Section 3:

MATERIALS
AND METHODS
OBJECTIVE: The study was designed to compare single and multiple doses pharmacokinetic bioequivalence of two marketed brands (Test) of Phenytoin 300 mg formulations with that of third marketed brand (Reference) of Phenytoin 300 mg capsule under fasting conditions in healthy, adult, male, human subjects. Also, various pharmacokinetic parameters were evaluated.

4. EXPERIMENTAL PROTOCOL

Experimental protocol is subdivided into two parts.

4.1. In-Vitro Dissolution Study

4.2. In-vivo Bioequivalence and Pharmacokinetic Study

4.1. IN-VITRO DISSOLUTION

In-vitro dissolution study was performed with the study preparations as per guidelines given by the CDER, US-FDA (Interim Guidance- Phenytoin/Phenytoin Sodium Capsules, Tablets and Suspension In Vivo Bioequivalence and In-Vitro Dissolution Testing, 1994). The conditions for the dissolution study are given in table 6

| Table 6: Conditions for in-vitro dissolution study of Phenytoin Sodium Preparations |
|---------------------------------|---------------------------------|
| Apparatus | USP XXII apparatus 1 (basket) |
| RPM | 50 |
| Medium | Distilled Water (37°C) |
| Volume | 900 ml |
| Sampling Times | 15, 30, 60, 90 and 120 min |
| Analytical | HPLC Method |
| Tolerance | NMT 40% in 30 min; 55% in 60 min; and NLT 70% in 120 min |

The percentage of label claim at each specified testing interval would be reported for each individual unit. The mean percentage dissolved, the range of dissolution and the coefficient of variation would also be reported.
4.2. IN-VIVO BIOEQUIVALENCE AND PHARMACOKINETIC STUDY

4.2.1. Objective
The objective of this study was to compare single and multiple dose (steady state) pharmacokinetic bioequivalence of two marketed brands (Test) of phenytoin 300 mg formulations with that of third marketed brand (Reference) of Phenytoin 300 mg capsule under fasting conditions in healthy, adult, male, human subjects.

4.2.2. Products to be evaluated
Reference (R)

Test (A)

Test (B)
Eptoin 300mg manufactured by Knoll Pharma, Batch No. F0092, Mfg Date- Sep 2004, Exp. Date- Aug 2007.

All the products to be evaluated were purchased from the retail market.

4.2.3. Study Design
The study was conducted as an open label, balanced, randomized, three-treatment, three-period, three-sequence, single and multiple dose cross over pharmacokinetic bioequivalence study comparing the bioequivalence of Epsolin and Eptoin with that of Dilantin under fasting conditions in healthy, adult, male, human subjects.

The consists of the following phases:
1. Single dose study (I): Oral administration of single dose of 300 mg phenytoin (study day 1) (Fasting)
2. Drug Free Day (II): Day 2
3. Steady state study (III); Following the single dose study, the subjects continued to take the medication during days 3-7. (Fasting)
4. Drug free days (IV): Days 8 and 9
5. After this a wash out of at least 7 days was given and same schedule was followed for two other formulations of phenytoin. Table 7 describes the study design.
4.2.4. Number of Subjects
The study was carried out in 15 healthy, adult, male human subjects. Subsequent dropouts were not replaced. Data is presented on all completed subjects.

4.2.5. Admission and Stay
Subjects were admitted and housed in the Clinical Pharmacology Unit from at least 12 hours before dose administration and discharged 24 hours after administration of the test and reference products on day 2 during each period, if the subjects did not suffer from any adverse drug reaction. The subjects were asked to come to the unit for ICF presentation and they were compensated for it.

4.2.5. Dose
A single 300mg dose of Phenytoin Sodium was administered once on day 1 and then daily for five days from day 3 during each period under supervision. Pre-dose sample was taken on all days. On the first day, the sample profile was taken from pre-dose till 24 hours. Thereafter, 24-hourly samples was taken from day 3 till day 9.

4.2.5.1. Reference (R)
Dilantin 300 mg (Parke-Davis) was administered in morning with 240 mL of water at ambient temperature, on days 1, 3, 4, 5, 6 and 7.

4.2.5.2. Test (A)
Epsolin 300mg Tablets (Zydus) was administered in morning with 240 mL of water at ambient temperature, on days 1, 3, 4, 5, 6 and 7.
4.2.5.3. Test (B)

Eptoin 300 mg Tablets (Knoll) was administered in morning with 240 mL of water at ambient temperature, on days 1, 3, 4, 5, 6 and 7.

4.2.6. Fasting/Meals

All subjects were required to fast overnight for at least 10 hours before the morning dose and for 4 hours post-dose. All subjects received standard meals - lunch, snacks and dinner 4.5, 9 and 13 hours, respectively, after drug administration. During housing, all meal plans were identical for all 3 periods. In case meals and blood sample collection coincide, samples were collected before meals are provided. Drinking water was not allowed from 1 hour before dosing until 2 hours post-dose. Thereafter, it was allowed at all times.

4.2.7. Sampling Schedule

A total of 22 blood samples (4ml each) per period were collected through an indwelling cannula placed in a forearm vein or through 5ml vacutainers. The blood samples were collected pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24 post dose. Then days 2, 3, 4, 5, 6, 7, 8, and 9 of study. The total amount of blood withdrawn was 363 ml spread over a period of 6 weeks. The sample on days 1, 3, 4, 5, 6 and 7 was taken before the morning dose administration of drug to the subject. Blood samples were collected on days 8 and 9 as well. The predose blood sample was collected within a period of 1 hour before dosing and the post dose samples were collected within two minutes of the scheduled time.

4.2.8. Washout Period

There was a washout period of at least seven days between each period.

4.2.9. Restrictions

Medications

Subjects should not have received any medications during 14-days period prior to the onset of the study. They were instructed during screening not to take any prescription and OTC medications subsequently until the completion of the study.

If drug therapy other than that specified in the protocol was required during the study or in the wash out period, decisions to continue or discontinue the subject would have been based on the following:

i) The pharmacology and pharmacokinetics of the non-study medication.
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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.

Diet

All subjects were instructed to abstain from any xanthine containing food or 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 instructed to remain seated or ambulatory for the first two hours following each drug administration. Thereafter, subjects were allowed to engage only in normal activities while avoiding severe physical exertion.

4.2.10. Assignment to Treatment Sequences

The order of receiving the study treatments for each subject during the three periods of the study was determined according to SAS-generated randomization schedule.

4.2.11. Clinical Safety Measurements

Vital signs of oral temperature, sitting blood pressure and radial pulse were measured during subject admission, prior to each dosing and 2, 4, 8, 12 and 24 hours after administration of study drug and at ambulatory visits in each study period. Vital signs to be measured prior to administration of the dose were taken within 1 hour of the scheduled dosing time. At all other times, vital signs were taken within 30 minutes of the scheduled times. In the event of detection of any abnormality during measurement of vital signs, the Clinical Pharmacologist or a medically qualified designate was consulted for necessary action that was recorded.

Brief clinical examination of the subject was conducted by a qualified medical designate on duty after subject admission, prior to dosing of study drug and before discharge. In the event of detection of any abnormality during clinical examination, the Clinical Pharmacologist or a medically qualified designate was consulted for necessary action, which was recorded.

Laboratory tests (haemoglobin, serum creatinine, blood urea nitrogen, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase
(ALP) and serum bilirubin were repeated at pre-dose in each study period (day 1) and discharge day (9) of all subjects. Any elevations in hepatic enzymes were followed up till they subsided.

4.2.12. Adverse Events
Subjects were monitored throughout the study period for adverse events. Subjects were informed to bring to the notice of the nurse or the doctor any adverse event that may have occurred during their stay at the site of investigation.
Subjects were also specifically asked about any adverse events throughout the study period every four hours.

4.2.13. Selection of subjects
Adequate number of subjects was selected randomly from the volunteer bank of Clinical Pharmacology Unit and underwent a standardized screening procedure. Fifteen healthy male subjects were selected on the basis of following inclusion and exclusion criteria:

**Inclusion criteria**
- Be in the age range of 18-45 yrs.
- Be neither overweight nor underweight
- Have voluntarily given written informed consent to participate in this study.
- Be of normal health as determined by the medical history and physical examination of the subject performed within 28 days prior to the commencement of the study.
- Absence of decease markers of HIV 1 and 2, Hepatitis B virus and syphilis infection.
- Be not clinically significantly different from normal reference ranges for haemoglobin, total white blood cells (WBC) count, differential WBC count and erythrocyte sedimentation rate (ESR).
- Be not clinically significantly different from normal reference ranges for creatinine, blood urea, serum aspartate, aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase, serum bilirubin, serum glucose (fasting) and serum cholesterol.
- Clinically normal chemical and microscopic examination of urine.

**Exclusion criteria:**
- History of allergy to Phenytoin Sodium and other antiepileptic drugs.
- Any evidence of organ dysfunction or any clinically significant deviation from the normal, in physical or clinical determinations.
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- History of serious gastrointestinal, hepatic, renal, cardiovascular, pulmonary, neurological disease, diabetes or pulmonary, neurological or haematological disease, diabetes or glaucoma.
- History of any psychiatric illness which may impair the ability to provide written informed consent.
- Regular smoker who smokes more than 20 cigarettes daily or has 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 two units of alcoholic beverages per day (one unit equivalent to half pint of beer or one glass of wine or one measure of spirit) or have difficulty in abstaining for the duration of each study period.
- Use of any enzyme modifying drugs within 30 days or any systemic medication (including OTC preparation) within 14 days prior to day one of this study.
- Participation in any clinical trial within six weeks preceding day one of this study.
- Hemoglobin concentration of less than 7% of lower limit of reference range e.g. 13 gm% for reference range of 14-18 gm at screening.
- Subject without adequate venous access in their left or right arm to allow collection of 57 blood samples via venous cannula in the three periods.

4.2.14. Ethical considerations

Basic Principles
This research was carried out as per the WHO guidelines for “Good Clinical Practice (GCP) for Trials on Pharmaceutical Products”, ICH (Step 5) “Guidance for Good Clinical Practice” and the principles enunciated in the Declaration of Helsinki (South Africa, 1996).

Institutional Review Board
This protocol and the corresponding informed consent form (ICF) were reviewed by the Jamia Hamdard Institutional Review Board and the study subjects were not dosed until the Board had approved the protocol and the ICF, as submitted or with modifications.

Informed Consent
The Clinical Pharmacologist or his designate informed the study subjects through an oral presentation regarding the purpose, procedures to be carried out, potential hazards, rights of the subjects. Subjects were required to understand and sign a consent form summarizing the
discussion prior to check-in for the study in Period I.

4.2.15. Drop-out/Withdrawal of Subjects from Study
Subjects were informed that they were free to dropout from the study at any time without stating any reason. Details of reasons for withdrawal of subjects were reported.

5. ANALYTICAL METHODOLOGY
Attached as Appendix 12.

6. PHARMACOKINETIC AND STATISTICAL ANALYSIS

6.1. Pharmacokinetic analysis
The following pharmacokinetic parameters were calculated for Phenytoin Sodium using WinNonlin Professional software version 1.5 (SCI, USA):
1. $T_{\text{max}}$: Time to maximum measured plasma concentration.
2. $C_{\text{max}}$: Maximum measured plasma concentration following each treatment.
3. $\text{AUC}_{\text{obs}}$: The area under the plasma concentration versus time curve from the time zero to the last measurable concentration, as calculated by the linear trapezoidal method.
4. $T_{\text{min}}$: The time of minimum concentration based on samples collected during a dosing interval will be calculated.
5. $C_{\text{avg}}$: The average steady state plasma concentration will be computed as $\text{AUC}(0-\text{Tau})/\text{Tau}$.
6. Percent Fluctuation: For steady state data: computed as $100\times(C_{\text{max}}-C_{\text{min}})/C_{\text{avg}}$.

6.2. Statistical analysis
The following analysis was conducted on least square means (LSM) of each pharmacokinetic component of the test product for all treatments using SAS software version 6.12 (SAS Institute Inc. Cary NC, USA)

Summary statistics
The following summary statistics (for the relevant pharmacokinetic parameters) was reported for both the test and reference products: the arithmetic means, the geometric means, standard
deviations and the coefficient of variation for the original (untransformed) data; and the arithmetic means, standard deviation and the coefficient of variation for the log (natural) transformed data. For the relevant pharmacokinetic parameters, the ratio of the test and reference product averages was also reported.

Ratio Analysis
Ratio of mean (in percentage) was calculated using the LSM for untransformed and log transformed \( C_{\text{max}} \), \( \text{AUC}_{0-t} \) and \( \text{AUC}_{0-\infty} \). The geometric mean values were reported for log transformed parameters only. Ratios of mean were expressed as a percentage of the LSM for the respective treatment comparisons.

Confidence Intervals
90% Confidence intervals (CI) was calculated for all the treatment comparisons for both untransformed and log transformed data.

ANOVA (Analysis of variance)
Analysis of variance was performed and a P value < 0.05 was considered statistically significant at 95% level of significance and \( \alpha = 0.05 \).

Intra subject variability
The Intra subject variability was reported for the Pharmacokinetic parameters \( C_{\text{max}} \), \( \text{AUC}_{0-t} \) and \( \text{AUC}_{0-\infty} \) for log transformed data.