CHAPTER-4

MATERIALS AND METHODS
4.0 MATERIALS AND METHODS

4.1 MATERIALS

The materials consist of the following headings:

4.1.1. Formulations evaluated  
4.1.2. Chemicals and reagents

4.1.1. Formulations evaluated

The following three marketed formulations were purchased from the local pharmacy for evaluation and stored as per labeled condition till use.


Product C: Gabata ® 300 mg capsule, containing 300 mg of gabapentin, manufactured by Alkem Laboratories Ltd, Village-Thana, Baddi, Himachal Pradesh - 173205, India. Batch No. GBC8004AB, Manufacturing date: August 2008, Expiry date: July 2010, Price: Rs 85.85/10 capsules.
* For the comparison purpose, Product A and Product C were considered as Test products and Product B was chosen as a comparator reference product due to the following reasons:

1. It was first launched in the Indian market along with the Intas Pharma.
2. Currently available in the US market and approved as therapeutically equivalent to the global innovator product by US FDA.
3. It can be used as a comparator product as per the WHO guidelines in Generic drug approval process due to marketing authorization in the ICH region.

4.1.2. Chemicals and reagents

The following chemicals and reagents were purchased from the Ranbaxy Research Laboratories for the study.

**Standards:** The gabapentin working standard with 99.9% purity and the amitriptyline internal standard with 100% purity were procured from US and were of USP standard. The gabapentin working standard with 99.2% purity (used for dissolution) was manufactured by Ranbaxy Research Laboratories Ltd, Dewas (in-house).

**Cartridge:** The extraction cartridge OASIS® MCX 1cc (30 mg) was manufactured by Water corporation, Massachusetts, Ireland.

**Analytical reagents:** The analytical grade ammonium acetate was procured from Thomas Baker (chemicals) Ltd, Mumbai whereas liquor ammonia and hydrochloric acid were from Qualigens Fine chemical Ltd, Mumbai and Merck Specialties Pvt. Ltd, Mumbai respectively. The HPLC grade methanol and acetonitrile were from Qualigens Fine Chemical Ltd, Mumbai and Spectrochem Pvt. Ltd, Mumbai respectively. HPLC grade water was prepared by Milli-Q apparatus supplied by Millipore USA. The potassium hydroxide was supplied by SISCO Research Laboratories, Mumbai. The Monobasic potassium phosphate was from Sigma Aldrich Chemie, Riedstrasse, Germany.
4.2. METHODOLOGY

A comparative *in-vivo* oral bioavailability study followed by the *in-vitro* dissolution method was carried out at Ranbaxy Research Laboratories facility.

4.2.1 *In-vivo* method (BA/BE study)

4.2.2 *In-vitro* method (Dissolution Test)

4.2.1 *IN-VIVO METHOD (BA/BE STUDY)*

A single-dose oral BA/BE study was conducted in healthy, adult, human male participants under fasting conditions for three marketed formulations of Gabapentin 300 mg, immediate release, capsule formulation and the study was carried out at Ranbaxy Clinical Pharmacology Unit (CPU), Majeedia hospital, New Delhi, India. The overall *in-vivo* methodology were divided into following headings:

4.2.1.1 Clinical study methodology

4.2.1.2 Bioanalytical methodology

4.2.1.3 Pharmacokinetics and statistical evaluation

The clinical part of the study was carried out at Ranbaxy Clinical Pharmacology Unit (CPU), Majeedia hospital and the bioanalytical part with pharmacokinetics and statistical evaluation were carried out at Ranbaxy Clinical Pharmacology and Pharmacokinetic Unit (CPP), Gurgaon.

4.2.1.1. Clinical Study Methodology

4.2.1.1.1 Clinical study objective

The objective of the study was to compare the single dose oral bioavailability of three marketed formulations of Gabapentin in healthy human male subjects under fasting condition...
and to determine the bioequivalence of two test products A and C with comparator product B and to check whether the test products may be substituted successfully for comparator product.

### 4.2.1.1.2 Study design

The study design was an open label, balanced, randomized, three-treatment, three-sequence, three periods, single-dose, cross-over oral bioavailability study in healthy, adult, human male subjects under fasting conditions. The schematic representation of study design is shown in the Figure 2.

![Schematic representation of study design](image)

### 4.2.1.1.3 Selection of participants

Adequate number of subjects were selected randomly from the volunteer data bank and the subjects underwent a standardized screening procedure.

### 4.2.1.1.4. Screening Assessments

Enough healthy human male participants were undergone a screening procedure from 14 October 2008 to 21 October 2008 to determine their healthy status which was concluded by demographic data including name, sex, age, body weight (kg), height (cm), smoking status,
tobacco use information, medical histories, physical examination and the laboratory tests of hematological, hepatic and renal function tests. The screening procedure is listed in Table 7.

**Table 7: Laboratory Tests carried out during the screening of subjects**

<table>
<thead>
<tr>
<th>TESTS</th>
<th>PARAMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematology</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td></td>
<td>Total Leukocyte Count</td>
</tr>
<tr>
<td></td>
<td>Differential Leukocyte Count</td>
</tr>
<tr>
<td></td>
<td>Platelet count</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>Blood urea Nitrogen</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
</tr>
<tr>
<td></td>
<td>Total Bilirubin</td>
</tr>
<tr>
<td></td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td></td>
<td>Aspartate aminotransferase (AST)</td>
</tr>
<tr>
<td></td>
<td>Alanine aminotransferase (ALT)</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>Physical Examination</td>
</tr>
<tr>
<td></td>
<td>Microscopic RBC</td>
</tr>
<tr>
<td></td>
<td>Drug screen Cannabinoids</td>
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<tr>
<td></td>
<td>Colour WBC</td>
</tr>
<tr>
<td></td>
<td>Appearance E. cells</td>
</tr>
<tr>
<td></td>
<td>Specific Gravity Crystals</td>
</tr>
<tr>
<td></td>
<td>Protein Casts</td>
</tr>
<tr>
<td></td>
<td>Glucose Others</td>
</tr>
<tr>
<td>Additional Tests</td>
<td>Human Immunodeficiency Virus (HIV I &amp; II)</td>
</tr>
<tr>
<td></td>
<td>Hepatitis B Antigen (HBsAg)</td>
</tr>
<tr>
<td></td>
<td>Hepatitis C virus (HCV)</td>
</tr>
<tr>
<td></td>
<td>Venereal Disease Research laboratory (VDRL)</td>
</tr>
</tbody>
</table>

Only medically healthy subjects with clinically normal laboratory profiles were selected, who met the following inclusion and exclusion criteria. Eighteen healthy human male subjects were selected based on the following inclusion and exclusion criteria.
4.2.1.1.5 Inclusion and exclusion Criteria

4.2.1.1.5.1. The inclusion criteria: The subjects were included if they fulfilled the following criteria:

- Male subjects in the age range of 18-45 years.
- Be neither overweight nor underweight for his height as per the Life Insurance Corporation of India height/weight chart for non-medical cases.
- Have voluntarily given written informed consent to participate in this study.
- Be 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.

4.2.1.1.5.2. Exclusion criteria: The subjects were excluded who had any of the following criteria:

- History of hypersensitivity to gabapentin.
- Past history of any CNS disorder including sleep disorders, movement disorders, disorders of gait or repeated episodes of unexplained dizziness.
- History of any cardiovascular illness including hypertension, ischemic heart disease and arrhythmias.
- History of musculoskeletal disorders including chronic joint and muscle pains.
- The subject has a history of tremors, pruritis, and backache.
- History of drug induced rash or urticaria.
- History of bleeding disorders, thyroid disorders, abnormal vision.
- History of seizures.
- 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 and 2, Hepatitis B and C viruses or syphilis infection.
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- Presence of values which are significantly different from normal reference ranges (as defined in Appendix 5) and/or judged clinically significant for haemoglobin, total white blood cells count, differential WBC count or platelet count.
- Positive for urinary screening test of drugs of abuse (opiates or cannabinoids).
- Presence of values, which are significantly different from normal reference ranges (as defined in Appendix 5) 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), E. Cell, glucose (positive) or protein (positive).
- Clinically abnormal ECG or Chest X-ray.
- History of serious gastrointestinal, hepatic, renal, cardiovascular, pulmonary, neurological or haematological 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/day 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.
- Subjects who, through completion of this study, would have donated and/or lost more than 350 mL of blood in the past 3 months.
4.2.1.1.6. Number of Subjects

Enough healthy adult male human subjects were enrolled to allow dosing of 18 subjects in the first period. Subsequent drop-outs were not replaced. Data is presented on all completed subjects.

4.2.1.1.7 Admission and stay

The 18 participants who fulfilled the study requirements based on inclusion and exclusion criteria were admitted and housed in the Clinical Pharmacology Unit from at least 12 hours before study drug administration and were discharged 24 hours after drug administration during each period and they were asked to come to the clinical unit for subsequent ambulatory blood sample collection after the discharge.

4.2.1.1.8 Assignment to treatment and dosing

The order of receiving study treatments for each subject during the three periods of the study were determined according to the SAS-generated balanced randomization schedule which is shown in Table 8.
Table 8: Randomization schedule

<table>
<thead>
<tr>
<th>#</th>
<th>Subject No.</th>
<th>Sequence</th>
<th>Treatment/Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>ABC</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>CAB</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>CAB</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>ABC</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>ABC</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>CAB</td>
<td>C</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>CAB</td>
<td>C</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>BCA</td>
<td>B</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>BCA</td>
<td>B</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>CAB</td>
<td>C</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>CAB</td>
<td>C</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>BCA</td>
<td>B</td>
</tr>
<tr>
<td>13</td>
<td>13</td>
<td>ABC</td>
<td>A</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td>ABC</td>
<td>A</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>BCA</td>
<td>B</td>
</tr>
<tr>
<td>16</td>
<td>16</td>
<td>BCA</td>
<td>B</td>
</tr>
<tr>
<td>17</td>
<td>17</td>
<td>BCA</td>
<td>B</td>
</tr>
<tr>
<td>18</td>
<td>18</td>
<td>ABC</td>
<td>A</td>
</tr>
</tbody>
</table>

A single oral dose of gabapentin 300 mg capsule was administered with 240 mL of drinking water at ambient temperature as per the randomization schedule during each period of the study under the supervision of a trained Medical Officer. The dosing was done next day morning of the admission day. The chronological time with respective dosing was similar for all the periods.

4.2.1.9 Assessment of compliance

Compliance was assessed by conducting a thorough examination of the oral cavity by trained study personnel after dosing each period and by measurement of plasma gabapentin during the analytical phase of the study.

4.2.1.10 Fasting/Meals

All subjects were fasted overnight for at least 10 hours before the morning dose and for 4 hours post-dose and received standard meals-lunch, snacks and dinner at 4, 9 and 13 hours after drug administration. During the housing, all meal plans and dosing schedules were identical for all 3 periods. In case meals and blood sample collection coincide, samples were
collected before providing meals. Drinking water was not allowed 1 hour before dosing and until 2 hours post-dose. Thereafter, it was allowed at all times.

4.2.1.11 Restrictions

Medications: All subjects were instructed not to take any other medications including OTC during the two week period prior to the onset of the study. Medication was advised only in case of medical emergencies.

Diet: All subjects abstained from any xanthine containing food, beverages or alcoholic products for 48 hours prior to dosing and throughout the sampling schedule during each period.

Activity: All subjects were dosed while seated and were asked to remain seated or ambulatory for the first 2 hours following each drug administration in each period. Thereafter, subjects were allowed to engage only in normal activities while avoiding severe physical exertion.

4.2.1.12 Blood sampling

Intravenous indwelling cannula was kept in situ as long as possible (until 24 hours post-dose) for the collection of blood samples. The cannula was maintained potent by injection of 1 mL of 5 IU/mL of heparin in normal saline solution. In such cases a blood sample was collected after discarding the first 0.5 mL of heparinised blood and heparin solution from the tubing. 4 mL of blood sample was collected pre-dose and at 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12, 16, 24, 36 and 48 hours post-dose during each period i.e. (19 draws/ period). Thus, there were 59 draws (including screening at the start of the study and safety sample at the end of the study) from each subject during the course of the study. For each subject, the total volume of blood drawn including 16 mL for screening, 10 mL for safety analysis at the end of study, 12 mL for duplicate sample at pre-dose, 4 mL of 57 draws for entire periods and 24 mL 'discarded' blood prior to venous cannula collections, did not exceeded 290 mL.
The pre-dose blood sample in each period was collected in a duplicate aliquot, within a period of approximately 90 minutes before dosing and the post-dose samples were generally collected in single aliquot within 2 minutes of the scheduled time. After collection, the blood samples were centrifuged at 4000 rpm under 4-8°C refrigeration as soon as possible to separate plasma. All plasma samples were transferred to suitably labeled poly propylene tubes and rechecked to ensure transfer of plasma to the correct tube. The plasma samples were then stored at -20°C or lower, till transfer to the bio-analytical facility for assay.

4.2.1.13. Safety

Clinical safety measurements: Vital signs of oral temperature, sitting blood pressure and radial pulse were measured during subject admission, prior to dosing, 2, 4, 8, 12, 24, 36 and 48 hr after administration of study drug in each period as shown in Fig. 3. Vital signs to be measured prior to administration of the dose were noted within 1.5 hours of the scheduled dosing time. At all other times, vital signs were noted within 45 minutes of the scheduled time. In the event of detection of any abnormality during measurement of vital signs, the Clinical Investigator was consulted for necessary action. Brief clinical examinations of the subject were conducted by a qualified medical personnel before subject admission, prior to dosing of study drug and at discharge in all the periods. In the event of detection of any abnormality during the study period by clinical, the Clinical Investigator was consulted for necessary action.

Monitoring Adverse Events: The Clinical Investigator or a Medical Officer was available at the site of investigation till 24 hours post-dose during each period. Subjects were monitored throughout the study period for adverse events. Subjects were informed to bring to the notice of the nurse or the doctor about any adverse event that may occur during the study period. Subjects were also specifically asked about any adverse events at every 4 hrs till 20 hrs post-dose and then till discharge and at every ambulatory sample as shown in Fig. 3
Figure 3: Schematic presentation of the clinical study

<table>
<thead>
<tr>
<th>Dinner</th>
<th>Vitals</th>
<th>Lunch</th>
<th>Vitals</th>
<th>Snacks</th>
<th>Vitals</th>
<th>Vitals</th>
<th>Vitals</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>AE</td>
<td>AE</td>
<td>AE</td>
<td>AE</td>
<td>AE</td>
<td>AE</td>
<td>AE</td>
</tr>
</tbody>
</table>

1500 2100 0730 0900 1100 1300 1500 1600 1800 2100 2200 0300 0900
-18 hr -12 hr -1.5 hr 0 hr 2 hr 4 hr 6 hr 8 hr 9 hr 12 hr 13 hr 18 hr 24 hr

Gabapentin 300 mg

Adverse events at every 4 hours till 2100 hrs & then at every 6 hours until discharge.

Washout Period (7 Days)

Safety measurements at the end of the study: The blood biochemistry test, renal function tests and liver function tests were conducted and included at the end of the study i.e., Last period blood collection, for safety of the study participants.

4.2.1.14. Discharge and Washout Period

All subjects were discharged 24 hours after administration of the study drug during each period. A washout period of seven days was enforced between dosing of each period.

4.2.1.15. Ethical Considerations

Basic Principles: This research was carried out according to the basic principles defined in US 21CFR Part 320, the ICH (62 FR25692, 09 May 1997)’ Guidance for ‘Good Clinical Practice’, Drugs and Cosmetic Rule of Schedule Y amendment 2005, Ethical guidelines
for Biomedical Research on Human Participants (ICMR 2006) and the principles enunciated in the Declaration of Helsinki (2004). The study was also carried out according to the relevant in-house Standard Operating Procedures (SOPs).

Institutional Review Board: The final protocol and the corresponding Informed Consent Form (ICF) were approved by Jamia Hamdard Institutional Review Board on 30th September 2008. Only the approved protocol and ICF were used throughout the clinical study procedure, however the study analysis part had the protocol violation which has an impact on the study result and minor changes in the clinical methodology. The description of protocol violation is discussed under the protocol violation heading.

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 Clinical Pharmacologist Unit for Period I. All the subjects provided formal written consent after attending an oral presentation and thoroughly reading and understanding the informed consent form before admission to the study.

4.2.1.1.16 Drop-out/Withdrawal of Subjects

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:

1. The subject suffers from significant intercurrent illness or undergoes surgery during the course of the study.
2. The subject experiences adverse event and withdrawal is in the best interest of the subject.
3. 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.
4.2.1.17 Volunteer Compensation:

The subjects were adequately compensated on account of their participation in the study and the details of compensation were discussed in the ICF according to the IRB instructions.

4.2.1.18 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.

4.2.1.19 Protocol deviation

There were no deviations in the clinical procedure. The IRB reviewed and approved final protocol was used throughout the study procedure, however the direction of the product comparison was changed in the analysis part. The approved one is focused on a comparison of high cost formulation with the low cost formulation and the final study procedure focused on comparison of standard of medicine which focused on proven quality, safety and efficacy of medicine. The deviation has minor/ no impact on the ethical principles to study participants and the changes can be read as follows throughout the study from the protocol.

The following protocol and ICF information have been changed throughout the study procedure

Reference: Gabalept ® 300 mg capsule, containing 300 mg of gabapentin, manufactured by Micro Labs Ltd, Hosur - 635126, India.
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Test A: Gabantin® 300 mg capsule, containing 300 mg of gabapentin, manufactured by Sun Pharmaceutical Industries, Dadra – 396191 or Kartholi, Jammu - 181133, India

Test B: Gabata® 300 mg capsule, containing 300 mg of gabapentin, manufactured by Alkem Laboratories Ltd, Village-Thana, Baddi, Himachal Pradesh – 173205, India

Changed to:

Product A: Gabalept® 300 mg capsule, containing 300 mg of gabapentin, manufactured by Micro Labs Ltd, Hosur - 635126, India

Product B*: Gabantin® 300 mg capsule, containing 300 mg of gabapentin, manufactured by Sun Pharmaceutical Industries, Dadra – 396191 or Kartholi, Jammu - 181133, India (* Comparator reference product)

Product C: Gabata® 300 mg capsule, containing 300 mg of gabapentin, manufactured by Alkem Laboratories Ltd, Village-Thana, Baddi, Himachal Pradesh – 173205, India

For comparison purpose, Product A and Product C are considered as Test products and Product B was chosen as a Comparator reference product as per the following reasons.

1. It is first launched in the India market along with the Intas Pharma.

2. Currently available in the US market and proven for therapeutic equivalency to the global innovator product and this information is freely available for public use through electronic Orange Book.

3. It can be considered as a comparator product as per the WHO guidelines of Generic drug approval process as approved by ICH region countries but the formulation is not perched from that country.

The above comparison has only difference in the product comparison status and not the product/formulation difference and therefore it may have minor/no impact in the ethical principles to the study participants.
4.2.1.2 BIOANALYTICAL METHODOLOGY

The Bioanalytical part of the study is started after the basic training followed by experience in bioanalytical procedure. The simple, rapid, economical, LC/MS/MS method employing a solid-phase extraction followed by MS (Mass Spectrometry) detection of gabapentin in human plasma was developed and validated to meet the acceptance criteria of FDA industrial guidance for the bio-analytical method validation\(^1\) by the Ranbaxy Research Laboratories (in-house procedure).

4.2.1.2.1 Instrumentation

High-pressure liquid chromatography (HPLC), combined Mass Spectrometric (MS) procedure such as LC-MS/MS system was used for the quantitative determination of gabapentin in the plasma which consists of Mass Spectrometry (MS # 23) from Applied Biosystem, MDS SCIEX, Toronto, Canada and HPLC system (HPLC # 42) of Agilent. The HPLC system has the following component:

- HPLC - Agilent (HPLC # 42)
- Degasser - Agilent (DGV-24)
- Pump - Agilent (P- 70)
- Auto Injector - Agilent (AI - 42)
- Sample Cooler - Agilent (SC-12)
- Column Oven - Agilent (CO- 35)
- Column - Hypersil Hypurity Advance C18 (4.6 X 50 mm, 5 \(\mu\)m)
- Mass Spectrometry - Applied Biosystem (MS # 23)

4.2.1.2.2 Preparation of reagents

i. Ammonium acetate (AR grade)

ii. Acetonitrile (HPLC grade)

iii. Methanol (HPLC grade)
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iv. Hydrochloric Acid (AR grade)  
v. Liquid Ammonia (AR grade)  
vi. Milli-Q water

10 ± 0.1 mM Ammonium acetate buffer (pH 6.6 ± 0.2): 0.770 ± 0.015 gm of ammonium acetate was transferred to a 1000 mL reagent bottle. 1000 mL of HPLC-grade water was added, mixed and degassed in the ultrasonic bath for 10 min. Then its pH was measured and it was within 6.6 ± 0.2. This solution was stored at room temperature and used within 5 days of its preparation.

*Diluent solution (Methanol and water (50:50 v/v)*: 50 mL of methanol was transferred to a 100 mL volumetric flask and made up to the volume with HPLC grade water, mixed well and degassed in an ultrasonic bath for 10 min. This solution was stored at room temperature and used within 7 days of its preparation.

0.5N Hydrochloric acid: 4.3 mL of hydrochloric acid was transferred into a 100 mL volumetric flask and made up to the volume with HPLC grade water and mixed well. Then sonicated and degassed in an ultrasonic bath. The solution was stored at room temperature and used within 5 days of its preparation.

5% Ammoniated methanol: 5 mL of liquor ammonia was transferred into 100 mL volumetric flask and the volume made up with HPLC grade methanol and mixed well. Then sonicated and degassed in an ultrasonic bath. The solution was used freshly every day.

*Methanol*: A HPLC grade methanol was used freshly every day.

Water: A HPLC grade Milli Q water was used freshly every day.
4.2.1.2.3. Mobile Phase:

200 mL of 10 ± 0.2 mM ammonium acetate buffer (pH 6.6 ± 0.2) was transferred into a 1000 mL reagent bottle and 800 mL of HPLC grade acetonitrile was added, mixed well and sonicated in an ultrasonic bath for 10 min. This solution was stored in room temperature and used within 3 days of its preparation.

4.2.1.2.4. Preparation of standard solution

4.2.1.2.4.1. Preparation of Internal Standard (IS) solution

Approximately 1 mg/mL of amitriptyline USP standard in methanol was prepared and stored in the refrigerator between 1-10°C. From this approximately 10μg/mL concentration was prepared and used freshly each time of the sample processing.

4.2.1.2.4.2. Preparation of Standard solution of Gabapentin

Approximately 1 mg/mL of the gabapentin USP standard in methanol was prepared and stored in the refrigerator between 1-10°C. This solution was used as a standard stock solution. From this a series of aqueous solution is prepared and used for preparation of CC and QC samples.

4.2.1.2.4.3 Preparation of CC and QC samples

The calibration curve (CC) standards of 6943.1, 5721.1, 4233.6, 2963.5, 1481.8, 444.5, 115.6, and 34.7 ng/mL solution in plasma were prepared from aqueous standard solution and named as STD H, STD G, STD F, STD E, STD D, STD C, STD B and STD A respectively. Similarly the quality control (QC) sample concentrations of 6943.1, 5721.1, 2963.5, 115.6 and 34.7 ng/mL solutions in plasma were prepared from the aqueous solutions and named as ULOQ, HQC, MQC, LQC and LOQ respectively. All the calibration standards and quality control samples were stored below -70°C and withdrawn at the time of sample preparation.
4.2.1.2.5 Sample preparation

The blank human plasma, calibration standards, quality control samples and unknown samples (subject’s samples) were taken from the cold room and allowed to thaw in room temperature. Then vortexed for 30s to ensure complete mixing of the contents. 500 μL of spiked plasma sample was aliquoted, 50 μL of solution amitriptyline (10μg/mL) was added as internal standard to all samples except blank plasma and vortexed for 1 min to ensure proper mixing. Cartridges (Oasis Mcx, 30 mg/lcc) are conditioned with 1 mL of methanol and 1 mL of water and were centrifuged in the refrigerated centrifuge at 1500rpm for 1 min respectively. Then the samples were loaded into the cartridges and centrifuged at 1500 rpm for 2 min. The cartridges were washed with 1 mL of 0.5 N HCL solution and 1 mL methanol solution for 1 min at 1500 rpm respectively. Then the samples from the cartridges were eluted two times with 2 mL of ammoniated methanol solution at 1500 rpm for 1 min. The eluted samples were dried under nitrogen at 50°C and 15 psi. The dried residues were reconstituted with 300 μL of mobile phase and transferred into a vial for drug analysis.

4.2.1.2.6 Chromatography condition

The chromatographic separation was achieved by using the Hypersil Hypurity Advance C18 (4.6 X 50 mm, 5 μm) column, maintaining temperature at 35°C± 1.0°C. A mixture of Acetonitrile and 10 mM ammonium acetate buffer pH 6.6 (20:80) was used as a mobile phase which is run into the column with flow rate of 0.6 mL/min. The final samples from the sample preparation method were loaded in the HPLC auto sampler tray, maintaining temperature at 10°C ± 1.0°C. 10μL of sample was injected into the column and the eluent was monitored for mass to charge ratio (m/z) by tandem mass spectrometry.

The ion transitions for gabapentin were 172.1 m/z (parent) and 137.2 m/z (product). Similarly for amitriptyline it was 278.3 m/z (parent) and 233.1 m/z (product) which was used as an internal standard. The total run time for an injection was 5 minutes. The instrument was washed with a mixture of methanol and water (50:50 v/v). The data acquisition was made
with Analyst Software version 1.4.1 and also used for data recording and processing. The respective peak area from the chromatogram was obtained. A summary of the chromatographic conditions was as described below.

**Table 9: Summary of chromatography condition**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Hypersil Hypurity Advance C18, (4.6 * 50 mm), 5 μm</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>20:80 ratio of 10 ± 0.2 mM Ammonium acetate buffer (pH 6.6 ± 0.2) &amp; Acetonitrile solution.</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.6 mL/minute</td>
</tr>
<tr>
<td>Detection</td>
<td>Gabapentin m/z 172.1 (parent) and 137.0 (product) Amitriptyline m/z 278.1 (parent) and 233.0 (product)</td>
</tr>
<tr>
<td>Mass Spectrometer</td>
<td>API 3000 LC-MS/MS System</td>
</tr>
<tr>
<td>Ion Source</td>
<td>Heated nebulizer in Positive Ion Mode</td>
</tr>
<tr>
<td>Column Oven Temperature</td>
<td>35°C ± 1.0°C</td>
</tr>
<tr>
<td>Sample Cooler Temperature</td>
<td>10°C ± 1.0°C</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>10 μL volumes</td>
</tr>
</tbody>
</table>
| Retention time             | Gabapentin: 0.8 to 1.8 minutes
Amitriptyline: 0.8 to 1.8 minutes |
| Rinsing Solution           | HPLC grade water: Methanol (50 : 50 v/v)        |
| Run Time                   | 5 minutes                                       |

**4.2.1.2.7. System suitability**

Prior to each analysis the system was checked for suitability. A set of test injections were performed. A high concentration of analyte solution was prepared in mobile phase. Similarly a mixture of analyte and internal standard concentration was prepared in aqueous medium. Both the solutions were injected into the chromatographic condition in the following manner and the peak response was recorded.

1. Single injection of analyte concentration.
2. Six replicate injections of aqueous mixture of analyte and internal standard solution.
3. Step 1 procedure repeated.

The system was suitable for analysis if it fulfils the following criteria:

- The % CV for peak response should be less than 4%
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- The % CV for retention time should be less than 5%
- The % difference of step 1 and step 3 should not exceed 0.1%

4.2.1.2.8. Method validation

A validation of analytical method was performed in order to evaluate the method in terms of selectivity, sensitivity, linearity, precision and accuracy. This method was already developed and validated according to FDA 2001 guideline of bio-analytical method validation procedure. The summary of full method validation was reported in the result.

The linearity of standard curve/calibration curve was performed on three batches of spiked plasma samples. Each batch of spiked plasma samples included one complete set of calibration standards (Blank, blank plus internal standard, LLOQ (STD A), ULOQ (STD H): each in duplicate, and STD-B, C, D, E, F & G) and six replicates of each quality control samples namely limit of quantification lower quality control-LQC, middle quality control-MQC and higher quality control-HQC. Calibration range was based on to cover the human plasma gabapentin concentration after single dose oral administration of Gabapentin 300 mg IR capsule.

4.2.1.2.8.1 Screening and selectivity

The five different blank plasma samples were screened for the interference at the retention times of analyte (gabapentin) and internal standard (amitriptyline). Blank plasma sample with no or minimum interference were spiked with standard gabapentin at the lower limit of quantification (LOQ) level i.e. 34.7 ng/ml and amitriptyline (IS) in six replicates, analyzed according to the proposed method and observed for interference at the retention times of gabapentin and internal standard (amitriptyline).

For the acceptance, the response of the interfering peak must be:

1. ≤ 20 % of the mean peak area response of the LOQ, at the retention time of the gabapentin and
2. ≤ 5% of the mean peak area response of the internal standard, at the retention time of the gabapentin.

At least 80% of the matrix should meet the above mentioned acceptance criteria.

4.2.1.2.8.2. Linearity of standard curve

Three calibration batches consisting of 10 standards (blank, blank with internal standard and standard A (LLOQ) to standard H (ULOQ) were analyzed and the respective peaks were calculated. The linear equation describing the relationship between concentration ratio and peak area ratio of gabapentin to amtriptyline determined by least-squares weighted (1/concentration²) regression method.

\[ Y = \frac{m}{X^2} + c \]

In which

- \( Y \) = chromatography response in terms of peak area ratio of gabapentin / IS
- \( X \) = Concentration ratio of gabapentin / IS
- \( m \) = Slope of the calibration curve
- \( c \) = Y-axis intercept at zero concentration.

For acceptance of linearity of calibration curves, at least 75% or a minimum of 6 Calibrators including LOQ and ULOQ must meet the following passing criteria:

1. Accuracy of calibrates (% nominal): within ± 15% of their nominal values (within ± 20% for LOQ).
2. Precision of calibrates (% CV): ≤ 15% (≤ 20% for LOQ)
3. In case first injected LOQ or ULOQ standard failed, lost or exhibited bad chromatography, their duplicates passed the above criteria.
4. Linear coefficient of correlation: ≥ 0.98.
4.2.1.2.8.3. Limit of quantification

The limit of quantification (LOQ) was 34.7 ng/mL. This was determined by 3 times lower the reported maximum concentration in oral bioavailability study.

4.2.1.2.8.4. Precision and accuracy

Three Precision and Accuracy batches (PA batches) each consisting of standard reference solution (aqsmix), 10 calibration standards (blank, blank with IS, standard A (LLOQ), standard H (ULOQ), standard-B, C, D, E, F and G) and 6 replicates of each QC (LQC, MQC and HQC) samples, interspersed within each other, were processed and analyzed according to the proposed method.

The back calculated concentration of gabapentin in each quality control sample was calculated using linear regression parameters of the corresponding calibration curve. The mean gabapentin concentration, SD, % CV (precision) and % nominal (accuracy) for each QC level of the three PA batches were calculated.

For the acceptance, between and within batch CVs for QC samples should be ≤ 15 % (≤ 20 % for LOQ QC) & % Nominal of QC samples should be within ± 15 % (within ± 20 % for LOQ QC).

4.2.1.2.9. Clinical study sample analysis

4.2.1.2.9.1. Objectives

The objective of this study was to analyze the clinical study samples using a validated HPLC method combined MS such as LC-MS/MS for the determination of gabapentin.
As per the protocol, a total number of 1026 blood samples were collected from 18 subjects during the study without any missing samples. All the subjects completed the clinical study procedure. The blood samples from each period were collected and samples from each time point were centrifuged to separate plasma and stored at $-20^\circ C$ in a Haereus deep freezer at the clinical facility on their respective dates of collection. Loss of samples during centrifugation and plasma separation stage at the clinical facility is avoided. All the time points, plasma samples were packed properly using dry ice, transported to the analytical facility and stored at below $-70^\circ C$ until analysis.

For each sample analysis, the following procedure was used. One set of calibration standards for quality control samples and subject plasma samples (all samples of one or more subjects). Samples were analyzed subject wise using one analytical batch at a time which consisted of an aqueous reference standard dilution, 10 calibration spiked standards (blank, blank with IS, standard A (LOQ), standard H (ULOQ) and standard-B, C, D, E, F and G), 57 samples from one subject (19 + 19 + 19; from three periods), 3 samples of predose with IS and 6 quality control samples interspersed between the subject samples (LQC, MQC & HQC; 2 samples each) thus, a total of 77 samples per batch. Whenever samples from 2 subjects were analyzed as a single batch, only one set of calibration spiked standards was included, however, 3 sets of quality control samples were interspersed between the subject samples instead of the 2, thus a total of 141 samples per batch in such cases.

The plasma samples were arranged batch wise according to the analytical run and processed for sample preparation. The prepared samples were injected into the column of an HPLC system. The chromatography data were recorded.
4.2.1.2.9.4 Calculation of gabapentin concentration in plasma

The chromatography data were acquired and processed batch wise using computer based Analyst software version 1.4.1. The best line using weighted \((1/X^2)\) least square linear regression analysis was obtained by peak area ratio of gabapentin and amitriptyline versus concentration ratio of gabapentin and amitriptyline. The concentrations of gabapentin in the unknown plasma samples and quality control samples were calculated using linear regression parameters of the corresponding calibration curve in every batch as follows:

\[ Y = m \frac{1}{X^2} + c \]

\[ Y = \text{Chromatography response in terms of peak area ratio of gabapentin / IS} \]
\[ X = \text{Concentration ratio of gabapentin / IS} \]
\[ m = \text{Slope of the calibration curve} \]
\[ c = \text{Y-axis intercept at zero concentration}. \]

4.2.1.2.9.5 Batch acceptance criteria

The acceptance of the single batch consists of passing the Calibration Concentration (CC) acceptance criteria, blank and blank with internal standard acceptance criteria, and quality control acceptance criteria. All the batches were evaluated rigorously and considered for the repeat analysis if failed with respect to any of the following criteria.

**Calibration curve acceptance criteria:** All the CC standards should pass the following criteria

1. Accuracy of calibrators: within ± 15% of their nominal values (within ± 20% for LOQ).
2. At least 75% or a minimum of 6 Calibrators including LOQ and ULOQ meet the above criteria.
3. In case first injected LOQ or ULOQ standard failed, lost or exhibited bad chromatography, their duplicates should pass the above criteria.
4. Linear coefficient of correlation: ≥0.98.
Blank and blank plus internal standard acceptance criteria: At least one blank and one blank + IS: free from significant interference i.e.

1. Peak area responses of the blanks at the retention time of the Gabapentin were < 20% of the peak area response of the LOQ standard and
2. Peak area responses of the blanks at the retention time of the internal standard were < 5% of the mean response of internal standards used in the calibration curve.

Quality control sample acceptance criteria:

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

4.2.1.2.9.6 Repeat analysis

Samples were subjected to repeat the analysis whenever bad chromatography (asymmetry > 1.5) was observed and failing of batch acceptance criteria.

4.2.1.3 Pharmacokinetic and Statistical Analysis

4.2.1.3.1 Pharmacokinetic analyses

The following pharmacokinetic parameters were calculated using WinNonlin software Version 5.0.1

1. $C_{\text{max}}$: $C_{\text{max}}$ was calculated as the maximum measured plasma concentration over the time span specified.
2. $T_{\text{max}}$: $T_{\text{max}}$ was calculated as time of the maximum measured plasma concentration.
3. $K_e$: Terminal elimination rate constant ($K_e$) was estimated from a semi-log plot of the plasma concentration versus time curve and calculated by
linear least square regression analysis using the last three (or more) non-zero plasma concentrations.

4. \( t_{1/2} \): The terminal half-life \( (t_{1/2}) \) was calculated by the formula \( 0.693/K_{el} \).

5. \( \text{AUC}_{0-4} \): The area under the plasma concentration versus time curve, from time zero to the last measurable concentration \( (\text{AUC}_{0-4}) \) was calculated by the linear trapezoidal method.

6. \( \text{AUC}_{0-\infty} \): The area under the plasma concentration versus time curve from the time zero to infinity \( (\text{AUC}_{0-\infty}) \) was calculated by sum of the \( \text{AUC}_{0-4} \) plus the ratio of the last measurable concentration to the elimination rate constant.

7. \( \text{AUC}_{0-4}/\text{AUC}_{0-\infty} \): The ratio of \( \text{AUC}_{0-4} \) to \( \text{AUC}_{0-\infty} \) \( (\text{AUC}_{0-4}/\text{AUC}_{0-\infty}) \) was calculated from \( \text{AUC}_{0-4} \) and \( \text{AUC}_{0-\infty} \) values.

### 4.2.1.3.2 Statistical Analyses

Statistical analyses were carried out using SAS software version 9.1.3 (SAS Institute Inc. Cary NC, USA). The analysis includes the data of 18 subjects who had completed the study. The Descriptive statistics i.e. Arithmetic mean, standard deviation, coefficient of variation were calculated for all pharmacokinetics parameters and geometric mean and percentage coefficient of variation of geometric mean was calculated for \( \text{AUC}_{0-4} \), \( \text{AUC}_{0-\infty} \) and \( C_{\text{max}} \).

#### 4.2.1.3.2.1 ANOVA

The log-transformed pharmacokinetic parameters \( (C_{\text{max}}, \text{AUC}_{0-4}, \text{AUC}_{0-\infty}) \) for Gabapentin was analyzed using a mixed effect ANOVA model using Type III sum of squares, with the main effects of sequence, period and formulations as fixed effects and subjects nested within the sequence as a random effect. A separate ANOVA model was used to analyze each of the parameters. The sequence effect was tested at the 0.10 level of significance using the subjects nested within sequence mean square as the error term. Treatment and period effects were tested at the 0.05 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 analysis was done using the PROC GLM, SAS procedure.

4.2.1.3.2.2 90% Confidence Interval

The percentage point estimate (ratio of the least square means) for the log transformed pharmacokinetic parameters \( C_{\text{max}} \), \( \text{AUC}_0\text{t} \), and \( \text{AUC}_0\text{r} \) were reported. The 90% CI of \( C_{\text{max}} \), \( \text{AUC}_0\text{t} \) and \( \text{AUC}_0\text{r} \) for the ratio of test and comparative reference product was calculated by first calculating the 90% CI for the difference in the averages (arithmetic means) of the log (natural) transformed data and then taking antilog of the obtained confidence limits to assess bioequivalence using 90% CI of 80 – 125%.

4.2.1.3.2.3 Non-parameteric test for \( T_{\text{max}} \) comparison

The \( T_{\text{max}} \) parameter was statistically evaluated using the non-parametric test Wilcoxon's Signed Rank Test at 5% level of significant.

4.2.1.3.2.4 Power of the test and Intra subject variability

The power of the test (%) and Intra subject variability (% CV) for the log transformed pharmacokinetic parameters \( C_{\text{max}} \), \( \text{AUC}_0\text{t} \) and \( \text{AUC}_0\text{r} \) were reported.

4.2.2 In-Vitro Method (Dissolution Test)

Drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution or volatilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, \textit{in vitro} dissolution may be relevant to the prediction of \textit{in vivo} performance. Based on this general consideration, \textit{in vitro} dissolution specifications are established to ensure batch-to-batch consistency and to signal potential
problems with in vivo bioavailability. In the case of a generic drug product, the dissolution specifications are generally the same as the reference listed drug (RLD) as per the dissolution guidelines.\(^5\)

The approaches for setting dissolution specifications for generic products fall into three categories, depending on whether an official compendium test for the drug product exists and on the nature of the dissolution test employed for the reference listed drug.\(^5\)

The three categories are:

1. USP drug product dissolution test available
2. USP drug product dissolution test not available; dissolution test for reference listed NDA drug product publicly available.
3. USP drug product dissolution test not available; dissolution test for reference listed NDA drug product not publicly available.

For all drugs even batch to batch formulations of all manufacturers should be compliant to Pharmacopoeias standards according to country specific as per GMP before releasing the finished product to the market.

4.2.2 1. Dissolution methodology

The method of pharmacopoeias standard for gabapentin capsule formulation is available in USP 32 (first approach categories).\(^6\) The test was carried out in the Department of Product and Development Research Unit (PDR), Ranbaxy Research Laboratories, Gurgaon.

The in-vitro dissolution study methodology consists of following headings.

4.2.2.1.1. Instrumentation and conditions
4.2.2.1.2 Preparation of solutions
4.2.2.1.3. Mobile phase
4.2.2.1.4 System suitability
4.2.2.1.5 Dissolution specifications
4.2.2.1.6 Sample analysis
4.2.2.1.7 Drug release

4.2.2.1.1 Instrumentation and conditions

An HPLC method with UV detection in 210-nm was used to estimate gabapentin and the chromatographic separation was achieved using 4.6 mm x 25-cm column that contains 5-μm packing L7. The column oven temperature was set at 45°C. The flow rate of mobile phase was 1.2 mL/min and it was equilibrated for about 30 minutes before the first injection. The injection volume of samples was 100 μL and sample tray maintained at ambient temperature. The gabapentin peak time was about 6 minutes and the run time was 10 minutes. Empower software used for data processing. The summary of chromatography conditions is given below:

**Column**: Hypersil Hypurity Advance C8, (4.6 * 25 mm), 5 μm

**Mobile Phase**: 940:60 ratio of monobasic potassium phosphate buffer (pH 6.9) & Acetonitrile solution.

**Flow Rate**: 1.2 mL/minute

**Detection**: UV at 210 nm

**Column Oven Temperature**: 45°C ± 1.0°C

**Sample Cooler Temperature**: 37°C ± 1.0°C

**Injection Volume**: 100 μL volumes

**Retention time**: about 6 minutes

**Rinsing Solution**: HPLC grade water: Methanol: Acetonitrile (40: 40: 40 v/v/v)

**Run Time**: 10 minutes

The solutions were filtered through nylon filter 45μm diameter thickness before injection into the chromatographic condition. Fresh mobile phase and standard solutions were used each day of the analytical run. The column was equilibrated with the mobile phase for 30
minutes before the analytical run. The chromatographic condition used in system suitability was not changed in the analytical run.

4.2.2.1.2 Preparation of solutions

The following solutions were prepared and used as a fresh solution on each day of the dissolution.

**Standard stock solution:** 55.75 mg of gabapentin working standard was taken into 50 mL volumetric flask and dissolved in 25 mL of dissolution medium, mixed well and made up to the volume with same and degassed in the ultrasonic bath for 5 min. From this stock solution, a 30 mL volume of solution was pipetted out to 100 mL volumetric flask and made up to the volume with dissolution medium, mixed well and this solution was used as standard solution.

**0.06N Hydrochloric acid solution (Dissolution medium):** 51 mL of hydrochloric acid was taken into 10 L volume container and dissolved in 1 L of water, stirred well with a rod and made up to the volume with same and used as dissolution medium.

**5N potassium hydroxide solution:** 20 g of potassium hydroxide pellet was dissolved in 100 mL volume of distilled water.

4.2.2.1.3 Mobile phase:

1.2 g of monobasic potassium phosphate was dissolved in 940 mL of water and pH was adjusted to 6.9 with 5N potassium hydroxide solution. 60 mL of acetonitrile was added and stirred well with a glass rod and finally filtered. It was then well degassed in sonicator and used as a mobile phase for analysis.
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4.2.2.1.4 System suitability

During the sample analysis the system was evaluated for suitability by five repetitive injections of standard solutions and the chromatogram was recorded. The system was suitable for analysis if and only if:

1. The column efficiency of Gabapentin was not less than 7000 theoretical plates
2. The tailing factor of Gabapentin was not more than 2.0
3. The relative standard deviation for replicate injections was not more than 2.0%

If the instrument was not suitable necessary corrections were made before analysis to fulfill the system suitability criteria.

4.2.2.1.5 Dissolution specifications

The dissolution method was carried out according USP Apparatus 2 (Paddle Type) with 50 rpm. The dissolution medium is 0.06N hydrochloric acid of 900 mL volumes at temperature 37°C ±0.5°C. 10 mL volumes of samples were withdrawn at 5, 10, 15, 20 and 30 min intervals and same volume was replaced with medium. The collected samples were filtered through 0.45μm nylon filter and transferred to HPLC vial for analysis.

4.2.2.1.6 Sample analysis

A single injection of 100 μL volumes of each time point’s samples injected into the HPLC system and the chromatogram was recorded. The test samples were run into the system if the system was found suitable from the standard solution injection. The run time of single injection was 10 minutes and Gabapentin retention time is about 6 minutes. Samples of one or more formulations were run into the system with batch organization. For each formulation a batch organization consists of 37 samples of following sequence:

1. Five injections of standard solution
2. Single injection of six units in the order of 1 to 6 units in increasing order time points till the last time points which was 30 samples of six units of five time points (5X6=30)

3. Two repetitive injections of standard solution injected at first.

The batch was accepted if the standard solution at beginning run and end run will give similar peak retention time and relative standard deviation less than 2.0%. The peak area was recorded with review of all system suitability requirements.

4.2.2.1.7 Drug release

The percentage of drug released from the dosage form calculated from the peak area of each time point for all units by the following formula

Gabapentin (%) of label claim dissolved = \( \frac{AT}{AS} \times \frac{DS}{DT} \times \frac{P}{100} \times 100 \times \frac{1}{C} \)

in which,

- **AT**: Area count of Gabapentin peak in the chromatogram of the sample solution.
- **AS**: Average area counts for Gabapentin peak in the chromatograms of the standard solutions as obtained under system suitability.
- **DS**: Dilution factor of standard solution.
- **DT**: Dilution factor of test solution.
- **P**: Percentage potency Gabapentin working standard.
- **C**: Label claim of Gabapentin per capsule.

The correction factor of the withdrawal volume was included in the % drug released values at each time points. The passing limit is not less than 80% of labeled amount (%Q) should dissolve within 20 minutes.