REFERENCE-SCALED AVERAGE BIOEQUIVALENCE STUDY OF PAROXETINE UNDER FED CONDITIONS: VALIDATION OF SAMPLE SIZE ESTIMATION BY BOOTSTRAPPING TECHNIQUE

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ABSTRACT

Objective: New technique was adopted and validated to estimate pivotal sample size from the pilot study data and to establish bioequivalence (BE) of highly variable drugs (HVD), paroxetine, a novel controlled release (CR) matrix tablets utilized ghatti Gum as a rate controlling membrane, in human subject under fed conditions by reference scale design.

Methods: Bootstrapping technique was adopted to calculate the pivotal sample size from pilot study data for HVD paroxetine. The reliability and validation of the method were tested in a semi replicate three sequence (RRT, RTR, and TRR where T stand for test drug and R stand for reference drug) cross-over BE study in 24 healthy subjects under fed conditions.

Results: The ratio of the pharmacokinetic (PK) metric obtained from the bootstrapping technique after log transformation was 1.04 for C_{max}, 1.23 for AUCT, and 1.21 for AUCI with corresponding power of the study which was greater than 80% from pilot study data simulation. The ratio of the PK metric obtained from the reference scaling design in the present study was 1.00 for C_{max}, 1.21 for AUCT, and 1.17 for AUCI. The upper limit of the C_{max}, AUCT, and AUCI at 95% confidence limit was −0.143, −0.136, and −0.17, respectively.

Conclusion: The test paroxetine CR formulation was bioequivalent with reference drug under fed conditions. The technique used for estimation of the sample size in the pivotal study was found reliable, and bootstrapping technique plays an important role in calculating sample size where intrasubject variability was immaterial.

Keywords: Reference scaling, Bootstrapping techniques, Sample size, Bioequivalence, Controlled release.

INTRODUCTION

Paroxetine hydrogen chloride (paroxetine [HCl]) is an antidepressant and belongs to the class of selective serotonin reuptake inhibitors (SSRIs) [1,2]. Paroxetine has the highest known affinity for the serotonin transporter (0.13 nanomoles) of any currently used antidepressant [3]. Paroxetine was previously reviewed as an antidepressant drug in 1991 and reported some amount of hepatic toxicity following the chronic use of the drug [4,5]. Paroxetine (HCl) is now used to treat obsessive-compulsive disorders and panic disorders [6,7]. Paroxetine (HCl) is a phenylpiperidine derivative and (-) enantiomer is optically active, used clinically [8,9]. Paroxetine (HCl) is not available in any combination preparations. The reason was to reduce acute toxicity and improved tolerability of SSRI compared to previously frequently used tri- and tetracyclic antidepressants [10,11]. Furthermore, controlled release (CR) formulation of paroxetine is highly safe and tolerable compared to immediate release formulation [12].

Controlled clinical trial on pharmacokinetics (PK) of paroxetine exhibited high variability within the subjects (>30% reference variability within subjects). The substantial change in the innovator formulation necessitates bioequivalence (BE) study as per scale-up and post-approval changes (SUPAC) guideline (FDA, Guideline for industry, 1997) for registration of the generic product [13]. FDA proposed scaled average BE (SABE) study in 2008 to establish BE of highly variable drugs (HVD) with a minimum subject of 24 [14,15]. The most important feature of the SABE study is that a fixed sample size is adequate to demonstrate BE regardless of within-subject variability. In the present study, bootstrap technique is used to fix sample size and to calculate test/reference (T/R) ratio and power of the study from pilot data. Hence, the aim of the current research was to estimate sample size calculation

and validation by bootstrapping techniques from the pilot study. The other aim of the study was to establish BE of paroxetine 37.5 mg CR tablets by adopting three-period, three-sequence, two-treatment reference replicate (SABE) proposed by regulatory agency [16].

MATERIALS AND METHODS

Materials

Paroxetine and fluoxetine were obtained from Lupin Bioresearch Center, Mumbai, India. Acetonitrile, n-hexane, and methanol were procured from Sigma-Aldrich, Bengaluru, India. Analytical grade formic acid, amyl alcohol, and sodium hydroxide were purchased from local chemical store. Water was purified using in-house Milli-Q-System. All chemicals used for method development and validation were of analytical grade.

The liquid chromatography (LC) system used was API4000 AB SCIEX (California, USA) LC system equipped with triple-quadrupole mass analyzers and XBridge OST (BEH) C 18 column (50 mm×4.6 mm, 2.5 μ particle size with a pore size of 130 A⁰). A Phenomenex SecurityGuard column (Bester, 4.0×2.0 mm) was used to protect the original column.

Methods

Formulation development

Extended-release (ER) matrix tablets were prepared by wet granulation technique [17]. The exact quantity of drug and inactive ingredients (Table 1) was passed through sieve #40 and mixed thoroughly in octagon blender. The blend was mixed with isopropyl alcohol to make a dough mass which was then transferred to rapid mixture granulator to get wet granules. The wet granules were dried at room temperature

70°C for about 15 min. The dried granules were then passed through sieve no 20. The extra-granular lubricant talc was mixed with granules and finally punched by a 27 station rotary tablet punching machine. The list of inactive ingredients used for the formulation of the tablets is given in Table 1.

High-performance LC method development for the quantification of paroxetine in human plasma
Various methods have already been established to quantify paroxetine in biological fluids [18-29]. Such methods include gas chromatography (GC) with nitrogen and mass spectrometry (MS) detection or simultaneous estimation with the same chemical category compounds by LC-tandem mass spectrometry (LC-MS/MS) or GC-MS or LC with voltammetric detection or LC with ultraviolet or MS analysis. However, in the present study, low solvent consuming and best recovery MS analysis method was applied to quantify picogram level of drug concentration in human plasma. The method was validated with respect to liquid-liquid extraction, reversed-phase LC, and MS/MS detection to perform the selective determination of paroxetine. Tandem MS was selected to improve the selectivity and sensitivity of the method of determination. The LC conditions, composition of mobile phase, and the MS/MS optimization were investigated to select the most appropriate operating conditions. The validation of the method was performed with respect to parameters such as linearity of the chromatographic response, precision, and accuracy which meets the accepted criteria for bioanalytical method validation and employed in BE study of two paroxetine 37.5 mg ER tablets.

Preparation of calibration curve
A stock solution of paroxetine was prepared in methanol to obtain a concentration of 1000 μg/L. An aliquot of the solution was evaporated to

dryness and reconstituted with blank plasma and vortexed for 15 min to get a final concentration of 1 μg/L. From this solution, eight calibration standard solutions containing 200, 500, 1000, 2000, 4000, 6000, 8000, and 10,000 ng/L and three quality control solutions at concentrations of 500, 5000, and 10,000 ng/L were prepared in blank plasma. The stock solution of fluoxetine was prepared in methanol and aliquot of the solution was evaporating to dryness and used as internal standard (IS). The dried IS was reconstituted with blank plasma and vortexed for 15 min to get a final concentration of 1 μg/L. The calibration curve of paroxetine in blank human plasma and fluoxetine as IS is given in Fig. 1.

Preparation of plasma sample for analysis
Extraction of paroxetine from human plasma sample was carried out following addition of 100 mg NaCl, 200 μl of 0.1 M NaOH, 5 ml hexane:isoamyl alcohol (99:1, v/v), and 20 μL (1 μg/L) of IS to 0.5 ml plasma sample. The mixture was vortexed for 15 min and centrifuged at 3000 rpm for 15 min at 4°C to separate an organic phase. 5 mL of the supernatant solution was transferred to 10 ml Eppendorf vial and evaporated to dryness after addition of 50 μL of 1N HCL. The dried mixture was reconstituted with mobile phase (solution of formic acid [0.1%] in acetonitrile:water [6:4 v/v] pumped at a flow rate of 0.15 mL/min).

Validation of the method
The recovery of paroxetine was determined in three different QC samples having concentrations of 500, 5000, and 10,000 ng/L in blank blood plasma. Plasma samples with the added drug at the concentrations specified above were extracted in triplicate. The concentrations of these samples were calculated on the basis of calibration curves constructed from the data for the drug not subjected to the extraction procedure.

To determine the intraassay precision, aliquots (n=6) of blank plasma containing the standard solution of paroxetine at concentrations of 500, 5000, and 10,000 ng/L were analyzed by the method proposed. To determine the interassay precision, blank plasma containing standard solution at the specified concentration was analyzed on 5 consecutive days. Linearity was obtained by analyzing control plasma samples (n=6) containing standard solutions of paroxetine at concentrations of 500–10,000 ng/L. The concentration range was estimated on the basis of the regression curve ($Y=1.004X+0.0045$, $R^2=0.999$) (Fig. 1) and correlation coefficient. The limit of quantification was determined by analyzing blank plasma samples enriched with decreasing concentrations of paroxetine standard solution. The limit of quantification was considered to be the lowest concentration quantified with an error of <10% for a sample size of 5. The selectivity of the method was evaluated by analyzing several drugs combined with paroxetine. The drugs that presented retention times close to those of the drugs under the study were submitted to the extraction procedure and rechromatographed.

Table 1: List of inactive ingredients used for the formulation of tablets

| Test drug	Reference drug
Hypromellose	Hypromellose
Lactose monohydrate	Lactose monohydrate
Ghatti gum	Polyethylene glycol
Purified talc	Purified talc
FD and C yellow No. 6 aluminum lake	FD and C yellow No. 6 aluminum lake
D and C yellow No. 10 aluminum lake	D and C yellow No. 10 aluminum lake
Ghatti gum	Polymethyl methacrylate
Polysorbate 80	Polysorbate 80
Sodium lauryl sulfate	Sodium lauryl sulfate
Titanium dioxide	Titanium dioxide
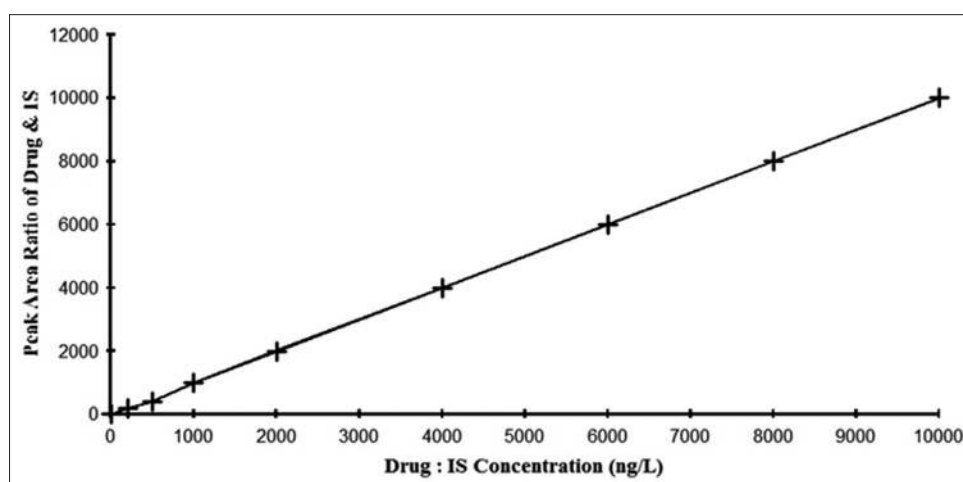
Fig. 1: Calibration curve of the paroxetine in blank human plasma using fluoxetine as internal standard in MRM mode ($Y=1.004X+0.0045$, $R^2=0.999$)

BE study

Twenty-six healthy male Indian volunteers aged between 18 and 45 years with body mass index (BMI) within 19–29.4 kg/m^2 were enrolled for the study after assessment of their health status by general physical examination and clinical laboratory evaluation (including electrocardiogram [ECG] and chest X-ray). The normal value range of the following laboratory tests: Albumin, alkaline phosphatase, aspartate aminotransferase (AST), aminotransferase (ALT), blood glucose, creatinine, μ-GT, total bilirubin, total protein, triglyceride, total cholesterol, hemoglobin, hematocrit, total and differential white cell counts, routine urinalysis, and negative serology for HIV, HBV, and HCV were determined before enrolling them in the study. All the subjects gave written informed consent, and the Madras Ethics Committee, Chennai, Tamil Nadu, India, approved the clinical study protocol. The study was conducted in accordance with ICH GCP, Indian GCP, ICMR guideline with the provisions of the Declaration of Helsinki (Seoul 2008).

The study was an open-labeled, randomized, three-sequence, three-period, single-dose, reference replicated three-way crossover design with 14 days' washout period between the doses. During each period, the volunteers were housed in a clinical pharmacology unit of Huclin Research Limited, Chennai, Tamil Nadu, India, on the eve of the dosing day. Following overnight fasting of about 10 h a high calorie, high fat breakfast was served 30 min before the study drug administration. A single dose of paroxetine (37.5 mg CR tablets of test or reference either formulation) was administered orally to each of the volunteers as per randomization code list at sitting posture with the aid of 240 ml of water in a staggered manner to easy sample collection. A mouth check following drug administration, with the help of a tongue depressor, was performed for each of the volunteers to ensure the compliance of the dosing activities. All the subjects were restricted to 2 h post-drug administration water intake and toileting, while at other time, water was given *ad libitum*. A standard meal was provided to all the volunteers at 4, 8, and 12 h post-drug administration. Vitals were recorded at 2, 4, 6, 8, 12, and 24 h post-drug administration for each volunteer to assess any health-related complication. No other food was permitted during the "in-house." Serial blood samples were collected from each of the volunteers at every period at pre-dose and at 04.00, 06.00, 08.00, 10.00, 12.00, 14.00, 16.00, 18.00, 20.00, 24.00, 28.00, 36.00, 48.00 72.00, and 96.00 h post-drug administration.

Sample size estimation for pivotal study

A pilot study was conducted among 12 subjects in three-period, three-sequence, cross-over design under fed conditions to establish sample size in the pivotal study. The within-subject reference variability of the area under the paroxetine plasma concentration-time curve from time 0 to the last measurable concentration (AUCT) and maximum drug concentration (C$_{max}$) was >0.3 and the T/R ratio of the drug was 1.27 and 1.3, respectively. Based on the result of the pilot study, 6 test and 12 reference subject data were randomly selected from the pool of 36 individual subject data for all the three periods. The resultant 18 (1:2) subjects were considered as a

boot sample and reanalysis the data to find the T/R ratio then simulate to 36, 45, 54, 60, 72, 90 and 108 boot samples and then recalculate the T/R ratio; this procedure was continued until T/R ratio was within 0.80–1.25. The simulation which produces the T/R ratio within the limit of 0.8 to 1.25 was considered the final sample size for the pivotal study ignoring the subjects and sequence effect in the bootstrap analysis. To confirm the sample size, a power calculation was done further by resampling up to 1000 times from the boot sample and individual calculation of PK metric to find the ratio (Fig. 2).

Finally, the power was calculated based on the percentage of the time, and the ratio was within 80–125 from thousand bootstrapping [30-34]. If the power was failed to meet 80%, then nearest boot sample was considered for further analysis to adjust the power of the study. In the present study, 72 boot samples were matched with the criteria of ratio between 80% and 125% which corresponds to a total number of subjects in the three-period design as 24 (72/3). Hence, 24 subjects were included in the study; the bootstrapping simulation of the data of 72 samples produced the T/R ratio of 1.23 for C$_{max}$ and 1.21 for AUCT after simulation.

PK analysis

Data from all subjects who completed all the three periods of the study were analyzed, and 24 subject data were used for PK and statistical analysis. The PK parameters of paroxetine were estimated using non-compartmental techniques [35]. The C$_{max}$ and the time elapsed to C$_{max}$ (T$_{max}$) were obtained directly from the data. The terminal-phase elimination rate constant was obtained using log-linear regression of the last four non-zero concentrations. The area under the paroxetine plasma concentration-time curve from time 0 to the last measurable concentration (AUCT) was computed using the linear trapezoidal rule. The area under the plasma concentration-time curve from time 0 to infinity (AUCI) was calculated as the sum of AUCT and C$_t$/K$_{el}$, where C$_t$ was the last measurable concentration. The elimination half-life was calculated as ln(2)/K$_{el}$. All the PK analyses were performed by WinNonlin Professional, version 5.0.1 (Pharsight Corporation, Mountain View, California).

Within subject reference variability

From three-period designs, sequence term was used to extract reference treatment effect and residual mean square for calculating reference variability assuming reference crossed with reference as like of conventional two-way cross-over study [36,37]. BE testing of paroxetine was performed using a partial replicate three-way cross-over design, and within-subject variability of primary PK parameters was calculated using mean square error (MSE) of log-transformed primary PK parameters (Equation 1).

$$\text{Within subject reference variability} = \left(\sqrt{\text{EXP}(\text{MSE}) - 1}\right) \times 100 \qquad (1)$$

The MSE was calculated by generalized linear modeling (GLM) procedure in SAS V 9.1.3 using sequence term to separate the cross-reference [38].
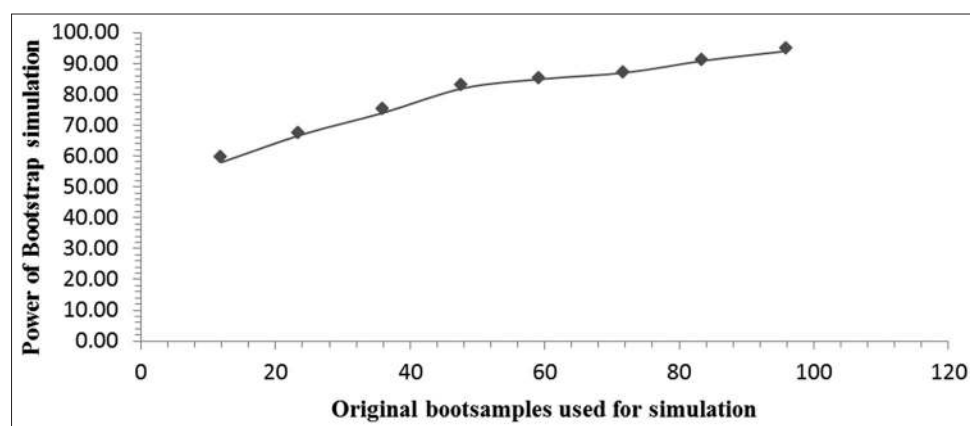

Fig. 2: Sample size and power estimation by 1000 boot samples analysis

The within-subject variability of paroxetine was used as critical criteria for its identification as a HVD and was an important parameter for the application of SABE and population bioequivalence [39].

Statistical analysis of PK data

The data were analyzed for normal average BE as well as reference scale average BE, and the statistical differences between the two approaches were evaluated. All the statistical analyses were performed by SAS V 9.2 (SAS Inc., Carry, USA). The primary PK parameters, namely $AUCT$, $AUCI$, and C_{max}, were log transformed to get parametric distribution, and log-transformed data were subjected to ANOVA analysis. A GLM procedure was used to evaluate formulation period and sequence effects on the primary PK parameters. The model included all primary PK parameters as main effect subjects nested within the sequence as random effect and sequence, period, and formulation as fixed effect [40]. The significance of all fixed effects was tested at alpha 0.05 and random effects at alpha 0.1 [41,42].

Reference variability between subjects and within-subjects was calculated by the use of SAS program for reference scaled approach as prescribed by the FDA for establishing the bioavailability of HVD.

The ratio of all primary PK parameter such as $AUCT$, $AUCI$, and C_{max} was also analyzed by the same program using PROC GLM model. The 90% confidence intervals (CIs) of the test/reference ratios for $AUCI$ and C_{max} were determined. Following national and international guidelines (including those of the FDA), the test and reference formulations were considered bioequivalent if the 90% CIs for the test/reference ratios of the log-transformed $AUCI$ and C_{max} mean values were between 0.80 and 1.25, and as per reference scaled approached point estimate of the ratio of PK parameters should fall within the 0.80–1.25 limit to show the BE as per FDA guidelines for HDV in a scale average BE approach and 95% CI upper limit of the test to reference PK metric will be less than zero.

In addition, a non-parametric analysis (Wilcoxon signed-rank test) was applied to compare T_{max} for both formulations. Non-parametric population characteristics were described with median and range.

Statistical evaluation of average BE

Average BE (ABE) was applied for BE assessment of paroxetine in 24 healthy human male subjects. ABE was assessed using a two one-sided hypothesis testing procedure as follows [43].

$$H_0: \log(1.25) \leq (\mu_T - \mu_R) \leq \log(0.80) \quad (2)$$

Versus

$$H_1: \log(0.8) < (\mu_T - \mu_R) < \log(1.25) \quad (3)$$

Where μT and μR are the mean for test and reference formulations adjusted for period and sequence effects on a log-transformed scale, respectively. ABE was concluded as the 90% CI for the ratio of test to reference limits within 0.80–1.25 for all PK parameters.

Statistical evaluation of SABE

SABE is also considered as scaled individual BE. There is no subject by formulation interaction and the same within-subject variability of two drug products. Difficulties arise to establish BE for that drug product which fall under the categories of within-subject reference variability ≥30%.

However, the scientists from FDA working group on HVD proposed the approach of reference SABER for the determination of BE in the case of HVD [44]. This method suggests that, if the expected intrasubject variability of a drug is <30%, the classic 0.80–1.25 limits should be applied. However, for drugs with CVWR exceeding 30%, a reference SABE approach should be used. These suggestions correspond to a switching coefficient of variation of CVw0=30%. It was proposed that three-period BE studies should be performed in which the reference

product will be administered twice and the test product once, i.e., the possible sequences are TRR, RTR, and RRT. SABE was used to establish the BE of paroxetine using a minimal number (n=24) subjects. The formula used to expand the BE limit is given below of the SABER criterion as described by equation 4.

$$\text{Upper / Lower BE limits} = \text{Exp}[\pm \ln(1.25) * \frac{swr}{swo}] \quad (4)$$

Where swr=Within-subject reference standard deviation (SD) and swo=Switching variability or regulatory limits.

SABE is concluded if the upper limit to the ratio of test to reference is zero or less than zero at 95% CI and the GMR of this parameter is within 0.8–1.25 to reduce the probability of type 1 error.

RESULTS

The generic paroxetine tablets were prepared using hypromellose, polyvinylpyrrolidone, ghatti gum, and glyceryl behenate. Polyethylene glycol and polymethyl methacrylate of the innovator formulation were replaced by ghatti gum. The list of other ingredients used in the development of the generic formula is given in Table 1. As per SUPAC guideline, changing one of the inactive ingredients from the innovator formula is considered to be major change. To obtain regulatory approval (USFDA) of the generic formulation with different inactive ingredients in comparison with reference listed drug require *in vivo* BE evaluation in human subjects. Hence, an *in vivo* BE study was conducted as per the FDA and EU guidelines to establish BE of developed generic formulation for marketing approval in the present study.

Twenty-six healthy adult male subjects were enrolled, 24 of whom completed all the periods of the study. The baseline demographic characteristics of the participants were expressed in terms of mean±SD (range): Age, 36.65±7.94 years (22–45); height, 169±12 cm (156–181); and body weight, 58.34±4.37 kg (45–72). One subject withdrew due to abnormal results on liver function tests (described later) before period 3. This subject's liver function test results returned to normal within 1 month. One subject discontinued the study prematurely for reasons other than investigational drug-related adverse events or serious adverse events.

The mean PK parameters observed after the administration of the various regimens are summarized in Table 2 for reference drug and Table 3 for test drug.

The mean plasma concentrations after the administration of paroxetine CR tablet with water are shown in Figs. 3 and 4.

A short lag time in maximum absorption after the administration of paroxetine CR tablets was observed for test drug. However, there was no difference in PK parameters observed for both the formulation.

Treatment-emergent adverse events were reported by 45.8% of the subjects (11/24) dosed during the study. Most of the adverse events were mild. Headache was the most common adverse event and was reported by 9.1% of subjects (2/22) with regimen A, 20.0% of subjects (4/20) with regimen B, 21.7% of subjects (5/23) with regimen C, and 20.0% of subjects (4/20) with regimen D. Before dosing in period 4, one subject was found to have abnormal liver function test results (AST, 270 U/L; alanine ALT, 875 U/L; lactate dehydrogenase, 658 U/L; and alkaline phosphatase, 252 U/L). At the final follow-up assessment, all levels had returned to within their respective normal ranges.

The linear mean plasma concentration versus time curves of test and reference paroxetine formulations to the 24 subjects under fed condition are given in Figs. 3 and 4, respectively. The primary and secondary PK parameters for both the formulations under fed condition are tabulated in Tables 2 and 3. The descriptive statistics for untransformed PK parameters for both the formulations under fed

Table 2: Descriptive statistics of pharmacokinetic parameters after reference treatment (fed conditions) (n=48)

Statistics	K_{el} (h^{-1})	T_{half} (h)	T_{max} (h)	C_{max} (ng/L)	AUCT (ng/L*h)	AUCI (ng/mL*h)
Mean	0.046	18.376	17.083	9701.246	297101.712	339057.078
SD	0.017	10.595	6.672	8266.247	267131.434	338282.858
Minimum	0.01	9.71	10.00	761.96	10630.02	11676.93
Median	0.05	13.26	15.00	7383.74	215353.70	218855.63
Maximum	0.07	52.65	28.00	35161.33	984853.59	1376373.59
CV%	37.3	57.7	39.1	85.2	89.9	99.8
Geometric mean	0.042	16.359	15.943	6635.088	179606.618	196337.926
95% CI lower	0.011	−3.541	3.281	−7398.790	−255501.763	−360734.331
95% CI upper	0.082	40.293	30.885	26801.281	849705.187	1038848.486

SD: Standard deviation, CI: Confidence interval, CV: Coefficient of variation

Table 3: Individual subject pharmacokinetics parameters after test treatment (fed conditions) (n=24)

Statics	K_{el} (h^{-1})	T_{half} (h)	T_{max} (h)	C_{max} (ng/L)	AUCT (ng/L*h)	AUCI (ng/L*h)
Mean	0.050	16.576	17.667	8195.932	272185.417	290550.049
SD	0.021	7.785	5.710	5091.449	145972.357	166821.804
Minimum	0.02	7.09	10.00	1046.68	23826.81	25542.89
Median	0.05	14.45	17.00	7171.07	258468.09	273805.94
Maximum	0.10	35.32	28.00	20242.99	534277.73	644989.53
CV%	41.7	47.0	32.3	62.1	53.6	57.4
Geometric mean	0.046	15.178	16.880	6663.217	217943.875	229617.563
95% CI lower	0.004	−0.558	5.099	−3010.272	−49097.573	−76622.265
95% CI upper	0.095	33.710	30.235	19402.135	593468.407	657722.364

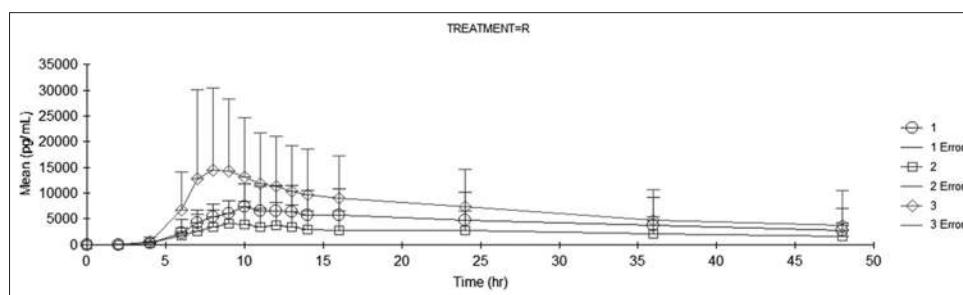SD: Standard deviation, CI: Confidence interval, CV: Coefficient of variation

Fig. 3: Linear mean plasma concentration of paroxetine reference product at three different periods under fed conditions (n=48)
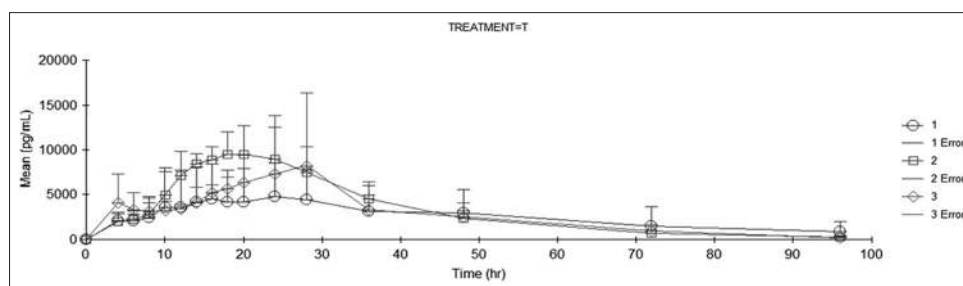

Fig. 4: Linear mean plasma concentration of paroxetine test product at three different periods under fed conditions (n=24)

condition is presented in Tables 2 and 3, respectively. The mean (±SD) C_{max} (ng/L) of test and reference formulations is 8195.9 (±5091.5) and 9701.3 (±8266.3), respectively. The mean (±SD) AUCT (ng/L*h) of test and reference is 272185 (±145972) and 297102 (±267131), while mean (±SD) AUCI (ng/L*h) of test and two reference formulations is 290550 (±166822) and 339057 (± 338283), respectively. The mean (±SD) of T_{max} (h), K_{el} (h^{-1}), and t_{half} (h) of test and reference formulations of paroxetine is 17.7 h (±5.7) and 17.1 h (±6.7), 0.05 h^{-1} (±0.21) and 0.05 h^{-1} (±0.17), and 11.0 h (±27.8) and 18.4 h (±10.6), respectively. The results of descriptive statistics of primary PK parameters of test and two reference products are presented in Tables 2 and 3, respectively. The reference variability (within-subject reference variability) of the primary PK parameters of C_{max}, AUCT (AUClast), and AUCI (AUC total)

is 0.58, 0.72, and 0.74, respectively. Details are presented in Table 4. The geometric mean ratio between two formulation and 95% CI for the primary PK parameters calculated from the ANOVA was 100%, −0.143113; 121%, −0.136539; and 117%, −0.170342, respectively (Table 4) meeting the criteria of scale average BE.

All the 24 subjects completed all the three periods of the study and there was no drop out or withdrawal. No serious adverse events were reported except abdominal pain in subject 01 and mild diarrhea in subject 02. However, such complications were resolved without sequel. There were no clinically significant changes in vital signs, clinical laboratory variables, ECG, X-ray, and general physical examination.

Table 4: Reference scaling of main PK parameters with 95% confidence interval

Parameter	S_{WR}	Point estimate	Standard error	Degrees of freedom	95% upper confidence limit
C_{max}	0.5808352	1.00	0.1137	21	−0.143113
AUCT	0.7223151	1.21	0.1331	21	−0.136539
AUCI	0.7358902	1.17	0.1338	21	−0.170342

DISCUSSION

The generic paroxetine 37.5 mg ER tablets were prepared using hypromellose, polyvinylpyrrolidone, ghatti gum, and glyceryl behenate. Polyethylene glycol and polymethyl methacrylate in the innovator formulation were replaced by ghatti gum. The list of other ingredients used in the development of the formula is given in Table 1. The ghatti gum was used to serve as a rate-controlling membrane for the ER formulation. Due to the change of the inactive ingredients, a BE study was needed to get regulatory approval of the drug for marketing. Keeping this in the mind, BE study was conducted under fed conditions. The inherent nature of the molecule was to behave differently in *in vivo* while dosing at the same individual at different occasion. Such variability was observed for all the primary PK parameters (GSK clinical trial registry study identifier, PCR: 112811 and 112812). Due to high variability observed in the PK parameters, it was needed more number of subjects to establish BE between dosage forms by a traditional average BE in two-way cross-over design. To avoid exploiting a large number of healthy volunteers, a reference scale approach was adopted to establish BE. The reference scale utilizes the reference variability >30%; if a PK metric shows that it variability between reference is <30%, then conventional BE approach will be adopted; otherwise, 95% CI test to reference ratio upper limit should be negative and test to reference ratio should be within 0.8–1.2 to claim BE. The method utilized two reference administration in the same subject at different periods and one test administration at another period; therefore, three-period, three-sequence, cross-over design was ideal to calculate reference variability.

This study was open-labeled, three-period, three-sequence, two-treatment cross-over design utilizing minimum subjects of 26 under fed conditions. A randomization was generated to eliminate the bias of selecting test and reference drug for subjects. The descriptive statistics of age, height, weight, and BMI of the 24 subjects participated in the study was within the limit of 18–45 years, 1.48–1.78 m, 55–75 kg, and 19–29.4 Kg/m², respectively. The drugs were dosed on the dosing day to the volunteers as per randomization code list 30 min after the completion of the high-fat high-calorie breakfast. The study-related procedure and blood collections were done at the scheduled time to quantify the plasma concentration of the drug. There were no reported serious adverse events, signifying that the Ghatti gum used for the development of the generic formulation is well tolerable in the human subject. Since the ghatti gum obtained from the natural sources, some microbial contamination will always be present after finest purification too. Therefore, it is obvious that there will be some amount of temperature rising following intake of the gum, but in our study, it was found that there were no such complications regarding fever to the subject which indicated the safety of the ghatti gum.

Both paroxetine and IS were extracted from plasma samples by solid-phase extraction using OASIS HLB extraction plates. Then, the plasma samples were washed further with a water:methanol (80:20, v/v) mixture. Mixtures containing varying percentages of methanol were tried. The 80:20 mixtures gave optimum results in terms of recovery and selectivity. The recovery of methanol:water mixture was not stable, and hence, the eluate was evaporated and the residue was reconstituted with mobile phase. A validated LC-MSMS method was used to quantify drug in the plasma samples. An eight-point calibration standard curve in human plasma, ranging from 200 to 10000 ng/L for paroxetine, was prepared in duplicate for each run. The standard curve was satisfactorily described by linear regression weighted by $1/x$, as the back calculated values were all within 61.5% of the nominal concentrations (62.0% at the LLOQ) in the three validation runs, which are not shown in the result. 26 of 24 enrolled subjects completed all the three periods of the study.

The PK profile of paroxetine, a single dose three-period, three-sequence, two-treatment cross-over reference replicate study with a minimum wash-out period of 14 days, carried out in fed conditions was considered to be adequate. The linear mean (±SD ng/L) concentration versus time graph of the reference formulation in three different periods is given in Fig. 3 and of test in Fig. 4. The descriptive statistics of PK parameters of test and reference drug are given in Tables 2 and 3, respectively. A test drug is considered to be pharmacokinetically equivalent, i.e., bioequivalent to a reference drug product if the 95% CI of the test and reference geometric mean ratios of the AUCs and C_{max} is found to be zero or less than zero and point estimates are in the range of 0.8–1.2. The appropriateness of the reference scaling approach was established from the S_{WR} values of the primary PK parameters (C_{max}, AUCT, and AUCI which were 0.58, 0.74, and 0.78, respectively); established primary PK parameters from this study at 95% CIs were within the pre-defined BE criteria (Table 4). The study results revealed that the two formulations of paroxetine were similar in PK characteristics among these healthy Indian subjects under fed conditions.

CONCLUSION

The sample size calculation for SABE from the pilot data using bootstrapping resampling techniques was adequate to estimate T/R ratio with only 1% deviation from the observed data. The LC-MSMS method used for the analysis of plasma samples was reliable with a good reproducibility of the results. The reference scale semi-replicate three-period, three-sequence, two-treatment *in vivo* clinical study was adequate to establish the difference of their geometric mean for all the primary PK metric.

There were no serious adverse events of the study and no change in the abnormal laboratory value for the subjects except diarrhea to the subjects 20 and 18 in period two and subject 13 in period three which were mild in nature and resolved without sequel.

The assumption of reference scaling for the BE study is proved since the within-subject reference variability (S_{WR}) for C_{max}, AUCT, and AUCI is 0.58, 0.72, and 0.73, respectively, under fed conditions; therefore, reference scaling approaches were used to establish BE and test product found BE with respect to the reference formulation, and hence, we can conclude that the ghatti gum can be used as rate-controlled membrane in certain concentration to develop CR formulation of other drugs.

AUTHORS' CONTRIBUTION

The authors were involved in study initiation, ethical committee approval, statistical and PK evaluation, and clinical study report.

CONFLICTS OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the corresponding author is the sole contact for the editorial process (including editorial manager and direct communications with the office). He is responsible for communicating with the other authors about progress, submissions of revisions, and final approval of proofs.

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