curtain gas: 30, CAD: High, ion source gas-1: 60, ion source gas-2: 60, ion spray voltage: -4,500 V and temperature: 650 °C.

Chromatographic separation was performed using INNOVAL C18 (150 × 4.0) mm, 5 µm, 100 Å column at 40 °C. The mobile phase consisted of acetonitrile grade HPLC and 0.1% formic acid buffer. The mobile phase was eluted at a flow rate of 1.0 mL/min in gradient mode as shown in Table 1, and each analysis required 5 min. The retention time were 3.7 min and 4.2 min for losartan and valsartan respectively [10]. Quantitation was achieved by measurement of the peak area ratio of the drug to the internal standard, using analyst 1.5.1 software.

The method was validated by following international guideline [11]. The calibration curves were validated over the concentration range of 50-5,000 ng/mL for valsartan in human plasma in the low limit of quantification LLOQ of 50 ng/mL.

2.7 Sample Preparation

A 200 µL internal standard (losartan, 5 µg/mL) was added to 200 µL plasma sample, then 100 µL of formic acid 1 M was added with 200 µL of acetonitrile and vortexed for 30 seconds and then centrifuged for 10 min at 10,000g. The supernatant was transferred to a

Table 1 Gradient mode.

Temps (min)	Phase A (%)	Phase B (%)
0.0	80	20
0.5	80	20
2.5	05	95
4.8	05	95
5.0	80	20

vial. 10 μ L of each sample was injected into the LC-MS/MS for analysis [12].

The procedure described here was applied not only to subject's samples, but also to the extraction of samples for calibration curve and QC (quality control) process.

2.8 Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by means of a model independent method using a Kinetica 4.4.1 computer program [13]. The elimination rate constant (k_z) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. The elimination half-life ($T_{1/2}$) was calculated as $0.693/k_z$. The area under the curve to the last measurable concentration AUC_{0-t} was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C_t/k_z$, where C_t is the last measurable concentration.

2.9 Statistical Analysis

For the purpose of bioequivalence analysis, AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} were considered as primary variables. The bioequivalence of the two products was assessed by means of an analysis of variance (ANOVA GLM procedure; Kinetica 4.4.1 Computer program) for crossover design and calculating standard 90% confidence intervals of the ratio test/reference (T/R) using log-transformed data. The products were considered bioequivalent if the difference between the two compared parameters was found statistically insignificant ($p \geq 0.05$) and 90% confidence intervals for these parameters fell within 80%-125% [10, 11].

3. Results and Discussion

All 15 participants successfully completed both phases of the study with no protocol violations. No serious adverse drug reaction or side effects were reported by the participants or observed by investigators during the study periods.

The relationship between concentration and peak area ratio was found to be linear within the range of 50-5,000 ng/mL with LLOQ of 50 ng/mL. As shown in Table 2, the intraday accuracy of the method ranged from 86.6% to 107.5% while the intraday precision ranged from 2.11% to 8.17%. The interday accuracy ranged from 90.91% to 101.18% while the interday precision ranged from 2.36% to 8.65%.

This reproductibility of valsartan was able to increase assay sensitivity. Therefore, simple protein precipitation procedure using formic acid 1M and acetonitrile has been successfully applied to the extraction of valsartan from human plasma. The developed method using the centrifugation technique offers the advantages of a simple and a safe sample preparation procedure without the matrix effect and high throughput with uniformity of extraction which is a critical challenge in LC-MS/MS method development.

Stability studies showed that valsartan was stable in plasma for 7 weeks when stored at -80 °C. The method had a total analysis time of 5 min, which is favored to analyze the samples on a large scale.

Both formulations were rapidly absorbed from the gastrointestinal tract and valsartan was measurable at the first sampling time (0.5 h) in all the volunteers. The peak concentration of 3,105.33 ng/mL and 3,412 ng/mL for valsartan was attained at 2.86 h and 2.476 h after administration of reference and test products, respectively and then declined and remained detectable up until 24 h. The half life elimination was 5.71 h and 5.61 h for reference and test products respectively. These values agree with the bibliographic data [5, 7]. Table 3 shows the pharmacokinetic parameters of valsartan for the two brands.

The relative bioavailability of valsartan test was 100,958% for AUC_{0-t} , 101,151 % for $AUC_{0-\infty}$, and 108,72% for C_{max} .

The 90 % confidence limits for AUC_{0-t} , $AUC_{0-\infty}$, and C_{max} as well as the results of the Schuirmann's two onesided *t*-tests are also shown in Table 4.

Table 2 Precision and accuracy of valsartan in human plasma.

Concentration ng/mL	Precision (CV%)		Accuracy (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
50 (LLOQ)	8.17	8.65	107.5	101.18
150	3.25	4.54	86.9	100.08
1,000	2.11	6.37	88.2	90.91
3,500	3.11	2.36	86.6	92.47
Mean $\pm$ SD	4.16 $\pm$ 2.72	5.48 $\pm$ 2.67	92.3 $\pm$ 10.15	96.16 $\pm$ 5.22

LLOQ = lower limit of quantification; CV = coefficient of variation = (SD/mean)*100. All the data were presented as arithmetic means.

Table 3 Pharmacokinetic parameters of valsartan coated breakable tablets (arithmetic mean $\pm$ standard deviation, n = 15).

Pharmacokinetic parameter	Test	Reference
C_{max} (ng/mL)	3412 $\pm$ 1760.38	3105.33 $\pm$ 1442.89
SSC_{0-t} (ng·h/mL)	17342.03 $\pm$ 8603.03	16791.59 $\pm$ 6967.44
$SSC_{0-\infty}$ (ng·h/mL)	18027.56 $\pm$ 9054	17455.67 $\pm$ 7482.67
t_{max} (h)	2.47 $\pm$ 1.01	2.86 $\pm$ 1.27
$t_{1/2}$ (h)	5.61 $\pm$ 1.95	5.71 $\pm$ 1.50

Table 4 The statistical evaluation of bioequivalence after oral dosage of 80 mg valsartan of each formulation.

	Geometric mean $\pm$ SD		CI	CV_{intra}	<i>t</i> -test	
	Test	Reference			Lower	Upper
AUC_{0-t} (ng/mL·h)	9.66 $\pm$ 0.45	9.65 $\pm$ 0.40	[85.824%,118.76%]	25.51%	2,133	2,4619
$AUC_{0-\infty}$ (ng/mL·h)	9.69 $\pm$ 0.45	9.68 $\pm$ 0.41	[86.098%,118.83%]	25.30%	2,402	2,4716
C_{max} (ng/mL)	7.84 $\pm$ 0.28	7.78 $\pm$ 0.26	[90.183%,131.07%]	29.52%	1,3218	2,9059

Fig. 2 Mean drug plasma concentration-time profiles of valsartan test and reference (ln transformation).

Mean drug plasma concentration-time profiles of valsartan (Fig. 2) were nearly identical, suggesting an equal *in vivo* performance of the two products.

Ratio $AUC_{0-t}/AUC_{0-\infty}$ of the two formulations were $> 80\%$, suggesting that the duration of sample collection was appropriate, covering $> 80\%$ of the

complete drug profile.

The mean and standard deviation of AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} of the two products did not differ significantly, suggesting that the blood profiles generated by valsartan test are comparable to those produced by TAREG. ANOVA (analysis of variance) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations either in periods, formulations or sequence, having p value greater than 0.05.

The 90% confidence interval of AUC_{0-t} and of $AUC_{0-\infty}$ were within the acceptable bioequivalence range of 80% to 125%, but the 90% CI of the C_{max} ratio did not fall within the regulatory range of 80% to 125%.

In this study, the intra-subject variation for AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} were calculated as 25.51%, 25.30% and 29.52% respectively. High variability was reported in previous valsartan studies [14-16]. It is clear that the number of subjects included in this study is insufficient to demonstrate bioequivalence, the number of subjects being proportional to the value of intra-subject variation.

The sample sizes required to generate > 80% power with error rate of 0.05 and high intra subject variability (30%), if the true difference is that equal or less than 20% is calculated as 52 subjects [17].

In order to demonstrate the high intrasubject variability of the both formulations of valsartan and to minimize the number of the included subject in this study, a replicate study designs could be recommended [18].

4. Conclusions

In this study, a simple, selective, accurate and reproducible LC-MS/MS method in negative ESI mode was developed and validated for the estimation of valsartan in human plasma. The method shows good performance with respect to all the validation parameters tested. In addition, the present method uses the protein precipitation extraction method and offers

high throughput because of a shorter run time. This approves the applicability of the method in our bioequivalence study.

The results of PK analysis suggested that the reference and test formulations of valsartan 80 mg coated breakable tablets were not bioequivalent during fasting state in these healthy algerian volunteers.

This pilot study led to evaluate the variability of valsartan. High intrasubject variability supports the previously reported high variability of valsartan [16, 19].

So, it's necessary to do a pivot study with an adequate sample size to overcome the intrasubject variability. Adequately designed studies to assess test formulation are needed.

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