CHAPTER 8.
APPENDICES
8.0 APPENDICES

Appendix I: IRB Permission and Protocol
Appendix II: Informed Consent Form
Appendix III: Randomization schedule
Appendix IV: Representative Chromatograms of Subjects, CC and QC samples
Appendix I: IRB Permission and Protocol
August 27, 2007

To:

The Chairman
Jamia Hamdard Institutional Review Board
Hamdard Nagar
NEW DELHI 110 062

Dear Sir,

Please refer to your letter dated 22/08/07 approving the Protocol number 006_JAMHAM_07, Version 1; 01 August 2007 including Informed Consent Form (ICF) English version entitled "An open label, balanced, randomized, three-treatment, three-period, three-sequence, single-dose, crossover, bioavailability study comparing three different marketed Metformin extended release tablets in healthy, adult, human male subjects under fed conditions".

Please find enclosed herewith the amended Protocol with List of Changes including ICF (English version) for above study (both Version 2; 24 August 2007) after incorporating the changes suggested by you/minor changes by Investigators.

Please approve.

Yours sincerely

Lakhvinder Singh Batolar
Investigator
An open label, balanced, randomized, three-treatment, three-period, three-sequence, single-dose, crossover, bioavailability study comparing three different marketed Metformin extended release tablets in healthy, adult, human male subjects under fed conditions.

Lakhvinder Singh Batolar

Department of Pharmaceutical Medicine, Faculty of Pharmacy, Jamia Hamdard, New-Delhi-110062, India
1.0 INVESTIGATORS’ DECLARATION

We, the undersigned have read and understood this protocol and hereby agree to conduct the study in accordance with this protocol and to comply with all requirements regarding the obligations of investigators and all other pertinent requirements of 21 CFR Part 320 and ICH (09 May 1997) 'Guidance on Good Clinical Practice'.

We agree to comply to all relevant SOPs required for the conduct of this study. We further agree to ensure that all associates assisting in the conduct of study are informed regarding their obligations.

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Ranbaxy Research Labs
Co-supervisor

Prof. P. L. Sharma
MD, PhD (Lond.), FAMS
Chief-supervisor
2.0 FACILITIES

2.1 Clinical Services and Clinical Laboratory

Clinical Pharmacology Unit
Majeedia Hospital (2nd Floor)
Jamia Hamdard (Hamdard University)
Hamdard Nagar
New Delhi 110 062
India

2.2 Alternate Clinical Laboratory

Dr. Lal PathLabs Pvt. Ltd.
Eskay House
54, Hanuman Road
New Delhi 110 001
India

2.3 Analytical, Pharmacokinetic & Statistical Services

Clinical Pharmacology and Pharmacokinetics
Ranbaxy Research Laboratories
Plot No. 20, Sector 18, Udyog Vihar Industrial Area
Gurgaon 122 015, Haryana
India
3.0 INTRODUCTION

Metformin, a biguanide oral antihyperglycemic, is well established as a therapeutic agent in patients with type 2 diabetes mellitus. It has a unique mechanism of action, proven efficacy, and a favorable safety profile. Metformin produces clinically significant improvements in glycemic control in patients with type 2 diabetes through its insulin-sensitizing actions on both the liver, where it reduces hepatic glucose overproduction, and peripheral tissues (particularly skeletal muscle), where it enhances glucose uptake (US Prescribing Information of Glucophage & Glucophage XR, Bristol-Myers Squibb Company, March 2004). Metformin hydrochloride is available worldwide in a number of countries in solid dosage form (immediate release and extended release tablets) and as oral solution (New Product Focus, 2004, IMS Health Incorporated).

Non-compliance with the pharmacological therapy in patients with type 2 diabetes can have serious short-term and long-term adverse effects. Short-term consequences include precipitation of diabetic ketoacidosis, a condition that can be life threatening. Long-term consequences of non-compliance include macrovascular, neurologic and microvascular complications: retinopathy, nephropathy, neuropathy, and cardiovascular disease. These complications cause major morbidity and mortality in diabetic patients. Indeed, intensive control of blood glucose in type 2 diabetics has been shown to reduce the progression of microvascular disease as well as cardiovascular disease (UK Prospective Diabetes Study Group, 1998).

An extended release formulation of metformin 500 mg is marketed in the USA (Metformin XR of Bristol Myers Squibb), and a 750 mg extended release formulation of the same manufacturer has been approved for the same manufacturer in the USA[3]. Metformin XR has been shown to produce systemic metformin exposures similar to those of IR and to be therapeutically as effective and as well tolerated on once daily dosing as equal daily doses of IR metformin given in divided doses. The maximum recommended dose of Metformin XR in the USA is 2000 mg/day (4 tablets of Metformin XR given once daily with the evening meal.)

Various manufacturers claim that their products are bioequivalent to the innovator’s formulation but it may or may not be by today’s standards. Most of the bioequivalence studies on which these claims are based did not use confidence intervals, a current DCGI requirement to document bioequivalence with the comparator drug formulation.

Hence, the objective of the proposed study is to compare the pharmacokinetic bioequivalence of three marketed brands of Metformin sustained release tablets 500 mg after Multiple dosing in healthy, adult, male human subjects under fed conditions.

4.0 OBJECTIVE

To compare single-dose oral bioavailability of three different marketed Metformin Extended release tablets in healthy, adult, human, male subjects under fed conditions.
5.0 PRODUCTS TO BE EVALUATED

5.1 Reference (R)
Cetapin XR 500mg sustained release tablets (containing Metformin 500mg) manufactured Aventis Pharmaceuticals, India.

5.2 Test (A)
Glycomet SR 500mg sustained release tablets (containing Metformin 500mg) manufactured by USV Pharmaceuticals Ltd, India.

5.3 Test (B)
Bigomet SR 500mg extended release tablets (containing Metformin 500mg) manufactured by Otsira Genetica Ltd, India.

6.0 PHARMACOLOGY

6.1 Absorption, Distribution, Metabolism and Excretion

Absorption:

The absolute bioavailability of a metformin 500 mg tablet given under fasting conditions is approximately 50-60%. Studies using single oral doses of metformin 500 mg to 1500 mg, and 850 mg to 2550 mg, indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination. Food decreases the extent of and slightly delays the absorption of metformin, as shown by approximately a 40% lower mean peak plasma concentration (C_max), a 25% lower area under the plasma concentration versus time curve (AUC), and a 35 minute prolongation of time to peak plasma concentration (T_max) following administration of a single 850 mg tablet of metformin with food, compared to the same tablet strength administered fasting. Following a single oral dose of metformin XR, C_max is achieved with a median value of 7 hours and a range of 4 hours to 8 hours.

Although the extent of metformin absorption (as measured by AUC) from the metformin XR tablet increased by approximately 50% when given with food, there was no effect of food on C_max and T_max of metformin. Both high and low fat meals had the same effect on the pharmacokinetics of metformin XR.

The apparent volume of distribution (V/F) of metformin following single oral doses of metformin 850 mg averaged 654±358 L. Metformin is negligibly bound to plasma proteins. Following oral administration, approximately 90% of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination half-life of approximately 6.2 hours. In blood, the elimination half-life is approximately 17.6 hours, suggesting that the erythrocyte mass may be a compartment of distribution.
Distribution:
The apparent volume of distribution (V/F) of metformin following single oral doses of METFORMIN 850 mg averaged 654±358 L. Metformin is negligibly bound to plasma proteins, in contrast to sulfonylureas, which are more than 90% protein bound. Metformin partitions into erythrocytes, most likely as a function of time. At usual clinical doses and dosing schedules of METFORMIN, steady state plasma concentrations of metformin are reached within 24-48 hours and are generally < 1 μg/mL. During controlled clinical trials of METFORMIN, maximum metformin plasma levels did not exceed 5 μg/mL, even at maximum doses.

Metabolism:
Intravenous single-dose studies in normal subjects demonstrate that metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) nor biliary excretion. Renal clearance is approximately 3.5 times greater than creatinine clearance, which indicates that tubular secretion is the major route of metformin elimination.

Excretion:
Following oral administration, approximately 90% of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination half-life of approximately 6.2 hours. In blood, the elimination half-life is approximately 17.6 hours, suggesting that the erythrocyte mass may be a compartment of distribution.

6.2 Adverse Effects

In a US double-blind clinical study of METFORMIN in patients with type 2 diabetes, a total of 141 patients received METFORMIN therapy (up to 2550 mg per day) and 145 patients received placebo. Adverse reactions reported in greater than 5% of the METFORMIN patients, and that were more common in METFORMIN- than placebo-treated patients, are listed in Table 1.

<table>
<thead>
<tr>
<th>Adverse Reaction</th>
<th>METFORMIN Monotherapy (n=141)</th>
<th>Placebo (n=145)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of Patients</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>53.2</td>
<td>11.7</td>
</tr>
<tr>
<td>Nausea/Vomiting</td>
<td>25.5</td>
<td>8.3</td>
</tr>
<tr>
<td>Flatulence</td>
<td>12.1</td>
<td>5.5</td>
</tr>
<tr>
<td>Asthenia</td>
<td>9.2</td>
<td>5.5</td>
</tr>
<tr>
<td>Indigestion</td>
<td>7.1</td>
<td>4.1</td>
</tr>
<tr>
<td>Abdominal Discomfort</td>
<td>6.4</td>
<td>4.8</td>
</tr>
<tr>
<td>Headache</td>
<td>5.7</td>
<td>4.8</td>
</tr>
</tbody>
</table>
Diarrhea led to discontinuation of study medication in 6% of patients treated with METFORMIN. Additionally, the following adverse reactions were reported in ≥1.0 - ≤5.0% of METFORMIN patients and were more commonly reported with METFORMIN than placebo: abnormal stools, hypoglycemia, myalgia, lightheaded, dyspnea, nail disorder, rash, sweating increased, taste disorder, chest discomfort, chills, flu syndrome, flushing, palpitation.

In worldwide clinical trials over 900 patients with type 2 diabetes have been treated with METFORMIN XR in placebo- and active-controlled studies. In placebo-controlled trials, 781 patients were administered METFORMIN XR and 195 patients received placebo. Adverse reactions reported in greater than 5% of the METFORMIN XR patients, and that were more common in METFORMIN XR- than placebo-treated patients, are listed in Table 2.

| Table 2: Most Common Adverse Reactions (>5.0 Percent) in Placebo-Controlled Studies of METFORMIN XR* |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Adverse Reaction                                | METFORMIN XR (n=781)                             | Placebo (n=195)                                 |
| Diarrhea                                        | 9.6                                              | 2.6                                              |
| Nausea/Vomiting                                 | 6.5                                              | 1.5                                              |

* Reactions that were more common in METFORMIN XR- than placebo-treated patients.

Diarrhea led to discontinuation of study medication in 0.6% of patients treated with METFORMIN XR. Additionally, the following adverse reactions were reported in ≥1.0% - ≤5.0% of METFORMIN XR patients and were more commonly reported with METFORMIN XR than placebo: abdominal pain, constipation, distention abdomen, dyspepsia/heartburn, flatulence, dizziness, headache, upper respiratory infection, taste disturbance.

6.3 Dosage

The usual starting dose of metformin XR (metformin hydrochloride extended-release tablets) is 500 mg once daily with the evening meal. Dosage increases should be made in increments of 500 mg weekly, up to a maximum of 2000 mg once daily with the evening meal. If glycemic control is not achieved on metformin XR 2000 mg once daily, a trial of metformin XR 1000 mg twice daily should be considered. If higher doses of metformin are required, metformin should be used at total daily doses up to 2550 mg administered in divided daily doses, as described above.
7.0 STUDY DESIGN

7.1 Summary

An open label, balanced, randomized, three-treatment, three-period, three-sequence, single-dose, crossover, bioavailability study comparing three different marketed Metformin sustained release in healthy, adult, human male subjects under fed conditions.

The study design has been schematically represented in Appendix 1 and a detailed summary of the protocol is presented in Appendix 2.

7.2 Number of Subjects

Eighteen (18) healthy, adult, human male subjects will be admitted in the first period of the study. Subsequent dropouts/withdrawals will not be replaced. Data will be presented on all completed subjects. If necessary, an unequal number of subjects per sequence will be used.

7.3 Admission and Stay

Subjects will be admitted and housed in the Clinical Pharmacology Unit from at least 10 hours before dose administration and will be discharged 24 hours after administration of the test or reference products during each period, if the subjects do not suffer from any adverse drug reaction. In case of an adverse event, the subject will be monitored until the event subsides.

7.4 Dose

The subjects will be divided into three groups with 6 subjects in each group. A single oral dose of Metformin 500mg sustained release tablet will be administered with 240 mL of drinking water at ambient temperature after standardized breakfast, during each period of the study under supervision of trained study personnel. The order of drug administration shall be randomized with SAS-generated randomization schedule.

7.4.1 Reference (R)

Cetapin XR 500mg sustained release tablets (containing Metformin 500mg) manufactured by Aventis Pharmaceuticals, India.

7.4.2 Test (A)

Glycomet SR 500mg sustained release tablets (containing Metformin 500mg) manufactured by USV Pharmaceuticals Ltd, India.

7.4.3 Test (B)

Bigomet SR 500mg extended release tablets (containing Metformin 500mg) manufactured by Otsira Genetica Ltd, India.
7.5 Fasting/Meals

All subjects will be required to fast overnight after admission for at least 10 hours. The study subjects will receive the study drug 30 minutes after the recommended meal in each period. The breakfast will be consumed within 30 minutes. They will receive standard meals—lunch, snacks and dinner at 4, 9 and 13 hours, respectively, after drug administration. During housing, all meal plans will be identical for both periods. Information on the amount of meal consumed and the time taken for consuming the meal will be recorded in the appropriate clinical raw data sheets. In case, meals and blood sample collection coincide, samples will be collected before meals are provided.

Drinking water will not be allowed from 1 hour before dosing until 2 hours post-dose. Thereafter, it will be allowed at all times.

7.6 Sampling Schedule

A total of sixty six, 4-mL blood samples will be collected from each subject in EDTA vacutainers during the course of the study through indwelling cannulae placed in forearm veins. The minimum blood sample volume required for analytical purpose is 4-mL in this study. The blood samples will be collected Pre-dose (within 1.5 hours of dosing) and at 0.5, 1, 2, 3, 4, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 12, 14, 16, 20, 24 and 48 hours post-dose in each period. The pre-dose blood sample in each period will be collected within a period of 1.5 hours before dosing and the post-dose samples will be generally within 2 minutes of the scheduled time. The actual end-point time of collection of each blood sample will be recorded. For each subject, the total number of blood draws during the study will be 66 and the total volume of blood drawn including 16 mL for screening and 30 mL 'discarded' blood prior to venous cannula collections, will not exceed 310 mL.

7.7 Washout Period

There will be a washout period of at least 7 days between the administrations of study drugs in each period.

8.0 RESTRICTIONS

8.1 Medications

Subjects should not have received any medication (except vitamins preparations) including over the counter medications (OTC) during the 2 weeks period prior to the onset of the study. They will be instructed during screening not to take any prescription and OTC medications until the completion of the study. If drug therapy other than that specified in the protocol is required during the study or in the washout period, decisions to continue or discontinue the subject will be 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.

8.2 Diet

All subjects will be instructed to abstain from alcoholic products for 48 hours prior to dosing and during in-house stay in each period. They will also abstain from tea, coffee, cigarette and any other xanthine containing food or beverages during in-house stay in each period.

8.3 Activity

All subjects will be dosed while seated and will be instructed to remain seated or ambulatory for the first 2 hours following each drug administration. Thereafter, subjects will be 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 will be placed in an appropriate position or will be permitted to lie down on their right side.

9.0 STUDY POPULATION

Eighteen healthy, adult, human, male subjects will be selected randomly from the Volunteer Bank of Clinical Pharmacology Unit and will undergo a standardized screening procedure as described in SOP No. CP-S02. Eighteen healthy human subjects will be selected on the following inclusion and exclusion criteria and will be documented in the CRITERIA CHECK form (Appendix 3).

9.1 Screening Assessments

Medical histories and demographic data, including name, sex, age, body weight (kg), height (cm) and tobacco use (including number of cigarettes smoked per day) will be recorded. Each subject will undergo physical examination and the laboratory tests of hematology, hepatic and renal functions as listed below. Only medically healthy subjects with clinically normal laboratory profiles will be enrolled in the study.
9.1.1 Laboratory Tests

**HEMATOLOGY**
- Hemoglobin
- Total leukocyte count
- Differential leukocyte count
- Platelet count

**BIO-CHEMISTRY**
- BUN
- Creatinine
- Total bilirubin
- Alkaline phosphatase
- AST
- ALT
- Glucose
- Cholesterol

**URINALYSIS**
- PHYSICAL EXAMINATION
  - Colour
  - Appearance
  - pH
  - Specific gravity
  - Protein
  - Glucose

**MICROSCOPIC EXAMINATION**
- RBC
- WBC
- Epithelial Cells
- Crystals
- Casts
- Others

**ADDITIONAL TESTS**
- HIV I & II
- HBsAg
- HCV
- VDRL
- Urine drug screen
  - cannabinoids
  - opioids

All the samples during screening will be collected and analyzed at clinical laboratory situated at Ranbaxy Clinical Pharmacology Unit. Dr. Lal Pathlabs will be used as a back-up laboratory for sample analysis whenever in-house clinical laboratory is out of stock of lab kits or whenever there is a malfunction in laboratory instruments.

9.2 Inclusion Criteria
- Be male and in the age range of 18-45 years.
- Be neither overweight nor underweight for his/her 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 14 days prior to the commencement of the study.

9.3 Exclusion Criteria
- History of allergy to metformin or other related antidiabetic biguanide preparations.
- Past history of Headache, Dizziness, recurrent Upper respiratory infections and Diarrhoea in the preceeding week.
- 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 5) and/or judged clinically significant for haemoglobin, total white blood cells count, differential WBC count or platelet count.

- Positive for urinary screen testing 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), 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 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.

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

10.0 SCHEDULE OF ASSESSMENTS

An overview of assessments during the whole study is given in Appendix 4.

11.0 STUDY MEDICATION
11.1 Handling, Storage and Accountability Procedures

Investigational products will be supplied in the original manufacturer's packing and the test products will be supplied in an appropriate package deemed to maintain the integrity of the products. At the clinical facility, the investigational products will be logged-in by the Registered Pharmacist and stored under prescribed storage conditions in a controlled access area in the drug store as described in SOP NO. CP-C24. The Investigator and the Registered Pharmacist will be accountable for the study drug products. Study drugs will be dispensed according to the randomization schedule as described in SOP No. CP-C04.

11.2 Assignment to Treatment Sequences

The order of receiving the test or reference products for each subject during the 3 period study will be determined according to a SAS-generated randomization schedule. The randomization will be balanced and the code will be kept under controlled access in the drug store. A working copy of the same will be provided to study personnel responsible for dosing. The Investigator, Registered Pharmacist, personnel involved in dispensing of study drugs and the dosing will be accountable for ensuring compliance to randomization schedule.
11.3 Assessment of Compliance

Compliance will be assessed by conducting a thorough examination of the oral cavity by trained study personnel after dosing in each period and by measurement of plasma metformin (during the analytical phase of the study).

12.0 PHARMACOKINETICS PROCEDURES

12.1 Blood Sampling

Blood samples will be collected within 2 minutes of the time specified under STUDY DESIGN. Intravenous indwelling cannula will be kept in situ as long as possible (until 16 hours post-dose) when multiple samples will be collected. The cannula will be maintained patent by injection of 1 mL of 5 IU/mL of heparin in normal saline solution. In such cases blood samples will be collected after discarding the first 0.5 mL of heparinised blood and heparin solution from the tubing.

Alternatively, blood samples may be collected by a fresh clean venipuncture using a disposable sterilised syringe and a needle.

All blood samples collected earlier or later than 2 minutes of the scheduled collection time as specified in section 7.0 under STUDY DESIGN will be reported as protocol deviations and the actual delay in collection will be adjusted during pharmacokinetic calculations.

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

12.2 Analytical Procedures

Samples from all subjects completing the crossover will be assayed for plasma metformin using chromatographic procedures developed and validated at Ranbaxy. If necessary, an unequal number of subjects per sequence will be used. No additional subjects will be enrolled. Samples from dropouts and/or withdrawn subjects will not be assayed.

Whenever possible, all samples from each subject will be analyzed on the same standard curve. Quality control samples will be distributed through each batch of study samples assayed. Samples with drug concentrations greater than the upper limit of the validated range of the assay will be diluted with the appropriate drug-free biological fluid and reassayed; those which are below the lower limit of this range will be reported as being below LOQ. The analysts will not have access to the randomization scheme.

Analytical results will be presented in tabular form in the final report and chromatographic and derived data will also be provided. Additionally, accuracy,
precision and linearity data for each standard curve and all quality control samples will be presented. Validation of analytic methods will be included in the final study report.

12.3 Pharmacokinetic Parameters

The following pharmacokinetic parameters will be calculated for metformin using WinNonlin-Node version 4.0 or above from Pharsight:

\[ AUC_{0\rightarrow t} \]: The area under the plasma concentration versus time curve, from time zero to the last measurable concentration, as calculated by the linear trapezoidal method.

\[ AUC_{0\rightarrow \infty} \]: The area under the plasma concentration versus time curve, from time zero to infinity. \( AUC_{0\rightarrow \infty} \) is calculated as the sum of \( AUC_{0\rightarrow t} \) plus the ratio of the last measurable plasma concentration to the elimination rate constant.

\[ AUC_{0\rightarrow t} / AUC_{0\rightarrow \infty} \]: The ratio of \( AUC_{0\rightarrow t} \) to \( AUC_{0\rightarrow \infty} \).

\[ MRT \]: The average amount of time spent by the drug in the body before being eliminated after each treatment.

\[ C_{\text{max}} \]: 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.

\[ K_{el} \]: 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 \( 0.693/K_{el} \).

No value of \( K_{el} \), \( AUC_{0\rightarrow \infty} \), or \( T_{1/2} \) will be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.
13.0 SAFETY

13.1 Clinical Safety Measurements

Vital signs of oral temperature, sitting blood pressure and radial pulse will be measured after subject admission, prior to dosing, 4, 8, 12 hours after administration of study drug, at discharge in each period and at ambulatory sample. Vital signs to be measured prior at predose will be taken within 1.5 hours of the scheduled dosing time. Post dose vital signs will be taken within 1 hour of the scheduled times. In the event of detection of any abnormality during measurement of vital signs, the Clinical Investigator must be consulted for necessary action, which will be recorded.

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

14.0 HANDLING OF SAFETY PARAMETERS

14.1 Adverse Events

The Clinical Investigator or a Medical Officer will be available at the site of investigation until 24 hours post-dose during each period. Subjects will be monitored throughout the study period for adverse events. Subjects will be informed to bring to the notice of the nurse or the doctor any adverse event that may occur during their stay at the site of investigation. Subjects will also be specifically asked about any adverse events every 4 hours (except at 20 hours post dose) during post-dose hours of in-house stay. Treatment of any adverse events will be done by a physician, either at the site of investigation or at a nearby hospital.

All adverse events and treatment administered will be recorded in the final report. Adverse events experienced will be followed until the events have resolved or stabilized. The study may be suspended or terminated depending on the seriousness of the adverse effects.

15.0 STATISTICAL ANALYSIS

Statistical analyses will be performed on plasma metformin using the SAS system for Windows, release 9.1 or above and WinNonlin PK Software, Version 4.0 or above. The analyses will include data from subjects 1 to 18 if all these subjects complete the study. In the event of dropouts and/or withdrawal, they will not be replaced. If necessary, an unequal number of subjects per sequence will be used.
15.1 Summary Statistics

Arithmetic means, standard deviations and coefficients of variation will be calculated for the parameters listed in section 13.3. Additionally, geometric means and percentage coefficient of variation of geometric means will be calculated for AUC\textsubscript{0-t}, AUC\textsubscript{0-\textinfty} and C\textsubscript{max}.

15.2 Analysis of Variance (ANOVA)

The log-transformed pharmacokinetic parameters (C\textsubscript{max}, AUC\textsubscript{0-t} and AUC\textsubscript{0-\textinfty}) will be 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 will be used to analyze each of the parameters. The sequence effect will be tested at the 10% level of significance using the subjects nested within sequence mean square as the error term. All other main effects will be 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 will include calculation of least-squares means, the difference between the adjusted formulation means and the standard error associated with the difference. The above analyses will be done using the appropriate SAS\textregistered procedure or the WinNonlin PK Software, Version 4.0 or above.

15.3 90% Confidence Intervals and Ratio Analysis

90% confidence interval for the ratio of the test and reference product averages (least square means) will be calculated for metformin 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 and B vs R, so ratio will be in the form of test/reference. Ratio of means will be calculated using the LSM for log-transformed C\textsubscript{max}, AUC\textsubscript{0-t}, and AUC\textsubscript{0-\textinfty}. Ratio of means will be expressed as a percentage of the LSM for the reference formulations.

For metformin, the 90% confidence interval for the ratio of test and reference product for C\textsubscript{max}, AUC\textsubscript{0-t} and AUC\textsubscript{0-\textinfty} should be between 80% and 125% for the log transformed data.

16.0 DEVIATIONS

All protocol deviations will be appropriately reviewed and documented in the raw data and those, which will affect the integrity of the study, will be reported in the final report. In addition, deviations from the original pharmacokinetic and statistical evaluation plan will be justified in the final report.
17.0 ETHICAL CONSIDERATIONS

17.1 Basic Principles

This research will be 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' and the principles enunciated in the Declaration of Helsinki (Edinburgh, October 2000) with notes of Clarification on Paragraph 29 and 30 added by the WMA General Assembly, Washington 2002, and by the WMA General Assembly, Tokyo 2004 respectively.

17.2 Institutional Review Board

This protocol and the corresponding informed consent form (ICF) used to obtain informed consent of study subjects will be reviewed by the Jamia Hamdard Institutional Review Board and the study subjects will not be dosed until the Board has approved the protocol and the ICF, as submitted or with modifications in subsequent version(s). The Board is constituted and operates in accordance with the Principles and requirements described in the US Code of Federal Regulations (21 CFR Part 56).

17.3 Informed Consent

The Clinical Investigator or his designate will inform the subjects before initiation of study through an oral presentation regarding the purpose, procedures to be carried out, potential hazards and rights of the subjects. Subjects will be required to understand and sign a consent form summarizing the discussion prior to admission for the study in Period 1. A copy of the informed consent statement will be provided in the final report.

17.4 Drop-out/Withdrawal of Subjects from Study

Subjects will be informed that they are free to dropout from the study at any time without stating any reason. The investigator may withdraw a subject from the study for any of the following reasons:

(i) The subject suffers from significant inter-current illness or undergoes surgery during the course of the study.

(ii) The subject experiences adverse event, when withdrawal would be in the best interest of the subject.

(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 will be recorded and reported. Every effort will be made to obtain a complete follow-up for any withdrawn subject.
17.5 Subject Compensation

The subjects will be 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 will be final and binding on both Ranbaxy Research Laboratories and the study subjects. The compensation in this study will be Rs. 6300/- per completed subject.

17.6 Medical Treatment for Injury

In case of research related injury, first aid will be available at the Ranbaxy Clinical Pharmacology Unit and treatment of adverse reactions requiring hospitalization will be undertaken at a nearby hospital and the expenses will be borne by Ranbaxy Research Laboratories.

18.0 TERMINATION OF THE STUDY

Ranbaxy Research Laboratories reserves the right to discontinue the trial at any time. Reasons for this termination will be provided to the subjects. The Clinical Investigator reserves the right to discontinue the study for safety reasons at any time.

19.0 STUDY DOCUMENTATION

All data generated during the conduct of the study will be directly entered in the raw data recording forms as governed by the SOPs of Department of Clinical Pharmacology & Development, Ranbaxy Research Laboratories except the analytical data of clinical laboratory of the Clinical Pharmacology Unit, which will be transcribed into the study related forms and the raw data retained by the laboratory for records. The computer-generated chromatograms will also be treated as raw data. All raw data and transcribed data forms will be completed by the study personnel assisting in the study and will be checked wherever applicable for completeness and logistics by the Clinical Investigator or his designate and the Laboratory Supervisor for the bioanalytical data. The Clinical Investigator and the Laboratory Supervisor will supervise compilation of data until ready for archiving.

20.0 CONFIDENTIALITY OF DATA

The data identifying each study subject by name will be kept confidential and will be accessible to the study personnel, and if necessary, to the Jamia Hamdard Institutional Review Board and various regulatory agencies.

21.0 PUBLICATION POLICY

The investigator may publish the results of the study.
22.0 REFERENCES


2. New Product Focus, 2004, IMS Health Incorporated


### 23.0 LIST OF APPENDICES

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<th>Description</th>
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<td>Informed Consent Form</td>
</tr>
<tr>
<td>10</td>
<td>List of Changes</td>
</tr>
</tbody>
</table>
## APPENDIX 1

### SCHEMATIC REPRESENTATION OF STUDY DESIGN

<table>
<thead>
<tr>
<th>Period I, II and III (n=18)</th>
<th>Dinner</th>
<th>Vitals</th>
<th>Vitals</th>
<th>Vitals</th>
<th>Snacks</th>
<th>Vitals</th>
<th>Dinner</th>
<th>Vitals and Discharge</th>
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<tbody>
<tr>
<td></td>
<td>1500</td>
<td>2100</td>
<td>730</td>
<td>900</td>
<td>1300</td>
<td>1700</td>
<td>1800</td>
<td>2100</td>
</tr>
<tr>
<td>78 hr Admission &amp; vitals</td>
<td>12 hr</td>
<td>1.5 hr</td>
<td>0 hr</td>
<td>4 hr</td>
<td>8 hr</td>
<td>9 hr</td>
<td>12 hr</td>
<td>13 hr</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24 hr</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE: Adverse Event</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Note: Dosing and subsequent sample time will be suitably staggered.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 2
PROTOCOL SUMMARY

1 Study Number: 006_JAMHAM_07

2 Description: Single-dose bioavailability study on three formulations of metformin 500 mg ER tablets under fed conditions.

4 Products to be Evaluated:

Reference (R)
Cetapin XR 500mg sustained release tablets (containing Metformin 500mg) manufactured Aventis Pharmaceuticals, India.

Test (A)
Glycomet SR 500mg sustained release tablets (containing Metformin 500mg) manufactured by USV Pharmaceuticals Ltd, India.

Test (B)
Bigomet SR 500mg extended release tablets (containing Metformin 500mg) manufactured by Otsira Genetica Ltd, India.

5 Dose: 500 mg with 240 mL of drinking water.

6 Study Design: Open label, balanced, randomised, three-treatment, three-period, three-sequence, crossover, single-dose fed bioavailability study.

7 Number of subjects: 18

8 Dietary Status: Breakfast will be provided 30 minutes prior to dose. Lunch, snacks, and dinner at 4, 9 and 13 hours post-dose, respectively. Water restricted 1 hour pre-dose to 2 hours post-dose.

9 Sampling Schedule: Pre-dose (within 1.5 hours of dosing) and at 0.5, 1, 2, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 12, 14, 16, 20, 24 and 48 hours post-dose in each period.

10 Housing: At least 10 hours prior to dose until 24 hours post-dose.

11 Visits: 1 ambulatory visits in each period for sampling of 48 hours.

12 Blood Loss: 4-mL/sample, 66 draws, 16 mL for screening and 30 mL discarded blood, 310 mL total per subject.

13 Compensation: Rs.6300/- per completed subject.

14 Washout Period: At least 7 days.

15 Clinical Safety Measurements: Vital signs - oral temperature, sitting BP and radial pulse at admission, within 1.5 hours pre-dose, 2, 4, 8, 24 and 48 hours post-dose in each period.

16 Analytical Procedure: Metformin in plasma quantitated using chromatographic procedures developed and validated at Ranbaxy.

17 Pharmacokinetic Parameters: AUC\text{0-4}, AUC\text{0-oo}, AUC\text{0-oo}/AUC\text{0-4}, C_{\text{max}}, T_{\text{max}}, K_{\text{el}} \text{ and } T_{1/2}.

18 Statistical Analysis: Summary statistics, ANOVA, 90% Confidence Intervals and Ratio Analyses.
Inclusion Criteria

1. The subject is male and in the age range of 18-45 years. Yes / No
2. The subject is neither overweight nor underweight for his height as per the Life Insurance Corporation of India height/weight chart for non-medical cases. Yes / No
3. The subject has voluntarily given written informed consent to participate in this study. Yes / No
4. The subject is of normal health as determined by medical history and physical examination of the subjects performed within 14 days prior to the commencement of the study.

Exclusion Criteria

1. The subject has history of allergy to metformin or other related antidiabetic biguanide preparations. Yes / No
2. Past history of Headache, Dizziness, recurrent Upper respiratory infections and Diarrhoea in the preceding week. Yes / No
3. The subject has evidence of organ dysfunction or any clinically significant deviation from the normal, in physical or clinical determinations. Yes / No
4. Investigations with blood samples of the subject shows presence of disease markers of HIV 1 or 2, Hepatitis B or C viruses or syphilis infection. Yes / No
5. Investigations with blood samples of the subject shows the presence of values which are significantly different from normal reference range (as defined in Appendix 5) and/or judged clinically significant for haemoglobin, total white blood cells count, differential WBC count or platelet count. Yes / No
6. Positive for urinary screen testing of drugs of abuse (opiates or cannabinoids). Yes / No
7. Investigations with blood samples of the subject shows the presence of values which are significantly different from normal reference range (as defined in Appendix 5) and/or judged clinically significant for serum creatinine, blood urea, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase, serum bilirubin, plasma glucose or serum cholesterol. Yes / No
8. Investigations with urine samples of the subject shows clinically abnormal chemical and microscopic examination of urine defined as presence of RBC, WBC (>4/HPF), glucose (positive) or protein (positive). Yes / No
9. Clinically abnormal ECG or Chest X-ray. Yes / No
10. The subject has history of serious gastrointestinal, hepatic, renal, pulmonary, cardiovascular, neurological or haematological disease, diabetes or glaucoma. Yes / No
11. The subject has history of any psychiatric illness which may impair the ability to provide written informed consent. Yes / No
12. The subject is a regular smoker who smokes more than 10 cigarettes daily or has difficulty abstaining from smoking for the duration of each study period. Yes / No
APPENDIX 3 (Continued)
CRITERIA CHECK

13. The subject has 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 has difficulty in abstaining for the duration of each study period.  Yes / No

14. The subject has used any enzyme modifying drugs within 30 days prior to Day 1 of this study.  Yes / No

15. The subject has participated in any clinical trial within 12 weeks preceding Day 1 of this study.  Yes / No

16. Subjects who, through completion of this study, would have donated and/or lost more than 350 mL of blood in the past 3 months.  Yes / No

Based on the above, the subject is SUITABLE / UNSUITABLE for the study.

CLINICAL INVESTIGATOR  DATE
## APPENDIX 4

### ASSESSMENT DURING THE STUDY

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Days -21 to 1 Screening Period</th>
<th>Reference R</th>
<th>Test T</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Safety</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demographics</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vital Signs</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Allergies and Medication History</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical History</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical Examination</td>
<td>X</td>
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<tr>
<td>Clinical Laboratory Tests</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECG</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest X-ray</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Criteria Check</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Adverse Events</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Written Informed Consent</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><strong>Pharmacokinetics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-dose blood sampling</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Drug Administration</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Blood sampling for metformin determination</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
## APPENDIX 5

**DEFINITION OF ABNORMAL TEST VALUES WHEN BASELINE VALUE WAS NORMAL OR MISSING**

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Clinically relevant direction*</th>
<th>Significant ≥</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (Hemoglobin)</td>
<td>Decrease</td>
<td>20 %</td>
</tr>
<tr>
<td>Platelets</td>
<td>Decrease</td>
<td>20 %</td>
</tr>
<tr>
<td>Total Leukocyte count</td>
<td>Either</td>
<td>20 %</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Either</td>
<td>25 %</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Either</td>
<td>25 %</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Increase</td>
<td>50 %</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Increase</td>
<td>50 %</td>
</tr>
<tr>
<td>Basophils</td>
<td>Increase</td>
<td>50 %</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>Increase</td>
<td>20 %</td>
</tr>
<tr>
<td>ALT</td>
<td>Increase</td>
<td>20 %</td>
</tr>
<tr>
<td>AST</td>
<td>Increase</td>
<td>20 %</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Increase</td>
<td>20 %</td>
</tr>
<tr>
<td>BUN</td>
<td>Increase</td>
<td>20 %</td>
</tr>
<tr>
<td>Creatinine **</td>
<td>Not Applicable</td>
<td>Not Applicable</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>Increase</td>
<td>20 %</td>
</tr>
<tr>
<td>Glucose, fasting ***</td>
<td>Not Applicable</td>
<td>Not Applicable</td>
</tr>
<tr>
<td>Glucose, random ***</td>
<td>Not Applicable</td>
<td>Not Applicable</td>
</tr>
</tbody>
</table>

(*) Only clinically relevant direction of abnormality considered and percent limits applied to the limit on normal range closest to that abnormality.

(**) Subjects with values more than the upper limit of the reference range will not be included in the study.

(***) Subjects with values outside the reference range will not be included in the study.
## APPENDIX 6

LIST OF LABORATORY REFERENCE RANGE VALUES – CLINICAL LABORATORY, RANBAXY CPU, MAJEEDIA HOSPITAL

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>UNIT</th>
<th>REF. RANGE</th>
<th>PARAMETER</th>
<th>REF. RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HAEMATOLOGY</strong></td>
<td></td>
<td></td>
<td><strong>URINE ANALYSIS</strong></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>g/dL</td>
<td>14 – 18</td>
<td>Total WBC Count</td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td></td>
<td></td>
<td>per mm³</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4000-10000</td>
<td>Colour</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pale straw/straw</td>
<td></td>
</tr>
<tr>
<td>Differential WBC Count</td>
<td></td>
<td></td>
<td>Appearance</td>
<td>Clear</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>%</td>
<td>40-75</td>
<td>pH</td>
<td>5.0 -6.5</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>%</td>
<td>20-45</td>
<td>Sp. Gr.</td>
<td>1.010-1.025</td>
</tr>
<tr>
<td>Monocytes</td>
<td>%</td>
<td>0-8</td>
<td>Glucose</td>
<td>Nil</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>%</td>
<td>0-6</td>
<td>Protein</td>
<td>Nil</td>
</tr>
<tr>
<td>Basophils</td>
<td>%</td>
<td>0-1</td>
<td>Microscopic</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>per mm³</td>
<td>150000-450000</td>
<td>RBC</td>
<td>Nil/HPF</td>
</tr>
<tr>
<td><strong>BIOCHEMISTRY</strong></td>
<td></td>
<td></td>
<td><strong>W.B.C.</strong></td>
<td></td>
</tr>
<tr>
<td>Glucose (Fasting)</td>
<td>mg/dL</td>
<td>70-110</td>
<td>E. Cells</td>
<td>0-3/HPF</td>
</tr>
<tr>
<td>Glucose (Random)</td>
<td>mg/dL</td>
<td>70-140</td>
<td>Crystals</td>
<td>Absent</td>
</tr>
<tr>
<td>Sr. Total Bilirubin</td>
<td>mg/dL</td>
<td>&lt; 1.00</td>
<td>Casts</td>
<td>Absent</td>
</tr>
<tr>
<td>Sr. AST</td>
<td>IU/L</td>
<td>15-37</td>
<td>Others</td>
<td>Absent</td>
</tr>
<tr>
<td>Sr. ALT</td>
<td>IU/L</td>
<td>30-65</td>
<td><strong>SEROLOGY</strong></td>
<td></td>
</tr>
<tr>
<td>Sr. Alk. Phosphatase</td>
<td>IU/L</td>
<td>50-136</td>
<td>HBs Ag</td>
<td>Non-Reactive</td>
</tr>
<tr>
<td>Sr. Creatinine</td>
<td>mg/dL</td>
<td>0.8 – 1.3</td>
<td>HIV 1&amp;2</td>
<td>Non-Reactive</td>
</tr>
<tr>
<td>- Male</td>
<td></td>
<td></td>
<td>HCV</td>
<td>Non-Reactive</td>
</tr>
<tr>
<td>Blood Urea Nitrogen</td>
<td>mg/dL</td>
<td>7-18</td>
<td>VDRL</td>
<td>Non-reactive</td>
</tr>
<tr>
<td>Sr. Cholesterol</td>
<td>mg/dL</td>
<td>&lt; 200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### APPENDIX 7

**LIST OF LABORATORY REFERENCE RANGE VALUES — DR LAL PATHLABS**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>UNIT</th>
<th>REF. RANGE</th>
<th>PARAMETER</th>
<th>REF. RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HAEMATOLOGY</strong></td>
<td></td>
<td></td>
<td><strong>URINE ANALYSIS</strong></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>g/dL</td>
<td>14 – 17.4</td>
<td>Routine</td>
<td></td>
</tr>
<tr>
<td>Total WBC Count</td>
<td>thou/ mm³</td>
<td>4.40 - 11.30</td>
<td>Colour</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>Differential WBC Count</td>
<td></td>
<td></td>
<td>Appearance</td>
<td>Clear</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>%</td>
<td>45.50-74</td>
<td>pH</td>
<td>5.0 -6.0</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>%</td>
<td>22.30-50</td>
<td>Sp. Gr.</td>
<td>1.015-1.025</td>
</tr>
<tr>
<td>Monocytes</td>
<td>%</td>
<td>0.70-10</td>
<td>Glucose</td>
<td>Nil</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>%</td>
<td>1-6</td>
<td>Protein</td>
<td>Nil</td>
</tr>
<tr>
<td>Basophils</td>
<td>%</td>
<td>&lt; 2.00</td>
<td>Microscopic</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>thou/ mm³</td>
<td>150,00 - 450.00</td>
<td>W.B.C.</td>
<td>&lt; 10 WBC/μL</td>
</tr>
<tr>
<td><strong>BIOCHEMISTRY</strong></td>
<td></td>
<td></td>
<td>E. Cells</td>
<td>Nil</td>
</tr>
<tr>
<td>Glucose (Fasting)</td>
<td>mg/dl</td>
<td>70 - 110</td>
<td>Crystals</td>
<td>Nil</td>
</tr>
<tr>
<td>Glucose (Random)</td>
<td>mg/dl</td>
<td>70 - 140</td>
<td>Casts</td>
<td>Nil</td>
</tr>
<tr>
<td>Sr. Total Bilirubin</td>
<td>mg/dl</td>
<td>&lt; 1.00</td>
<td>Others</td>
<td>Nil</td>
</tr>
<tr>
<td>Sr. AST</td>
<td>U/L</td>
<td>&lt; 38</td>
<td>SEROLOGY</td>
<td></td>
</tr>
<tr>
<td>Sr. ALT</td>
<td>U/L</td>
<td>&lt; 41</td>
<td>HBs Ag</td>
<td>Non-Reactive</td>
</tr>
<tr>
<td>Sr. Alk. Phosphatase</td>
<td>U/L</td>
<td>95-270</td>
<td>HIV 1 &amp;2</td>
<td>Non-Reactive</td>
</tr>
<tr>
<td>Sr. Creatinine</td>
<td>mg/dL</td>
<td>0.5 – 1.20</td>
<td>HCV</td>
<td>Non-Reactive</td>
</tr>
<tr>
<td>Blood Urea Nitrogen</td>
<td>mg/dL</td>
<td>7-20</td>
<td>VDRL</td>
<td>Non-reactive</td>
</tr>
<tr>
<td>Sr. Cholesterol</td>
<td>mg/dL</td>
<td>144-200</td>
<td></td>
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</table>
APPENDIX 10

List of Changes

1.0 Headers and text on all pages of Protocol and ICF

006_JAMHAM_07, Version 1; 01 August 2007

Changed to

006_JAMHAM_07, Version 2; 24 August 2007

2.0 Page 1 of Protocol and ICF

Supersedes: Not Applicable

Changed to

Supersedes: Version 1; 01 August 2007

4.0 Page 13 of 23 of protocol (9.3 Exclusion Criteria)

The following exclusion criteria’s have been included:

“Past history of Headache, Dizziness, recurrent Upper respiratory infections and Diarrhoea in the preceding week”

5.0 Appendices 3 and 9 have been modified accordingly, and Appendix 10 has been included.

Reason for change of Version

As suggested by Jamia Hamdard IRB at its meeting held on 22.08.07.
INFORMED CONSENT FORM

PROTOCOL NO: 006_JAMHAM_07
SUPERSEDES: Version 1; 01 August 2007

TITLE: A randomised, three-treatment, three-period, three-sequence, single-dose, crossover pilot bioavailability study on three metformin formulations in healthy, adult, male, human subjects under fed conditions

BEFORE AGREEING TO PARTICIPATE IN THE PRESENT STUDY IT IS IMPORTANT THAT YOU READ AND UNDERSTAND THE FOLLOWING INFORMATION

AN ORAL PRESENTATION OF THIS DOCUMENT WILL BE HELD. IF YOU HAVE ANY QUESTIONS/CLARIFICATIONS PLEASE DISCUSS DURING THE PRESENTATION.

YOU WILL BE PROVIDED TWO COPIES OF THIS FORM. PLEASE SIGN THE ORIGINAL COPY AND SUBMIT TO US FOR OUR RECORDS. PLEASE RETAIN THE DUPLICATE COPY FOR YOUR REFERENCE AND RECORDS.

DURING YOUR PARTICIPATION IN THE CLINICAL STUDY, YOU WILL ACT AS AN INDEPENDENT CONTRACTOR, AND NOT AS AN AGENT, PARTNER OR EMPLOYEE OF RANBAXY LABORATORIES LIMITED.

INTRODUCTION

This statement describes the purpose, procedures, benefits, risks/discomforts of the study, alternative procedures that are available to you and your right to withdraw from the study at any time.

PURPOSE

This study involves research to evaluate the amount of drug in the blood after administration of a new formulation of metformin. The drug is effective in reducing elevated blood glucose concentrations in patients with diabetes, but it does not increase insulin secretion. There is no blood-glucose-lowering effect in non-diabetic subjects. The usual starting dose of metformin is 500 mg once daily given with meals. The maximum dose recommended should not exceed a daily dose of 2550 mg.

ELIGIBILITY

A total of 18 healthy male volunteers, aged 18 to 45 years, neither overweight nor underweight will be recruited for the study.

You cannot participate in this study if:

Signature ______________________
you have a history of allergy to metformin and other related antidiabetic biguanide preparations.

Past history of Headache, Dizziness, recurrent Upper respiratory infections and Diarrhoea in the preceeding week.

you have any evidence of any significant abnormality in physical or clinical examination.

you have a history of serious gastrointestinal, liver, kidney, heart, lung, neurological or blood diseases, diabetes or glaucoma.

you have presence of disease markers of HIV 1 and 2, Hepatitis B and C viruses, syphilis infection, values which are clinically significantly different from normal reference ranges for haemoglobin, total white blood cells count, differential WBC count and platelet count.

you have presence of +ve for urinary screen testing of drugs of abuse (opiates and cannabinoids), values which are significantly different from normal reference ranges for serum creatinine, blood urea, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase, serum bilirubin, plasma glucose and serum cholesterol.

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

you have a history of any psychiatric illness which may impair your ability to provide written consent.

you are a regular smoker of more than 10 cigarettes a day or you have difficulty abstaining from smoking for the duration of each study period.

you have any 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 one glass of wine or one measure of spirit) or have difficulty in abstaining for the duration of each study period.

you have taken any enzyme modifying drugs within 30 days prior to Day 1 of this study.

you have participated in any clinical trial within 6 weeks preceding Day 1 of this study.

you have a haemoglobin concentration of less than 7 % of lower limit of reference range e.g. 13 gm % for reference range of 14-18 gm at screening.

you have difficult venous access in left and right forearms to allow collection of 66 blood samples via venous cannula in the 3 periods.

you have problem(s) in complying with the study protocol.

STUDY PROCEDURES

In this study (cross-over design), you will receive one of the following formulations orally in the first period. Subsequently, you will receive the other formulations in the subsequent periods. The order in which you will receive each treatment will be randomly determined:

Reference (R)
Cetapin XR 500mg sustained release tablets (containing Metformin 500mg) manufactured Aventis Pharmaceuticals, India.

Signature ____________________
Test (A)
Glucomet SR 500mg extended release tablets (containing Metformin 500mg)
manufactured by USV Pharmaceuticals Ltd, India.

Test (B)
Bigomet XR 500mg extended release tablets (containing Metformin 500mg)
manufactured by Otsира Genetica Ltd, India

You must not have taken any medications including: aspirin, non steroidal anti-
inflammatories (Brufen®, Voveran®), acetaminophen (Crocin®) or any other pain
medication, antihistamines, anti-acidity medicines (Zantac®, Pepfiz®), cough syrup
for 14 days before and throughout the study. Also you should not have consumed
certain drugs prescribed by doctors, like antiulcer medicines (Tagamet®, Lomac®),
antitubercular medications (INH, rifampicin) and antiinfective medications like
erthromycin, ketoconazole for 30 days before and throughout the study. For 48
hours before and during the stay at CPU, you must not consume any alcohol or any
products that contain alcohol (beverages, marinades, medicines, etc), or caffeine or
any products that contain caffeine (chocolate, coffee, cola, medications, tea).

The study consists of 3 in-house stays of approximately 42 hours each at the
Ranbaxy Clinical Pharmacology Unit (CPU), Majeedia Hospital (2nd floor), Hamdard
Nagar, New Delhi 110 062. Each phase of the study will be separated by a period of
at least 7 days to ensure that the study drug(s) taken during the initial phase is (are)
no longer in your body when the next phase begins (see study schedule for dates).

On the evening before receiving the study drug(s), after your admission, you will
receive a standard evening meal. This will be approximately 12 hours before you
receive the study drug (dosing). The study drug(s) will be taken the next morning
with approximately 240 mL of water. You will be served with a standard high-fat
breakfast 20 minutes prior to dosing in all periods. The breakfast is to be consumed
within 20 minutes. Standard meals - lunch, snacks and dinner will be provided 4, 9
and 13 hours, respectively, after drug administration (for detailed meal plan see study
schedule). During your stays at the centre, you must consume all food and drink
provided and no other food or drink will be permitted. After receiving the study drug,
you will be required to sit upright or remain ambulatory for 2 hours, thereafter you can
resume only normal activities while avoiding vigorous exercise. However, should
medical events occur at any time during housing you will be placed in an appropriate
position or will be permitted to lie down on your right side. Drinking water will not be
allowed from 1 hour before dosing until 2 hours post-dose.

Blood will be collected through a disposable needle and tube which will be inserted
into a blood vessel and kept fixed at that site. The needle will not be allowed to get
blocked by introduction of a very dilute solution of heparin (which is a normal body
constituent). One millilitre of heparinised blood will be discarded before the sample is
collected. Alternatively, blood samples can be collected directly with a sterile
disposable needle and syringe every time a sample is to be collected. However, in
this study, direct collection of blood sample will not be done unless considered
essential by the Clinical Pharmacologist.

Signature __________________
A total of Sixty six 4-mL blood samples will be collected during the course of the study. The blood samples will be collected Pre-dose (within 1.5 hours of dosing) and at 0.5, 1, 2, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 12, 14, 16, 20, 24 and 48 hours post-dose in each period.

- 4 mL per sample
- 66 collections in 3 periods
- total of 310 mL in 3 periods including 16 mL for screening, 30 mL for safety as discarded blood prior to venous cannula collection.

Laboratory tests (haemoglobin, serum creatinine, blood urea nitrogen, serum AST, serum ALT, serum ALP and serum bilirubin) will be repeated at pre-dose in each study period.

Pain, swelling and/or numbness of the arm may occasionally result from the multiple blood collections during this study. This procedure may also occasionally trigger vagal reactions (light-headedness, fainting). These reactions are usually benign, of short duration and limited to a feeling of weakness, accompanied by sweating and decrease in heart beats. Vital signs of oral temperature, sitting blood pressure and pulse rate will be measured and recorded during subject admission, before dosing, 2, 6, 12, 24 and 48 hours post-dose in each period. Brief clinical examination of the subject will be conducted by a qualified medical designate on duty after subject admission, prior to dosing of study drug and before discharge.

You can leave the study site approximately 24 hours after receiving the study drug(s) in each period.

SIDE EFFECTS OF STUDY DRUG(S)

As with any drug, the study drug(s) may cause side effects. The most commonly reported adverse effects of metformin are: diarrhoea, nausea, vomiting, abdominal bloating, flatulence and anorexia.

If you feel unwell or experience any uneasiness, please bring to the notice of the Medical Officer/Nurse/staff on duty immediately.

BENEFITS

Since you do not require treatment with the study drug(s), you will receive no medical benefit from this study, other than the benefit of a free medical check-up and the satisfaction of serving the interests of ailing human beings.

NEW FINDINGS

Any new and important information which may be discovered during the study which may influence your willingness to continue in the study will be made available to you as soon as possible.

ALTERNATIVE TREATMENT

Signature ___________________
Since this study is for research only, the alternative would be not to participate.

A compensation package of Rs. 6300/- (rupees seven thousand one hundred only) will be paid to you at the completion of the study. This is to compensate you for discomfort and inconvenience. If you refuse to have your baggage searched at admission or you are uncooperative during conduct of the study procedures you will be discharged without any payment.

INSURANCE POLICY

You are insured under the insurance policy and you will be compensated in case of a trial related injury.

MAINTENANCE OF DISCIPLINE

You are expected to observe various rules of the CPU and maintain discipline during your stay in the unit. In case of any misconduct in the CPU or in the JNA Medical campus, you will be (a) dropped from the study without any payment and (b) debarred from participating in all future studies.

DETERMINATION OF FINANCIAL COMPENSATION DUE IN CASES NOT COMPLETING THE STUDY

1. Withdrawn from the study by the Clinical Investigator on objective medical grounds to safeguard your health
   Full payment on completion of study/follow-up visits

2. Dropped-out of the study, on your own accord, after initiation of medication
   50% of proportionate payment due

3. Dropped from the study on compassionate grounds, with the permission of Clinical Investigator
   Proportionate payment due in full

4. Dropped from the study by the Clinical Investigator after signing the consent form but before receiving any medication due to your failure to comply with the requirements of the study
   No payment

5. Dropped from the study by the Clinical Investigator because of your wilful withholding of information regarding your past or present medical illness(es) relevant to the study
   No payment

6. Non-compliance with the prescribed time-schedule for the follow-up visit in follow-up visits (where applicable)
   50% of the payment due for that visit

CONFIDENTIALITY

Signature ____________________
Records of your participation in this study will be confidential so far as permitted by law. However, the confidential data, which identifies you by name, will be available to the study personnel, Quality Assurance Auditor during audits and to the Jamia Hamdard Institutional Review Board (IRB) & various regulatory agencies, as it becomes necessary. Any publication of the data will not identify you by name. By signing this consent form, you authorize the Study Director to release your study related medical records, to the regulatory authorities and the IRB. Clinical Investigator's representatives/ designates shall act as data custodian for this study till it is sent for archiving.

MEDICAL TREATMENT FOR INJURY

In case of research related injury, first aid will be available at the Ranbaxy Clinical Pharmacology Unit and treatment of adverse reactions requiring hospitalisation will be undertaken at a nearby hospital and the expenses will be borne by Ranbaxy Research Laboratories.

CONTACTS FOR ADDITIONAL OR EMERGENCY INFORMATION

At any time before, during or after the study, you can obtain further information about this drug research. You may also consult your personal doctor at any time during the study. If you wish to contact someone concerning possible risks related to the study or have additional questions about the study, please contact the Clinical Pharmacologist directly at the following location:

Ranbaxy Clinical Pharmacology Unit
Majeedia Hospital 2nd Floor
Hamdard Nagar, New Delhi 110 062
Telephone: 2995-6721

In case of medical emergencies during the study, or if you have any urgent questions concerning discomfort or injury associated with the study, please telephone the Clinical Investigator at 2995-8529 (office), 9810771534 (residence).

If you have questions regarding your rights as a research subject, you may call Prof. P.L. Sharma, Chairman, Jamia Hamdard Institutional Review Board, Convention Center First Floor, Jamia Hamdard, Hamdard Nagar, New Delhi 110 062, Telephone 26059688, Extn 440.

VOLUNTARY NATURE OF PARTICIPATION

You are free to participate or refuse to volunteer for this study. Your refusal to participate or withdrawal from the study will involve no penalty or loss of medical benefits to which you would otherwise be entitled and will not affect your selection for any future studies. You are advised to contact the Study Director or the Clinical Pharmacologist if you decide to withdraw from the study. They will explain the best way for you to withdraw from the research study. The Clinical Pharmacologist can stop your participation in the study at any time without your consent if it appears to be medically harmful to you. You may also be withdrawn from the study at any time.

Signature ____________________
without your consent if you fail to follow directions for participating in the study, or if it is discovered that you have withheld some vital information pertaining to your past medical history or you do not meet the study requirements or if the study is cancelled for administrative reasons.

Signature __________________________
STUDY SCHEDULE

The following is the schedule for the study:

**Period I, II and III**

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<th>Date</th>
<th>Time</th>
<th>Activity</th>
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<td>Day 1</td>
<td>3.00 p.m.</td>
<td>Admission of volunteers to Ranbaxy Clinical Pharmacology Unit</td>
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<td>9.00 p.m.</td>
<td>Dinner</td>
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<td>Bed-time</td>
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<td>Day 2</td>
<td>8.00 a.m.</td>
<td>Pre-dose blood sampling with cannulation &amp; vitals</td>
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<td>8.40 a.m.</td>
<td>Breakfast</td>
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<td>Drug administration followed by post-dose blood sampling at 0.5, 1, 2, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 12, 14, 16, 20, 24 and 48 hours post-dose in each period.</td>
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<td>Vitals</td>
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<td>10.00 p.m.</td>
<td>Dinner</td>
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<td>Day 3</td>
<td>9.00 a.m.</td>
<td>24 hour sampling, vital and discharge</td>
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<td>Day 4</td>
<td>9:00 a.m.</td>
<td>48 hr sampling and vitals</td>
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DECLARATION

Title of the study: An open label, balanced, randomized, three-treatment, three-period, three-sequence, single-dose, crossover, bioavailability study comparing three different marketed sustained release Metformin extended release tablets in healthy, adult, human male.

Protocol Number: 006_JAMHAM_07
Status of Study Drug: Available for clinical use

I hereby declare that:

- My participation in this study is voluntary.
- I have been provided a copy of informed consent.
- This study provides me no medical benefits.
- I have the right to be provided with answers to questions arising during the course of the study.
- I can withdraw from the study at any time without prejudice to future medical care or selection for future studies.
- I can be dropped from the study at any time if I violate the study protocols or to protect my health.
- My date of birth is ___________ and I am more than 18 years but less than 45 years of age.
- My reference number with respect to volunteer enrolment of Ranbaxy Research Laboratories is ___________.
- I currently require no medical treatment or care.
- I have not bled more than 350 mL of blood in the past 3 months including the blood that I will be losing during this study.
- I have withheld no information regarding my past medical history and current drug intake.
- I have read the consent form and any questions I had about the study, possible side effects or the consent form, have been answered to my satisfaction.
- I voluntarily give my consent for my personal data related to any information relating to me, as I have provided in the enrollment form, or as it is generated during screening and study procedures, including identification number, or factors specific to my physical, physiological, mental, economic, cultural or social identity, to be processed as required for the study requirements. I also voluntarily give my consent for the processing of data.

Signature ____________________
I am aware that my biological samples shall be anonymized or destroyed as per the requirements of the procedures of the Study.

It is my right to obtain information at reasonable intervals and without excessive delay regarding whether or not data relating to me are being processed.

It is my right that, unless required by Law, or while fulfilling a contract, with suitable measures to safeguard my legitimate interests: "No automated processing of my personal data shall be done which makes me subject to a automated decision, produces legal effects concerning me or significantly affects me."
"No automated processing of my personal data shall be done to evaluate certain personal aspects relating to me, such as my performance at work, creditworthiness, reliability, conduct, etc."

During the past 12 weeks I have not participated in any experimental studies conducted here or elsewhere.

I will have to maintain discipline during my stay at the Jamia Hamdard campus.

If I have any further questions regarding this research study or in the event of research related injury, I may contact Dr. Monika Tandon, Clinical Investigator (Telephone number 2995-8529). I may contact Dr. P.L. Sharma, Chairman, Jamia Hamdard Institutional Review Board (Telephone number 2605-9688. Extn. 440), if I have any questions regarding my rights as a volunteer.

My signature confirms that consent is based on information provided and that I had freely chosen to participate without prejudice.
Appendix III: Randomization schedule
Randomisation Code (seed=16112007) N= 18

Ref. R = Cetapin XR 500mg sustained release tablets (containing Metformin 500mg)
Batch No. C17017, Expiry Date: Nov 2009, Mfd by Aventis Pharmaceuticals, India

Test A = Glycomet SR 500mg extended release tablets (containing Metformin 500mg)
Batch No.: 28000650, Expiry Date: December 2008, Mfd. by USV Pharmaceuticals Ltd, India

Test B = Bigomet XR 500mg extended release tablets (containing Metformin 500mg)
Batch No.: 2860307, Expiry Date: March 2009, Mfd. by Otsira Genetica Ltd, India

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Prepared By:
(Vikesh K. Shrivastav)
(Biostatistician)
Appendix IV: Representative Chromatograms of Subjects, CC and QC samples
**Results Path:** D:\Analyst

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Sample Solution

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Operator: Anejad Hadi Khan Shervani

Project: E06_JAHAN_07

Workstation: OAHICP0023