BIOEQUIVALENCE STUDY OF GABAPENTIN

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Abstract:
The study was performed to compare the bioavailability of Gabapentin capsules USP 400 mg Test formulation with Neurontin 400 mg from Pfizer, USA as reference formulation in 48 male human volunteers. The study was conducted open Label with randomized two period crossover designs and a two week wash out period. Plasma samples were obtained over a 48 hour interval. The Gabapentin was analyzed by LC/MS/MS technique, in the presence of Gabapentin-D4 as an internal standard. With plasma concentration vs. time curves, data obtained from this analyte, the following pharmacokinetics parameters were obtained: C_{max}, AUC_{0-t}, and AUC_{0-∞}. The 90% confidence intervals in fasting conditions for C_{max} were 95.23% - 107.93%, for AUC_{0-t} were 93.42% – 110.42% and for AUC_{0-∞} were 94.34% – 109.58%, respectively. The 90% confidence intervals in fed conditions for C_{max} were 99.86% - 106.09%, for AUC_{0-t} were 98.31% - 105.35% and for AUC_{0-∞} were 98.44% - 105.23, respectively. Since the 90% confidence intervals for C_{max}, AUC_{0-t} and AUC_{0-∞} were within the 80 – 125% interval proposed by Food and Drug Administration, it was concluded that Gabapentin capsules USP 400 mg was bioequivalent to Neurontin 400 mg from Pfizer, USA according to both the rate and extent of absorption in fasting and fed conditions.

Key words: Gabapentin; Bioequivalence; Bioavailability; Pharmacokinetics.

Introduction:
Gabapentin [1-(aminomethyl)cyclohexaneacetic acid] is an antiepileptic drug structurally similar to GABA. Gabapentin (brand name Neurontin) is originally developed for the treatment of epilepsy however, presently; Gabapentin is widely used to relieve pain, especially neuropathic pain. Potential uses include monotherapy of refractory partial seizure disorders, and treatment of spasticity in multiple sclerosis, tremor. Mood disorders and attenuation of disruptive behaviors in dementia. Gabapentin is structurally related to the neurotransmitter Gamma Amino Butyric Acid (GABA). It is indicated as adjunctive therapy in the treatment of partial seizures with and without secondary generalization in adults with epilepsy and for the management of post herpetic neuralgia.

Gabapentin can be actively transported across the brain–blood barrier and the gut via the L-system amino acid transporter, which recognizes L-isoleucine, L-leucine, L-phenylalanine and L-valine. Gabapentin interacts with cortical neurons at auxillary subunits of voltage-sensitive calcium channels. Gabapentin increases

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the synaptic concentration of GABA, enhances GABA responses at non-synaptic sites in neuronal tissues, and reduces the release of mono-amine neurotransmitters. One of the mechanisms implicated in this effect of Gabapentin is the reduction of the axon excitability measured as an amplitude change of the presynaptic fibre volley (FV) in the CA1 area of the hippocampus. This is mediated through its binding to presynaptic NMDA receptors. Other studies have shown that the antihyperalgesic and antiallodynic effects of Gabapentin are mediated by the descending noradrenergic system, resulting in the activation of spinal alpha2-adrenergic receptors. Gabapentin has also been shown to bind and activate the adenosine A1 receptor.

**Theory/ Literature review:**
Various studies have been shown the mechanism of action of Gabapentin i.e. antiepileptic and antinociceptive activity\textsuperscript{[1]-[3]}, treatment of chronic musculoskeletal problems \textsuperscript{[4]}. Most of the investigator evaluated the pharmacokinetics of Gabapentin \textsuperscript{[5-9]}, but still fruitful data are yet to still avail. Additional double-blind, placebo-controlled clinical studies have suggested a potential role for Gabapentin in the treatment of restless legs syndrome \textsuperscript{[10]}, anxiety disorders \textsuperscript{[11]} and other neuropathies \textsuperscript{[12]}. However, Gabapentin has inherent pharmacokinetic deficiencies that may limit its effectiveness. Plasma exposure to Gabapentin after oral dosing is highly variable and unpredictable, apparently due to saturation of its absorption pathway, a low-capacity transporter found only in the upper small intestine \textsuperscript{[13, 14]}. Maximum plasma Gabapentin concentrations (mean) are attained 2 to 3 hours after a single oral 300 mg dose in healthy volunteers.

The objective of this study was to compare in healthy volunteers, the pharmacokinetics profiles and evaluate the bioequivalence of one test formulation of 400 mg capsule of Gabapentin and the test formulation was compared with one commercial formulation “Neurontin”.

**Material and Methods:**
**Study protocol:** The study was performed in accordance with the Helsinki Declaration and Good Clinical Practice Guideline, and informed consent was obtained from participants prior to study commencement.

**Subjects:** 48 Volunteers aged from 18-45 years with a body mass index (BMI) of 18.67-23.85 kg/m\(^2\) were enrolled according to the inclusion and exclusion criteria. They were assessed to be in healthy condition based on medical, systemic and physical examination including vital signs (blood pressure, pulse rate, oral temperature and respiratory rate) and normal laboratory tests results (haematology, biochemistry, urine analysis, 12-lead ECG and Chest X-ray (PA view)) including negative HIV-1 & 2, Hepatitis B, Hepatitis C. All the subjects provided written informed consent to participate after explaining the nature and purpose of the study. The study protocol was approved by the independent Ethics Committee.

**Drug Products:** The test formulation used was Gabapentin Capsules USP 400 mg and the reference formulation used was Neurontin® (Gabapentin Capsules USP 400 mg) of Pfizer, USA. The study was
conducted in an open randomized, balanced, two-treatment, two-period, two-sequence, single dose, two way crossover design with a wash out period of 14 days between the doses.

**Study Design:** Balanced, open label, randomized, two treatment, two period, two sequence, single dose, cross over comparative oral bioavailability study in healthy, adult, human male subjects under fasting or fed conditions.

**Study Duration:** Duration of clinical phase was 17 days including a wash out period of at least 14 days.

**Sample Size:** Sufficient number of subjects was enrolled to dose 48 subjects.

**Screening:** Demographic data, medical and medication history, physical examination, 12 lead ECG, vital signs, hematology, Biochemistry, HIV I & II, Hepatitis B & C, urine analysis and Chest X-ray PA view. Drugs of abuse and breathe alcohol test was performed before check in and each ambulatory samples in each period.

**Confinement:** In each period, the subjects were housed from at least 12 hours before drug administration to 24 hours after drug administration. Subjects visited the clinical facility for 36.0 and 48.0 hrs ambulatory samples.

**Drug Administration Procedure:** In each period, after an overnight fast of at least 10 hours, subjects received a single dose of Test (T) or Reference (R) product while in sitting posture with about 240 ml of water at ambient temperature according to the randomization schedule. But in case of fed study, high fat and high calories breakfast was provided before dosing.

**PK Sampling:** Total 17 samples was collected from each subject per period at pre-dose (0.0) and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 8.0, 12.0, 24.0, 36.0, 48.0 hrs post dose.

**Total Blood Loss:** Total 34 samples of 5 ml each (170 ml), 5.6 ml discarded blood, 14 ml blood for pre-study screening and 8 ml blood for post study test, amount to total blood loss of approximately 197.6 ml.

**Plasma Separation:** Blood samples were centrifuged to separate plasma as soon as possible. The samples were centrifuged at 4000 rpm, between 8°C -10°C for 10 minutes.

**Bioanalytical Procedure:** The concentration of Gabapentin in plasma was quantified using LC-MS/MS method according to the regulatory guidelines.

**Pharmacokinetic Parameters:** Employing the estimated Plasma concentration time profile of Gabapentin following Pharmacokinetic parameters was calculated Using SAS Statistical Software (9.1.3 or higher, SAS institute Inc., USA). Primary PK Parameters: C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> and Secondary PK Parameters: K<sub>el</sub>, T<sub>max</sub> and T<sub>1/2</sub>. Descriptive statistics like minimum, maximum, mean, geometric mean, median, standard deviation and coefficient of variation for all pharmacokinetic parameters was calculated.

**Statistical Evaluation:** Summary statistics, ANOVA, intra subject variability, 90% confidence intervals and power was calculated by non compartmental method using SAS® statistical software (9.1.3 or higher, SAS institute Inc., USA). Bioequivalence between the Test and reference
formulations was assayed by the calculation of the 90% confidence Interval of test / reference ratio (Least Square Mean) for Cmax, AUC$_{0-t}$ and AUC$_{0-\infty}$ based on Gabapentin (after log transformation) for Gabapentin.

**Bioequivalence Criteria:** Considered bioequivalent, T/R ratio & the 90% confidence interval of the primary parameters was fall within the interval 80.00% to 125.00%. The power of the ANOVA to detect a 20% difference ($\alpha=0.05$) between formulations was determined.

**Results and Discussion:**

**Gabapentin under Fasting Condition:**

Forty eight (46+2) healthy male subjects were enrolled in the study. All 48 subjects were randomized to receive either of the sequence of administration of the investigational drug product. Out of 48 subjects, 46 subjects were completed both the periods. The plasma samples of 46 subjects who completed both the periods were analyzed for Gabapentin plasma concentration and the data of the 46 subjects receiving reference and test product were used for pharmacokinetic and statistical analysis.

After oral administration, Cmax was attained for Gabapentin within 1.0 to 6.0 hours for both reference product and test product. There was no pre-dose concentration detected for any subject in both the periods. The drug was detected in plasma for 48.0 hours post dose. Period effect, treatment effect and sequence effect are found to be statistically insignificant at 5% level of significance.

**Pharmacokinetic and statistical analysis:**

Central and Dispersible Central and dispersion measures for all pharmacokinetic parameters for both formulations are shown in Tables presents the ratios and the respective confidence intervals for bioequivalence analysis.

**Table-1: Mean Pharmacokinetic Parameters under fasting condition**

<table>
<thead>
<tr>
<th></th>
<th>Reference Product</th>
<th>Test Product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arithmetic Mean</td>
<td>SD</td>
</tr>
<tr>
<td>C$_{max}$ (ng/mL)</td>
<td>3379.8912</td>
<td>908.5617</td>
</tr>
<tr>
<td>AUC$_{0-t}$ (ng/mL).hr</td>
<td>34305.2370</td>
<td>11434.9404</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (ng/mL).hr</td>
<td>35418.7432</td>
<td>11373.1332</td>
</tr>
<tr>
<td>T$_{max}$ (hrs)</td>
<td>3.2826</td>
<td>1.3360</td>
</tr>
<tr>
<td>K$_{el}$ (hr$^{-1}$)</td>
<td>0.1261</td>
<td>0.0225</td>
</tr>
<tr>
<td>T$_{1/2}$ (hr)</td>
<td>5.6396</td>
<td>0.8403</td>
</tr>
</tbody>
</table>
Table-2: Geometric mean Pharmacokinetic Parameters under fasting condition

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reference Product Geo LSM</th>
<th>Test Product Geo LSM</th>
<th>Ratio</th>
<th>Intra_CV</th>
<th>Power</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>3260.1212</td>
<td>3305.1417</td>
<td>101.38</td>
<td>18.01</td>
<td>99.99</td>
<td>95.23</td>
<td>107.93</td>
</tr>
<tr>
<td>AUC₀₋ₜ (ng/mL).hr</td>
<td>32345.3378</td>
<td>32851.8686</td>
<td>101.57</td>
<td>24.2</td>
<td>99.13</td>
<td>93.42</td>
<td>110.42</td>
</tr>
<tr>
<td>AUCᵢ₅₋ₗ (ng/mL).hr</td>
<td>33621.5773</td>
<td>34183.7073</td>
<td>101.67</td>
<td>21.62</td>
<td>99.77</td>
<td>94.34</td>
<td>109.58</td>
</tr>
</tbody>
</table>

The number of volunteers must always ensure enough statistical power to ensure the reliability of the results of the bioequivalence study. The mean (± SD) plasma concentration time profile of the two formulations under fasting were shown in figures.

Results of Gabapentin under Fasting Condition

Results of Gabapentin under Fed Condition:
Forty eight (46+2) healthy male subjects were enrolled in the study. All 48 subjects were randomized to receive either of the sequence of administration of the investigational drug product. Out of 48 subjects, 46 subjects were completed both the periods. The plasma samples of 46 subjects who completed both the periods were analyzed for Gabapentin plasma concentration and the data of the 46 subjects receiving reference and test
product were used for pharmacokinetic and statistical analysis. After oral administration, \( C_{\text{max}} \) was attained for Gabapentin within 1.50 to 5.00 hours for reference product and 1.50 to 5.00 hours for test product. There was no predose concentration detected for any subject in both the periods. The drug was detected in plasma for 48.0 hours post dose.

**Pharmacokinetic and statistical analysis:**

Central and Dispersible Central and dispersion measures for all pharmacokinetic parameters for both formulations are shown in Tables presents the ratios and the respective confidence intervals for bioequivalence analysis.

### Table-3: Mean Pharmacokinetic Parameters under fed condition

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reference Product</th>
<th>Test Product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arithmetic Mean</td>
<td>SD</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>3753.7626</td>
<td>926.9555</td>
</tr>
<tr>
<td>( \text{AUC}_{0-t} ) (ng/mL).hr</td>
<td>39154.5004</td>
<td>9303.2784</td>
</tr>
<tr>
<td>( \text{AUC}_{0-\infty} ) (ng/mL).hr</td>
<td>40291.8999</td>
<td>9180.8050</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (hrs)</td>
<td>3.9891</td>
<td>0.7637</td>
</tr>
<tr>
<td>( K_{\text{el}} ) (hr(^{-1}))</td>
<td>0.1223</td>
<td>0.0135</td>
</tr>
<tr>
<td>( T_{1/2} ) (hr)</td>
<td>5.7406</td>
<td>0.7012</td>
</tr>
</tbody>
</table>

### Table-4: Geometric mean Pharmacokinetic Parameters under fed condition

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reference Product Geo LSM</th>
<th>Test Product Geo LSM</th>
<th>Ratio</th>
<th>Intra_CV</th>
<th>Power</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>3644.9325</td>
<td>3751.6311</td>
<td>102.93</td>
<td>8.65</td>
<td>100</td>
<td>99.86</td>
<td>106.09</td>
</tr>
<tr>
<td>( \text{AUC}_{0-t} ) (ng/mL).hr</td>
<td>38078.6019</td>
<td>38752.0969</td>
<td>101.77</td>
<td>9.88</td>
<td>100</td>
<td>98.31</td>
<td>105.35</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{inf}} ) (ng/mL).hr</td>
<td>39279.4647</td>
<td>39978.2376</td>
<td>101.78</td>
<td>9.52</td>
<td>100</td>
<td>98.44</td>
<td>105.23</td>
</tr>
</tbody>
</table>

The number of volunteers must always ensure enough statistical power to ensure the reliability of the results of the bioequivalence study. The mean (± SD) plasma concentration time profile of the two formulations under fasting were shown in figures.
Results of Gabapentin under Fed Condition:

The bioavailability of a pharmaceutical form refers to the extent and speed of absorption of the active principle in contained it. Two pharmaceutical forms are said bioequivalent when, to be administered to the same individual, in the same experimental conditions and at the same dose, showed no significant differences in relation to bioavailability. In this study two formulations of Gabapentin had been evaluated. The mean ratio of parameters $C_{\text{max}}$ and AUC$_{0-t}$ and 90% confidence intervals of correspondents were calculated to determine the bioequivalence.

The statistical comparison of $C_{\text{max}}$, AUC$_{0-t}$ and AUC$_{0-\infty}$ clearly indicated no significant difference in the two formulations of Gabapentin 400 mg capsule. 90% confidence intervals for the mean ratio (T/R) of $C_{\text{max}}$, AUC$_{0-t}$ and AUC$_{0-\infty}$ were entirely within the US Food and Drug Administration acceptance range. Based on the pharmacokinetic and statistical results of this study, we can conclude that Gabapentin 400 mg capsule, test formulation is bioequivalent to Neurontin® 400 mg capsule (Pfizer, Brazil), and that then the test product can be considered interchangeable in medical practice. Both the drug products were well tolerated by all the subjects. All the biochemical parameters showed without any clinical relevant alterations. No adverse effects and serious adverse effects were either reported or observed.

Conclusion:
The result of the study indicates that the test product Gabapentin capsules USP 400 mg is bioequivalent with the reference product Neurontin 400 mg of Pfizer, USA with respect to the rate and extent of absorption under fasting condition. Both the study formulations were well tolerated by all the subjects in the study.

Figure-3: Mean graph for plasma concentration profile of Gabapentin under fed condition

Figure-4: Semi log graph for plasma concentration profile of Gabapentin under fed condition
References:

8. Toufigh Gordi, Eddie Hou, Sreeneeranj Kasichayanula, and Bret Berner (2008), Pharmacokinetics of Gabapentin After a Single Day and at Steady State Following the Administration of Gastric-Retentive–Extended-Release and Immediate-Release Tablets: A Randomized, Open-Label, Multiple-Dose, Three-Way Crossover, Exploratory Study in Healthy Subjects, Clinical Therapeutics/Volume 30, Number 5, 909-616.
of Gabapentin Enacarbil Extended-Release Tablets in Patients With Varying Degrees of Renal Function Using Data From an Open-Label, Single-Dose Pharmacokinetic Study, Clinical Therapeutics/Volume 34, Number 1, 201-212.


