Evaluation of gender difference in pharmacokinetics of Candesartan cilexetil in the fasted state by RP-HPLC: A single dose comparative study

Hafiz Awais Nawaz[1], Muhammad Irfan Masood[1], Mateen Abbas[2]*, Muhammad Usman[1], Abdul Muqeet Khan[2], Huma Rasheed[1] and Sualeha Riffat[3]

[1]Institute of Pharmaceutical Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Quality Operations Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan
[3]Bioequivalence Study Center, University of Veterinary and Animal Sciences, Lahore, Pakistan

Abstract: To compare the pharmacokinetics of candesartan cilexetil in healthy male and female volunteers in order to identify possible influence of gender and to improve therapeutic outcomes, an HPLC method for the quantification of candesartan cilexetil was developed and validated. Total of 16 volunteers (8 male and 8 female) were registered. Candesartan cilexetil 16 mg was administered orally to all the volunteers and blood samples were collected at different time intervals between 0-72 hours. Plasma was separated and analysed by HPLC method. Pharmacokinetic parameters were calculated by using APO software MW/PHARM version 3.02 and compared in male and female volunteers. The developed HPLC method fulfils the criteria for linearity, accuracy and precision described in EMA guideline. The values for absorption rate constant (Ka), maximum plasma concentration (C_{max}), volume of distribution (Vd) and Clearance (CL) were similar in male and female volunteers. No influence of gender was observed on overall pharmacokinetics of candesartan cilexetil. Therefore, no need for dose optimization while administering candesartan cilexetil in male and female patients was found based on the results of this study.

Keywords: Candesartan cilexetil, pharmacokinetics, hypertension, gender.

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of deaths worldwide with approximately 31% of deaths globally and 19% in Pakistan (WHO-CVDs, 2015; WHO-NCD, 2015) CVDs may involve angina pectoris, atherosclerosis, heart attack, thrombo-embolism and the most significantly hypertension, which alone is responsible for 7.5 million (12.8%) deaths globally (WHO-GHO, 2015). In the last few decades, a great research has been conducted to find promising therapies to treat such problems. Among these, angiotensin receptor blockers (ARBs) have proved themselves to be first line therapy for treating hypertension due to lesser side effects and once daily dosing (De Leeuw, 1999). When compared with angiotensin converting enzyme inhibitors (ACEIs) and calcium channel blockers, the ARBs have shown less withdrawals due to fewer side effects (Li, 2014; Fukui, 2003) Among ARBs, candesartan cilexetil proved to have greater pharmacodynamic effects when compared with losartan (Fuchs *et al*., 2000). However, treatment of early stage of stroke with candesartan cilexetil is not recommended in a recent study (Hornslien *et al*., 2015). Animal data suggest that ARBs not only enhance coronary flow reserve but also help to minimize glomerular sclerosis, coronary resistance, albuminuria and cardiac hypertrophy (Unger *et al*., 1998; Nunez *et al*., 1997; Bohm *et al*., 1996).

**Corresponding author:* e-mail: mateen.abbas@uvas.edu.pk

Candesartan cilexetil is an ARB and is inactive form that metabolizes to its active form, Candesartan (CT) by intestinal esterases after oral administration and competitively inhibits the attachment of angiotensin-II with angiotensin-1 (AT1) receptors (Ferreiros *et al*., 2007; Easthope *et al*., 2002). Bioavailability of candesartan Cilexetil after oral administration is around 40 % due to poor absorption and more than 99% of systemically available drug binds to plasma proteins. The major route for elimination of candesartan is kidney and to some extent intestine (Gleiter and Gundert-Remy, 1996). Pharmacokinetic parameters may vary among the patients, depending on patient related factors such as age, race, gender and disease conditions (Mahmood and Chamberlin, 1998). The pharmacokinetics of candesartan cilexetil has been investigated in children of less than 6 years (Schaefer *et al*., 2010) and under various clinical situations such as patients receiving haemodialysis (Schulz *et al*., 2009), patients with CYP2C91/3 genotype (Uchida *et al*., 2003), in hypertensive patients (Pfister *et al*., 1999) and also in patients with impaired liver and kidney functions (De Zeeuw *et al*., 1997). The pharmacokinetics of candesartan has been compared between young and elderly volunteers (Hubner *et al*., 1997), between subjects with normal and impaired kidney functions (Buter *et al*., 1999) and also between healthy volunteers and patients with liver impairment (Hoogkamer *et al*., 1998). The rate of absorption and elimination of certain drugs may vary in different genders

due to different extents of gastric acids secretion (Gleiter and Gundert-Remy, 1996) and different GFR (Abbas *et al*., 2014). Despite of substantial literature available, no study was found for the comparison of pharmacokinetics of candesartan cilexetil in healthy male and female volunteers.

The aim of this was study was to investigate the possible effect of gender on the pharmacokinetics of candesartan cilexetil in order to establish recommendation for safe administration of candesartan cilexetil in male and female patients. Moreover, an HPLC method for the quantification of candesartan cilexetil in human plasma was also developed and validated.

MATERIALS AND METHODS

Study design and sample collection

A comparative study was designed to observe the influence of gender on the pharmacokinetics of candesartan cilexetil. A total number of sixteen (eight male and eight female) healthy, non-smoking adult volunteers between the age of 18 to 25 years were registered for the study. Volunteers were enrolled through the open advertisement and they were briefed over the possible side effects of the drug, the purpose and the conduct of the study before allowing them to decide for willful participation in the study. A complete physical and medical examination was conducted and only those volunteers were included who were found healthy and have signed the written informed consent. The volunteers were re-evaluated clinically before the clinical trial and were housed in the supervised premises of Bioequivalence Study (BeSt) Centre, a night before the trial. Drug was administered after a minimum of 10 hours fasting with a baseline sample taken 15 minutes before the administration of the drug. All volunteers were treated in the uniform manner and received a standard meal after 4 hours of drug administration. Records of all deviation and adverse effects were made during the course of the trial. The volunteers were allowed to leave after 12 hours of sampling and the last three samples were taken on out-patient basis. Record of all the sampling times were made on the individual volunteer record sheet in accordance with the ICH guidelines (ICH Harmonised Tripartite Guideline, 2005). The same guidelines were used to ensure proper documentation and record of the clinical trial.

Creatinine clearance (CLcr) was calculated from serum creatinine (SeCR) levels by using Cockroft and Gault's equation (Cockcroft and Gault, 1976). The study was conducted in Bioequivalence Study (BeSt) Center of University of Veterinary and Animal Sciences (UVAS) Lahore, Pakistan after getting approval from independent Ethical Committee of UVAS (Letter No.BeSt/007).

A single dose of candesartan cilexetil 16 mg was administered orally to all the volunteers and 5 ml of blood samples were drawn in heparinized tubes at different time intervals (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 24, 48 and 72 hours) after drug administration. The plasma was separated by centrifugation within 0.5 hours of sample collection and stored at -80°C until the analysis was carried out. A back up sample was also maintained by dividing the separated plasma into two well labelled polypropylene tubes.

Analysis of candesartan

A reverse phase HPLC method for quantification of candesartan cilexetil was developed and partially validated. Stock solution (1mg/mL) was prepared by dissolving 10 mg of candesartan cilexetil in 10mL of q.s methanol. The calibration standard solutions were prepared in plasma directly from stock solution as 20, 40, 80, 120, 160 and 200ng/mL. The QC samples of 60, 100 and 180ng/mL were also prepared in plasma from an independent stock solution as lower quality control (LQC), median quality control (MQC) and higher quality control (HQC) respectively. A liquid-liquid extraction method was used for extraction of candesartan cilexetil from plasma samples. For this purpose, equal amounts (0.5 mL) of plasma and acetonitrile were mixed in a polypropylene tube and centrifuged at 10,000 g for 10 minutes after vortex mixing for 5 minutes. The supernatant layer was filtered through 0.2 µm filter and 55 µL was injected to HPLC.

The HPLC system comprised of Shimadzu 20-A (Japan) with a photodiode array detector (DAD) and an auto-sampler. The mobile phase used was a mixture of Potassium dihydrogen phosphate buffer (10mM)-methanol (25: 75, v/v). The pH of buffer was adjusted to 3 with orthophosphoric acid (10%). The separation was carried out at an isocratic flow rate of 1mL/min on a Merck LiChrosepher C_{18} column (250mm × 4.6mm, particle size 5µm) with detection at 260nm and 30°C column temperature. A partial validation of method was performed by following European Medicine Agency (EMA) guideline (EMA, 2011). The method was validated for linearity, sensitivity, accuracy and precision (Inter-day and intra-day).

Pharmacokinetic analysis

All the samples collected from volunteers were analysed by above described HPLC method and a plasma concentration-time data were obtained. The pharmacokinetic parameters of candesartan cilexetil in all volunteers were measured by using APO software MW/PHARM version 3.02 as already used in few studies (Rehman *et al*., 2012; Usman *et al*., 2013). The bioavailability of candesartan cilexetil was considered as 1. The pharmacokinetic parameters calculated for comparison were absorption rate constant (Ka),

elimination half-life ($t_{1/2}$), maximum plasma concentration (C_{max}), volume of distribution (Vd) and Clearance (CL).

STATISTICAL ANALYSIS

Statistical analysis of the data was performed by using SPSS 17.0 software. An un-paired t-test with a level of significance (α=0.05) was used for the comparison of pharmacokinetic parameters in male and female volunteers.

RESULTS

HPLC method validation

The calibration standard curve was plotted between spiked candesartan concentrations (20-200ng/mL) in plasma and corresponding peak areas. It was linear with coefficient of determination $R^2 \geq 0.999$. The signal to noise ratio for lowest level in the calibration curve was greater than 5. Therefore, it was considered as lower limit of quantification (LLOQ). The mean concentration (±SD) found for LLOQ after back calculation from five calibration curves (n=6) was 20.1±3.05 ng/mL with percentage coefficient of variation (CV %) 15.2 (table 1).

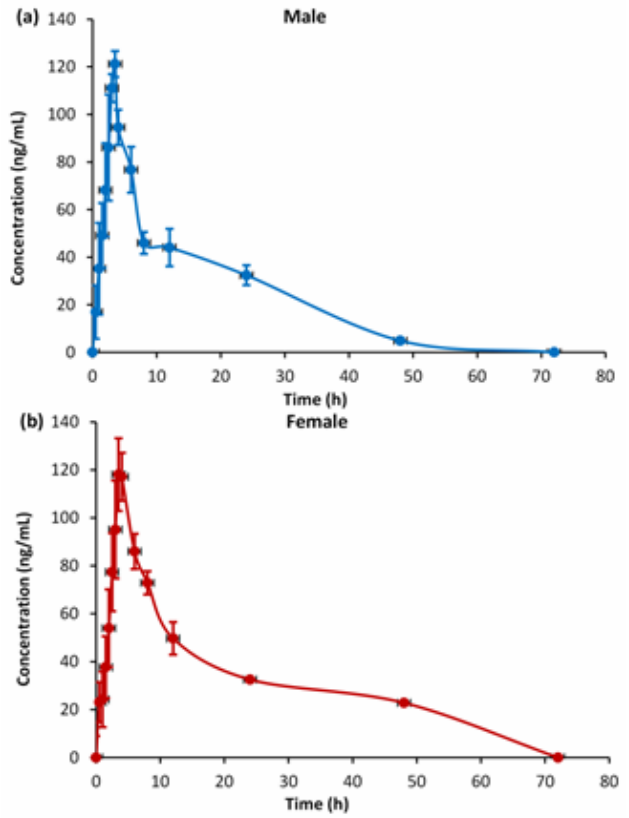

Fig. 1: Plasma concentration-time curves of candesartan after oral administration in male (a) and female (b) volunteers.

Six replicates of quality control samples of LQC (60 ng/mL), MQC (100ng/mL) and HQC (180ng/mL) were used for calculation of accuracy and precision (table 2). For intra-day accuracy the concentrations of candesartan cilexetil in QC samples were calculated through a simultaneously prepared calibration standard curve. The values for mean percentage recovery were 103.3%, 96.9% and 98.6% for LQC, MQC and HQC, respectively. The values for mean percentage recovery of LQC, MQC and HQC calculated on six different days through calibration curves prepared on respective days were 97.2%, 101.7% and 97.7%, respectively for inter-day accuracy. The maximum value of CV observed for intra and inter-day precisions was 7.24%.

Pharmacokinetic analysis

This comparative pharmacokinetic study was conducted on 16 healthy volunteers. Age of all the volunteers ranged from 18-25 years (table 3). A total number of 15 samples were collected from each volunteer. The plasma concentration-time curves of candesartan cilexetil in both genders are shown in fig 1. A two compartment model was used for calculation and comparison of pharmacokinetic parameters. The parameters calculated for comparison were Ka, C_{max}, $t_{1/2}$, Vd and CL. The mean ±SD values of these parameters in both genders are shown in table 4. An un-paired t-test was used for comparison with a level of significance (α=0.05). The results are expressed in term of P-value. No statistically significant difference was observed for all the observed parameters between male and female volunteers.

DISCUSSION

A limited number of methods were found for quantification of candesartan cilexetil in human plasma which includes UHPLC and LC-MS/MS methods. Therefore, a new HPLC method was developed and validated for quantification of candesartan cilexetil in human plasma and applied for pharmacokinetic estimation. Our method is more simple and sensitive with LLOQ 20ng/mL when compared with method developed by Kumar *et al*., (2012) with LLOQ 90 ng/mL. However, Bharati *et al*., (2012) developed and validated a more sensitive but expensive LC-MS/MS method where LLOQ is 1ng/mL. The calibration standard curve was linear with coefficient of determination $R^2 \geq 0.999$ and the LLOQ (20 ng/mL) was reproducible with mean percentage recovery of 100.4% and CV=15.2% (table 1). The results for accuracy and precision (table 2) were also with-in the acceptable range described by EMA guideline, (2011) where the percentage recovery for accuracy should be between 85% to 115% and CV (%) value for precision should not exceed 15% for LQC, MQC and HQC. However, for LLOQ these values are 80% to 120% for accuracy and 20% for precision.

Successful therapeutic outcomes can be achieved with appropriate dose of a particular drug. Dose

Table 1: Back calculated concentrations of calibration standards in plasma

Back calculation (n = 6)	Nominal Concentration (ng/L)					
	200	160	120	80	40	20
Mean (mg/L)	200	158.8	121.3	81.0	38.8	20.1
SD ±	1.35	2.65	2.88	4.03	1.91	3.05
Recovery (%)	100	99.3	101.1	101.3	97.0	100.4
CV (%)	0.67	1.67	2.38	4.98	4.93	15.2

Table 2: Accuracy and precision

Accuracy & precision (n = 6)	Nominal Concentration (ng/mL)		
	180 (HQC)	100 (MQC)	60 (LQC)
Intra-day[a]			
Mean (mg/L)	177.5	96.9	61.9
SD ±	2.03	0.34	0.36
Recovery (%)	98.6	96.9	103.3
CV (%)	1.14	0.35	0.58
Inter-day[b]			
Mean (mg/L)	175.8	101.7	58.3
SD ±	5.28	1.33	4.22
Recovery (%)	97.7	101.7	97.2
CV (%)	3	1.3	7.24

[a] Analyzed on same day
[b] Analyzed on six different days.

Table 3: Demographic data of volunteers

Volunteer's Demographic data	Male	Female
No. of volunteers (Male/Female)	8	8
Age (Years)	21 (18-24)	20 (18-24)
Weight (Kg)	64.5 (59-75)	60 (50-69)
Height (cm)	168 (160-175)	159 (149-169)
BMI (kg/m^2)	23 (20-29.3)	24.4 (19.5-26.1)
SeCr (mg/dL)	0.87 (0.40-1.30)	0.63 (0.20-1.01)
CRCL (mL/Min)	110.2 (98.1-122.7)	85.7 (71.3-96.1)
No. of samples per volunteer	15	15

Note: All the values are mentioned in median (range) except for number of volunteers and samples

Table 4: Comparison of pharmacokinetic parameters of Candesartan cilexetil in male and female volunteers

Pharmacokinetic Parameters	Male	Female	P Value
	Mean ± SD	Mean ± SD	
Ka (h^{-1})	0.33 ± 0.07	0.31 ± 0.05	0.471 [NS]
C_{max} (ng/ml)	93.4 ± 9.91	95.1 ± 7.53	0.723 [NS]
$t_{1/2}$ (h)	29.6 ± 13.9	30.6 ± 14.9	0.585 [NS]
Vd (L/kg)	0.47 ± 0.15	0.38 ± 0.06	0.145 [NS]
CL (L/h)	0.61 ± 0.24	0.49 ± 0.11	0.16 [NS]

[NS]Non-Significant

individualization can be determined by understanding the possible variability of pharmacokinetic parameters and the special features of target population. Pharmacokinetic parameters may vary among the patients, depending on patient related factors such as age, race, and gender (Mahmood and Chamberlin, 1998). Gender differences may influence not only the pharmacokinetics of a drug but also affect the therapeutic outcomes. Until now, to the best of our knowledge, no study is available to describe the possible influence of gender on the pharmacokinetics of candesartan cilexetil in healthy volunteers. We have conducted this comparative study in 8 healthy male and 8 healthy female volunteers. No significant difference was observed between male and female volunteers for Ka, $t_{1/2}$,

C_{max}, Vd, and CL. The Ka was almost similar in male and female volunteers with value of $0.33\pm0.07h^{-1}$ and $0.31\pm0.05h^{-1}$, respectively. The C_{max} was observed in male volunteers as 93.5±9.91ng/mL and in female volunteers as 95.1± 7.53ng/mL. A similar value for C_{max} (95.2ng/mL) in healthy subjects was observed by Hoogkamer *et al*. (1998). The higher values for C_{max} were observed by Tjandrawinata *et al*. (2013) in a bioequivalence study as 155.5±52.6 and 146.3±50.9 respectively for test and reference brands of candesartan cilexetil. The mean values for $t_{1/2}$ of candesartan cilexetil were observed as 29.6±13.9h and 30.6±14.9h in male and female volunteers respectively. However, shorter half-life 10.2±2.48h and 9h were reported by Tjandrawinata *et al*. (2013) and Hübner *et al*. (1997), respectively. This may be due to the reason that they have used wide range of age (19-55 years) for volunteers. The Vd calculated were 0.47±0.15L/kg and 0.38±0.06L/kg respectively for male and female volunteers. Females have lower value for Vd which may be due to higher percent body fat than males because Vd of certain drugs may be influenced by body fats (Anderson, 2008). Total body CL was observed as 0.61±0.24 L/h in male and 0.49±0.11L/h in female volunteers. Greater activity of enzyme CYP2D6 in males and slow GFR in females may be the reason of rapid CL in males as compared to females (Abbas *et al*., 2014).

CONCLUSION

An HPLC method for quantification of candesartan cilexetil in human plasma was successfully developed and validated. After comparative analysis of pharmacokinetic parameters in male and female volunteers all the observed parameters were similar in both genders. It can be concluded that there is no need for dose adjustment while administering candesartan cilexetil in male and female patients.

ACKNOWLEDGEMENTS

The authors are highly thankful to BeSt Center and Quality Operations Laboratory (QOL), UVAS for facilitating sampling and plasma storage and providing lab facilities for analysis of samples.

REFERENCES

World Health Organization (WHO), Cardiovascular Diseases (CVDs), (2015). Available from: http://www.who.int/mediacentre/factsheets/fs317/en/. [cited 2015 13 Aug].

World Health Organization (WHO), Noncommunicable Diseases (NCD), (2015). *Country Profiles*. Available from: http://www.who.int/nmh/countries/pak_en.pdf. [cited 2015 13 Aug].

World Health Organization (WHO), Global Health Observatory (GHO) (2015). Available from: http://www.who.int/gho/ncd/risk_factors/blood_pressure_prevalence_text/en/. [cited 2015 13 Aug].

De Leeuw PW (1999). How do angiotensin II receptor antagonists affect blood pressure? *Am. J. Cardiol*., **84**: 5K-6K.

Li EC, Heran BS, and Wright JM (2014). Angiotensin converting enzyme (ACE) inhibitors versus angiotensin receptor blockers for primary hypertension. *Cochrane Database Syst. Rev*., **8**: CD009096.

Fukui T, Rahman M, Hayashi K, Takeda K, Higaki J, Sato T, Fukushima M, Sakamoto J, Morita S, Ogihara T, Fukiyama K, Fujishima M and Saruta T (2003). Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) trial of cardiovascular events in high-risk hypertensive patients: rationale, design and methods. *Hypertens Res*., **26**: 979-990.

Fuchs B, Breithaupt-Grogler K, Belz GG, Roll S, Malerczyk C, Herrmann V, Spahn-Langguth H and Mutschler E (2000). Comparative pharmacodynamics and pharmacokinetics of candesartan and losartan in man. *J. Pharm. Pharmacol*., **52**: 1075-1083.

Hornslien AG, Sandset EC, Wyller TB, and Berge E (2015). Effects of candesartan in acute stroke on activities of daily living and level of care at 6 months. *J. Hypertens.*, **33**:1487-1491.

Unger T, Culman J and Gohlke P (1998). Angiotensin II receptor blockade and end-organ protection: pharmacological rationale and evidence. *J. Hypertens. Suppl*., **16**: S3-9.

Nunez E, Hosoya K, Susic D and Frohlich ED (1997). Enalapril and losartan reduced cardiac mass and improved coronary hemodynamics in SHR. *Hypertension*, **29**: 519-524.

Bohm M, Lippoldt A, Wienen W, Ganten D and Bader M (1996). Reduction of cardiac hypertrophy in TGR (mREN2) 27 by angiotensin II receptor blockade. *Mol Cell Biochem*., **163-164**: 217-221.

Ferreiros N, Dresen S, Alonso RM and Weinmann W (2007). Hydrolysis and transesterification reactions of candesartan cilexetil observed during the solid phase extraction procedure. *J. Chromatogr. B. Analyt Technol. Biomed. Life Sci*., **855**: 134-138.

Easthope SE and Jarvis B (2002). Candesartan cilexetil: An update of its use in essential hypertension. *Drugs*, **62**: 1253-1287.

Gleiter CH and Gundert-Remy U (1996). Gender differences in pharmacokinetics. *Eur. J. Drug Metab. Pharmacokinet*., **21**:123-128.

Mahmood I and Chamberlin N (1998). A limited sampling method for the estimation of AUC and Cmax of carbamazepine and carbamazepine epoxide following a single and multiple dose of a sustained-release product. *Br. J. Clin. Pharmacol*., **45**: 241-246.

Schaefer F, van de Walle J, Zurowska A, Gimpel C, van Hoeck K, Drozdz D, Montini G, Bagdasorova IV, Sorof J, Sugg J, Teng R and Hainer JW (2010). Efficacy, safety and pharmacokinetics of Candesartan

cilexetil in hypertensive children from 1 to less than 6 years of age. *J. Hypertens*., **28**: 1083-1090.

Schulz EG, Bahri S, Schettler V, Popov AF and Hermann M (2009). Pharmacokinetics and antihypertensive effects of Candesartan cilexetil in patients undergoing haemodialysis: an open-label, single-centre study. *Clin. Drug Investig*., **29**: 713-719.

Uchida S, Watanabe H, Nishio S, Hashimoto H, Yamazaki K, Hayashi H and Ohashi K (2003). Altered pharmacokinetics and excessive hypotensive effect of Candesartan in a patient with the CYP2C91/3 genotype. *Clin. Pharmacol. Ther*., **74**: 505-508.

Pfister M, Schaedeli F, Frey FJ and Uehlinger DE (1999). Pharmacokinetics and haemodynamics of Candesartan cilexetil in hypertensive patients on regular haemodialysis. *Br. J. Clin. Pharmacol*., **47**: 645-651.

De Zeeuw D, Remuzzi G and Kirch W (1997). Pharmacokinetics of Candesartan cilexetil in patients with renal or hepatic impairment. *J. Hum. Hypertens*., **11**(2): S37-42.

Hubner R, Hogemann AM, Sunzel M and Riddell JG (1997). Pharmacokinetics of Candesartan after single and repeated doses of Candesartan cilexetil in young and elderly healthy volunteers. *J. Hum. Hypertens*., **11**(2): S19-25.

Buter H, Navis GY, Woittiez AJ, de Zeeuw D and De Jong PE (1999). Pharmacokinetics and pharmaco-dynamics of candesartan cilexetil in patients with normal to severely impaired renal function. *Eur. J. Clin. Pharmacol*., **54**: 953-958.

Hoogkamer JF, Kleinbloesem CH, Ouwerkerk M, Hogemann A, Nokhodian A, Kirch W and Weidekamm E (1998). Pharmacokinetics and safety of Candesartan cilexetil in subjects with normal and impaired liver function. *Eur. J. Clin. Pharmacol*., **54**: 341-345.

Abbas M, Khan AM, Riffat S, Tipu MY, Nawaz HA and Usman M (2014). Assessment of sex differences in Pharmacokinetics of carvedilol in human. *Pak. J. Pharm. Sci*., **27**: 1265-1269.

ICH Harmonised Tripartite Guideline, (2005). Validation of Analytical Procedures: Text and Methodology Q2(R1), Current Step 4 version, Published in the Federal Register, **62**(96): 27463-27467.

Cockcroft DW and Gault MH (1976). Prediction of creatinine clearance from serum creatinine. *Nephron*., **16**: 31-41.

European Medicines Agency (EMA). Guideline on bioanalytical method validation (2011). Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf. [cited 2015 06 July].

Rehman ZU, Ashraf M, Khan MA, Jabbar MA and Rasheed MA (2012). Pharmacokinetics of ketoprofen in healthy horses in Pakistan. *The Journal of Animal & Plant Sciences*, **22**(3): 584-587.

Usman M, Ashraf M, Khokhar MI, Ashiq B, Masood MI, Afzal S, Omer O, Ali M and Qadir MI (2013). Comparative pharmacokinetics of levofloxacin in healthy volunteers and in patients suffering from typhoid Fever. *Iran J. Pharm Res.,* **12**: 147-154.

Kumar ND, Babu KS, Gosada U and Sharma N (2012). A validated ultra high-pressure liquid chromatography method for separation of Candesartan cilexetil impurities and its degradents in drug product. *Pharm Methods*, **3**: 31-39.

Bharathi DV, Hotha KK, Chatki PK, Satyanarayana V and Venkateswarlu V (2012). LC-MS/MS method for simultaneous estimation of Candesartan and hydrochlorothiazide in human plasma and its use in clinical pharmacokinetics. *Bioanalysis*, **4**: 1195-1204.

Tjandrawinata RR, Setiawati E, Yunaidi DA, Simanjuntak R, Santoso ID and Susanto LW (2013). Bioequivalence study of two formulations of Candesartan cilexetil tablet in healthy subjects under fasting conditions. *Drug Des. Devel Ther*., **7**: 841-847.

Anderson GD (2008). Gender differences in pharmacological response. *Int. Rev. Neurobiol*., **83**: 1-10.