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Assessment of The Bio-Equivalence by Comparing the Single Oral Dose Bioavailability of Pioglitazone Usp Tablets 45 Mg Compare with Reference Product Actos 45mg Tablet in Healthy Adult

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ABSTRACT

The present study was open labelled, balanced, randomized, two treatment, two sequence, two period,single dose, cross over comparative oral Bio-equivalence of pioglitazone 45mg tablet study on 16 healthy, adult, human subjects under fasting condition. Test product and treatment with reference drug are done during every period of study. Two-period study is designed over 2 periods with wash out period of 10 days in between and single-dose study because each subject receives only a single dose in each period.

Keywords: Pioglitazone

INTRODUCTION

Single- source drug products are drug products for which the patent has notyet expired or has certain exclusivities so that only one manufacturer can make it. These products are usually brand- name (Innovator) drug products. After the patentand other exclusivities for the brand- name drug expires, a pharmaceutical firm may manufacture a generic drug product that can be substituted for the branded drug product.

Earlier there was duplication of trails, wherein the generic drug manufacturer had to go through all the tedious procedure as of Innovator Company to market the drug. Hence "Drug Price Competition & Patent Restoration Act, 1984" informally known as the "Hatch-Waxman Act" came in picture. As per this act the generic company doesn"t have to go through the tedious procedure instead prove that the generic compound is Bioequivalent to the Innovator compound and market the drug. Generic substitution thus provides the means of supplying the market with inexpensive, efficacious, & safe drug products without the need to repeat an entire clinical & clinical pharmacology development following patent expiration. Multiple companies may produce & market similar formulations to the original marketed product, provided they can demonstrate bioequivalence to the original product. Bioequivalence studies are carried out under the guidelines of CDSCO (Central Drug Standard Control Organization). Ensuring uniformity in standards of quality, efficacy and safety of pharmaceutical products is the fundamental Reasonable assurance has to be provided that various products, containing same active ingredients, marketed by different licensees, are clinically equivalent and interchangeable[(1)]. Bioavailability is a pharmacokinetic term that describes the rate

and extent to which the active drug ingredient is absorbed from a drug product and becomes available at the site of drug action. Since pharmacological response is generally related to the concentration of drug at the receptor site, the availability of a drug from a dosage form is a critical element of a drug product‟s clinical efficacy. However, drug concentrations usually cannot be readily measured directly at the siteaction. Therefore, most bioavailability studies involve the determination of drug concentration in the blood or urine. This is based on the premise that the drug at the site of action is in equilibrium with drug in the blood. It is therefore possible to obtain an indirect measure of drug response by monitoring drug levels in the blood or urine. Thus, bioavailability is concerned with how quickly and how much of a drug appears in the blood after a specific dose is administered. The bioavailability of a drug product often determines the therapeutic efficacy of that product since it affects the onset, intensity and duration of therapeutic response of the drug. In most cases one is concerned with the extent of absorption of drug, (that is, the fraction of the dose that actually reaches the bloodstream) since this represents the “effective dose" of adrug. This is generally less than the amount of drug actually administered in the dosage form.

Conduct of the study

Housing

The study participants were admitted in the CPU at Piramal Clinical Research and housed from at least 12 hours prior to drug administration until 48 hours after dosing in every period. Inclusion and exclusion criteria of the study participants.

Inclusion criteria

- Healthy human subjects within the age range of 18 - 45 years
- Non-smokers since at least six months
- Willingness to provide written informed consent to participate in the study
- Body-mass index of 18.5 kg/m^2 and 24.9 kg/m^2 with body weight not less than 50 kg
- Absence of significant disease or clinically significant abnormal laboratory values on laboratory evaluations, medical history or physical examination during the screening
- Normal 12-lead ECG or one with abnormality considered to be clinically insignificant
- Normal chest X-ray Posterior Anterior view
- Comprehension of the nature and purpose of the study and compliance with the requirement the protocol

Female Subjects of child bearing potential practicing an acceptable method of birth control for the duration of the study as judged by the investigator(s), such as condoms, foams, jellies, diaphragm, intrauterine device (IUD) or abstinence,or postmenopausal for at least 1 year, or surgically sterile (bilateral tubal ligation, bilateral oophorectomy, or hysterectomy) has been performed on the subject.

Exclusion criteria

- Personal/family history of allergy or hypersensitivity to Niacin or allied drugs.
- Past history of anaphylaxis or angioedema.
- Any major illness in the past three months or any clinically significant ongoing chronic medical illness (e.g. CCF (heart failure), hepatitis, pancreatitis etc.)
- Presence of any clinically significant abnormal values during screening e.g. significant abnormality of Liver Function Test (LFT), Renal (kidney) Function Test (RFT) etc.
- Any cardiac, renal or liver impairment any other organ or system impairment
- History of seizures or psychiatric disorders
- Presence of disease markers of HIV 1 and 2 and hepatitis B and C virus.
- Consumption of alcohol for more than two years, or consumption of more than three alcoholic drinks per day or consumption of alcohol within 48 hours prior to dosing and during the study.
- Consumption of xanthine containing derivatives (coffee, tea, cola drinks, chocolate) within 48 hours before check-in of each period.
- Use of any recreational drug or a history of drug addiction Participation in any clinical trial within the past 3 months
- Inaccessibility of veins in left and right arm
- Donation of blood (one unit or 350 mL) within 3 months prior to receiving the first dose of study medication
- Receipt of any prescription drug therapy within four weeks or over-the-counter (OTC) drugs within two week prior to receiving the first dose of study medication or repeated use of drugs within the last four weeks

An unusual diet, for whatever reason e.g. low sodium diet, for two weeks prior toreceiving any medication and throughout subject‟s participation in the study.

Consumption of grapefruit- containing food or beverages within 7 days prior to receiving the first dose of study medication in all the three periods. Recent history of dehydration from diarrhea, vomiting or any other reason within a period of 7 days prior to the study.

Female volunteers demonstrating a positive pregnancy screen or currently breast-feeding.

Restrictions

Smoking and chewing tobacco

Study participants are instructed to not to smoke, chew or consume tobacco containing products for at least 48 hours before dosing in each period and will be prohibited from doing so throughout their stay at CPU and until last PK sample has been collected in each period.

Medications

Study participants were asked about their medication history in the last two weeks or earlier before screening date with a view to identify drugs likely to affect pharmacokinetics of the study drug , and are instructed not to take any medications (either prescribed or over-the-counter) from the date of screening till completion of the study. Check lists of likely drugs interacting with Pioglitazone 30mg Tablet were assessed while taking medication history. It should specificallyinclude drugs known

to interact like amiodarone, amphetamines, Dextromethorphan, Fluoxetine, Glimepiride, Glipizide, Lidocaine, Mirtazapine, Phenytoin, Warfarin.. Other drugs if taken by study participant and reported wereassessed for likely interactions before subject is admitted in the study.

If drug therapy other than that specified in the protocol is required prior to or during the study or in the washout period, decisions shall be taken by the investigator to continue or discontinue the study participant based on the following:

The pharmacology and pharmacokinetics of the non-study medication. The likelihood of a drug–drug interaction, thereby affecting pharmacokineticcomparison of the study medications. The time and duration of administration of the non-study medication.

Water and Fluids

All participants are instructed to abstain from consumption of grapefruit, xanthine containing food or beverages (tea, coffee, chocolates, soft drinks etc.) oralcoholic products for at least 72 hrs. prior to dosing in each period and were prohibited from consuming above mentioned products during their stay at the CPU and until the last PK blood samples has been collected in each period. Drinking water was not allowed from 1 hour before dosing until 01 hour post- dose except while administration of the dose.

Study Design and conduct

Design

An open label, balanced, randomized, two-period, two-sequence, single dose, crossover bioequivalence study with at least 7 days washout period between eachadministration under fed conditions.

Open-label

This study is an open label study. The study participants and the investigator are not be blinded towards the identity of the study medications. The analyst was blinded towards the identity of the study medications.

Treatment

Treatment R (Reference): Single oral dose of ACTOS (containing Pioglitazone45mg) under fed condition

Treatment T (Test): Single oral dose ofPioglitazone USP 45mg (containingPioglitazone 45mg) under fed conditions.

Randomization

Study participants were randomized to the two treatment sequences in a random order .00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00, 72.00, 96.00, 120.00, 144.00, 168.00 and 192.00 hours post-dose.

The cannulation was carried out in the study subjects before pre-dose sample collection and was kept till the 12.00 hour sample. Later on, the cannula was removed and sample collection was done by direct venipuncture.Blood sample collection was done at bedside.

For each subject, the total volume of blood withdrawn was not exceeded 189 mL (160 mL for estimation of plasma drug levels [5 mL per draw and a total of 32 draws- 16 in each period], 8 mL for screening, 8 mL for post-study safety assessment and 13 mL discarded heparinized blood).

Sampling Procedure

Blood samples are collected by means of intravenous cannula placed in a forearmvein. To ensure the patency of intravenous cannula, 0.5 mL 5 IU/ml of heparin was injected into the cannula after each sample withdrawal. All vacutainers used to collect blood samples for pharmacokinetic analysis were pre-labelled with thestudy number, study participant identification, period and sampling time point forcollection.

Processing

After collection the blood samples were centrifuged at 4000 rpm for 10 minutes at 4ºC for separating the plasma. Centrifugation of all samples will be done within 30 minutes after each sample draw time point. All plasma samples in cryovials were separated and are aliquoted into two set(s) and are transferred to deep freezer ,maintained at -20□C $\pm$ 5□C from 0.00 (pre-dose) to 12.00 hours post-dose, after which the samples were directly transferred to a deep freezer maintained at -70ºC $\pm$ 10ºC.

Dietary plan

After check-in, subjects received a standard meal (Day 0), at dinner consisting of approximately 1000-1200 calories, after which they were kept fasted overnight(for at least 10 hours). Thereafter, standard meals comprising of 2200-2400 calories per day was provided at 4.00, 8.00 and 13.00 hours post-dose (i.e. lunch, snacks, dinner respectively) on Day 1 during both the periods. In addition to this, 20% oral glucose solution in water (240 mL with dosing and 60 mL every 15 minutes up to 4 hours of post-dose) comprising of 960 calories was given.

Safety assessments

Medical Investigator/ Study physician were available on site for monitoring thestudy participants for the first 08 hours after dosing in each period, and then afterwards he was available on call. Trained paramedic(s) were present during entire period of housing for monitoring and for taking vitals prior to and after dosing as specified.

Vital signs: Subjects were ensured safe time to time by checking the vital signs at intervals of 1 hr. prior to drug administration and 2.00, 6.00, 12.00, 24.00, 36.00,48.00 hrs. post dose. Subject questionnaire: Each and every subject was questioned about their wellbeing at intervals of every 4.00hrs. They were asked to report any adverse event occurred apart from the observation.

Adverse events: No Adverse event was observed.

Washout period: 21 days of washout was given between period one and period two.

Period II

After the specified washout period, the second period was started. Same procedure is carried out as period-one, such that the two periods are identical in every manner. In period two, the subjects those who had received test T drug during period one received reference R drug and the subjects who had

received reference R drug during period one received test T drug .

Post study evaluation

After the completion of two periods blood sample of 9ml was collected from subjects for the clinical laboratory tests for the post study evaluation to ensure that the subject has same clinical conditions after the study as he was before the study.

BIOANALYTICAL PROCEDURE

A validated LC/MS/MS method was employed for the estimation of Pioglitazone hydrochloride in plasma. All samples from a maximum of 72 subjects completing both the periods were analysed. During estimation of Pioglitazone hydrochloride, quality control samples were distributed throughout each batch of study samples.

Plasma samples from dropout subjects would not be analysed, unless suchdropouts or withdrawals are due to adverse events related to study drug. In these cases the samples would be analysed only for safety issues. During estimation of drug quality control samples will be distributed throughout each batch of study samples.

Whenever possible, samples from each subject would be analysed on the same standard curve. Samples with drug concentration greater than upper limit ofthe validated range of the analysis should be diluted with the appropriate drugfree biological fluid and reanalysed as per the method validation report. Samples, which are below the lower limit of quantification (LLOQ) should be set to zero for all pharmacokinetic and statistical evaluation and reported as below limit of quantification (BLQ). Any missing sample or un reportable concentration value will be reported as „missing" and should not be included for pharmacokinetic andstatistical analysis. Time point deviations (more than 2 minutes) will be incorporated while PK calculation

Metoprolol Succinate concentrations in subjects were determined by high performance liquid chromatography mass spectrometry using solid phase extraction technique. The eluted samples were chromatographed on Hypurity advance 4.6 x 50 mm, 5 µm (Make: Thermo) column using a mobile phase consisting of HPLC Grade Acetonitrile: 5mm ammonium acetate (80:20 v/v).

The method was validated over a concentration range of 15.00 ng/mL to 3000.00 ng/mL for Pioglitazonehydrochloride .Samples, which are below the lower limit of quantification (LLOQ), were set to zero for all pharmacokinetic and statistical evaluation and reported as below limit of quantification (BLQ).

Bio analytical Methodology

A validated LC-MS/MS method will be employed for the estimation of Pioglitazone and its active metabolite M-IV in Plasma. All the samples from subjects treated for the study would be analysed. During estimation of Pioglitazone and its active metabolite M-IV, quality control samples will be distributed throughout each batch of study samples.

Whenever possible, samples from each subject will be analysed on the same standard curve. Samples with drug concentration greater than upper limit of the validated range of the analysis will be diluted using the appropriate drug free biological fluid and/ or reanalysed by using partial volume. Samples, which are below the lower limit of quantification (LLOQ), will be set to zero for all pharmacokinetic and statistical evaluation and reported as below limit of quantification (BLQ). Any missing sample(s) value will be reported as „M" and un reportable concentration value will be reported as „NR" and will not be included for pharmacokinetic and statistical analysis.

The accuracy, precision and linearity data for each standard curve and all quality control samples will be presented. Representative chromatograms and standard curve graphs will be included from the 20% of serially selected subjects completing the study will be reported in the final report as per Current Version of SOP No. CR-GS-XX "Generation of Randomization for BA/BE Studies". If during clinical phase, 3 consecutive samples in any phase i.e. (Absorption, Distribution and Metabolism / Excretion) are found to be missing then samples ofthat subject will not be analysed except for safety reasons. Incurred sample reanalysis will be performed in accordance with AnaCipher Clinical Research Organisation current version SOP No: CR-BP-XX and the results will be included in the final study report.

The estimation of Pioglitazone and the active metabolite M-IV will be done asper the below mentioned table:

Table 1: Estimation of Pioglitazone and the active metabolite

S.No	Samplingpoint	Pioglitazone	Active metabolite M-IV
1	0.00	√	√
2	0.25	√	-
3	0.50	√	√
4	0.75	√	-
5	1.00	√	√
6	1.25	√	-
7	1.50	√	-
8	1.75	√	-
9	2.00	√	√
10	2.33	√	-
11	2.67	√	-
12	3.00	√	√

13	3.50	√	-
14	4.00	√	√
15	6.00	√	√
16	8.00	√	√
17	10.00	√	√
18	12.00	√	√
19	16.00	√	√
20	24.00	√	√
21	36.00	√	√
22	48.00	-	√
23	72.00	-	√

Sample Preparation

The thawed samples were vortexed to ensure complete mixing of the contents. 70µL of the plasma sample was pipetted into RIA vials, 20 µL (15 µg/mL of Carbamazepine) Internal Standard spiking solution was added to it and vortexed, except in blank plasma samples where 20 µL diluent was added to it and vortexed. Then 500 µL of 100 mm Ammonium Acetate Buffer in HPLC grade (or) Milli-Q water was added and vortexed.

Sample Processing

Oasis HLB, 30 mg/ 1CC SPE cartridges (New cartridge for each sample) were taken and Conditioned with 1.0ml HPLC grade methanol, followed by 1.0mL Milli-Q water (or) HPLC grade water. Taken care that cartridge doesn't get dry. Applied Sample, which is already prepared in RIA vials. Maximum pressure wasapplied to remove the sample from the catridge. When the sample was removed, the catridges are washed with 1.0mL of 100 mm Ammonium Acetate Buffer and followed by 2.0 mL of HPLC grade (or) Milli-Q water. The sample is eluted with mL of Mobile Phase. Transferred into auto sampler vials. Loaded into auto sampler.

Bio analytical Conditions

HPLC: Shimadzu Prominance
Column: Hypurity advance, 50 x 4.6 mm, 5 µm
Mobile phase: HPLC Grade Acetonitrile: 5 mM Ammonium Acetate buffer (80:20 v/v)
Rinsing solution: HPLC Grade Acetonitrile: Milli Q water (50:50, v/v)Flow rate: 1.000 mL/minute
Split ratio: 50:50
Sample Cooler Temperature: 15°C Injection volume : 15 µL
Detection: Positive ion mode (API 3000)
Retention time: pioglitazone hydrochloride 1.40 ± 0.3 minutes
Run Time: 2.50 minutes

Determination

The results of selectivity, matrix effect, sensitivity, linearity, precision and accuracy, stabilities, recovery, dilution integrity and partial volume test within the range for bio analytical batch acceptance criteria USFDA acceptance range asper Guidance for Industry–Bio analytical Method Validation given by CDER. The analytical method described above is valid for the estimation of MetoprololSuccinate, in human plasma over a range of 15.00 ng/mL to 3000.00 ng/mL with the detection of pioglitazone hydrochloride

PHARMACOKINETIC ANALYSIS

Pharmacokinetic parameters of pioglitazone hydrochloride were calculated using the WinNonlin Version 5.2.1. The parameters estimated were $T_{max,}$ $C_{max,}$ $AUC_{0-t,}$ $AUC_{0-\infty}$, K_{el} and $T_{1/2}$.

STATISTICAL ANALYSIS

Statistical analysis was performed using the SAS system version 9.1.3. for Windows, (SAS Institute Inc. USA)

Summary Statistics

The summary statistics (for relevant pharmacokinetic parameters) was reported for Metoprolol Succinate. The reported parameters are the arithmetic means, standard deviations and the coefficient of variation for Tmax, Cmax, AUC0-t and$AUC_{0-\infty}$

Analysis of Variance (ANOVA)

The untransformed pharmacokinetic parameters (C_{max}, AUC_{0-t}, $AUC_{0-\infty}$) will be analyzed using an ANOVA model with the main effects of sequence and treatment. A separate ANOVA model will be used to analyze each of the parameters. A 5% level of significance between subject comparisons (i.e., sequence). Each analysis of variance will include calculation of mean square error, coefficient of variance and the associated degree of freedom.

RESULTS

Sixteen (males) are enrolled in the present study. Pioglitazone 45mg was well tolerated. No SOP deviation and No protocol deviation was seen. None of the subjectshad reported adverse events [Except subject ID: 08 (loose stools) and 13 (vomiting)]. Significant differences was observed in the analysed pharmacokinetic parameter. The generic formulation had a C_{max} at 1282.379 ± 478.2052 ng/ml, a T_{max} at 4.114 (1.8143-6.4137) hr while the original formulation (reference product) had a C_{max} at 1252.9171 ± 461.7369 ng/ml, T_{max} at 5.928 (2.957-8.898) hr respectively.

Table 2: Comparison of mean values of test and reference

PK Parameters	Pioglitazone	
	Mean ± SD	
	T	R
T_{max} (hr)	4.114 (1.8143-6.4137)	5.928 (2.957-8.898)
C_{max} (ng/mL)	1282.379 ± 478.205	1252.9171 ± 461.7369
AUC_{0-t} (ng.hr/mL)	18112.812 ± 6455.822	17628.542 ± 6289.628
$AUC_{0-\infty}$ (ng.hr /mL)	18372.007 ± 6542.1139	17942.049 ± 6230.0209
$T_{½}$ (hr)	6.473 ± 1.3693	6.658 ± 1.9866
k_{el} (1/hr)	0.1117 ± 0.0237	0.11233 ± 0.03080
AUC_% Extrap obs	1.4331 ± 0.6975	2.1942 ± 1.9119

The sample size for using in the study was suggested by Sponsor Company which wasthe maximum number (n=16). In this study, the 90%confidence intervals for C_{max},AUC_{0-t},$AUC_{0-\infty}$were not corresponding to the bioequivalence criteria. intra subject coefficient of variation from log ANOVA of C_{max},AUC_{0-t},$AUC_{0-\infty}$values were 29.30, 22.05, 20.97 respectively. The 90% confidence intervals for the log transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of Pioglitazone are within the 80.00-125.00 % acceptance criteria.

Table 3: Statistical Process

Statistical data processing of Pioglitazone									
Parameter	**Least Square Mean Reference**	**Lea st Squ are Mean Test**	**Geom etric Least Square Mean Refere nce**	**Geometric Least Square MeanTest**	**(T/R) Ratio**	**Intra Subject Variability**	**Po wer**	**90% Confidence Interv al**	**Bio- equival ence**
Log	7.0546	7.09	1158.2	1211.2	104.	29.30	66.5	(87.46 -	No
(C_{max})		94	011	383	58		9	125.04)	
Log	9.7087	9.74	16460.	17020.	103.	22.05	86.2	(90.28 -	Yes
(AUC_{0-t})		22	1775	7420	41		3	118.43)	
Log	9.7311	9.75	16832.	17268.	102.	20.97	88.9	90.16 -	Yes
($AUC_{0-\infty}$)		66	5386	6259	59		5	116.74)	

The sample size for using in the study was suggested by Sponsor Company which wasthe maximum number (n=16). In this study, the 90%confidence intervals for C_{max}, AUC_{0-t},$AUC_{0-\infty}$were not corresponding to the bioequivalence criteria. Intra subject coefficient of variation from log ANOVA of C_{max},AUC_{0-t},$AUC_{0-\infty}$values were 29.30, 22.05, 20.97 respectively.90% confidence interval of $C_{max}$is 87.46 – 125.04 (lower & upper limit) respectively. The upper limit of Cmax is <125. So that, it is consider as pioglitazone USP tablet (test product) is not bioequivalent to ACTOS 45mg (reference product). Based on results, it has been concluded that the test product, Pioglitazone tablets USP45 mg of sponsor company is not bioequivalent to the Reference product, Actos (pioglitazone) Tablets 45 mg of Takeda Pharmaceuticals America, Inc., Deerfield, IL 60015, in healthy, adult, male subjects, under fasting conditions.

DISCUSSION

After proper screening of BMI, vitals, serology and hematology all the relevant parameters explained in methodology, 18 subjects were enrolled for study; they were admitted into the clinical facility 11hrs before the dose administration. Being a fastingstudy the subjects were fasted for 10 hrs, and they were dosed with pioglitazone USP 45mg with 240±2ml of 20% glucose solution, Subjects will be instructed not to chew or crush the tablet but to consume it as a whole. Compliance for dosing will be assessed by a thorough check of the oral cavity immediately after dosing. 60 ml of 20% oral glucose solution in water every 15 minutes up to 4 hour. Administration of investigational products will be carried out while the subjects are in sitting postureand they will be instructed to remain seated for two hours after dosing in each period except when clinically indicated. The subjects have received the dose according to randomization schedule i.e. test (A) and reference (B) treatments mentioned in the protocol. Drinking water was prohibited for one hour before and one hour after dosing. At other times, drinking provided adlibitum. Diet provided was at 04^{th}hr. lunch, 08^{th}hrsnacks, 13^{th}hr. dinner in day1. 25^{th}hr Breakfast, 29^{th}hrlunch, 33^{th}hr snacks, 37^{th}hr dinner in day2 after dosing in each period. Twenty two (23) blood samples were collected from each subject during each period. The venous blood samples (5 ml) were withdrawn at pre-dose (before dosing, in the morning of the day of dosing). The venous blood samples (1 x 5ml each)

were withdrawn at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.33, 2.67, 3.00,
3.50, 4.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00 and 72.00 hours post dose and were collected in to the vacutainers which is having K_2EDTA as anti-coagulantand The samples will be collected in wet ICE bath with sufficient quantity of a mixture of ice and water at each time point. Within 30 minutes of the sample collection, samples will be placed in a refrigerated centrifuge and then centrifuged at 4□C and 4000 rpm for 10 minutes to separate plasma, after receiving the blood samples from all the subjects.The plasma was separated into pre-labeled polypropylene tubes in two aliquots and sorted according to subject wise and sent to bio analytical for concentration estimation.
Clinical Examination was carried out and recorded at check-in and prior to check out and at post study. Vital signs measurement (seated blood pressure, radial pulse rate, and axillary temperature) was carried out and recorded at check-in, prior to dosing and at checkout and at the termination of the study. In house Vital signs measurements (seated blood pressure and radial pulse rate) was carried out and recorded at 1.00, 3.00, 6.00, 10.00hourspost dose within ±45 minutes to the schedule time. 26, 31, 35, 48 hours within ±1 hr and 72±2 hrs. Blood glucose will be monitored using glucometer at 1.00, 2.00, 4.00, 8.00 and 10.00 (with a window period of ± 15min).
There were no serious adverse events, so the data represented in this study is for 16subjects to establish the bio equivalence of tablet. The ratios for geometric least square means lie within the acceptance ranges 80-125%for log transformed C_{max}, AUC_{0-t}, $AUC_{0-□}$. The 90% confidence interval for the ratio of geometric least square mean values werenot within acceptance limit of 80%-125% for log transformed C_{max}, AUC_{0-t}, AUC_{0-} pioglitazone 45mg. Hence the test product, pioglitazone USP 45mg are not bioequivalent with the reference product, ACTOS 45mg (Contain 45mg of pioglitazone) in terms of rate and extent of absorption under fasting conditions.
In-vivo data was predicted by Using Protein extraction procedure and concentration werefound out through LCMS/MS detection instrument. The Pharmacokinetic parameter sassessed were AUC0-t, AUC0-□, C_{max}, T_{max}, the bioequivalance criteria was based on the 90%confidence intervals whose acceptance range in between 80% -125%.Test and Reference ratio is C_{max} (104.58%), AUC_{0-t} (103.41%), $AUC_{0-□}$ (102.59%) respectively.

CONCLUSION

Pioglitazone is an antidiabetic agent that has a good result for glycaemic control and improves serum lipid profile. The analytical method (LC-MS/MS) utilized to determine the concentrations of pioglitazone in human plasma demonstrated good precision and accuracy.The present study employed a randomized, single dose, two treatments, two periods, two sequences crossover design to study the bioequivalence in 16 healthy volunteers. The study design and sample size are considered most appropriate and standard for this type of study.
The pharmacokinetic parameters of test product assessed were within the acceptable limits of Bioequivalence 80-125%. *Hence it is inferred that test drug pioglitazone USP 45mg tablet is not bioequivalent to reference drug ACTOS 45mg (Each tablet contains pioglitazone 45mg).* If this study had to go through clinical studies, it would have still yielded the same result but with great expenditure and long time. The concept of expedite testing has taken over the conventional clinical testing BA & BE studies assume that the results observed reflects in the general patient population. The tabulated results were carefully derived, scrutinized and documented at appropriate times without any delay.
Various parameters were assessed in comparison with Standard drug. Final discretion of results in terms of log transformed C_{max}, AUC $_{0-t}$ and AUC $_{0-\infty}$ is found to be in the range of 80-125 % of standard pioglitazone formulation. Confidence interval was maintained at 90%. The significance of errors noted was found to have 0.005 % impact on the final values. Thus safety and protocol instructions were strictly executed

Summary

The present study was open labelled, balanced, randomized, two treatment, two sequence, two period,single dose, cross over comparative oral Bio-equivalence of pioglitazone 45mg tablet study on 16 healthy, adult, human subjects under fasting condition. Test product and treatment with reference drug are done during every period of study. Two-period study is designed over 2 periods with wash out period of 10 days in between and single-dose study because each subject receives only a single dose in each period.
The study was conducted in 16 subjects and all 16 subjects completed the study in accordance with overnight fasting of at least 10 hours before dosing (a food- free period) was maintained in each study period. After an overnight fast for at least hours, subjects will be administered as per the randomization schedule, eithersingle dose of one tablet of Test, pioglitazone USP 45 mg or ACTOS 45mg (Each capsule contains 45mg of pioglitazone) with 240±2 ml of 20% oral glucose solution. A twenty three blood samples will be collected from each subject during each period. The venous blood samples (5ml) will be withdrawn at pre-dose (before dosing, in the morning of the day of dosing). The venous blood samples (1x 5mleach) will be withdrawn at0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.33, 2.67, 3.00,3.50, 4.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00 and 72.00 hours post-dose. 5mL of blood per sample was collected using syringe and transferred to pre-labelK_2EDTA sample collection vaccutainer. After completion of each period, these plasma samples were transferred to Bio analytical section and were stored at –75° C.Plasma samples of 16 subjects who completed the study were analyzed usingvalidated

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