Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(8):645-651

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Pharmacokinetic and bioequivalence comparison between levetiracetam extended release tablets 750mg: An open label, balanced, randomized-sequence, single-dose, two-period crossover study in healthy male volunteers

Nageswara Rao Pilli[a*], Swetha Savakula[b], S. Sai Satyanaraya Reddy[c] and Ravindra Reedy S.[c]

[a]*Bioanalytical Dept., Piramal Clinical Research, Hyderabad, Telangana, India*
[b]*Clinical Dept., ClinSync Clinical Research Pvt Ltd, Hyderabad, Telangana, India*
[c]*Information Technology, Vardhaman College of Engineering, Hyderabad, Telangana, India*

ABSTRACT

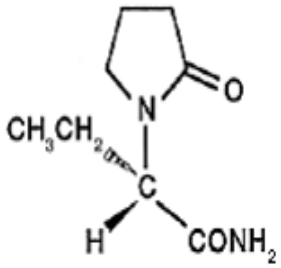
This present bioequivalence study was designed to determine the pharmacokinetic, bioavailability and bioequivalence of Levetiracetam 750mg extended release tablets in comparison with Keppra™ XR Levetiracetam 750mg tablets after single dose administration under fed conditions in healthy adult male subjects. Therefore the design of an open label, balanced, randomized, two-sequence, single dose, two way crossover study with a wash-out period of at least 7 days was used. An open-labeled, balanced, single-dose with food, two-treatment, two-period, two-sequence, randomized crossover study was conducted in 20 healthy male volunteers. Each volunteer received a 750mg extended release tablet of the reference or test drug respectively. On the day of dosing, blood samples were collected before dosing and at various time points up to 36 hours after dosing. Analysis of Levetiracetam concentrations was performed using a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) method. The pharmacokinetic parameters including C_{max}, AUC_{0-t}, AUC_{0-inf}, T_{max}, $t_{1/2}$ and K_{el} were analyzed using the non-compartmental model. Drug safety and tolerability were assessed. The pharmacokinetic parameters including C_{max}, AUC_{0-t}, AUC_{0-inf}, T_{max}, $t_{1/2}$ and K_{el} were analyzed using the non-compartmental model. Drug safety and tolerability were assessed. The primary pharmacokinetic parameters (C_{max}, AUC_{0-t} and AUC_{0-inf}) 90%CI were within the 80 to 125% interval required for bioequivalence as stipulated in the current regulations of the USFDA acceptance criteria. The geometric mean ratios (Test/Reference) between the two products of 750mg tablets under fed condition were 120.0% (91.85%-119.51%) for C_{max} ratios, 105.914% (94.95%-109.9%) for AUC_{0-t} ratios and 105.798% (93.73%-110.7%) for AUC_{0-inf} ratios of Levetiracetam. Twenty volunteers had completed both treatment periods. There was no significant difference of the T_{max} parameter between the two formulations ($p > 0.05$). No serious adverse events related to the study drugs were found. This single dose study found that the test formulation Levetiracetam 750mg extended release tablets are bioequivalent to the reference formulation Keppra™ XR Levetiracetam 750mg Tablets in terms of extent and rate of absorption, under fed condition in healthy adult male volunteers according to the USFDA regulatory guidance.

Keywords: Levetiracetam, Bioavailability, Bioequivalence, Intrasubject Variability

---

INTRODUCTION

Levetiracetam [1-2] is an antiepileptic drug available as 500 mg and 750 mg extended-release tablets for oral administration. The chemical name of Levetiracetam, a single enantiomer, is [-]-[S]-α-ethyl-2-oxo-1-pyrrolidine acetamide. Levetiracetam is chemically unrelated to existing antiepileptic drugs [AEDs]. It has the empirical formula $C_8H_{14}N_2O_2$ with a molecular weight of 170.21 g/mol, and the following structural formula:



Levetiracetam is a white to off-white crystalline powder with a faint odor and a bitter taste. It is very soluble in water. It is freely soluble in chloroform and in methanol, soluble in ethanol, sparingly soluble in acetonitrile and practically insoluble in n-hexane.

The exact mechanism by which Levetiracetam acts to treat epilepsy is unknown [3]. However, the drug binds to a synaptic vesicle protein, SV2A which is believed to impede nerve conduction across synapses. Absorption of Levetiracetam is rapid and peak plasma concentrations occur in about four hrs [4-7]. The time to peak plasma concentrations is about three hrs longer with extended-release Levetiracetam than with immediate-release tablets. Intake of a high fat, high calorie breakfast before the administration of extended-release Levetiracetam tablets resulted in a higher peak concentration, and longer median time to peak. The median time to peak [T_{max}] was two hrs longer in the fed state [8].

Levetiracetam is not extensively metabolized in humans. The major metabolite is inactive in animal seizure models. Two minor metabolites were identified as the product of hydroxylation of the 2-oxo-pyrrolidine ring [2% of dose] and opening of the 2-oxo-pyrrolidine ring in position 5 [1% of dose]. There is no enantiomeric interconversion of levetiracetam or its major metabolite.
Levetiracetam plasma half-life in adults is 7±1 hour and is unaffected by either dose or repeated administration. Levetiracetam is eliminated from the systemic circulation by renal excretion as unchanged drug which represents 66% of administered dose.

The rationale of this present bioequivalence study for two formulations of 750mg Levetiracetam Extended Release Tablets was examined between generic drug Levetiracetam 750MG Extended Release tablets as the test product and Keppra^{TM} XR (UCB INC) as the reference product. This bioequivalence study could give assurance when prescribing less expensive generic drugs as alternatives with similar efficacy and safety.

The study objectives of this present study are to assess the single dose bioequivalence of Levetiracetam 750mg Extended Release Tablets with Keppra^{TM} XR (UCB INC) in healthy, adult, human study participants under fed conditions and to monitor the clinical status, adverse events, laboratory investigations and assess relative safety and tolerance of Levetiracetam formulations under fed conditions.

EXPERIMENTAL SECTION

According to the USFDA Regulatory individual product recommendations, two studies (Fed and Fasting) to be done with 750mg Levetiracetam Extended Release Tablets to obtain marketing authorization in USA.

USFDA Waiver request of in-vivo testing [9]: 500 mg strength based on (i) acceptable bioequivalence studies on the 750 mg strength, (ii) acceptable in vitro dissolution testing of all strengths, and (iii) proportional similarity of the formulations across all strengths.

Study drugs
Levetiracetam 750mg Extended Release Tablets and Keppra^{TM} XR from UCB INC were used as the test and the reference products respectively. Both products were prepared as Levetiracetam Extended Release Tablets equivalent to Levetiracetam 750mg. Both the products were stored at controlled room temperature 25˚C (77 ˚F).

Study population
The study was carried out at ClinSync clinical Research Private Limited, India. The study protocol was approved by the Ethics Committee. In addition, the protocol was performed in accordance with the Declaration of Helsinki Principles [10] as outlined in the ICH-E6 Guidelines for Good Clinical Practice (GCP) [11]. All subjects were given a detailed description of the study and written informed consent was obtained prior to the enrollment.

The sample size was estimated based on, Coefficient of variation (C.V.) of the drug, sufficient statistical power to detect 20% difference with the power of 0.8 in C_{max} and AUC between the test and reference product, Regulatory requirements.

Sample size was based on estimates obtained from reported literature and previous studies. Assuming a formulation ratio (T/R) ranging from 0.95-1.05 a sample of 20 subjects including dropouts would be sufficient to show bioequivalence between the two formulations with a power of at least 80%. Hence sample size of 20 subjects was enrolled in the study.

Twenty healthy male volunteers between the ages of 18-45 years with a body mass index between 18.5 kg/m^2 and 24.9 kg/m^2, with body weight equal to or not less than 50 kg were assessed to be in good physical condition by a complete medical screening including a medical history, physical examination and laboratory screening test for hematologic and blood biochemistry parameters. Subjects with a history of hypersensitivity to any ingredients in the Levetiracetam products and/or related drugs or its constituents or who were taking any medication or alcohol for a 21-day period prior to the study were excluded. Subjects who had a history of cardiovascular, hepatic, renal, gastrointestinal or hematologic disease were excluded from the study.

Study design

The study was an open-labeled, single-dose, study taken with food, two-treatment, two-period, two-sequence randomized two way crossover with at least one week washout period. Subjects were randomly allocated to two groups by the sequence of product administered [Test-Reference (TR) and Reference-Test (RT) group]. In each period, 1X750MG extended release tablet of Levetiracetam of the test or reference product was administered 30 minutes after starting a high fat, high calorie breakfast at the same time in the morning before dosing. Subjects were housed 12 hours prior to dosing in the clinical facility from a time adequate to ensure 10 hours supervised fasting before consuming high fat breakfast and were allowed to leave the facility after 24.00 hours post-dose sample in each period. The subjects received a standard meal at about 4.0, 9.0 and 13.0 hours after dosing in each period. During housing, all meal plans were identical for all the periods. Drinking water was not allowed from one hour before dosing till one hour post-dose (except for 240 ± 02 mL of drinking water given for dosing). Before and after that, drinking water was allowed at *ad libitum*. After a minimum of 1 week washout period, the subjects were crossed over to the next treatment following the same procedure as conducted in the 1st period.

Sample collection

During dosing day in each period, 21 blood samples (6 mL each) will be collected as per the following schedule:

Pre dose sample (0.00 hr) within 02 hrs prior to drug administration and the others at 0.15, 0.30, 0.45, 0.60. 0.75, 0.90, 1.05, 1.30, 1.60, 2.00, 3.00, 4.00, 6.00, 8.00, 9.00, 10.00, 12.00, 16.00, 24.00 and 36.00 hours post dose. The total volume collected per study participant in this study will not exceed approximately 321 mL including up to 9 mL for screening, and 7-9 mL for post clinical assessment of lab parameters and 18 mL for discarded blood sample resulting from use of intravenous cannula for 12 hours and 2-9 mL was collected for repeat/additional lab tests, if required. For separating plasma, all blood samples were centrifuged at 3800 RPM for 10 minutes at 4°C ± 2°C.

Centrifugation of all samples was done as early as possible after each sample draw time point. After centrifugation, plasma samples were aliquoted into two sets in properly labeled polypropylene tubes and immediately stored at about -60°C or colder.

Levetiracetam analysis by LC-MS/MS

The published LC-MS/MS method [12] was validated according to USFDA regulations [13] for quantification of Levetiracetam from extracted subject plasma samples. A plasma sample (0.150 mL) was pipetted into a 2-mL centrifuge tube, 500 µL of IS working solution (500 ng/mL) were added. After vortex mixing for 10 s, a 1.0-mL Acetonitrile was added and the sample was vortex-mixed for 10 s. Centrifuged the centrifuge tubes at 14000 rpm at 10^oC for 10 min, transferred approximately 0.8mL of supernatant to HPLC vials and a 10-µL aliquot was injected into the chromatographic system.

HPLC was carried isocratically at room temperature using a Thermo BDS Hypersil C18 (4.6 x 50mm) column. The mobile phase consisted of 45:45:10 ACN: MeOH: 10 mM Ammonium acetate Buffer. The flow-rate was 0.8 ml/min. The duration of the analytical time was 3.0 min. The analytical column effluent is directed through the divert valve to a thermo electron TSQ quantum discovery mass spectrometer[14-16].

Chromatograms were acquired on a TSQ tandem mass spectrometry (Thermo Finnegan, Sanjose, CA, USA) equipped with Electrospray ionization (ESI) and connected to a PC runs with the standard software Xcalibur 2.0.7

and LC Quan 2.5.6 [17-19]. Mass spectroscopic detection was performed on a Triple quadrapole instrument (Thermo, TSQ Quantum Discovery Max). The calibration curve is constructed by weighted $1/x^2$ least-square linear regression analysis of the peak area ratio (drug/ISTD) vs. the concentration of drug [20].

Pharmacokinetic and statistical analysis [21-23]

For the purpose of Average Bioequivalence analysis C_{max}, AUC_{0-t} and AUC_{0-inf} were considered as the primary variables and T_{max}, $t_{1/2}$ and K_{el} were considered as the secondary variables. General Linear Model for analysis of variance (ANOVA) for crossover design was performed for log-transformed data and used to assess the effect of formulations, periods, sequences and subjects nested in sequence on these parameters. The difference between two related parameters was considered statistically significant for a *p*-value equal to or less than 0.05. 90% confidence interval (CI) for the ratios of geometric mean Test/Reference (T/R) for C_{max}, AUC_{0-t} and AUC_{0-inf} was calculated based on least squares means from the ANOVA of log-transformed data.

The 90% geometric CI of the ratio (T/R) of least squares means from the ANOVA of the log-transformed C_{max}, AUC_{0-t} and AUC_{0-inf} should be within 80.00% to 125.00%.

Tolerability assessment

Physical examination and measurement of vital signs (Blood Pressure, Pulse Rate and Oral Temperature) were examined at the time of Check-in, prior to administration of the each study drug (0.00 hr), 1.00, 3.00, 6.00, 12.00, 24.00 and 36.00hours post dose and during the entire study period. Adverse events were monitored throughout the study and recorded by physicians.

RESULTS

Study population

Twenty healthy male adults eligible for the study enrollment were randomly divided into 2 groups [Test-Reference (TR) and Reference-Test (RT)] according to the sequence of drug administration. All the subjects had completed both the periods. Thus, this study was balanced in each sequence and the results from 20 volunteers were used for pharmacokinetic and statistical analysis. Table 1 demonstrates the demographic characteristics of the volunteers.

Table 1: Demographic characteristics

Category		Treatment		Total
		Test (T)	Reference (R)	
Age (years)	Mean ± SD	23.84 ± 4.10	23.84 ± 4.00	23.84 ± 4.05
	Range	18.0 – 36.0	19.0 – 36.0	18.0 – 36.0
	Median	23.0	23.0	23.0
	N	20	20	40
Age Groups	< 18	00	00	00
	18 – 40	20	20	20
	41 – 64	00	00	00
	65 – 75	00	00	00
	> 75	00	00	00
Gender	Female	00	00	00
	Male	20	20	40
Race	American	00	00	00
	Hispanic	00	00	00
	Caucasian	00	00	00
	Asian	20	20	40
Height (cm)	Mean ± SD	163.52 ± 5.69	164.24 ± 5.67	165.48 ± 5.67
	Range	157.0 – 174.0	159.0 – 177.0	157.0 – 177.0
	N	20	20	40
Weight (kg)	Mean ± SD	58.96 ± 6.24	61.56 ± 6.43	60.26 ± 6.41
	Range	52.0 – 70.0	52.0 – 77.0	52.0 – 77.0
	N	20	20	40
BMI (kg/m^2)	Mean ± SD	21.86 ± 1.46	22.10 ± 1.79	21.98 ± 1.62
	Range	20.1 – 24.8	20.0 – 24.9	20.0 – 24.9
	N	20	20	40

Bioanalysis and pharmacokinetics

The Mass instrument is operated in the positive ion mode. The precursor ions at m/z 171.543 for Levetiracetam and m/z 179.668 for ISTD are selected by the first quadrupole (Q1). After collision-induced fragmentation in Q2, the product ions at m/z 126.134 for Levetiracetam and m/z m/z 163.136 ISTD are monitored in Q3. A resolution of one unit (at half peak height) is used for both Q1 and Q3. The method was fully validated using these Q1 and Q3 masses for both compounds with satisfactory results. *Linear calibration curves* were obtained with a coefficient of

correlation (r^2) usually higher than 0.995 in range of 1–40 µg/mL. For each calibration standard level, the concentration was back calculated from the linear regression curve equation.

No significant difference was observed in any of the analyzed pharmacokinetic parameters for Levetiracetam was shown in Table 2.

Table 2: Pharmacokinetic Parameters of Levetiracetam for Both Formulations

PK Parameters	Formulation [Levetiracetam]	
	Test	Reference
C_{max} [ng/mL]	22764.965	18975.553
AUC_{0-t} [ng.h/mL]	222856.551	210412.82
AUC_{0-inf} [ng.h/mL]	245910.505	232433.303
T_{max} [H]	1.067	0.963
K_{el} [H^{-1}]	0.088	0.091
$T_{1/2}$ [H]	8.053	7.811

Bioequivalence analysis

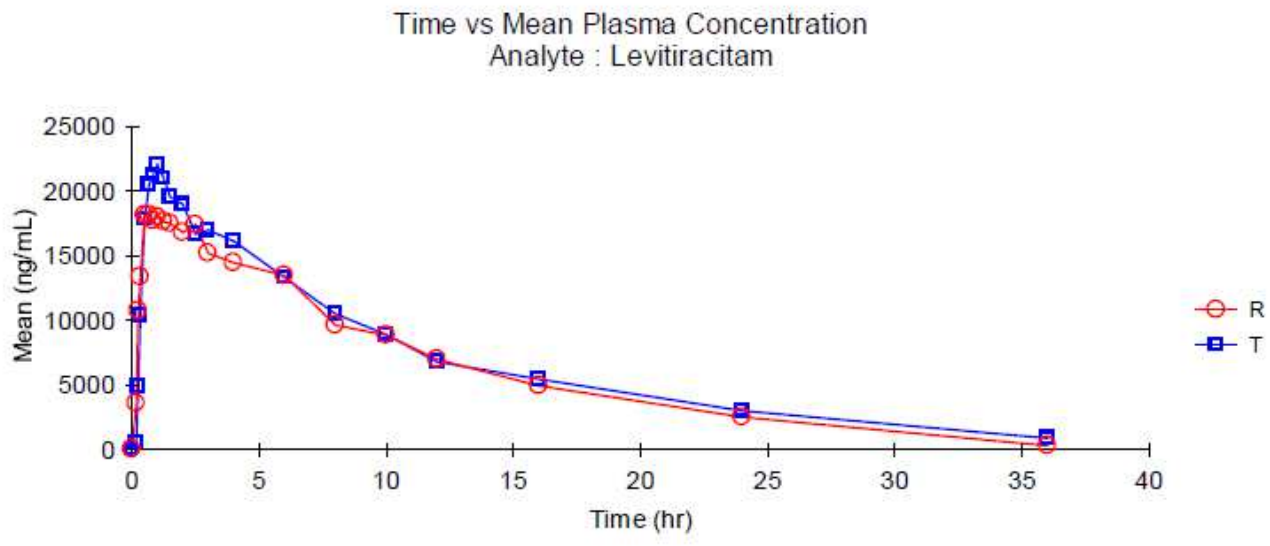
Ninety percent confidence interval of geometric mean ratios of bioavailability parameters between the test and reference formulation are presented in Table 3. The statistical analysis obtained from this study showed that the point estimate (90% CI) of the geometric mean ratio (GMR) (T/R) of C_{max}, AUC_{0-t} and AUC_{0-inf} was entirely within the equivalence criteria (80.00-125.00%) which was 120.0% (91.85%-119.51%) for C_{max} ratios, 105.914% (94.95%-109.9%) for AUC_{0-t} ratios and 105.798% (93.73%-110.7%) for AUC_{0-inf} ratios of Levetiracetam.

Table 3: Bioequivalence Parameters for Levetiracetam

Parameter	Levetiracetam		
	C_{max}	AUC_t	AUC_{inf}
90% CI Lower Limit	91.85	94.95	93.73
90% CI Upper Limit	119.51	109.9	110.7
T/R Ratio (%)	120.0	105.914	105.798
Power	0.91	1	1
Intra Subject Variability	10.19	4.6	5.7
Inter Subject Variability	28.33	49.12	43.12
ANOVA (p-Value)			
Sequence	0.13	0.15	0.19
Period	0.9	0.5	0.4
Treatment	0.9	0.4	0.6

In addition, no significant difference of the T_{max} parameter between the two studied formulations was observed ($p > 0.05$). Therefore, it was concluded that the two formulations of Levetiracetam were bioequivalent in terms of rate and extent of absorption for the drug. The mean plasma concentration vs time profiles were given in Fig 1.

Fig 1: Time vs. Mean Plasma Concentration Graph of Levetiracetam



Tolerability

Almost all volunteers taking both Levetiracetam formulations were noted for mild adverse events. Most common events were drowsiness, nausea and loss of appetite. However, no subject had any severe adverse event or withdrew from the study because of an adverse event.

DISCUSSION

An open-labeled, single-dose with food, two-treatment, two-period, two-sequence randomized two way crossover design in 20 healthy adult volunteers was considered appropriate and standard for bioequivalence evaluation of the generic and the reference products. The study simulates real life conditions including the influence of meals as well as circadian effects on the performance of the product. For a safety reason, co-administration of the drug with food can reduce nausea, a common side effect of Levetiracetam.

In general, the pharmacokinetic parameters for both formulations were similar to the pharmacokinetic parameters of Levetiracetam in previous published data. This study demonstrated that 90% CI of the logarithmic transformed of parameters C_{max}, AUC_{0-t} and AUC_{0-inf} were contained in 80.00-125.00%. In addition, no significant differences of the T_{max} values between the two formulations were observed ($p>0.05$). Therefore, the two extended release formulations of Levetiracetam are considered bioequivalent in terms of the rate and extent of absorption. Moreover, both formulations were well tolerated. Hence, the test (Levetiracetam) and reference (Keppra^{TM} XR) formulations of Levetiracetam 750mg ER are bioequivalent.

CONCLUSION

This single dose study found that the test formulation Levetiracetam Extended Release Tablets is bioequivalent to the reference formulation Keppra^{TM} XR Levetiracetam Extended Release Tablets the extent and the rate of absorption, of 750mg under fed condition in healthy adult male volunteers according to the USFDA regulatory guidance.

REFERENCES

[1] C.A.Hovinga. *Pharmacotherapy*., **2001**, 21[11], 1375-88.
[2] E.A. Wilson, M.J. Brodie. *Baillieres Clin. Neurol*., **1996**, 5[4], 723-47.
[3] William E. Rosenfelda, Selim Benbadisb, Pascal Edrichc, Carlo Alberto Tassinarid, Edouard Hirsche. Levetiracetam as add-on therapy for idiopathic generalized epilepsy syndromes with onset during adolescence: Analysis of two randomized, double-blind, placebo-controlled studies, **2009**, 85[1], 72-80.
[4] E. Perucca, M. Bialer, *Clin. Pharmacokinet*., **1996**, 31[1], 29-46.
[5] Eric Snoecka, Philippe Jacqmina, Maria Laura Sargentini-Maierb. Modeling and simulation of intravenous levetiracetam pharmacokinetic profiles in children to evaluate dose adaptation rules., **2007**, 76[2], 140-147.
[6] Nathan B. Fountaina, Joan A. Conryb, Ildefonso Rodriguez-Leyvac, Juvenal Gutierrez-Moctezumad, Edubijes Salase, Rene Coupezf, Armel Stockisf, Zhihong [Sarah] Lue. Prospective assessment of levetiracetam pharmacokinetics during dose escalation in 4- to 12-year-old children with partial-onset seizures on concomitant carbamazepine or valproate., **2007**, 74[1], 60-69.
[7] N. Isoherranen, B. Yagen, S. Soback, M. Roeder, V. Schurig and M.Bialer. *Epilepsia*., **2001**, 42, 825-830.
[8] R. Coupez, R. Straetemans, G. Sehgal, A. Stockis, Z.S. Lu, J. *Clin.Pharmacol*., **2003**, 43[12], 1370-6.
[9] Draft Guidance on Levetiracetam, Recommended Feb **2010**.
[10] World Medical Association Declaration Of Helsinki, Ethical Principles For Medical Research Involving Human Subjects, 59th WMA General Assembly, **2008**
[11] International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use, Guideline For Good Clinical Practice, E6, **1996**.
[12] Y.K.N, Raghunadha Reddy S., Koteswara Rao Divi, M.P. Kalyan Reddy, I. Sarath Chandiran and K.N. Jayaveera. *Research J. Pharm. and Tech*., **2010**, 3(3), 847-853..
[13] U.S. Department of Health and Human Services Food and Drug Administration, Bioanalytical Method Validation, 2001.
[14] I.Sarath Chandiran, K. N. Jayaveera and Raghunadha Reddy. S., *Journal of Chemical and Pharmaceutical* Research, , **2011**, 3[2]:341-353.
[15] I.Sarath Chandiran, K. N. Jayaveera and Ragunadha Reddy. S. *Der Pharmacia Lettre*, **2011**, 3[2]: 316-328.
[16] Raghunadha Reddy. S, I. Sarath Chandiran, K. N. Jayaveera and Koteswara Rao. Divi. *Journal of Chemical and Pharmaceutical Research*, **2010**, 2(3), 59-69.
[17] Raghunadha Reddy Seelam, Sarath Chandiran I, Koteswara Rao Divi, Jayaveera K. N., *International journal of pharmaceutical sciences and Drug Research*, **2010**, 2(3), 188-192.

[18] Raghunadha Reddy.S, I.Sarath chandiran, K.N. Jayaveera and Koteswara Rao.Divi. *archives of applied research*, **2010**, 2(3), 101-111.
[19] Raghunadha Reddy.S, Koteswara Rao.Divi, Y.K.Naidu, I.Sarath Chandiran, k.N. Jayaveera and M.P.Kalyan Reddy. *journal of chemical and pharmaceutical sciences*, **2010**, 3(2).
[20] Raghunadha Reddy.S, M.P. Kalyan Reddy, Y.K.Naidu, Koteswara Rao.Divi, I.Sarath Chandiran and K.N. Jayaveera. *International Journal of Pharma Research and Development*, **2010**, 2(2) , 1-9.
[21] I.Sarath Chandiran K.N. Jayaveera and Raghunadha Reddy.S, *International Research Journal Of Pharmacy*, **2011**, 2[3], 262-269.
[22] U.S. Department of Health and Human Services Food and Drug Administration, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations, **2003**.
[23] U.S. Department of Health and Human Services Food and Drug Administration, Statistical Approaches to Establishing Bioequivalence, **2001**.