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Bioequivalence Study of Tramadol + Paracetamol (37.5 + 325 mg) In Healthy Human Volunteers in Fasting Condition

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ABSTRACT

The bioequivalence between test and reference Tramadol and Paracetamol (37.5 + 325 mg) tablets was determined in 36 healthy subjects after a single dose in a randomized crossover study under fasting condition. Plasma concentrations were monitored over a period of 24 hour after the drug administration by validated LC/MS/MS analytical method. The pharmacokinetic parameters C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, AUC_{0-t} / $AUC_{0-\infty}$, T_{max}, K_{el} and $t_{½}$ were determined from plasma concentration time profile of both formulations and found to be acceptable. The calculated pharmacokinetic parameters were compared statistically to evaluate bioequivalence between the test and reference products. The analysis of variance did not show any significant difference between the two formulations and 90 % confidence intervals for the ratio of C_{max} (92.29 -104.18 %), AUC_{0-t} (99.52 - 104.11 %) and $AUC_{0-\infty}$ (99.05 - 104.22 %) for tramadol and C_{max} (93.56 - 110.27 %), AUC_{0-t} (96.37 - 102.70 %) and $AUC_{0-\infty}$ (97.22-103.28 %) for paracetamol test and reference products were within the 80 – 125 % interval, satisfying the bioequivalence criteria the US Food and Drug Administration Guidelines. These results indicate that the test and the reference products of Tramadol and Paracetamol are bioequivalent.

Keywords: Bio-equivalence, Body Mass Index, Confidence interval, LC/MS/MS.

INTRODUCTION

Tramadol and Paracetamol fixed-dose combination is indicated for the symptomatic treatment of pain. [1] This combination is marketed in several countries worldwide. In Europe, the combination tablet is indicated in the treatment of moderate to severe pain in adolescents (>12 years) and adults and in USA, it is recommended for the short-term (≤ 5 days) management of acute pain in adults (>16 years). The combination provided effective analgesia in patients with various types of pain, such as osteoarthritis flare pain, chronic lower back pain, postsurgical orthopedic pain, and diabetic peripheral neuropathy pain. The recommended dosage of tramadol 37.5 mg and paracetamol 325 mg is two tablets 4 - 6 times per day as needed for pain relief and up to a maximum of 8 tablets per day. Due to the relatively short duration of action this is taken 4 - 6 times per day. [2]

Tramadol is well tolerated and effective analgesic used to treat moderate, severe and chronic pain. [3] This drug is a synthetic, centrally acting analgesic which possesses opioid agonist properties and activates monoaminergic spinal inhibition of pain. [4] Tramadol binds weakly to μ- and δ-opioid receptors and also interferes with neuronal release and reuptake of serotonin (5-HT) and nor-epinephrine in the descending inhibitory pathway of the central nervous system. Tramadol is a racemic mixture of 2 enantiomers, (–)-tramadol and (+)-tramadol; the latter being more pharmacologically potent than the former. [2]

Paracetamol is classified in the group of aspirin like or NSAID like drugs. [5] It is commonly used for the pain relief. The analgesic and antihyperalgesic effects rely on cyclooxygenase inhibition, nitric oxide synthesis blockade or reinforcement of the serotonergic system. [6-7]

The combination of analgesic drugs with different pharmacological properties show better efficacy and less side effects. Tramadol and Paracetamol combination shows synergistic analgesic effect in animal model [8] and healthy human subjects. [9] In clinical studies, the combination of tramadol and paracetamol is found to be safe, efficacious and is well tolerated in various pain conditions. [10] The benefit/risk is more for combination than individual. [2]

The bioavailability of a drug product has been defined as the rate and extent to which the active ingredient or therapeutic moiety is absorbed and becomes available at the site of drug action. Two drug products are considered bioequivalent if they are pharmaceutical equivalents (i.e. similar dosage forms made, perhaps, by different manufacturers) or pharmaceutical alternatives (i.e. different dosage forms) and if their rates and extents of absorption do not show a

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significant difference when administered at the same molar dose of the therapeutic moiety under similar experimental conditions. [3]

The overall cost of health care is rising due to increased cost of medicines. Introduction of the generic drugs has tremendously reduces the price of medicines without compromising the quality. [11] The genegic market has saved about $8.8 billion of drug expenditure for adults in US. [12] The FDA's definition of "therapeutic equivalence" says that the generic formulation is (among other things) bioequivalent to the innovator formulation and indicates the FDA's expectation that the formulations are likely "to have equivalent clinical effect and no difference in their potential for adverse effects". [13] "In vivo equivalence" or "bioequivalence" studies are carried out to assess the "interchangeability" between the innovator and generic products. [11]

The aim of the present study is to evaluate the bioequivalence between two fixed dose combination tablets of Tramadol and Paracetamol (37.5 + 325 mg) tablets in normal, healthy, adult, human subjects under fasting condition.

MATERIALS AND METHODS

Independent Ethics Committee approval was obtained before conducting the study. The study was conducted in accordance with the ethical principles that have their origin in the World Medical Association Declaration of Helsinki and in compliance with ICH E6 GCP and the local ethical guidelines of Indian Council of Medical Research. Subjects were enrolled in the study after obtaining written informed consent. The subjects were under medical supervision throughout their stay in the clinical facility to ensure safety and well being of the subjects in GVK Biosciences Pvt. Ltd.

Test product: Tramadol Hydrochloride 37.5 mg and Acetaminophen 325 mg Tablets, Batch No: TAAG001, Manufactured by MICRO LABS LIMITED, INDIA.

Reference product: ULTRACET® (37.5 mg tramadol HCl/325 mg acetaminophen tablets), Batch No: 8HG824-X, Manufactured by Janssen Ortho, NJ 08869.

The study was a randomized, open label, balanced, two-treatment, two-sequence, two-period, single-dose, crossover oral bioequivalence study in normal, healthy, adult, human subjects under fasting condition. A total of 36 normal, healthy, adult, human subjects were enrolled in the study. 35 subjects completed both the periods of the study and the data from 35 subjects were included in the pharmacokinetic and statistical analysis.

All subjects were examined for inclusion and exclusion criteria based on clinical examinations, recording of 12-lead electrocardiogram and laboratory investigations of blood and urine within the 21 days prior to first dosing. Radiological investigations (Chest X-ray) were performed not more than 6 months prior to first dosing. The subjects were healthy male human subjects aged between 18 to 55 years, BMI between 18.5 to 24.9 Kg/m^2 but the body weight not less than 45 kg.

The total duration of the study was 10 days including the 07 days of washout period. In both the study periods, subjects were housed at the clinical facility from not less than 10.5 hours pre-dose till at least 24 hours post-dose in each period.

Subjects fasted overnight for at least 10 hours prior to scheduled time for dosing. As per the randomization schedule generated by statistical software SAS® Version 9.1.3, one tablet of test or reference product was administered to each subject in sitting posture with 240 mL of water at ambient temperature by trained study personnel in each period. Subjects were instructed not to chew or crush the tablet but to consume it as a whole. Compliance for dosing was assessed by a thorough check of the oral cavity immediately after dosing. Subjects remained seated for two hours after dosing in each period except when clinically indicated to change the posture or in case of any natural exigency. Thereafter, the subjects were allowed to engage in normal activities while avoiding severe physical exertion. Equal allocation of treatments or balanced randomization was ensured.

Blood sampling and processing

Twenty nine venous blood samples (each 5ml) were collected from each subject during each period at pre-dose (within 1 hour before dosing) and at 0.167, 0.33, 0.50, 0.67, 0.83, 1.00, 1.167, 1.33, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.25, 3.50, 4.00, 4.50, 5.00, 6.00, 7.00, 8.00, 10.00, 12.00, 16.00, 20.00 and 24.00 hours after dosing within an window period of ±2 minutes. Post-dose samples collected beyond ± 2 minutes from the scheduled sampling time were reported as protocol deviations.

Heparin-lock technique (about 1 mL of 5 IU/mL heparin in normal saline solution was injected into the cannula after each sample collection) was used to prevent clotting of the blood in the indwelling cannula. Blood samples were collected after discarding the first 0.5 mL of heparinised blood from the tubing of the cannula. Collected blood samples were transferred to properly labeled vacuettes®/ vacutainers® containing K_2EDTA as anticoagulant.

Blood samples were centrifuged under refrigeration with machine set at 3000 rpm for 10 minutes at 4°C. After centrifugation, the plasma were separated and transferred to prelabeled polypropylene tubes in two aliquots. These polypropylene tubes were stored below -20°C for a maximum period of 12 hours and then they were stored at -70°C ± 20°C until analysis.

Statistical and pharmacokinetic evaluation

Plasma samples were analysed to quantify the concentrations of Tramadol and Paracetamol using LC/MS/MS bioanalytical method. The bioanalytical method was validated at the analytical facility for sensitivity, specificity, linearity, accuracy and precision (repeatability and reproducibility), ruggedness, recovery, dilution integrity, stability of samples (freeze-thaw stability, bench-top stability, auto sampler stability, dry extract stability, post extract stability, intermediate term stability, short-term and long-term stock solution stability of drug and internal standard) and matrix effect.

For Tramadol

The linearity range of Tramadol during method validation was 0.996 ng/mL to 402.351 ng/mL and the limit of quantification was 0.996 ng/mL. The subject samples were analysed with the linearity range of 1.011 ng/mL to 400.930 ng/mL and the limit of quantification was 1.011 ng/mL

For Paracetamol

The linearity range of Paracetamol during method validation was 199.974 ng/mL to 10001.186 ng/mL and the limit of quantification was 199.974 ng/mL. The subject samples were analysed with the linearity range of 200.277 ng/mL to 9992.672 ng/mL and the limit of quantification was 200.277 ng/mL.

Table 1: Summary Statistics of Pharmacokinetic Parameters For Tramadol in Reference (R) Formulation

PK parameter (Units)	N*	Mean	SD	Minimum	Maximum	Median	CV%	Geometric Mean
Kel (hr^{-1})	35	0.0967	0.0199	0.057	0.142	0.1005	20.6	0.0946
$t_{1/2}$(hr)	35	7.4901	1.6671	4.873	12.223	6.8948	22.3	7.3242
T_{max} (hr)	35	1.617	0.7197	0.67	3.25	1.500	44.5	1.462
C_{max} #	35	191.3023	48.6896	133.093	334.837	172.6360	25.5	186.1500
AUC_{0-t} ##	35	1523.7305	394.0632	915.392	2373.399	1455.0437	25.9	1475.8904
AUC_{0-INF} ##	35	1734.0037	535.0818	979.995	3167.675	1626.1292	30.9	1658.9307
AUC_{0-t}/ AUC_{0-INF}(%)	35	89.097	4.8045	74.93	95.84	90.508	5.4	88.966

*N= Total number of samples, #expressed in (ng/mL), ## expressed in (ng.hr/mL)

Table 2: Summary Statistics of Pharmacokinetic Parameters For Tramadol in Test (T) Formulation

PK parameter (Units)	N*	Mean	SD	Minimum	Maximum	Median	CV%	Geometric Mean
Kel (hr^{-1})	35	0.0978	0.0201	0.052	0.137	0.0940	20.6	0.0956
$t_{1/2}$(hr)	35	7.4319	1.7949	5.043	13.452	7.3732	24.2	7.2483
T_{max} (hr)	35	1.733	0.7403	0.50	3.50	1.750	42.7	1.574
C_{max} #	35	188.1868	48.4363	101.012	317.059	177.6450	25.7	182.2394
AUC_{0-t} ##	35	1552.2417	420.4755	906.447	2453.933	1436.6880	27.1	1499.8658
AUC_{0-INF} ##	35	1763.4758	569.0672	988.726	3288.820	1563.5003	32.3	1682.9453
AUC_{0-t}/ AUC_{0-INF}(%)	35	89.268	5.0369	72.63	95.28	91.133	5.6	89.121

*N= Total number of samples, #expressed in (ng/mL), ## expressed in (ng.hr/mL)

Table 3: Summary Statistics of Pharmacokinetic Parameters For Paracetamol In Reference (R) Formulation

PK parameter (Units)	N*	Mean	SD	Minimum	Maximum	Median	CV%	Geometric Mean
Kel (hr^{-1})	35	0.2928	0.0833	0.129	0.502	0.2706	28.5	0.2820
$t_{1/2}$(hr)	35	2.5535	0.7424	1.380	5.353	2.5611	29.1	2.4581
T_{max} (hr)	35	1.026	0.5883	0.33	2.25	0.830	57.4	0.871
C_{max} #	35	4103.0050	1151.2171	2544.901	6963.543	3630.3310	28.1	3958.8520
AUC_{0-t} ##	35	13033.4669	3446.6678	6708.489	20638.414	13864.2188	26.4	12569.3593
AUC_{0-INF} ##	35	14023.3538	3697.4393	7334.080	22431.113	15077.7486	26.4	13528.6210
AUC_{0-t}/ AUC_{0-INF}(%)	35	92.926	1.7759	89.49	96.47	93.324	1.9	92.909

*N= Total number of samples, #expressed in (ng/mL), ## expressed in (ng.hr/mL)

Table 4: Summary Statistics of Pharmacokinetic Parameters For Paracetamol in Test (T) Formulation

PK parameter (Units)	N*	Mean	SD	Minimum	Maximum	Median	CV%	Geometric Mean
Kel (hr^{-1})	35	0.2878	0.0681	0.167	0.450	0.2911	23.7	0.2801
$t_{1/2}$(hr)	35	2.5448	0.6219	1.541	4.151	2.3815	24.4	2.4748
T_{max} (hr)	35	1.076	0.5585	0.33	2.75	1.000	51.9	0.946
C_{max} #	35	4181.1049	1204.3891	2017.367	6528.856	3971.7950	28.8	4011.8101
AUC_{0-t} ##	35	12938.3732	3354.0484	7504.984	19508.024	13464.8636	25.9	12503.4514
AUC_{0-INF} ##	35	14015.9442	3611.6087	8440.917	21995.913	14439.7168	25.8	13554.1302
AUC_{0-t}/ AUC_{0-INF}(%)	35	92.273	2.1565	88.69	96.05	92.123	2.3	92.248

*N= Total number of samples, #expressed in (ng/mL), ## expressed in (ng.hr/mL)

Table 5: Statistical Results of Log Transformed PK Parameters For Tramadol

PK Parameters	Geometric Least Square Mean		Ratio T/R	90%	Power	ISCV
	T	R	(%)	Confidence Interval	(%)	(%)
C_{max}	182.7000	186.3234	98.06	92.29 - 104.18	100.0	15.0
AUC_{0-t}	1502.3094	1475.8395	101.79	99.52 - 104.11	100.0	5.6
AUC_{0-INF}	1685.1902	1658.6371	101.60	99.05 - 104.22	100.0	6.3

Table 6: Statistical Results of Log Transformed Pk Parameters For Paracetamol

PK Parameters	Geometric Least Square Mean		Ratio T/R	90%	Power	ISCV
	T	R	(%)	Confidence Interval	(%)	(%)
C_{max}	4016.4602	3954.2061	101.57	93.56 - 110.27	99.7	20.5
AUC_{0-t}	12515.0567	12579.5725	99.49	96.37 - 102.70	100.0	7.9
AUC_{0-INF}	13566.5882	13538.9321	100.20	97.22 - 103.28	100.0	7.5

Pharmacokinetic Analyses

Non-compartmental model was used for the estimation of pharmacokinetic parameters C_{max}, AUC_{0-t}, AUC_{0-INF}, t_{max}, K_{el} and $t_{1/2}$ from plasma concentration time profiles of Tramadol and Paracetamol using WinNonlin® Version 4.1.

The area under the curve from time '0' to the last time point with measurable plasma concentrations was computed using linear trapezoidal rule.

RESULTS

The maximum concentration of tramadol was achieved at 1.733 ± 0.7403 hours, 1.617 ± 0.7197 hours and of paracetamol at 1.076 ± 0.5585, 1.026 ± 0.5883 hours for test and reference product respectively. The $t_{1/2}$ of tramadol was observed to be 7.43 hours for test and 7.49 hours for reference where as the $t_{1/2}$ for paracetamol was 2.544 hours for test and 2.553 hours for reference.

Summary statistics of pharmacokinetic parameters (K_{el}, $t_{1/2}$, T_{max}, C_{max}, AUC_{0-t}, AUC_{0-INF}, AUC_{0-t}/ AUC_{0-INF}) for reference (R) and test (T) formulation of tramadol and paracetamol are presented in Table-1, Table-2, Table-3 and Table-4.

A total of 35 subjects completed both the periods of the study. The mean pharmacokinetic parameters (C_{max}, AUC_{0-t} and AUC_{0-INF}) estimated for Tramadol and Paracetamol for both the test and reference products are represented in Table-5 and Table-6 respectively.

The mean of C_{max}, AUC_{0-t} and AUC_{0-INF} of tramadol were 182.7000, 1502.3094, 1685.1902 for test and 186.3234, 1475.8395, 1658.6371 for reference respectively. For paracetamol the mean of C_{max}, AUC_{0-t} and AUC_{0-INF} were

4016.4602, 12515.0567, 13566.5882 for test and 3954.2061, 12579.5725, 13538.9321 for reference respectively.

The 90% confidence interval of C_{max} (92.29 - 104.18), AUC_{0-t} (99.52 - 104.11) and AUC_{0-INF} (99.05 - 104.22) for tramadol and the 90% confidence interval of C_{max} (93.56 - 110.27), AUC_{0-t} (96.37 - 102.70) and AUC_{0-INF} (97.22 - 103.28) for paracetamol were within the range of 80.00 – 125.00%.

The mean and logarithmic Plasma concentrations versus Time curve for tramadol test (T) and reference (R) formulations on linear scale and logarithmic scale are presented in Figure-1 and Figure-2 respectively and for paracetamol in Figure-3 and Figure-4 respectively.

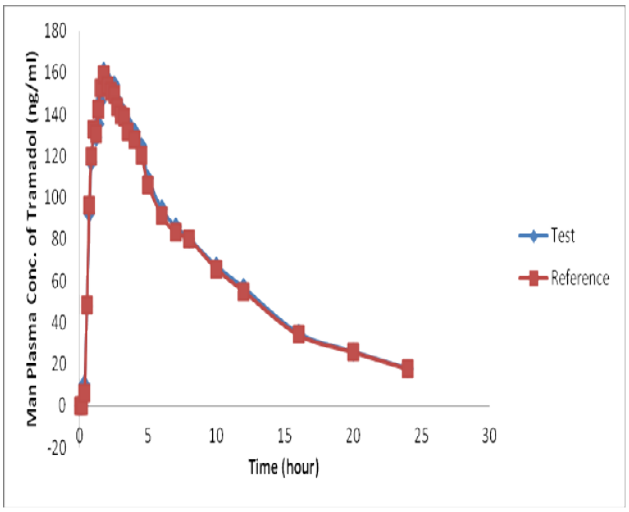

Fig. 1: Mean plasma concentrations versus Time curve for tramadol Test (T) and Reference (R) formulations on linear scale

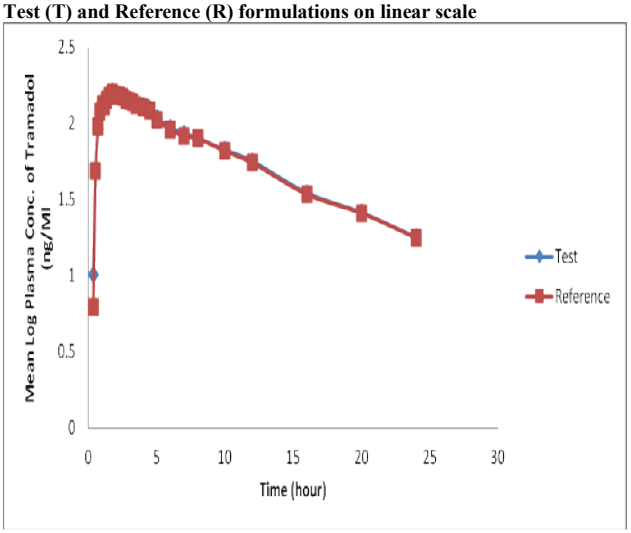

Fig. 2: Log Mean plasma concentrations versus Time curve for Tramadol Test (T) and Reference (R) formulations on logarithmic scale

DISCUSSION

This bioequivalence study conducted on healthy subjects shows that there is no significant difference for C_{max}, AUC_{0-t} and AUC_{0-INF} between the two formulations. The 90% confidence interval for the geometric least square mean ratio for above parameters indicates that the reported values are entirely within the bioequivalence acceptance range of 80 - 125% (Log transformed data). [14,15] Based on the pharmacokinetic and statistical results of this study, we can conclude that Tramadol Hydrochloride 37.5 mg and Paracetamol 325 mg Tablets (MICRO LABS LIMITED, INDIA) is bioequivalent to ULTRACET® (37.5 mg tramadol HCl/325 mg acetaminophen tablets, Janssen Ortho, NJ 08869) and the two products can be interchangeable in the medical use.

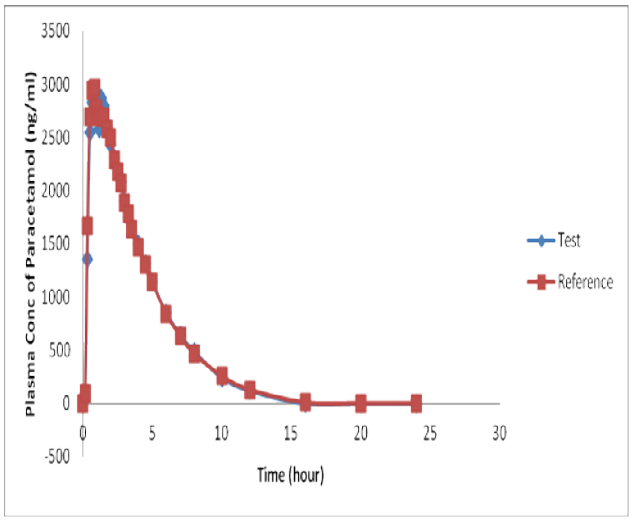

Fig. 3: Mean plasma concentrations versus Time curve for paracetamol test (T) and reference (R) formulations on linear scale

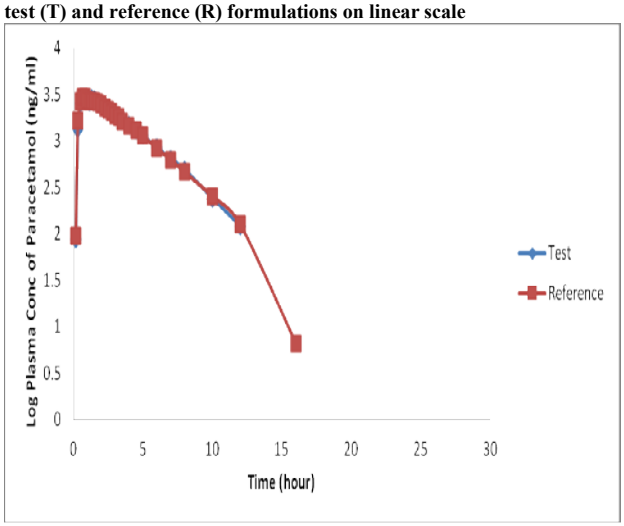

Fig. 4: Log Mean plasma concentrations versus Time curve for paracetamol test (T) and reference (R) formulations on logarithmic scale.

Since the test product was found to be bioequivalent to the reference product, both the products can be considered to have similar efficacy and safety. US FDA, EMEA, TGA and some other countries regulatory bodies recommended that the generic product should be bioequivalent to the innovator product/ marketed product to exclude any difference in clinical efficacy and pharmacokinetic profile and implementing different guidelines to ensure quality of the product. This study was conducted in fasting condition. So the bioequivalence need to be established in fed condition.

Multiple dose studies may be conducted to explore any difference in pharmacokinetic profile and may be done in patient population to access pharmacodynamic similarities.

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