3.0 Materials and Methods

The experimental design has been divided into five parts:

1. Clinical study methodology
2. Analytical Methodology
3. Pharmacokinetic analysis
4. Statistical analysis
5. In vitro In Vivo Correlation

3.1 Clinical Study Methodology

Clinical part of the study was carried out at Ranbaxy Clinical Pharmacology Unit (CPU) Majeedia hospital. The two batches of the test product Pregabalin 600 mg extended release tablet and the reference product Lyrica 300 mg immediate release capsules (each dose containing Pregabalin 300 mg) and all the other clinical trial supplies were provided by Ranbaxy Research Laboratories. The drug details are given in the Table MM 1.

3.1.1 Products evaluated

Reference (R)

Lyrica capsules (containing Pregabalin 300 mg) of Pfizer GmbH, Germany.

Test (A)

Pregabalin 600 mg extended release tablet of Ranbaxy Laboratories Limited, India.

Test (B)

Pregabalin 600 mg extended release tablet of Ranbaxy Laboratories Limited, India.

Details of drug product for study are listed in Table MM 1.

<table>
<thead>
<tr>
<th>Product</th>
<th>Batch. No.</th>
<th>Mfg. Date</th>
<th>Expiry date</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyrica 300 mg capsules</td>
<td>0460106U</td>
<td>N/AP</td>
<td>09/2009</td>
<td>Pfizer GmbH, Germany</td>
</tr>
<tr>
<td>Pregabalin ER 600 mg (Test A)</td>
<td>DM (169D)21</td>
<td>09/2008</td>
<td>08/2010</td>
<td>Ranbaxy Laboratories Limited, India.</td>
</tr>
<tr>
<td>Pregabalin ER 600 mg (Test B)</td>
<td>VC (178D)19</td>
<td>02/2009</td>
<td>01/2011</td>
<td>Ranbaxy Laboratories Limited, India.</td>
</tr>
</tbody>
</table>
3.1.2 Study design
The study design was an open label, balanced, randomized, three-treatment, three-period, three-sequence, crossover bioavailability study comparing single dose of two batches of Pregabalin 600 mg extended release tablet of Ranbaxy Laboratories Limited, with two oral doses of Lyrica 300 mg capsules (each dose containing Pregabalin 300 mg administered 12 hourly; total dose 600 mg, of Pfizer GmbH) in healthy adult, human, male subjects under fed condition as shown in the Fig. MM 1 & MM 2.

3.1.3 Number of subjects
Eighteen (18) healthy male adult human subjects were admitted in the first period. Subsequent dropouts and/or withdrawn subjects were not replaced. Data was presented on all completed subjects. If necessary, an unequal number of subjects per sequence was used.

3.1.4 Selection of subjects
Adequate numbers of subjects were selected randomly from the volunteer bank of Clinical Pharmacology Unit and the subjects underwent a standardized screening procedure.

3.1.4.1 Screening Assessments
Medical histories and demographic data, including name, sex, age, body weight (kg), height (cm) and number of cigarettes smoked per day were recorded. Each subject underwent physical examination and the laboratory tests of hematology, hepatic and renal functions as listed in the Table MM 2. Only medically healthy subjects with clinically normal laboratory profiles were selected who met following inclusion criteria. Eighteen healthy male human subjects were selected based on the following inclusion and exclusion criteria.
Table MM 2: Laboratory Tests carried out during screening of subject

<table>
<thead>
<tr>
<th>TEST</th>
<th>PARAMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematology</strong></td>
<td>Hemoglobin&lt;br&gt;Total Leukocyte Count&lt;br&gt;Differential Leukocyte Count&lt;br&gt;Platelet count</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td>Blood urea Nitrogen&lt;br&gt;Creatinine&lt;br&gt;Total Bilirubin&lt;br&gt;Alkaline Phosphatase&lt;br&gt;Aspartate aminotransferase (AST)&lt;br&gt;Alanine aminotransferase (ALT)&lt;br&gt;Glucose&lt;br&gt;Cholesterol</td>
</tr>
<tr>
<td><strong>Urinalysis</strong></td>
<td>Physical Examination&lt;br&gt;Microscopic&lt;br&gt;Drug screen&lt;br&gt;Colour&lt;br&gt;Appearance&lt;br&gt;PH&lt;br&gt;Specific Gravity&lt;br&gt;Protein&lt;br&gt;Glucose&lt;br&gt;Cannabinoids&lt;br&gt;Opioids&lt;br&gt;E. cells&lt;br&gt;Crystals&lt;br&gt;Casts&lt;br&gt;Others</td>
</tr>
<tr>
<td><strong>Additional Tests</strong></td>
<td>Human Immunodeficiency Virus (HIV I &amp; II)&lt;br&gt;Hepatitis B Antigen (HBsAg)&lt;br&gt;Hepatitis C virus (HCV)&lt;br&gt;Venereal Disease Research laboratory (VDRL)</td>
</tr>
</tbody>
</table>
Fig. MM 1: Schematic representation of study design for Reference

**Period I, II and III (n=18)**

<table>
<thead>
<tr>
<th>Reference:</th>
<th>D</th>
<th>V</th>
<th>B</th>
<th>Dosing</th>
<th>V</th>
<th>L</th>
<th>V</th>
<th>V</th>
<th>D</th>
<th>Dosing</th>
<th>V</th>
<th>V &amp; B</th>
<th>L</th>
<th>S</th>
<th>V &amp; Di</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>2100</td>
<td>0730</td>
<td>0815</td>
<td>0900</td>
<td>1100</td>
<td>1300</td>
<td>1500</td>
<td>1900</td>
<td>2015</td>
<td>2100</td>
<td>2300</td>
<td>0900</td>
<td>1300</td>
<td>1700</td>
</tr>
<tr>
<td>Admission &amp; vitals</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Blood sample</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Predose Lyrica 300 mg capsule</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>
| Note: Blood samples Prior to morning dose, 0.500, 1.000, 1.333, 1.667, 2.000, 2.333, 2.667, 3.000, 3.333, 3.667, 4.000, 4.500, 5.000, 6.000, 8.000, 10.000, 12.000, 12.500, 13.000, 13.333, 13.667, 14.000, 14.333, 14.667, 15.000, 15.333, 15.667, 16.000, 16.500, 17.000, 18.000, 20.000, 24.000, 30.000, 36.000 and 48.000 hrs post morning dose in each period D= Dinner, B= Breakfast, L= Lunch, S= Snacks, V= Vitals, Di= Discharge Dosing and subsequent sampling timings will be suitably staggered.

Fig. MM 2: Schematic representation of study design for Test Product

<table>
<thead>
<tr>
<th>Test:</th>
<th>D</th>
<th>V</th>
<th>B</th>
<th>Dosing</th>
<th>V</th>
<th>L</th>
<th>V</th>
<th>V</th>
<th>D</th>
<th>V</th>
<th>V &amp; B</th>
<th>L</th>
<th>S</th>
<th>V &amp; Di</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>2100</td>
<td>730</td>
<td>830</td>
<td>900</td>
<td>1100</td>
<td>1300</td>
<td>1500</td>
<td>1900</td>
<td>2030</td>
<td>2300</td>
<td>0900</td>
<td>1300</td>
<td>1700</td>
</tr>
<tr>
<td>Admission &amp; vitals</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Blood sample</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Predose Pregabalin 600 mg extended release tablet</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>
| Note: Blood samples: Predose and at 1.000, 2.000, 2.500, 3.000, 3.500, 4.000, 4.500, 5.000, 5.500, 6.000, 6.500, 7.000, 7.500, 8.000, 8.500, 9.000, 10.000, 12.000, 14.000, 16.000, 18.000, 24.000, 30.000, 36.000 and 48.000 hrs post morning dose in each period D= Dinner, B= Breakfast, L= Lunch, S= Snacks, V= Vitals, Di= Discharge Dosing and subsequent sampling timings will be suitably staggered.

Post dose Adverse Event monitoring at admission, pre dose, every 4 hours post dose until discharge and at every ambulatory visit during each period. The subjects will make 1 visit to the Clinical Pharmacology Unit for collection of further blood samples and vitals at 48 hours post-dose in each period.
3.1.4.2 Inclusion Criteria

- Were in the age range of 18-45 years.
- Were neither overweight nor underweight for his/her height as per the Life Insurance Corporation of India height/weight chart for non-medical cases.
- Had voluntarily given written informed consent to participate in this study.
- Were of normal health as determined by medical history and physical examination of the subjects performed within 21 days prior to the commencement of the study.
- Were non-vegetarian

3.1.4.3 Exclusion Criteria

The subjects were excluded who had any of the following exclusion criteria and finally eighteen healthy male human subjects were selected.

- History of hypersensitivity to Pregabalin or any other drug.
- History of dizziness, ataxia, or in-coordination.
- History of recurrent headache.
- History of excessive somnolence / narcolepsy.
- History of drug induced rashes/pruritis.
- History of confusion, or abnormal thinking.
- History of peripheral edema, or dry mouth.
- History of blurred vision.
- Abnormal fundoscopic findings at the time of admission of any period of the study.
- History of myopathy, myalgia.
- PR interval > 200 msec at the time of screening.
- Any evidence of organ dysfunction or any clinically significant deviation from the normal, in physical or clinical determinations.
- Presence of disease markers of HIV 1 or 2, hepatitis B or C viruses or syphilis infection.
- Presence of values, which are significantly different from normal, reference ranges (as defined in Appendix 4) and/or judged clinically significant for hemoglobin, total white blood cells count, differential WBC count or platelet count.
Positive for urinary screen testing of drugs of abuse (opiates or cannabinoids).

Positive for breath alcohol test.

Presence of values, which are significantly different from normal reference ranges (as defined in Appendix 4) and/or judged clinically significant for serum creatinine, blood urea nitrogen, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase, serum bilirubin, plasma glucose or serum cholesterol.

Clinically abnormal chemical and microscopic examination of urine defined as presence of RBC, WBC (>4/HPF), epithelial cells (>4/HPF), glucose (positive) or protein (positive).

Clinically abnormal ECG or chest x-ray.

History of serious gastrointestinal, hepatic, renal, cardiovascular, pulmonary, neurological or hematological disease, diabetes or glaucoma.

History of any psychiatric illness, which may impair the ability to provide, written informed consent.

Regular smokers who smoke more than 10 cigarettes daily or have difficulty abstaining from smoking for the duration of each study period.

History of drug dependence or excessive alcohol intake on a habitual basis of more than 2 units of alcoholic beverages per day (1 unit equivalent to half pint of beer or 1 glass of wine or 1 measure of spirit) or have difficulty in abstaining for the duration of each study period.

Use of any enzyme modifying drugs within 30 days prior to Day 1 of this study.

Participation in any clinical trial within 12 weeks preceding Day 1 of this study (except for the subjects who dropout/withdrawn from the previous study prior to period I dosing).

Subject had hemoglobin concentration of less than 13 gm%.

Subjects who, through completion of this study, would have donated and/or lost more than 350 mL of blood in the past 3 months.
3.1.5 Admission and stay

Subjects were admitted and housed in the clinical pharmacology unit at least 10 hours before dose administration and were discharged 36 hours after administration of study drug during each period, if the subjects do not suffer from any adverse drug reaction. In case of an adverse event, the subjects were monitored until the event subsides. The subjects made one visit to the clinical pharmacology unit for vitals and collection of further blood sample at 48 hours post-dose in each period.

Period I: 17 March 2009 to 20 March 2009
Period II: 23 March 2009 to 26 March 2009
Period III: 30 March 2009 to 02 April 2009

3.1.6 Fasting/Meals

For Reference: After an overnight fast of at least 10 hours, subjects started the standard meal 45 minutes prior to administration of the morning dose. Study subjects ate this meal in 30 minutes or less. The dose was administered 45 minutes after start of the meal with 240 mL of water.

The second dose was administered at 12.00 hrs after the morning dose. The standard meals were served at 45 minutes prior to second dose. Study subjects ate this meal in 30 minutes or less. The dose was administered 45 minutes after start of the meal with 240 mL of water.

No food was allowed for at least 4 hours each post-dose.

For Test: After an overnight fast of at least 10 hours, subjects started the standard meal 45 minutes prior to administration of the study drug. Study subjects ate this meal in 30 minutes or less. The dose was administered 45 minutes after start of the meal with 240 mL of water. The standard meal was also served at 11.25 hrs. No food was allowed for at least 4 hours post-dose.

Apart from the standard meals, subjects received lunch, breakfast, lunch and snacks at 4, 24, 28 and 32 hours, respectively, after drug administration. During housing, all meal plans were identical for all the periods. Information on the amount of meal consumed and the time taken for consuming the meal was recorded in the appropriate clinical raw data.
sheets. In case meals and blood sample collection time coincide, samples were collected before meals are provided. Drinking water was not allowed from 1 hour before dosing until 2 hours post-dose (for test product and for first and second dose of reference product) except 240 ml of water given during administration of the dose. Thereafter, it was allowed at all times.

3.1.7 Assignment to treatment

The order of receiving the test A or test B or reference product for each subject during the 3 periods of the study was determined according to a SAS-generated randomization schedule [Annexure IV]. The randomization was balanced and the code was kept under controlled access in the drug store. A working copy of the same was provided to study personnel responsible for dosing. The investigator, registered pharmacist, personnel involved in dispensing of study drugs and the dosing was accountable for ensuring compliance to randomization schedule.

Either two oral doses of reference product, each containing Pregabalin 300 mg administered at 12-hour intervals or test (A) or test (B) products containing Pregabalin 600 mg was administered with 240 mL of drinking water under fed condition in each period under supervision of trained personnel.

Reference (R)

Two oral doses of Lyrica capsules (each containing Pregabalin 300 mg) manufactured by Pfizer GmbH, Germany, was administered at an interval of 12-hours with 240 mL of drinking water at an ambient temperature, 45 minutes after starting of a standard meal. The first dose was administered after an overnight fast of at least 10 hours. The second dose was administered 12.0 hours after the first dose, 45 minutes after starting of a standard meal.

Test (A)

A single oral dose of Pregabalin 600 mg extended release tablet manufactured by Ranbaxy Laboratories Limited, was administered with 240 mL of drinking water at an ambient temperature 45 minutes after starting of a standard meal.
Test (B)
A single oral dose of a Pregabalin 600 mg extended release tablet manufactured by Ranbaxy Laboratories Limited, was administered with 240 mL of drinking water at an ambient temperature 45 minutes after starting of a standard meal.

3.1.8 Assessment of compliance
Compliance was assessed by conducting a thorough examination of the oral cavity by trained study personnel after dosing in each period and by measurement of plasma Pregabalin (during the analytical phase of the study).

3.1.9 Restrictions
3.1.9.1 Medications
All subjects were instructed not to take any other medications including OTC during the 2 weeks period prior to the onset of the study. The medication was advised only in case of medical emergencies. Decisions to continue or discontinue the subject was based on the following:
   i) The pharmacology and pharmacokinetics of the non-study medication.
   ii) The likelihood of a drug-drug interaction, thereby affecting pharmacokinetic comparison of the study medication.
   iii) The time of administration of the non-study medication.

3.1.9.2 Diet
All subjects were instructed to abstain from alcoholic products and grape fruit juice for 48 hours prior to dosing and during in-house stay in each period. They also abstained from any other xanthine containing food or beverages during in-house stay in each period.

3.1.9.3 Activity
All subjects were dosed while seated and were instructed to remain seated or ambulatory for the first 2 hours following each drug administration. Thereafter, subjects were allowed to engage only in normal activities while avoiding severe physical exertion. However, should any adverse medical event occur at any time during housing the subjects were placed in an appropriate position or were permitted to lie down on their right side.
3.1.10 Blood sampling

A total of ninety (90) 4-mL blood samples {including duplicate predose samples (2 X 4 mL)} were collected from each subject in CPDA vacutainers during the course of the study through indwelling cannulae placed in forearm veins.

Reference (R): Prior to morning dose (in duplicate) and at 0.5, 1.0, 1.33, 1.67, 2.0, 2.33, 2.67, 3.0, 3.33, 3.67, 4.0, 4.5, 5.0, 6.0, 8.0, 10.0, 12.0, 12.5, 13.0, 13.3, 13.67, 14.0, 14.33, 14.67, 15.0, 15.33, 15.67, 16.0, 16.5, 17.0, 18.0, 20.0, 24.0, 30.0, 36.0 and 48.0 hours after the morning dosing in each period. The sampling at 12.0 h post first dose was done 2 minutes before scheduled time to enable second dosing on time.

Test (A & B): Predose (in duplicate) and at 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 12.0, 14.0, 18.0, 24.0, 30.0, 36.0 and 48.0 hours post dose in each period.

The pre-dose (for test product) / prior to morning dose (for reference product) blood sample in each period were collected within a period of 1.5 hours before dosing and the post-dose samples were generally within 2 minutes of the scheduled time. These predose samples were collected in duplicate. The actual end time of collection of each blood sample were recorded. For each subject, the total number of blood draws during the study were 87 and the total volume of blood drawn, including 16 mL for screening, 37.5 mL 'discarded' blood prior to venous cannula collections, 06 mL for safety analysis at the end of study; will not exceed 419.5 mL, including duplicate predose samples.

After collection of blood samples from all the subjects at each time-point, one of the study personnel or an attendant transferred all the collection tubes to a sample processing room at the Clinical Pharmacology Unit. Thereafter the blood samples were centrifuged under refrigeration as soon as possible to separate plasma. All plasma samples were divided into 2 aliquots and transferred to suitably labeled tubes and re-checked to ensure transfer of plasma to the correct tube. The plasma were stored at below -15° C, until transferred to the analytical facility for assay.
3.1.11 Safety

3.1.11.1 Clinical Safety Measurements

Vitals signs recording

Vital signs (oral temperature, sitting blood pressure and radial pulse) were measured during subject admission, prior to dosing, 2, 6, 10, 14, 24, 36 and 48 hours after administration of investigational products in each period. Vital signs to be measured prior to administration of the dose were taken within 1.5 hours of the scheduled dosing time. Post-dose, vital signs were taken within one hour of the scheduled time. In the event of detection of any abnormality during measurement of vital signs, the investigator must be consulted for necessary action, which was recorded. Fundoscopy of each subject was done at the time of admission of each period of the study.

Clinical examination

Brief clinical examination of the subject was conducted by a qualified medical designate on duty after subject admission, prior to dosing and thereafter approximately every 12 hours until discharge. In the event of detection of any abnormality during clinical examination, the investigator must be consulted for necessary action, which was recorded.

Laboratory Evaluations for Safety

Laboratory parameters were repeated at the end of the study. Any laboratory parameter outside acceptable limits was termed as laboratory abnormality and followed up, if deemed necessary by investigator.

3.1.11.2 Adverse Events

The Investigator or a medical officer was available at the site of investigation until 36 hours post-dose during each period. A medically qualified designate was on call for the remaining period of the study. Subjects were monitored throughout the study period for adverse events. Subjects were informed to bring to the notice of the nurse or the physician, any adverse event that may occur during their stay at the site of investigation. Subjects were also be specifically asked about any adverse events at the time of admission, predose and at 4, 8, 12, 16, 20, 24, 36 and 48 hours post dose in each period.
Treatment of any adverse events was done by a physician, either at the site of investigation or at a nearby hospital as shown in Fig. MM 2.

### 3.1.12 Discharge & Washout Period

All subjects were discharged 36 hours after administration of the study drug during each period. A washout period of six and seven days was enforced between dosing of period I and II and period II and III respectively.

### 3.1.13 Ethical Considerations

#### 3.1.13.1 Basic Principles

This research was carried out in accordance with the basic principles defined in US 21 CFR Part 320, the ICH (62FR 25692, 09 May 1997) ‘Guidance for good clinical practice’, ICMR 'ethical guidelines for biomedical research on human participants (2006)', CDSCO 'guidance on good clinical practices for clinical research in India' and the principles enunciated in the Declaration of Helsinki (WMA General Assembly, Seoul 2008) respectively.

#### 3.1.13.2 Institutional Review Board

This protocol and the corresponding informed consent form (ICF) used to obtain informed consent of study subjects were reviewed and approved by the Jamia Hamdard Institutional Review Board and the study subjects were not dosed until the board approved the protocol and the ICF, as submitted or with modifications. The version one of the protocol and the ICF for this study were reviewed and approved by the Jamia Hamdard Institutional Review Board on 17 March 2009.

#### 3.1.13.3 Informed Consent

The purpose of the study, procedures to be carried out, potential hazards and rights of the subjects were described to the subjects in non-technical terms before the subjects were admitted to the Ranbaxy CPU for Period I. All the subjects provided formal written consent after attending an oral presentation and after thoroughly reading the informed consent form.
3.1.13.4 Drop-out/ Withdrawal of Subjects from Study

Subjects were informed that they are free to drop-out from the study at any time without stating any reason. The decision of withdrawal of a subject from the study was considered for any of the following reasons:

(i) The subject suffers from significant intercurrent illness or undergoes surgery during the course of the study.
(ii) The subject experiences adverse event and withdrawal is in the best interest of the subjects.
(iii) The subject fails to comply with the requirements of the protocol. This would include pre-study directions regarding alcohol and drug use, fasting or if the subject is uncooperative during the study.

Details of reasons for withdrawal of subjects were recorded and reported. Every effort was made to obtain a complete follow-up for any withdrawn subject.

3.1.13.5 Volunteer Compensation

The subjects were adequately compensated on account of their participation in the study. In case of drop-out/withdrawal of a subject before completion of the study, the guidelines issued by the Jamia Hamdard Institutional Review Board were final and binding on both Ranbaxy Research Laboratories and the study subjects.

3.1.14 Study documentation

All data generated during the conduct of the study was directly entered in the raw data recording forms except the analytical data of clinical laboratory of the clinical pharmacology unit, which was transcribed into the study related forms and the raw data retained by the laboratory for their records. The computer-generated chromatograms were also treated as raw data. All raw data and transcribed data forms were completed by the study personnel assisting in the study and were checked wherever applicable for completeness and logistics by the investigator.

3.2 Analytical Methodology

Estimation of Pregabalin in human plasma was done at department of clinical pharmacology and pharmacokinetics, Ranbaxy Laboratories Limited, using Pregabalin-d10 as internal standard.
Validated method was used for analysis of study samples.

3.2.1 Chemicals and Reagents
Pregabalin was purchased from varda biotech (Mumbai, India). Pregabalin d-10 was procured from TLC pharma chem. Acetonitrile was purchased from spectrochem pvt. ltd. (Mumbai, India). Ammonium Acetate was purchased from fluka analytical, netherland. Hydrochloric acid, liquor ammonia and methanol were purchased from qualigens fine chemicals (A division of GSK Ltd, Mumbai, India). Human plasma containing citrate phosphate dextrose adenine (CPDA) as an anticoagulant was collected in-house which were free from HIV and hepatitis.

3.2.2 Instrumentation and conditions
Chromatographic separation was achieved by using the acquity UPLC BEH C18 column (2.1 X 50mm, 1.7µm) maintaining the column oven temperature at 35ºC. A mixture of acetonitrile, ammonium acetate buffer (80:20;v/v) was used as the mobile phase with flow rate of 0.25 mL/min. The samples were loaded in the UPLC auto-sampler and temperature of the auto sampler was set at 10ºC. 2µl of sample extract was injected. Quantification was performed by waters quattro premier mass spectrometer using electro-spray ionization in positive ion mode. The chromatographic data was acquired and processed using computer based mass lynx software version 4.1. Ion transitions (m/z) were monitored 160.04 m/z (parent) and 141.98 m/z (product) for Pregabalin and 170.04 m/z (parent) and 152.03 m/z (product) for Pregabalin-d10 with the capillary voltage set at 3.75 kV. The source and desolvation temperature was set at 120ºc and 350ºc respectively. Cone and desolvation gas flow were maintained at 40 and 600 L/hr respectively. The argon gas collision induced dissociation was used with the energy 10 eV. The total run time for analysis was 1.5 minutes.

3.2.3. Stock solution, Calibration Curve (CC) and Quality Control (QC) sample preparation
Approximately 1 mg/ml of stock solutions for Pregabalin were prepared using HPLC grade methanol. This solution was diluted to make a series of standard working solutions in the range from 50.8 to 18167.0 ng/mL. All the working solutions were freshly
prepared and stored protected from light at 1-10°C. Calibration standard and QC samples in plasma were prepared by spiking corresponding working solutions in the drug free human plasma. The final concentrations of calibration standard in plasma were 50.8, 133.7, 607.6, 1446.6, 3013.7, 13080.2, 18167.0 ng/mL, respectively. The final concentrations of QC in plasma were 51.0 (LOQQC), 151.8 (LQC), 1446.2 (M1QC), 6287.7 (MQC), 13099.4 (HQC) ng/mL, respectively. All the plasma samples were stored at below −15°C.

3.2.4. Sample Preparation for plasma
Aliquots of human plasma containing both analyte and its internal standard (Pregabalin-d10) were extracted by solid phase extraction (HLB cartridges, 30 mg/1cc) method. 50 µL of internal standard stock dilution (Pregabalin-d10, approximately 10000 ng/mL) and 400 µL aliquot of each sample were added into polypropylene tubes. 200 µL of solution 1 (HCL 4.3 ml + HPLC grade water) was added and vortexed. The cartridges were conditioned with methanol (1ml) followed by 1 ml of HPLC grade water on positive pressure solid phase extraction at about 15 psi. The samples were loaded onto cartridges and the cartridges were washed with 1 mL of solution 1 followed with 1 mL of HPLC grade water and eluted the samples twice with 1 ml of methanol. The eluates were evaporated to dryness at 50°C under nitrogen gas pressure. Dried residues were then reconstituted with 400 µL of mobile phase (80:20 v/v acetonitrile: ammonium acetate buffer).

3.2.5 Validation Parameters
A validated liquid chromatography mass spectroscopic (LC-MS/MS) assay was used to determine Pregabalin concentrations in human plasma.

The linearity, precision and accuracy evaluations were performed on three batches of spiked plasma samples. Each batch of spiked plasma samples included one complete calibration curve and Six QC samples.
3.3 Clinical Study Sample Analysis

3.3.1 Objective
The objective of the study was to analyze the clinical study samples using a validated LC-MS/MS method for the determination of Pregabalin.

3.3.2 Sample collection and storage
The blood samples for period I, II and III were collected from 18 March 2009 to 20 March 2009, 24 March 2009 to 26 March 2009, 31 March 2009 to 02 April 2009 respectively. Blood samples collected from each period were centrifuged to separate plasma and stored at −15°C in a deep freezer at the clinical facility on their respective dates of collection. All plasma samples from three periods were then packed properly using dry ice, transported to the analytical facility on 06 April 2009 (Aliquot-I) and 10 April 2009 (Aliquot-II) stored at −15°C until analysis.

3.3.3 Sample analysis
As per the protocol a total number of 1620 samples were collected from 18 subjects during the three periods. As subject number 6 in period 1, subject number 03, 10 in period II and subject number 02 in period III was withdrawn from the study as the subject did not report for period II, a total of 1381 samples collected from the subjects during the whole study. Samples were analyzed subject wise using one analytical batch at a time, which consisted of an aqueous reference standard dilution, 10 calibration spiked standards (standard blank, standard blank+internal standard, standard A (LOQ), B, C, D, E, F, G and H (ULOQ), 1 subject was analyzed as a single batch, only one set of calibration spiked standards was included, however, 8 quality control samples (LQC, MQC, M1QC & HQC; 2 samples each) were interspersed between the subject samples 90 samples from one subject (30 + 30 + 30; from three periods) thus, a total of 109 samples per batch.

3.3.4 Batch acceptance criteria
All the batches were evaluated rigorously and considered for the repeat analysis if failed with respect to any of the following criteria.
3.3.4.1 Calibration curve acceptance criteria

All the calibration curves were evaluated for the following passing criteria-

- Accuracy of calibrators: within ± 15 % of their nominal values (within ± 20 % for LOQ).
- At least 75% of calibrators including LOQ and ULOQ meet the above criteria.
- Linear coefficient of correlation: \( \geq 0.98 \).

3.3.4.2 Blank and blank plus internal standard acceptance criteria

Blank and Blank + IS: free from significant interference. i.e.

- Peak area responses of the blank at the retention time of the Pregabalin were < 20 % of the peak area response of the LOQ standard and
- Peak area responses of the blank at the retention time of the internal standard were < 5% of the mean response of internal standards used in the calibration curve.

3.3.4.3 Quality control sample acceptance criteria

Batch acceptance required that back calculated concentrations of at least 50 % of each QC sample (LQC, MQC, M1QC & HQC) and 67% overall were within ± 15 % of their nominal values.

3.4 Pharmacokinetic and Statistical Analysis

3.4.1 Pharmacokinetic analyses

The following pharmacokinetic parameters were calculated for Pregabalin using WinNonlin-Node version 5.0.1 from pharsight:

- \( AUC_{0-t} \): The area under the plasma concentration versus time curve, from time zero to the last measurable concentration, as calculated by the linear trapezoidal method.
- \( AUC0-24 \): The area under the plasma concentration versus time curve, from time zero to 24 h.
AUC\(_{0-\infty}\): The area under the plasma concentration versus time curve, from time zero to infinity. AUC\(_{0-\infty}\) is calculated as the sum of AUC\(_{0-t}\) plus the ratio of the last measurable plasma concentration to the elimination rate constant.

AUC\(_{0-t}\) / AUC\(_{0-\infty}\): The ratio of AUC\(_{0-t}\) to AUC\(_{0-\infty}\).

C\(_{\text{max}}\): Maximum measured plasma concentration over the time span specified.

T\(_{\text{max}}\): Time of the maximum measured plasma concentration. If the maximum value occurs at more than 1 time point, T\(_{\text{max}}\) is defined as the first time point with this value.

K\(_{\text{el}}\): Apparent first-order terminal elimination rate constant calculated from a semi-log plot of the plasma concentration versus time curve. The parameter will be calculated by linear least-square regression analysis using the maximum number of points in the terminal log-linear phase (e.g. three or more non-zero plasma concentrations).

T\(_{1/2}\): The apparent first-order terminal elimination half-life will be calculated as 0.693/K\(_{\text{el}}\).

No value of K\(_{\text{el}}\), AUC\(_{0-\infty}\) or T\(_{1/2}\) were reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.

### 3.4.2 Statistical Analyses

Statistical analyses were performed on plasma Pregabalin using the SAS system for Windows, release 9.1.3 or above or WinNonlin PK Software, Version 5.0.1. The analyses included data from subjects 1 to 18 except subject number 02, 03, 06 and 10. In the event of dropouts and/or withdrawals, they were not replaced.
3.4.2.1 Summary statistics
Arithmetic means, standard deviations and coefficients of variation were calculated for the parameters listed in section 14.3. Additionally, geometric means and percentage coefficient of variation of geometric means were calculated for $AUC_{0-t}$, $AUC_{0-\infty}$, $AUC_{0-24}$ and $C_{\text{max}}$.

3.4.2.2 Analysis of Variance (ANOVA)
The log-transformed pharmacokinetic parameters ($C_{\text{max}}$, $AUC_{0-t}$, $AUC_{0-24}$, and $AUC_{0-\infty}$) for Pregabalin were analyzed using a mixed effects ANOVA model using Type III sum of squares, with the main effects of sequence, period and formulations as fixed effects and subjects nested within sequence as random effect. A separate ANOVA model was used to analyze each of the parameters. The sequence effect was tested at the 10% level of significance using the subjects nested within sequence mean square as the error term. All other main effects was tested at the 5% level of significance against the residual error (mean square error) from the ANOVA model as the error term. Each analysis of variance included calculation of least-squares means, the difference between the adjusted formulation means and the standard error associated with the difference. The above analyses was done using the appropriate SAS® procedure or the WinNonlin PK Software, Version 5.0.1.

3.4.2.3 90% Confidence Intervals and Ratio Analyses
90% confidence interval for the ratio of the test and reference product averages (least square means) for $AUC_{0-24}$ and $AUC_{0-t}$ was calculated for Pregabalin by first calculating the 90% confidence interval for the differences in the averages (arithmetic means) of the log-transformed data and then taking the antilogs of the obtained confidence limits. The comparison of interest is A vs R & B Vs R, so the ratios were of the form: Test/Reference. Ratio of means were calculated using the LSM for log-transformed $C_{\text{max}}$, $AUC_{0-t}$, $AUC_{0-24}$ and $AUC_{0-\infty}$ for Pregabalin. Ratio of means was expressed as a percentage of the LSM for the reference formulations.
3.5 In Vitro In Vivo Correlation Methodology

In vitro Dissolution

For the in vitro dataset, two preliminary prototypes and a reference product tested in a pilot bioequivalence study (Study no. 323_PREGA_09) were used. In the study, Test A [DM (169D) 21] and Test B [VC (178D)19] each containing Pregabalin 600mg ER tablets of Ranbaxy Laboratories Ltd., India were compared to the Reference product Lyrica 300 mg IR capsules of Pfizer [0460106U], also containing Pregabalin 300mg. The dissolution study was conducted using USP Apparatus II (Paddle method) at 50 rpm. The dissolution medium was phosphate buffer (pH 6.8; 900 ml). Samples were collected at predetermined intervals up to 12.0 h for Test products A & B after the start of study. Six units of each prototype were used.

The mean dissolution data for Test A and Test B are provided below in Table IVIVC1:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>% Dissolved</td>
<td>Fraction dissolved</td>
<td>% Dissolved</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>0.57</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>0.7</td>
</tr>
<tr>
<td>8</td>
<td>87</td>
<td>0.87</td>
</tr>
<tr>
<td>12</td>
<td>99</td>
<td>0.99</td>
</tr>
</tbody>
</table>

In vivo Studies in Healthy Volunteers

Data from the study, as described below, were used to define in vivo functions:

Primary dataset for IVIVC Model Development

An open label, balanced, randomized, three-treatment, three-period, three-sequence, crossover bioavailability study comparing single dose of two batches of Pregabalin 600 mg extended release tablet of Ranbaxy Laboratories Limited, with two oral doses of Lyrica 300 mg capsules (each dose containing Pregabalin 300 mg administered 12 hourly; total dose 600 mg, of Pfizer GmbH) in eighteen healthy adult, human, male subjects under fed condition was conducted. Each treatment was administered with 240 ml of water. Standard meals were served at 45 minutes prior to each dose. No food was allowed for at least 4 hours post dose. Each treatment was separated by a 5 days wash-out.
period. Plasma samples were collected for Test (A & B): Predose (in duplicate) and at 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 12.0, 14.0, 18.0, 24.0, 30.0, 36.0 and 48.0 hours post dose in each period. Plasma samples from volunteers completing the study were assayed for Pregabalin using chromatographic procedures developed and validated at Ranbaxy. The following pharmacokinetic parameters were calculated for Pregabalin using WinNonlin-Node version 5.0.1 from Pharsight: $AUC_0-t$, $AUC_{0-24}$, $AUC_{0-\infty}$, $AUC_0-t / AUC_{0-\infty}$, $C_{max}$, $T_{max}$, $K_{el}$ and $T_{1/2}$.

Table IVIVC 2: Showing Mean of the $C_{max}$ and $AUC$ for Pregabalin after administration of an oral dose of 600 mg ER tablets for Test product A and B

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$T_{max}$ (Hr) Mean (% CV)</th>
<th>$C_{max}$ (ng/ml) Mean (% CV)</th>
<th>$AUC_{0-24}$ (Hr*ng/ml) Mean (% CV)</th>
<th>$AUC_{0-\infty}$ (Hr*ng/ml) Mean (% CV)</th>
<th>$AUC_{0-t}$ (Hr*ng/ml) Mean (% CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.28 (40.3)</td>
<td>10329.29 (20.4)</td>
<td>110857.05 (10.9)</td>
<td>122321.27 (11.6)</td>
<td>121288.63 (11.6)</td>
</tr>
<tr>
<td>B</td>
<td>6.39 (35.7)</td>
<td>11295.47 (17.7)</td>
<td>109804.72 (12.5)</td>
<td>123095.85 (14.1)</td>
<td>121893.42 (14.1)</td>
</tr>
</tbody>
</table>

**Secondary dataset (IR Formulation) for defining the Weighting function**

An IR (Immediate Release) formulation is required to compute the Weighting Function [also referred to as the Unit Impulse Response (UIR)] for deconvolution and convolution steps. For this purpose, the reference formulation R which was an immediate release formulation was used for generation of UIR. IR was dosed twice. But, in vivo profile of first 12 hour was used for in vivo generation.

Blood samples were collected for Reference (R): Prior to morning dose (in duplicate) and at 0.500, 1.000, 1.333, 1.667, 2.000, 2.333, 2.667, 3.000, 3.333, 3.667, 4.000, 4.500, 5.000, 6.000, 8.000, 10.000, 12.000, 12.500, 13.000, 13.333, 13.667, 14.000, 14.333, 14.667, 15.000, 15.333, 15.667, 16.000, 16.500, 17.000, 18.000, 20.000, 24.000, 30.000, 36.000 and 48.000 hours after the morning dosing in each period. The treatments were assigned to study volunteers according to a SAS generated randomization schedule. A washout period of one week was included between each period. Plasma samples from
fourteen volunteers who completed the study were assayed for Pregabalin using a validated LC-MS/MS method.

Table IVIVC 3: Showing mean of the Cmax and AUC for Pregabalin after administration of an oral dose of 300 mg bid for Reference product R

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tmax (Hr) Mean (% CV)</th>
<th>Cmax (ng/ml) Mean (% CV)</th>
<th>AUC0-24 (Hr*ng/ml) Mean (%)</th>
<th>AUC0-inf (Hr*ng/ml) Mean (%)</th>
<th>AUC0-t (Hr*ng/ml) Mean(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>3.40 (22.5)</td>
<td>7722.21 (14.7)</td>
<td>99662.16 (9.4)</td>
<td>126958.53 (10.8)</td>
<td>125090.65 (10.6)</td>
</tr>
</tbody>
</table>

**In vitro - In vivo Correlation (IVIVC) Models: Level A Correlation**

In vitro Dissolution Model

In Vitro dissolution model was generated using Test A [DM (169D) 21] and Test B [VC (178D) 19] each containing Pregabalin 600mg of Ranbaxy Laboratories Ltd., India and Reference product Lyrica 300 mg IR capsules of Pfizer [0460106U].

The dissolution study was conducted using USP Apparatus II (Paddle method) at 50 rpm. The dissolution medium was phosphate buffer (pH 6.8; 900 ml). Samples were collected at predetermined intervals up to 12.0 h for Test Products (A & B) after the start of study. Six units of each prototype were used. These time points were chosen to represent early, middle, and late stages of the in vitro dissolution profiles.

In the modeling window of the software program, the Test A and Test B were chosen as the internal validation batches.
The dissolution profiles for Test A and Test B are provided in Figure IVVC 1.

![Dissolution Profiles](image)

The in-vitro data fitting was performed using raw data. Model selection was done using AIC values. AIC (Akaike's information criterion) is a measure of goodness-of-fit for an estimated statistical model. The AIC values observed for Test A and Test B was -20.05 and -14.84 respectively.

**In vivo Data Modeling:** Use of UIR for estimation of Input Functions

Plasma concentration-time data of the immediate-release test formulation (Reference R) were first evaluated for generating the Unit Impulse Response (Weighting function). The deconvolution procedure in the software program (WinNonlin IVIVC-Toolkit) was used to obtain in vivo input functions for each ER dosage form. This deconvolution of ER datasets was conducted using IR data as the weighting function.
3.5.3.3. Development and Validation of the IVIVC Model

A correlation model was evaluated between the in vitro fraction dissolved and the in vivo input function using the following equation in the software program:

\[ T = A_2 \times X^{B_1-2.5} \]

IF \( T > 0 \) THEN

\[ \text{DISS} = \text{IVINTERP}(T) \]

ELSE

\[ \text{DISS} = 0 \]

ENDIF

\[ F = A_1 \times \text{DISS} \]
An r-square value of 0.9621 was obtained in this relationship.

The mean in vivo input functions for each formulation were then convoluted by the software program to obtain predicted plasma concentrations. Mean pharmacokinetic parameter estimates were calculated from the predicted profiles. A comparison of these mean parameter estimates for each formulation with mean observed estimates was conducted to validate the correlation.

**Internal Predictability of IVIVC models:** To assess the predictability and the validity of the correlations, we determined the observed and IVIVC model predicted Cmax and AUC for each formulation from the bioavailability study. Prediction errors for the observed and predicted Cmax and AUC were calculated for each formulation to determine the accuracy of the IVIVC models in characterizing the rate and extent of
Pregabalin absorption. The percent prediction errors for Cmax and AUC were calculated as follows:

\[
\% \text{ PE Cmax} = \frac{\text{Cmax (obs)} - \text{Cmax (pred)}}{\text{Cmax (obs)}} \times 100 \quad \text{[Amidon G., 1995]}
\]

\[
\% \text{ PE AUC} = \frac{\text{AUC (obs)} - \text{AUC (pred)}}{\text{AUC (obs)}} \times 100 \quad \text{[Rekhi G.S., 1997]}
\]

Where \( \text{Cmax (obs)} \) and \( \text{Cmax (pred)} \) = the observed and IVIVC model predicted maximum plasma concentration profiles, respectively; and \( \text{AUC (obs)} \) and \( \text{AUC (pred)} \) = the observed and IVIVC model predicted AUC for the plasma concentration profiles, respectively. The IVIVC was considered valid if the average absolute % prediction error is < 10 for Cmax and AUC and if the % prediction error for each formulation does not exceed 15%.