Bioequivalence Studies

2.1. Title Page

2.1.1. Study Code: PK-07-108

2.1.2. Study title:

A Randomised, Open Label, Two-Period, Two-Treatment, Two-Sequence, Crossover, Single Dose Bioequivalence Study of Carvedilol 25mg Tablets (Test) [Torrent Pharmaceuticals Ltd., India] Versus Carvedilol 25mg Tablets (Coreg®) (Reference) [Produtos Roche Quimicos e Farmaceuticos S.A., Brazil] In Healthy Human Subjects Under Fasted State

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Village Bhat,
District Gandhinagar,
Gujarat, India

2.1.5. Date: May 08, 2008

Study code: PK-07-108

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2.2 FOLIO OF SIGNATURE

2.2. FOLIO OF SIGNATURES

2.2.1. Study Code: PK-07-108

2.2.2. Study Title: A Randomised, Open Label, Two-Period, Two-Treatment, Two-Sequence, Crossover, Single Dose Bioequivalence Study of Carvedilol 25mg Tablets (Test) [Torrent Pharmaceuticals Ltd., India] Versus Carvedilol 25mg Tablets (Coreg®) (Reference) [Produtos Roche Quimicos e Farmaceuticos S.A., Brazil] In Healthy Human Subjects Under Fasted State

2.2.3. Study Site: Bio Evaluation Centre,
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Gujarat, India

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30 / 06 / 08
Date (dd/mm/yy)

2.3 SUMMARY

2.3. SUMMARY

2.3.1. Study Title: Study Title: A Randomised, Open Label, Two-Period, Two-Treatment, Two-Sequence, Crossover, Single Dose Bioequivalence Study of Carvedilol 25mg Tablets (Test) [Torrent Pharmaceuticals Ltd., India] Versus Carvedilol 25mg Tablets (Coreg®) (Reference) [Produtos Roche Quimicos e Farmaceuticos S.A., Brazil] In Healthy Human Subjects Under Fasted State

2.3.2. Sponsor: Torrent Pharmaceuticals Limited,
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2.3.5. Clinical Laboratory: **Hematology Biochemistry and Urine**
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Serology and Drugs of Abuse
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2.3.6. Objective

The Primary objective of the study was to assess the bioequivalence of Carvedilol 25mg tablets (Test formulation; Torrent Pharmaceuticals Ltd., India) versus Coreg® 25mg tablet (Reference formulation; Produtos Roche Quimicos e Farmaceuticos S.A., Brazil) after a single dose administration in healthy human volunteers under fasting condition.

The Secondary objective of the present study was to investigate the safety of the formulation on the basis of clinical and laboratory examinations at the beginning and at the end of the study and registration of adverse events and/or adverse drug reactions.

2.3.7. Design

The study was conducted as an Open-Label, Randomised, Two-Period, Two-Treatment, Two-Sequence, Crossover, Single Dose Study under fasting condition, with a clinical stay of 32 hours after dosing. A wash-out period of 7 days was kept between the two periods.

As per the randomization schedule, a single oral dose of carvedilol 25mg tablet of the test (A) or Coreg® 25mg tablet of the reference (B) formulation was administered under fasting conditions in each study period, which was followed by blood samplings for pharmacokinetic analysis. The same procedure was repeated after 7 days (second period) which was the planned washout period required for the study.

2.3.8. Volunteers

A total of 26 healthy adult male volunteers were completed the study. As per the protocol, samples from 26 volunteers were sent to bioanalytical laboratory for analysis.

2.3.9. Drugs

2.3.9.1. Test

Test Product: (A)	
Generic Name	Carvedilol 25mg
Pharmaceutical Form	Tablet
Batch No.	B9717002
Manufacturing Date	February 2007
Expiry Date	January 2009
Manufactured By	Torrent Pharmaceuticals Ltd., India

2.3.9.2. Reference

Reference Product: (B)	
Generic Name	Carvedilol 25 mg
Trade Name	Coreg®
Pharmaceutical Form	Tablet
Batch No.	RJ0382
Manufacturing Date	--
Expiry Date	July 2009
Manufactured By	Produtos Roche Quimicos e Farmaceuticos S.A., Brazil

2.3.10. Dosage

A single oral dose of either test (Carvedilol 25mg of Torrent Pharmaceuticals Ltd., India) or reference (Coreg® 25mg of Produtos Roche Quimicos e Farmaceuticos S.A., Brazil) as per the randomization were administered with 200 ml water under fasting conditions.

2.3.11. Confinement of the volunteers

The volunteers enrolled in the study were housed at the Pharmacokinetic Unit of BE Center, at least 12 hours (except Enrollment no. C-23) confined before dosing to 32 hours post dose.

2.3.12. Administration of Drugs

Volunteers were dosed in two periods. In Period I, 26 Volunteers were dosed in the morning of January 24, 2008 and in Period II, 26 Volunteers were dosed in the morning of January 31, 2008, between 08:00 and 08:26 hours at two dosing stations respectively (Station 01: volunteer numbers 01-14) and (Station 02: volunteer numbers 15-26).

The dosing was done at a gap of 2 minutes between each volunteer at each dosing station. Volunteers were administered the test or reference medication (as per the randomization scheme) as a single oral dose of 1 tablet containing carvedilol 25 mg with 200 ml water under fasting conditions.

2.3.13. Washout Period

The washout period was 7 days between two periods.

2.3.14. Fasting and feeding schedule

During the residential stay in the Pharmacokinetic Unit, food intake was standardized and identical for all volunteers in both the periods. Study drugs were administered under identical conditions to all volunteers in both the periods.

Standard and controlled meals with respect to quantity, as per pre planned menu were served and finished between 20:00 to 21:00 hours on 23 January, 2008 in period I and between 20:05 to 20:46 hours on 30 January, 2008 in period II subsequent to check-in. After supervised overnight fasting of at least 10 hours, they received study drug with 200ml of water according to the randomization schedule. All the volunteers received lunch after 4 hours, snacks after 8 hours, dinner after 12 hours, breakfast after 24 hours, lunch after 28 hours and snacks after 32 hours post dose in both periods for entire duration of stay in the facility. The menu served was identical in both periods.

Volunteers were restricted for water intake 1 hour Predose and 2 hours post dose except water taken with dosing in both the periods. They were not allowed to smoke or consume tobacco in any form for at least 48 hours before dosing and during study. They were prohibited from smoking or consuming tobacco during their entire stay in Pharmacokinetic Unit, Bio Evaluation Centre. The use of xanthine containing beverages (tea, coffee, cola drinks), grapefruit juice and foods (chocolates) were prohibited for 48 hours before dosing and throughout their stay in Pharmacokinetic Unit, Bio Evaluation Center. Volunteers were abstained from alcohol for 48 hours prior to dosing and throughout the conduct of the study. They were restricted from taking any medication (including over the counter products), throughout the study, unless authorized by the Clinical Investigator.

2.3.15. Chronogram for Collection of Samples

Twenty one venous blood samples were collected from each volunteer during each period. In the morning of dosing day after vitals measurement, a pre-dose blood sample (5ml) was taken 30 minutes before dosing. The other venous blood samples (5ml each) were withdrawn at 0.17, 0.33, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 6.00, 8.00, 12.00, 18.00, 24.00, 32.00 and 48.00 hours post dose in each period. About 3 ml of blood was collected for post-study safety analysis from each of the volunteers at the time of last sample of period II.

Blood sampling up to ± 2 minutes of the planned time of in-house sampling and ± 1 hour of ambulatory sample were considered as an acceptable deviation. Beyond that, time deviation was taken into consideration for further pharmacokinetic parameters, except for pre dose samples, which always be reported as zero (0) hour. All deviations related to blood samples were recorded in CRFs. During collection of blood sample at each time point the mid-point of the minute was considered to calculate the nearest minute, which was recorded on the appropriate CRF.

2.3.16. Procedures for Sample Handling

After collection of blood samples from all the volunteers at each time point, tubes containing blood samples were kept in box containing coolant and transferred for centrifugation. The centrifugation was carried out at 2000 RPM for 10 minutes at 20°C. The plasma samples then separated in pre-labeled 5ml polypropylene tubes, were subsequently stored at -70°C ± 10°C until withdrawn for analysis. Timings of samples received, separation and storage were documented in the form "Centrifugation and Storage of Samples".

2.3.17. Dropout/ Withdrawal of Volunteers in each period

No dropout or withdrawal during study; all 26 volunteers completed both the periods of study.

2.3.18. Bio-analytical Method for estimation of Carvedilol in human plasma

2.3.18.1. Bio-analytical technique

LC-MS/MS technique was followed.

The summary of the chromatographic conditions were mentioned in section 17.0 of SOTP/Protocol No. BA/08/003.

2.3.18.2. Detection

Mass spectrometer (API 4000) detection was used.

2.3.18.3. Internal Standard

Olanzapine was used as a internal standard for carvedilol.

2.3.18.4. Biological Source

Heparinised blank human plasma matrix was procured from Pharmacokinetic Unit for preparation of the plasma calibration standards and quality control samples. Study samples were received from Pharmacokinetic unit of Bio Evaluation Centre.

2.3.18.5. Anticoagulant

Heparin was used as an Anticoagulant for the study samples received from Pharmacokinetic unit; where as heparinised blank human plasma procured from Pharmacokinetic Unit contained heparin as anticoagulant.

2.3.18.6. Type of Extraction

Type: Solid Phase Extraction Method

Procedure: Solid phase extraction technique was followed and its procedure was mentioned in section 16.0 of SOTP/Protocol No.BA/08/003.

2.3.18.7. Linearity Group

The calibration curves were linear from 0.500 ng/ml to 150.000 ng/ml for carvedilol.

2.3.18.8. Quantification Parameter

The quantification parameters used were as per the Analyst software- Version -1.4.1

2.3.18.9. Detection parameters

The summary of the detector parameters was mentioned in section 18.0 of SOTP/Protocol No. BA/08/003.

2.3.18.10. Working Standards

1) Name	: Carvedilol
Batch No.	: CAN0030208
Validity date	: 28/02/2009
Name and address of manufacture	: Symed Lab Ltd. India
2) Name	: Olanzapine (Internal Standard)
Batch No.	: OLA3/FORM-I/XII/007
Validity date	: MAY-2008
Name and address of manufacture	: Torrent Research Ltd., India

2.3.19. Date of Initiation and Completion of Analytical Phase

Date of Initiation	:	08th May 2008
Date of Completion	:	13th May 2008

2.3.20. Statistical Analysis for pharmacokinetic parameters:

All 26 volunteers' data, has taken for statistical analysis of carvedilol, who completed the study.

Plasma concentration vs. time data of carvedilol were provided with mean, standard deviation and %CV for each sampling time point for both the formulations. To compare the bioavailability after administration of study drugs, the pharmacokinetic parameters [Tmax, Cmax, AUClast, AUCINF, AUC_%Extrap, Lambda_z, Lambda_z_lower, Lambda_z_upper, HL_Lambda_z, MRTlast, MRTINF] were calculated for each volunteer for carvedilol. Descriptive statistics were calculated for all pharmacokinetic parameters of carvedilol.

ANOVA and 90% Confidence interval were performed on the log-transformed data of Cmax, AUClast and AUCINF for carvedilol.

The actual values of Tmax for test and reference were compared by non-parametric Wilcoxon signed rank test for carvedilol.

All pharmacokinetic and bioequivalence analyses were carried out using WinNonlin® (Version 5.2) and SAS® (Version 9.1.3), licensed software available at Bio Evaluation Centre, Torrent Pharmaceuticals Ltd.

2.3.21. Summary of Deviation from Protocol

Deviations from the protocol were limited which did not have any significant impact on the quality and pharmacokinetic evaluation of the study. Following deviations were reported during the study.

- Volunteer replacement
- Late confinement of volunteer
- Sample Time Point Deviation
- Missing Sample Deviation

2.3.22. Adverse Events (AEs)

All the volunteers were monitored for adverse events as specified in the protocol. None of the volunteer experienced adverse event, however, according to the post study safety evaluation, total 7 volunteers (Enrolment No. 02, 06, 08, 14, 16, 21 and 26) were found with abnormal laboratory parameters which were considered clinically significant. These 7 Volunteers were advised to come for follow up to the pharmacokinetic unit. Out of these 7, four were followed with laboratory values and rest 3 volunteers were not reported for the repeat analysis.

Upon conclusion of the clinical portion of the study, both the test and reference drugs were well tolerated.

Results of blood analysis were not indicative of any adverse effects of the study medication.

2.3.23. Results

The descriptive statistical analysis of mean plasma concentrations for carvedilol is presented in Table 1. Linear and semi log-transformed mean plasma concentration versus time curve are plotted in Figure 1a, 1b for carvedilol.

Descriptive analysis of the pharmacokinetic parameters for carvedilol is presented in Table 2. Summary Tables of ANOVA Results for carvedilol are listed in Table 3 and Bioequivalence analyses for Pharmacokinetic parameters for carvedilol is presented in Table 4. Non-parametric Wilcoxon signed rank test for Tmax analysis is presented in Table 5 for carvedilol.

2.3.24. Discussion and Conclusion

The study was planned and conducted as an open label, randomised, two-period, two-treatment, two-sequence, crossover, single dose, bioequivalence study in 26 healthy male volunteers under fasting conditions. No changes in the protocol were made after starting off the study and no major deviations from the protocol were observed.

A total of 26 healthy adult male volunteers were enrolled in the study and all 26 volunteers completed the study. Bio-analysis and subsequent pharmacokinetic and statistical analysis were carried out for all 26 volunteers who completed the study.

The objective of this study was to assess the bioequivalence of Carvedilol 25mg tablet (Test) [Torrent Pharmaceuticals Ltd., India] versus Coreg® 25mg tablet (Reference) [Produtos Roche Quimicos e Farmaceuticos S.A., Brazil], in healthy human volunteers under fasting conditions. The design of the study was adequate to determine the pharmacokinetic end points of the test and reference formulations. All clinical work was performed according to GCP guidelines, in-house SOPs, local regulatory requirements and the current declaration of Helsinki.

The quantification of carvedilol in plasma samples was performed in accordance with in-house SOP requirements. The analytical methods by LCMS-MS allowed specific and sensitive determination of carvedilol in plasma. The calibration ranges validated for the analysis of plasma samples showed linearity between 0.500 ng/ml to 150.000 ng/ml for carvedilol and the validation parameters of the method fulfilled regulatory requirements for method validation.

Data of all 26 volunteers completed both the period of study were considered for final statistical analysis.

Plasma concentrations of carvedilol were presented with mean, standard deviation and percentage coefficient of variation for each sampling time point for both the formulations.

Descriptive statistical analysis was presented for all primary and secondary [Cmax, AUClast, AUCINF, Tmax, Lambda_z (K_{el}) and HL_Lambda_z ($T_{1/2}$)] pharmacokinetic parameters.

Analysis of variance of log-transformed primary pharmacokinetic parameters [Cmax, AUClast and AUCINF] of carvedilol revealed that there was no statistical significant effect of variation due to sequence and formulation at 5% level of significance. A statistical significant effect due to period was observed in AUClast and AUCINF except Cmax at 5% level of significance.

As the cross-over design exploits the fact that period effect is orthogonal to treatment effect i.e. the true treatment effect is not affected if a statistically significant period effect is

encountered, therefore even though ANOVA reveals a significant period effect for log-transformed AUClast and AUCINF, it may not affect the bioequivalence assessment of carvedilol.

It was found that the 90% confidence interval of the intra-individual mean ratios (test/reference) of log-transformed primary pharmacokinetic parameters [Cmax, AUClast and AUCINF] for carvedilol were within the acceptance range of 80.00%-125.00%.

Wilcoxon signed rank test for un-transformed Tmax of carvedilol revealed that there was no statistically significant difference between the both the formulations.

Based on these results, it can be concluded that test formulation (carvedilol 25 mg tablet manufactured by Torrent Pharmaceuticals Limited, India) is bioequivalent with the reference formulation (Coreg® 25 mg tablet manufactured by Produtos Roche Quimicos e Farmaceuticos S.A., Brazil) and is also well tolerated after single dose administration in healthy, adult, male, human volunteers under fasting condition.

There was no adverse event reported during study.

Post study laboratory values were out of normal range for some volunteers but based on clinical investigator/physician's judgment they were clinically not significant except for Enrolment no. 02, 06, 08, 14, 16, 21 and 26. As per physician assessment the volunteers were asked to follow up within 7-10 days. The repeat analyses were found within normal ranges for four volunteers (Enrolment no. 08, 14, 16 and 21) and other volunteers did not tern up to the centre after repeated follow ups. The laboratory and clinical screening revealed no indications for adverse events or adverse drug reactions related to drug.

2.3.25. Date and Signature of the Chief Investigator

I, the undersigned, declare that I have reviewed data summaries, results and conclusions in this report, and that to the best of my knowledge the report is consistent with the raw data and is scientifically rational.

Dr. Jogesh Mahajan, MBBS

Date: 30/06/2008

2.4. APPENDICES

2.4.1. PROTOCOL OF THE STUDY

Study Code: PK-07-108

STUDY PROTOCOL

A Randomised, Open Label, Two-Period, Two-Treatment, Two-Sequence, Crossover, Single Dose Bioequivalence Study of Carvedilol 25mg Tablets (Test) [Torrent Pharmaceuticals Ltd., India] Versus Carvedilol 25mg Tablets (Coreg®) (Reference) [Produtos Roche Quimicos e Farmaceuticos S.A., Brazil] In Healthy Human Subjects Under Fasted State

Version No	01
Date	12/10/07
Supersedes No	-
Date	-

SPONSOR	**STUDY SITE**
Torrent Pharmaceuticals Ltd., (Torrent Research Centre), Torrent House, Off Ashram Road, Ahmedabad - 380009 Gujarat, India.	**Bio Evaluation Centre, Torrent Pharmaceuticals Ltd., Village Bhat, Gandhinagar -382428 Gujarat, India.**

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Fax +91 79 26582100

Sponsor's Representative:

24/10/07
Date (dd/mm/yy)

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24/10/07
Date (dd/mm/yy)

CONTENT INDEX

LIST OF ABBREVIATIONS

ADL	Analytical Development Laboratory
ADR	Adverse Drug Reaction
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
ANVISA	Agencia Nacional de Vigilancia Sanitaria
AST	Aspartate Aminotransferase
AUCINF	Area under the plasma concentration extrapolated to infinite time (AUC_{0-inf})
AUClast	Area under the plasma concentration curve from administration to last observed concentration time(AUC_{0-t})
BLQ	Below Limit of Quantification
BMI	Body Mass Index
BP	Blood Pressure
Ca^{++}	Calcium
Cmax	Maximum Plasma Concentration
Conc.	Concentration
CRF	Case Record Form
CV	Coefficient of Variation
ECG	Electrocardiogram
F-test	Variance Ratio Test
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
HbsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HL_Lambda_z	Elimination Half–life ($T_{1/2}$)
hrs	Hours
HCT	Haematocrit
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICF	Informed Consent Form
IEC	Institutional Ethics Committee
K^{+}	Potassium
Lambda_z	Elimination Rate constant (K_{el})
LLOQ	Lower Limit Of Quantification
Ln	Natural Logarithm to the base e
LOD	Limit of Detection
LOQ	Limit of Quantification
max	Maximum Value Found
mg	Milligram (10^{-3} g)
ml	Millilitre (10^{-3} l)

mmHg	Millimeter of Mercury
Mol	Mole
MRT	Mean Residence Time
µl	Microlitre
N	Sample Size
NA	Not Applicable
Na^+	Sodium
PK	Pharmacokinetic
QA	Quality Assurance
QC	Quality Control
RPM	Revolutions Per Minute
SAE	Serious Adverse Event
SAS®	Statistical Analyst System (software)
SD	Standard Deviation
SOP	Standard Operating Procedure
Subject	Volunteer
Tmax	Time to reach the peak of the maximum plasma concentration of the drug
TPL	Torrent Pharmaceuticals Limited
TRC	Torrent Research Centre
T/R	Test over Reference ratio
WinNonlin®	Statistical software for Pharmacokinetic calculations

1.0 Project Title

A Randomised, Open Label, Two-Period, Two-Treatment, Two-Sequence, Crossover, Single Dose Bioequivalence Study of Carvedilol 25mg Tablets (Test) [Torrent Pharmaceuticals Ltd., India] Versus Carvedilol 25mg Tablets (Coreg®) (Reference) [Produtos Roche Quimicos e Farmaceuticos S.A., Brazil] In Healthy Human Subjects Under Fasted State

2.0 Protocol Number/ Study Code & Date

Study code: **PK-07-108**
Date: **October 12, 2007**

3.0 Principal Researcher/ Chief Investigator/ Principal Investigator

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4.0 Clinical Researcher/ Clinical Investigator

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Physicians In-charge
Dr. Tejas Talati, *MBBS*, **Dr. Chirag Shah,** *MBBS*, **Dr. Vishal Shah,** *MBBS*, **Dr. Alpesh Parmar,** *MBBS*, **Dr. Shreyansh Shah,** *MBBS*
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5.0 Analytical Phase In-charge

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6.0 Statistical Phase In-charge

Ms. Ankita Shah, *M. Sc., M.Phil.*
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7.0 Equipments

7.1 Clinical Phase

Site: Bio Evaluation centre, Village Bhat, District Gandhinagar		
Sr. No	**Name of Equipment (PKU)**	**Make of the equipment**
1	Central Oxygen and Suction Line	Datex Ohmeda
2	Defibrillator	Hewlett Packard Code Master XL
3	Electrocardiograph	BPL
4	Sphygmomanometer	Diamond
5	Stethoscope	Littmann
6	Boyle Apparatus	BOC India Ltd
7	Ambu Bag	Silicon
8	Incubator	Tempo
9	Refrigerator	Samsung Ice World
10	Micro Pipettes	Eppendorf
11	Cooling Centrifuge	Heareus
12	Laryngoscope	Anaesthetics
13	Tracheotomy Box	-
14	Deepfreeze	Heraeus
15	Multiparameter monitor	BPL MPM 563
16	Auto strip washer	Bio Tek ELX 50
17	ELISA processor	Dad Beharing
18	Clinical Thermometer	BD
19	Infusion Syringe Pump	B/Braun
20	Staturemeter	---
21	Breath alcohol analyzer	Manish & Sun
22	Glucometer	B/Braun
23	Nebulizer	Cicobay

Other Facilities

1	Ambulance with Oxygen Cylinder
2	Crash Carts with emergency medicines
3	Emergency Trolley
4	Emergency alarm indicator
5	Synchronized clock
6	Thermohygrometer

7.2 Analytical Phase

7.2. a Analytical Development Laboratory

Site: Village Bhat, District Gandhinagar		
Sr. No	**Name of Equipment**	**Make of the equipment**
1	HPLC	Shimadzu
2	GC	Perkin Elmer
3	UV/VIS Spectrophotometer	Jasco
4	Dissolution Apparatus	Electrolab

5	PH meter	Labindia
6	Analytical Balance	Mettler
7	Analytical Balance	Sartorius
8	Micro Balance	Afcoset
9	Analytical Balance	Afcoset
10	Oven	Labline
11	Vacuum Oven	Labline
12	Muffle Furnace	Labline
13	KF Autotitrator	Metrohm
14	Shaker	Orbitek
15	Centrifuge	Remi
16	Centrifuge	Sigma
17	Water Bath	Labline
18	Hot Plate	Labline
19	Milli Q-Water Puri. System	Millipore
20	GC Q	Finnigan mat
21	Vacuum Concentrator	Sawant
22	Sonicator	Meltronics
23	Hamo Autowasher T-420	Hamo
24	Refrigerator	Samsung
25	Autoclave	Labline
26	Fuming Cupboard	-
27	Dry Heat Sterilizer	Labline
28	Glassware Drying Oven	Labline
29	Heating Block	ATC
30	Solvent Evaporator	Zymark

7.2.b Bioanalytical Laboratory

Site: Village Bhat, District Gandhinagar		
Sr. No	**Name of Equipment**	**Make of the equipment**
1	LCMS/MS (TSQ, Discovery)	Finnigan Mat
2	LCMS/MS(TSQ, Quantum)	Finnigan Mat
3	LCMS/MS (API-4000)	Sciex
4	LCMS/MS (LCQ)	Finnigan Mat
5	LCMS (LXQ)	Finnigan
6	LCMS (TSQ Ultra)	Finnigan
7	HPLC	Surveyor
8	Centrifuge	Heraeus Multifuge 3S-R
9	Centrifuge	Heraeus Multifuge 3S
10	Multipulse Vortexer	Glas-Col
11	Plasma Extractor (Rugged Rotor)	Model 099RD 4512 & Rd 4524
12	Plasma Extractor (Rugged Rotor)	Model 099RD 4512 & Rd 4525
13	LV Solvent Evaporator	Zymark
14	Turbo Vap 96 Well Plate	Caliper Life Sciences
15	Nitrogen generator	Dominick Hunter

16	Nitrogen generator	Peak Scientific Instruments
17	Refrigerator	Samsung
18	Deep Freezer (-20 C°)	Vest frost
19	Deep Freezer (-70 C°)	Heraeus
20	Deep Freezer (-70 C°)	New Brunswick Scientific
21	Data Logger (32 Channel)	Envic
22	Analytical Balance	Mettler Toledo
23	Analytical Balance Printer	Mettler Toledo
24	Microbalance (UMX 2)	Mettler Toledo
25	Microbalance Printer	Mettler Toledo
26	Liquid Handling System (LHS)	Tecan
27	Analytical Balance for LHS (SAG 285)	Mettler Toledo
28	pH meter	Labindia
29	pH analyzer	Analab Scientific
30	Milli Q water system	Millipore
31	Ultrasonicator	Jeio Tech
32	Transonic Digital Ultrasonicator	Elma
33	Fuming Cupboard	--
34	Fuming Cupboard	--
35	Safety Shower and Eye Washer	Unicare
36	Solid Phase Assembly	Waters
37	Micro pipettes	Eppendorf
38	Synchronized Clock	Microsystem
39	Thermo hygrometer	Mex Tech TH-103
40	Thermo hygrometer	Humitherm 842 C
41	Digital Stop Watch	Racer
42	Photo Tachometer	Lutron

7.3 Statistical Phase

Site: Bio Evaluation Centre, Village: Bhat, District -Gandhinagar		
1	Pharmacokinetic and Statistical Software	WinNonlin® (Version 5.2 or higher) and SAS® (Version 9.1.3 or higher)

7.4 Clinical Evaluation

Site: Bio Evaluation Centre, Village: Bhat, District -Gandhinagar		
Sr. No	**Name of Equipment**	**Make of the Equipment**
1	Electrocardiograph BPL 108 (2)	BPL
2	Electrocardiograph BPL 8408 (2)	BPL
3	MPM Bed-side monitor (2)	BPL
4	Multipara Monitor (Life Plus)	Life Plus
5	MPM Bed-side monitor (3)	Philips
6	Defibrillator	HP Codemaster
7	Boyles Apparatus	BOC India
8	Oxygen & Suction	

9	Electronic Weighing Machine (2)	Bhagyoday Sales
10	Electronic Digi Scale	IOTA
11	Digital clocks-1 Master clock	Microsystem
12	Electronic Digital clock (4)	Ajanta
13	Sphygmomanometer (12)	Diamond
14	Refrigerator Samsung (2)	Samsung
15	Thermometer (2)	Zeal
16	Thermo Hygrometer (7)	Mextech
17	Digital Thermometer (4)	BD
18	Infusion Syringe Pump (2)	B/BRAUN
19	Stature meter (2)	
20	Glucometer (2)	B/BRAUN

7.5 Clinical Laboratory Tests

7.5a

Site: Biochemical Laboratory, Village: Bhat, District -Gandhinagar		
Sr. No	**Name of Equipment**	**Make of the equipment**
1	Deep Freeze (-20)	Blue Star
2	Deep Freeze (-70)	Kendro
3	Deep Freeze (-70)	(New Brunswick scientific)
4	Cooling Centrifuge Heraeus (two)	Heraeus
5	Autostrip Washer	ELX 5018
6	Incubator	Tempo
7	Refrigerator	Samsung
8	Micropipettes (four)	Eppendorf
9	BEP2000 Advance (Fully Automatic ELISA Processor)	Dade Beharing
10	Digital Thermo Hygrometer (two)	MEXTECH

7.5b

Site: Clinical Pathology laboratory, Village: Bhat, District -Gandhinagar		
Sr. No.	**Name of Equipment**	**Make of the equipment**
1	Urine Analyzer	SIEMENS Medical Solutions Diagnostics Ltd.
2	Micropipette 2-20 mic.1	Eppendorf
3	Micopipette 20-200 mic.1	Eppendorf
4	Micropipette 20-100 mic.1	Eppendorf
5	Micropipette 100-1000 mic.1	Eppendorf
6	Micropipette 1-5ml	Eppendorf
7	Clinical chemistry analyzer	Olympus Inc.
8	Refrigerator	LG Electronics
9	Thermometer-Dry-Wet bulb	Zeel
10	Cyclomixer	Remi
11	Centrifuge	Eppendorf
12	Micropipette 0.1 to 2.5 mic. L	Eppendorf

13	Micropipette 2 to 20 mic. L	Eppendorf
14	Micropipette 20 to 200 mic. L	Eppendorf
15	Micropipette 100 to 1000 mic. L	Eppendorf
16	Micropipette 500 to 5000 mic. L	Eppendorf
17	Thermometer	Zeel
18	Hematology Analyzer	Abbott Healthcare
19	Water Purification System	Millipore

7.5c

Sr. No	Name of Equipment	Site
1	X-ray	Kanoria Hospital and Research Centre, Gandhinagar

8.0 Objective of the study

Primary objective of the study is to compare the bioavailability of Carvedilol 25mg tablet (Test) [Torrent Pharmaceuticals Ltd., India] versus Coreg® 25mg tablet (Reference) [Produtos Roche Quimicos e Farmaceuticos S.A., Brazil], in healthy human volunteers under fasting conditions.

Secondary objective of the present study is to investigate the safety of the formulations on the basis of clinical and laboratory examinations at the beginning and at the end of the study and registration of adverse events and/or adverse drug reactions.

9.0 Study Summary/ Outline of the Study

Name of Sponsor	Torrent Pharmaceuticals Ltd., India.
Title of Study	A Randomised, Open Label, Two-Period, Two-Treatment, Two-Sequence, Crossover, Single Dose Bioequivalence Study of Carvedilol 25mg Tablets (Test) [Torrent Pharmaceuticals Ltd., India] Versus Carvedilol 25mg Tablets (Coreg®) (Reference) [Produtos Roche Quimicos e Farmaceuticos S.A., Brazil] In Healthy Human Subjects Under Fasted State
Study Code	PK- 07- 108
Study Design	An Open-Label, Randomised, 2-Period, 2-Treatment, 2-Sequence, Crossover, Single-Dose Bioequivalence under fasting conditions
Objective	**Primary objective** of the study is to compare the bioavailability of Carvedilol 25mg tablets (Test formulation; Torrent Pharmaceuticals Ltd., India) versus Coreg® 25mg tablet (Reference formulation; Produtos Roche Quimicos e Farmaceuticos S.A., Brazil) in Healthy Human volunteers under fasting conditions. **Secondary objective** of the present study is to investigate the safety of the formulations on the basis of clinical and laboratory

	examinations at the beginning and at the end of the study and registration of adverse events and/or adverse drug reactions.
Study Site	Bio Evaluation Centre, Torrent Pharmaceuticals Ltd., Village: Bhat, District Gandhinagar-382 428, Gujarat, India.
Planned Sample Size	26 (24+2) Volunteers
Main Selection Criteria	Healthy Human Volunteers, aged 18-45 years, body mass index within 18-27(both inclusive) kg/m^2
Screening Procedure	Demographic data, medical and medication histories, physical examination, height, weight, BMI, ECG, vital signs, haematology, biochemistry, serology, X-ray chest (If not done with in last 6 months) and urine analysis carried out for the screening. Urine test of drug abuse and Breath alcohol test will be conducted prior to each study periods.
Confinements & visits	For each period, volunteer will be confined to study area at least 12 hours before study drug administration until 32 hours after study drug administration. At 48 hour post dose volunteer will come back for ambulatory blood draw.
Washout Period	Minimum of 7 days
Test Medication **Generic Name** **Formulation** **Company** **Dose**	 Carvedilol 25 mg Tablet Torrent Pharmaceuticals Limited, India 25mg as a single dose
Reference Medication **Generic name** **Formulation** **Company by)** **Dose**	 Carvedilol 25 mg (Coreg®) Tablet Produtos Roche Quimicos e Farmaceuticos S.A., Brazil 25mg as a single dose
Drug Administration	A single oral dose of test or reference formulations (1 x 25mg tablet) will be given with 200ml of drinking water under fasting conditions in each period.
Blood Sample Collection	A total (21 x 5 ml) of venous blood samples will be taken at sampling time points are: Pre-dose, 0.166, 0.333, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 6.00, 8.00, 12.00, 18.00, 24.00, 32.00 and 48.00 hours (post-dose). Blood sample for post dose 48.0 hours will be taken as ambulatory sample. All in-house samples will be collected from indwelling cannula and an extra 0.5ml heparinised blood sample will be discarded before each in-house sample collection. Heparin-lock technique will be used to prevent clotting of the blood in the indwelling cannula. If for any reason the indwelling cannula is blocked or must be removed for practical reasons, or on volunteer request, direct vein puncture will be done.
Total Blood Loss	~ 233 ml

Volunteer Safety	Vital sign measurement at pre-dose, 1.00, 2.00, 4.00 and 8.00 hours post-dose ± 30 minutes of scheduled time (except at pre-dose) and whenever necessary.
Post Study Procedure	Haematology, biochemistry and adverse events.
Bio-analytical Method	The estimation of carvedilol in plasma samples will be carried out by validated LC-MS/MS method.
Primary Pharmacokinetic Parameters	Cmax, AUClast, AUCINF
Secondary Pharmacokinetic Parameters	Tmax, Lambda_z (K_{el}) and HL_Lambda_z ($T_{1/2}$)
Additional Pharmacokinetic Parameters	AUC_%Extrap, Lambda_z_lower, Lambda_z_upper, MRTlast, MRTINF
Statistical Analysis	Descriptive statistics of plasma concentration vs. time data and all pharmacokinetic parameters will be provided. ANOVA will be performed on log-transformed data of primary pharmacokinetic parameters [Cmax, AUClast and AUCINF]. A non-parametric test will be used to compare actual values of Tmax.
BE Acceptance Criteria	Acceptance range for bioequivalence will be 80.00%- 125.00% for Cmax, AUClast and AUCINF
Evaluation of Bioequivalence	90% confidence interval of the intra-individual mean ratios (test/reference) for log-transformed primary pharmacokinetic parameters [Cmax, AUClast and AUCINF] will be determined and if the limits fall between acceptance ranges then the product would be considered bioequivalent.

BACKGROUND INFORMATION[1]

Carvedilol is an arylethanolamine β- adrenoceptor antagonist with venodialating properties. These properties are due to blockade of α_1- adrenoceptors along with weak β_1- selective blockade. This dual mode of action avoids the reflex tachycardia due to excessive vasodilation and the peripheral vasoconstriction due to β-blockade.

The antagonism of β_2 adrenoceptors exists, although to a lesser extent. Carvedilol has no Intrinsic Sympathomimetic Activity, and only weak Membrane Stabilizing Activity. Carvedilol is cardio protective in animal models. It is also anti-mitogenic on vascular smooth muscle in vitro, and protects against neuronal damage in in-vitro and in vivo models of brain ischaemia.

Single oral doses of Carvedilol as low as 12.5 mg reduce resting and exercise induced blood pressure in healthy volunteers without effect on heart rate or cardiac index. In patients with Coronary Artery Disease, Carvedilol improves the exercise capacity and left ventricular function, and increases ejection fraction. Left Ventricular Hypertrophy regression has been observed in some cases of hypertension.

Pharmacokinetics

Absorption

Carvedilol is rapidly and extensively absorbed following oral administration, with absolute bioavailability of approximately 25% to 35% due to a significant degree of first-pass metabolism. Following oral administration, the apparent mean terminal elimination half-life of carvedilol generally ranges from 7 to 10 hours.

After single oral dose of 25 mg Carvedilol in healthy volunteers, maximum plasma concentration (Cmax) was found to range from 21-67 µg/L. Similar corresponding variations were also observed in the values of mean area under the plasma concentration time curve (AUC). AUC was found to range from 157-337 µg/L.h after single dose of 25 mg in healthy volunteers. Cmax and AUC of Carvedilol were found to increase linearly with dose. However, the time to achieve maximum plasma concentration (Tmax) was within the range of 1-2 hrs for 25 mg as well as 50 mg dose in healthy volunteers and hypertensive patients.

Plasma concentrations achieved are proportional to the oral dose administered. When administered with food, the rate of absorption is slowed, as evidenced by a delay in the time to reach peak plasma levels, with no significant difference in extent of bioavailability. Taking carvedilol with food should minimize the risk of orthostatic hypotension.

Distribution

Carvedilol is a basic, lipophilic compound with a steady-state volume of distribution of approximately 115 L, indicating substantial distribution into extravascular tissues. Plasma clearance ranges from 500 to 700 mL/min. Carvedilol is more than 98% bound to plasma proteins, primarily with albumin. The plasma-protein binding is independent of concentration over the therapeutic range.

Metabolism & Excretion

Carvedilol is rapidly and extensively metabolised with less than 2% of the dose recovered as unchanged drug in urine. About 60% of the metabolites are excreted into bile and are eliminated in faeces.

Carvedilol is metabolized primarily by aromatic ring oxidation and glucuronidation. The oxidative metabolites are further metabolized by conjugation via glucuronidation and sulfation. The metabolites of carvedilol are excreted primarily via the bile into the feces. Demethylation and hydroxylation at the phenol ring produce three active metabolites. Compared to carvedilol, the three active metabolites exhibit weak vasodilating activity. Plasma concentrations of the active metabolites are about one-tenth of those observed for carvedilol and have pharmacokinetics similar to the parent.

SIDE EFFECTS

Carvedilol is well tolerated as a once daily 25 mg dose. Overall incidence of withdrawal due to adverse effects was reported only in 7% of patients studied. The

most common adverse effects causing discontinuation of treatment were vertigo (1.7%), headache (1.4%), and bronchospasm, fatigue and skin reactions (0.5% each). In 50 mg dose, the adverse event incidence was 31%. Other adverse events rarely reported were loose stools, dry mouth, mucosal swelling, depression, constipation, itching and/or rash. The incidence of syncope or orthostatic hypotension was relatively low (<1%).

CONTRAINDICATIONS

Carvedilol is contraindicated in the following conditions:

- Bronchial asthma or related bronchospastic conditions. Deaths from status asthmaticus have been reported following single doses of Carvedilol.
- Second- or third-degree AV block
- Sick sinus syndrome
- Severe bradycardia (unless a permanent pacemaker is in place)
- Patients with cardiogenic shock or who have decompensated heart failure requiring the use of intravenous inotropic therapy. Such patients should first be weaned from intravenous therapy before initiating Carvedilol
- Patients with severe hepatic impairment
- Patients with a history of a serious hypersensitivity reaction to carvedilol (e.g. Stevens- Johnson syndrome)

9.1 Type/ Study Design

This study will be monocentric, open-label, randomised, two-period, two-treatment, crossover bioequivalence study under fasting conditions. A wash-out period of at least 7 days is planned between the two periods. Each of the volunteers will be randomly assigned to one of two possible dosing sequences (AB or BA). A total of 26 volunteers will be enrolled in the study.

The study design is chosen according to the recommendation of the ANVISA Guidelines for Relative Bioavailability/ Bioequivalence Tests on Drugs Resolution - Re N° 1170, April 19, 2006.

Discussion of study design

In the process of development of bioequivalent formulation of any product, it is important to investigate the relative bioavailability of the new product in comparison with a market standard. The reference product Coreg® 25mg tablet (Carvedilol) is already registered and commercially available for years in Brazil. For the purpose of approval the efficacy and safety of this drug have been proven in clinical trials. This drug will therefore serve as reference and basis for comparison for the test formulation Carvedilol 25mg tablet; company responsible for placing the product on the market: Torrent Pharmaceuticals, India. This study will be conducted with the aim to investigate whether differences concerning rate and extent of absorption exist between the test product and the reference product.

Type of Study

Bioequivalence study of Carvedilol 25mg tablet will be conducted under Fasting Conditions according to Lista 1 of Resolution - Re N° 1170, April 19, 2006.

Choice of Volunteers

Bioavailability is generally recommended to be examined in volunteers of both the sexes. The objective of this study is, however, not the investigation of the pharmacokinetics of different drugs, but the comparison of the pharmacokinetic profiles of the drug entities itself. As, on the one hand, the group of volunteers under investigation should be homogeneous as possible, and on the other hand an interaction between galenics and sex of the volunteer is highly unlikely, the volunteers will be recruited males only. Moreover, the risk to women of childbearing potential is considered.

Choice of duration of sampling

The duration of sampling was chosen taking into account the mean plasma elimination half-life of Carvedilol which is 7-9 hours. The sampling period corresponds thus to more than 4 of the registered half-lives.

Choice of Wash out period

The minimum wash-out period of at least 7 days was also chosen according to the terminal elimination half-life. After more than 7-8 half lives a pharmacokinetic carry-over effect can be excluded.

Choice of Analytes

The parent drug (Carvedilol) will be detected in plasma samples according to Lista 2 of Resolution - Re N° 1170, April 19, 2006.

Choice of dosage

Selection of the study dose (25mg) corresponds to daily recommended dose, without regard to meals.

Blinding

Mostly all the bioequivalence studies are open-label. Only the bio-analyst will be held blind in respect of both test and reference products to minimize bias.

9.2 Identification of the Test and Reference Product

Test Product	
Generic Name	Carvedilol 25 mg
Pharmaceutical Form	Tablet
Batch No.	B9717002
Manufacturing Date	February 2007
Expiry Date	January 2009
Manufactured By	Torrent Pharmaceuticals Ltd., India
Reference Product	
Generic Name	Carvedilol 25 mg
Trade Name	Coreg®
Pharmaceutical Form	Tablet
Batch No.	RJ0382
Manufacturing Date	--

Expiry Date	July 2009
Manufactured By	Produtos Roche Quimicos e Farmaceuticos S.A., Brazil

Receipt, Storage and retention

The study drugs will be received in a sufficient quantity with certificate of analysis (COA) for the needs of whole study and retention. The Pharmacist will confirm receipt of study drugs and keep it in pharmacy for storage. The receipt and delivery of study drugs is to be documented by Pharmacist.

The Investigator will be responsible for proper storage of the study drugs. All the study drugs must be stored in the pharmacy at controlled temperature specified on drug label. All unused study drugs will be returned to the pharmacy.

A minimum sample quantity sufficient for the complete study of pharmaceutical equivalence, a re-test and crosschecking shall be kept as retention for the validity period of both products plus one year.

Study drug shall be stored in zip lock bag containing following label:

"FOR CLINICAL RESEARCH PURPOSE ONLY" torrent	
Study Code:	Batch No.
Generic Name:	
Brand Name:	Storage Condition:
Expiry date/Use by date/Retest date:	
No. of units transferred.	
Remarks:	

Method of Assigning Volunteers to Treatment Group

Volunteers who are eligible for study will be first thoroughly informed about he aims and details of the study. Each of the volunteer will be randomly assigned to one of the two possible sequence of administration of the study medication.

One copy of randomization will be provided to pharmacist for dispensing of medication and same copy will be preserved in pharmacy. The dispensing record generated by pharmacist will be checked by responsible study personnel and kept in study file.

Drug Dispensing

The study formulations, test and reference drugs will be dispensed on a day of enrolment or shall be completed atleast 30 min before the dosing in each period in an opaque, white polypropylene container duly labelled in presence of study personnel and will be delivered to study centre 30 ± 10minutes before dosing by the Pharmacist, till that time dispensed study drugs will be kept under control access in pharmacy. One extra unit of test and reference product each shall be dispensed in each period. The labels of the containers consist of two segments, a fixed and a flag-

tear off segment. The dispensing record generated by pharmacist will be checked by study personnel and kept in study file.

Each label will contain study code, date of dosing, enrolment no., period, randomization code, drug name and signature of pharmacist. In case of extra dispensing, enrolment no. shall be kept as blank.

Study Code:	Period		Study Code:	Period:
Enrollment No	Randomization code:		Enrollment No:	Randomization code:
Drug name:			Drug name:	
Date of dosing:	Sign:		Date of dosing:	Sign:
FOR CLINICAL RESEARCH PURPOSE ONLY			FOR CLINICAL RESEARCH PURPOSE ONLY	

Randomization and Blinding

Randomization schedule for all 26 volunteers will be generated before the start of study. Volunteers will be administered each treatment (A or B) during the two period of the study according to the randomization schedule. A total of 26 volunteers will be divided in two batches. All 26 healthy volunteers will be dosed per period.

The randomization will be balanced and the code will be kept under controlled access. Randomization generated by a statistician will be available in the study file. One copy of randomization will be provided to pharmacist for dispensing of medication and the same copy will be preserved in pharmacy. The bio-analyst will be held blind in respect of both test and reference products to minimize bias.

Drug Accountability

The drug accountability will be maintained by pharmacist through out study and documented in study drug accountability form.

All the investigational products (i.e. extra dispensed, not dosed, un dispensed except retention) returned from bio-study shall be sent back to the pharmacy, disposed off after completion of study and record shall be maintained in "Drug Disposal Record Logbook" and "Study Drug Accountability and Retention Record. The investigator will not allow the study drugs to be used for purposes other than specified in protocol.

9.3 Dosage

In each period the volunteers will be administered a single oral dose of either test product (Carvedilol 25 mg tablet) or reference product (COREG® 25 mg tablet) as per the randomization schedule.

The volunteers will be asked to swallow whole tablet with 200ml drinking water. The dosing will be done in the morning of dosing day. Clock times are valid for first volunteer in the each group. The clock times for other volunteers will be moved forward in steps of 2 minutes.

Dosing will be done in the presence of the Chief investigator/Clinical researcher/ Sub investigator/Study coordinator/Physician. To ensure the subject has swallowed the drug a "mouth check" will be done. The personnel administering investigational product will stick the detachable part of the label in to the Volunteer's case record form (Section 'Drug Dosing') with signature of person involved in the dosing, the signature of the volunteer and dosing supervisor. All blood sampling times will be with reference to the drug administration time.

9.4 Place and Form of Confinement of the Volunteers

Volunteers will arrive at Pharmacokinetic Unit a day before drug dosing and will stay at least for 32 hours after drug administration in each period. Each volunteer will be asked about his health status particularly about any significant changes from pre-study/ screening examination and questionnaire for compliance will be applied. Volunteers who will satisfy the inclusion and exclusion criteria checklist will be enrolled in the study. Enrolled volunteers would be confined to the Pharmacokinetic Unit at least 12.00 hours before drug administration. Total stay of volunteer in Pharmacokinetic unit is approximately 44 hours in each period.

9.5 Fasting and Feeding Schedule

Volunteer will be served dinner from 20.00 hrs and would be ask to finish their dinner before 22.00 hrs and then after they will be under supervision for overnight fasting.

The first main meal (lunch) of the dosing day will be taken not less than four hours after investigational product administration and the subsequent meals will be given at appropriate times i.e. 8, 12, 24, 28 and 32 hours after administration of investigational product (snacks, dinner, breakfast, lunch and snacks respectively).

Meals will be prepared and served individually and the volunteers will be asked politely to finish the meal completely and the same will be recorded in "meal distribution record". Exactly the same meals will be served in both the periods of the study to all volunteers. Dietary content and procedures during each study period will be identical.

9.6 Schedules for Collection of Samples

A series of 21x5 ml venous blood samples will be collected over a period of 48 hours in each period. To create a secure peripheral venous access, the indwelling intravenous cannula will be used for collecting blood sampling by syringe and transferred into 10 ml plastic tubes containing diluted heparin in normal saline. Heparin-lock technique will be used to prevent clotting of the blood in the indwelling cannula. Before each in house blood sample, 0.5 ml of blood will be discarded so as to prevent the heparin in the cannula from interfering with the analysis. If for any reason the indwelling cannula is blocked or must be removed for practical reasons, or on volunteer request, direct vein puncture will be done. Other blood samples will be collected through direct venous puncture. After every blood sample collection through indwelling cannula, 0.5 ml of heparinised saline will be

injected to intravenous cannula. Tubes will be shaken gently to ensure the proper mixing of blood with anticoagulant. Sampling will relate to drug administration time. In the morning of dosing day after recording vitals, a pre-dose blood sample will be taken at least 30 minutes before study drug administration. Other blood samples will be withdrawn at following times: 0.166, 0.333, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 6.00, 8.00, 12.00, 18.00, 24.00, 32.00 and 48.00 hours of post dose. Post dose sample for 48.00 hour will be taken as ambulatory sample.

Blood sampling up to ± 2 minutes of the planned time of in-house sampling and up to ±1 hr in ambulatory sample will be considered as an acceptable deviation. Beyond that, time deviation will be taken in to consideration for further pharmacokinetic parameters, except for pre dose samples, which will always be reported as zero hour sample (0).

Total blood loss will be of approximately 233 ml during study. This amount includes blood samples during study (21x5 per period); 0.5ml heparinised blood at each in-house time point and 3 ml blood for post study safety evaluation.

Minimum 7 days washouts will be maintained between dosing of Period I and II.

9.7 Sample Handling Procedures

The blood samples will be collected in pre-labelled 10ml polypropylene tubes and containing 5IU diluted heparin for each ml of blood. Each sample will be centrifuged as soon as possible at 2000 RPM for 10 minutes at 20°C to separate plasma. The plasma then will be transferred in pre-labelled 5ml polypropylene tubes, which will be subsequently frozen at - 70 ±10 °C as soon as possible until it is analysed.

Timing of samples received, separated and stored will be documented in the form "Centrifugation and Storage of Samples". Sample will be stored at bio-chemical laboratory during the study and will be transferred to bio-analytical department for analysis in vials placing in a thermocol box containing dry ice / ice cubes.

Both the blood collection tubes and plasma storage tubes will be pre labeled. Each label shall contain Study code, Enrolment no., Period no, Time point, Date of dosing and Sign.

10.0 STUDY POPULATION

10.1 Detailed Description

Total 26 (24+2) healthy males, 18 to 45 years of age with BMI 18-27 kg/m^2 (both inclusive) will be included in the study. The volunteers will be selected based on their good health confirmed by complete clinical, haematological, serological and biochemical tests. Analysis for carvedilol will be carried out for all the volunteers who complete both periods of the study.

10.2 Selection of Volunteers

The health status of the volunteers will be evaluated prior to study drug administration. Demographic data, medical and medication histories, clinical physical examination, height, weight, BMI, ECG, vital signs, haematology, biochemistry, serology, X-ray chest (if not done during last 6 months) and urine analysis will be carried out for the screening. The screening evaluation will be valid for maximum of 28 days. Test for urine drugs of abuse and breath alcohol test will be performed before each study period.

Volunteers found positive to the drug abuse check and breath alcohol will not be allowed to participate.

10.3 Clinical Evaluation

Medical Case History

- Demography
- Subject's medical case history
- Subject's surgical history
- Tea, caffeine, cola, alcohol and tobacco consumption patterns
- Significant past and family history

Clinical Physical Examinations

The following pre-study examinations regarding to the physical status will be done:

Age	Skin and lymphatic nodes
Height	Head, eyes, ears, nose, throat
Weight	Cardiovascular system
BMI	Respiratory system
ECG	Musculoskeletal system
Pulse rate	Gastrointestinal system
Blood pressure	Endocrine system
General examination	Neurological system

10.4 Clinical Laboratory Tests

X-ray & ECG: If needed new X-ray will be carried out as per discretion of Clinical Investigator, otherwise validity of X-ray is six months and ECG will be taken at the time of screening to check normal function of chest & heart respectively.

Pre-study Laboratory Examinations

The following clinical laboratory tests will be performed.

Hematology:	**Biochemistry:**	**Urine:**
Hemoglobin	Blood Sugar (Random)	Physical examination
White blood cells (WBC)	Aspartate aminotransferase	RBCs

Differential WBC count	Alanine aminotransferase	Albumin
Red blood cells	Alkaline phosphatase	Casts and crystals
Hematocrit	Total bilirubin	Epithelial cells
Platelets	Creatinine	Sugars
Serological Test HbsAg, HCV, HIV test	Total protein	Bile salts and bile pigments
	Sodium	
	Potassium	Pus cells
	Chloride	
	Calcium	
	GGT, Total cholesterol, Tryglicerides	
Drug of Abuse Screen Test: (Opium, Tetra Hydrocannabinoid, Amphetamine, Barbiturates, Benzodiazepines, Cocaine)		
Breath Alcohol Test		

Post Study Clinical Evaluation

At the time of discharge in each period physical examination will be carried out. The post-study safety evaluation includes physical examination and laboratory examination i.e. Haematology and Biochemistry. The end of the study clinical laboratory tests will not include HIV, HBsAg, HCV, ECG, Urine examination.

10.5 Criteria for Inclusion

Subjects must meet all of the following criteria in order to be included in the study:

- Sex: male
- Age: 18 -45 years.
- Volunteer with BMI of 18-27 (inclusive both) kg/m^2 with minimum of 50 kg weight.
- Healthy and willing to participate in the study.
- Signed Written Informed Consent for Screening and study.
- Non-smokers or smoking less than 10 cigarettes a day and willing to break smoking during the course of the study.

10.6 Criteria for Exclusion

- Clinically relevant abnormal physical findings at the screening examination, which would interfere with the objectives of the study.
- Clinically relevant abnormalities in the results of the laboratory screening evaluation.
- Systolic blood pressure less than 100 mmHg or more than 140 mmHg and diastolic blood pressure less than 60 mmHg or more than 90 mmHg
- Pulse rate less than 50/minute or more than 100/minute
- Oral temperature less than 95°F or more than 98.6°F
- Respiratory rate less than 12/minute or more than 20/minute
- Clinically significant abnormal ECG or Chest X-ray
- Habituation of tobacco necessitating uninterrupted tobacco consumption

- Addiction to alcohol or history of any drug abuse.
- History of kidney or liver dysfunction.
- History of allergy to the test drug or any drug chemically similar to the drug under investigation.
- Administration/ Intake of any prescription medication for two weeks or OTC medication for one week before the study.
- Patients suffering from any chronic illness such as arthritis, asthma etc.
- HIV, HCV, HBsAg positive volunteers.
- Opium, tetrahydro cannabinoids, amphetamine, barbiturates, benzodiazepines, cocaine positive volunteers based on urine test.
- Breath alcohol test positive
- Subjects suffering from any psychiatric (acute or chronic) illness.
- Administration of any investigational drug in the period 0 to 3 months before entry to the study.
- Intake of barbiturates or any enzyme-inducing drug in last three months.
- History of significant blood loss due to any reason, including blood donation in the past 12 weeks.
- History of any bleeding disorder.
- Existence of any surgical or medical condition, which, in the judgment of the clinical investigator, might interfere with the absorption, distribution, metabolism or excretion of the drug or likely to compromise the safety of volunteers.
- Serious adverse reaction or hypersensitivity to study drug or any of the excipients.
- Inability to communicate or co-operate with the investigator due to language problem, poor mental development or impaired cerebral function.

If some minor deviations as regards to the laboratory results are detected, Chief Investigator and / or clinical investigator will assess their relevance to the purpose of the study and to the volunteer inclusion. If some minor deviations like 2 to 4 mm Hg fluctuation in blood pressure, shall be consider based on the decision of Chief Investigator and / or clinical investigator.

In case of marked deviations of health status leading to Volunteer's non-inclusion in the study, wherever possible the Volunteer will be explained about the same with recommendation to visit his physician.

10.7 Restrictions and Prohibitions

Clinical Residential Stay:
Volunteer enrolled would be confined to the BE Centre from 12 hours before the dosing to a minimum of 32 hours post dosing.

Food, Beverages and Smoking:
At least 10 hours fasting will be required before dosing and 4 hours post dose in each period.
Volunteers will be restricted from drinking water 1 hour before and 2 hours after dosing in each study period.

Volunteers will be requested to abstain from alcohol for 48 hours prior to check-in in each period and until the end of each study period. Volunteers will be instructed to avoid beverages containing caffeine or xanthines (coffee, tea, cola drinks), food (chocolates) and grape fruit juice for 48 hours prior to check-in in each period and until the end of each study period. Volunteers will be instructed to avoid tobacco in any form for 48 hours prior to check-in in each period and until the end of each study period.

Medications:
Volunteers should take no prescribed medications beginning two weeks prior to and no OTC medications beginning one week prior to initiation of study until after the study is completed. If drug therapy other than that specified in the protocol is required prior to study or during the study or in the washout period, decision shall be taken by the Chief Investigator /clinical investigator whether to continue or discontinue the volunteer on the basis of the following:

- The pharmacology and pharmacokinetics of the non-study medication.
- The likelihood of drug-drug interaction, thereby affecting pharmacokinetic comparison of the study drugs.
- The time and duration of administration of the non-study medication and likelihood of interference in bio-analysis.
- The safety, well-being and clinical judgment about the volunteer.

Physical Activity:
Volunteers will be asked to remain in sitting position for at least first 4 hours after study drug administration except for blood sampling and toilet purpose wherein they are allowed to walk for a brief period. However, should medical events occur at any time volunteers may be placed in an appropriate position. The Volunteers will be restricted from doing any sort of stressful physical activity during the entire period of stay at Pharmacokinetic unit.

The sponsor/ sponsor's representative will monitor the study with prior intimation.

10.8 Criteria for Discontinuation or Withdrawal of Volunteers from the Study

Volunteers will be informed that they are free to withdraw from the study at any time without giving any reason for doing so. The decision to withdraw/ discontinue the volunteer if vomiting occurs will be taken by the chief investigator considering the frequency and amount of vomiting and after assessing the general well being of volunteer's health status.

Volunteers may be discontinued from the study for any of the following reasons:
1. Volunteers not wishing to continue with the study, irrespective of the reason.
2. Adverse event during the study.
3. Any illness requiring medication during the study.
4. Violation of the protocol by the volunteer.

Any volunteer may be discontinued from the study for any reason beneficial to his well-being. The Chief Investigator, as well as the study Sponsor, will decide to discontinue any volunteer's participation in the study if, in their judgment,

continuation in the study may prove harmful to the volunteer. Such a decision may be precipitated by adverse events, including changes in vital signs, physical examination and ECG. The Chief Investigator may also discontinue a volunteer due to poor compliance to the study protocol.

Volunteer Replacement

There will be no replacement.

11.0 Adverse Reactions (including classification method) and emergency procedures

Definitions:
Adverse Event (AE): Any untoward medical occurrence in a patient or clinical investigation volunteer administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

Abnormal laboratory values will be reported as adverse events under the following circumstances:

i. When the abnormal lab report is accompanied with associated symptoms.
ii. When medical/surgical intervention is required.
iii. Leads to a serious adverse event.
iv. When it is considered by Principal Investigator as an adverse event.

Adverse Drug Reaction (ADR): All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions.

Unexpected Adverse Drug Reaction: An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational medicinal product).

Serious Adverse Event (SAE): A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:

- Results in death,
- Is life threatening,
- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- In a congenital abnormally/birth defect
- Any other medical event required medical or surgical intervention and judged as serious adverse events by treating physician or Chief Investigator.

Intensity of adverse events:
Intensity of adverse events will be assessed as per the following classification:

Mild: An adverse event, usually transient in nature and generally not interfering with normal activities.
Moderate: An adverse event, which is sufficiently discomforting to interfere with normal activities.
Severe: An adverse event, which is incapacitating and prevents normal activities.

Relationship to the Study drugs

Relationship	Description
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. The event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. The patients' clinical condition, other concomitant treatments).
Possibly	There is some evidence to suggest a causal relation ship (e.g. Because the event occurs within a reasonable time after administration of the trial medication). However the influence of other factors may have contributed to the event (e.g. The patients' clinical condition, other concomitant treatments).
Probable	There is a evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definite	There is a clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Unassessable	There is insufficient or incomplete evidence to make a clinical judgment of the casual relationship

Reporting and Documentation of Adverse Events

Chief Investigator/physician in charge will be available during housing in the Pharmacokinetic Unit. Volunteers will be monitored throughout the study period for occurrence of adverse events. They will be advised to report the investigator/ Physician in charge of any inconvenience or adverse event that may occur during their stay at the Pharmacokinetic Unit and after Post discharge.

Chief Investigator will notify Sponsor about any adverse events that is both serious (fatal or life threatening) and unexpected within 24 hours after its appearance using phone, fax or email. The Ethics committee will be notified within 7 days through its chairman.

All other serious adverse events that are not fatal or life threatening must be reported not later than 14 days to the sponsor. All remaining adverse events will be collected, recorded and announced to the sponsor and to relevant authorities by the final report. Relationship/ causality used for reporting adverse events will be based on Chief Investigator's assessment of the event as unlikely, possibly, probable, definite, and inaccessible (as given in the table above).

All adverse events that are reported will be properly documented on the adverse event form. In particular information will induce description of the event details of the timing of the event to administration of the study medication, frequency of adverse event, description of the severity of adverse event, any treatment or diagnostic step taken in relative to the event, description of the outcome of the event, judgment by the chief Investigator/physician in charge of any relationship of the event to study medication or procedures and outcome of a repeated dose of the medication of the subject.

Serious Adverse Events

Serious adverse events will be reported to the local ethics committee, sponsor and to the regulatory health authorities of the study site by the Chief Investigator according to the local legal requirements as per mentioned.

Emergency Procedures

Emergency equipments and drugs will be available within the clinical unit. In case emergency treatment will be necessary, the treatment and the drugs used during the emergency should be documented. To handle Emergency, 24 hrs ambulance will be kept ready during study period and if necessary, volunteer will be transferred with necessary precaution to contract hospital for further management.

Safety Monitoring

During the entire stay in the facility, volunteers will be observed for any adverse event. Vitals (pulse and BP) will be recorded and monitored at pre-dose, 1.0, 2.0, 4.0 and 8.0 hrs post-dose ± 30 minutes of scheduled time (except at predose) and whenever necessary. Each volunteer will be asked for well being at the time of vital monitoring. Volunteers shall be instructed to report any side effect (nature, severity, onset and disappearance) whenever it appears. In the case of an adverse event, Chief Investigator and/or clinical investigator will decide whether to withdraw the volunteer from the study and to initiate appropriate treatment. If unwell, they will be evaluated clinically and their health status will be followed up till recovery. Treatment will be given if necessary at the Pharmacokinetic Unit or at a hospital identified by the Torrent Pharmaceuticals Limited. Clinical biochemistry and hematology tests will be repeated as part of safety monitoring at the end of study.

Post Study Safety Evaluation

The post-study safety evaluation includes haematological and biochemistry tests which will be recorded in "Post study evaluation section of during study case record form".

- Post study safety evaluation will be done within 7days after the withdrawal of last blood sample of the study.
- In case of discontinuers or dropouts by themselves who have taken the investigational product, they will be informed to come for post study safety evaluation. For those who report at clinical site within 7 days of discontinuation, Post study safety evaluation will be carried out.
- In case of withdrawal due to adverse events, post-study examination will be performed in the course of 24 hours and Volunteer will be observed until adverse event is not apparent or is stabilized on clinically acceptable level.

If the results of these examinations are found to have any clinically significant abnormality as decided by chief investigator, then the same will be recorded in the individual's AE form and reported.

Follow Up

Any adverse event, which occurs in the course of the study, should be monitored and followed up until it resolved according to the judgment of the Investigator.

All adverse events including serious adverse events which occur within 7 days after completion of the clinical study, which are considered to be related to the study must also be reported and treated accordingly.

12.0 Ethical Considerations

12.1 Basic Principles

The study would be performed in accordance with the principles of Declaration of Helsinki (Appendix 16.5). The Ethics committee shall approve study protocol, CRF and ICF. No volunteer will be enrolled in the study before ethics committee approval. The Chief Investigator will be responsible for informing Ethics Committee about any revision to protocol, CRF, ICF and any other written information provided to volunteer and subsequent approval for the same.

Written Informed Consent

Prior to being enrolled into the study, the volunteer must have consented to participate in response to a complete written and verbal explanation of the nature, scope and possible consequences of the study by a trained person in presence of physician in a form understandable to him and in case of any doubt it will be cleared by the physician.

The volunteers must be able to understand the full implications of their decision.

ICF will be available in vernacular language (Gujarati). It will explain the nature of the study, its objectives and potential risks. In addition, the following points must also be covered:

- a description of the aims of the study and how it will be organized
- the type of treatment and the way in which the volunteers will be allocated to treatment (e.g. by randomization)
- any negative effects possibly attributable to the study treatments
- the freedom to ask for further information at any time
- the volunteer's right to withdraw from the study at any time without giving reasons and without jeopardizing the further course of treatment
- the existence of volunteer insurance cover
- the right of the monitor and an independent authorized person to look into personal data.

Personal information will be treated as strictly confidential and not be publicly available.

The translated forms will be used for confirmation of the volunteer's consent by the signature of the responsible person delegated by investigator and the volunteer.

As required by the ICH-GCP guideline the volunteer authorizes in written form that the Volunteer's original medical records may be audited by the monitor(s), the auditor(s), and the regulatory authorities by direct access in accordance with the applicable laws and regulations.

Original personally signed and dated version of the informed consent will be left at the investigator's site and the photocopy of the same will be forwarded to the volunteer.

Obligations of Volunteers

The volunteers undertake the obligation to fulfill all study requirements if they consent to participate (except of valid medical reason for terminating their participation). However, all volunteers will be informed that they can withdraw at any time, whatever the reason.

Confidentiality and Data Protection

In order to maintain volunteer's confidentiality, all data recorded during the course of the study will be identified by volunteer's screening and Enrollment number. Data relating to the study will be stored in such a way as to prevent their communication to the third party, except if required by regulatory, Ethics committee, or Sponsors designated persons.

13.0 Analytical Method

13.1 Description

The samples of all the volunteers completing both the periods in the study will be analyzed. The concentrations of Carvedilol in plasma will be determined by means of an LC-MS-MS method at Bio-analytical department of Bio Evaluation Centre of Torrent Pharmaceuticals Limited.

The analyst will be held blind with respect to identification of the test or reference dosing sequence. The test and reference preparation will not be identified as such on the labels. The method will be validated for all respective parameters and be able to determine Carvedilol concentrations with sufficient Sensitivity and accuracy. A limit of quantification will be set to quantify the levels of drug adequately in plasma. The method validation has to include a pre-study validation with determination of stability of the stock solutions and of the analyte(s) in the biological matrix under processing conditions and during the entire period of storage, specificity, accuracy, precision, limit of quantification, and response function, as well as online validation with control samples at three concentration levels.

All samples of the same volunteer have to be measured in a single analytical run in order to eliminate the influence of the inter-assay variance on the assessment. The analyst had to provide a final analytical report with tables and chromatograms for all Volunteers who completed the study according to study protocol. The final

analytical report will be part of the final study report and will contain 20% of the chromatograms.

13.2 Validation Parameter

The validation will be carried out based on following parameters:

1. Specificity
2. Sensitivity
3. Carry over check
4. Linearity
5. Lower Limit of quantification
6. Between run accuracy: QC % nominal concentration
7. Between run precision: QC % CV
8. Within run accuracy: QC % nominal concentration
9. Within run precision: QC % CV
10. Recovery of analyte: QC mean
11. Recovery of internal standard: QC mean
12. Stock solution stability at 2-8°C
13. Stock solution stability of an internal standard at 2-8°C
14. Stability of matrix at room temperature
15. Stability of matrix at -20°C and -70°C
16. Stability of matrix after Freeze-Thaw cycles at -20°C and -70°C
17. Stability following sample processing
18. Matrix effect

14.0 Statistical Treatment

14.1 Plans for Pharmacokinetic Analysis

The following pharmacokinetic parameters would be estimated:

a)	Cmax	Maximum concentration of drug observed in plasma.
b)	Tmax	Time required to reach maximum concentration of drug in plasma.
c)	AUClast	Area under the plasma concentration vs time curve from time zero to the last measurable concentration time t (AUC_{0-t}).
d)	AUCINF	Area under the plasma concentration vs time curve from time zero to time infinity (AUC_{0-inf}).
e)	AUC_%Extrap	Extrapolated AUC percentage of total AUC.
f)	Lambda_z	Elimination Rate constant (K_{el}).
g)	Lambda_z_lower	The time point where log-linear elimination slope begins
h)	Lambda_z_upper	The sampling time of the last quantifiable concentration used to estimate the log-linear elimination slope
i)	HL_Lambda_z	Time taken by plasma concentration to reduce to 50% during the elimination phase ($T_{1/2}$).
j)	MRTlast	Mean Residence Time for which a drug molecule resides in body (MRT_{0-t}).

k)	MRTINF	Mean Residence Time when the drug concentration profile is extrapolated to infinity ($MRT_{0\text{-}inf}$).

- Tmax and Cmax are observed values.
- The AUClast values will be calculated using linear trapezoidal method.
- AUCINF = AUClast + C_t/Lambda_z (where C_t is the last concentration of the drug determined through experimentation).
- Apparent first order elimination or termination rate constant (Lambda_z) will be calculated as the negative slope of the log-linear terminal portion of the plasma concentration-time curve. This parameter will be calculated by the linear least square regression analysis using the last three (or more) non-zero plasma concentration.
- The elimination half-life (HL_Lambda_z) is obtained by dividing 0.693 by Lambda_z.
- MRTlast and MRTINF will be calculated by using following formula:

  MRTlast = AUMClast/AUClast

  MRTINF=AUMCINF/AUCINF

14.2 Sample Size Justification

According to regulatory requirements minimum required sample size is 24. Hence, total 26 volunteers will be an appropriate sample size considering 2 volunteers as potential dropouts to achieve a sufficient power. Drop out subjects would not be replaced.

14.3 Definition of the Acceptance Interval of the Pharmacokinetic Parameters

Descriptive statistics will be calculated for demography and safety parameters of Carvedilol.

Plasma concentration vs. time data of Carvedilol will be provided for all the volunteers who will complete both period of the study. To compare the bioavailability after administration of study drugs, the pharmacokinetic parameters [Tmax, Cmax, AUClast, AUCINF, AUC_%Extrap, Lambda_z, Lambda_z_lower, Lambda_z_upper, HL_Lambda_z, MRTlast, MRTINF] will be calculated for each volunteer. Descriptive statistics will be calculated for concentration vs. time data and all pharmacokinetic parameters.

ANOVA will be performed on the log-transformed data of Cmax, AUClast and AUCINF. The sequence, subject within sequence, period and formulation will be considered as sources of variation. The sequence effect will be tested using the subject within sequence effect as the error term. The formulation and period effects will be tested against the residual mean square error. Probability (p) values will be derived from Type III sum of squares and effects will be considered statistically significant if the probability is less than 0.05.

Inter-subject and Intra-subject percentage coefficient of variance (%CV) will be calculated for Cmax, AUClast and AUCINF.

Ratio analysis of test/Reference formulation will be calculated for primary pharmacokinetic parameters Cmax, AUClast and AUCINF.

The bioequivalence acceptance interval is set to 80.00% to 125.00%. To justify the bioequivalence claim; the 90% confidence interval of the intra-individual mean ratio (Test/Reference) and Anderson & Hauck test will be computed for the log-transformed primary pharmacokinetic parameters [Cmax, AUClast and AUCINF].

The actual values of Tmax for test and reference will be compared by non-parametric test.

All pharmacokinetic and bioequivalence analysis will be carried out using WinNonlin® (Version 5.2 or higher) and SAS® (Version 9.1.3 or higher), licensed software available at Torrent Pharmaceuticals Ltd.

14.4 Outlier Detection Method

Subject outliers will be defined in bioequivalence studies, if subjects will have discordant values of one or more pharmacokinetic parameters, when compared with other values for remaining subjects of the test versus reference response in the study.

Outliers will not be dropped from the analysis of the data solely on the basis of statistical test. Outliers will provide scientific evidence or explanations to justify the exclusion of the subject(s) data from statistical analysis. In this case, study results will be submitted with and without outlier.

14.5 Missing and BLQ Values

Missing values such as sample not submitted (SNS) or sample not analyzed (SNA) will be ignored from the calculations of pharmacokinetics parameters. If a below limit of quantification value (BLQ) occurs anywhere in between two detectable plasma concentration values then those values will be considered as missing and will be ignored from the calculation of pharmacokinetic parameters. If BLQ values are observed in the initial absorption phase or at the terminal elimination phase they will be treated as zero.

14.6 Missing samples

Missing sample can be due to withdrawal of volunteer, accidental spillage of samples or due to non-reporting of volunteer for ambulatory samples. The clinical data will clearly identify the missing samples. The individual missing samples will be dealt as per case to case and the chief investigator will evaluate its impact on the data.

Volunteers who are dropped out or withdrawn due to any reason will not be considered for pharmacokinetic and statistical analysis.

If the vomited volunteer is continued in the study than the statistical data will be provided with and without the data of vomited volunteer.

14.7 Pre-dose concentration

If the pre-dose concentration is ≤ 5% of Cmax value, the subject's data without any adjustment will be included in all pharmacokinetic measurements and calculations. If the pre-dose value is ≥ 5% of Cmax, the subject will be dropped from all BE study evaluations.

15.0 Acceptance Criteria for Deviation from Protocol

All the deviations from approved protocol (an unintended departure from the study plan after study initiation) and SOPs will be recorded according to in-house SOP and will be recorded in the Deviation Record Form. Acceptance criteria for deviation in the protocol for collection time point are up to ± 2 min for in-house samples and up to ±1 hr for ambulatory sample.

16.0 APPENDIXES

16.1 Form for Study Drug Accountability and Retention Record
16.2 Model of Free and Informed Consent Agreement (Informed Consent Form)
16.3 Adverse Event Form
16.4 Randomization Schedule
16.5 World Medical Association, Declaration of Helsinki
16.6 Food Menu
16.7 Laboratory Normal Ranges

References for Background Information:

1. Prescribing information: Coreg®

References for Protocol Preparation:

1. ANVISA Guidelines for relative bioavailability / bioequivalence tests Resolution - RE nº 1170, dated 19th April 2006.
2. ANVISA Guidelines for drafting of the protocol and technical report of the bioequivalence study Resolution - RE nº 894, dated 29th May 2003
3. ANVISA Guidelines for drafting of the technical report of the relative bioavailability/bioequivalence study Resolution - RE nº 895, dated 29th May 2003
4. ANVISA Guidelines for planning and execution of the statistical phase. Resolution - RE nº 898, dated 29th May 2003
5. ANVISA Guidelines for the validation of analytical and bioanalytical methods Resolution - RE nº 899, dated 29th May 2003
6. ANVISA Guidelines for the execution of the study and drafting of the report of pharmaceutical equivalence Resolution - RE nº 900, dated 29th May 2003
7. Manual for Good Bioavailability and Bioequivalence Practices. Volume I, Brazilian Sanitary Surveillance Agency. Brasilia 2002
8. ICH Harmonized Tripartite Guideline for GCP 1996

16.1 SAMPLE RETENTION & INVENTORY OF DRUGS USED IN THE STUDY

Study Code:		Test / Reference:	
Generic Name: Strength/Formulation		Manufacturer /Country:	
Brand name:		No. of units received:	
Batch no.		Quantity to be retained:	
Expiry date/ Retest date/ Use by date		Retention storage identification:	
Storage condition:		To be retained up to:	
Storage Location:		Sign / Date:	

DESCRIPTION OF USE:

Date	Opening Balance	Period	Qty Used	Closing Balance	Extra dispensed	Un Dispensed (a)	Withdrawal By	Checked By

❖ Remarks:

QUANTITY RETURENED FROM BIO-STUDY:

Date	Period	Extra Dispensed (b)	Un dosed (c)	b + c	Remarks	Received By	Total Qty d=(a+b+c)

❖ No. of unused drug disposed after BA/BE study (d):

❖ Comments:

	Prepared By (Pharmacist)	Checked By (Sub-Investigator)	Approved By (Chief- Investigator)
Name:			
Sign /Date			

16.2 MODEL OF FREE AND INFORMED CONSENT AGREEMENT

STUDY CODE: PK-07-108

INFORMED CONSENT FORM

For

Carvedilol 25mg Single Dose Fasted State Bioequivalence Study

Version No.:	01
Date:	October 12, 2007
Supersedes Version No.:	Not Applicable
Date:	Not Applicable

Dear Volunteer,

You are being invited to participate in a research study. You are being asked to volunteer since you meet the requirements for enrollment into this study. Your participation is voluntary which means you can choose whether or not you want to participate. If you choose not to participate, there will be no loss of benefits to which you are otherwise entitled. Before you can make your decision, you will need to know what the study is about, the possible risks and benefits of being in this study, and what you will have to do in this study. The research team is going to talk to you about the research study, and they will give you this consent form to read. You may find some of the medical language difficult to understand, please ask the study doctor and/or the research team about that. If you decide to participate, you will be required to sign this form.

It may become necessary to seek details of your health and family history. Since this is mostly for your own safety, please do not hide any facts. You can rest assured that all your personal details and your identity will be kept strictly confidential at Bio-Evaluation Centre, except it demanded by Governmental Regulatory Authorities, an Institutional Ethics Committee or the Sponsor.

In case of medical emergencies during the study or if any urgent questions related to the study needs advice, you may contact the following personnel:

Dr. Jogesh Mahajan, *MBBS*, Chief Investigator, Bio-Evaluation Centre, Torrent Pharmaceuticals Limited, village Bhat, Gandhinagar, 079-23969100 (Extn: 270)
Dr. Sushil Kumar Anand, *MBBS*, Clinical Investigator, Bio-Evaluation Centre, Torrent Pharmaceuticals Limited, village Bhat, Gandhinagar, 079-23969100 (Extn: 280/281)

If you have questions regarding your rights as human volunteer, you are free to contact, Institutional Ethics Committee (IEC), at the following contact number,

Dr. R. K. Dikshit *MD*, Chairman, IEC, Phone: 079-22683721 Ext: 1275
Dr. A. J. Singh *MD, DNB, D.Pharm. Med(UK)*, Member, IEC, Phone: 079-26577625

Volunteer's Signature: _____

Dear Volunteer,

We thank you for showing interest in participating in this study as a subject. This is a single dose bioequivalence study of Carvedilol tablet containing Carvedilol 25mg [Torrent Pharmaceuticals Ltd., India] Versus Coreg® tablet containing Carvedilol 25mg [Produtos Roche Quimicos e Farmaceuticos S.A., Brazil]. But before going to the specific details about the Carvedilol study, let us explain you about bioavailability and bioequivalence.

Introduction

Whenever we eat anything, say food, it gets fragmented to smaller and smaller particles, then gets dissolved and finally goes into our blood. In the case of food, splitting it into tiny fragments is called digestion and getting into the blood is called absorption. In the same way, when we take a medicine (e.g. capsule or tablet or solution), it finally gets absorbed into blood.

What are drug formulations?

When we talk about a medicine, it is practically a dosage form that contains the active ingredient (drug) (which actually gives the therapeutic effect) and some other substances called excipients. Excipients are needed to help to form a specific shape (to the tablet) or to add some other feature, for example taste or color. The specific quantity of the active ingredient and the specific proportions of the excipients together form a Formulation.

Some medicines are administered directly into the blood as injections; in such cases the entire drug enters into the blood at once, so its bioavailability is 100%. However, when a drug is taken orally, it behaves in quite a different manner. Drug enters into the blood slowly from the stomach or intestine. Whole quantity of drug never gets absorbed into blood; instead only part of it gets absorbed. Oral bioavailability is the amount of drug taken orally and fraction of that finally gets absorbed into blood. Thus, the amount of the drug (that you swallowed) in a given volume of your blood (called concentration) increases slowly as more and more drug enters into the blood. This concentration reaches a peak or maximum around the time when drug has been fully absorbed from the gut. Then, the concentration slowly declines as the body eliminates and modifies the drug through various means. The change in the drug concentration in blood over a period of time is called the plasma drug concentration profile of the medicine.

What are bioequivalence studies?

If two formulations administered in same molar dose and with same route of administration and bioavailability of both the formulations are similar they are called bioequivalent of each other and this phenomenon is known as bioequivalence. These studies are carried out in healthy, adult, human subjects. General explanation of these studies is given below:

A group of subjects are chosen based on their healthiness by carrying out various screening, diagnostic tests and medical examination. Total number of subjects is decided on the basis of property of the drug and purpose of study.

The chosen group is splitted randomly into 2 subgroups and each subgroup is randomly assigned a sequence: RT (for Reference-Test) or TR (for Test-Reference).

Volunteer's Signature: _____

The entire group is admitted into a clinic; the length of duration for the admission depends on the study. During their admission into the clinic, subjects have to follow certain restrictions, which are clearly explained to them. During this first admission (or first period), one group is given the Reference formulation and one group is given the Test formulation. Thereafter, blood samples are withdrawn from each subject at pre-determined times and collected blood samples (either as it is or after some processing) are stored in a deep freezer. The number of blood samples drawn and the timing for these samples are determined on the basis of properties of the medicine.

Then the entire group is discharged to go home with instructions to return on another specific date at a specific time for another admission. Subjects lead a normal life during this time. The entire group returns to the clinic at the specified time for the second period. This time the one group is given the Test formulation who received reference formulation and one group is given the reference formulation who received test formulation. Once again blood samples are withdrawn from each subject at pre-specified times like in the earlier period.

A number of blood samples are drawn and not simply a single sample. This is because we are looking to determine the bioavailability profile (concentration as it varies over time) and not simply the concentration at one specific time. Indeed in some studies, subjects are asked to return to the clinic even after their discharge for giving blood samples. Such samples are called Ambulatory Samples.

Completion of all admissions and any ambulatory samples concludes the clinical phase of the project. Then comes the Bio-analytical phase in which the samples are analysed to determine the concentrations of the medicine. Finally, statistical tests are applied to compare the bioavailability profiles of the two formulations. If the profiles are similar, the two formulations are deemed bioequivalent to each other; otherwise they are not.

Why are bioequivalence studies conducted?

In many parts of the world, medicines are protected by patents. This means no one else than innovator (the company which originally discovered the medicine) can market the drug. However patents are valid only for a limited period of time, the duration depends on the country. If someone wants to sell the drug before the patent expires, they have to obtain permission from the innovator company. But after the patent expires, anyone can market the medicine. Such "copies" of innovator medicine is called Generic. A company that wishes to sell a generic version of a medicine has to prove that their formulation is as good as the original innovator medicine. To prove this, two kinds of tests are done: First, some simple chemical tests are done to prove the generic has the same substance in same quantity as the innovator medicine. Second, it has to be proved that the generic medicine reaches the blood at same extent as innovator after being administered.

In other words, bioequivalence has to be proven between the innovator medicine (called Reference formulation) and the Generic medicine (called Test formulation). Governmental agencies carefully examine the results of study. If they are satisfied (that the two formulations are bioequivalent), the Generic company may get permission to sell their formulation.

This elaborate procedure is meant to safeguard of patients using this type of medicine. Due to this procedure, patients buying medicines can be confident that it will be effective without

Volunteer's Signature: _____

regard to the company that manufactured it. The prices of these types of medicines are very high because no one else is allowed to sell the medicine during the patent lifetime. Therefore, bioequivalence studies benefiting mankind by lowering the overall cost of medicines.

Background information

Carvedilol is a beta adrenergic receptor antagonist. It causes a dose-dependent, long-lasting reduction in arterial blood pressure.

Use and Dosage

Carvedilol should be taken with food to slow the rate of absorption and reduce the incidence of orthostatic effects. Dosage should be individualized.

Heart failure: Start at 3.125 mg twice daily and increase to 6.25, 12.5, and then 25 mg twice daily over intervals of at least 2 weeks. Maintain lower doses if higher doses are not tolerated.

Left ventricular dysfunction following myocardial infarction: Start at 6.25 mg twice daily and increase to 12.5 mg then 25 mg twice daily after interval of 3 to 10 days. A lower starting dose or slower titration may be used.

Hypertension: Start at 6.25 mg twice daily and increase if needed for blood pressure control to 12.5 mg then 25 mg twice daily over intervals of 1 to 2 weeks.

Adverse Effects, Risk and Discomforts Associated with Drug

Carvedilol is well tolerated as a once daily 25 mg dose. Overall incidence of withdrawal due to adverse effects was reported only in 7% of patients. The most common adverse effects causing discontinuation of treatment were dizziness/vertigo, headache, hypotension, bronchospasm, fatigue and skin reactions. Other adverse events rarely reported were loose stools, dry mouth, depression, constipation, itching and/or rash. If you experience any of the adverse effects mentioned above or any other unusual symptoms, notify the same to the study physician.

In addition there is a small but real risk of allergic reactions with any medication. These reactions usually arise shortly after dosing as skin itching, redness and difficulty to breath, and may be severe in some cases. Among other known risks are those, related to discomfort or redness of the skin punctured for indwelling cannula. The amount of blood collected in the course of the study cannot do any harm to a healthy person. The amount of blood will be withdrawn is less than the amount of blood withdrawn at blood donation. After taking medicine do not undergo any surgical procedure or tooth extraction. If, it is unavoidable then, please inform to physician before deciding to participate in the study.

Dose for the Subject in this Study

Single tablet of Carvedilol 25mg will be administered with 200 ml of water in each period.

Purpose of this research study

This clinical research study involves 26 healthy male volunteers aged 18-45 years. This study is non-therapeutic clinical study on a drug called carvedilol, which is used for the treatment

Volunteer's Signature: _____

of mild to severe chronic heart failure, Left ventricular dysfunction following myocardial infarction in clinically stable patients and hypertension. The goal of this study is to assess the bioequivalence after single dose administration.

Torrent Pharmaceuticals Ltd., India has developed Carvedilol 25mg tablet. This preparation will be compared with reference Coreg® 25mg tablet manufactured by Produtos Roche Quimicos e Farmaceuticos S.A., Brazil, in respect to their absorption after single dose drug administration in healthy volunteers. To compare them, the quantity of drug found in the biological fluids (e.g. blood, urine etc) will be measured.

Study procedure

This is a two period, two treatment, single dose crossover study in fasting conditions.

Each period will be involving a stay of approximately 44 hours at the pharmacokinetic unit. During stay meals, vitals, water, study drug administration time etc are as per the schedule mentioned on page no. 45.

You will be admitted to the research facility a day before dose administration (at least 12 hours before) in each period. Your time of reporting to the facility will be reported and detail explanation of study medication and procedure will be given, thereafter you will be given informed consent form (In period I) to read and sign. Brief medical examination, vitals monitoring and history will be taken for your eligibility for study. Compliance questionnaire will be applied for taking history. Urine test will be performed for the checking of drugs of abuse. If you will be eligible then ID card and cloths will be provided you. Dinner will be served to you between 20:00 hrs to 21:00 hours and you will not be allowed to eat anything till the next morning. Breakfast, lunches, snacks and dinner will be served as per the schedule mentioned on page no 45.

Clinical examination will be done at the time of admission and at the time of discharge. Vital will be monitored at regular intervals specified in protocol (as per schedule page no. 45).

At scheduled time the study drug will be administered as per the protocol (as per schedule page no. 45). The type of study drug first received by you will be determined as per randomization. A physician will be present during your stay in the clinical facility and will check your health condition. Food intake will be standardized during your staying in the clinical facility.

The in-house samples will be withdrawn by indwelling cannula in your arm, while ambulatory samples are withdrawn by direct venous puncture. You may feel slight pain during these procedures.

A total of [(Each period: 21 samples of 5ml each + 20 x 0.5ml heparinized blood) + 3ml for safety evaluation at the end of second period] =233ml blood will be collected during study using indwelling intravenous cannula. Blood samples will be collected at the following times: Pre-dose, 0.166, 0.333, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 6.00, 8.00, 12.00, 18.00, 24.00, 32.00 and 48.0 hours post dose after drug administration. You will be discharged at the end of 32 hours of the drug administration if found to be in good health. After completion of both the period, physical examination, biochemical and haematological tests will be performed.

Volunteer's Signature: _____

Each period will be separated by at least 7 days (gap) to ensure that the drug taken in the previous period is no longer in the body system. After the washout period the next part of the study will be carried out and all procedures will be repeated in the same way, using the comparator study drug.

Total blood loss

During study total blood loss is approximately 233ml. The blood loss during blood donation is around 350ml at single period of time. While in case of study your total blood loss is less than blood donation loss and over a period of time.

Restrictions during study

During the study:

1. You will have to comply with study procedure & medical personal instructions.
2. You will be required to be available at the bed or dosing station before drug administration.
3. You will not be allowed to drink water for 1 hour before and 2 hours after drug administration.
4. You will be in sitting or semi-reclining position for 4 hrs after the drug administration. Thereafter, you will be free to move but not out of pharmacokinetic unit
5. You will have to avoid doing any sort of physical stressful activity.
6. You will be restricted from taking any medication (including over-the-counter products), throughout the study, unless authorized by the chief investigator.
7. You will abstain from consuming alcohol, tobacco in any form and smoking for at least 48 hours before enrollment and during the entire stay in Pharmacokinetic Unit.
8. You will only consume the food that is served to you and will be strictly prohibited from taking tobacco, grape fruit juice, fruit beverages, alcohol and xanthine containing food items, tea, coffee and chocolates before 48 hours of check in till end of the study.

Safety monitoring during study

You will be discharged at the end of 32 hours after drug administration, if you are in good health and not suffering from any adverse events. In case of any adverse events, you will be given proper medical care and kept under observation until recovery. All drug and/ or study related adverse events would be treated by the attending physician either at the Pharmacokinetic Unit or at a suitable nearby hospital at no extra cost to you. You do not give up any of your legal rights by participating in this study. You must immediately contact any of person listed on front page if you believe you have injury caused by the study.

Benefits of study

Since you do not require treatment with any of the study drug medications, you are unlikely to be benefited by taking these medications. By participating in this study you will get a free medical check-up, a study participation fee plus your satisfaction of serving the interest of drug research.

Volunteer's Signature: _____

Insurance

Bio-Evaluation Centre, Torrent Pharmaceuticals Limited has taken insurance coverage in accordance with Indian laws. The volunteers are therefore insured for damage resulting from the study.

Withdrawal

Your participation in this study is completely voluntary. You may decide to discontinue your participation in the study at any time, without any penalty and without giving any reason. The investigator may also withdraw you from the study at any time if he considers it necessary for health reasons or due to your violation of requirements of the study or because of your willful misinformation on present and/ or past medical illness/ history.

Confidentiality

All information about your participating in this study will be kept strictly confidential. However, your original clinical records may be inspected by representatives of the study sponsors, by the Institutional ethics committee or other regulatory agencies for verification of study procedures. If the results of the study are published your identity will remain confidential.

Use of study data in future

Occasionally, the same or another researcher will request the permission to review or use previously gathered data from a completed research project for a different project. If confidentiality of the data is protected and if a human subject protection committee has approved the study, would you be willing to give your permission to the release of your data collected from your participation in the current study without prior notification?

Responsibility during study

You will be requested to co-operate with the Pharmacokinetic Unit staff. On arrival and departure, you and your baggage will be searched. During your participation in this study you will be expected to abide by the rules of the organization and maintain discipline during the course of stay for the study. You will be requested to read and understand informed consent form carefully and sign all pages. All the clarifications and questions will be asked during the verbal explanation of informed consent form.

Compensation

You will be getting Rs. 4300/- for the complete study. Total payment will be split on a 20:80 (2-period basis). The volunteers shall give a duly signed stamped receipt for the payment received by them. The payment is subject to the condition that you will follow all instructions and study protocol exactly. In case of premature withdrawal from the study the volunteer will be entitled to the following compensation.

Volunteer's Signature: _____

Reasons of withdrawal from the study	Compensation
Withdrawn from the study on medical decision, for the volunteer's health interest by the attending physician/investigator	100% proportionate participation dues or as per IEC guidelines
After initiation of the study if the volunteer withdraws on his/her free will	Proportionate participation dues or as per IEC guidelines
If volunteer withdrawn from the study by the investigator due to his/her violation of requirements of the study or non-adherence to the study restrictions or because of his wilful misinformation on present and/or past medical illness/history	No payment

What if new information becomes available about the study?

During the course of this study, we may find more information that could be important to you. This includes information that, once learned, might cause you to change your mind about being in the study. We will notify you as soon as possible if such information becomes available.

Volunteer's Signature: _____

SCHEDULE

Below mentioned schedule is the tentative time table of each period during study. These activities will be repeated after 7 days of wash-out period.

Time in relative to dosing(Hrs)	Time (Hrs)	Day	Events
-20.0 to -14.0	12:0 to 18:0	D-0	Reporting for informed consent, ICF presentation and obtaining written consent (period-I only), criteria check (period-I only) and compliance assessment
-14.0 to -12.0	18:0 to 20:0	D-0	Clinical examination, Urine drug screen and Check-in
-12.0 to -10.0	20:0 to 21:0	D-0	Dinner
-10.0 to -9.0	22:0 to 23:0	D-0	Bed time
-2.50	05:30	D-1	Wake up call
-2.0 to -0.50	06:0 to 07:30	D-1	Pre-dose Vital signs, Cannulation, Pre-dose blood sample collection
0.00	08:00	D-1	Study drug administration **(Carvedilol 25mg)**
0.166	08:10	D-1	Blood Draw
0.333	08:20	D-1	Blood Draw
0.50	08:30	D-1	Blood Draw
0.75	08:45	D-1	Blood Draw
1.00	09:00	D-1	Blood Draw **followed by vitals**
1.25	09:15	D-1	Blood Draw
1.50	09:30	D-1	Blood Draw
1.75	09:45	D-1	Blood Draw
2.00	10:00	D-1	Blood Draw **followed by vitals**
2.50	10:30	D-1	Blood Draw
3.00	11:00	D-1	Blood Draw
3.50	11:30	D-1	Blood Draw
4.00	12:00	D-1	Blood Draw **followed by vitals** followed by **lunch**
6.00	14:00	D-1	Blood Draw
8.00	16:00	D-1	Blood Draw **followed by vitals** followed by **snacks**
12.00	20:00	D-1	Blood Draw followed by **dinner**
18.00	02:00	D-2	Blood Draw
24.00	08:00	D-2	Blood Draw followed by **breakfast**
28.00	12:00	D-2	**Lunch**
32.00	16:00	D-2	Blood Draw followed by **snacks** followed by medical examination and discharge
48.00	08:00	D-3	Ambulatory Blood Draw

Day-0: Entry day, Day-1: Drug administration day, Day-2: Discharge day, Day-3: Ambulatory Sample
Note: Medical examination will be carried out if adverse effect occurs or physician requires.

Volunteer's Signature: _____

VOLUNTEER DECLARATION AND SIGNATURE

Please read the declarations mentioned below and if you feel comfortable about taking part in the study, put your signature at the specified space of this document. Please note that there is no pressure of any kind from Bio-Evaluation Centre on you to participate. Please take part only if you are fully satisfied that you would like to do so.

Declarations by the Volunteer

- I have read this informed consent document, it is explained to me to my satisfaction, and I have understood it. Where I had doubts or questions, I had clarified them by study personnel.
- I understand that I am deemed medically fit enough to participate in this project and I will not gain any therapeutic benefit from participating in this study and its only for the purpose of research. However, if the test formulation is found to be bioequivalent, this could lead to lowering of prices for the drug due to increased competition, which is a possible benefit to mankind.
- I understand that I will not take up any financial encumbrance as a result of taking part in this study. All diagnostic costs and expenses related to any hospitalizations will be borne by torrent pharmaceuticals Ltd.
- I understand the risks to me of taking part in this study as explained in the adverse effects section of "Background information". I understand that these risks include possible hospitalization.
- I understand that I have to be present at clinical facility of Bio-Evaluation Centre as specified in timetable of events and to comply with other instructions.
- I declare that I did not take part in a drug trial study at any company in the past 3 months.
- I understand that taking part in these studies more frequently than once in 3 months is injurious to my health.
- I agree not to commit any misbehaviour or misconduct with any study personnel or with any member of staff and disobeying that will make me liable for legal consequences.
- I agree not to cause any damage or loss of any property of Bio-Evaluation Centre.
- I am 18 years or older. I have given facts to the best of my knowledge to study personnel about my medical and family history.
- I am aware that my identity and personal details will be kept confidential and will not be revealed to anyone except the IEC, the Regulatory Agency (ies) and the Sponsor's inspectors/auditors.
- I am aware that I can withdraw my consent from this project at any time during the course of the project even without disclosing the reason(s) thereof and that I shall not be deprived of any medical care that I should get for participation in this project and this will not take away my right for future participation in such projects.
- I am giving my consent voluntarily and absolutely free from duress of any kind.
- I am aware that one photocopy of this signed document will be provided to me, if I asked for the same.
- I am provided with the contact details of all the relevant persons whom I can contact for any project-related query or queries pertaining to my rights as a subject.

Volunteer's Signature: _____

I am Mr./Ms._____,

my father's name is _____,

my mother's/gardian's name is _____,

and my full residential address is _____,

_____

and my phone number is: _____,

I hereby give my voluntary free consent (means without any. coercion, misrepresentation and fraud) for including myself as a subject in the single dose bioequivalence study of Carvedilol 25mg in healthy human subjects under fasting conditions.

Signature of the volunteer:	Date:
Name of witness:	
Address of witness:	
Phone No.:	
Signature of the witness:	Date:
Medical query resolved by:	Date:
Written informed consent obtained by:	Date:
ICF Checked by:	Date:
Allotted Enrollment No.:	
Photocopy of the signed ICF received by:	Date:

16.3 FORM FOR RECORDING OF ADVERSE EVENTS

Study Code: PK-	Period No.	Volunteer ID. PK-
Date:	Reporting Time:	Enrollment No.

Details of Adverse Event:

Onset Date & Time	Date & Time of last dose

Present complaint/(s):_____

_____

_____

_____

Relevant history (if any): _____

_____

_____

_____

Examination Findings:_____

_____

_____

_____

Management:_____

_____

_____

_____

(If any medication given please enter details in "Concomitant Medication")

_____

(Physician's Signature & Date)

Follow-up of the Volunteer:

(If any medication given please enter details in "Concomitant Medication")

Adverse Event Assessment:

Adverse Event	Date & Time of onset	Date & Time of last Dose (hh:mm)	Duration of AE (H-hrs M-Min)	Current status 1=continuing 2=recovered w/o sequelae 3= recovered w/sequelae 4=indeterminate	Severity 1=Mild 2=Moderate 3=Severe	Seriousness 1=not serious 2=serious, not expected 3= serious, expected	Date & Time of resolution	Study drug related 1=Unlikely 2=Possible 3=Probable 4=Definite 5=Unassesable	Course of Adverse Event 1=disappeared w/study drug 2=tolerated w/study drug 3=study drug discontinued 4=other	Treatment required 1=None 2=OTC 3=Prescription Drug 4=Hospitalization 5=Non-Drug Therapy	If drug discontinued date of reinstitution dd/mm/yy

Physician's Sign & Date: _____ Chief Investigator's Sign & Date: _____

Scales for Adverse Event Reporting:

Relationship	Description
Unlikely	There is little evidence to suggest there is a causal relationship.(e.g. The event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. The patient's clinical condition, other concomitant treatments).
Possible	There is some evidence to suggest a causal relation ship. (e.g. Because the event occurs within a reasonable time after administration of the trial medication).However the influence of other factors may have contributed to the event (e.g. The patients clinical condition, other concomitant treatments).
Probable	There is a evidence to suggest a causal relationship and the influence of other factors is unlikely
Definite	There is a clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Inaccessible	There is insufficient or incomplete evidence to make a clinical judgment of the causal relationship
Severity	**Description**
Mild:	An adverse event, usually transient in nature and generally not interfering with normal activities
Moderate:	An adverse event, which is sufficiently disconforting to interfere with normal activities
Severe:	An adverse event, which is incapacitating and prevents normal activities

CONCOMITANT MEDICATION DURING STUDY

Sr. no	Date	Generic name	Brand name	Batch no	Expiry Date	Quantity Used	Physician's Sign

Comment on drug interaction with study drug: _____

_____

Chief Investigators sign & date: _____

16.4 RANDOMIZATION LIST

Study Code : PK-07-108

Randomization Schedule : Carvedilol 25mg Fasted BE Study

Volunteer No.	Period-1	Period-2
1	B	A
2	A	B
3	A	B
4	A	B
5	B	A
6	B	A
7	B	A
8	A	B
9	B	A
10	A	B
11	B	A
12	A	B
13	B	A
14	A	B
15	B	A
16	B	A
17	A	B
18	A	B
19	B	A
20	B	A
21	A	B
22	A	B
23	A	B
24	B	A
25	A	B
26	B	A
A =	13	13
B =	13	13

Product: A (Test)	Carvedilol
Brand Name:	-
Formulation:	Tablet
Batch No:	B9717002
Expiry:	January 2009
Mfg. By:	Torrent Pharmaceuticals Limited, India
Dose	25mg
Product: B (Reference)	Carvedilol
Brand Name:	Coreg®
Formulation:	Tablet
Batch No:	RJ0382
Expiry:	July 2009
Mfg. By:	Produtos Roche Quimicos e Farmaceuticos S.A., Brazil
Dose	25mg

16.5 WORLD MEDICAL ASSOCIATION, DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975, 35th WMA General Assembly, Venice, Italy, October 1983, 41st WMA General Assembly, Hong Kong, September 1989, 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996, and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000, Note of Clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002, Note of Clarification on Paragraph 30 added by the WMA General Assembly, Tokyo 2004

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
4. Medical progress is based on research, which ultimately must rest in part on experimentation involving human subjects.
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
12. Appropriate caution must be exercised in the conduct of research, which may affect the environment, and the welfare of animals used for research must be respected.
13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
20. The subjects must be volunteers and informed participants in the research project.
21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.
23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.
25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.
26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.
27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.
29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.[1]
30. At the conclusion of the study, every patient entered into the study should be assured of access to the best-proven prophylactic, diagnostic and therapeutic methods identified by the study.[2]
31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.
32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

[1]Note of clarification on paragraph 29 of the WMA Declaration of Helsinki

The WMA hereby reaffirms its position that extreme care must be taken in making use of a placebo-controlled trial and that in general this methodology should only be used in the absence of existing proven therapy. However, a placebo-controlled trial may be ethically acceptable, even if proven therapy is available, under the following circumstances:

- Where for compelling and scientifically sound methodological reasons its use is necessary to determine the efficacy or safety of a prophylactic, diagnostic or therapeutic method; or
- Where a prophylactic, diagnostic or therapeutic method is being investigated for a minor condition and the patients who receive placebo will not be subject to any additional risk of serious or irreversible harm.

All other provisions of the Declaration of Helsinki must be adhered to, especially the need for appropriate ethical and scientific review.

[2]Note of clarification on paragraph 30 of the WMA Declaration of Helsinki

The WMA hereby reaffirms its position that it is necessary during the study planning process to identify post-trial access by study participants to prophylactic, diagnostic and therapeutic procedures identified as beneficial in the study or access to other appropriate care. Post-trial access arrangements or other care must be described in the study protocol so the ethical review committee may consider such arrangements during its review.

16.6 FOOD MENU FOR FASTED STATE STUDY

CHECK-IN DAY			
SNACKS			
Items	**No.**	**Amount (gms)**	**Kcal**
Wada pau	2	150	376.44
Chutney		15	50.61
			427.05
DINNER			
Items	**No.**	**Amount (gms)**	**Kcal**
Chapati	6	120	463.20
Rice	1 plate	120	259.5
Curd	1 small bowl	50	30.00
Guj.Tuar Dal	2 bowl	200	146.5
Red Gram (Tuar Whole)	1 bowl	100	101.00
Cabbage Mutter	1 bowl	100	90.00
Papad (Black gram)	1	7	24.02
			1114.22
STUDY DAY			
LUNCH			
Items	**No.**	**Amount (gms)**	**Kcal**
Chapati	6	120	463.20
Lemon Rice	1 plate	120	268.05
Curd	1 small bowl	50	30.00
Guj Tuar Dal	2 bowl	200	146.5
Paneer Tikka Masala	1 bowl	100	201.44
Mung dhal palak	1 bowl	100	80.56
Salad	Cabbage, Cucumber, Carrot	50	10.01
			1199.76
SNACKS			
Peas Sandwitch	2	140	268.56
Milk	1cup	150	158.68
			427.24
DINNER			
Chapati	6	120	462.85
Hariyala Pulao	1 plate	100	236.37
Kofta curry	2 bowl	200	135.78
Cauliflower Masala	1 bowl	100	57.87
Chhole	1 bowl	100	114.21
Khir	1 bowl	100	156.10
			1200.92

BREAKFAST			
Items	**No.**	**Amount (gms)**	**Kcal**
Bread Vada	3	120	219.46
Milk	1 cup	150	158.68
			378.14
LUNCH			
Chapati	6	120	462.85
Rice	1 plate	120	259.5
Guj.Tuar Dal	2 bowl	200	146.5
Butter Milk	1 cup	100	15.00
Mix Vegetable	Potato, Carrot Peas, Cauliflower	100	121.2
Salad	Cabbage, Cucumber, Carrot	50	10.01
Rajmah Masala	1 bowl	100	120.40
Papad (Black gram)	1	7	24.02
			1159.48
SNACKS			
Kachori	2	100	291.22
Chutney		30	101.35
			392.57

Reference: Gopalan C. *et al* (2004). Nutritive value of Indian foods, NIN, Indian Council of Medical Research, Hyderabad.

16.7 NORMAL LABORATORY VALUES

TORRENT RESEARCH CENTRE

PRECLINICAL SAFETY EVALUATION DEPARTMENT, PATHOLOGY LABORATORY

HEMATOLOGICAL & BIOCHEMICAL ANALYSIS REPORT

Project code:		Compound ID:	
Requested by:		Date of sampling:	
Volunteer/Employee ID No.:		Date of Analysis:	
PARAMETER	Normal range* MALE	FEMALE	Observed values
HEMATOLOGY			
WBC x K/ µl	4.0 - 10.0	4.0 - 10.0	
Neutrophils %	40.0 - 80.0	40.0 - 80.0	
Lymphocytes %	20.0 - 40.0	20.0 - 40.0	
Monocytes %	2.0 - 10.0	2.0 - 10.0	
Eosinophils %	1.0 - 6.0	1.0 - 6.0	
Basophil %	< 1 - 2	< 1 - 2	
RBC x M/ µl	4.5 - 5.5	3.8 - 4.8	
Hb[g/dl]	12.5 - 17.0	12.0 - 15.0	
HCT%	40.0 - 50.0	37.0 - 46.0	
Platelets x K/ µl	150 - 400	150 - 400	
BIOCHEMISTRY			
Albumin (g/dl)	3.5-5.2	3.5-5.2	
Total Proteins (g/dl)	6.4-8.3	6.4-8.3	
Globulin (g/dl)*	2.3-3.5	2.3-3.5	
ALT [SGPT](U/L)	<46	<34	
Total Bilirubin (mg/dl)	0.3-1.2	0.3-1.2	
AST [SGOT] (U/L)	<35	<31	
Serum Alkaline Phosphatase U/L	30-120	30-120	
Random Blood Sugar (mg/dl)	45-130	45-130	
Serum Creatinine (mg/dl)	0.84-1.25	0.66-1.09	
Triglyceride (mg/dl)	<150	<150	
Cholesterol (mg/dl)	150-250	150-250	
Calcium (mg/dl)	8.8-10.6	8.8-10.6	
Sodium (mEq/L)	136-145	136-145	
Potassium (mEq/L)	3.5-5.1	3.5-5.1	
Chloride (mEq/L)	98-106	98-106	
GGT (U/L)	<55	<38	
URINALYSIS			
Glucose	Negative	Negative	
Bilirubin	Negative	Negative	
Ketone	Negative	Negative	
Sp.Gr.	1.001 - 1.035	1.001 - 1.035	
Blood	Negative	Negative	
pH	5 - 9	5 - 9	
Protein	Negative	Negative	
U.bil.gen E.U./dL*	0.2 - 1.0	0.2 - 1.0	
Nitrite	Negative	Negative	
Leucocytes	Negative	Negative	
URINALYSIS: Physical and Microscopic			
Quantity		Pus cell	
Color		RBC	
Appearance		Cast	
Clarity		Crystals	
Epithelial cell		Others	

Abbriviations used:WBC-White blood corpuscles,RBC-Red blood corpuscles,Hb-Hemoglobin,HCT-Hematocrit
ALT-Alanine Amino Transferase,AST-Aspartate Amino Transferase.

* Normal ranges for hematology are given as per reference: Dacie & Lewis Practical Haematology, 9th Edition, Page-12.
* Globulin values are calculated from Total protein & Albumin.
* Normal Ranges for biochemistry are given as per kit literature
  - Blood glucose range (Random sampling): Clinical diagnosis and management by laboratory methods, Henry et al, Ch.9, p.199
  - S. Electrolytes normal range as per Teitz textbook of clinical chemistry
  - Normal range for Globulin is given as per Clinical diagnosis and management by laboratory methods,Henry et al, 19th edition.
  - Normal ranges of urine parameters are provided according to kit manufacturers
* Normal ranges for hematology are given as per reference: Dacie & Lewis Practical Haematology, 9th Edition.
* Normal range for Cholesterol given as per Clinical Diagnosis and Management by Laboratory Methods, Henry et al, 19th edition

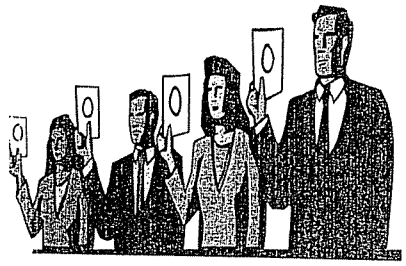
Pathologist

Dr.Sunil Advani (Pathologist)

2.4.2. APPROVAL REPORT OF THE COMMITTEE FOR ETHICS IN RESEARCH

INSTITUTIONAL ETHICS COMMITTEE

UNDER ICMR GUIDELINES ON "ETHICAL GUIDELINES FOR BIO-MEDICAL RESEARCH ON HUMAN SUBJECT" - 2000



Correspondence
Torrent Research Centre
Torrent Pharmaceuticals Ltd.
Village Bhat-382 428
Gandhinagar
Ph. No.079-239 69 100
Fax No.079-239 69 135

Certificate of Approval

1st November, 2007

The committee reviewed the following project:

Title: A Randomised, Open Label, Two-Period, Two-Treatment, Two-Sequence, Crossover, Single Dose, Bioequivalence Study of CARVEDILOL 25mg Tablets (TEST) [Torrent Pharmaceuticals Ltd., India] Versus CARVEDILOL 25mg Tablets (COREG®) (REFERENCE) [Produtos Roche Químicos e Farmacêuticos S.A., Brazil] In Healthy Human Subjects Under Fasted State.

STUDY CODE: PK-07-108 (Version – I)

Study Site**: - Bio Evaluation Centre, Village: Bhat, Dist: Gandhinagar.**

Documents Reviewed: -

1. Study Protocol
2. Consent Form

Subsequent to the fulfillment of the procedural requirements and other conditions imposed by the committee the project is hereby:

Granted an approval without any change	☑
Granted an approval with some changes	☐
Not Approved	☐

The principal investigator and the sponsors are required to read and comply with the following:

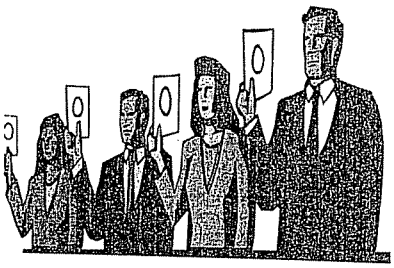
- The Committee has approved the ethical aspects of the proposed work. However, all other concerns related to the work (e.g. scientific, procedural, legal, financial and regulatory etc) remain the sole responsibility of the principal investigator and/or the sponsor(s).
- The committee must be informed from time to time about the progress of the work and a summary report of the project should be submitted within one month of the end of this work.
- Any serious/unexpected side effects must be communicated to the committee and any further instructions thereupon should be duly followed.

Dr. R.K. Dikshit

520

INSTITUTIONAL ETHICS COMMITTEE

(UNDER ICMR GUIDELINES ON "ETHICAL GUIDELINES FOR BIO-MEDICAL RESEARCH ON HUMAN SUBJECT" - 2000)



Correspondence
Torrent Research Centre
Torrent Pharmaceuticals Ltd.
Village Bhat-382 428
Gandhinagar
Ph. No.079-239 69 100
Fax No.079-239 69 135

STUDY CODE: PK-07-108 (Version – I)

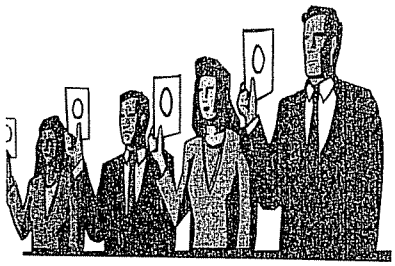
Title: **A Randomised, Open Label, Two-Period, Two-Treatment, Two-Sequence, Crossover, Single Dose, Bioequivalence Study of CARVEDILOL 25mg Tablets (TEST) [Torrent Pharmaceuticals Ltd., India] Versus CARVEDILOL 25mg Tablets (COREG®) (REFERENCE) [Produtos Roche Químicos e Farmacêuticos S.A., Brazil] In Healthy Human Subjects Under Fasted State.**

The following members were present during the Ethics Committee meeting held on **1st November, 2007.**

	Name & Designation	Signature
1.	**Dr. R. K. Dikshit** Professor & Head of the Department Department of Pharmacology B. J. Medical College, Civil Hospital, Ahmedabad.	
2.	**Shri. J. M. Vyas,** Director, Forensic Science Laboratory, Gandhinagar.	
3.	**Dr. Harshad Gandhi,** Consultant Physician & Cardiologist, Ahmedabad.	
4.	**Smt. Daxaben Bhatt,** Social Worker, Hospital for Mental Health, Ahmedabad.	

INSTITUTIONAL ETHICS COMMITTEE

(UNDER ICMR GUIDELINES ON "ETHICAL GUIDELINES FOR BIO-MEDICAL RESEARCH ON HUMAN SUBJECT" - 2000)



Correspondence
Torrent Research Centre
Torrent Pharmaceuticals Ltd.
Village Bhat-382 428
Gandhinagar
Ph. No.079-239 69 100
Fax No.079-239 69 135

01.11.2007

5. **Smt. Tejalben Thakore,**
Social Worker,
B. M. Institute of Mental Health,
Ahmedabad.

6. **Dr. Bashir Ahmadi,**
Consulting Neuro-Physician,
Ahmedabad.

7. **Dr. A. J. Singh,**
Associate Professor Pharmacology Dept.,
NHL Medical College,
V. S. Hospital,
Ahmedabad.

8. **Dr. Bharat Shah,**
Consultant Physician & Cardiologist
Ahmedabad.

9. **Dr. Atul Parikh,**
Consultant Physician,
Ahmedabad.

10. **Dr. Harsha Parikh,**
Consultant Physician,
Ahmedabad.

11. **Mr. Nehul L. Dave**
Advocate – Gujarat High Court
Ahmedabad.

2.4.3. CURRICULUM VITAE OF THE CHIEF RESEARCHER AND THOSE RESPONSIBLE FOR THE CLINICAL, ANALYTICAL AND STATISTICAL PHASES

Ref SOP No. QA-018

torrent

Form No. QAU/GEN/01

CURRICULUM VITA

1.	NAME	Dr. (Maj) Jogesh Mahajan
2.	DATE OF BIRTH	16 July 1965
3.	SEX	Male
4.	DATE OF JOINING	26 May 2006
5.	QUALIFICATION	MBBS
6.	PROFESSIONAL EXPERIENCE	

	PERIOD	NAME OF COMPANY	RESPONSIBILITY
i)	Sep 1997 - Jan 2006	Indian Army (AMC)	Cl 1 Gazetted Medical Officer, Adjutant, Officer Commanding
ii)	Jan 1997 – Sep 1997	Dharamshila Cancer Hospital Delhi	Medical Officer Medical Oncology
iii)	July 1993 – Jan 1997	Pvt Practice Mumbai	
iv)	Oct 1992 – July 1993	Jaslok Hospital & Research Center Mumbai	Medical Officer (Medicine) RMO
v)	Feb 1992 – August 1992	MGM Hospital Mumbai	Medical Officer (Medicine) RMO
vi)	August 1991 – Jan 1992	LTMG Hospital , Mumbai	Medical Officer (Gastroenterology) RMO

7.	TRAINING TAKEN			
	DATE	TOPIC	CONDUCTED BY	CONDUCTED AT
i)				
ii)				

8.	DETAILS OF MEMBERSHIP/WORKSHOP/CONFERENCE ATTENDED
i)	Member – Maharashtra Medical Council Member - Karnataka Medical Council
9.	DETAILS OF PUBLICATION

10.	PHOTOCOPY ATTACHED	☐ Degree Certificate ☐ Training Certificate ☐ Membership Certificate (If any) ☐ Abstract of the publication

Sign of Staff:

Date: 01 June 2006

(Dr. Jogesh Mahajan)

Updated on : –

Ref SOP No. QA-018

torrent

Form No. QAU/GEN/01

CURRICULUM VITAE

1.	NAME	Nikesh Patel
2.	DATE OF BIRTH	20th December 1978
3.	SEX	Male
4.	DATE OF JOINING	6th February 2003
5.	QUALIFICATION	B. Pharm, M.Sc. (Pharmacology)
6.	PROFESSIONAL EXPERIENCE	

	PERIOD	NAME OF COMPANY	RESPONSIBILITY
i)	14th June 2002- 4th February 2003	Claris Life sciences Limited. Ahmedabad	Designing, monitoring, report preparation and regulatory submission of Clinical studies.
ii)	6th February 2003- till date	Torrent Research Centre, Torrent Pharmaceuticals Ltd.	Designing, monitoring, report preparation and regulatory submission of Clinical studies.
iii)			

7.	TRAINING TAKEN			
	DATE	TOPIC	CONDUCTED BY	CONDUCTED AT
i)				
ii)				

8.	DETAILS OF MEMBERSHIP/WORKSHOP/CONFERENCE ATTENDED
	• **1st International conference** of Ayurveda held at Dhanvantri Institute of Ayurveda Jamnagar. • **2nd International conference** of Ayurveda held at Dhanvantri Institute of Ayurveda Jamnagar. • **National conference** of Indian Pharmacological society held in "K.B. institute of pharmacy Gandhinagar. • **Gujarat Chapter** of Indian Pharmacological society held in "A.R. College of Pharmacy Gandhinagar • **International workshop on Advanced GCP**, Bioinformatics centre at, Pune

9.	DETAILS OF PUBLICATION	
	Sarin A[1], Nagpal J [2], Bohra NK[3],. Jiloha R.C[4], Rao GP[5], Sharma SK[6], Vaishnav M [7]. Vaya L[8], Karan RS[9], **Patel NK[10]** and Patel R[11] ; Open Labeled, Randomized, Switch Over Study Of Two Fixed Doses Of Aripiprazole: To Evaluate Its Safety And Efficacy In The Treatment Of Schizophrenia. Ind. J. of Psy.: March 2004:46:64-71	
10.	**PHOTOCOPY ATTACHED**	☐ **Degree Certificate** ☐ **Training Certificate** ☐ **Membership Certificate (If any)** ☐ **Abstract of the publication**

Sign of Staff: _____ **Date:** 30/06/06

Updated on : 30/06/06

CURRICULUM VITAE

1.	NAME	DR.SUSHIL KUMAR ANAND
2.	DATE OF BIRTH	17/11/1969
3.	SEX	MALE
4.	DATE OF JOINING	05/02/05
5.	QUALIFICATION	M.B.B.S ; P.G.C.I.H, P.G.D.H.M.
6.	PROFESSIONAL EXPERIENCE	

	PERIOD	NAME OF COMPANY	RESPONSIBILITY
i)	FEB-2005 TO TILL DATE	TORRENT RESEARCH CENTER, GANDHINAGAR	SCIENTIST-I
ii)	JULY- 2004 TO FEB - 2005	APOLLO TYRES LTD.	INDUSTRIAL PHYSICIAN
iii)	JULY-2003 TO JULY-2004	INDIAN PETROCHEMICALS CORPORATIN LTD.	MEDICAL OFFICER
iv)	DEC – 2002 TO DEC - 2004	OUR LADY OF PILLAR HOSPITAL.	MEDICAL OFFICER
v)	SEP – 2001 TO SEP – 2002	MINISTRY OF HEALTH, LIBYA	MEDICAL OFFICER
vi)	NOV- 99 TO SEP – 2001	GOVT.OF GUJARAT	MEDICAL OFFICER

7.	TRAINING TAKEN			
	DATE	TOPIC	CONDUCTED BY	CONDUCTED AT
i)	15/FEB/2004	WOUND HEALING SYMPOSIUM	INTERNATIONAL WOUND HEALING FOUNDATION	VADODARA
8.	DETAILS OF MEMBERSHIP/WORKSHOP/CONFERENCE ATTENDED			
	1.THE ANNUAL CONFERENCE OF CARDIOLOGY NEXT AT BARODA HEART INSTITUTE & RESEARCH INSTITUTE.			

9.	DETAILS OF PUBLICATION	
10.	PHOTOCOPY ATTACHED	☑ Degree Certificate ☑ Training Certificate ☐ Membership Certificate (If any) ☐ Abstract of the publication

Sign of Staff:

(Dr. Sushilkumar Anand)

Date: 23/04/07

Updated on : 23/04/07

CURRICULUM VITAE

1.	NAME	GUNTA SUBBAIAH
2.	DATE OF BIRTH	01-07-1948
3.	SEX	Male
4.	DATE OF JOINING	19-09-1995
5.	QUALIFICATION	M.Sc., Ph.D
6.	PROFESSIONAL EXPERIENCE	

	PERIOD	NAME OF COMPANY	RESPONSIBILITY
i)	Dec.1971 to Dec.1972	SSNRM College	Lecturer
ii)	Dec.1972 to Sep.1985	IDL Chemicals Ltd.	Research Chemist
iii)	Sep.1985 to Sep.1995	Hoechst India Ltd.	Research Scientist
iv)	Sep.1995 to till now	Torrent Research Centre	Chief, Analytical

	7. TRAINING TAKEN			
	DATE	TOPIC	CONDUCTED BY	CONDUCTED AT
i)	12-02-1998	Mass Spectrometry	Kratos	Manchester
ii)	20-09-1985	Mass Spectrometry	Jeol	Tokyo
iii)	05-08-2002	NABL Assessor's Course	IIQM	Jaipur
iv)	19-04-1998	Gen.Mgmt.Programme	IIM, A'bad	Ahmedabad

	8. DETAILS OF MEMBERSHIP/WORKSHOP/CONFERENCE ATTENDED
I	Workshop on MRI/MRS by NMR, AIMS, Delhi.
ii	Seminar on Recent Advances in LCMS by Waters India, Mumbai.
iii	Seminar on Recent Trends in NIR Systems.
iv	Pharmaceutical Analysts convention – IDMA PAC every year.
v	8th Indian Society for Mass Spectrometry Symposium.
vi	Recent advances in Elemental analysis.

9	DETAILS OF PUBLICATION
i	"Identification and characterization of major degradation products of risperidone in bulk drug and pharmaceuticals dosage form" Journal of Pharmaceutial and biomedical analysis, 36(2004)231-235.
ii	"Determination of Rosuvastatin in prescence of degradation products by Stability Indicating LC method." Journal of Association of Analytical Communities published in July/August issue,05 (Reprints awaited).
iii	"Determination of Cefdinir by Stability indicating High Performance LC Method" Journal of Association of Analytical Communities. (Accepted and it will be published in Nov-Dec '05 issue.
iv	"Determination of Inorganic impurity from Nicorandil and its tablet dosage form by simple reverse phase chromatographic method" Journal of chromatography A (Accepted for publication).

Sign of Staff: _____ Date: 01 June 2006

CURRICULUM VITA

1.	NAME	ANKITA SHAH
2.	DATE OF BIRTH	16/6/1981
3.	SEX	FEMALE
4.	DATE OF JOINING	1/6/2006
5.	QUALIFICATION	M.PHIL. (STATISTICS) - DISSERTATION TITLE "A STUDY ON SOME STATISTICAL TESTS", M.SC. (STATISTICS)
6.	PROFESSIONAL EXPERIENCE	

	PERIOD	NAME OF COMPANY	RESPONSIBILITY
i)	2 YEARS	GUJARAT CANCER & RESEARCH INSTITUTE, AHMEDABAD	CODING OF PERFORMAS, CHECKING THE DATA FOR COMPLETION, COMPILATON & COLLECTION OF DATA, SUPERVISION OF ALL FIELD STAFF, STATISTCAL ANALYSIS OF THE DATA, MAKING STATISTICAL REPORTS.
ii)			
iii)			

7.	TRAINING TAKEN			
	DATE	TOPIC	CONDUCTED BY	CONDUCTED AT
i)				
ii)				
8.	DETAILS OF MEMBERSHIP/WORKSHOP/CONFERENCE ATTENDED			

A.B.Shah

	CONFERECES & WORKSHOPS ATTENDED: 1. WORK SHOP ON "BIO MEDICAL STATISTICS" ORGANIZED BY SARDAR PATEL UNIVERSITY, VALLABH VIDHYANAGAR IN FEBRUARY, 2006. 2. I HAVE ATTENDED "PRE-ARM WORKSHOP & ANNUAL REVIEW MEETING (ARM)" ORGANISED BY NCRP (NATIONAL CANCER REGISTRY PROGRAM) - ICMR HELD AT KOLKATA IN NOVEMBER, 2005. 3. I HAVE VISITED "RURAL CANCER REGISTRY PROJECT-NARGIS DUTT MEMORIAL HOSPITAL"-BARSHI (MAHARASHTRA) AND "KIDWAI MEMORIAL INSTITUTE OF ONCOLOGY-PBCR" – BANGALORE FOR ORIENTATION OF WORK IN SEPTEMBER, 2005. 4. I HAVE ATTENED "PRE-ARM WORKSHOP & ANNUAL REVIEW MEETING (ARM)" ORGANISED BY NCRP (NATIONAL CANCER REGISTRY PROGRAM) - ICMR HELD AT GANGTOK (SIKKIM) IN DECEMBER, 2004. 5. ATTENDED AND PRESENTED IN 24th ANNUAL CONFERENCE OF GSA (GUJARAT STATISTICAL ASSOCIATION) HELD AT BHAVNAGAR IN NOVEMBER 2003.
9	**DETAILS OF PUBLICATION**

A. B. Shah

10.	PHOTOCOPY ATTACHED	☑ Degree Certificate ☐ Training Certificate ☐ Membership Certificate (If any) ☐ Abstract of the publication

Sign of Staff: **Date:** 30/04/17

A. B. Shah

MS. ANKITA SHAH
(BIO STATISTICIAN)

Updated on : 30/04/17

3.0. CLINICAL REPORT

3.1. Title Page

3.1.1. Study Code: PK-07-108

3.1.2. Study Title: A Randomised, Open Label, Two-Period, Two-Treatment, Two-Sequence, Crossover, Single Dose Bioequivalence Study of Carvedilol 25mg Tablets (Test) [Torrent Pharmaceuticals Ltd., India] Versus Carvedilol 25mg Tablets (Coreg®) (Reference) [Produtos Roche Quimicos e Farmaceuticos S.A., Brazil] In Healthy Human Subjects Under Fasted State

3.1.3. Clinical Report

3.1.4. Study Site

Bio Evaluation Centre,
Torrent Pharmaceuticals Ltd.,
Village Bhat, Gandhinagar-382 428,
Gujarat, India

3.2. Table of Content

Physician In-charge:

Tejas. 30/06/08

Dr. Tejas Talati, MBBS
Bio Evaluation Centre,
Torrent Pharmaceuticals Ltd.,
Tel. +91- 079-23969100 **Extn.:** 280/281

Date (dd/mm/yy)

V Shah 30/06/08

Dr. Vishal Shah, MBBS
Bio Evaluation Centre,
Torrent Pharmaceuticals Ltd.,
Tel. +91- 079-23969100 **Extn.:** 280/281

Date (dd/mm/yy)

30/06/08

Dr. Chirag Shah, MBBS
Bio Evaluation Centre,
Torrent Pharmaceuticals Ltd.,
Tel. +91- 079-23969100 **Extn.:** 280/281

Date (dd/mm/yy)

30/06/08

Dr. Alpesh Parmar, MBBS
Bio Evaluation Centre,
Torrent Pharmaceuticals Ltd.,
Tel. +91- 079-23969100 **Extn.:** 280/281

Date (dd/mm/yy)

Shreyansh 30/06/08

Dr. Shreyansh Shah, MBBS
Bio Evaluation Centre,
Torrent Pharmaceuticals Ltd.,
Tel. +91- 079-23969100 **Extn.:** 280/281

Date (dd/mm/yy)

3.3. Folio of Signatures

Study code: PK-07-108

Study Title: A Randomised, Open Label, Two-Period, Two-Treatment, Two-Sequence, Crossover, Single Dose Bioequivalence Study of Carvedilol 25mg Tablets (Test) [Torrent Pharmaceuticals Ltd., India] Versus Carvedilol 25mg Tablets (Coreg®) (Reference) [Produtos Roche Quimicos e Farmaceuticos S.A., Brazil] In Healthy Human Subjects Under Fasted State

Study Site: Bio Evaluation Centre,
Torrent Pharmaceuticals Ltd.,
Village Bhat, Gandhinagar-382 428,
Gujarat, India

Principal Researcher
(Chief Investigator):

30 /06 /08
Date (dd/mm/yy)

Dr. Jogesh Mahajan, MBBS
Assistant General Manager
Bio Evaluation Centre,
Torrent Pharmaceuticals Ltd.,
Tel. +91- 079-23969100 **Extn.:** 270

Clinical Researcher
(Clinical Investigator):

30 /06 /08
Date (dd/mm/yy)

Dr. Sushil Kumar Anand, MBBS
Scientist I
Bio Evaluation Centre,
Torrent Pharmaceuticals Ltd.,
Tel. +91- 079-23969100 **Extn.:** 280

Sub-Investigator:

30 /06 /08
Date (dd/mm/yy)

Mr. Nikesh Patel, M. Sc. Pharmacology
Research Associate
Bio Evaluation Centre,
Torrent Pharmaceuticals Ltd.,
Tel. +91- 079 -23969100 **Extn.:** 295

In-charge Statistical Phase :

A B. Shah

30 /06 /08
Date (dd/mm/yy)

Ms. Ankita Shah, M. Sc., M. Phil
Bio-Statistician
Bio Evaluation Centre,
Torrent Pharmaceuticals Ltd.,
Tel. +91- 079 -23969100 **Extn.:** 293

3.4. Glossary/List of Abbreviations

ADL	Analytical Development Laboratory
ADR	Adverse Drug Reaction
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
ANVISA	Agencia Nacional de Vigilancia Sanitaria
AST	Aspartate Aminotransferase
AUCINF	Area under the plasma concentration extrapolated to infinite time ($AUC_{0\text{-}inf}$)
AUClast	Area under the plasma concentration curve from administration to last observed concentration time($AUC_{0\text{-}t}$)
BLQ	Below Limit of Quantification
BMI	Body Mass Index
BP	Blood Pressure
Ca^{++}	Calcium
Cmax	Maximum Plasma Concentration
Conc.	Concentration
CRF	Case Record Form
CV	Coefficient of Variation
ECG	Electrocardiogram
F-test	Variance Ratio Test
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
HbsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HL_Lambda_z	Elimination Half–life ($T_{1/2}$)
hrs	Hours
HCT	Haematocrit
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICF	Informed Consent Form
IEC	Institutional Ethics Committee
K^{+}	Potassium
Lambda_z	Elimination Rate constant (K_{el})
LLOQ	Lower Limit Of Quantification
Ln	Natural Logarithm to the base e
LOD	Limit of Detection
LOQ	Limit of Quantification
max	Maximum Value Found
mg	Milligram (10^{-3} g)
ml	Millilitre (10^{-3} l)

mmHg	Millimeter of Mercury
Mol	Mole
MRT	Mean Residence Time
µl	Microlitre
N	Sample Size
NA	Not Applicable
Na^+	Sodium
PK	Pharmacokinetic
QA	Quality Assurance
QC	Quality Control
RPM	Revolutions Per Minute
SAE	Serious Adverse Event
SAS®	Statistical Analyst System (software)
SD	Standard Deviation
SOP	Standard Operating Procedure
Subject	Volunteer
Tmax	Time to reach the peak of the maximum plasma concentration of the drug
TPL	Torrent Pharmaceuticals Limited
TRC	Torrent Research Centre
T/R	Test over Reference ratio
WinNonlin®	Statistical software for Pharmacokinetic calculations

3.5. Introduction

Estimates worldwide prevalence for hypertension may be as much as 1 billion individuals, and approximately 7.1 million deaths per year may be attributable to hypertension. The World Health Organization reports that suboptimal BP (>115 mm Hg SBP) is responsible for 62% of cerebrovascular disease and 49% of ischemic heart disease, with little variation by sex. In addition, suboptimal blood pressure is the number one attributable risk for death throughout the world.

Carvedilol is an arylethanolamine β- adrenoceptor antagonist with venodialating properties. These properties are due to blockade of α_1- adrenoceptors along with weak β_1- selective blockade. This dual mode of action avoids the reflex tachycardia due to excessive vasodilation and the peripheral vasoconstriction due to β-blockade.

The antagonism of β_2 adrenoceptors exists, although to a lesser extent. Carvedilol has no Intrinsic Sympathomimetic Activity, and only weak Membrane Stabilizing Activity. Carvedilol is cardio protective in animal models. It is also anti-mitogenic on vascular smooth muscle in vitro, and protects against neuronal damage in in-vitro and in-vivo models of brain ischaemia.

Single oral doses of carvedilol as low as 12.5 mg reduce resting and exercise induced blood pressure in healthy volunteers without effect on heart rate or cardiac index.

BASIC PHARMACOKINETIC PROPERTIES

Absorption

Carvedilol is rapidly and extensively absorbed following oral administration, with absolute bioavailability of approximately 25% to 35% due to a significant degree of first-pass metabolism. Following oral administration, the apparent mean terminal elimination half-life of carvedilol generally ranges from 7 to 10 hours.

After single oral dose of 25 mg Carvedilol in healthy volunteers, maximum plasma concentration (Cmax) was found to range from 21-67μg/L. Similar corresponding variations were also observed in the values of mean area under the plasma concentration time curve (AUC). AUC was found to range from 157-337μg/L.h after single dose of 25 mg in healthy volunteers. Cmax and AUC of Carvedilol were found to increase linearly with dose. However, the time to achieve maximum plasma concentration (Tmax) was within the range of 1-2 hours for 25 mg as well as 50 mg dose in healthy volunteers and hypertensive patients.

Plasma concentrations achieved are proportional to the oral dose administered. When administered with food, the rate of absorption is slowed, as evidenced by a delay in the time to reach peak plasma levels, with no significant difference in extent of bioavailability. Taking carvedilol with food should minimize the risk of orthostatic hypotension.

Distribution

Carvedilol is a basic, lipophilic compound with a steady-state volume of distribution of approximately 115 L, indicating substantial distribution into extravascular tissues. Plasma clearance ranges from 500 to 700 mL/min. Carvedilol is more than 98% bound to plasma proteins, primarily with albumin. The plasma-protein binding is independent of concentration over the therapeutic range.

Metabolism & Elimination

Carvedilol is rapidly and extensively metabolised with less than 2% of the dose recovered as unchanged drug in urine. About 60% of the metabolites are excreted into bile and are eliminated in faeces.

Carvedilol is metabolized primarily by aromatic ring oxidation and glucuronidation. The oxidative metabolites are further metabolized by conjugation via glucuronidation and sulfation. The metabolites of carvedilol are excreted primarily via the bile into the feces. Demethylation and hydroxylation at the phenol ring produce three active metabolites. Compared to carvedilol, the three active metabolites exhibit weak vasodilating activity. Plasma concentrations of the active metabolites are about one-tenth of those observed for carvedilol and have pharmacokinetics similar to the parent.

Potential Adverse Effects of Study Medications

Carvedilol is well tolerated as a once daily 25 mg dose. Overall incidence of withdrawal due to adverse effects was reported only in 7% of patients studied. The most common adverse effects causing discontinuation of treatment were vertigo (1.7%), headache (1.4%), and bronchospasm, fatigue and skin reactions (0.5% each). In 50 mg dose, the adverse event incidence was 31%. Other adverse events rarely reported were loose stools, dry mouth, mucosal swelling, depression, constipation, itching and/or rash. The incidence of syncope or orthostatic hypotension was relatively low (<1%).

Contraindications

Carvedilol is contraindicated in the following conditions:

- Bronchial asthma or related bronchospastic conditions. Deaths from status asthmaticus have been reported following single doses of Carvedilol.
- Second- or third-degree AV block
- Sick sinus syndrome
- Severe bradycardia (unless a permanent pacemaker is in place)
- Patients with cardiogenic shock or who have decompensated heart failure requiring the use of intravenous inotropic therapy. Such patients should first be weaned from intravenous therapy before initiating Carvedilol
- Patients with severe hepatic impairment
- Patients with a history of a serious hypersensitivity reaction to carvedilol (e.g. Stevens- Johnson syndrome)

3.6. Objective

Primary objective: To assess the bioequivalence of Carvedilol 25mg tablet (Test) [Torrent Pharmaceuticals Ltd., India] versus Coreg® 25mg tablet (Reference) [Produtos Roche Quimicos e Farmaceuticos S.A., Brazil], in healthy human volunteers under fasting condition.

Secondary objective: To investigate the safety of the formulations on the basis of clinical and laboratory examinations at the beginning and at the end of the study and registration of adverse events and/or adverse drug reactions.

3.7. Design

Bioequivalence studies are general requirements for registration of generic and similar products. A single center, randomized, crossover design is typically employed in bioequivalence studies, and was considered to be the most appropriate for this study.

The study was open-label in nature, because blood concentration levels can not be influenced by the knowledge of the identity of the treatment.

This was a two period, two sequence, two treatment study with a washout period of 7 days between two dosing. The duration of the study starting from check-in of period I to the last in-house blood draw of period II was 10 days. The washout was sufficient to allow the complete elimination of the drug before subsequent dosing and to avoid carry over effects.

Twenty six healthy male volunteers were enrolled in the study, all of them had completed study. Sampling was done up to 48 hours such that plasma concentration could be measured for 5 half-lives of carvedilol.

The volunteers were administered a single oral dose of carvedilol 25mg of either the test (A) or reference (B) product in period I and period II in two sequence AB or BA.

3.8. Randomization List

The order of receiving the test and reference product for each volunteer during each period of the study was determined according to the randomization schedule. The volunteers were randomly assigned to one of the 2 possible administration sequences AB or BA. Randomization was done in such a way that equal number of sequence allocations was ensured. The randomization schedule and the dispensing record were kept in the pharmacy under controlled access. The Investigator and study personnel involved in the dispensing of study products were accountable for ensuring compliance to randomization schedule.

Table 1: Randomization Schedule Generated for Carvedilol 25 mg Tablet under Fasting Condition

Study Code: PK-07-108

Subject No.	Period-1	Period-2
1	B	A
2	A	B
3	A	B
4	A	B
5	B	A
6	B	A
7	B	A
8	A	B
9	B	A
10	A	B
11	B	A
12	A	B
13	B	A
14	A	B
15	B	A
16	B	A
17	A	B
18	A	B
19	B	A
20	B	A
21	A	B
22	A	B
23	A	B
24	B	A
25	A	B
26	B	A
A =	B	A
B =	A	B

Product: A (TEST)	Carvedilol Tablet
Batch No	B9717002
Expiry Date	January 2009
Mfg. By	Torrent Pharmaceuticals Ltd, India
Product: B (REFERENCE)	Coreg® (Carvedilol) Tablet
Batch No	RJ0382
Expiry Date	July 2009
Mfg. By	Produtos Roche Quimicos e Farmaceuticos S.A., Brazil

3.9. Drugs

3.9.1. Test

Test Product (A)	
Generic Name	Carvedilol 25 mg
Pharmaceutical Form	Tablet
Batch No.	B9717002
Manufacturing Date	February 2007
Expiry Date	January 2009
Manufactured By	Torrent Pharmaceuticals Ltd., India

3.9.2. Reference

Reference Product (B)	
Generic Name	Carvedilol 25 mg
Trade Name	Coreg®
Pharmaceutical Form	Tablet
Batch No.	RJ0382
Manufacturing Date	--
Expiry Date	July 2009
Manufactured By	Produtos Roche Quimicos e Farmaceuticos S.A., Brazil

3.9.3. Analytical Certificate of the Drugs

The complete certificate of analysis for both test and reference drug was obtained from the analytical department of Torrent Pharmaceuticals Ltd. (Appended in Appendix I).

3.9.4. Samples for Retention of Drugs for the Study

One hundred and twenty eight units of test and reference medication were archived for bioequivalence study of Carvedilol 25 mg (PK-07-108) in Pharmacy.

The reference and test medications were stored below 25°C and separately from normal practice stocks, locked and only accessible for authorized personnel, in accordance with the manufacturer's instructions.

Pharmacist will store the retention samples to the validity period of the product plus one year (July, 2010) taking a parameter the validity of the most recent product (Reference formulation).

Records of the receipt and dispensing of study products were made to provide complete accountability of the disposition of both study drugs.

3.9.5. Inventory of the Drug in the Study

The study medications were provided in a sufficient quantity for the needs of the whole study. The appropriate amount of each study medication was stored in pharmacy in order to allow repeated pharmaceutical analysis and retention. Reference products were supplied in the original manufacturer's packing and the test products were supplied in box deemed to maintain the integrity of the products.

The temperature and the humidity in the storage room were continuously monitored. The storage conditions were checked by the study personnel. All supplies were accounted at the end of the study. A drug accountability form was completed for this purpose and will be maintained by the pharmacist at the study site for a minimum of 15 years following completion of the study. (Annexure - II)

3.10. Population of Study

A total of 26 healthy males, having 19 to 40 years of age with BMI 18.25-26.64 kg/m^2 were included in the study. The volunteers were selected based on their good health confirmed by complete clinical, haematological, serological and biochemical tests.

3.10.1. Individual Details

Table 2: Volunteer Demographics

Enrolment No.	Volunteer ID PK-	Volunteer Initials	Sex	Age (Yrs)	Height (cm)	Weight (Kg)	*BMI	Race	Smoking	Date of Screening
1	C-518	MKC	Male	33	164.0	63.57	23.64	Asian	None	08/01/08
2	F-266	NSB	Male	21	158.5	51.56	20.52	Asian	None	11/01/08
3	G-630	SDP	Male	23	169.5	66.62	23.19	Asian	None	08/01/08
4	F-050	NIS	Male	31	172.5	78.32	26.32	Asian	None	31/12/07
5	G-669	BLP	Male	21	168.0	52.20	18.49	Asian	None	16/01/08
6	D-616	NGP	Male	23	169.0	68.84	24.10	Asian	None	11/01/08
7	G-627	RRK	Male	26	163.5	52.27	19.55	Asian	None	08/01/08
8	G-571	MSV	Male	30	173.5	63.90	21.23	Asian	None	28/12/07
9	C-938	MKB	Male	33	156.0	62.50	25.68	Asian	None	11/01/08
10	G-603	DBR	Male	26	167.5	71.42	25.46	Asian	None	04/01/08
11	D-206	SSM	Male	29	174.5	70.45	23.14	Asian	None	16/01/08
12	G-665	FNP	Male	25	173.0	73.19	24.45	Asian	None	11/01/08
13	G-663	LBC	Male	29	171.5	68.81	23.40	Asian	Ex-smoker (discontinued >3 months ago)	11/01/08
14	G-676	SKG	Male	25	175.0	76.45	24.96	Asian	None	17/01/08
15	G-670	BIP	Male	36	168.0	51.65	18.30	Asian	None	16/01/08
16	G-667	SAV	Male	39	164.0	65.67	24.42	Asian	None	11/01/08
17	F-706	BNV	Male	21	173.5	61.85	20.55	Asian	None	11/01/08
18	G-664	DJS	Male	21	166.0	51.43	18.66	Asian	None	11/01/08
19	G-654	HLD	Male	40	175.0	66.66	21.77	Asian	Current smoker (cigarettes 1-09)	11/01/08
20	G-580	MBP	Male	31	168.0	66.74	23.65	Asian	Current smoker (cigarettes 1-09)	31/12/07

Enrolment No.	Volunteer ID PK-	Volunteer Initials	Sex	Age (Yrs)	Height (cm)	Weight (Kg)	*BMI	Race	Smoking	Date of Screening
21	F-038	SGU	Male	35	169.5	64.75	22.54	Asian	None	11/01/08
22	E-156	NJS	Male	25	158.5	66.92	26.64	Asian	None	08/01/08
23	E-297	RSG	Male	22	173.0	55.85	18.66	Asian	None	11/01/08
24	G-346	HPS	Male	29	159.0	55.47	21.94	Asian	None	08/01/08
25	G-662	ASV	Male	19	171.5	53.67	18.25	Asian	None	11/01/08
26	E-162	CRZ	Male	31	160.0	59.05	23.07	Asian	None	04/01/08
Mean				**27.85**	**167.77**	**63.07**	**22.41**			
Standard Deviation				**5.86**	**5.75**	**8.05**	**2.62**			
Maximum				40	175	78.32	26.64			
Minimum				19	156	51.43	18.25			
****%CV**				21.0551	3.4253	12.7561	11.6785			

* BMI-Body Mass Index
** % CV- Percentage Co-efficient of Variance

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3.10.2. Selection

Screening of the volunteers was done and those found to be healthy and met all inclusion and none of the exclusion criterion were included in the study. The screening procedure consisted of clinical examination, reading of electrocardiogram, radiological investigation (chest X-ray, if not done in the past 6 months and if clinically indicated), and laboratory investigation of blood and urine conducted not more than 28 days prior to first dosing.

3.10.2.1. Clinical Evaluation

Screening assessment comprised of detailed medical history followed by general physical examination and laboratory investigations (hematology, biochemistry, urine analysis and serology) ECG (done 28 days prior to study start) and X-ray (within 6 months of the study start).

All the volunteers enrolled in the study met all the inclusion and none of the exclusion criteria as described in the protocol and were judged eligible for the study based on medical history, demographic data (sex, age, weight, height, body mass index), medication history, physical examination, vital signs (blood pressure, heart rate and pulse rate), ECG and clinical laboratory tests (hematology, biochemistry, urine analysis, chest X-ray, and serology like HIV, HBsAg, HCV). All volunteers enrolled were negative for screening test for Drug abuse and breath alcohol test just before enrollment. Samples were collected for biochemical and hematological tests at the time of last sample of period II.

3.10.2.2. Clinical Laboratory Tests

Following tables represents laboratory values from all 26 volunteers enrolled.

Table 3: Hematology: - Screening and Post Study

En. No.	Volunteer ID PK-	Time	WBC	Neut	Lymp	Mono	Eosi	Baso	RBC	Hb	HCT	PLT
			K/µl	%	%	%	%	%	M/µl	g/dl	%	K/µl
Normal Range			4-10	40-80	20-40	2-10	1-6	<1-2	4.5-5.5	12.5-17	40-50	150-400
1	C-518	T0	7.16	52.200	35.300	7.200	4.590	0.669	5.40	15.40	43.10	291.00
		T1	5.12	55.200	33.500	7.520	2.320	1.400	**6.70**	15.90	**53.80**	272.00
2	F-266	T0	8.26	56.500	29.500	8.150	5.000	0.819	4.58	15.20	41.80	383.00
		T1	**12.80***	63.300	26.900	4.800	4.770	0.248	4.90	15.50	49.70	371.00
3	G-630	T0	7.19	48.800	36.200	**10.200**	4.160	0.783	**4.44**	13.50	**37.50**	221.00
		T1	6.77	48.400	39.000	5.900	**6.550**	0.080	4.77	13.50	44.30	277.00
4	F-050	T0	5.67	41.900	**46.500**	5.470	5.390	0.741	4.67	12.80	**36.60**	282.00
		T1	5.63	46.400	**44.400**	2.860	5.710	0.652	4.78	13.30	**36.70**	245.00
5	G-669	T0	8.63	64.900	24.400	4.410	5.860	0.508	**5.59**	14.80	42.30	251.00
		T1	6.34	45.400	**40.600**	5.430	**7.730**	0.810	5.44	13.90	45.30	225.00
6	D-616	T0	7.03	52.900	35.400	6.470	4.640	0.617	5.23	13.90	40.10	399.00
		T1	7.95	60.100	28.500	5.070	5.630	0.625	5.36	13.70	45.30	356.00
7	G-627	T0	5.93	62.600	28.900	6.190	1.650	0.651	**6.18**	15.70	45.90	310.00
		T1	6.22	52.500	**42.300**	3.110	2.120	0.017	**6.00**	14.90	48.50	308.00
8	G-571	T0	6.39	60.000	32.200	4.860	2.040	0.878	**5.53**	14.70	42.30	249.00
		T1	6.21	56.600	38.100	2.660	1.940	0.751	**5.60**	14.20	47.10	327.00
9	C-938	T0	9.21	62.700	24.200	6.230	**6.030**	0.896	**6.41**	16.50	47.00	**401.00**
		T1	8.74	53.300	30.900	**1.530**	**14.200**	0.013	**6.22**	15.50	**50.50**	311.00
10	G-603	T0	5.42	60.600	28.600	7.860	2.440	0.473	**6.24**	14.10	41.50	300.00
		T1	6.16	60.800	28.200	7.000	3.360	0.668	**6.16**	13.10	44.80	293.00
11	D-206	T0	5.41	63.700	27.700	6.210	1.910	0.476	5.25	13.50	**39.80**	294.00
		T1	7.36	67.100	23.600	6.000	2.860	0.359	5.30	13.10	44.10	277.00
12	G-665	T0	7.07	54.900	33.900	6.090	3.600	1.580	**5.56**	13.60	40.30	342.00
		T1	7.12	59.200	33.300	5.090	2.150	0.185	**5.60**	13.00	44.20	282.00
13	G-663	T0	8.24	46.400	35.800	8.830	**8.240**	0.695	5.13	15.50	43.20	354.00
		T1	8.02	49.500	32.200	7.570	**9.810**	0.872	5.11	14.80	47.60	378.00

En. No.	Volunteer ID PK-	Time	WBC	Neut	Lymp	Mono	Eosi	Baso	RBC	Hb	HCT	PLT
			K/µl	%	%	%	%	%	M/µl	g/dl	%	K/µl
Normal Range			4-10	40-80	20-40	2-10	1-6	<1-2	4.5-5.5	12.5-17	40-50	150-400
14	G-676	T0	6.60	48.900	35.200	6.050	**8.860**	1.010	5.23	14.20	40.10	282.00
		T1	7.50	47.300	35.700	6.900	**8.760**	1.290	**5.54**	14.40	46.30	306.00
15	G-670	T0	6.85	62.200	26.900	7.530	2.300	1.070	5.38	15.10	43.10	320.00
		T1	7.22	49.600	**40.700**	5.620	2.920	1.200	5.08	13.90	45.00	319.00
16	G-667	T0	6.45	54.600	38.100	4.200	1.710	1.380	4.86	15.70	43.00	329.00
		T1	6.77	56.600	35.700	3.830	2.900	0.966	4.73	14.60	45.50	311.00
17	F-706	T0	6.79	60.700	31.300	5.850	1.720	0.479	4.93	13.30	**38.60**	269.00
		T1	7.85	51.600	**41.000**	5.140	1.610	0.637	5.14	13.60	44.60	279.00
18	G-664	T0	8.40	62.200	28.000	6.150	3.080	0.567	**6.36**	16.20	47.10	306.00
		T1	7.69	51.400	36.000	7.770	3.610	1.170	**6.25**	15.20	**50.70**	283.00
19	G-654	T0	6.92	54.500	36.500	6.180	1.990	0.807	4.95	16.20	**37.80**	262.00
		T1	7.43	60.900	29.700	5.410	2.980	0.970	5.10	15.00	48.20	**425.00**
20	G-580	T0	8.58	54.800	37.800	3.760	3.060	0.640	5.29	15.50	44.00	346.00
		T1	9.22	62.900	30.300	2.930	3.190	0.690	**5.83**	16.20	**51.70**	376.00
21	F-038	T0	8.61	54.200	35.200	6.030	3.700	0.861	5.41	13.70	40.60	283.00
		T1	6.57	45.400	33.400	8.470	**11.500**	1.250	**5.82**	14.00	48.30	306.00
22	E-156	T0	7.85	70.800	**19.400**	7.690	1.310	0.848	4.74	13.30	**38.30**	343.00
		T1	8.85	68.800	23.000	5.150	2.590	0.445	4.71	12.50	41.50	297.00
23	E-297	T0	6.94	50.700	**42.400**	4.360	1.960	0.561	5.25	13.10	**38.30**	266.00
		T1	7.98	44.100	**42.300**	8.700	3.940	0.915	**5.66**	13.40	45.30	292.00
24	G-346	T0	6.83	56.800	35.100	5.920	1.600	0.602	4.59	12.50	**35.90**	260.00
		T1	4.57	**37.400**	**53.500**	2.620	5.730	0.696	5.01	**11.50**	**39.00**	320.00
25	G-662	T0	6.63	59.300	32.800	4.680	2.990	0.276	4.97	14.20	**39.80**	278.00
		T1	6.19	58.400	31.500	5.550	4.090	0.492	5.21	14.20	46.00	300.00
26	E-162	T0	7.71	57.000	31.200	9.500	1.660	0.681	**6.12**	15.00	44.30	380.00
		T1	7.42	60.000	29.400	7.670	1.930	1.010	**6.34**	15.10	50.00	340.00

Note: Values in bold were out of normal range.

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En. No.	Enrollment Number	Neut	Neutrophils
T0	At the time of Screening	Lymp	Lymphocytes
T1	Post Study	Mono	Monocytes
WBC	White Blood Cells	Eosi	Eosinophils
RBC	Red Blood Cells	Baso	Basophils
Hb	Hemoglobin	g/dl	Gram per deciliter
HCT	Hematocrit	PLT	Platelets
*	Values which were considered clinically significant & suggested for follow up		

Table 4: Biochemistry: - Screening and Post Study

En. No.	Volunteer ID PK-	Time	Alb. g/dl	T.Pro. g/dl	Glob. g/dl*	ALT U/L	T.Bil. mg/dl	AST U/L	ALP U/L	RBS mg/dl	Cret. mg/dl	Trigly mg/dl	Choles mg/dl	Ca mg/dl	Na mEq/L	K mEq/L	Cl mEq/L	GGT U/L
Normal Range			**3.5-5.2**	**6.4-8.3**	**2.3-3.5**	**<45**	**0.3-1.2**	**<35**	**30-120**	**45-130**	**0.84-1.25**	**<150**	**150-250**	**8.8-10.6**	**136-145**	**3.5-5.1**	**98-106**	**< 55**
1	C-518	T0	4.38	7.09	2.7	18.96	0.54	24.41	90.10	85.23	1.15	92.13	153.38	9.09	138.90	4.50	103.70	22.50
		T1	4.53	7.32	2.8	24.60	0.32	28.18	109.03	84.01	1.20	114.97	**140.87**	8.95	142.00	4.60	105.60	27.00
2	F-266	T0	4.59	7.06	2.5	16.70	0.48	21.00	78.18	82.84	1.12	62.12	**104.90**	8.95	138.70	4.10	103.00	20.90
		T1	4.98	7.81	2.8	21.06	0.75	21.70	97.18	98.08	1.17	**198.00***	**130.40**	9.57	139.10	4.00	101.70	25.30
3	G-630	T0	4.69	7.34	2.7	25.02	0.77	27.25	76.67	77.48	1.20	57.58	**109.94**	9.38	138.00	3.80	102.00	14.10
		T1	4.60	7.54	2.9	33.29	0.37	26.09	86.83	98.46	1.00	**157.84**	**129.76**	8.91	138.90	4.30	105.10	16.00
4	F-050	T0	4.59	7.97	3.4	43.49	0.82	**36.73**	60.58	86.99	1.07	144.37	**264.53**	9.19	**135.80**	4.40	99.70	29.30
		T1	4.72	8.03	3.3	37.93	0.55	32.00	60.82	118.62	1.12	**179.07**	240.88	9.59	136.50	4.30	100.30	25.10
5	G-669	T0	5.00	8.18	3.2	29.33	1.04	25.33	80.30	87.90	0.99	41.74	**103.62**	9.37	**135.20**	4.30	101.50	16.80
		T1	5.12	**8.36**	3.2	25.87	0.60	23.43	70.74	90.92	1.00	91.33	**122.47**	9.43	137.20	4.10	102.30	15.10
6	D-616	T0	4.78	7.54	2.8	11.71	0.41	15.92	94.94	81.44	1.05	126.17	201.80	8.90	136.00	4.50	99.70	20.80
		T1	4.78	7.64	2.9	15.51	**0.29**	17.56	105.34	89.49	1.05	**217.50***	193.51	9.34	138.30	4.30	101.00	20.80
7	G-627	T0	5.12	8.09	3.0	18.49	0.52	24.65	79.32	90.35	1.02	64.68	**137.32**	9.78	137.90	4.80	100.60	18.10
		T1	**5.22**	**8.43**	3.2	26.36	0.36	27.11	80.97	105.19	1.05	139.69	165.28	9.91	138.80	4.10	100.40	21.00
8	G-571	T0[28/12/07]	4.66	7.64	3.0	29.61	0.66	31.71	75.14	99.91	1.12	**182.47****	163.13	9.05	137.80	4.00	102.60	21.00
		T00[04/01/08]	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	108.11	N.A	N.A	N.A	N.A	N.A	N.A
		T1[02/02/08]	4.97	**8.33**	3.4	22.43	0.62	27.96	84.47	118.02	1.14	**253.66***	201.34	9.27	137.70	4.10	100.80	22.80
		T2[12/02/08]	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	100.62	N.A	N.A	N.A	N.A	N.A	N.A
9	C-938	T0[11/01/08]	4.61	7.60	3.0	24.70	0.60	25.11	62.09	79.08	1.11	**154.58****	233.84	9.13	137.10	4.30	**97.40**	**55.30**
		T00[21/01/08]	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	111.91	N.A	N.A	N.A	N.A	N.A	N.A
		T1	4.62	7.71	3.1	27.13	0.36	24.88	68.20	87.13	1.09	107.69	243.34	9.34	138.30	4.70	99.30	**63.00**
10	G-603	T0	4.70	7.93	3.2	27.44	0.65	21.32	87.80	82.78	1.05	44.71	173.39	9.46	136.30	4.20	98.90	17.40
		T1	4.69	7.80	3.1	25.43	0.48	19.83	81.51	87.47	1.01	85.92	175.66	9.68	136.80	4.40	98.30	16.10
11	D-206	T0	4.66	7.83	3.2	16.50	0.54	25.89	78.97	99.90	1.03	88.29	183.52	8.88	136.20	4.20	101.10	16.20

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En. No.	Volunteer ID PK-	Time	Alb. g/dl	T.Pro. g/dl	Glob. g/dl*	ALT U/L	T.Bil. mg/dl	AST U/L	ALP U/L	RBS mg/dl	Cret. mg/dl	Trigly mg/dl	Choles mg/dl	Ca mg/dl	Na mEq/L	K mEq/L	Cl mEq/L	GGT U/L
Normal Range			**3.5-5.2**	**6.4-8.3**	**2.3-3.5**	**<45**	**0.3-1.2**	**<35**	**30-120**	**45-130**	**0.84-1.25**	**<150**	**150-250**	**8.8-10.6**	**136-145**	**3.5-5.1**	**98-106**	**< 55**
		T1	4.86	7.97	3.1	23.19	0.50	29.01	73.53	100.85	1.03	122.71	221.75	9.45	138.50	4.60	101.20	18.20
12	G-665	T0	4.84	7.86	3.0	20.41	0.88	21.23	93.49	86.63	1.02	69.71	181.77	8.99	138.10	4.50	101.90	29.40
		T1	4.97	8.27	3.3	21.18	**0.29**	22.90	106.74	103.37	0.93	97.36	188.96	9.06	139.10	4.50	102.60	39.50
13	G-663	T0	4.91	8.04	3.1	27.74	0.89	28.89	67.66	77.38	1.07	78.41	184.25	9.30	137.70	4.60	102.10	37.80
		T1	4.81	8.25	3.4	31.74	0.42	33.91	72.86	94.73	1.03	68.79	176.02	9.49	139.70	4.90	103.90	37.50
14	G-676	T0	4.49	7.03	2.5	28.42	0.51	23.53	59.73	76.08	0.92	147.25	**123.73**	**8.56**	137.10	4.90	102.60	30.80
		T1[02/02/08]	4.81	7.55	2.7	26.92	0.41	24.09	59.67	90.12	0.94	**203.63***	**125.83**	9.42	138.40	4.70	102.60	29.50
		T2[12/02/08]	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	**150.13**	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
15	G-670	T0	4.68	7.90	3.2	14.53	0.48	27.47	65.78	87.23	1.22	106.06	176.18	9.24	136.00	4.40	100.80	9.30
		T1	4.50	7.64	3.1	13.79	0.31	25.37	59.73	94.84	1.12	111.87	162.63	8.98	137.80	5.10	101.20	9.40
16	G-667	T0[11/01/08]	4.83	7.84	3.0	25.18	0.83	25.81	60.34	112.91	1.10	**182.53****	185.35	8.98	138.90	4.20	100.90	25.80
		T00[21/01/08]	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	109.02	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
		T1[02/02/08]	4.72	7.78	3.1	21.36	0.51	22.20	67.90	**207.43***	1.06	**412.76***	199.98	9.16	136.40	4.10	99.20	26.40
		T2[13/02/08]	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	47.92	N.A.	74.62	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
17	F-706	T0	4.60	7.28	2.7	9.54	0.49	14.00	70.99	78.36	0.98	59.72	160.19	8.94	136.90	4.40	100.50	12.20
		T1	4.99	8.08	3.1	12.84	0.37	16.60	72.01	123.20	0.98	92.81	177.03	9.55	139.60	4.60	101.20	12.70
18	G-664	T0	5.00	7.53	2.5	20.96	0.47	18.61	67.23	78.67	0.90	98.76	165.42	9.49	141.20	4.20	102.20	26.70
		T1	4.87	7.62	2.8	21.76	0.33	17.99	78.49	91.90	0.95	155.22	**158.99**	9.90	141.90	4.60	102.70	34.40
19	G-654	T0	4.68	7.37	2.7	10.80	1.17	25.94	113.81	92.19	0.91	82.37	193.99	**8.76**	138.90	4.70	100.00	34.10
		T1	4.46	7.22	2.8	26.00	0.76	26.76	**123.55**	**139.66**	0.87	119.11	204.90	**8.48**	137.80	4.20	101.10	45.70
20	G-580	T0 [31/12/07]	4.54	7.51	3.0	28.14	0.49	30.76	103.11	77.99	0.88	**232.09****	235.21	9.09	139.10	4.40	105.90	25.00
		T00 [04/01/08]	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	137.22	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
		T1	4.88	7.86	3.0	20.36	0.41	20.09	102.50	112.78	0.95	**174.81**	227.03	9.45	140.00	4.10	103.20	24.80
21	F-038	T0	4.64	7.67	3.0	20.89	0.67	20.74	75.95	76.34	0.91	85.49	224.94	**8.61**	137.10	3.70	99.90	25.50
		T1[02/02/08]	4.88	**8.38**	3.5	19.45	**0.29**	18.35	84.60	87.66	0.96	**315.92***	202.44	9.19	138.10	4.60	101.60	22.60
		T2[12/02/08]	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	95.92	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

En. No.	Volunteer ID PK-	Time	Alb. g/dl	T.Pro. g/dl	Glob. g/dl*	ALT U/L	T.Bil. mg/dl	AST U/L	ALP U/L	RBS mg/dl	Cret. mg/dl	Trigly mg/dl	Choles mg/dl	Ca mg/dl	Na mEq/L	K mEq/L	Cl mEq/L	GGT U/L
Normal Range			**3.5-5.2**	**6.4-8.3**	**2.3-3.5**	**<45**	**0.3-1.2**	**<35**	**30-120**	**45-130**	**0.84-1.25**	**<150**	**150-250**	**8.8-10.6**	**136-145**	**3.5-5.1**	**98-106**	**< 55**
22	E-156	T0	4.77	7.88	3.1	22.35	0.68	21.01	73.77	99.73	0.91	82.83	**130.52**	9.55	136.30	4.80	103.40	14.60
		T1	4.38	7.50	3.1	23.42	0.37	18.19	74.13	**137.39**	0.84	94.51	**133.40**	8.82	136.40	**5.20**	104.50	13.90
23	E-297	T0	4.64	7.40	2.8	18.06	0.59	19.58	76.73	95.42	**0.83**	49.49	**149.16**	**8.65**	**135.50**	3.60	100.10	13.80
		T1	4.95	**8.42**	3.5	24.97	0.54	19.29	88.04	98.91	0.91	67.56	166.92	9.60	137.50	4.70	100.60	16.60
24	G-346	T0	4.65	7.35	2.7	13.87	0.41	16.87	87.74	103.59	0.87	131.51	200.05	9.33	139.30	4.10	100.00	25.40
		T1	4.20	7.00	2.8	37.08	0.34	33.17	109.09	97.75	**0.82**	122.13	231.58	9.48	139.70	4.90	103.70	44.70
25	G-662	T0	4.85	6.99	**2.1**	15.88	0.48	27.36	81.39	88.84	**0.76**	78.68	**131.53**	8.89	138.30	4.10	100.60	9.80
		T1	5.15	7.67	2.5	17.57	0.52	31.81	89.44	81.81	**0.78**	111.51	**130.58**	9.45	140.60	4.20	102.80	11.10
26	E-162	T0	4.79	7.90	3.1	29.58	1.05	29.25	92.46	79.18	1.03	113.72	183.77	9.48	**134.10**	4.60	**94.40**	52.20
		T1	4.90	8.11	3.2	29.90	0.40	31.20	89.13	85.44	1.11	**196.82***	193.88	9.21	138.40	4.00	**96.80**	44.40

Note: Values in bold were out of normal range

En. No.	Enrollment Number	ALP	Alkaline Phosphtase	AST	Aspartate Amino Transferase
Alb	Albumin	RBS	Random Blood Sugar	K	Potasium
T. Pro.	Total Proteins	Cret	Creatinine	Na	Sodium
Glob.	Globulin	Trigly	Triglycerides	Cl	Chloride
ALT	Alanine Amine Transferase	Choles	Choesterol	GGT	Gama glutamine transferace
T. Bil.	Total Bilirubin	Ca	Calcium	*	Values which were considered clinically significant & suggested for follow up
T0, T00	At the time of Screening	T1, T2	Post Study	**	Values which were considered clinically significant in screening visit

3.10.2.3. Criteria for Inclusion

Volunteers meeting following criteria were enrolled:

- Sex: male
- Age: 18-45 years.
- Volunteer with BMI of 18-27 (inclusive both) kg/m^2 with minimum of 50 kg weight.
- Healthy and willing to participate in the study.
- Signed Written Informed Consent for Screening and study.
- Non-smokers or smoking less than 10 cigarettes a day and willing to break smoking during the course of the study.

3.10.2.4. Criteria for Exclusion

Volunteers were excluded from the study based on the following criteria:

- Clinically relevant abnormal physical findings at the screening examination, which would interfere with the objectives of the study.
- Clinically relevant abnormalities in the results of the laboratory screening evaluation.
- Systolic blood pressure less than 100 mmHg or more than 140 mmHg and diastolic blood pressure less than 60 mmHg or more than 90 mmHg
- Pulse rate less than 50/minute or more than 100/minute
- Oral temperature less than 95°F or more than 98.6°F
- Respiratory rate less than 12/minute or more than 20/minute
- Clinically significant abnormal ECG or Chest X-ray
- Habituation of tobacco necessitating uninterrupted tobacco consumption
- Addiction to alcohol or history of any drug abuse.
- History of kidney or liver dysfunction.
- History of allergy to the test drug or any drug chemically similar to the drug under investigation.
- Administration/ Intake of any prescription medication for two weeks or OTC medication for one week before the study.
- Patients suffering from any chronic illness such as arthritis, asthma etc.
- HIV, HCV, HBsAg positive volunteers.
- Opium, tetrahydro cannabinoids, amphetamine, barbiturates, benzodiazepines, cocaine positive volunteers based on urine test.
- Breath alcohol test positive
- Subjects suffering from any psychiatric (acute or chronic) illness.
- Administration of any investigational drug in the period 0 to 3 months before entry to the study.
- Intake of barbiturates or any enzyme-inducing drug in last three months.
- History of significant blood loss due to any reason, including blood donation in the past 12 weeks.
- History of any bleeding disorder.

- Existence of any surgical or medical condition, which, in the judgment of the clinical investigator, might interfere with the absorption, distribution, metabolism or excretion of the drug or likely to compromise the safety of volunteers.
- Serious adverse reaction or hypersensitivity to study drug or any of the excipients.
- Inability to communicate or co-operate with the investigator due to language problem, poor mental development or impaired cerebral function.

The minor deviations in laboratory results, blood pressure were considered clinically not significant based on the decision of chief investigator and/or clinical investigator/physician.

3.10.3. Restrictions and Prohibitions: Before, During and After the Study

Clinical Residential Stay: All the enrolled volunteers were confined to the Pharmacokinetic Unit at least 12 hours before dosing and 32 hours post dosing except one volunteer (Enrollment no. C-23). He had been enrolled at 20:49 on enrollment day and given dinner ensuring 10 hours fasting till study drug administration in period-I.

Physical Activity: Volunteers were asked to remain in sitting position for a period of 4 hours post dose. The volunteers were restricted from doing any sort of stressful/ vigorous physical activity during the entire period of stay.

Volunteers were not allowed to smoke or consume tobacco in any form for at least 48 hours before dosing and during study. They were prohibited from smoking or consuming tobacco during their entire stay in Pharmacokinetic Unit, Bio Evaluation Centre. The use of xanthine containing beverages (tea, coffee, cola drinks), grapefruit juice and foods (chocolates) were prohibited for 48 hours before dosing and throughout their stay in Pharmacokinetic Unit, Bio Evaluation Center. Volunteers were abstained from alcohol for 48 hours prior to dosing and throughout the conduct of the study. They were restricted from taking any medication (including over the counter products), throughout the study, unless authorized by the Clinical Investigator.

Volunteers were fasted overnight for atleast 10 hours. No fluid was allowed for 1 hour before and 2 hours post dose except water provided during dosing.

During the residential stay in the BE Centre, food intake was standardized and identical for both the Periods. Both the study drugs were administered under identical conditions in each period.

Volunteers were asked not to take prescribed medications beginning two weeks prior to and no OTC medications beginning one week prior to initiation of study and until after the study completed. No concomitant drug therapy was given during study to all the volunteers.

All the procedures in the study were carried out as per the in-house standard operating procedure.

3.10.4. Criteria for Discontinuation or Withdrawal of the Volunteers in the Study

Volunteers were informed that they were free to withdraw from the study at any time without giving any reason for doing so. The chief investigator had the right to withdraw a volunteer from the study for any of the following reason:

1. Volunteers not wishing to continue with the study, irrespective of the reason.
2. Adverse event during the study.
3. Any illness requiring medication during the study.
4. Violation of the protocol by the volunteer.

3.10.5. List of the Cases Withdrawn or Cancelled

A total of 26 healthy, adult male volunteers were enrolled in the study. Total 26 volunteers completed the study. Samples from 26 volunteers completing both the periods were analyzed and their plasma concentration data were included in pharmacokinetic and statistical analysis.

3.11. Confinement of Volunteers

For the purpose of dosing, the volunteers reported to the Pharmacokinetic Unit atleast 12 hours before dosing on enrollment day (day 0 in each study period) and stayed there for 32 hours after dosing for the respective blood samplings. Enrollment No. C-23 reported to the clinic at 20:49 hours in period I.

The physician had checked well being of each volunteers prior to discharge from the clinic. For 26 volunteers post-study laboratory tests were carried out. Blood sample for post-study evaluation was collected at the time of last blood sample in period-II.

The dates and time of the beginning and end of confinement in both study periods were recorded in respective logbooks and case record forms in pharmacokinetic unit, Bio Evaluation Centre.

3.11.1. Place

BE Centre, Torrent Pharmaceuticals Ltd.,
Village Bhat, Gandhinagar-382 428, Gujarat, India

3.11.2. Description of the Conditions, Restrictions, Exercises

The volunteers were restricted from drinking water 1 hour before and 2 hours after dosing except at the time to dosing. They were asked to stay in sitting position for at least 4 hours post dose. The volunteers were restricted from doing any sort of vigorous physical activity during the entire period of stay.

3.11.3. Periods: Date and Time of Entry and Exit in Each Period

Volunteers entered and left the facility on following dates

	Enrollment Date & Time	**Discharge Date & Time**
Period I	23rd January, 2008, 16:52-20:49	25th January, 2008, 16:21 - 17:15
Period II	30th January, 2008, 18:48 - 19:59	01st February, 2008, 16:23 -17:16

3.12. Schedule in Fasting Condition and Consumption of Food

Standard and controlled meals with respect to quantity, as per pre planned menu were served and finished between 20:00 to 21:00 hours on 23 January, 2008 in period I and between 20:05 to 20:46 hours on 30 January, 2008 in period II subsequent to check-in. After supervised overnight fasting of at least 10 hours, they received study drug with 200ml of water according to the randomization schedule. All the volunteers received lunch after 4 hours, snacks after 8 hours, dinner after 12 hours, breakfast after 24 hours, lunch after 28 hours and snacks after 32 hours post dose in both periods for entire duration of stay in the facility. The menu served was identical in both periods.

3.13. Standard Diet and Consumption of Liquids

Standard diet as per the Appendix 16.6 of the protocol was served to volunteers after dosing in each period.

Meals were prepared and served individually and the volunteers were politely asked to consume the complete meal. The meal starting time and ending time for each volunteer was recorded in section 'Meal Distribution Record' for period I and II respectively.

No water was allowed for 1 hour before and 2 hours after study drug administration then after ad libitum except at the time of study drug administration.

3.14. Administration of the Drugs

In Period I, all 26 volunteers were dosed on January 24, 2008 at morning and in Period II also, all 26 Volunteers were dosed January 31, 2008 at morning. In both the periods dosing was done between 08:00 and 08:26 hours at two dosing stations (Station 01: enrolment numbers 01-14) and (Station 02: enrolment numbers 15-26). The dosing was done at a gap of 2 minutes between each volunteer at each dosing station. Volunteers were administered the test or reference medication (as per the randomization scheme) as a single oral dose of 1 tablet containing carvedilol 25mg with 200 ml water under fasting condition.

Volunteers were seated upright for the first four hours following administration of the study product except for blood draws and toilet purpose. They were prohibited from

doing any sort of stressful physical activity during the entire period of stay at Pharmacokinetic unit.

3.14.1. Dosage

Test Drug- Single tablet of carvedilol 25mg manufactured by Torrent Pharmaceuticals Ltd., India was administered with 200 ml of water.

Batch No. B9717002

Reference Drug- Single tablet of Coreg® (Carvedilol 25mg) manufactured by Produtos Roche Quimicos e Farmaceuticos S.A., Brazil was administered with 200 ml of water.

Batch No. RJ0382

3.14.2. Washout Period

The washout period was 07 days between two periods.

3.14.3. Table with Administration Dates and Schedules for All Individuals

Table 5: Dosing Schedule

(Period I)

Date of Dosing	Dosing time	Enrolment No.	
24th January, 2008	8:00	1	15
24th January, 2008	8:02	2	16
24th January, 2008	8:04	3	17
24th January, 2008	8:06	4	18
24th January, 2008	8:08	5	19
24th January, 2008	8:10	6	20
24th January, 2008	8:12	7	21
24th January, 2008	8:14	8	22
24th January, 2008	8:16	9	23
24th January, 2008	8:18	10	24
24th January, 2008	8:20	11	25
24th January, 2008	8:22	12	26
24th January, 2008	8:24	13	
24th January, 2008	8:26	14	

(Period II)

Date of Dosing	Dosing time	enrolment No.	
31st January, 2008	8:00	1	15
31st January, 2008	8:02	2	16
31st January, 2008	8:04	3	17
31st January, 2008	8:06	4	18
31st January, 2008	8:08	5	19
31st January, 2008	8:10	6	20
31st January, 2008	8:12	7	21
31st January, 2008	8:14	8	22
31st January, 2008	8:16	9	23
31st January, 2008	8:18	10	24
31st January, 2008	8:20	11	25
31st January, 2008	8:22	12	26
31st January, 2008	8:24	13	
31st January, 2008	8:26	14	

3.15. Chronogram for Sample Collection

Twenty one venous blood samples were collected from each volunteer during each period. In the morning of dosing day after vitals measurement, a pre-dose blood sample (5ml) was taken 30 minutes before dosing. The other venous blood samples (5ml each) were withdrawn at 0.17 (0.166), 0.33, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 6.00, 8.00, 12.00, 18.00, 24.00, 32.00 and 48.00 hours post dose in each period. About 3 ml of blood was collected for post-study safety analysis from each of the volunteers at the time of last sample of period II.

Blood sampling up to ± 2 minutes of the planned time of in-house sampling and ± 1 hour of the planned time of ambulatory samples were considered as an acceptable deviation. Beyond that, time deviation was taken into consideration for further pharmacokinetic parameters, except for pre dose samples, which always be reported as zero (0) hour. All deviations related to blood samples were recorded in CRFs. During collection of blood sample at each time point the mid-point of the minute was considered to calculate the nearest minute, which was recorded on the appropriate CRF.

3.17. Collection, Handling, Storage and Transportation of the Samples

After collection of blood samples from all the volunteers at each time point, tubes containing blood samples were kept in box containing coolant and transferred for centrifugation. The centrifugation was carried out at 2000 RPM for 10 minutes at 20°C. The plasma samples then separated in pre-labeled 5ml polypropylene tubes, were subsequently stored at - 70°C ± 10°C until withdrawn for analysis. Timing of sample received, separation and storage was documented in form "Centrifugation and Storage record of study samples".

Plasma Samples were stored in 5ml polypropylene tubes pre labeled as shown below:

PK-07-108 (Carvedilol 25mg)
P-* En: * Dt: *
TP: * Sign:

* Respective period, enrollment number, date and time point (hh:mm)

At the end of the study the samples were kept in box containing coolant and transferred to bio-analytical department for analysis. The samples received at the analytical facility were frozen and in good condition.

3.18. Adverse Events and Emergency Procedures

All the volunteers were monitored for adverse events as specified in the protocol. No adverse event occurred during study.

Post study laboratory values were out of normal range for some volunteers but based on clinical investigator/physician's judgment they were clinically not significant except for Enrollment No. 02, 06, 08, 14, 16, 21 and 26 as mentioned below.

Enrollment No. 02: At the time of post study safety analysis, it was observed that his Triglyceride (198.00 mg/dl) and WBC (12.8 K/µl) values were high against reference normal ranges. As per physician assessment the volunteer was asked to follow up within 7-10 days, but the volunteer did not report to the centre after repeated follow ups.

Enrollment No. 06: At the time of post study safety analysis, it was observed that his Triglyceride (217.50 mg/dl) value was high against reference normal range. As per physician assessment the volunteer was asked to follow up within 7-10 days, but the volunteer did not report to the centre after repeated follow ups.

Enrollment No. 08: At the time of post study safety analysis, it was observed that his Triglyceride (253.66 mg/dl) value was high against reference normal range. As per physician assessment the volunteer was asked to follow up within 7-10 days. The repeat analysis of volunteer's Triglyceride (i.e. 100.62 mg/dl) was found within normal range.

Enrollment No. 14: At the time of post study safety analysis, it was observed that his Triglyceride (203.63 mg/dl) value was high against reference normal range. As per physician assessment the volunteer was asked to follow up within 7-10 days. The repeat analysis of volunteer's Triglyceride (i.e. 150.13 mg/dl) was clinically non-significant as judge by physician.

Enrollment No. 16: At the time of post study safety analysis, it was observed that his Triglyceride (412.76 mg/dl) and RBS (207.43 mg/dl) values were high against reference normal ranges. As per physician assessment the volunteer was asked to follow up within 7-10 days. The repeat analysis of volunteer's Triglyceride (i.e. 74.62 mg/dl) and RBS (47.92 mg/dl) values were found within normal ranges.

Enrollment No. 21: At the time of post study safety analysis, it was observed that his Triglyceride (315.92 mg/dl) value was high against reference normal range. As per physician assessment the volunteer was asked to follow up within 7-10 days. The repeat analysis of volunteer's Triglyceride (i.e. 95.92 mg/dl) was found within normal range.

Enrollment No. 26: At the time of post study safety analysis, it was observed that his Triglyceride (196.82 mg/dl) value was high against reference normal range. As per physician assessment the volunteer was asked to follow up within 7-10 days, but the volunteer did not report to the centre after repeated follow ups.

Upon conclusion of the clinical portion of the study, both the test and reference drugs were well tolerated.

3.19. Standard Operating Procedures (SOPS) in the Clinical Phase

The clinical phase of the study was carried out according to the in-house SOPs.

3.20. Deviation from Protocol and the Respective Degree of Impact in the Clinical and Pharmacokinetic Results

Following deviations were reported during the study. The below mentioned deviations do not have any significant impact on the quality and pharmacokinetic evaluation of the study.

- Volunteer replacement
- Late confinement of volunteer
- Sample Time Point Deviation
- Missing Sample Deviation

Volunteer replacement: Enrollment No. 23 withdrew voluntarily on a day before dosing in period I. Thus new volunteer had been enrolled at 20:49 hours and given the enrollment no. C-23, as per the in-house SOP no. BE-P-038.

Late confinement of volunteer: Enrollment No. C-23 had been confined to Pharmacokinetic Unit at 20:49 hours on enrollment day (33 minutes late).

Table 12: Sample Time Point Deviation

Enrolment No.	Period	Sampling Time point (hr)	Planned Sampling Time (hh:mm)	Actual Sampling Time (hh:mm)	Deviation (hh:mm)
02	II	48:00	08:02	09:24	01:22
25	II	48:00	08:20	09:27	01:07

Table 13: Missing sample Deviation

Enrolment No.	Period	No. of Missing samples	Sampling time point	Reason
21	I	1	48:00	Did not come for ambulatory sample

3.21. Discrepancies

Following discrepancy was noted in the study but did not have any impact on the outcome of the study.

As per the protocol page 35 of 60 (6^{th} Reference for protocol preparation) – "RE n^0 900 dated 29^{th} May, 2003" should be read as "RE n^o 310 dated 1^{st} September, 2004.

ANNEXURE – I
(CERTIFICATE OF ANALYSIS)

TORRENT PHARMACEUTICALS LTD.

RESEARCH CENTRE
ANALYTICAL DEVELOPMENT

CERTIFICATE OF ANALYSIS

Name of Product	: COREG®	Batch No :	RJ0382
Generic Name	: Carvedilol 25	Mfg Date :	July, 2006
Label Claim	: Each Uncoated Tablet contains : Carvedilol Ph. Eur 25mg	TRF No. :	FDV 9125/06-1
Manufactured By	: Roche	Report Date :	23.11.2006

Sr. No	Test	Results
1.	Appearance	White Circular biconvex tablet debossed D 5 on side separated by break line and debossed B M on other side separated by break line
2.	Average weight	82.0mg
3.	Water by Kf	4.0%
4.	Disintegration	3 minutes, 89 seconds
5.	Dissolution in 45 minutes	97.3%
6.	Uniformity of Dosage Unit	Min.-98.2, Max.-106.2, Mean : -102.8 % RSD : 2.7%
7.	Assay (By HPLC) (Each uncoated tablet contains : Carvedilol Ph. Eur....25mg)	99.6% of label claim
8.	Related Substances (By HPLC) Impurity A Impurity C Any other impurity Total Impurities	 Not detected Not detected 0.04% 0.12%

	Prepared By	*Checked By*	*Approved By*
Signature			
Name	Jalpa Trivedi	Mr. Umesh Khandelwal	Mr. Hitesh Jogia
Date	23.11.2006	23.11.2006	23.11.2006

Torrent Pharmaceuticals Ltd.
Works :
Indrad, Tal. Kadi,
Ahmedabad-Mehsana Highway, Gujarat, India.
Phone : (02764) 233671, 233678, 233680
Fax : (02764) 233676

Torrent House
Off. Ashram Road,
Ahmedabad-380 009.
India.
Phone : (079) 2658 5090
2658 3060
Fax : (079) 2658 2100

Quality Control Laboratory

Certificate of Analysis

SEMI FINISHED PRODUCT

Name Of Product : KARVIL 25 (FOR BRAZIL)	Specification No. : 10016668
Material No. : 4001650	Version No. : 1
Generic Name : CARVEDILOL TABLETS	Mfg.Lic.No. : G/926
:	Inspection Lot No. : 040000018924
Composition : Each uncoated tablet	Batch Size : 100000.000 TAB
: contains:	Date Of Receipt : 21.02.2007
: Carvedilol Ph. Eur...25 mg	Manufacturing Date : FEB-2007
:	Expiry Date : JAN-2009
Batch No : B9717002	Sample Quantity : 150.00 TAB
Manufactured By : Torrent Pharmaceuticals Ltd.	Analytical Report No.: 040000018924
Analysis as per : RLT/721	Date Of Report : 03.03.2007

TESTS	LIMITS	RESULTS
Appearance	White to off-white colored, round, flat, uncoated tablets with breakline on one side and plain on other side.	Off-white colored, round, flat, uncoated tablets withbreak line on one side and plain on other side.
Identification (By HPLC)	The retention time of the Carvedilol peak in the chromatogram shouldexhibit same retention time as that in the standard preparation.	The retention time of the Carvedilol peak in the chromatogramshould exhibit same retention time that in the standardpreparation.
Average weight	172.0 mg ± 5 % (163.4 to 180.6 mg)	172.1 mg
Uniformity of weight	Average weight ± 7.5 %	-2.1% to +2.0%
Hardness	80 N to 160 N	88 N
Friability	Not more than 1.0 %	0.01 %
Water by KF	Not more than 7.0 %	5.1 %
Disintegration	Not more thah 15 minutes	05 min
Dissolution in 45 minutes	Not less than 75.0 % (Q); of label claim	1)101% 2)102% 3)102%4)101% 5)101% 6)103%Mean:103%
Uniformity of Dosage Unit	Not less than 85.0 % and not more than 115.0%; of label claim (RSD: Notmore than 6.0 %)	1)102.0% 2)101.8% 3)101.3%4)103.6% 5)102.9% 6)103.7%7)101.8% 8)102.0% 9)103.5%10)102.2%Mean:102.5% RSD:0.8%
Assay (BY HPLC) (Each uncoated tablet contains:Carvedilol Ph. Eur. ...25 mg)	Not less than 95.0 % and not more than 105.0 %; of label claim	101.1 %

Remarks : Complies as per RLT/721 Specification.
Sampling Plan : Z001
Issued To Plant Q.A.

Prepared by :	Checked by :	Approved by :
Date : 21/02/08	Date : 21/02/08	Date : 21/02/08

4.0. BIOANALYTICAL REPORT

Bio-Evaluation Centre
Torrent Pharmaceuticals Limited
Village: Bhat, Dist Gandhinagar – 382 428
Gujarat, India

QUALITY ASSURANCE STATEMENT

Study Code: PK-07-108

Title: ***A Randomised,Open label, Two-period, Two-treatement, Two-sequence, Crossover, Single Dose Bioequivalence study of Carvedilol 25mg tablets (Test) [Torrent pharmaceuticals Ltd., India] versus Carvedilol 25mg tablets (Coreg®) (Reference) [Produtos Roche Quimicos E Farmaceuticos S.A., Brazil] in healthy Human subjects under fasted state.***

The Quality Assurance Unit audited the study sample analysis. The result presented in this report accurately reflects the raw data and is in compliance with the relevant SOPs and Protocol. The documentation of data is consistant with GLP guideline.

The in process and retrospective audit of this study sample analysis is conducted based on the QA procedures.

The dates on which audits were performed are given below:

Activity	Dates of Audit	Reporting Date
In-Process Audit	09/05/08 and 12/05/08	12/05/08
Retrospective Audit	20/05/08 and 21/05/08	22/05/08

Authorized by: Mr. Gopal Joshi
(Head –Quality Assurance)

Signature: _____

Date: 29/05/08

4. BIO ANALYTICAL STUDY REPORT

(FOR CARVEDILOL)

4.1 TITLE PAGE

4.1.1 IDENTIFICATION CODE OF THE STUDY (STUDY CODE: PK-07-108)

4.1.2 TITLE

A RANDOMISED, OPEN LABEL, TWO-PERIOD, TWO-TREATMENT, TWO-SEQUENCE, CROSSOVER, SINGLE DOSE BIOEQUIVALENCE STUDY OF CARVEDILOL 25mg TABLETS (TEST) [TORRENT PHARMACEUTICALS LTD., INDIA] VERSUS CARVEDILOL 25mg TABLETS (COREG®) (REFERENCE) [PRODUTOS ROCHE QUIMICOS E FARMACEUTICOS S.A., BRAZIL] IN HEALTHY HUMAN SUBJECTS UNDER FASTED STATE

4.1.3 ANALYTICAL REPORT DATE (MAY-2008)

4.1.4 NAME AND ADDRESS OF THE BIOEQUIVALENCE CENTRE

BIOANALYTICAL LABORATORY
BIO-EVALUATION CENTRE
TORRENT PHARMACEUTICALS LTD.
VILLAGE BHAT, GANDHINAGAR-382 428
GUJARAT, INDIA

4.2

INDEX

A) List of Abbreviations

Abbreviation	Description
BLQ	Below Limit of Quantification
CV	Coefficient of Variance
EN	Enrollment/ Volunteer/Subject
Hrs or hr	Hours
HQC	High Quality Control
LC-MS	Liquid Chromatography – Mass Spectrometry
LLOQ	Lower Limit of Quantification
LQC	Low Quality Control
MS	Mass Spectrometric
MQC	Medium Quality Control
NA	Not Applicable
ng	nanogram
P-I	Period one
P-II	Period two
QC	Quality Control
r	Correlation Coefficient
SD	Standard Deviation
SOP	Standard Operating Procedure
SOTP/Protocol	Standard Operating Test Procedure
ULOQ	Upper Limit of Quantification
µ	Micron
%	Percentage

B) List Of Figures

C) List of Tables

D) List of Annexure

Annexure No.	Title
4.12.1.1	Method validation report of Carvedilol
4.12.2	Certificate of analysis for Carvedilol and Olanzapine
4.12.3	SOTPs/Protocol and SOPs
4.12.3.1	Method for estimation of Carvedilol in Human Plasma over concentration range of 0.500ng/ml to 150.000ng/ml using High Performance Liquid Chromatography-Mass Spectrometric detection. [SOTP/ Protocol No. BA/08/003(T1)].
4.12.3.2	Method for estimation of Carvedilol in Human Plasma over concentration range of 0.500ng/ml to 150.000ng/ml using High Performance Liquid Chromatography-Mass Spectrometric detection [SOTP /Protocol No. BA/08/003].
4.12.3.3	Preparation, Identification and verification for spiking stock solutions of Calibration Standards and Quality Control Samples for Bioavailability (BA) and Bioequivalence (BE) studies. (SOP No. BE-A-012).
4.12.3.4	Pre method validation, full method validation and partial method validation of Bioanalytical methods related to Bioavailability (BA) and Bioequivalence (BE) studies. (SOP No. BE-A-013)
4.12.3.5	Sample preparation, analytical run/batch organization, re-injection, reassaying and reporting of the study samples results related to Bioavailability (BA) and Bioequivalence (BE) studies. (SOP No. BE-A-014)
4.12.3.6	Preparation and control of Standard Operating Test Procedures (SOTPs)/protocol (SOP No. BE-A-015)
4.12.3.7	Assessing quality of chromatograms by proper peaks integration, generation and verification of chromatograms, and their acceptance criteria. (SOP No. BE-A-016)

4.12.3.8 Identification, receipt, storage, use, restorage and disposal of all biological samples received in Bioanalytical laboratory. (SOP No. BE-A-010)

4.12.3.9 Sequence of Bioanalytical courses and related documentation. (SOP No. BE-A-011)

4.12.3.10 Copy of complete series of chromatogram for Volunteer Number: 19,20,21,22,23 & 24

4.12.3.11 Copy of complete series of specificity check chromatograms (Method Validation)

4.3 FOLIO OF SIGNATURES

The analysis of study samples and process derived data of Carvedilol were performed by

Name	: Mr. Shailesh Ghataliya	Name	: Mr. Tushar Bhavsar
Designation	: Scientist – II	Designation	: Scientist – II
Qualification	: M.Sc.	Qualification	: M.Sc.
Function	: Analyst	Function	: Analyst
Date	: 29/05/08	Date	: 29/05/08
Signature	: Ghataliya S.P.	Signature	: Tusshar
Name	: Ms. Sapna Shah	Name	: Mr. Kavan Mudiyanda
Designation	: Scientist – II	Designation	: Scientist – II
Qualification	: M.Sc.	Qualification	: M.Sc.
Function	: Analyst	Function	: Analyst
Date	: 29/05/08	Date	: 29/05/08
Signature	: S.P.Shah	Signature	:
Name	: Mrs. Heena Patel	Name	: Mr. Suni Khubchandani
Designation	: Technical Assistant	Designation	: Technical Assistant
Qualification	: B.Pharm.	Qualification	: B.Pharm.
Function	: Analyst	Function	: Analyst
Date	: 29/05/08	Date	: 29/05/08
Signature	: Patel	Signature	: Suni.V

I, the undersigned, declare that, to best of my knowledge, I had reviewed this analytical report for the compliance with the Bio-Evaluation Centre implemented SOPs and that raw data presented in this report were accurate and authentic.

Name	: Dr. Deepak Jain	Name	: Mr. Nitesh Patel
Designation	: Scientist-I	Designation	: Research Associate
Qualification	: M.Sc., Dip - R & D, MDBA, Ph.D	Qualification	: M.Sc.
		Function	: Supervisor
Function	: Supervisor	Date	: 29/05/08
Date	: 29/05/08	Signature	: pkdave
Signature	:		

I, the under signed, declare that, to the best of my knowledge, I had reviewed this analytical report for compliance with Bio-Evaluation Centre implemented SOPs and that I had scientifically evaluated statements and conclusions of this report.

Name	: Dr. Gunta Subbaiah
Designation	: Head of Department
Qualification	: Ph.D
Function	: Chief Researcher
Date	: 29/05/08
Signature	: Gsubbu

4.4 GLOSSARY

Accuracy:

The degree of closeness of mean test results obtained by the method to the true value (concentration) of analyte(s).

Anticoagulant:

An anticoagulant is a substance that stops blood from clotting.

Bioavailability:

Rate and extent to which a drug is absorbed or is otherwise available to the treatment site in body.

Bioequivalence:

Scientific basis on which generic and brand named drugs are compared. To be considered bioequivalent, the bioavailability of two products must not differ significantly when the two products are given in studies at the same dosage under similar conditions. Some drugs, however, are intended to have a different absorption rate.

Blank Plasma:

Plasma sample without Carvedilol and internal standards.

Calibration curve:

Plot of Drug response vs. known concentration obtained by analyzing a set of standard samples and measuring the response. The curve may use the response for the sample (height or area) or the ratio of the response to that of an internal standard.

Internal standard:

Test compound(s) (e.g. structurally similar analog, stable labeled compound) added to both calibration standards and samples at known and constant concentration to facilitate quantification of the target analyte (s).

Method validation:

Bioanalytical method validation includes all the procedure that demonstrate that a particular method used for quantitative measurement of analyte(s) in a given

biological matrix such as blood, plasma, serum or urine is reliable and reproducible for intended use.

Precision:
Precision is the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed condition.

Quality control sample:

A sample added to a batch to estimate the data quality and accuracy of unknown samples in the batch. The QC sample has a known concentration but is treated as an unknown so that the measured concentration can be compared to the actual value.

Standard operating procedure (SOP):

Standard elaborate written instructions to achieve uniformity of performance in the management of clinical study or analytical work. SOPs provide a general framework for the efficient implementation and performance of all the functions and activities related to a particular study.

Standard operating test procedure (SOTP/Protocol):

Describe the analytical methodology to perform the test.

Zero standard:

Blank plasma with internal standard only.

4.5 BIO-ANALYTICAL METHOD FOR ESTIMATION OF CARVEDILOL IN HUMAN PLASMA

4.5.1 Bio-Analytical technique

LC-MS/MS technique was followed.

The summary of the chromatographic conditions were mentioned in section 17.0 of SOTP/Protocol No. BA/08/003.

4.5.2 Detection

Mass spectrometer (API 4000) detection was used.

4.5.3 Internal standards

Olanzapine was used as a internal standard for Carvedilol.

4.5.4 Biological source

Heparinised blank human plasma matrix was procured from pharmacokinetic unit for preparation of the plasma calibration standards and quality control samples. Study samples were received from pharmacokinetic unit of Bio-Evaluation Centre

4.5.5 Anticoagulant

Heparin was used as an Anticoagulant for the study samples received from pharmacokinetic unit, where as Heparinised blank human plasma procured from pharmacokinetic unit of Bio-Evaluation Centre containing Heparin as an Anticoagulant.

4.5.6 Type of extraction and procedure

Solid phase extraction technique was followed and its procedure was mentioned in section 16.0 of SOTP/Protocol No.BA/08/003.

4.5.7 Linearity group

The calibration curves were linear from 0.500 ng/ml to 150.000 ng/ml for Carvedilol.

4.5.8 Quantification parameter

The quantification parameters used were as per the Analyst software- Version -1.4.1.

4.5.9 Detection parameters

The summary of the detector parameters was mentioned in section 18.0 of SOTP/Protocol No. BA/08/003.

4.6 WORKING STANDARDS

4.6.1)	Name	:Carvedilol
	Batch No.	: CAN0030208
	Validity date	: 28/02/2009
	Name and address of manufacture	: Symed Lab Ltd. India
4.6.2)	Name	: Olanzapine (Internal Standard)
	Batch No.	: OLA3/FORM-I/XII/007
	Validity date	: MAY-2008
	Name and address of manufacture	: Torrent Research Ltd., India

4.7 PREPARATION OF THE CALIBRATION STANDARDS AND QUALITY CONTROL SAMPLES

The preparation of the calibration standards and quality control samples were done with heparinised blank human plasma according to the method SOTP/Protocol No. BA/08/003.

4.8 RECEIPTS, LABELING AND STORAGE OF SAMPLES

The study samples were received from pharmacokinetic unit under freezed condition on 29/02/08 and were stored below –70°C in deep freezer at the bio-analytical facility of Bio-Evaluation Centre.

Labeling: The samples were labeled to indicate the Study code, Date, Enrollment No., Period, Sample collection Time point and Signature of person authorized by the Chief Investigator.

4.9 CALCULATION OF THE SAMPLE CONCENTRATION

The concentration of the standards and the unknowns were calculated from the following equation using regression analysis of spiked plasma calibration standard with weighting factor of $1/x^2$.

$$y = mx + c$$

Where → y = peak area ratio of drug to IS
m = slope of the calibration curve
x = Concentration of drug

c= y-axis intercept of the calibration curve

4.10 SOP deviation

No deviation

4.11 TABLES

See page number 16 to 29

4.12 ANNEXURE

The annexure comprises of Method validation report and Certificate of analysis (Carvedilol and Olanzapine), SOTPs/Protocol and SOPs.

4.13 RANGE OF CONCENTRATION EMPLOYED IN THE STANDARD CURVE

The range of the concentration employed in the standard curve for Carvedilol was 0.500 ng/ml to 150.000 ng/ml.

4.14 DATE OF PREPARATION OF CALIBRATION STANDARDS AND QUALITY CONTROL SAMPLES

The calibration curve standards and Quality control samples were bulk spiked on 29/04/08 and stored at -70°C for the study samples and DQC were freshly prepared during reanalysis.

4.15 DURATION OF STUDY SAMPLE STORAGE AND ANALYSIS

Date of first study sample collected was on 24/01/08 and the date of last study sample analyzed on 13/05/08. The time period between the first study sample collected and last study sample analyzed was 111 days.

4.16 CALIBRATION CURVES

Representative calibration curves for Carvedilol were given in Figure 1.
Summary of calibration curve parameters for Carvedilol were given in Table No. 4.11.3

4. 17 REANALYSIS

Observing the results of study samples, 9 samples were reanalyzed for Carvedilol. The reanalysis of the study samples was done as per SOP No. BE-A-014 and the results were represented in Table No. 4.11.2 for Carvedilol.

Total % of reanalyzed samples for Carvedilol was 0.82 (Table No. 4.11.6.1).

Observing the results of study samples, 1 sample were repeated for Carvedilol. The repeat analysis of the study samples were done as per SOP No. BE-A-014 and the results were represented in Table No. 4.11.2 for Carvedilol.

Total % of repeat sample for Carvedilol was 0.09. (Table No. 4.11.6.2).

4.18 RESULTS

4.18.1 Precision and accuracy data of quality control samples

Quality control samples at four different concentrations were analyzed randomly interspersed with the study samples.

The % CV for LQC, MQC, HQC and DQC samples of Carvedilol were 7.13, 9.00, 6.79 and 1.50 respectively. (Table No. 4.11.5).

The mean % nominal concentration for LQC, MQC, HQC and DQC samples of Carvedilol were 99.49, 101.52, 100.00 and 94.84 respectively (Table No. 4.11.5).

4.18.2 Back calculated calibration curve concentrations

A total of ten calibration curves were generated for analysis of study samples for Carvedilol.

The calibration curves were linear from 0.500 ng/ml to 150.000 ng/ml for Carvedilol (Table No. 4.11.4). Calibration lines of chromatographic response versus concentration were determined by weighted least square regression analysis with a weighting factor of $1/x^2$, the coefficient of correlation (r) was consistently ≥ 0.9973 for all calibration curves. (Table No. 4.11.3)

4.19 STUDY SAMPLE CONCENTRATIONS

Obtained concentrations of Carvedilol (ng/ml) in Enrollment samples for Period –I and Period-II were given in Table No. 4.11.1.1 to 4.11.1.4.

4.20 HISTORY OF CHANGE IN VERSION OF STUDY REPORT

Version No.	Date	Page No.	Section	Modification
01	16/05/08	-	-	Original report

BIO-EVALUATION CENTRE
TORRENT PHARMACEUTICALS Ltd.
Village Bhat, Gandhinagar-382 428
Gujarat, India.

ANNEXURE No. 4.12.1.1

METHOD VALIDATION REPORT OF CARVEDILOL

Bio-Evaluation Centre
Torrent Pharmaceuticals Limited
Village: Bhat, Dist Gandhinagar – 382 428
Gujarat, India

QUALITY ASSURANCE STATEMENT

MV No.: ***BA/MV/003/08***

Title: ***Method for Estimation of Carvedilol In Human Plasma over concentration Range 0.500 ng/ml to 150.00 ng/ml using high performance liquid chromatography-mass spectrometric detection.***

The Quality Assurance Unit audited the above method validation. The result presented in this report accurately reflects the raw data and is in compliance with the relevant SOPs and Protocol. The documentation of data is consistant with GLP guideline.

The in process and retrospective audit of this method validation is conducted based on the QA procedures.

The dates on which audits were performed are given below:

Activity	Dates of Audit	Reporting Date
In process audit	17/04/08	17/04/08
Retrospective Audit	25/04/08, 28/04/08, 29/04/08	29/04/08

Authorized by: Mr. Gopal Joshi
(Head Quality Assurance)

Signature: _____

Date: 29/04/08

METHOD FOR ESTIMATION OF CARVEDILOL IN HUMAN PLASMA OVER CONCENTRATION RANGE OF 0.500 ng/ml TO 150.000 ng/ml USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRIC DETECTION.

BIOANALYTICAL METHOD VALIDATION REPORT (APRIL-2008)

BIOANALYTICAL LABORATORY
BIO-EVALUATION CENTRE
TORRENT PHARMACEUTICALS LTD.
VILLAGE BHAT, GANDHINAGAR-382 428
GUJARAT, INDIA

INDEX

A) List of Abbreviations

Abbreviation	Description
CV	Coefficient of Variance
Comp. Sample	Comparison Sample
Conc.	Concentration
Contd.	Continued
hrs.	Hours
HQC	High Quality Control
IS	Internal Standard
LC-MS/MS	Liquid Chromatography-Mass Spectrometry/ Mass Spectrometry
LLOQ	Lower Limit of Quantification
LQC	Low Quality Control
ml	Milliliter
MS	Mass Spectrometric
MQC	Medium Quality Control
µg	Microgram
No.	Number
ng	Nanogram
QC	Quality Control
r	Correlation Coefficient
SD	Standard Deviation
SOP	Standard Operating Procedure
SOTP	Standard Operating Test Procedure
temp./TEMP.	Temperature
ULOQ	Upper Limit of Quantification
%	Percentage

B) List of Figures

C) List of Tables

1.0 INVESTIGATORS

FOLIO OF SIGNATURES

The analysis of validation samples and process derived data of Carvedilol were performed by

Name	: Mr. Shailesh Ghataliya	Name	: Mr. Tushar Bhavsar
Designation	: Scientist – II	Designation	: Scientist – II
Qualification	: M.Sc.	Qualification	: M.Sc.
Function	: Analyst	Function	: Analyst
Date	: 29/04/08	Date	: 29/04/08
Signature	: Ghataliya S.P	Signature	: Tusher
Name	: Ms. Sapna Shah	Name	: Mr. Kavan Mudiyanda
Designation	: Scientist – II	Designation	: Scientist – II
Qualification	: M.Sc.	Qualification	: M.Sc.
Function	: Analyst	Function	: Analyst
Date	: 29/04/08	Date	: 29/04/08
Signature	: S.P.Shah	Signature	: [illegible]
Name	: Mrs. Heena Patel	Name	: Mr. Suni Khubchandani
Designation	: Technical Assistant	Designation	: Technical Assistant
Qualification	: B.Pharm.	Qualification	: B.Pharm.
Function	: Analyst	Function	: Analyst
Date	: 29/04/08	Date	: 29/04/08
Signature	: [signature]	Signature	: SUNI

We, the undersigned, declare that, to best of our knowledge, we had reviewed this analytical report for the compliance with the Bio-Evaluation centre implemented SOPs and that raw data presented in this report were accurate and authentic.

Name	: Dr. Deepak Jain	Name	: Mr. Nitesh Patel
Designation	: Scientist-I	Designation	: Research Associate
Qualification	: M.Sc., Dip - R & D, MDBA,Ph.D	Qualification	: M.Sc.
Function	: Supervisor	Function	: Supervisor
Date	: 29/04/08	Date	: 29/04/08
Signature	: [signature]	Signature	: [signature]

I, the under signed, declare that, to the best of my knowledge, I had reviewed this analytical report for compliance with Bio-Evaluation centre implemented SOPs and that I had scientifically evaluated statements and conclusions of this report.

Name	: Dr. Gunta Subbaiah
Designation	: Head of Department
Qualification	: Ph.D
Function	: Chief Researcher
Date	: 29/04/08
Signature	: [signature]

2.0 OBJECTIVE

The objective of this work was to validate specific LC-MS/MS for the determination of Carvedilol in human plasma for bioequivalence study of Carvedilol.

3.0 SUMMARY

LC-MS/MS method for the determination of Carvedilol i n human plasma was carried out according to SOTP No.BA/08/003(T1). Carvedilol were extracted from human plasma using solid phase extraction technique. The final eluent was injected into a liquid chromatograph equipped with mass detector. Quantification was performed by peak area ratio method. A weighting factor $1/X^2$ was used to determine the concentration of the drug.

3.1 BIO-ANALYTICAL METHOD FOR ESTIMATION OF CARVEDILOL IN HUMAN PLASMA

3.1.1 Reported Literature

Estimation of carvedilol in human plasma by using HPLC-fluorescence detector and its application to pharmacokinetic study

J Chromatogr B Analyt Technol Biomed Life Sci. 2007 Jul 22; : 17702675 (P,S,E,B,D)
Rajeshwari Rathod, L Poorna Chandra Prasad, Shubha Rani, Manish Nivsarkar, Harish Padh

A simple, precise and sensitive high performance liquid chromatography proccdurc has been developed for determination of carvedilol in human plasma. The method was developed on Lichrosphere R CN column using a mobile phase of acetonitrile/20mM ammonium acetate buffer with 0.1% triethylamine (pH adjusted to 4.5) (40/60, v/v). The peaks were detected by using fluorescence detector (excitation wavelength 282nm and emission wavelength 340nm). Carvedilol and domperidone (internal standard) were extracted by liquid-liquid extraction procedure using dichloromethane. This method was specific and had a linearity range of 1-128ng/ml with intra- and inter-day precision (%C.V.) less than 15%. The accuracy ranges from 87.3 to 100.88% and the recovery of carvedilol was 69.90%. The stability studies showed that carvedilol in human plasma was stable during short-term period for sample preparation and analysis. This method was used to assay the carvedilol in human plasma samples obtained from subjects who had been given an oral tablet of 12.5mg carvedilol

3.1.2 Bio-Analytical technique

LC-MS/MS technique was followed.
The summary of the chromatographic conditions are mentioned in section 17.0 of SOTP No. BA/08/003(T1).

3.1.3 Detector Parameters

The summary of the detector parameters are mentioned in section 18.0 of SOTP No. BA/08/003(T1).

3.1.4 Internal Standard

Olanzapine was used as internal standard for Carvedilol.

3.1.5 Biological Source

Heparinised control plasma, heamolysed and lipemic plasma were procured from pharmacokinetic unit department.

3.1.6 Anticogulant

Anticoagulant includes heparin.

3.1.7 Type of extraction

Solid phase extraction technique was followed and its procedure was mentioned in Section 16.0 of SOTP No. BA/08/003(T1).

3.1.8 Linearity Group

The calibration curves were linear from 0.500 ng/ml to 150.000 ng/ml for Carvedilol.

3.1.9 Quantification Parameter

The quantification parameters were performed as per Analyst software, version-1.4.1

3.2 Working Standards

3.2.1)	Name	: Carvedilol
	Batch No.	: CAN0351206
	Validity date	: 20/04/08
	Name and address of manufacture	: Symed Lab Ltd. India
3.2.2)	Name	: Olanzapine (Internal Standard)
	Batch No.	: OLA3/FORM-I/XII/007
	Validity date	: MAY-2008
	Name and address of manufacture	: Torrent Research Ltd., India

3.3 PREPARATION OF CALIBRATION STANDARDS AND QUALITY CONTROL SAMPLES.

Calibration standards and quality control samples were bulk spiked as per SOTP No. BA/08/003(T1) on 16/04/08 and store at -70°C and -20°C deepfreeze.

3.4 LABELING AND STORAGE

3.4.1 Aqueous stock solutions

The stock solutions were labeled to indicate the analyte name, standard identification (calibration standard or quality control sample) and date of preparation.

3.4.2 Plasma samples

Blank plasma was labeled to indicate the lot number and stored in deep freeze (-20°C and / or -70°C).

3.5 CALCULATION OF THE SAMPLE CONCENTRATION

The concentration of the analyte was calculated from the following equation using regression analysis of spiked plasma calibration standard with the weighting factor of $1/x^2$: $y = mx + c$

Where → y = peak area ratio of analyte to internal standard
m = slope of the calibration curve
x = concentration of analyte
c = y-axis intercept of the calibration curve

3.6 SOP DEVIATION

No deviation

4.0 VALIDATION AND CHARACTERISTICS OF THE METHOD

4.1 Chromatography

Representative chromatograms of system suitability, blank plasma, zero standard, LLOQ, ULOQ, LQC, MQC, HQC samples and calibration curve for Carvedilol were represented in figure No. 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8 and 7.9 respectively.

4.2 Specificity

Six different lots of heparinised plasma, one lot of haemolised plasma and one lot of lipemic plasma were chromatographed and the area observed at the RT of carvedilol was ≤ 20% of

LLOQ area response and the area observed at the RT of I.S. in blank plasma was ≤ 5% of IS area response (Table No.8.1)

4.3 Sensitivity

The LLOQ was 0.500 ng/ml for Carvedilol.

The % CV of Carvedilol (Table No.8.2) at LLOQ was found to be 6.47.

The % nominal concentration for LLOQ samples of Carvedilol were ranged from 93.80 to 107.40 (Table No. 8.2).

4.4 Linearity

The Linearity of the method was determined by a weighted least square regression analysis of standard plots associated with a ten-point standard calibration curve. Best-fit calibration curve of peak area ratio versus concentration were drawn. The calibration curve for Carvedilol were linear from 0.500 ng/ml to 150.00 ng/ml with correlation coefficient of $r \geq 0.9980$(Table No. 8.3 and 8.4)

4.5 Accuracy

4.5.1 Within-batch or intra batch accuracy

The % nominal concentration for LLOQ, LQC, MQC and HQC samples of Carvedilol were ranged from 93.80 to 107.40, 91.87 to 110.40, 92.13 to 97.61 and 91.75 to 95.78 respectively. (Table No. 8.5.1)

4.5.2 Between-batch or inter-batch Accuracy

The % nominal concentration for LLOQ, LQC, MQC and HQC samples of Carvedilol were ranged from 93.80 to 118.80, 91.87 to 110.40, 85.48 to 102.54 and 85.68 to 106.96 respectively. (Table No. 8.5.2)

4.6 Precision

4.6.1 Within-batch or intra- batch Precision

The % CV for LLOQ, LQC, MQC and HQC samples of Carvedilol were 6.47, 6.32, 2.07 & 1.67 respectively. (Table No. 8.5.1)

4.6.2 Between-batch or inter-batch Precision

The % CV for LLOQ, LQC, MQC and HQC samples of Carvedilol were 5.89, 3.53, 4.08 & 6.49. (Table No. 8.5.2)

4.7 Recovery

The percentage recovery of Carvedilol was determined by comparing the mean peak area of Carvedilol in extracted LQC, MQC and HQC samples with freshly prepared unextracted LQC, MQC and HQC samples.

The mean % recovery for LQC, MQC and HQC samples of Carvedilol were 97.76, 91.51 & 89.53 respectively. (Table No. 8.6.1).

For Olanzapine (IS) mean peak areas of eighteen extracted samples were compared to the mean peak area of unextracted internal standard solution. The mean percentage recovery value for Olanzapine (IS) was 88.51 (Table No. 8.6.3).

The %CV of Unextracted LQC, MQC and HQC samples of analyte were 3.60, 1.84 and 2.19 respectively (Table No. 8.6.1).

The %CV of Extracted LQC, MQC and HQC samples of analyte were 3.60, 2.62 and 2.24 respectively (Table No. 8.6.1).

The % CV of recovery across QC levels for Carvedilol was 4.62 (Table No 8.6.2).

The % CV within IS concentration of unextracted and extracted samples for Olanzapine (IS) was 6.31 and 4.13 respectively (Table No. 8.6.3).

4.8 Dilution Integrity

Dilution integrity experiment was carried out at six replicate of two times diluted 2xULOQ (½ dilution), four times diluted 2xULOQ (¼ dilution) samples were prepared and its concentrations were calculated against the freshly prepared calibration curve.

The %CV for ¼ dilution and ½ dilution samples of Carvedilol were 1.07 and 2.64 respectively (Table No 8.7).

The % nominal concentration for ¼ dilution and ½ dilution samples were ranged from 97.41 to 100.00 and 89.38 to 96.42 respectively (Table No 8.7)

4.9 Matrix Effect

In order to ensure the effect of matrix through out the application of the method, plasma blanks obtained from six different lots (04 normal heparinised, 01 haemolysed and 01lipemic) were spiked with Carvedilol and Olanzapine at LQC and HQC level. Three quality control samples

at each level along with the set of calibration standards were analyzed and the % Nominal conc. of the samples analyzed was within ± 15 % for Carvedilol. (Table No. 8.8.)

4.10 Stability

4.10.1 Stock solution stability

Stock solution stability was determined by comparing the peak areas of freshly prepared solutions (comparison sample) with stability samples.

4.10.1.1 Main Stock solution stability of Carvedilol and Olanzapine (IS) at room temperature for 27 hrs.

Main stock of Carvedilol and Olanzapine (IS) stock solution were freshly prepared and aliquots of Carvedilol and Olanzapine (IS) were kept at room temperature for 27 hours (stability samples). Aqueous equivalent highest calibration standard of Carvedilol and Olanzapine (IS) were prepared from stability samples and were analysed. Area of stability samples and freshly prepared samples were compared to determine % mean change during stability period.

The %CV for comparison sample and stability sample of Carvedilol were 1.29 and 0.49 respectively. (Table No. 8.9.1.1)

The %CV for comparison sample and stability sample of Olanzapine (IS) solution were 1.00 and 0.17 respectively. (Table No. 8.9.1.2)

Carvedilol main stock solution was found to be stable at room temperature for 27 hrs with % mean change of -8.73 (Table No.8.9.1.1).

Olanzapine (IS) main stock solution was found to be stable at room temperature for 27 hrs with % mean change of 1.85 (Table No.8.9.1.2).

4.10.1.2 Spiking Stock solution stability of Carvedilol and Olanzapine (IS) at room temperature for 27 hrs.

Spiking stock of Carvedilol and Olanzapine (IS) stock solution were freshly prepared and aliquots of Carvedilol and Olanzapine (IS) were kept at room temperature for 27 hours (stability samples). Aqueous equivalent highest calibration standard of Carvedilol and Olanzapine (IS) were prepared from stability samples and were analysed. Area of stability samples and freshly prepared samples were compared to determine % mean change during stability period.

The %CV for comparison sample and stability sample of Carvedilol were 1.29 and 1.65 respectively. (Table No. 8.9.1.3)

The %CV for comparison sample and stability sample of Olanzapine (IS) solution were 1.00 and 0.65 respectively. (Table No. 8.9.1.4)

Carvedilol Spiking stock solution was found to be stable at room temperature for 27 hrs with % mean change of -2.84 (Table No.8.9.1.3).

Olanzapine (IS) Spiking stock solution was found to be stable at room temperature for 27 hrs with % mean change of 0.73 (Table No.8.9.1.4).

4.10.1.3 Main Stock Solution Stability of Carvedilol and Olanzapine at 2-8°C temperature for 27 Hrs.

Main stock of Carvedilol and Olanzapine (IS) stock solution were freshly prepared and aliquots of Carvedilol and Olanzapine (IS) were kept at 2-8°C for 27 hours (stability samples). Aqueous equivalent highest calibration standard of Carvedilol and Olanzapine (IS) were prepared from stability samples and were analysed. Area of stability samples and freshly prepared samples were compared to determine % mean change during stability period.

The %CV for comparison sample and stability sample of Carvedilol were 1.29 and 1.37 respectively. (Table No.8.9.1.5)

The %CV for comparison sample and stability sample of Olanzapine were 1.00 and 0.85 respectively. (Table No. 8.9.1.6)

Carvedilol stock solution was found to be stable at 2-8°C for 27 Hrs with % Mean change of 0.08 (Table No. 8.9.1.5)

Olanzapine stock solution was found to be stable at 2-8°C for 27 Hrs with % Mean change of -1.44 (Table No.8.9.1.6)

4.10.1.4 Main Stock Solution Stability of Carvedilol and Olanzapine at 2-8°C temperature for 3 days.

Main stock of Carvedilol and Olanzapine (IS) stock solution were freshly prepared and aliquots of Carvedilol and Olanzapine (IS) were kept at 2-8°C for 3 days stability samples). Aqueous equivalent highest calibration standard of Carvedilol and Olanzapine (IS) were prepared from stability samples and were analysed. Area of stability samples and freshly prepared samples were compared to determine % mean change during stability period.

The %CV for comparison sample and stability sample of Carvedilol were 0.92 and 0.48 respectively. (Table No.8.9.1.7)

The %CV for comparison sample and stability sample of Olanzapine were 0.38 and 0.64 respectively. (Table No. 8.9.1.8)

Carvedilol stock solution was found to be stable at 2-8°C for 3 days with % Mean change of -1.11 (Table No. 8.9.1.7)

Olanzapine stock solution was found to be stable at 2-8°C for 3 days with % Mean change of 0.14 (Table No.8.9.1.8)

4.10.2 Bench Top Stability of Carvedilol in human plasma at room temperature for 27 hours

Bulk spiked Samples at LQC and HQC levels were retrieved and kept at room temperature for 27 hours and were analyzed along with freshly prepared LQC and HQC samples. Concentrations were calculated to determine % mean change during stability period.

Carvedilol was found to be stable at LQC and HQC samples for 27 hours at room temperature with % mean change of -2.56 and -4.05 respectively (Table No 8.9.2).

4.10.3 Process Stability of Carvedilol at 4°C in autosampler 43 hours

LQC and HQC samples were prepared and processed. These processed samples were kept in autosampler for 43 hours at about 4°C. These samples were analyzed after 43 hours along with freshly prepared LQC and HQC samples. Concentrations were calculated to determine % mean change during stability period.

Carvedilol was found to be stable at LQC and HQC samples for 43 hours at about 4°C in autosampler with % mean change of 3.41 and 5.88 respectively (Table No.8.9.3.)

4.10.4.1 Freeze and Thaw Stability of Carvedilol in human plasma after 3rd cycles at at –20°C

Bulk spiked Samples at LQC and HQC levels were frozen at -20°C. Six samples from each concentration were subjected to three freeze and thaw cycles (stability samples). These samples were processed after 3rd cycle and analyzed along with freshly prepared LQC and HQC samples (comparison samples). Concentrations were calculated to determine % mean change after 3rd cycle.

Carvedilol was found to be stable in LQC and HQC samples after 3rd cycle at -20°C with % mean change of 0.11and 5.35 respectively (Table No.8.9.4.1).

4.10.4.2 Freeze and Thaw Stability of Carvedilol in human plasma after 4th cycles at at –70°C

Bulk spiked Samples at LQC and HQC levels were frozen at -70°C. Six samples from each concentration were subjected to four freeze and thaw cycles (stability samples). These samples were processed after 4th cycle and analyzed along with freshly prepared LQC and HQC samples (comparison samples). Concentrations were calculated to determine % mean change after 4th cycle.

Carvedilol was found to be stable in LQC and HQC samples after 4th cycle at -70°C with % mean change of 14.39 and -0.50 respectively (Table No.8.9.4.2).

5.0 CONCLUSION

The results of the Method Validation for Carvedilol were summarized in (Table No.8.10). The analytical method was valid for the analysis of Carvedilol with a calibration range of 0.500 ng/ml to 150.000 ng/ml in human plasma using Olanzapine as internal standard.

6.0 HISTORY OF CHANGE IN VERSION OF VALIDATION REPORT

Version No.	Date	Page No.	Section	Modification
01	22/04/08	-	-	Original report

ANNEXURE No. 4.12.3.3

PREPARATION, IDENTIFICATION AND VERIFICATION FOR SPIKING STOCK SOLUTIONS OF CALIBRATION STANDARDS AND QUALITY CONTROL SAMPLES FOR BIOAVAILABILITY (BA) AND BIOEQUIVALENCE (BE) STUDIES.

(SOP No. BE-A-012).

UNCONTROLLED COPY CONTROLLED STAMP	**Title: Preparation, Identification, and verification for spiking stock solutions of Calibration Standards and Quality Control Samples for Bioavailability (BA) and Bioequivalence (BE) studies.**	SOP No.	:	BE-A-012
		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	09/01/2008
		Review Period	:	2 Years

References: - -

Revision History:

Version	Section	Revision Summary	Reason for revision
04	7.0	Procedure modified	Up gradation
		Procedure for Stock identity is included	

	Name	Designation & Department	Signature	Date
Prepared by	Shuja Khan	Scientist II – Bioanalytical Laboratory	Shuja	05/01/08
Reviewed by	Dr.Deepak Jain	Scientist I – Bioanalytical Laboratory		07/01/08
	Hemang Pathak	Research Associate – Quality Assurance	Pathak H.U.	07/01/08
Approved by	Dr.G.Subbaiah	General Manager- Bioanalytical Laboratory	G Subbaiah	08/01/08

UNCONTROLLED COPY CONTROLLED STAMP	**Title: Preparation, Identification, and verification for spiking stock solutions of Calibration Standards and Quality Control Samples for Bioavailability (BA) and Bioequivalence (BE) studies.**	SOP No.	:	BE-A-012
		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	09/01/2008
		Review Period	:	2 Years

1.0 PURPOSE

To provide procedure for the preparation, identification and verification for stock solutions of calibration standards and quality control samples for BA and BE studies.

2.0 DEFINITIONS

2.1 Analyte(s): A specific, unique chemical moiety in the form(s) it would be found in a matrix.

2.2 Analyte(s) main stock solution: It is the most concentrated analyte(s) solution. It is used for the preparation of intermediate or spiking stock solution with lower concentration compared with main stock solution.

2.3 Analyte(s) intermediate solutions: These are the solutions at a concentration lower than the analyte(s) main stock solutions. The intermediate solution will be used to prepare the spiking stock solutions of calibration standard and quality control samples.

2.4 Analyte(s) spiking solutions are solutions at concentration lower than the analyte(s) intermediate solution, used for the preparation of the aqueous and plasma calibration standards and quality control samples.

2.5 Internal standard (IS): A compound (s) added to a sample in known concentration and used as a qualifier in an analytical method.

2.6 Internal standard main stock solution: It is the most concentrated internal standard solution; it is used for the preparation of the intermediate and /or spiking internal standard solution.

2.7 Internal standard intermediate solution: It is solution at a concentration lower than internal standard main stock solutions. It is used for the preparation of internal standard spiking solution. Any volume of internal standard can be added as per the method requirement.

2.8 The internal standard spiking solution: It is a solution at a concentration lower than the internal standard intermediate solution, and is used for spiking the aqueous and matrix samples.

3.0 SCOPE

This SOP is applicable for the preparation of stock solution during method development, pre method validation; full method validation and study sample analysis samples for BA and BE studies performed at Bioanalytical laboratory.

4.0 POLICIES

4.1 It is the policy of the laboratory to provide identification of the stock solutions used in the planned experiments during the course of different bioanalytical stages.

5.0 RESPONSIBILITY

5.1 All analysts of Bioanalytical laboratory involved in stock solution preparation activities.

5.2 Supervisor for the overall compliance and adherence to this SOP.

6.0 MATERIAL

Not Applicable

7.0 PROCEDURE

7.1 ANALYTE (s) MAIN STOCK SOLUTION

7.1.1 Preparation

Analyst should prepare analyte(s) main stock solution by separately weighing analyte(s) using calibrated weighing balance and dissolve it in an appropriate solvent using clean class A volumetric glassware. Record the weight taken as per the procedure given in the respective balance SOPs in project journal/respective forms.

Note: 1) Refer to current valid certificate of analysis for correction of water content, purity and salt content.

2) Use USP/EP/BP/IP – reference standard, if available, but not mandatory. For a non – USP reference standard, a currently valid certificate of analysis should be available.

3) For multi-Analyte(s) method, each Analyte(s) should be weighed and recorded individually.

4) The stock solutions can be stored at room temperature and or in refrigerated condition as per project requirement.

7.1.2 Identification

The analyte main stock solution and or the container holding them should be identified as follows,

torrent

Project Name :
Analyte(s) name :
Stock identity (ID) :
Concentration :
Solvent used :
Z of Y :
Prepared by :
Date of Preparation :
Storage condition :
Use Before :

Project Name: It represents the name of the project (study code) for which the stocks are prepared.

Analyte(s) name: Name of the analyte(s)

Stock identity (ID): SOTP/Protocol No / XX / YY

Where,

XX : indicates the respective section or subsection number of that SOTP/Protocol.

YY : indicate the preparation number in increment order.

Preparation number is defined as: The first preparation of stock solution on a particular day is recorded as 01. Subsequent preparations of the same stock solution within the same day or different day are recorded with an increment of 01, e.g. 02.

Concentration: Concentration of the analyte(s) main stock solution i.e. mg/ml, µg/ml, ng/ml etc.

Solvent used: Solvent used in preparation of analyte(s) stock solution.

Z of Y: Z is the number of aliquot and Y is the total number of aliquots.(if applicable)

Prepared by: Name of analyst by whom solution was prepared.

Storage Condition: Condition at which it solution is intended to be stored.

Use Before: The date should be defined based on the established stability during method validation.

Note : As per requirement, aliquot the volume of main stock in eppendorf for daily use and for performing stability experiments as per SOP No.BE-A-013.

7.1.3 Documentation

The weighing of analyte(s) shall be done as per the respective balance SOPs, and preparation of analyte(s) main stock solutions should be documented in project journal / respective forms, or as per the attachment I. All the activities of storage, retrieval, restorage and disposal shall be done in the same form as per SOP No.BE-A-011.

7.2 ANALYTE (s) INTERMEDIATE SOLUTIONS

7.2.1 Preparation

Analyte(s) intermediate solutions are prepared by diluting each analyte(s) main stock solution in appropriate solvent using clean class A volumetric glassware.

Note: 1) The stock solutions can be stored at room temperature and or in refrigerated condition as per project requirement.

2) This stock could be a mixture of two or more analytes with respect to method requirement.

7.2.2 Identification

The analyte(s) intermediate solutions and or the container holding them should be identified as follows:

torrent

Project Name :
Analyte(s) name :
Stock identity (ID) :
Concentration :
Solvent used :
Z of Y :
Prepared by :
Date of Preparation :
Storage condition :
Use Before :

Project Name: It represents the name of the project (study code) for which the stocks are prepared.

Analyte (s) name: Name of the analyte(s)

Stock identity (ID): SOTP/Protocol No / XX / YY

Where XX indicates the respective section or subsection number of that SOTP/Protocol.

YY indicate the preparation number in increment order.

Preparation number is defined as: The first preparation of stock solution on a particular day is recorded as 01. Subsequent preparations of the same stock solution within the same day or different day are recorded with an increment of 01, e.g. 02.

Concentration: Concentration of the analyte(s) intermediate solution i.e.

µg/ml, ng/ml etc.

Solvent used: Solvent used in preparation of analyte(s) intermediate solution.

Z of Y: Z is the number of aliquot and Y is the total number of aliquots (if applicable)

Prepared by: Name of analyst by whom solution was prepared.

Storage Condition: Condition at which solution is intended to be stored.

Use Before: The date should be defined based on the established stability during method validation.

Note : 1) If mixed intermediate stock is prepared then refer section 7.3.2 (b), (c) for analyte name labeling on the apparatus.

2) For mixed stocks, the mentioning of concentration on label is not mandatory, for this, the respective SOTP/Protocol should be referred.

7.2.3 Documentation

Preparation of analyte(s) intermediate stock solutions should be noted in project journal and or the respective document form, or as per the attachment I. The analyst preparing the stock should sign the preparation and also by person witnessing it or get it reviewed by supervisor All the activities of storage, retrieval, restorage and disposal shall be done in the same form as per SOP No. BE-A-011.

7.3 ANALYTE (s) SPIKING SOLUTIONS

7.3.1 Preparation

Analyte(s) spiking solution are prepared by diluting analyte(s) main stock solution and or intermediate solutions in appropriate solvent using clean class A volumetric glassware.

Note: 1) The stock solutions can be stored at room temperature and or in refrigerated condition as per project requirement.

2) This stock could be a mixture of two or more analytes with respect to method requirement.

3) The concentration should be adjusted such that spiking volume should not exceed 2-10% v/v in biological samples.

7.3.2 Identification

The analyte(s) spiking solutions and or the container holding them should be identified as follows:

torrent

Project Name :
Analyte(s) name :
Stock identity (ID) :
Concentration :
Solvent used :
Z of Y :
Prepared by :
Date of Preparation :
Storage condition :
Use Before :

a) When only one Analyte "ABC" was monitored

ABCSSCSXX or ABCSSPQC

Where,

ABC : First three alphabet of analyte

SS : Spiking solution.

CS : Calibration standard

XX : Levels

PQC : Quality control standards (LQC, MQC, HQC).

b) When only one Analyte "ABC" was monitored in presence of other Analyte (DEF), shown in parenthesis

ABC (DEF) SSCXX or ABC (DEF) SSPQC

Where,

ABC : First three alphabet of analyte

DEF : First three alphabet of second analyte

SS : Spiking solution.

CS : Calibration standard.

XX : Levels

PQC : Quality control standards (LQC, MQC, HQC).

c) When two or more Analyte "ABC +DEF+..." were monitored.

ABC + DEF+... SSCSXX or ABC + DEF+... SSPQC

Where,

ABC : First three alphabet of analyte

DEF : first three alphabets of second analyte and so on.

SS : Spiking solution.

CS : Calibration standard.

XX : Levels

PQC : Quality control standards (LQC, MQC, HQC).

Stock identity (ID): SOTP/Protocol No / YY/ ZZ

Where YY indicates the respective section or subsection number of that SOTP/Protocol.

ZZ indicate the preparation number in increment order.

Preparation number is defined as: The first preparation of stock solution on a particular day is recorded as 01. Subsequent preparations of the same stock solution within the same day or different day are recorded with an increment of 01, e.g. 02.

Concentration: Concentration of the analyte(s) intermediate solution i.e.

µg/ml, ng/ml etc.

Note : For mixed stocks, the mentioning of concentration on label is not mandatory, for this, the respective SOTP/Protocol should be referred.

Solvent used: Solvent used in preparation of analyte(s) intermediate solution.

Z of Y: Z is the number of aliquot and Y is the total number of aliquots (if applicable)

Prepared by: Name of analyst by whom solution was prepared.

Storage Condition: Condition at which it solution is intended to be stored.

Use Before: The date should be defined based on the established stability during method validation.

Note: For proper identification of analyte/Internal standard stock solutions which have same first three alphabets, then use the next alphabet, which is uncommon, for identifying the stock solutions.

(Example. Ramipril + Ramiprilat should be written as RAM+RAA)

7.3.3 Documentation

The preparation of analyte(s) and quality control spiking solutions should be recorded in project journal and or the respective document form or as per the attachment I. The analyst preparing the stock should sign the preparation and also by person witnessing it or get it reviewed by supervisor. All the activities of storage, retrieval, restorage and disposal shall be done in the same form as per SOP BE-A-011.

7.4 INTERNAL STANDARD MAIN STOCK SOLUTION

7.4.1 Preparation

Prepare internal standard main stock solution by separately weighing compound that will be used for internal standard using calibrated weighing balance and dissolve in a appropriate solvent using clean class A volumetric glassware. Record the weight taken as per the procedure given in the respective balance SOPs in project journal/respective forms as per SOP No.BE-A-011.

Note:
1) Refer to current valid certificate of analysis for correction of water content, purity and salt content.
2) Use USP – reference standard, if available, but not mandatory. For a non – USP reference standard, a currently valid certificate of analysis should be available.
3) For multi-internal standard (s) method, each internal standard (s) should be weighed and recorded individually.
4) The stock solutions can be stored at room temperature and or in refrigerated condition as per project requirement.

7.4.2 Identification

The internal standard [IS] main stock solution and or the container holding should be identified as follows,

torrent

Project Name :
IS(s) name :
Stock identity (ID) :
Concentration :
Solvent used :
Z of Y :
Prepared by :
Date of Preparation :
Storage condition :
Use Before :

Project Name: It represents the name of the project (study code) for which the stocks are prepared.

Internal standard name: Name of the internal standard.

Stock identity (ID): SOTP/Protocol No / XX / YY

Where XX indicates the respective section or subsection number of that SOTP/Protocol.

YY indicate the preparation number in increment order.

Preparation number is defined as: The first preparation of stock solution on a particular day is recorded as 01. Subsequent preparations of the same stock solution within the same day or different day are recorded with an increment of 01, e.g. 02.

Concentration: Concentration of the internal standard stock solution i.e. mg/ml,µg/ml, ng/ml etc.

Z of Y: Z is the number of aliquot and Y is the total number of aliquots. (if applicable)

Solvent used: Solvent used in preparation of internal standard stock solution.

Prepared by: Name of analyst by whom solution was prepared.

Storage Condition: Condition at which it solution is intended to be stored.

Use Before: The date should be defined based on the established stability during method validation.

Note: As per requirement, aliquot the volume of main stock in eppendorf for daily use and for performing stability experiments as per SOP No.BE-A-013

7.4.3 Documentation

The weighing of analyte(s) as internal standard shall be done as per the respective balance SOPs, and preparation of analyte(s) main stock solutions should be documented in project journal / respective forms, or as per the attachment I. The analyst preparing the stock should sign the preparation and also by person witnessing it or get it reviewed by supervisor. All the activities of storage, retrieval, restorage and disposal shall be done in the same form as per SOP No. BE-A-011.

7.5 INTERNAL STANDARD INTERMEDIATE SOLUTIONS

7.5.1 Preparation

Intermediate internal standard solution is prepared by diluting the internal standard main stock solution in appropriate solvent using clean class A volumetric glassware as described in respective method SOTP.

Note: 1) The stock solutions can be stored at room temperature and or in refrigerated condition as per project requirement.

2) This stock could be a mixture of two or more internal standard(s) with respect to method requirement.

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		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	09/01/2008
		Review Period	:	2 Years

7.5.2 Identification

The internal standard [IS] intermediate stock solution and or the container holding should be identified as follows,

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Project Name :
IS (s) name :
Stock identity (ID) :
Concentration :
Solvent used :
Z of Y :
Prepared by :
Date of Preparation :
Storage condition :
Use Before :

Project Name: It represents the name of the project (study code) for which the stocks are prepared.

Internal standard name: Name of the internal standard.

Stock identity (ID): SOTP/Protocol No / XX / YY

Where XX indicates the respective section or subsection number of that SOTP/Protocol.

YY indicate the preparation number in increment order.

Preparation number is defined as: The first preparation of stock solution on a particular day is recorded as 01. Subsequent preparations of the same stock solution within the same day or different day are recorded with an increment of 01, e.g. 02.

Concentration: Concentration of the internal standard stock solution i.e. mg/ml,µg/ml, ng/ml etc.

Z of Y: Z is the number of aliquot and Y is the total number of aliquots. (if applicable)

Solvent used: Solvent used in preparation of internal standard stock solution.

Prepared by: Name of analyst by whom solution was prepared.

Storage Condition: Condition at which it solution is intended to be stored.

Use Before: The date should be defined based on the established stability during method validation.

Note : If mixed intermediate stock is prepared then refer section 7.6.2 (a) for labeling the apparatus.

7.5.3 Documentation

Preparation of analyte(s) (as internal standard) intermediate stock solutions should be noted in project journal and or the respective document form or as per the attachment I. The analyst preparing the stock should sign the preparation and also by person witnessing it or get it reviewed by supervisor. All the activities of storage, retrieval, restorage and disposal shall be done in the same form as per SOP BE-A-011.

7.6 INTERNAL STANDARD SPIKING SOLUTION

7.6.1 Preparation

An internal standard working solution is prepared by diluting an internal standard main stock or intermediate solution in an appropriate solvent as described in respective method SOTP.

Note: 1) The stock solutions can be stored at room temperature and or in refrigerated condition as per project requirement.

2) This stock could be a mixture of two or more internal standard(s) with respect to method requirement

7.6.2 Identification

The internal standard spiking solution and or the container holding them should be identified as follows,

torrent

Project Name :
IS (s) name :
Stock identity (ID) :
Concentration :
Solvent used :
Z of Y :
Prepared by :
Date of Preparation :
Storage condition :
Use Before :

Project Name: It represents the name of the project (study code) for which the stocks are prepared.

Internal standard name: Name of the internal standard.

a) When two or more IS"ABC +DEF+..." were monitored.

ABC + DEF +... SS

Where,

ABC : First three alphabet of analyte.

DEF : first three alphabets of second analyte and so on.

SS : Spiking solution.

Stock identity (ID): SOTP/Protocol No / YY / ZZ

Where YY indicates the respective section or subsection number of that SOTP/Protocol.

ZZ indicate the preparation number in increment order.

Preparation number is defined as: The first preparation of stock solution on a particular day is recorded as 01. Subsequent preparations of the same stock solution within the same day or different day are recorded with an increment of 01, e.g. 02.

Concentration: Concentration of the internal standard stock solution i.e. mg/ml,µg/ml, ng/ml etc.

Z of Y: Z is the number of aliquot and Y is the total number of aliquots. (if applicable)

Solvent used: Solvent used in preparation of internal standard stock solution.

Prepared by: Name of analyst by whom solution was prepared.

Storage Condition: Condition at which it solution is intended to be stored

Use Before: The date should be defined based on the established stability during method validation.

7.6.3 Documentation

Preparation of analyte(s) (as internal standard) intermediate stock solutions should be noted in project journal and or the respective document form or as per the attachment I. The analyst preparing the stock should sign the preparation and also by person witnessing it or get it reviewed by supervisor. All the activities of storage, retrieval, restorage and disposal shall be done in the same form as per SOP No.BE-A-011.

7.7 VERIFICATION OF STANDARD, QUALITY CONTROL AND INTERNAL STANDARD STOCK SOLUTIONS

7.7.1 In case of bulk spiking it is recommended to verify that the intermediate, spiking solution of analyte(s) and internal standards are correctly prepared. The verification test is not mandatory. The verification test is performed according to the following procedures.

7.7.2 Procedure for the stock solutions verification test

a) Refer the method SOTP/Protocol for the preparation of different stocks

b) Inject aqueous or extracted calibration standards at a concentration equivalent to plasma calibration standard.

c) LLOQ and ULOQ should be injected in singlet only.

d) Inject in triplicate aqueous or extracted quality control sample of each level i.e. LQC, MQC and HQC at a concentration equivalent to plasma quality control sample.

7.7.3 Acceptance Criteria

a) A correlation coefficient (r) of the calibration curve should be $\geq$ 0.9900.

b) The back-calculated concentration of the lowest calibration standard (CS1) should be within 80.00-120.00% of its nominal concentration.

c) The back-calculated concentrations of all other calibration standards should be within 85.00-115.00% of their nominal concentrations.

d) The LLOQ (CS-1) and ULOQ standards cannot be rejected, and should meet the acceptance criteria.

e) The back calculated concentration of all the quality control samples should be within 85.00-115.00%.

Note: 1) If the acceptance criteria of either calibration standard or quality control sample do not meet then, prepare fresh stock for the respective level and reinject it.

2) If still the acceptance is not met then check the preparation of stock preparation and after rectification, re-verify the same. The verification in any case is done by re-injecting the old stock or injecting the freshly prepared stock (from same main stock / intermediate stock or a new stock).

3) The acceptance of the spiking stock or the spiked & processed samples should be based on scientific judgment by the analyst and or supervisor.

7.7.4 Documentation

The conclusion of the experiment should be recorded in the journal and should be reviewed by the supervisor.

7.7.5 Details of stock preparation should be recorded in excel sheet format and should be pasted in respective project journal or attach with the applicable forms.

8.0 REFERENCE

8.1 SOP for "Sequence of Bioanalytical Courses and related documentation"- BE-A-011

8.2 SOP for "Pre method validation, full method validation and partial method validation of Bioanalytical methods related to Bioavailability (BA) and Bioequivalence (BE) studies."- BE-A-013

9.0 ATTACHMENTS

Attachment No.	Form No.	Title	No. of Pages
Attachment I	NA	Excel Sheet format for Stock Preparation	2

ATTACHMENT – I

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TITLE: FORMAT FOR STOCK PREPARATION IN EXCEL SHEET					
Form No.:	NA	Reference SOP No.	BE-A-012	SOP Version No.	04

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Project Name :
Activity : **Date :**

Compound Name:		**Weight (equivalent to10mg)**	
Batch No.:		**Weigh taken (mg)**	
Formula Weight:		**Total Volume Prepared (ml)**	
Molecular Weight:		**Concentration (µg/ml):**	
Assay:		**Solvent used for stock preparation**	
Main Stock ID:		**Solution used for dilution**	

Intermediate Stock Dilution 01

Stock ID	Conc.(µg/ml)	Volume Taken(ml)	Final Volume(ml)	Final Conc.(µg/ml)	Intermediate Stock ID

Intermediate Stock Dilution 02

Stock ID	Conc.(µg/ml)	Volume Taken(ml)	Final Volume(ml)	Final Conc.(µg/ml)	Intermediate Stock ID

Spiking Solution Preparation for Calibration Standards and Quality Control Samples

Intermediate Stock ID	Intermediate Stock Conc.(µg/ml)	Stock Volume(ml)	Final Volume(ml)	Spiking Solution Conc.(ng/ml)	SS ID

TITLE: FORMAT FOR STOCK PREPARATION IN EXCEL SHEET					
Form No.:	NA	Reference SOP No.	BE-A-012	SOP Version No.	04

Preparation for Calibration Standards and Quality Control Samples

SS ID	Spiking Solution Conc.(ng/ml)	Spiking Volume	Blank Plasma Volume	Final Conc. In Plasma(ng/ml)	Sample ID

Internal Standard (IS)

Compound Name:		**Weight (equivalent to10mg)**	
Batch No.:		**Weigh taken (mg)**	
Formula Weight:		**Total Volume Prepared (ml)**	
Molecular Weight:		**Concentration (µg/ml):**	
Purity/Assay:		**Solvent used for stock preparation**	
Main Stock ID:		**Solution used for serial dilution**	

IS Spiking Solution Preparation

Stock ID	Conc.(µg/ml)	Volume Taken(ml)	Final Volume(ml)	Final Conc.(µg/ml)	ISTD Spiking Stock ID

Note: Use appropriate units for Weight and concentration.

Prepared by : **Checked by :**
Date : **Date :**

Note: 1) Mention only applicable parameters.

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TITLE: REVISION SUMMARY FORM					
Form No.:	QA-001/05	Reference SOP No.	QA-001	SOP Version No.	05

SOP No. : BE-A-012

Title of SOP : Preparation, Identification, and verification for spiking stock solutions of Calibration Standards and Quality Control Samples for Bioavailability (BA) and Bioequivalence (BE) studies

Ver.No.	Date Revised	Section	Revision Summary
01.	12/06/2006	--	Change in Title
		--	Replacement of "glass B" with "glass A"
		5.7.3	Value of correlation coefficient changed
02.	24/08/2006	3.0	Section modified
		5.0	Procedure elaborated
		6.0 & 7.0	Section included
03	22/01/2007	--	Change in format as per SOP for SOP (QA-001)
		7.0	Procedure modified

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ANNEXURE No. 4.12.3.4

PRE METHOD VALIDATION, FULL METHOD VALIDATION AND PARTIAL METHOD VALIDATION OF BIOANALYTICAL METHODS RELATED TO BIOAVAILABILITY (BA) AND BIOEQUIVALENCE (BE) STUDIES.

(SOP NO. BE-A-013)

UNCONTROLLED COPY CONTROLLED STAMP	**Title: Pre method validation, full method validation and partial method validation of Bioanalytical methods related to Bioavailability (BA) and Bioequivalence (BE) studies.**	SOP No.	:	BE-A-013
		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	23/02/2007
		Review Period	:	2 Years

References: USFDA and Brazilian guidelines for Bioanalytical method validation

Revision History:

Version	Section	Revision Summary	Reason for revision
04	2.31	Definition added.	Up gradation
	7.1.4	Added one more precision & accuracy experiment.	
	7.2.2	Procedure modified.	
	7.5	Reinjection criteria added.	
	9.0	Attachment-II and III modified.	

	Name	Designation & Department	Signature	Date
Prepared by	Deepak Jain	Scientist I –Bioanalytical Laboratory		17/02/07
Reviewed by	Jignesh Kotecha	Scientist I –Bioanalytical Laboratory	Kotecha	19/02/07
	Hemang Pathak	Scientist II –Quality Assurance	Pathak H.C.	20/02/07
Approved by	Dr.G.Subbaiah	General Manager-Bioanalytical Laboratory		21/02/07

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		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	23/02/2007
		Review Period	:	2 Years

1.0 PURPOSE

To define procedures and acceptance criteria for validation of chromatographic and spectrometric methods for the analysis of analyte(s)/metabolite(s) in biological matrix.

2.0 DEFINITIONS

2.1 Accuracy: The degree of closeness of mean test results obtained by the method to the true value (concentration) of analyte(s).

2.2 Analyte(s): A specific chemical moiety being measured or monitored, which can be intact drug (Standard, internal standard, etc.), bio-molecule or its derivative, metabolite and /or degradation product in a biological matrix.

2.3 Analytical run (or batch): A complete set of analytical samples with appropriate number of standards and quality control samples for their validation. Several runs (or batches) may be completed in one day, or one run (or batch) may take more than a day to complete.

2.4 Anticoagulant: An anticoagulant is a substance that stops blood from clotting; the anticoagulants commonly used are Heparin, citrate-phosphate-dextrose (CPD), EDTA.

2.5 Biological matrix: A discrete material of biological origin that can be sampled and processed in a reproducible manner. Examples are blood, serum, plasma, urine, feces, saliva, sputum, and various discrete tissues.

2.6 Calibration standard: A biological matrix to which a known amount of analyte(s) has been added or spiked. Calibration standards are used to construct calibration curves from which the concentration of analyte(s) in quality control samples and in unknown study samples are determined.

2.7 Haemolysed plasma: The plasma obtained from the blood with ruptured erythrocytes.

2.8 Internal standard: Test compound(s) (e.g. structurally similar analog, stable labeled compound) added to both calibration standards and samples, at known and constant concentration to facilitate quantification of the target analyte(s).

2.9 Limit of detection (LOD): The lowest concentration of an analyte(s) that the Bioanalytical procedure can reliably differentiate from background noise.

2.10 Sensitivity/Lower limit of quantification (LLOQ): The lowest amount of an analyte(s) in a sample that can be quantitatively determined with suitable precision and accuracy

2.11 Lipemic plasma: The plasma with high lipid contents compared to the lipid contents in normal plasma.

2.12 Matrix effect: The direct or indirect alteration or interference in response due to the presence of unintended analyte(s) (for analysis) or other interfering substances in the sample.

2.13 Method: A comprehensive description of all procedures used in sample analysis.

2.14 Multiplexed LC-MS/MS: Two HPLC coupled with one MS/MS.

2.15 Outlier / Excluded: A data point that fails to meet its defined acceptance criteria.

2.16 Precision: The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions.

2.17 Processed sample: The final extract (prior to instrumental analysis) of a sample that has been subjected to various processing steps (e.g.: extraction, dilution, etc).

2.18 Quantification range: The range of concentration, including upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) that can be reliably and reproducibly quantified with accuracy and precision through the use of a concentration- response relationship.

2.19 Recovery: The extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte(s) carried through the sample extraction and processing steps of the method.

2.20 Reproducibility: The precision between two laboratories. It also represents precision of the method under the same operating conditions over a short period of time.

2.21 Sample: A generic term encompassing controls, blanks, unknowns, and processed samples, as described below.

2.22 Blank solution: It is the solution in which the analyte (s) is finally subjected to injection after processing.

2.23 Blank matrix: A sample of biological matrix (plasma, urine, blood etc) to which no analyte(s) has been added that is used to assess the specificity of the bioanalytical method.

2.24 Zero Standard: Blank matrix with internal standard(s) only.

2.25 Quality control (QC) sample: A spiked sample used to monitor the performance of a Bioanalytical method and to assess the integrity and validity of the results of the unknown samples analyzed in an individual batch. The QC samples include Low quality control (LQC), Medium quality control (MQC) and High quality control (HQC) samples.

2.26 Unknown sample: A biological sample that is the subject of the analysis

2.27 Selectivity/Specificity: The ability of the Bioanalytical method to measure and differentiate the analyte(s) in the presence of components that may be expected to be present. These could include metabolites, impurities, degradants, or matrix components. It also includes an individual search of the signal of an exclusive analyte(s) species.

2.28 SOTP/Protocol: Standard operating test procedure/Protocol. It refers the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include, but is not limited to, the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

2.29 Stability: The chemical stability of an analyte(s) in a given matrix under specific conditions for given time intervals.

2.30 Standard curve: The relationship between the experimental response value and the analytical concentration (also called calibration curve) or

A set of at least six calibration standards defined the assay range. The standard with the lowest concentration defines the LLOQ for the assay while the standard with the highest concentration defines the ULOQ. If possible, the range of the curve should cover the range of concentration expected in study samples. An appropriately chosen regression analysis of the data from the calibration standard samples is used to characterize the standard line. The resulting standard line is used to back calculate the concentration of the samples including the sample with only IS. The application software program calculates the slope, intercept and r- squared (Coefficient of determination) or r (Correlation coefficient) value.

A specific calibration standard point is excluded if there is an identifiable chromatographic, instrument or procedural problem. Data point whose back calculated values are not within the acceptance criteria in the final curve calculation is also excluded.

2.31 Power for Weights: The power for weights (PW) is the slope obtained by evaluating the linear dependence of the logarithm of the standard deviations of peak area/height ratio on the logarithm of sample concentration, i.e.:

$$PW = \sum_i (\ln Conc_i - Mean\ \ln Conc)\ \ln SD_i / \sum_i (\ln Conc_i - Mean\ \ln Conc)^2$$

2.32 System suitability: Determination of instrument performance (e.g. response and chromatographic retention) by analysis of a reference standard prior to running the analytical batch.

2.33 Upper limit of quantification (ULOQ): The highest amount of analyte(s) in a sample that can be quantitatively determined with precision and accuracy.

2.34 Pre method validation: Assuring of sample preparation methodology, which involves the process of extraction, identification and quantification of the drug in the biological matrix, before proceeding for full validation.

2.35 Full method validation: Establishment of all validation parameters to apply to the analysis for the Bioanalytical method for each analyte(s).

2.36 Partial method validation: Modification of Validated Bioanalytical methods that do not necessarily call for full revalidation, OR

A validation performed to substantiate the modification of a validated method. The minimum requirement is one intra run accuracy and precision determination including a calibration curve, six replicate at each LLOQ, LQC, MQC and HQC level. The acceptance criteria are that of a validation run.

3.0 SCOPE

- This SOP is applicable for the validation of bioanalytical methods, (by any kind of extraction techniques: precipitation, liquid-liquid extraction, solid phase extraction, etc.) executed in bioanalytical laboratory at Bio-Evaluation (BE) centre of Torrent Research Centre.
- These criteria apply to all methods used in support of bioavailability / bioequivalence and regulated validation studies requiring (e.g. HPLC and LC-MS, LC-MS/MS) quantitative analysis of samples.
- During validation of a bioanalytical method, the performance of the procedure is evaluated across the range of intended sample concentration (if possible). Method validation includes determination of the accuracy and precision of the test procedures and stability of the analytes (s) in solution and the biological matrix.

4.0 POLICIES

4.1 Any deviation (either planned or unplanned) from the written procedure during the course of any Bioanalytical stage shall be documented and reported in the analytical reports.

4.2 Any additional information or the documentation of the activity that are to be used to document the outcome of the procedure must be attached as templates in the form of Note to File (Attachment-III).

5.0 RESPONSIBILITY

5.1 All analysts of Bioanalytical laboratory involved in analytical method validation activities.

5.2 Supervisor - to check the overall compliance and adherence to the SOP.

5.3 Head of the department - responsible for the approval of final results.

6.0 MATERIAL

Not Applicable

7.0 PROCEDURE

7.1 PRE METHOD VALIDATION (PMV)

Pre method validation is performed after developing a Bioanalytical method, to check the entire sample analysis methodology.

Parameters to be performed during pre method validation:

7.1.1 Selectivity/Specificity: Appropriate biological blank matrix (plasma, urine, or other matrix) obtained from at least six different sources is to be checked during pre method validation. Refer section-7.2.1 for sample preparation and its acceptance criteria.

7.1.2 Sensitivity: The lowest standard of the calibration should be accepted as the LLOQ.Refer section-7.2.3 for sample preparation and its acceptance criteria.

7.1.3 Calibration curve/Linearity: Linearity shall be decided from the reported literature / pilot study data and thus checking the developed method results for the required LLOQ and ULOQ and hence the quality control sample concentration can be decided. Refer section-7.2.2).

7.1.3.1 Selection criteria for quality control samples:

a) LQC: Concentration approximately equivalent to 3 times of the lowest calibration standard (CS-1) or concentration between the second calibration standard (CS-2) and the third calibration standard (CS-3).

b) MQC: Concentration should be average between the LQC and HQC concentration ± 20%.

c) HQC: Concentration between 75-90% of the ULOQ.

(Refer section-7.2.2 for sample preparation and acceptance criteria)

Note: At least three linearity experiments should be performed during pre method validation.

7.1.4 Accuracy and Precision: Accuracy and Precision are determined at LLOQ, LQC, MQC, and HQC levels and should be measured by minimum of six determinations per QC sample level.

7.1.4.1 At least three accuracy and precision experiments should be performed during pre method validation.

7.1.4.2 Single accuracy and precision experiment includes calibration curve, and six replicates of each LLOQ, LQC, MQC and HQC samples.

(Refer sections-7.2.4 & 7.2.5 for sample preparation and acceptance criteria)

7.1.5 Recovery: The recovery of an analyte does not need to be 100% however; the extent of recovery should be consistent, precise and reproducible. Recovery experiment should be performed at LQC, MQC and HQC levels.

(Refer section-7.2.6 for sample preparation and acceptance criteria)

7.1.6 Carry over check: Carry over check experiment should be performed to confirm that there is no carry over of analyte (s) from the previous injection. The experiment is performed at ULOQ level. Blank matrix used during the performance of experiment should be screened for its selectivity and specificity.

7.1.6.1 PROCEDURE

7.1.6.1.1 Process all the samples of blank matrix, LLOQ and ULOQ as per the optimized method.

7.1.6.1.2 Appropriately dilute working/reference standard solution of the analyte(s) to get concentration equivalent to extracted LLOQ and ULOQ standard nominal concentration.

7.1.6.1.3 Inject samples in the sequence of extracted LLOQ sample, extracted LLOQ sample, extracted blank matrix sample, extracted ULOQ sample, extracted blank matrix sample, extracted ULOQ sample, and extracted blank matrix sample.

7.1.6.1.4 Carry over check experiment in 96 well and/or its multiple formats (384, 1536) is to be performed as given below. (ULOQ Cluster with Blank solution).

	ULOQ		CS1	CS8	LLOQ	MQC	HQC	MBL6		ULOQ	
ULOQ	**BS**	**ULOQ**	CS1	CS9	LQC	MQC	HQC	LLOQ1	ULOQ	**BS**	ULOQ
	ULOQ		CS2	CS9	LQC	MQC	HQC	LLOQ2		ULOQ	
		MB	CS3	LLOQ	LQC	MQC	MBL1	LLOQ3			
		ZS	CS4	LLOQ	LQC	MQC	MBL2	LLOQ4			
	ULOQ		CS5	LLOQ	LQC	HQC	MBL3	LLOQ5		ULOQ	
ULOQ	**BS**	ULOQ	CS6	LLOQ	LQC	HQC	MBL4	LLOQ6	ULOQ	**BS**	ULOQ
	ULOQ		CS7	LLOQ	MQC	HQC	MBL5			ULOQ	

7.1.6.1.5 MB = Matrix Blank, ZS = Zero standard, MBL = Matrix Blank Lot-1,2.., BS = Blank solution

7.1.6.2 ACCEPTANCE CRITERIA

a) If any peak is present at the retention time of analyte(s), its area response should be ≤ 20.00% of mean response of an extracted lowest plasma calibration standard i.e. LLOQ standard.

b) If any peak is present at the retention time of an internal standard, its area response should be ≤ 5.00% of the area response of an extracted internal standard (i.e. the concentration to be used in method validation).

c) The carry over in the blank solution processed in between the ULOQ Cluster (as shown in the above 96 format example), should not be more than 20% of the average LLOQ area.

Also if any peak is present at the retention time of an internal standard, its area response should be ≤ 5.00% of the area response of an extracted internal standard (i.e. the concentration to be used in method validation).

d) If carry over is observed, autosampler rinsing cycle should be changed accordingly.

e) The precision and accuracy acceptance criteria remains same as defined in section 7.2.4 & 7.2.5

Note: a) Pre method validation should be repeated, incase of any problem observed with extraction procedure, detection, or in chromatography after optimizing the method and the same should be documented in journal or in the respective documentation format.

b) Stock solution stability and long term stability samples preparation can be started during method development or pre method validation experiments as per the requirement. It

should meet the documentation criteria with respect to number of aliquots, aliquots identification, their storage and retrieval at and from various locations and their usage as per SOP No. BE-A-011 and SOP No. BE-A-012 respectively.

c) The pre method validation data should be reviewed and approved by the supervisor. The conclusion of the pre method validation should be written in the journal or in the respective documentation format followed by preparation of tentative SOTP/Protocol, which will be reviewed by supervisor and QA (with the run summary raw data sheet and Instrument methodology) and approved by head of the department.

7.2 FULL METHOD VALIDATION

Bioanalytical method validation includes all the procedures that demonstrate that a particular method used for quantitative measurement of analyte(s) in a given biological matrix such as blood, plasma, serum or urine is reliable and reproducible for intended use.

Measurement of each analyte(s) in the biological matrix should be validated. In addition, the stability of the analyte(s) in spiked samples should be determined.

During method validation activity, use of freshly prepared stock solutions should be made or their stability should be established later at the end of the validation.

Full Bioanalytical method validation should be performed for following parameters:

- Selectivity / Specificity
- Sensitivity
- Calibration curve/Linearity
- Accuracy and Precision
- Recovery
- Stock solution stability and stability of analyte(s) in spiked matrix samples (i.e. Bench top stability, auto sampler stability, dry state stability, freeze and thaw stability and long term stability)
- Dilution Integrity
- Matrix effect
- Anticoagulant effect (if applicable)

Note: a) When validation of Analyte is to be performed in presence of one or more analyte(s), add one or more analyte(s) in all samples of validation experiments without quantifying them. Use the added analyte(s) concentration of at least the expected Cmax concentration or its ULOQ level of analyte(s) concentration.

b) The criteria below are the minimum required to validate an assay procedure for use in a study: however it may be necessary to modify the validation criteria for a particular study

c) If the batch/sequence run stops during the performance of method validation, then refer section 7.5 for performance of re-injection of the batch/sequence.

7.2.1 SELECTIVITY / SPECIFICITY

7.2.1.1 PROCEDURE

a) Obtain samples of the relevant biological matrix (e.g., plasma, serum, blood, etc.) collected under controlled conditions.

b) For plasma matrix include 08 normal plasma lots (04 different plasma lots with the anticoagulant to be used during method validation and 04 different plasma lots with the anticoagulant to be used for study), 0l lipemic plasma and 01 Haemolysed plasma lot.

c) Process and analyze one sample each of the above mentioned 10 plasma lots at Blank and of LLOQ level (preferably from the same lots) as per the procedure described in tentative method SOTP/Protocol.

Note: a) In case of HPLC only for blank matrix sample keep the acquisition stop time for at least 3 times the retention time of the analyte(s)/internal standard (which ever is farthest), to detect any long runner.

b) If anticoagulant used for validation and study sample is same, then use only 04 normal plasma lots with respective anticoagulant. But use of lipemic and haemolyzed plasma is to be included in the specificity. Thus in such cases atleast six different lots will be checked for selectivity/specificity.

7.2.1.2 ACCEPTANCE CRITERIA

a) No interfering peaks from endogenous matrix components, decomposition product etc., should be present at the retention time of an analyte(s) and an internal standard in blank matrix.

b) If any peak is present at the retention time of analyte(s) in blank matrix, its area response should be $\leq$20.00% of area response of an extracted lowest plasma calibration standard i.e. LLOQ standard of the same lot.

c) If any peak is present at the retention time of an internal standard in blank matrix, its area response should be $\leq$5.00% of the area response of an extracted internal standard concentration of the same lot.

d) At least 75.00% of the buffered plasma and heparinised plasma should meet the acceptance criteria.

e) Both lipemic and Haemolysed plasma should meet the above criteria (a to c) for the interference at the respective retention time of analyte and internal standard. If the

experiment fails, repeat the experiment and then change the methodology if required.

f) If the method is intended to quantify more than one analyte, then above criteria is applicable to all intended analytes and internal standards.

7.2.2 CALIBRATION CURVE/LINEARITY

a) A calibration curve (standard curve) is the relationship between the response of the instrument and known concentrations of the analyte(s).

b) A calibration curve should be prepared for each analyte(s) in the sample.

c) A sufficient number of standards should be used in order to properly define the relationship between concentration and response.

d) A calibration standard should be prepared in the same biological matrix same as that of the study samples, by spiking with known concentrations of the analyte(s).

e) A calibration curve should be comprised of a "blank matrix" (matrix processed without analyte and internal standard), a "zero standard" (blank matrix processed only with internal standard) and six or more calibration standards covering the expected range, including the LLOQ and ULOQ.

7.2.2.1 PROCEDURE:

a) LLOQ should cover at least 4-5 half-life of the reported Cmax.

b) ULOQ should cover the expected Cmax value. When no reference is available, it can be decided on basis of results of development studies.

UNCONTROLLED COPY CONTROLLED STAMP	**Title: Pre method validation, full method validation and partial method validation of Bioanalytical methods related to Bioavailability (BA) and Bioequivalence (BE) studies.**	SOP No.	:	BE-A-013
		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	23/02/2007
		Review Period	:	2 Years

c) Calibration curve must contain minimum six calibration standards. Selection of calibration standard expressed as multiple of LLOQ and percent of ULOQ are as follows:

A 8 Standard Calibration Curve	CODE	A 9 Standard Calibration Curve
LLOQ	Standard 1 – CS-1	LLOQ
5% ULOQ or 2 X LLOQ	Standard 2 – CS-2	2 X LLOQ
10% ULOQ	Standard 3 – CS-3	2% to 5% ULOQ
20% ULOQ	Standard 4 – CS-4	10% ULOQ
40% ULOQ	Standard 5 – CS-5	20% ULOQ
60% ULOQ	Standard 6 – CS-6	40% ULOQ
80% ULOQ	Standard 7 – CS-7	60% ULOQ
100% ULOQ	Standard 8 – CS-8	80% ULOQ
	Standard 9 – CS-9	100% ULOQ

d) Prepare LLOQ and ULOQ calibration standards in duplicate (add suffix "D" in sample name of duplicate standard), so that if first does not meet acceptance criteria, then only second standard of the same concentration level should be used in regression analysis. If both are within acceptance criteria consider the first standard for regression analysis.

e) Process and analyze blank matrix, zero standard and calibration standards as per the procedure described in tentative method SOTP/Protocol.

f) Prepare summary of the curve fitting parameters and of back-calculated concentration at each standard level from each calibration curve.

g) Calculate mean, standard deviation and % coefficient of variation of back-calculated concentration at each calibration level as per formula, described in Attachment-I, to determine precision at each calibration level.

h) Calculate % nominal concentration of back-calculated value at each calibration level to determine accuracy at each calibration level.

Note: a) The above selection criteria (mentioned in c of above section 7.2.2.1) of calibration curve range can be changed as per the requirement.

b) To maximize matrix integrity during preparation of calibration standards and quality control samples, a maximum of 2 % to 10 % (v/v) spiking solutions shall be added to

blank matrices (e.g., 0.2ml of spiking solution in 9.8ml of blank plasma for 2% spiking).

7.2.2.2 Weighting for the best curve fitting in linear regression is decided as follows

The standard curves (blanks are not included) from pre method validation, (in total three linearities) will be evaluated to determine if they need to be fitted by weighted regression. The evaluation for weighted linear regression is performed in the following manner:

a) Compute the standard deviation of the peak area or height ratios at each standard concentration from the between-run experiments (three linearity from pre method validation).

b) Compute the natural logarithms of the values for the standard deviations and the concentration.

c) Fit the data from above using an Excel spreadsheet and un weighted linear regression.

d) Obtain the power for weights from the un weighted report as defined in the section 2.31 for "power for weights".

e) Round the value for the power for weights to determine the weighting as follows:

- <0.250, the value is 0 (use un weighted regression);
- 0.250 to 0.750, the value is 0.5 (use 1/concentration for the regression);
- > 0.750, the value is 1 (use 1/concentration^2 for the regression) ;

Note :

a) Standard curve fitting is determined by applying the simplest model to the calibration curve standards that adequately describes the concentration-response relationship using appropriate weighting (e.g. 1/X, 1/X^2, etc).

b) The selection of regression and weighting should primarily be based on the criterias defined in section 2.31 and 7.2.2.2, but the final selection and acceptance should be done by the supervisor based on the practical experience and on the available scientific justification also. For the selected criteria, a complete supporting raw data and or documentation should be available with the pre method validation raw data and the conclusion should be mentioned in the journal or in the respective documentation format.

c) Use the standard curve fittings to calculate the concentration of all quality control samples required in validation parameters.

d) Minimum five calibration curves should be included in method validation data.

e) Bulk spiking of calibration standards can be done.

f) Documentation of the calibration standard storage, retrieval, restorage, and disposal should be done as mentioned in the SOP No. BE-A-011.

7.2.2.3 ACCEPTANCE CRITERIA

a) A correlation coefficient (r) of the calibration curve must be $\geq$ 0.9900.

b) Among the analyzed blank matrix and zero standard, at least one should meet the specificity criteria as per section 7.2.1.2

c) The back-calculated concentration of the lowest calibration standard (CS1) must be within 80.00-120.00% of its theoretical concentration.

d) The back-calculated concentrations of all other calibration standards must be within 85.00 - 115.00% of their theoretical concentrations.

e) The curve must contain at least 75% of the calibration standards for evaluation of curve fitting.

f) No two adjacent (or consecutive) calibration standards can be rejected.

g) Both replicates each of LLOQ and ULOQ standard cannot be rejected, either of the two replicate must meet the acceptance criteria.

7.2.3 SENSITIVITY

7.2.3.1 PROCEDURE

Process and analyze blank matrix, zero standard, calibration standards and six sets of matrix sample spiked at LLOQ concentration using blank matrix lot, as per the procedure described in tentative method SOTP/Protocol.

7.2.3.2 ACCEPTANCE CRITERIA

a) The analyte(s) area response at the LLOQ should be at least 5 times the response compared to blank response.

b) Analyte(s) calculated concentration should be identifiable and reproducible with a precision of 20.00% and accuracy of 80.00 - 120.00%

7.2.4 ACCURACY

Accuracy is further subdivided into:

A) Within-batch or intra-batch accuracy: It measures accuracy during a single analytical run.

B) Between-batch or inter-batch accuracy: It measures accuracy with time, i.e. with respect to different analytical batches in same day or on different days.

A single accuracy experiment includes calibration curve, and six samples each of LLOQ, LQC, MQC and HQC.

Note: a) The bulk spiking of quality control samples for accuracy and precision experiments can be done with established stability or the stability can be established after the completion

of the experiment.

b) Documentation of the precision and accuracy sample storage, retrieval, restorage, and disposal should be done as mentioned in the SOP No. BE-A-011.

7.2.4.1 PROCEDURE

A) Within-batch or Intra-batch accuracy

a) Process and analyze calibration standards and six replicates each of LLOQ, LQC MQC and HQC samples as per the procedure described in tentative method SOTP/Protocol.

b) Calculate % nominal concentration of back-calculated value for LLOQ, LQC, MQC and HQC, analyzed in single analytical batch, as per formula, described in Attachment-I, to determine within-batch or intra-batch accuracy.

B) Between-batch or Inter-batch accuracy

a) Process and analyze calibration standards and six replicates each of LLOQ, LQC, MQC and HQC samples as per the procedure described in tentative SOTP/Protocol.

b) A minimum of five different batches on same day or on different days is to be performed.

c) At least one batch should be processed by one different analyst.

d) At least one different batch should be analyzed using a different serial number/batch/lot of the same column used in method validation.

e) Prepare summary of above accuracy batches.

f) Calculate % nominal concentration of back-calculated value for LLOQ, LQC, MQC and HQC samples, analyzed on five different batches on same day or on different days, as per formula, described in Attachment- I, to determine between-batch or inter-batch accuracy.

7.2.4.2 ACCEPTANCE CRITERIA

a) The back calculated concentrations of all QC samples (LQC, MQC, and HQC) must be within 85.00 - 115.00% of their nominal concentration except at LLOQ sample where it should not deviate by more than 80.00-120.00% of its nominal concentration.

b) At least 67.00 % quality control samples must fall within above-mentioned criteria at each LLOQ, LQC, MQC, and HQC levels.

7.2.5 PRECISION

Precision is further subdivided into:

a) Within-batch or Intra-batch precision: It measures precision during a single analytical batch.

b) Between-batch or Inter-batch precision: It measures precision with time, i.e. with respect

to different analytical batch in same day or on different days.

c) Single precision experiment includes calibration curve, and six replicates of LLOQ, LQC, MQC and HQC samples.

d) Documentation of the precision and accuracy sample storage, retrieval, restorage, and disposal should be done as mentioned in the SOP No. BE-A-011.

7.2.5.1 PROCEDURE

A) Within-batch or Intra-batch precision

a) Process and analyze calibration standards and six replicates each of LLOQ, LQC, MQC and HQC samples as per the procedure described in tentative method SOTP/Protocol.

b) Calculate mean, standard deviation and % coefficient of variation for LLOQ, LQC, MQC and HQC samples, analyzed in single analytical batch, as per formula, described in Attachment- I, to determine within-batch or intra-batch precision.

B) Between-batch or Inter-batch precision

a) Process and analyze calibration standards and six replicates of LLOQ, LQC, MQC and HQC samples as per the procedure described in tentative SOTP/Protocol.

b) A minimum of five different batches on same day or on different days is to be performed.

c) At least one batch should be processed by one different analyst.

d) At least one different batch should be analyzed using a different serial number/batch/lot of the same column used in method validation.

e) Prepare summary of above precision batch.

f) Calculate mean, standard deviation and % coefficient of variation for LLOQ, LQC, MQC and HQC samples, analyzed on five different batches on same day or on different days as per formula, described in Attachment- I, to determine between-batch or inter-batch precision.

7.2.5.2 ACCEPTANCE CRITERIA

a) The %CV of the back-calculated concentrations of all QC samples (LQC, MQC, and HQC) must be within 15.00%, except for LLOQ, which should be within 20.00%.

b) At least 67.00 % quality control samples must fall within above-mentioned criteria at each LLOQ, LQC, MQC and HQC levels.

7.2.6 RECOVERY

Recovery for analyte and internal standard is performed by comparing the area of extracted samples at three different concentrations (LQC, MQC, and HQC) with un-

extracted standards area that represents 100% recovery.

7.2.6.1 PROCEDURE

a) Recovery of an analyte(s) is determined at LQC, MQC HQC levels and recovery of an internal standard is determined at concentration to be used during method validation and study sample analysis.

b) Process and analyze six replicates each of LQC, MQC, and HQC samples as per the procedure described in tentative method SOTP/Protocol. Consider these as extracted samples for recovery.

c) To prepare comparison samples for recovery, use extracted solutions of blank matrix, for the preparation of equivalent unextracted samples, to nullify the matrix effect while calculating the recovery.

d) For the recovery comparison samples, spike the spiking stock of standard solution of an analyte(s) and internal standard in the extracted blank matrix solution to achieve concentration equivalent to extracted samples nominal concentration.

e) Process and analyze unextracted standard solution of an analyte(s) (at LQC, MQC and HQC level) and internal standard of concentrations equivalent to concentration of extracted samples.

f) Compare the area for extracted LQC, MQC, HQC samples and internal standard with areas of unextracted standards and internal standard that represent 100 % recovery (as mentioned above in c and d).

g) Calculate % recovery of an analyte(s) at LQC, MQC and HQC samples and an internal standard as per formula described in Attachment- I.

Note: % Recovery for extracted HQC, MQC and LQC samples should not exceed 115.00%, if it exceeds then reporting should be justified.

7.2.6.2 ACCEPTANCE CRITERIA

a) Recovery of analyte(s) and internal standard should be consistent, precise and reproducible.

b) Variability within areas at each QC levels for analyte(s) should be within % CV of 15.00%.

c) Variability of the analyte recoveries across all the three QC level should be within % CV of 20.00%.

d) Variability between areas of IS found with each extracted QC levels should be within % CV of 20.00%.

e) If any one sample area is found to be inconsistent for either extracted or unextracted, it

should be rejected, with appropriate justification.

7.2.7 STABILITY

a) Drug stability in a biological matrix is a function of the storage conditions, the chemical properties of the drug, the matrix, and the container system.

b) Stability procedures should evaluate the stability of the analyte(s) for the duration of sample collection and handling, short-term storage (bench top, room temperature), long-term storage (frozen at the intended storage temperature), going through the freeze and thaw cycles and after the analytical process.

c) All stability determinations should use a set of samples prepared from a freshly prepared stock solution or stock solutions with proven stability of the analyte(s) and internal standard in the appropriate analyte(s)-free, interference-free biological matrix. Stock solutions of the analyte(s) for stability evaluation should be prepared in an appropriate solvent at known concentrations.

d) If the defined acceptance criteria are not met for the performed experiment, then the experiment(s) should be repeated if on investigation any processing error is found or should be repeated for relevant shorter storage period or storage temperature

e) Documentation of all type of the stability samples storage, retrieval, restorage, and disposal should be done as mentioned in the SOP No. BE-A-011. Document filled for the stability experiment should include either of the autosampler loading time, processing time which is required to calculate the actual respective stability period.

7.2.7.1 STOCK SOLUTION STABILITY

7.2.7.1.1 PROCEDURE:

a) Prepare fresh stock solution of an analyte(s) and an internal standard as per the procedure described in tentative method SOTP/Protocol, and allocate the stock identification number (ID) as per SOP No.-BE-A- 012.

b) Store freshly prepared main stock solutions aliquots of analyte(s) and internal standard solution at 2-8°C in refrigerator or in freezer if required for a relevant period for short-term and long-term stability.

c) Store freshly prepared main stock solutions aliquots of analyte(s) and internal standard solution at room temperature for at least 6 hrs or relevant short-term period.

d) After relevant stability period prepare fresh stock comparison solution of analyte(s) and an internal standard as per the procedure described in tentative method SOTP/Protocol.

e) Retrieve the main stock aliquots from refrigerator and from room temperature and note down the time of withdrawal in journal or in respective documentation format.

f) Appropriately dilute stock solution of analyte(s) to get concentration equivalent to ULOQ nominal concentration for both comparison and stability sample solution.

g) Perform six injections of freshly prepared diluted analyte(s) and internal standard comparison solution and stability solutions.

h) Compare the analytical area results of stability solutions with those of freshly prepared solutions area results.

i) After relevant long-term stability period at 2-8°C prepare fresh stock comparison solution of analyte(s) and an internal standard as per the procedure described in tentative method SOTP/Protocol.

j) Repeat above steps (d), (e), (f) and (g) for long-term solution stability

k) Compare the area results of long term stability solutions with those of freshly prepared comparison solutions.

l) Calculate % mean change for analyte(s) and internal standard as per formula given in Attachment –I.

Note : a) If the diluent used for main stock, intermediate and or spiking stock is same then the established stability for the main stock should be considered applicable also for the spiking stock, and no need to establish separate stability.

b) Stock solution stability need to be established for each and every analyte and internal standard.

c) If mixed stock is to be used for the intended experiment then stock solution stability needs to be established for the mixed stock also even though if the individual stock solutions are established stable.

7.2.7.1.2 ACCEPTANCE CRITERIA

a) % Mean change between freshly prepared stock solution (comparison samples) area results and stability samples area results must be within ± 10.00% for analyte(s).

b) If %CV of area of Analyte (s) and Internal Standard (s) for both stability and comparison sample is greater than 5.0% than out of six only one inappropriate sample should be rejected.

Note: If above acceptance criteria are not met, then experiment should be repeated if on investigation any processing error is found or should be repeated for relevant shorter storage period or storage temperature.

7.2.7.2 BENCH TOP STABILITY

Bench top stability of analyte(s) in matrix is determined at LQC and HQC levels.

7.2.7.2.1 PROCEDURE

a) Spike six aliquots each of LQC and HQC samples or withdraw from the deepfreezer the spiked and stored samples for bench top stability. Keep these samples at room

temperature for minimum 4 hours. (Based on the expected duration that samples will be maintained at room temperature in the intended study).

b) In case of spiking and keeping the bench top stability samples at room temperature, the bench top stability period should be considered between the time of keeping at room temperature and the time of start of processing those samples.

c) In case of retrieval of bulk spiked samples for bench top stability, the bench top stability period should be considered between the time of withdrawal of samples from deep-freezer and the beginning of sample processing.

d) Process and analyze calibration standards and six replicates each of LQC and HQC comparison samples prepared by spiking with freshly prepared stock solutions or stock solutions with proven stability as per the procedure described in tentative method SOTP/Protocol. Record the processing time of samples in journal or in the respective documentation format.

e) Process and analyze the stability samples after relevant period.

f) Compare the mean back-calculated concentration of stability samples with those of freshly prepared comparison samples

g) Calculate % mean change as per formula described in Attachment –I.

Note : a) The valid stability period for the stock used in spiking can be established

7.2.7.2.2 ACCEPTANCE CRITERIA

a) The back-calculated concentrations of all LQC and HQC samples must be within 85.00-115.00% of their theoretical concentration.

b) At least 67.00 % QC samples must fall within above-mentioned criteria at each LQC and HQC levels.

c) % Mean change must be within ± 15.00%.

Note: If above acceptance criteria are not met, then experiment should be repeated if on investigation any processing error is found or should be repeated for relevant shorter storage period or storage temperature.

7.2.7.3 PROCESS STABILITY/AUTO SAMPLER STABILITY

Process stability of analyte(s) is determined at LQC and HQC levels.

7.2.7.3.1 PROCEDURE

a) Process and analyze calibration standards and six replicates each of LQC and HQC comparison samples prepared by spiking with freshly prepared stock solutions as per the procedure described in tentative method SOTP/Protocol or use the stored samples.

b) Keep the above QC samples in the autosampler for relevant period to measure the stability of the processed samples in the autosampler. Note down in journal or in the respective documentation format, the sample storing time in autosampler.

c) Analyze the process stability samples after relevant period. Along with calibration standards and six replicates each of LQC and HQC comparison samples prepared by spiking with freshly prepared stock solutions or stock solutions with proven stability as per the procedure described in tentative method SOTP/Protocol.

d) Note down the time of last injection of the process stability sample in journal or in the respective documentation format.

e) The process stability period should be considered between the time of storing of samples in autosampler or refrigerator and the time of analysis of last process stability sample.

f) If the injection volume is not sufficient enough for re-injection (for extended period stability) then repeat the step-a to e and keep them in autosampler for the process stability period.

g) Compare the mean back-calculated concentration of stability samples with those of freshly prepared comparison samples.

h) Calculate % mean change as per formula described in Attachment - I

7.2.7.3.2 ACCEPTANCE CRITERIA

a) The back-calculated concentrations of all LQC and HQC samples must be within 85.00-115.00% of their nominal concentration.

b) At least 67.00% of quality control samples must fall within above-mentioned criteria at each LQC and HQC levels

c) % Mean change must be within ± 15.00%.

Note: If above acceptance criteria are not met, then experiment should be repeated if on investigation any processing error is found or should be repeated for relevant shorter storage period or at relevant storage temperature.

7.2.7.4 DRY STATE STABILITY

Dry state stability of analyte(s) is determined at LQC and HQC levels.

Dry state stability experiment is applicable for only those methods in which the sample treatment procedure requires the reconstitution of dried matrix with reconstitution solution prior to injection.

7.2.7.4.1 PROCEDURE

a) Prepare and process six replicates each at LQC and HQC samples for dry state stability prepared by spiking with freshly prepared stock solutions or stock solutions with proven stability as per the procedure described in tentative method SOTP/Protocol.

b) Store the dried residue of stability samples for minimum 4 hrs in refrigerator/deepfreezer.

c) Prepare process and analyze calibration standards and six replicates each at LQC and HQC comparison samples.

d) Withdraw the dry state stability sample and immediately reconstitute the dried residue of stability samples after relevant period and analyze along with freshly prepared comparison samples.

e) The dry state stability period should be considered between the time of storing of samples in refrigerator/deepfreezer and the time of withdrawal from the refrigerator/deepfreezer.

f) Compare the mean back-calculated concentrations of stability samples with those of freshly prepared comparison samples

g) Calculate % mean change as per formula described in Attachment –I.

7.2.7.4.2 ACCEPTANCE CRITERIA

a) The back calculated concentrations of all LQC and HQC must be within 85.00 - 115.00% of their nominal concentration.

b) At least 67.00% of QC samples must fall within above-mentioned criteria at each LQC and HQC levels.

c) % Mean change must be within ± 15.00%

Note: If above acceptance criteria are not met, then experiment should be repeated if on investigation any processing error is found or should be repeated for another relevant storage period or storage temperature.

7.2.7.5 FREEZE AND THAW STABILITY

Freeze and thaw stability of analyte(s) is determined after three freeze and thaw cycles at LQC and HQC levels.

7.2.7.5.1 PROCEDURE:

a) Spike three sets (set-1, set-2 and set-3) each having six replicates each of LQC and HQC sample of freeze and thaw stability by spiking with freshly prepared stock solutions or stock solutions with proven stability.

b) Store all the samples at –20°C and/or –70°C for at least 24 hrs and after this period thaw

all the three sets of samples unassisted at room temperature.

c) When completely thawed, set-1, set-2 and set-3 are refrozen for at least 12 hours under the same conditions.

d) Keep set-1 in frozen condition to use for second freeze and thaw cycle when analytical results of third freeze and thaw cycle are not within the acceptance criteria.

e) The second freeze - thaw cycle is repeated for set-2 and set-3.

f) Thaw the frozen set-2 and set-3 for the third freeze and thaw cycle. Refreeze the set-3.

g) Prepare, process and analyze calibration standards and six replicates each LQC and HQC comparison samples by spiking with freshly prepared stock solutions or stock solution with proven stability as per the procedure described in tentative method SOTP/Protocol.

h) Process and analyze set-2 stability samples after third freeze and thaw cycle along with freshly prepared low and high quality control comparison samples.

i) Repeat fourth freeze and thaw cycle for set-3 if required

j) Compare the mean back-calculated concentrations of stability samples with those of freshly prepared comparison samples

k) Calculate % mean change as per formula described in Attachment –I.

Note : The withdrawal of set-2 and set-3 in (e, f) for subsequent freeze and thaw cycles will be based on project requirement and supervisors decision.

7.2.7.5.2 ACCEPTANCE CRITERIA

a) The back-calculated concentrations of all LQC and HQC samples must be within 85.00 - 115.00% of their nominal concentration.

b) At least 67.00% of QC samples must fall within above-mentioned criteria at each LQC and HQC levels.

c) % Mean change must be within ± 15.00%.

Note: If above acceptance criteria are not met, then experiment should be repeated if on investigation any processing error is found or should be repeated for another relevant storage period or storage temperature.

7.2.7.6 STABILITY AT –20°C (± 5°C) and AT–70°C (± 10°C)

Stability at –20°C and -70°C is determined by freezing six aliquots each of the LQC, MQC and HQC samples under the same conditions as that of the study samples.

The storage time in a long-term stability evaluation should exceed the time between the date of first study sample collection and the date of last sample analysis.

7.2.7.6.1 PROCEDURE:

a) Spike five sets each having six replicates each of LQC, MQC and HQC samples with freshly prepared stock solutions or stock solutions with proven stability.

b) Store 2 sets at –20°C and 3 sets at -70°C for relevant period to measure the long-term stability of analyte(s) in biological matrix.

c) Prepare, process and analyze calibration standards and six replicates each of LQC, MQC and HQC as per the procedure described in tentative method SOTP/Protocol.

d) After relevant storage period process and analyze stability samples with comparison samples.

e) The long-term state stability period should be considered between the time of storing of samples in deepfreezer and the time of withdrawal from the deepfreezer.

f) Long term stability should be performed for at least 7 days.

g) Compare the mean back-calculated concentrations of long-term stability samples with those of comparison samples.

h) Determine % mean change in stability samples as per formula described in Attachment–I.

7.2.7.6.2 ACCEPTANCE CRITERIA

a) The back-calculated concentrations of all LQC, MQC and HQC samples must be within 85.00 - 115.00% of their nominal concentration.

b) At least 67.00% of quality control samples must fall within above-mentioned criteria at each LQC, MQC and HQC levels.

c) % mean change must be within ± 15.00%.

Note: If above acceptance criteria are not met, then experiment should be repeated if on investigation any processing error is found or should be repeated for another relevant storage period.

7.2.8 DILUTION INTEGRITY

7.2.8.1 PROCEDURE

a) Prepare the stock solutions of analyte(s) and internal standard as per the procedure described in tentative method SOTP/Protocol.

b) Spike analyte(s) spiking stock solution in blank plasma to get concentration equivalent to 2 times of ULOQ.

c) Dilute above spiked samples (2xULOQ) with blank plasma to get 1/2 and 1/4 concentrations of the spiked sample or as per requirement.

d) Process and analyze calibration standards and six aliquots each of diluted samples (1/2 and 1/4 dilutions) as per the procedure as described in tentative method SOTP/Protocol.

e) Calculate %CV for the back calculated concentration as described in Attachment-I.

f) Calculate % accuracy for the back calculated concentration as described in Attachment-I.

7.2.8.2 ACCEPTANCE CRITERIA

a) Back calculated concentration accuracy should be within 85.00 to 115.00% of theoretical concentration.

b) Precision (% CV) for the back calculated concentration should be within 15.00%.

c) At least 67.00% of total dilution samples at each dilution level should fall within above-mentioned criteria.

Note : If above acceptance criteria are not met, then experiment should be repeated

7.2.9 MATRIX EFFECT

Matrix effect should be measured in six different lots of same matrix, out of which 04 should be normal buffered / heparinized / EDTA plasma, and out of other two, 01 lipemic plasma and 01 haemolyzed plasma with heparin anticoagulant to ensure that precision, selectivity, and sensitivity is not affected in/by different lots of matrix.

7.2.9.1 PROCEDURE:

a) Process and analyze the matrix calibration standards and three replicates from all lots described above each at LQC and HQC levels as per the procedure described in tentative method SOTP/Protocol.

b) Determine accuracy as per formula described in Attachment -I.

7.2.9.2 ACCEPTANCE CRITERIA

a) The back calculated concentrations of LQC and HQC must be within 85.00 - 115.00% of their theoretical concentration.

b) At least 67.00 % of LQC and HQC samples must fall within above-mentioned criteria for each lot of matrix.

c) At least 75.00% of the buffered plasma / heparinized/EDTA plasma should meet the acceptance criteria.

d) Both Lipemic and Haemolysed plasma should meet the above criteria (a & b), if the experiment fails repeat the experiment once or change the methodology.

7.2.10 ANTICOAGULANT EFFECT

Anticoagulant effect is performed to check the effect of different anticoagulant on analytical results.

7.2.10.1 PROCEDURE

a) Process and analyze calibration standards (prepared using buffered plasma/heparinised plasma / EDTA plasma) and six replicates each of LLOQ, LQC, MQC and HQC samples (QC samples should be prepared with different anticoagulant other than that used for preparing calibration standard and that is to be used in study) prepared as per tentative method SOTP/Protocol.

b) Calculate % CV for LLOQ, LQC, MQC and HQC samples, as per formula, described in Attachment-I.

Note: If anticoagulant used for validation and study samples is same, then anticoagulant effect should not be performed during Method Validation.

7.2.10.2 ACCEPTANCE CRITERIA

a) The back calculated concentrations of all LQC, MQC and HQC must be within 85.00 - 115.00% of their nominal concentration except at LLOQ sample where it should not deviate by more than 80.00-120.00% of its nominal concentration.

b) The %CV of the back calculated concentrations of all QC samples (LQC, MQC, and HQC) must be within 15.00%, except for LLOQ, which should be within 20.00%.

c) At least 67.00% QC samples must fall within above-mentioned criteria at each LLOQ, LQC, MQC and HQC levels.

7.3 SAMPLE SEQUENCE FOR METHOD VALIDATION IN MULTIPLEXING MODE

HPLC 1	HPLC 2	MS/MS
Blank solution.	Blank solution.	Blank solution (from HPLC 1) Blank solution (from HPLC 2)
System Suitability.	System Suitability.	System Suitability (from HPLC 1) System Suitability (from HPLC 2)
Blank matrix.	Blank matrix.	Blank matrix (from HPLC 1) Blank matrix (from HPLC 2)
Zero standard (ZS).	Zero standard (ZS).	Zero standard (ZS)(from HPLC 1) Zero standard (ZS) (from HPLC 2)
Calibration standards (CS)	Calibration standards (CS)	Calibration standards (CS) (from HPLC 1) Calibration standards (CS) (from HPLC 2)
Validation samples	Validation samples	Validation samples (from HPLC 1) Validation samples (from HPLC 2)

Note: 1) Submission of sequence or batch for the first time should have blank solution followed by system suitability and followed by validation samples.

2) Total number of samples in a sequence should be same in HPLC 1 and HPLC 2. If not, add blank solution sample.

3) For single LC-MS/MS, the batch organization is to be considered as that in either of the HPLC-1 / HPLC-2 with respect to planned validation parameters.

7.4 PARTIAL VALIDATION

a) Partial validations are modifications of already validated Bioanalytical methods. A validation performed to substantiate the modification of a validated method. The minimum requirement is one intra run accuracy and precision determination including a calibration curve, six replicate at each LLOQ, LQC, MQC and HQC level. The acceptance criteria are that of a validation run.

b) When changes are to be made to a previously validated method then these modifications should be partially validated to ensure suitable performance of the analytical method.

c) Analyst should exercise scientific judgment giving due weightage to the factors affecting the already established full method validation, as to how much additional validation is needed.

d) The judged parameters should be discussed and reviewed by the supervisor for its application.

e) All these details should be documented in journal or in the respective documentation format.

f) Partial validation parameters to be performed can range from as little as one intra-assay accuracy and precision determination to a nearly full validation.

7.4.1 PROCEDURE

Partial validation for Bioanalytical methods should be carried out in following conditions not limited to,

a) Bioanalytical methods transfer between laboratories and analysts- all validation parameters other than stabilities (Stabilities need to be added if matrix is changed).

b) Change in analytical methodology. (e.g. change in detection systems within same laboratory).

(e.g. HPLC to LC-MS/MS) – Specificity, sensitivity, and three linearities and three precision and accuracy batches.

c) Change in Chromatographic conditions such as Mobile phase, Column, Wavelength etc. – Specificity, three Linearity and three precision and accuracy batch.

d) Change in flow rate – Specificity and one linearity and one precision and accuracy batch.

e) Change in sample processing procedures e.g. buffering of plasma, extraction solvent, extraction technique, reconstitution or elution solution – Specificity, recovery, three linearity and three precision and accuracy, but process stability will be required if there is change in reconstitution solution or elution solution.

f) Change in relevant concentration range – If LLOQ is still reduced then perform specificity, sensitivity, two linearity and two precision-accuracy batches. When LLOQ and ULOQ are increased then perform specificity, sensitivity, carry over check, two linearity and two precision and accuracy batches.

g) Change in instrument make – Specificity, Sensitivity, two linearity, two precision and accuracy batch and carry over check.

h) Change in software platforms – One linearity and one precision and accuracy batch.

i) Change from single LC-MS to Multiplexing LC-MS system or vice versa – two precision

and accuracy batch, carry over check.

j) Change in anticoagulant in harvesting biological fluid – specificity, one linearity and one precision and accuracy batch, also stability (bench top, freeze thaw and long term) to be performed.

k) Selectivity demonstration of an analyte in the presence of concomitant medications – specificity, sensitivity, one linearity and one precision and accuracy batch and matrix effect.

l) Change in matrix within species (Human plasma to human urine) – Full validation

m) Change in species within matrix (e.g. rat plasma to mice plasma) – Full validation

n) Rare matrices – Specificity, linearity, two precision and accuracy batches with all stability experiments.

o) If for any other reason a partial method validation need to be performed, and then the parameters to be performed should be scientifically judged by the supervisor and should be discussed with head of the department for the scientific basis of selection criteria.

7.5 REINJECTION OF VALIDATION BATCH / SAMPLES

7.5.1 PROCEDURE

7.5.1.1 The batch / samples can be reinjected in cases not limited to:

a) System stopped in between

b) Un acceptable Chromatography (Problem related to column, etc)

c) Pumping problem

d) Autosampler problem (Needle blockage)

e) Mass spectrometry problem

f) Any other events which is scientifically judged by the supervisor for reinjection.

g) The procedure of reinjection should be scientifically decided by the analyst(s) and or supervisor

h) The found reason should be documented in journal or in respective documentation format.

7.5.1.2 The file name of the reinjected sample should have suffix R1 after the file name.

Note : Necessity of any additional experiment should be judged by the supervisor.

7.6 Reporting of Data Generated During the Method Validation Experiment.

7.6.1 The validation data generated during the method validation experiment shall be described

as per the format of Attachment- II. This method validation reporting format is subjected to modification as per the method, regulatory or GLP requirement.

7.6.2 Any deviation in Validation experiments should be reported as per SOP No. BE-008.

7.6.3 Any additional information or the documentation of the activity that are to be used to document during validation experiments shall be attached as templates in the form of Note to File as per attachment III.

7.6.4 Reporting of failing parameters of Validation experiments will be done in project journal or in the respective documentation format while electronic copy of all the raw data of the failing experiment will be stored in the computer system or in its accessories. (Backup devices/server).

7.6.5 A unique Method Validation report number should be given to full method validation report of that project. The same method validation report number should be kept for all its supplement reports which are generated on the performance of additional parameters, long term stability or performance of partial validation for that project. The method validation report number is given as :

BA/MV/XXX/YY

Version No:

Where,

BA : Bioanalytical

MV : Method Validation

XXX : Report no. (given in increment starting from 001,002, -----)

YY : Last two digit of the year in which report was prepared.

7.6.6 Draft report shall be prepared with appropriate QC check and given for QA review. Only after incorporating QA comments, version 01 is prepared. The first analytical report will become the main Analytical Report.

7.6.7 Version No. should be 01 if the report was prepared first time. Subsequent version number 02 will be given to the validation report or to the supplement report if a change is to be done or found in the report (report already QA approved). Thus the new subsequent version number is applicable only for approved result. The main report number of Validation and Supplements should remain the same throughout its lifecycle of the project. Reason for change in Validation Report and Supplement Reports Version No. should be reported in brief as per format given in Attachment II.

7.6.8 Supplement report preparation should be done as per Attachment-II and its version No. which includes all the necessary and applicable sections with respect to the experiments/validation parameters performed. Before mentioning the title of supplement on the first page, it should mention the Supplement No. Ex: SUPPLEMENT-II (Version No.:01)

UNCONTROLLED COPY CONTROLLED STAMP	**Title: Pre method validation, full method validation and partial method validation of Bioanalytical methods related to Bioavailability (BA) and Bioequivalence (BE) studies.**	SOP No.	:	BE-A-013
		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	23/02/2007
		Review Period	:	2 Years

Note Addition or removal of sections in report can be done based on regulatory requirement.

8.0 REFERENCE

8.1 SOP for "Issuing, identification and control of all documents of Bioavailability (BA) and Bioequivalence (BE) studies generated in Bioanalytical laboratory" - BE-A-003

8.2 SOP for "Procurement, storage, usage, retest and disposal of working standards, pharmacopoeial and non pharmacopoeial reference standards"-BE-A-007.

8.3 SOP for "Procedure for the separation of plasma and serum from blood, its procurement, identification and storage of blank and stability samples of Bioavailability (BA) and Bioequivalence (BE) studies in deepfreezer"-BE-A-009

8.4 SOP for "Sequence of Bioanalytical Courses and related documentation"-BE-A-011

8.5 SOP for "Preparation, Identification and verification for spiking stock solutions of Calibration Standards and Quality Control Samples for Bioavailability (BA) and Bioequivalence (BE) studies"-BE-A-012.

8.6 SOP for "Assessing the quality of chromatogram by proper peaks integration, generation and verification of chromatograms, and their acceptance criteria"-BE-A-016

8.7 SOP for "Handling of deviations"- BE-008

9.0 ATTACHMENTS

Attachment No.	Form No.	Title	No. of Pages
Attachment I	NA	Formula to be used in validation.	1
Attachment II	NA	Validation Report Format	36
Attachment III	NA	Note to file Format	1

torrent

TITLE: FORMULA TO BE USED IN VALIDATION					
Form No.:	NA	Reference SOP No.	BE-A-013	SOP Version No.	04

Mean (X): $\frac{\text{Sum of all values}}{\text{Number of values}}$

Standard Deviation (S.D (±)): $\sqrt{\frac{n\sum x^2 - (\sum x^2)}{n(n-1)}}$

(S.D is calculated using Excel spread sheet)

Precision: Coefficient of variation (CV %): $\frac{\text{Standard deviation}}{\text{Mean}} \times 100$

% Accuracy: % Nominal concentration: $\frac{\text{Concentration found}}{\text{Theoretical concentration}} \times 100$

Concentration ES : $\frac{\text{Mean peak response (ES) x Concentration (US)}}{\text{Mean peak response (US)}}$

Percent of recovery: $\frac{\text{ES x CF}}{\text{US}} \times 100$

ES: Mean Extracted standard sample area
US: Mean Unextracted sample area

CF: Concentration factor = $\frac{\text{Initial processed sample volume}}{\text{Final processed sample volume}}$

Note : If CF is not applicable then "1" should be used.

% Mean Change:

$$\left[\frac{\text{Mean calculated concentration of stability samples}}{\text{Mean calculated concentration of comparison samples}} - 1\right] \times 100$$

% Bias: $\left[\frac{\text{Calculated concentration of stability samples}}{\text{Calculated concentration of comparison samples}} - 1\right] \times 100$

% Mean change of stock solutions:

$$\left[\frac{\text{Mean area response of stability samples}}{\text{Mean area response comparison samples}} - 1\right] \times 100$$

UNCONTROLLED COPY

torrent

TITLE: VALIDATION REPORT					
Form No.	NA	Reference SOP No.	BE-A-013	SOP Version No.	04

Note:

a) For Attachment II, Format of Header should be as below

torrent

TORRENT RESEARCH CENTRE
Village: Bhat, Dist Gandhinagar,
India
Validation Report No. (As per SOP) Version No. :(As per SOP)

b) For Attachment II, Format of Footer should be in the center of the page, as below

---- of -----

TITLE: VALIDATION REPORT					
Form No.	NA	Reference SOP No.	BE-A-013	SOP Version No.	04

TITLE
(as per Method SOTP/Protocol)

BIOANALYTICAL METHOD VALIDATION REPORT
(REPORT DATE)

BIOANALYTICAL LABORATORY
TORRENT RESEARCH CENTRE,
TORRENT PHARMACEUTICALS LTD.
VILLAGE: BHAT
DIST: GANDHINAGAR
INDIA

Note this page is applicable only for the Supplement reports

SUPPLEMENT –I
(Version No.:01)

TITLE
(as per Method SOTP/Protocol)

BIOANALYTICAL METHOD VALIDATION REPORT
(REPORT DATE)

BIOANALYTICAL LABORATORY
TORRENT RESEARCH CENTRE,
TORRENT PHARMACEUTICALS LTD.
VILLAGE: BHAT
DIST: GANDHINAGAR
INDIA

INDEX

5.0 Conclusion

6.0 History of change in Version of Validation Report

7.0 Figures

8.0 Tables

A) List of Abbreviation

Abbreviation	**Description**
ADL	Analytical Development Laboratory
Comp. sample	Comparison Sample
conc.	Concentration
CS	Calibration Standard
CV	Coefficient of Variance
FDC	Fixed Dose Combination
HPLC	High Performance Liquid Chromatography
HQC	High Quality Control
hrs.	Hours
IS/ISTD	Internal Standard
i .d	Internal Diameter
LC-MS	Liquid Chromatography-Mass Spectrometry
LC-MS/MS	Liquid Chromatography-Mass Spectrometry- Mass Spectrometry
LLOQ	Lower Limit of Quantification
LQC	Low Quality Control
mm	Millimeter
ml	Milliliter
mM	Milli molar
MS	Mass Spectrometric
MQC	Medium Quality Control
min.	Minute
ng	Nanogram
No.	Number
QC	Quality Control
r	Correlation Coefficient
RT	Retention Time
SD	Standard Deviation
Sec.	Second
SOP	Standard Operating Procedure
SOTP/Protocol	Standard Operating Test Procedure/Protocol
STD	Standard
SYS SUIT	System Suitability

temp./TEMP.	Temperature
ULOQ	Upper Limit of Quantification
V/V	Volume/Volume
V	Volts
WS	Working Standard
ZSTD	Zero Standard
µ	Micron
µl	Microlitre
%	Percentage
arb	Arbitory unit

B) List of Figures

C) List of Tables

1.0 INVESTIGATORS

FOLIO OF SIGNATURES

The analysis of validation samples and process derived data of ----- were performed by

Name	:	Name	:
Designation	:	Designation	:
Qualification	:	Qualification	:
Function	: Analyst	Function	: Analyst
Date	:	Date	:
Signature	:	Signature	:

I, the undersigned, declare that, to best of my knowledge, I had reviewed this analytical report for the compliance with the Torrent Research Centre implemented SOPs and that raw data presented in this report are accurate and authentic.

Name	:
Designation	:
Qualification	:
Function	: Supervisor
Date	:
Signature	:

I, the under signed, declare that, to the best of my knowledge, I had reviewed this analytical report for compliance with Torrent Research Centre implemented SOPs and that I had scientifically evaluated the statements and conclusions of this report.

Name	:
Designation	: Head of Department
Qualification	:
Function	:
Date	:
Signature	:

2.0 OBJECTIVE

The objective of this work was to validate specific ----- method for the determination of Analyte in blank matrix for bioavaibility /bioequivalence study of Analyte.

3.0 SUMMARY

------ method for the determination of Analyte in -----matrix was carried out according to SOTP/Protocol No.------.
Analyte were extracted from ------- matrix using ----- extraction technique. The final eluent was injected into a liquid chromatograph equipped with ---- detector. Quantification was performed by peak area ratio method. A weighting factor --- was used to determine the concentration of the drug.

3.1 BIOANALYTICAL METHOD FOR ESTIMATION OF ANALYTE IN ------ MATRIX.

3.1.1 Reported literature

3.1.2 Bioanalytical technique

-------- Technique was followed.

The summary of the chromatographic conditions are mentioned in section --- of SOTP/Protocol No. which has been enclosed as a part of Annexure No.

3.1.3 Detector Parameters

The summary of the detector parameters are mentioned in section --- of SOTP/Protocol No. which has been enclosed as a part of Annexure No. ----.

3.1.4 Internal Standard

------ was used as Internal standard for Analyte.

3.1.5 Biological Source

Buffered blank plasma was procured from blood bank for the preparation of plasma calibration standards and quality control samples. For specificity check experiment haemolysed, lipemic and heparinised plasma were procured from Pharmacokinetic Unit or other concerned Department of Torrent Research Centre. For anticoagulant experiment, heparinised plasma was used for preparation of quality control samples that was procured from Pharmacokinetic Unit or other concerned Department of Torrent Research Centre.

3.1.6 Anticoagulant

Anticoagulants include heparin and buffers. Buffers include mixture of Citrate, Phosphate and Dextrose (CPD).

3.1.7 Type of extraction

---- Extraction technique was followed and its procedure was mentioned in section -- of SOTP/Protocol No.----- which has been enclosed as a part of Annexure No.-----

3.1.8 Linearity Group

The calibration curves were linear from ---- to ---- for Analyte.

3.1.9 Quantification Parameter

The quantification parameters were performed as per ---- software, version ----.

3.2 REFERENCE/WORKING STANDARDS

3.2.1 Reference/working Standard

Name :
Batch No. :
Exp. date/Retest date/Validity date:
Name and address
of manufacture :

3.3 PREPARATION OF THE CALIBRATION STANDARDS AND QUALITY CONTROL SAMPLES.

Calibration standards and quality control samples were prepared as per SOTP/Protocol No.----

3.4 LABELLING AND STORAGE

3.4.1 Aqueous stock solutions

The stock solutions were labeled to indicate the analyte name, standard identification (Calibration standard or quality control sample) and date of preparation. These solutions were stored at 2-8°C.

3.4.2 ---- samples

Blank ----- was labeled to indicate the lot No. and stored at -70°C / -20°C

The bulk spiked/freshly prepared samples were labeled to indicate the analyte name, standard identification (calibration standard or quality control sample) and date of spiking. These samples were stored at -70°C / -20°C deepfreezer.

3.5 CALCULATION OF THE SAMPLE CONCENTRATION

The concentration of the analyte was calculated from the following equation using linear regression analysis of spiked plasma calibration standard with the reciprocate of the drug concentration as a weighting factor
e.g.1/concentration, i.e. 1/x: $y = mx + c$

Where → y = peak area ratio of analyte to internal standard
m = slope of the calibration curve
x = concentration of analyte
c = y-axis intercept of the calibration curve

3.6 SOP DEVIATION

4.0 VALIDATION AND CHARACTERISTICS OF THE METHOD

4.1 Chromatography

Representative chromatograms of aqueous system suitability, blank plasma, zero standards, LLOQ, ULOQ, LQC, MQC, HQC samples and calibration curve for analyte were represented in Figure No. ---, ---, ---, ---, ----, and --- respectively.

4.2 Specificity

Four different lots of --------, four different lots of --------, one lot of ---- and one lot of ---- were chromatographed and no area response was observed or area response less than 20% and 5% was observed at the RT of analyte as well as Internal standard (IS) respectively (Table No. ---).

4.3 Sensitivity

The LLOQ was --- for analyte.
The % CV of analyte (Table No.---) at LLOQ was found to be ----
The % nominal concentration for LLOQ samples of analyte were ranged from -- to – (Table No. ---)

4.4 Linearity

The Linearity of the method was determined by a weighted ----- regression analysis of standard plots associated with a ----- standard calibration curve. Best-fit calibration curves of peak area ratio versus concentration were drawn. The calibration curves were

linear from --- to --- with correlation coefficient of r ≥ --- for analyte (Table No. --- and Table No. ---)

4.5 Accuracy

4.5.1 Within-batch or intra-batch Accuracy

The % nominal concentration for LLOQ, LQC, MQC and HQC samples of analyte were ranged from --- to ---, --- to --- , --- to --- and --- to --- respectively. (Table No. ---).

4.5.2 Between -batch or inter-batch Accuracy

The % nominal concentration for LLOQ, LQC, MQC and HQC samples of analyte were ranged from --- to ---, --- to ---, --- to --- and --- to --- respectively. (Table No. ---).

4.6 Precision

4.6.1 Within-batch or intra-batch Precision

The % CV for LLOQ, LQC, MQC and HQC samples of analyte were ---, ---, --- and --- respectively. (Table No. ---).

4.6.2 Between -batch or inter-batch Precision

The % CV for LLOQ, LQC, MQC and HQC samples of analyte were ---, ---, --- and --- respectively (Table No. ---).

4.7 Recovery

The percentage recovery of analyte was determined by comparing the mean peak area of analyte in extracted LQC, MQC and HQC samples with freshly prepared unextracted LQC, MQC and HQC samples respectively.

The mean % recovery for LQC, MQC and HQC samples of analyte were ---, --- and --- respectively (Table No. ---).

For Internal standard, mean peak area of --- extracted samples were compared to the mean area peak of --- Internal standard solutions. The mean percentage recovery for Internal standard was --- (Table No. ---).
The % CV Unextracted for LQC, MQC and HQC samples of analyte were ----, ----- and ----- respectively. (Table No. ----).

The % CV Extracted for LQC, MQC and HQC samples of analyte were ----, ---- and ----- respectively. (Table No. -----).

The % CV of recovery across QC level for analyte was --- (Table No. ---).

The % CV within IS concentration of extracted samples was --- (Table No. ---).

4.8 Anticoagulant Effect

The precision and accuracy experiment was performed using buffered plasma for calibration standards, and heparinised plasma for QC samples.

The % C.V for LLOQ, LQC, MQC and HQC samples of analyte were ---, ---, --- and --- respectively (Table No. ---).

The % nominal concentration for LLOQ, LQC, MQC and HQC samples of analyte were ranged from --- to ---, --- to ---, --- to --- and --- to --- respectively. (Table No. ---)

4.9 Dilution Integrity

Dilution integrity experiment was carried out at five replicate of two times diluted 2xULOQ (½ dilution), four times diluted 2xULOQ (¼ dilution) samples were prepared and its concentrations were calculated against the freshly prepared calibration curve.

The % CV for ½ dilution and ¼ dilution samples of analyte were --- and --- respectively (Table No. ---).

The % nominal concentration for ½ dilution and ¼ dilution samples of analyte were ranged from --- to --- and --- to --- respectively (Table No. ---).

4.10 Matrix effect

In order to ensure the effect of matrix through out the application of the method, matrix blanks obtained from six different lots (04 normal, 01 Lipemic and 01 Haemolysed) were spiked with analyte and internal standard at LQC and HQC level. Three quality control samples at each level along with the set of calibration standards were analyzed and the % Nominal conc. of the samples analyzed was represented in (Table No. ---) for analyte.

4.11 Stability

4.11.1 Stock solution stability.

Stock solution stability was determined by comparing the peak areas of freshly prepared solutions (comparison samples) with stability samples.

4.11.1.1 Stock solution stability of Analyte and Internal Standard at room temperature for ---- hours.

Main stock solution of Analyte and Internal Standard were freshly prepared and aliquots of stocks were kept at room temperature for --- hours (stability sample). Aqueous equivalent highest calibration standards of Analyte and Internal Standard were prepared from the stability samples and analyzed. Areas of stability samples and freshly prepared samples were compared to determine % Mean change during stability period. Analyte stock solution was found to be stable at room temperature for --- hours with % Mean change of --- (Table No. ---).

Internal Standard stock solution was found to be stable at room temperature for --- Hours with % Mean change of --- (Table No. ---).

4.11.1.2 Stock solution stability of Analyte and Internal Standard at ---°C for --- hours.

Main stock solution of Analyte and Internal Standard were freshly prepared and aliquots of stocks were kept at ---°C for --- hours (stability sample). Aqueous equivalent highest calibration standards of Analyte and Internal Standard were prepared from the stability samples and analyzed. Areas of stability samples and freshly prepared samples were compared to determine % Mean change during stability period.

Analyte stock solution was found to be stable at ---°C for --- hours with % Mean change of --- (Table No. ---).

Internal Standard stock solution was found to be stable at ---°C for --- hours with % Mean change of --- (Table No. ---).

4.11.1.3 Stock solution stability of Analyte and Internal Standard at ---°C for --- Days.

Main stock solution of Analyte and Internal Standard were freshly prepared and aliquots of stocks were kept at ---°C for --- Days (stability sample). Aqueous equivalent highest calibration standards of Analyte and Internal Standard were prepared from the stability samples and analyzed. Areas of stability samples and freshly prepared samples were compared to determine % Mean change during stability period.

Analyte stock solution was found to be stable at ---°C for --- Days with % Mean change of --- (Table No. ---).

Internal Standard stock solution was found to be stable at ---°C for --- Days with % Mean change of --- (Table No. ---).

4.11.2 Bench Top Stability of Analyte (at room temperature for --- hours.)

LQC and HQC samples were spiked in --- matrix and were kept at room temperature for --- hours and were processed and analyzed along with freshly prepared calibration standards, LQC and HQC samples. Concentrations were calculated to determine % Mean change during stability period.

Analyte was found to be stable in LQC and HQC samples for ----hours at room temperature with % Mean change of --- and --- respectively (Table No. ---).

4.11.3 Process Stability of Analyte at ---°C in autosampler for --- hours

LQC and HQC samples were prepared and processed. These processed samples were kept in auto sampler for --- hrs at ---°C, these samples were analyzed along with freshly prepared calibration standards, LQC and HQC samples. Concentrations were calculated to determine % Mean change during stability period.

Analyte was found to be stable in LQC and HQC samples for --- hours at ---°C in autosampler with % Mean change of --- and --- respectively (Table No. ---).

4.11.4 Freeze and Thaw Stability of Analyte (after --- cycle at -----)

Samples were prepared at LQC and HQC levels, aliquoted and frozen at ----°C. Six samples from each concentration were subjected to --- freeze and thaw cycles (stability samples). These samples were processed after --- cycle and analyzed along with freshly prepared calibration standards, LQC and HQC samples (comparison samples). Concentrations were calculated to determine % Mean change after --- cycle.

Analyte was found to be stable in LQC and HQC samples after --- cycle at ------°C with % Mean change of --- and --- respectively (Table No. ---).

4.11.5 Long term stability of Analyte (at ------°C for --- Days)

Samples were prepared at LQC, MQC and HQC levels, aliquoted and frozen at ------ °C. Six samples of each concentration were analyzed after --- days (stability samples) along with freshly prepared calibration standards, LQC, MQC and HQC samples (comparison samples). Concentrations were calculated to determine % Mean change during stability period.

Analyte was found to be stable in human plasma at ------ for --- days in LQC, MQC and HQC samples with % Mean change of ---, --- and --- respectively. (Table No. ---).

5.0 CONCLUSION

The results of the Method Validation for Analyte were summarized in (Table No. ---). The analytical method was valid for the analysis of Analyte with a calibration range of --- to --- in --- matrix using --- as internal standard.

6.0 HISTORY OF CHANGE IN VERSION OF VALIDATION REPORT

Note: It should be in tabular form describing in brief reasons for change in Validation Report.

Version No.	Date	Page No.	Section	Modification

7.0 Figures

BIO-EVALUATION CENTRE
TORRENT PHARMACEUTICALS Ltd.
Village Bhat, Gandhinagar-382 428
Gujarat, India.

ANNEXURE No. 4.12.3.5

SAMPLE PREPARATION, ANALYTICAL RUN/BATCH ORGANIZATION, RE-INJECTION, REASSAYING AND REPORTING OF RESULTS OF THE STUDY SAMPLES RELATED TO BIOAVAILABILITY (BA) AND BIOEQUIVALENCE (BE) STUDIES.

(SOP No. BE-A-014)

UNCONTROLLED COPY CONTROLLED STAMP	**Title: Sample preparation, analytical run / batch organization, re-injection, reassaying and reporting of the study samples results related to Bioavailability (BA) and Bioequivalence (BE) studies.**	SOP No.	:	BE-A-014
		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	23/02/2007
		Review Period	:	2 Years

References: USFDA guidelines and ANVISA guidelines.

Revision History:

Version	Section	Revision Summary	Reason for revision
04	7.0	Procedure modified	Up gradation
	7.4	Added additional Reanalysis code	
	7.6	Procedure modified	
	9.0	Attachment I –Reanalysis code P added	
		Attachment VI modified	

	Name	Designation & Department	Signature	Date
Prepared by	Deepak Jain	Scientist I –Bioanalytical Laboratory		17/02/07
Reviewed by	Jignesh Kotecha	Scientist I –Bioanalytical Laboratory		19/02/07
	Hemang Pathak	Scientist II –Quality Assurance	Pathak H.M.	20/02/07
Approved by	Dr.G.Subbaiah	General Manager-Bioanalytical Laboratory		21/02/07

UNCONTROLLED COPY CONTROLLED STAMP	**Title: Sample preparation, analytical run / batch organization, re-injection, reassaying and reporting of the study samples results related to Bioavailability (BA) and Bioequivalence (BE) studies.**	SOP No.	:	BE-A-014
		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	23/02/2007
		Review Period	:	2 Years

1.0 PURPOSE

To provide a procedure for sample preparation, analytical run /batch organization, re-injection, reassaying and reporting of the study samples results related to BA and BE studies.

2.0 DEFINITIONS

2.1 Analytical run (batch): A complete set of analytical samples with appropriate number of calibration standards, quality control samples and study samples that is taken through preparation, extraction, and analysis. Several runs/ batches may be completed in one day, or one run /batch may take more than a day to complete.

2.2 SOTP/Protocol: Standard operating test procedure/Protocol. It refers the way of performing the analysis. It describes in detail the steps necessary to perform each analytical test. This may include, but is not limited to, the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

2.3 Study sample: A study sample is a liquid or solid biological matrix collected from a study specimen during a study performed at the Clinical Research Department/other concerned department of Torrent Research Centre or externally and requiring the quantitative determination of analyte(s).

2.4 Positive Control: Those study samples, (other than the QC and repeat samples of that batch) selected from the already run batch which is accepted as per the acceptance criteria, run with repeat samples of that batch only, repeat analyzed to judge the status of the initial analysis of that complete batch.

2.5 Aliquot: An aliquot is a portion of a sample.

2.6 Additional Quality Control (AQC): Addition of an appropriate (judged by the analyst/supervisor) calibration standard processed and analyzed along with other quality control (LQC,MQC,HQC) samples if required to cover the achieved study samples concentration by the use of this additional quality control samples.

2.7 Dilution Quality Control (DQC): A quality control sample defined as dilution quality control sample achieved after the dilution of 2ULOQ concentration (diluted with the same dilution factor as applied for the study samples). DQC need to be run with the dilution repeat samples only.

2.8 Multiplexed LC-MS/MS: Two HPLC coupled with one MS/MS.

2.9 Processed sample: The final extract (prior to instrumental analysis) of a sample that has been subjected to various treatments. (e.g., extraction, dilution, concentration)

2.10 Sample re-injection: Sample re-injection is defined as a second injection (or more) on a chromatographic system of the same processed sample.

2.11 Re-analysis: Samples with improper results re-processed and re-analyzed to achieve the proper results are considered as samples for re-analysis. Following criteria should be considered for the judging the improper results and hence re-analysis: Poor chromatography (for e.g. very high baseline, peak splitting, peak tailing), unacceptable internal standard response/area, technical error (functioning technical hindrance to complete the analysis), software communication error. Such samples need to be re-analyzed in singlet. The original values of the re-analysis results in report should be reported as NA (not applicable), since the original results are not complete.

2.12 Repeat analysis: Samples which give unexpected results are considered for the repeat analysis. The reasons for repeat analysis is not limited to predose sample with calculated concentration greater than LLOQ, unexpectedly very high or very low concentration in between samples.

2.13 Pharmacokinetic repeat: Repeat analysis done on a sample because its reported value is valid analytically but inconsistent with the pharmacokinetic profile of the drug. This would be requested by the statistician only.

2.14 Unknown Samples: BA/BE study samples from the Enrolled subjects / Volunteers who had participated in the BA/BE study.

3.0 SCOPE

This SOP is applicable for all study samples analyzed chromatographically (HPLC / LC-MS/MS) in Bioanalytical laboratory at Bio-Evaluation (BE) centre of Torrent Research Centre.

4.0 POLICIES

Not Applicable

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		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	23/02/2007
		Review Period	:	2 Years

5.0 RESPONSIBILITY

5.1 All persons from Bioanalytical laboratory involved in study sample analysis related to BA and BE studies must follow the procedure described in this SOP.

5.2 Supervisor is to check the overall compliance and adherence to the SOP.

5.3 Head of the department is for the approval of final results.

6.0 MATERIAL

Not Applicable

7.0 PROCEDURE

7.1 SAMPLE PREPARATION

7.1.1 PROCEDURE

7.1.1.1 Withdraw the blank matrix lot for preparation of the calibration and quality control samples and or withdraw the pre stored calibration and quality control samples from deep freezer and make entry in respective activity forms and thaw them un assisted at room temperature.

7.1.1.2 Withdraw the necessary type of stock solution batch for spiking the samples with either calibration or the addition of the internal standard and make entry in respective activity forms and thaw them unassisted at room temperature.

Note : Sufficient Calibration and QC samples should be bulk spiked (Preparation of large quantity of Calibration and QC samples) and required quantity of aliquots should be stored in the same deepfreeze where the respective received study samples are stored. This procedure of bulk spiking of calibration and quality control samples should be done only after completion of the respective method validation activity. In case study sample are received before the completion of method validation, then in that case, the bulk spiking of the calibration and quality control samples should be done before start of the study samples.

7.1.1.3 Take out all the samples to be analyzed from the deep freezer (sequentially) and make entry in the same form for study samples storage, retrieval, restorage and disposal as mentioned in SOP No. BE-A-011, and thaw them un assisted at room temperature. Process and analyze all the samples as per the respective approved method SOTP/Protocol.

7.1.1.4 Follow the procedures for study sample withdrawal, processing and analysis as per the established stability parameters during method validation.

7.2 ANALYTICAL RUN/BATCH/SEQUENCE ORGANIZATION

7.2.1 PROCEDURE

a) Samples of an analytical run/batch/sequence should be analyzed in the following order:

- Blank solution.
- System Suitability.
- Blank matrix.
- Zero standard (ZS).
- Calibration standards (CS)
- Quality control samples (LQC, MQC, HQC)
- Unknown study samples or Coded samples
- Quality control samples (LQC, MQC, HQC)
- Unknown study samples or Coded samples
- Quality control samples (LQC, MQC, and HQC) and so on.

b) Additional quality control samples in addition to LQC, MQC and HQC can be injected along with the QC set. This selected Additional QC (s) (AQC) should be from the calibration standard only.

c) The decision of injecting the Additional QC sample and its selection should be done by the analyst and or supervisor either right before the beginning of the study sample analysis or in between the study sample analysis.

d) The decision of injecting the Additional QC samples is made to justify the batch acceptance in the achieved concentration range.
Example: If the linearity range set is for 100mg IR dose and now the same range is to be used for 50mg IR dose, and if the concentration levels achieved are within LQC and MQC concentration then an additional QC point (point between CS-2,CS-3,CS-4) selected from the linearity range can be used to actually judge the batch acceptance.

e) A dilution quality control (DQC) samples in addition to LQC, MQC and HQC set and or additional QC can be injected along with the QC set (LQC, MQC, HQC). This selected dilution QC (s) (DQC) should be prepared by diluting the 2ULOQ as done for the re-analysis dilution samples.

f) The QC qualification and batch acceptance criteria will still remain the same as defined in section 7.2.3.

g) All the unknown samples from an Enrolled subject / Volunteer should preferably be analyzed in a single batch/run.

h) Unknown samples will be appropriately arranged in an analytical run/batch considering the total number of samples in particular period per volunteer / Enrolment and accordingly sufficient quality control samples will be placed.

i) The planned batch/run sequence for the study samples analysis should be prepared and print out should be taken. The analyst should cross check the sample identification and the sample position while placing the samples with the printed batch/sequence and should sign it for the match before analysis. This should be counter singed by other analyst or by the supervisor. No additional volunteer samples can be added to this predefined batch. If they need to be added then they need to be run with new batch, which will also follow the above rule for the planned batch.

j) The total number of Quality Control samples (irrespective of any number of multiples) should not be less than 5% of the total unknown study samples or six total Quality control samples (including two Low, two Medium and two High) whichever is greater in an analytical run /batch.

k) For arranging the samples in HPLC-1 and HPLC-2 batch/run of multiplexing refer Attachment –II

Note : Coded samples (barcoded samples) are the study samples which are blinded {i.e. the identity of the volunteer, formulation type (test and reference) and or the time point is hidden} as per the specific requirements.

7.2.2 Criteria for starting an analytical run/batch

a) Chromatographic conditions of analyte (s) and internal standard(s) are to be similar to those defined in respective Method SOTP/Protocol.

b) The analyst should first check the system suitability by running the test system suitability sample as described in SOTP/Protocol , if the system is found suitable and stabilized, i.e. after achieving the required precision value for Area or Area ratio, submit the analytical run/batch as per sequence described in section 7.2.1.

c) %CV of area ratio of analyte(s) to Internal standard (s) should be less than or equal to 5.0%. If the method requires specific acceptance criteria then it should be mentioned in the respective approved method SOTP/Protocol.

Note: If any one of the above criteria is not met than the analysis of study samples should not be started. Corrective actions should be taken prior to sample analysis to resolve

the anomaly. After sorting out the problem again give five replicate injections from system suitability vial before starting an analytical run/batch.

7.2.3 Acceptance Criteria for an Analytical run /Batch.

A) For blank matrix & zero standard

a) Analyte area should be less than 20.00% of the LLOQ area and area of IS (Internal standard) should be less then 5% of the IS area for blank matrix.

b) At least the area of analyte in any one of the blank matrix or the zero standard should be less then 20.00% of the accepted LLOQ area.

B) Calibration curve

a) The back-calculated concentration of the lowest calibration standard (CS1) must be within 80.00-120.00% of its nominal concentration.

b) The back-calculated concentrations of all other calibration standards must be within 85.00-115.00% of their nominal concentrations.

c) The curve must contain at least 75.00% of the calibration standards for evaluation of curve fitting e.g. 6 out of 8 calibration standards (Not to consider those calibration standards which are run in duplicate and one is excluded e.g. at LLOQ and ULOQ level).

d) No two adjacent (or consecutive) calibration standards can be rejected.

e) If LLOQ or ULOQ fails then the calibration curve is redefined by considering the next acceptable calibration standard after LLOQ as new LLOQ and or the acceptable concentration before ULOQ as the new ULOQ.

f) If LLOQ or ULOQ fails then the calibration curve is redefined and the samples concentration below the new LLOQ (as mentioned in e) and those sample concentration above the new ULOQ (as mentioned in e) should be repeated for the actual results with the pre defined linearity.

g) The correlation coefficient (r) of the calibration curve must be $\geq$ 0.9900 using the same weighting factor established for method validation.

C) Quality control samples

a) In an analytical run/batch for all samples, at least 66.67% of the total QC samples should be within 85.00-115.00 % of their nominal concentration and not more than 33.33% of the QCs at respective QC level (all replicates at same conc.) should not deviate from 85.00-115.00% of their nominal concentration {i.e. at least 66.67% of the respective QCs (all replicates at same

concentration) should be within 85.00-115.00%.}

b) In case of batch run with only two QC sets (i.e. total two set of LQC, MQC and HQC) with unknown samples sandwiched in between, at least 66.67% (four out of six) of the total QC samples should be within 85.00-115.00 % of their nominal concentration and not more than 50.00% of QC at respective QC level (all replicates at same conc.) should deviate from 85.00-115.00% of their nominal concentration.

c) In an analytical run/batch for all samples, at least 66.67% of the total AQC samples should be within 85.00-115.00 % of their nominal concentration

d) In case of batch run with only two Additional QCs (AQC) along with the defined QC set (LQC, MQC and HQC) with unknown samples sandwiched in between, at least 50.00% (one out of two AQC) should be within 85.00-115.00 % of their nominal concentration.

e) In an analytical run/batch for all samples, at least 66.67% of the total DQC samples should be within 85.00-115.00 % of their nominal concentration.

f) In case of batch run with only two Dilution QCs (DQC) along with the defined QC set (LQC, MQC and HQC) with unknown samples sandwiched in between, at least 50.00% (one out of two DQC) should be within 85.00-115.00 % of their nominal concentration.

g) Sample analysis runs are valid only if at least two-thirds (2 / 3) of overall number of quantifying QC samples (total LQC, MQC, HQC and other AQC and or DQC samples) are within 85.00-115.00%.

h) The position of the failed QC samples in a batch run is not relevant for run or individual sample acceptance.

D) Unknown samples

a) If the predose concentration is less than or equal to 2.00 % of Cmax value but greater than LLOQ concentration, then the subject's data can be reported in the test result sheet.

b) If the predose concentration is exceeding the 2.00 % of Cmax value, then that sample need to be taken for repeat sample analysis.

c) Estimation of concentration in unknown samples by extrapolation of standard curves below LLOQ or above the ULOQ is not recommended. Instead, the standard curve should be redefined and or samples with higher concentration

should be diluted and reassayed.

d) It is preferable to analyze all study samples from a subject in a single run.

Note : At any point of time/situation the batch acceptance / rejection (for the above mentioned criteria of section 7.2.3 in A, B, C and D) can be judged by the supervisor and the scientific justification for the acceptance / rejection activity should be noted in the journal or in the respective documentation format.

7.3 SAMPLE RE-INJECTION

7.3.1 Re-injection reasons:

a) Analytical instrument power failure.

b) Faulty instrument setting.

c) Technical problem such as, analytical instrument or analytical column malfunctions, unexpected chromatography (resolution between peaks reduced, not suitable for quantification) or any other problem justifiable with scientific reasoning.

d) Software error.

7.3.2 Procedure

a) Partial re-injection of an analytical run/batch: Re-injected sample(s) must be analyzed starting with an already acquired QC set of LQC, MQC and HQC taken from the same analytical run/batch. The last sample of the re injected run/batch should be a quality control sample.

b) If the analytical run/batch stops in between the calibration standard, then the batch should be re-injected starting from the beginning.

c) Sample(s) requiring re-injection must be analyzed within established stability period reported in method validation report or should be established for the relevant period after the study as scientifically judged by the supervisor after reviewing the validation data.

d) Scientific reason(s) for partial or total re-injection of an analytical run/batch and description of re-injected samples should be documented in respective project journal or in the respective documentation format, dated, signed and witnessed by the analyst and co-analyst, indicating the consensus for the given scientific reason.

e) Data files of re-injected samples must not overwrite the original data file. Analytical run/batch sequence must be modified accordingly. The re-injected samples file name should end with R1 for first re-injection, second time re-injection it should be given R2 and so on. (e.g. 270507001R1, 270507001R2...). This specific way of naming the

re-injection files is to identify the re-injected sample. The prefix "270507001" can be the same date of first injection or the new date of batch re-injection.

f) In case of complete batch re-injection the batch name should have R1 as suffix indicating the re-injection of the total batch Example: 220107R1 is the new batch name, while the file name of the re-injected samples should be different than the original file name.

g) File all the re-injected chromatograms along with the run summary. The original data file should remain intact in the system or at storage location.

7.3.3 Acceptance criteria

a) Partial re-injection of an analytical run/batch: QC sample(s) re-injected must meet quality control acceptance criteria, as mentioned in section 7.2.3.

b) Total re-injection of an analytical run/batch: Calibration standards and QC samples must meet calibration and QC acceptance criteria.

Note: When analytical run/batch stops in-between due to reasons not limited to as described in section 7.3.1, the run/batch should be started by injecting already acquired QC set from the same run/batch followed by either injecting from the same sample which is not completely acquired or from where the batch/sequence is stopped or all the samples can be re-injected with a new batch name with suffix R1 Example: 22JAN07R1 is the new batch name.

7.4 RE-ANALYSIS AND REPEAT ANALYSIS

a) Study samples should be reanalyzed and or repeat analyzed using the remaining sample.

b) Prior to re-analysis and or repeat analysis, the probable samples for the repeat analysis with appropriate repeat codes should be discussed by the analyst with supervisor.

c) Not more than 20.00% of the total study samples should be reanalyzed and or repeat analyzed. Complete rejected batch reanalysis and the positive controls samples run with the repeat analysis should not be considered in this 20.00% criterion.

7.4.1 SAMPLE RE-ANALYSIS

Re-analysis: Samples with improper results re-processed and re-analyzed to achieve the proper results are considered as samples for re-analysis. Following criteria should be considered for the judging the improper results and hence re-analysis: Poor chromatography (for e.g. very high baseline, peak splitting, peak tailing), unacceptable internal standard response/area, technical error, software communication error. Such samples need to be re-analyzed in singlet. The original values of the re-

analysis results in report should be reported as NA (not applicable), since the original results are not true values.

a) Sample re-analysis required for analytical reasons are to be coded, approved, signed and dated by the supervisor of Bioanalytical laboratory in project journal or in the respective documentation format.

A study sample can be re-analyzed for the following reasons:

A : Poor chromatography.

B : Unacceptable Internal standard response.

D2 : ½ Diluted sample analysis of sample above ULOQ

D4 : ¼ Diluted sample analysis of sample above ULOQ.

E : Rejected analytical run.

P : Batch reanalyzed for positive control failure

G : Incomplete Analysis

7.4.1.1 Poor chromatography (Code: A)

a) Poor chromatography includes merging peaks, broad or large peak, peaks on drifting baseline, maximum peak response (saturation), minimum analyte(s) peak response etc. (refer SOP BE-A-016)

b) Samples reanalyzed for poor chromatography should be coded 'A' in the reanalysis table of the analytical report.

7.4.1.2 Un acceptable Internal Standard Response (Code: B)

a) Any study samples analysis presenting internal standard response beyond ± 50% (For HPLC and LCMS /MS) of mean internal standard response of accepted. Calibration standards and Quality control samples must be rejected and document technical reasons or specific criteria e.g.

- Loss of extraction solvent.
- Improper volume of reconstitution solution.
- Half, double, or no addition of internal standard during sample pretreatment.

b) Samples reanalyzed for Un-acceptable Internal Standard Response should be coded 'B' in the reanalysis table of analytical report.

7.4.1.3 Sample concentration above upper limit of quantification (Code: D2, D4)

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		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	23/02/2007
		Review Period	:	2 Years

a) Sample for which concentration exceeds the nominal concentration of the upper limit of quantification of the calibration curve must be re-assayed after appropriate dilution with blank biological matrix.

b) Refer the respective SOTP/Protocol for the dilution criteria of study sample analysis.

c) Samples reanalyzed as falling above the upper limit of quantification should be coded 'D2', 'D4', in the reanalysis table of the analytical report.

d) Where D2 is for ½ dilution, D4 is for ¼ dilution.

e) Analyze the dilution QC (one number) with the QC set while repeating the dilution reanalysis samples analysis.

f) The dilution QC will be prepared using 2ULOQ concentration which will be diluted in similar fashion as that of the dilution procedure (same dilution factor) followed for the dilution study samples.

Example: If dilution reanalysis samples are diluted 4 times, then the dilution QC should be diluted four times using the same control matrix and the matrix lot used should be mentioned in the respective project journal or in the respective documentation format.

g) Acceptance criteria should be same as that for the QC samples, i.e. atleast 66.67% of the dilution QC should be within 85.00-115.00% of their nominal concentration. If only two dilutions QC are analyzed then at least 50.00% of the analyzed dilution QC should be within 85.00-115.00%.

Note: a) Only one type of dilution (i.e one dilution factor) will be applied throughout the dilution reanalysis samples.

b) After dilution the concentration of diluted samples should be within the quantified calibration range.

7.4.1.4 Rejected analytical run/batch (E)

a) A run/batch is rejected when the acceptance criteria for calibration standards and quality control samples, described in section 7.2.3 are not met. Results of the samples will not be presented in the report. The batch should be reanalyzed with batch name having suffix "E" indicating the rejected batch reanalysis. Example: 220107E as batch name. The prefix "220107" can be of same date of first injection batch or new date of batch injection.

b) A run/batch may also be rejected even if acceptance criteria of calibration standards and quality control samples are met but most of the chromatograms exhibit poor

chromatography, interferences, baseline drifting etc, (Refer SOP No. BE-A-016) in that case, batch rejection reasons must be documented, dated by the analyst and approved by supervisor.

7.4.1.5 Batch rejected for positive control failure (P)

A run/batch is rejected when the acceptance criteria for the selected positive control samples, run with the repeat analysis, are not met, as described in section 7.4.2. Results of the samples will not be presented in the report. The batch should be reanalyzed with batch name having suffix "P" indicating the rejected batch reanalysis analysis. Example: 220207P as batch name. The prefix "220207" can be of same date or new date of batch injection.

7.4.1.6 Incomplete analysis (Code G)

An incomplete analysis may be defined as sample for which conc. cannot be obtained due to technical reasons such as-

a) Loss of sample during processing.

b) Equipment Failure (Laboratory equipment, analytical instrumentation, Computer, Server backup system, gas panel supply, column or tubing pressure, Power failure).

c) Samples reanalyzed for Incomplete analysis should be coded "G" in re analysis table of the analytical report.

Note : Occurrence of any such event as in section 7.4.1.6 should be noted in the project journal or in the respective documentation format.

7.4.2 SAMPLE REPEAT ANALYSIS

Repeat analysis: Samples which give unexpected results are considered for the Repeat analysis. The reasons for repeat analysis is not limited to predose sample with calculated concentration greater than LLOQ, unexpectedly very high or very low concentration in between samples. Such samples need to be repeated with positive control.

a) Sample repeat-analysis required for analytical reasons are to be coded, approved, signed and dated by the supervisor of bioanalytical laboratory in project journal or in the respective documentation format.

b) All samples for repeat analysis should be analyzed in single.

c) Selection of six positive control samples (other than the repeat analysis samples) should be selected at random from the batch in which some of the sample(s) require repeat analysis.

Example: If only two samples of two different batch are to be considered for repeat analysis, then in total 12 positive control samples (six from first batch and six from second batch) need to be processed and analyzed with the repeat samples.

d) Acceptance Criteria of Positive controls: Out of six positive controls four positive control should be within 85.00-115.00% of their initially back calculated concentration.

e) If the above mentioned criteria in (d) is not met then the total batch need to be reanalyzed for the true value meeting the defined batch acceptance criteria. The batch name should have the suffix "P". Example: 22JAN07P which indicate that it is a batch reanalyzed where the positive control did not meet the acceptance criteria on repeating some of the samples for the repeat analysis. The reportable value will be of this repeat batch.

f) Reporting of the positive control in the report should be done as per Attachment-VI

g) A study sample can be repeat-analyzed for the following reasons:

C : Sample volume not sufficient for repeat analysis.

H : Sample reanalyzed to obtain confirming value on analytical reasons.

F : Pharmacokinetics repeats

7.4.2.1 Sample volume not sufficient for repeat analysis (Code C)

Such study samples should be diluted for:

a) The sample volume available for repeat analysis is less than the sample volume specified in the Bioanalytical method SOTP/Protocol.

b) Dilution factors of study samples are to be recommended by the supervisor/Head of the department (based on validation data, i.e. Atleast the sample volume should not be less than $1/4^{th}$ of actual processing volume) in order to obtain the concentration within the calibration range. If the selected sample for the repeat analysis with initial sample concentration below or near LLOQ, then their dilution should not be carried out and the initial concentration should not be reported.

c) Study sample dilutions should be performed with blank biological matrix.

d) The identification lot number of biological matrix used for dilutions should be documented in project journal or in the respective documentation format.

e) The final concentration of a diluted sample is the calculated concentration multiplied

by the dilution factor.

7.4.2.2 Sample reanalyzed to obtain confirming value for following reasons (Code H)

a) Predose sample presenting a quantifiable conc. (higher than the lower limit of quantification but in consensus with that defined in section 7.2.3 (D)).

b) A sample presenting no conc. while quantifiable conc. was obtained with the two adjacent sampling time points or vice versa.

c) Improper sample identification. (Processing tubes position interchanged, Auto sampler vial position interchanged, incorrect Analytical run/batch sequence schedule)

d) Samples can be repeat analyzed to confirm the first analyzed value.

e) Samples repeat analyzed to obtain confirming value should be coded "H" in repeat analysis table of the analytical report.

Note: The compiled tabular form for the summary of reanalysis and repeat analysis with code, is given in Attachment-I.

7.4.2.3 Pharmacokinetic repeat (Code F)

a) Pharmacokinetic repeats are defined as repeat analysis done on a sample inconsistent with the Pharmacokinetic profile of the drug. Pharmacokinetic repeats are to be determined by the bio-statistician.

b) Samples repeat analyzed, for Pharmacokinetic reasons should be coded 'F' in the repeat analysis table of the Bioanalytical report.

c) Pharmacokinetic repeat analysis should be performed in single with positive controls. Incase of insufficient amount of sample dilute the samples with interference free biological matrix such that diluted concentration must be within the quantifiable Calibration range. If falling below LLOQ after dilution then it will be reported as Non Reportable Value, NRV'.

7.5 PROCEDURE FOR SAMPLES NOT ANALYZED

Other codes can be used in the analytical reports for the reason encountered during the first assay and or re-assay:

7.5.1 CODIFICATION

I: Sample with insufficient volume for a first analysis.

J: Sample received at the analytical facility in damaged or unacceptable condition was not observed during random check.

SLP: Sample lost during processing.

SNS: Sample not submitted

SNA: Sample not analyzed

BLQ: Values falling below lower limit of quantification (LLOQ) should be reported as "BLQ" (below limit of quantification) e.g. LLOQ = 100 ng/ml and value less then 100 ng/ml= "BLQ".

7.6 REPORTING OF FINAL CONCENTRATIONS

7.6.1 Reporting of the final concentration values should be done in respective "Test Result Sheet" formats as per attachment-III, IV and V. After completion of all study sample analysis, the Bioanalytical laboratory should compile the results in Microsoft excel sheet (or appropriate software) on the path defined on the server. The test result sheet should have the names of the compiler and reviewers of the Test Results Sheet. The names include any one analyst, supervisor, and head of the department. Other than the analyst, all other persons have read only access for their respective activities. This test result sheet is with name and date only. Since it is an electronic copy and no signature should be done on this sheet. The "Reviewed By" and "Approved By" of the result sheet on the server will be done by the supervisor and Head of the Department. An alternative mode of communication (written or verbal) for the approved result sheet on the server should be acceptable.

7.6.1.1 Test result sheet number should be given as:
For BA or BE studies, "Report No." should be given as:
BA/Study Code

Where,
BA : Bioanalytical
Study Code : Number available on the Test Request Sheet supplied by the requestor.

7.6.1.2 Bioanalytical (BAN) Reference No. in Test Result Sheet should be given as:
Project Journal No. /Date on which experiment performed (start to end). Date should be documented in ddmmyy format. (eg. 001/300706-310706)

7.6.2 Reporting of the pharmacokinetic repeat values should be done in "Pharmacokinetic Reanalysis Test Result Sheet" as per attachment-IV. The preparation of the PK repeat sheet should follow the same procedure as mentioned in above section 7.6.1.

7.6.2.1 For PK repeat Test result sheet number should be given as:

BA/Study Code/RN

Where,
BA : Bioanalytical
Study Code : Number available on the Test Request Sheet supplied by the requestor.
R : Repeat analysis.
N : Repeat analysis number (No. given in increment, starting from 1, 2 ---)

7.6.2.2 Bioanalytical (BAN) Reference No. in "Pharmacokinetic Repeat analysis Test Result Sheet" should be given:

Project Journal No. /Date on which experiment performed (start to end). Date should be documented in ddmmyy format. (e.g. 001/300706-310706)

7.6.3 Reporting of the coded sample values should be done in "Coded samples Test Result Sheet" as per attachment-V. The preparation of this test result sheet should follow the same procedure as mentioned in above section 7.6.1.

7.6.3.1 Coded study sample Test result sheet number and
BA or BE studies, "Report No." should be given as:
BA/Study Code

Where,
BA : Bioanalytical
Study Code : Number available on the Test Request Sheet supplied by the requestor.

7.6.3.2 Bioanalytical (BAN) Reference No. in Test Result Sheet should be given as:

Project Journal No. /Date on which experiment performed (start to end).Date should be documented in ddmmyy format. (e.g. 001/300706-310706).

7.6.4 Reporting of the total study details as Bioanalytical Study Report should be done as per attachment –VI.

Note : Among the list of Annexure as mentioned in Attachment-VI, the section 4.12.3.2 and onwards need not be attached with the non regulatory submission studies analytical report. All attachments other than above mentioned need to be attached with the analytical report. The analytical report format should vary with respect to the regulatory requirement and as per the respective project requirement.

7.6.5 Criteria for the reporting final value in Bioanalytical Study Report should be as follows:

7.6.6 First reportable value

a) Incase of no repeat analysis, the initial value is accepted and reported as such.

b) First analysis (initial value) value should be reported if after the repeat analysis, the second analysis values show deviation within 85.00-115.00 % with respect to the initial values.

7.6.7 Second reportable values

7.6.7.1 Second reanalyzed value is to be reported for all the reanalysis samples.

7.6.7.2 Second repeat analysis (all kind of the repeat values) values is to be reported for repeat analysis if the second analysis values show deviation more than 85.00-115.00% with respect to the initial values.

Note : In case of second repeat analysis, if any abnormal event or function had occurred or observed then the analyst should proceed for the third or further repeat analysis in consensus with the supervisor. The reporting of then achieved analytical value should fall in similarity as that defined in section 7.6.7.2.

7.6.8 Non reportable values (NRV)

a) If during the initial analysis, the value is not acceptable (scientifically or not in consensus with the other values) and sample is lost or not available during subsequent analysis, then the value should not be reported and should be coded as "NRV".

7.6.9 Study Report No. should be given same as that "Report No." given on the "Test Result Sheet".

a) Draft report shall be prepared with appropriate QC check and given for QA review. After incorporating QA comments only version 01 is prepared. The first analytical report will become the main Analytical Report.

b) Version No: should be given in increment from 01, 02, ---.

c) Version No. should be 01 if the report was prepared first time.

d) Subsequent version number 02 will be given to the analytical report if a change is to be done or found in the report (report already QA approved). Thus the new subsequent version number is applicable only for approved result. The Analytical Report No. of Study should remain the same.

e) Reason for change in Analytical Report Version No. should be reported in brief as per format given in Attachment VI.

UNCONTROLLED COPY CONTROLLED STAMP	**Title: Sample preparation, analytical run / batch organization, re-injection, reassaying and reporting of the study samples results related to Bioavailability (BA) and Bioequivalence (BE) studies.**	SOP No.	:	BE-A-014
		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	23/02/2007
		Review Period	:	2 Years

8.0 REFERENCE

8.1 SOP for "Issuing, identification and control of all documents generated in Bioanalytical laboratory" BE-A-003.

8.2 SOP for "Procurement, storage, usage, retest and disposal of working standards, pharmacopoeial and non pharmacopoeial reference standards"-BE-A-007.

8.3 SOP for "Procedure for the separation of plasma and serum from blood, its procurement, identification and storage of blank and stability samples of Bioavailability (BA) and Bioequivalence (BE) studies in deep freezer"- BE-A-009.

8.4 SOP for "Sequence of Bioanalytical Courses and related documentation"- BE-A-011.

8.5 SOP for "Preparation, Identification, and verification for spiking stock solutions of Calibration Standards and Quality Control Samples"- BE-A-012.

8.6 SOP for "Assessing the quality of chromatogram by proper peaks integration, generation and verification of chromatograms, and their acceptance criteria"- BE-A-016.

9.0 ATTACHMENTS

Attachment No.	Form No.	Title	No. of Pages
Attachment I	NA	Summary for reanalysis and repeat analysis	2
Attachment II	NA	Sample Sequence for Multiplexing.	1
Attachment III	NA	Test Result Sheet format (soft copy)	1
Attachment IV	NA	Pharmacokinetic repeat analysis test result sheet format (soft copy)	1
Attachment V	NA	Coded Samples Test Result Sheet format (soft copy)	1
Attachment VI	NA	Format of Bioanalytical study report	22

ATTACHMENT –I

TITLE: SUMMARY FOR REANALYSIS AND REPEAT ANALYSIS					
Form No.:	NA	Reference SOP No.	BE-A-014	SOP Version No.	04

Sr. No.	REASON FOR REANALYSIS	EXAMPLES	CODE
1	Poor Chromatography	Merging peaks, broad or large peak, peaks on drifting baseline, interfering peaks in, maximum peak response (saturation), minimum analyte (s) peak response	A
2	Unacceptable internal standard response	Loss of extraction solvent, Improper volume of reconstitution solution, Half, double, or no addition of internal standard during sample pretreatment	B
4	Sample concentration above upper limit of quantification	Sample for which concentration exceeds the nominal concentration of the upper limit of quantification of the calibration curve	D2, D4
5	Rejected analytical batch	Calibration standards not meeting acceptance criteria, Quality control samples are not within acceptance criteria, acceptance criteria of Calibration standards and Quality control samples are met but most of the chromatograms exhibit poor chromatography, interferences, baseline drifting etc	E
6	Batch reanalyzed for positive control failure	Positive control samples do not meet the acceptance criteria	P
7	Incomplete analysis	Loss of sample during processing, Equipment Failure (Laboratory equipment, analytical instrumentation, Computer, Server backup system, gas panel supply, column or tubing pressure, Power failure).	G

TITLE: SUMMARY FOR REANALYSIS AND REPEAT ANALYSIS					
Form No.:	NA	Reference SOP No.	BE-A-014	SOP Version No.	04

Sr. No.	REASON FOR REPEAT ANALYSIS	EXAMPLES	CODE
1	Sample volume not sufficient for repeat analysis	This is applicable only if the sample volume left is Atleast 1/4th of the actual processing volume, and which after dilution will give concentration within the calibration range.	C
2	Sample reanalyzed to obtain confirming value	Predose sample showing quantifiable conc. (higher than the lower limit of quantification), sample presenting no conc. while quantifiable conc. was obtained with the two adjacent sampling time points and vice versa, Improper sample identification. (Processing tubes position interchanged, Auto sampler vial position interchanged, incorrect Analytical run/batch sequence schedule)	H
3	Pharmacokinetic repeat	Sample inconsistent with the Pharmacokinetic profile of the drug.	F

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TITLE: SAMPLE SEQUENCE FOR MULTIPLEXING					
Form No.	NA	Reference SOP No.	BE-A-014	SOP Version No.	04

HPLC 1	HPLC 2	MS/MS
Blank solution.	Blank solution.	Blank solution (of HPLC 1) Blank solution (of HPLC 2)
System Suitability	System Suitability.	System Suitability (of HPLC 1) System Suitability (of HPLC 2)
Blank matrix	Blank matrix.	Blank matrix (of HPLC 1) Blank matrix (of HPLC 2)
Zero standard (ZS)	Zero standard (ZS)	Zero standard (ZS) (of HPLC 1) Zero standard (ZS) (of HPLC 2)
Calibration standards (CS)	Calibration standards (CS)	Calibration standards (CS)(of HPLC 1) Calibration standards (CS)(of HPLC 2)
Quality control samples (LQC, MQC, HQC)	Quality control samples (LQC, MQC, HQC)	LQC from HPLC 1 LQC from HPLC 2 MQC from HPLC 1 MQC from HPLC 2 HQC from HPLC 1 HQC from HPLC 2
Unknown study samples	Unknown study samples.	Unknown study samples (of HPLC 1) Unknown study sample (of HPLC 2)
Quality control samples (LQC, MQC, HQC)	Quality control samples (LQC, MQC, HQC)	LQC of HPLC 1 LQC of HPLC 2 MQC of HPLC 1 MQC of HPLC 2 HQC of HPLC 1 HQC of HPLC 2
Unknown study samples	Unknown study samples.	Unknown study sample (of HPLC 1) Unknown study sample (of HPLC 2)
Quality control samples (LQC, MQC, HQC) and so on	Quality control samples (LQC, MQC, HQC) and so on	LQC of HPLC 1 LQC of HPLC 2 MQC of HPLC 1 MQC of HPLC 2 HQC of HPLC 1 HQC of HPLC 2

BIO-EVALUATION CENTRE
TORRENT PHARMACEUTICALS Ltd.
Village Bhat, Gandhinagar-382 428
Gujarat, India.

ANNEXURE No. 4.12.3.6

PREPARATION AND CONTROL OF STANDARD OPERATING TEST PROCEDURES (SOTPs)/PROTOCOL

(SOP No. BE-A-015)

UNCONTROLLED COPY CONTROLLED STAMP	**Title: Preparation and control of Standard Operating Test Procedures (SOTPs)/Protocol.**	SOP No.	:	BE-A-015
		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	09/01/2008
		Review Period	:	2 Years

References: - -

Revision History:

Version	Section	Revision Summary	Reason for revision
04	2.0	Section elaborated	Up gradation
	7.0	Procedure modified	
	9.0	Attachment I and II content and format modified	
		Attachment VI added	
		SOTP/Protocol request form included.	
		SOTP/Protocol training record excluded.	Procedure has been defined in a separate SOP

	Name	Designation & Department	Signature	Date
Prepared by	Shuja Khan	Scientist II – Bioanalytical Laboratory.	Shuja	05/01/08
Reviewed by	Dr.Deepak Jain	Scientist I – Bioanalytical Laboratory.		07/01/08
	Hemang Pathak	Research Associate – Quality Assurance.	Pathak H.U.	07/01/08
Approved by	Dr.G.Subbaiah	General Manager- Bioanalytical Laboratory.		08/01/08

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		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	09/01/2008
		Review Period	:	2 Years

1.0 PURPOSE

The purpose of this SOP is to provide procedure for the preparation of SOTP/Protocol for Bioanalytical methods and for chromatographic purity.

2.0 DEFINITIONS

2.1 Tentative SOTP/Protocol: A written down Standard Operating Test Procedure/Protocol. It refers the way of performing the analysis for that particular methodology for carrying out method validation and or chromatographic purity activity(s). It should describe in detail the steps necessary to perform each analytical test. This may include, but is not limited to, the sample, the reference standard and the reagents preparations, use of the apparatus, plasma samples preparation, chromatographic condition, MS parameters, generation of the calibration curve, use of the formulae for the calculation, etc.

2.2 Approved SOTP/Protocol: A written down Standard Operating Test Procedure/Protocol. It refers the way (full method validation) of performing the analysis for that particular methodology for carrying out subject sample analysis. It should describe in detail the steps necessary to perform each analytical test. This may include, but is not limited to, the sample, the reference standard and the reagents preparations, use of the apparatus, plasma samples preparation, chromatographic condition, MS parameters, generation of the calibration curve, use of the formulae for the calculation, etc.

2.2 Alternative SOTP/Protocol: A substitute written down Standard Operating Test Procedure/Protocol. It refers the way of performing the analysis for that particular methodology for carrying out subject sample analysis. It should describe in detail the steps necessary to perform each analytical test. This may include, but is not limited to, the sample, the reference standard and the reagents preparations, use of the apparatus, plasma samples preparation, chromatographic condition, MS parameters, generation of the calibration curve, use of the formulae for the calculation, etc. This substitute SOTP/Protocol is prepared when the earlier version is also in use and the substituted SOTP / Protocol will also be in use for the intended application.

2.3 Obsolete SOTP/Protocol: A written down Standard Operating Test Procedure/Protocol which is no longer in general use or is discarded or which describes outmoded, out of date methodology for carrying out a particular experiment etc., or the existing SOTP/Protocol is obsoleted when the methodology described in this particular document is changed and a revision is implemented for this procedure. Reasons not limited to fail of validation experiments.

UNCONTROLLED COPY CONTROLLED STAMP	**Title: Preparation and control of Standard Operating Test Procedures (SOTPs)/Protocol.**	SOP No.	:	BE-A-015
		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	09/01/2008
		Review Period	:	2 Years

3.0 SCOPE

This SOP is applicable for the preparation of both tentative SOTPs/Protocol and approved SOTPs/Protocol within the Bioanalytical Laboratory.

4.0 POLICIES

4.1 It is the policy of the laboratory to prepare the required SOTP/Protocol for the respective activity and give its training to the persons involved before starting the activity. Also the controlled copy of the respective SOTP/Protocol will be made available to the analyst for its ready reference during the performance of the activity.

4.2 If the analyst finds any typographical error in the SOTP/Protocol, then the analyst can cut and correct the error and should sign and date for the acceptance by the analyst, an other witness and or by the supervisor in the original document. The earlier controlled copy shall be withdrawn and a new copy shall be issued for use.

5.0 RESPONSIBILITY

5.1 All analysts for preparation and follow of SOTPs/Protocol for performing the respective activity.

5.2 Supervisor for review of SOTPs/Protocol.

5.3 Quality assurance for review and controlling of SOTPs/Protocol.

5.4 Head of Department /assigned alternative for final approval of SOTPs/Protocol.

6.0 MATERIAL

Not Applicable

7.0 PROCEDURE

7.1 Preparation of SOTPs /Protocol.

7.1.1 Bioanalytical Method SOTP/Protocol

7.1.1.1 After pre method validation of bioanalytical method, a tentative SOTP/Protocol should be prepared which is to be used for the full or partial method validation while after full method validation an approved SOTP/Protocol should be prepared which shall be used for study sample analysis. In case of partial method validation, first a tentative SOTP/Protocol should be prepared and following it partial method

validation should be performed, after completion of the validation activity an approved SOTP/Protocol should be prepared.

7.1.2 Chromatographic purity SOTP/PROTOCOL

7.1.2.1 After optimizing a chromatographic method a tentative SOTP/Protocol should be prepared. After completion of the planned chromatographic purity experiment the same tentative SOTP should be used for the subsequent chromatographic purity testing / retesting of that respective analyte (since the procedure remains the same).

7.1.3 Contents of header and footer

7.1.3.1 Header of the SOTPs/Protocol :

1) Company Logo: Insert logo of the company on the right top corner of each page.

2) Shall mention "Restricted Circulation" using bold letters and left alignment.

3) Shall mention "Standard Operating Test Procedure / Protocol" using bold letters and left alignment.

4) SOTP/Protocol No. :

a) For Bioanalytical Method SOTP/Protocol No shall be given as :

Example 1. For Tentative SOTP/Protocol

Tentative SOTP/Protocol should be prepared after completion of pre-method validation and before starting full method validation or any further method validation activity, and should be numbered as,
AB/YY/XXX (T1)

Where,

AB :

i) BA: Bioanalytical activity carried out for Bioavailability / Bioequivalence studies or other studies in humans.

ii) TK: Bioanalytical activity for Toxicokinetic studies.

iii) For Bioanalytical activity for any other studies conducted in animals shall be abbreviated using abbreviations other than described above.

YY : Last two digit of the year in which SOTP/Protocol is prepared

XXX: SOTP/Protocol No. (In increment 001,002...)

T1 : Tentative (In increment T1, T2 and so on)

Note: If Tentative SOTP/Protocol is prepared for the first time it should be numbered as T1. If tentative SOTP/Protocol is to be prepared for the second time or multiple times and it should be numbered as T2, T3 and so on. The same tentative SOTP/Protocol should be used for any type of validation activity after full method validation. The SOTP/Protocol should be OBSOLETED if and only if a new tentative SOTP/Protocol comes into existence superseding the initial tentative SOTP/Protocol.

Example 2: For Approved SOTP/Protocol

After completion of validation experiments and validation report preparation approved SOTP/Protocol should be prepared by making changes if required in the Tentative SOTP/Protocol. This approved SOTP/Protocol should be used for the study sample analysis. Approved SOTP/Protocol should be numbered as :
AB / YY/XXX:

Where,

AB :

i) BA: Bioanalytical activity carried out for Bioavailability / Bioequivalence studies or other studies in humans.

ii) TK: Bioanalytical activity for Toxicokinetic studies.

iii) For Bioanalytical activity for any other studies conducted in animals shall be abbreviated using abbreviations other than described above.

YY : Last two digit of the year in which SOTP/Protocol is prepared

XXX: SOTP/Protocol No. (In increment 001,002...)

Example 3.For Alternative SOTP/Protocol

Alternative SOTPs/Protocol should only be prepared when more than one SOTP/Protocol needs to be kept in use for a particular drug as per reason but not limited to any partial validation criteria such as change in extraction procedure, change of instrument, and change in concentration range. Alternative SOTPs/Protocol No. should be numbered as :
AB/YY/XXX (a_1)

Where,

AB :

i) BA: Bioanalytical activity carried out for Bioavailability / Bioequivalence studies or other studies in humans.

ii) TK: Bioanalytical activity for Toxicokinetic studies.

iii) For Bioanalytical activity for any other studies conducted in animals shall be abbreviated using abbreviations other than described above.
XXX: SOTP/Protocol No. (In increment 001,002...)

YY : Last two digit of the year in which SOTP/Protocol is prepared

a_1 : Alternative (In increment a_1, a_2....)

b) For Chromatographic purity SOTP/Protocol No should be given as:

Example 1. For Tentative SOTP/Protocol

AB/C/YY/XXX (T1)

Where,

AB :

i) BA: Bioanalytical activity carried out for Bioavailability / Bioequivalence studies or other studies in humans.

ii) TK: Bioanalytical activity for Toxicokinetic studies.

iii) For Bioanalytical activity for any other studies conducted in animals shall be abbreviated using abbreviations other than described above.
C : Chromatographic Purity

YY : Last two digit of the year in which SOTP/Protocol is prepared

XXX: SOTP/Protocol No. (In increment 001,002...)

Note: When drug name or drug combination changes SOTP/Protocol number should change.
T1 : Tentative (In increment T1, T2 and so on)

Note: If Tentative SOTP/Protocol is prepared for the first time it should be numbered as T1. If tentative SOTP/Protocol is to be prepared for the second time or multiple times and it should be numbered as T2, T3 and so on. The SOTP/protocol should be OBSOLETED if and only if a new tentative SOTP/Protocol comes into existence superseding the initial tentative SOTP/Protocol.

5) Revision No. : shall be in two digits

For original, revision no should be 00 and when SOTP/Protocol has been revised revision no should be 01 and so on in increment. When revised SOTP/Protocol is in use, previous revision should be obsolete. SOTP/Protocol is revised reasons not limited to fail of validation experiments.

6) Effective Date: Effective date shall be printed in dd/mm/yy format.

7.1.3.2 Footer of the SOTPs/Protocol :

7.1.3.2.1 Shall mention page no. as "Page X of Y" in the centre of the page.

7.1.4 Contents of cover page

7.1.4.1 As per format given in Attachment I and II. following are the lists of information that are required on the cover page:

1) Title
2) Superseded No. :(Previous no. of SOTP/Protocol being superseded)
3) Reference Tentative SOTP/Protocol No.
4) Names and designations of person(s) involved in "Prepared by", "Reviewed by" and "Approved by" shall be mentioned with date and their signatures.

7.1.5 Contents of body pages

7.1.5.1 Contents of the tentative and approved method SOTPs/Protocol of both Bioanalytical methods and chromatographic purity SOTPs/Protocol should be as per sequence described below

1) Objective: It should described for the estimation of drug (s) and or metabolite (s)
2) Scope: Defines applicability of the SOTPs/Protocol (in concurrence to the respective SOPs)
3) Abbreviations: Abbreviations used in a particular SOTP/Protocol.
4) Responsibility: Defines who should follow the procedure in accordance with the compliance. This should be defined by title of functions and not specific name.
5) Method Summary.
6) Chemical structure of analyte (s).
7) Chemical Structure of Internal standard (s).

   (Not applicable for SOTP/Protocol of chromatographic purity).
8) Instrumentation.

   This section shall be in tabular form describing Instrument to be used, its brand/model and Manufacturer/ Supplier.
9) Reagents and Chemicals

   This section should also be in tabular form describing Chemicals to be used, its Grade and Manufacturer/ Supplier.
10) Apparatus.

11) Preparation of solutions.

12) Preparation of analyte solutions.

13) Preparation of Internal standard solution.

(Not applicable for SOTP/Protocol of chromatographic purity).

14) Preparation of calibration standard and Quality control spiking solutions.

(Not applicable for SOTP/Protocol of chromatographic purity)

15) Preparation of biological matrix calibration and quality control samples in matrix.

(Not applicable for SOTP/Protocol of chromatographic purity)

16) Sample treatment.

Detailed procedure of the sample pretreatment method should be mentioned. (Not applicable for SOTP/Protocol of chromatographic purity)

17) Chromatographic conditions

Detailed information of chromatographic condition should be mentioned.

18) Detectors Parameters: Detailed information should be mentioned.

19) System Suitability: Detailed procedure for vial preparation for system suitability should be mentioned.

20) Acceptance criteria for calibration curve & quality control samples.

(Not applicable for SOTP/Protocol of chromatographic purity)

21) Analytical batch organization.

22) Quantification.

23) Calculations.

24) Precautions.

25) Storage of biological matrix and samples.

(Not applicable for SOTP/Protocol of chromatographic purity)

26) Representative Chromatograms.

(applicable to approved SOTP/Protocol only)

27) History.

7.1.6 Format for Headings and text

7.1.6.1 The whole tentative SOTP/Protocol shall be printed with black ink.

7.1.6.2 SOTPs/Protocol shall be on letter size/A 4 size plain/ bond paper

7.1.6.3 Times New Roman and 12 point font shall be used for the text and tables of SOTP/Protocol with single line spacing.

7.1.6.4 Each Heading should be in uppercase, in bold and numbered separately as 1.0, 2.0, 3.0…and so on.

7.1.7 The author shall submit the SOTP/Protocol to the supervisor for reviewing.

7.1.8 Once the supervisor has reviewed, the SOTP/Protocol shall be given to quality assurance personnel for reviewing for compliance with the documentation and formatting and make corrections, if necessary.

7.1.9 After it is reviewed, effective date shall be assigned to the SOTP/Protocol.

7.1.10 Final version of the SOTP/Protocol must be signed and dated by the author, reviewer (s) and approver (Head of the Department/ assigned alternative).

7.1.11 Allowed deviations

7.1.11.1 Tube lens offset, Declustering potential (DP), Detector voltages etc. instrument parameters that are supposed to change for that specific compound after conduction of performance check can be modified as per method requirement at any point of time.

Note : A "Note to File" shall be generated for the above said deviations and kept along with the departmental SOTP/Protocol controlled copy for ready reference at the time of application.

7.1.12 Analyst involved in the project should be trained for that particular SOTP/Protocol and it shall be documented as per SOP No. BE-A-011. This record should be kept along with the raw data file of that particular experiment.

7.1.13 The training of the SOTP/Protocol shall be conducted before the start of the particular activity.

7.1.14 When the training is conducted for the first time author/reviewer shall be responsible for training of the anticipated users.

7.1.15 Incase the author / reviewer is not available before the start of that particular activity than any validated personnel from the same group to which the activity has been allotted can impart training to the anticipated users.

UNCONTROLLED COPY CONTROLLED STAMP	**Title: Preparation and control of Standard Operating Test Procedures (SOTPs)/Protocol.**	SOP No.	:	BE-A-015
		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	09/01/2008
		Review Period	:	2 Years

Note: Flow of events for SOTP/Protocol preparation has been described in attachment VI.

7.2 Controlling of SOTPs/Protocol Copies

7.2.1 Controlling of both tentative and approved SOTPs/Protocol copies should be done by the quality assurance department.

7.2.2 Required number of copies shall be requested from the quality assurance department as per the attachment III

7.2.3 SOTP/Protocol's number shall be allotted by the document in charge or analyst authorized by the supervisor.

7.2.4 All the details of numbers allotted for SOTPs/Protocol shall be maintained with the documentation incharge or analyst authorized by the supervisor of the laboratory as soft copy as per attachment IV and V.

8.0 REFERENCE

Not Applicable

9.0 ATTACHMENTS

Attachment No.	Form No.	Title	No. of Pages
Attachment I	NA	Bioanalytical Method Standard Operating Test Procedure (SOTP) / Protocol.	12
Attachment II	NA	Standard Operating Test Procedure/Protocol for determination of chromatographic purity.	6
Attachment III	NA	SOTP/Protocol request.	1
Attachment IV	NA	SOTP/Protocol Index (soft copy).	1
Attachment V	NA	Chromatographic purity SOTP/Protocol Index (soft copy).	1

UNCONTROLLED COPY CONTROLLED STAMP	**Title: Preparation and control of Standard Operating Test Procedures (SOTPs)/Protocol.**	SOP No.	:	BE-A-015
		Version No.	:	04
		Supersedes	:	03
		Effective Date	:	09/01/2008
		Review Period	:	2 Years

Attachment VI	NA	Flow of events for SOTP/Protocol preparation.	1

ATTACHMENT –I

TITLE: BIOANALYTICAL METHOD STANDARD OPERATING TEST PROCEDURE (SOTP) / PROTOCOL PREPARATION					
Form No.	NA	Reference SOP No.	BE-A-015	SOP Version No.	04

NOTE:

a) For Attachment I, Format of Header should be as below,

Restricted Circulation

Standard Operating Test Procedure/Protocol

SOTP/Protocol No.: BA/YY/XXX	Revision No:	00	Effective Date:	dd/mm/yy

b) For Attachment I, Format of Footer should be in the center of the page, as below

Page x of y

TITLE: BIOANALYTICAL METHOD STANDARD OPERATING TEST PROCEDURE (SOTP) / PROTOCOL PREPARATION					
Form No.	NA	Reference SOP No.	BE-A-015	SOP Version No.	04

c) For Attachment I, cover page content and format should be as below,

Title: Method for estimation of Drug (s) X and or metabolite (s) in biological matrix over concentration range (unit) using High Performance Liquid Chromatography ---------- detection			
Superseded No.		**Reference Tentative SOTP/Protocol No.:**	

	NAME	**DESIGNATION**	**SIGNATURE**	**DATE**
Prepared By				
Reviewed By				
Reviewed By (QA)				
Approved By				

TITLE: BIOANALYTICAL METHOD STANDARD OPERATING TEST PROCEDURE (SOTP) / PROTOCOL PREPARATION					
Form No.	NA	Reference SOP No.	BE-A-015	SOP Version No.	04

c) For Attachment I, body pages contents and format should be as below,

1.0 OBJECTIVE

To describe standard operating test procedure for estimation of Drug (s) and or metabolite(s) in biological matrix.

2.0 SCOPE

This standard operating test procedure/Protocol is to be read and applied by Bioanalytical Laboratory personnel for the analysis of Method Validation samples (in concurrence with SOP No. BE-A-013) / for the analysis of Study samples (in concurrence with SOP No. BE-A-014).

3.0 ABBREVIATIONS

Note : Include applicable abbreviations and add any further as per the terms used in the procedure.

Abbreviation		Description
BA	:	Bioanalytical
conc.	:	Concentration
CS	:	Calibration Standard
CV	:	Coefficient of Variance
HPLC	:	High Performance Liquid Chromatography
HQC	:	High Quality Control
i .d	:	Internal Diameter
IS	:	Internal Standard
LLOQ	:	Lower Limit of Quantification
LQC	:	Low Quality Control
mm	:	Millimeter

	mL	:	Milliliter
	mM	:	Millimolar
	mg	:	Milligram
	MS	:	Mass Spectrometric
	MQC	:	Medium Quality Control
	Min.	:	Minute
	ng	:	Nanogram
	QC	:	Quality Control
	r	:	Correlation Coefficient
	Sec.	:	Second
	SOTP/Protocol	:	Standard Operating Test Procedure/Protocol
	Temp.	:	Temperature
	ULOQ	:	Upper Limit of Quantification
	Vol	:	Volume
	v/v	:	Volume/Volume
	V	:	Volts
	µ	:	Micron
	µg	:	Microgram
	µl	:	Micro liter
	%	:	Percentage

4.0 RESPONSIBILITY

4.1 Analyst

The analyst (s) involved in the method validation / study sample analyses are responsible for adherence to this SOTP/Protocol.

4.2 Group leader/Supervisor

Group Leader / Supervisor will be responsible to ensure fulfillment of all the requirements of this SOTP/Protocol.

4.3 Quality Assurance Personnel

Quality assurance personnel will be responsible to review and controlling.

4.4 Head of the Department/alternate designee

The Department head /alternate designee will be responsible for overall compliance of this SOTP/Protocol.

5.0 METHOD SUMMARY

Biological matrix	:
Sample volume required	:
Anticoagulant	:
Analyte	:
Internal standard	:
Calibration curve range	:
Detection mode	:
Analytical technique	:
Sample treatment	:
Quantitation method	:
Weighting factor for Calibration curve	:

6.0 CHEMICAL STRUCTURE OF ANALYTE (s)

Name of Standard	:
Molecular weight of Standard	:
Name of Analyte	:
Molecular weight of analyte	:

7.0 CHEMICAL STRUCTURE OF INTERNAL STANDARD (s)

Name of Standard	:
Molecular weight of Standard	:

Name of Analyte :

Molecular weight of analyte :

8.0 INSTRUMENTATION

Instrument	Brand/Model	Manufacturer/ Supplier

Note : This section should be in tabular form describing Instrument to be used, its brand/model and Manufacturer/ Supplier.

9.0 REAGENTS AND CHEMICALS

Chemicals/Reagents	Grade	Manufacturer/ Supplier

Note : Equivalent grade reagents and chemicals may be used.

: This section should also be in tabular form describing Chemicals to be used, its Grade and Manufacturer/ Supplier.

10.0 APPARATUS

Apparatus	Grade/Capacity	Manufacturer/ Supplier

Note : This section should also be in tabular form describing apparatus to be used, its grade/capacity and Manufacturer/ Supplier.

TITLE: BIOANALYTICAL METHOD STANDARD OPERATING TEST PROCEDURE (SOTP) / PROTOCOL PREPARATION					
Form No.	NA	Reference SOP No.	BE-A-015	SOP Version No.	04

11.0 PREPARATION OF SOLUTIONS

Note : Describe in brief the procedure for preparing solutions to be used, other than analyte and internal standard stock and working solutions.

12.0 PREPARATION OF ANALYTE SOLUTIONS

12.1 Preparation of analyte main stock and or intermediate stock solutions:

Note :

a) Consider percentage purity/ assay of standard while weighing.

b) Consider salt equivalency while weighing.

c) Weight and volume of solution may change as per the requirement without altering the concentration for analysis.

13.0 PREPARATION OF INTERNAL STANDARD SOLUTION

13.1 Preparation of Internal standard main stock and or intermediate stock and or spiking stock solutions :

Note :

a) Consider percentage purity/ assay of standard while weighing.

b) Consider salt equivalency while weighing.

c) Weight and volume of solution may change as per the requirement without altering the concentration for analysis.

14.0 PREPARATION OF CALIBRATION STANDARD AND QUALITY CONTROL SPIKING SOLUTIONS

Details of Main stock and Intermediate Stock solution used			Diluent (unit)	Spiking stock Solution	
ID	Conc. (unit)	Volume (unit)		Conc. (unit)	ID

15.0 PREPARATION OF BIOLOGICAL MATRIX CALIBRATION AND QUALITY CONTROL SAMPLES IN MATRIX

Take out the blank biological matrix from –20°C/-70°C deep freeze and keep at room temperature for thawing and vortex adequately before pipetting.

15.1 Preparation of blank sample

To -------- of analyte free plasma in micro tube add --------- of diluent (refer section ----) and vortex to mix. Ad d--- of ---- during sample treatment as per section 16.0.

Add ------ of ---------and follow the sample treatment procedure as per section 16.0.

15.2 Preparation of zero standard

To -------- of analyte free plasma in micro tube add --------- of diluent (refer section ----) and vortex to mix. Add ------ of ---------and follow the sample treatment procedure as per section 16.0.

15.3 Preparation of calibration standard and quality control samples in biological matrix with respect to -------% spiking.

Details of Main stock and Intermediate Stock solution used			Biological Matrix Volume (unit)	Conc. (unit)	ID
ID	Conc. (unit)	Volume (unit)			

Note : Sufficient quantity of calibration and quality control samples shall be bulk spiked (large quantity of calibration and quality control samples) and aliquots are stored along with study samples in same deep freezer.

16.0 SAMPLE TREATMENT

17.0 CHROMATOGRAPHIC CONDITIONS

* Retention time of analyte(s) and internal standard (s) may vary between run/batch.

18.0 DETECTOR PARAMETERS

19.0 SYSTEM SUITABILITY

19.1 Procedure

19.2 Acceptance criteria

% CV for the area ratio of analyte to Internal standard should be ≤5.0%.

20.0 ACCEPTANCE CRITERIA FOR CALIBRATION CURVE & QUALITY CONTROL SAMPLES

a) Correlation coefficient (r) for biological matrix standards should be ≥ 0.99

b) Back calculated concentration for calibration standards should be within ±20% of nominal concentration for CS-1 and ±15% of nominal concentration for CS-2 to CS-9 / CS-10.

c) Back calculated concentrations for Quality control samples should be within ±15% of nominal concentration at low, medium and high quality control samples.

Note : Refer the respective applicable SOP for the other defined acceptance criterias.

21.0 ANALYTICAL BATCH ORGANIZATION

21.1 For Validation Experiments.

Inject Blank solution, system suitability and validation samples.

OR

21.1 For Study samples analysis.

Inject Blank solution, system suitability, blank biological matrix, zero standard, biological matrix calibration standards, QC samples, study samples, QC samples and so on.

22.0 QUANTIFICATION

22.1 Quantification is performed using respective software.

22.2 Construct chromatogram of Analyte and Internal standard and calculate area under the peak for Quantification.

22.3 Calculate area ratio of Analyte to that of Internal standard and plot against the respective concentration to construct the calibration curve.

22.4 Use weighting factor (1/X, $1/X^2$ etc) to construct calibration curve.

22.5 Construct calibration curve for every batch / run and use the same for calculation of concentration of Drug.

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23.0 CALCULATION

Concentration of Drug (s) and or its metabolite (s) in unknown samples is calculated using linear equation (by respective software) :

$y = mx + c$

Where,

y = Area ratio of Drug (s) and or its metabolite(s) to Internal standard.

m = Slope of calibration curve.

x = Concentration in ---/ml of Drug (s) and or its metabolite(s).

c = Intercept of Calibration curve.

Note : Other equations like Quadratic may be used.

24.0 PRECAUTIONS

24.1 Always wear hand glove, mask and safety goggle while handling biological matrix samples.

24.2 Handle the glass test tubes and other glass apparatus with care.

24.3 Always use calibrated micropipettes.

24.4 Use and process the samples within the established stability period or establish the stability for appropriate/sufficient period.

25.0 STORAGE OF BIOLOGICAL MATRIX AND SAMPLES

Store blank biological matrix in deepfreeze (–20°C / -70°C) and study samples in -70°C deepfreeze or as per protocol, until use.

26.0 REPRESENTATIVE CHROMATOGRAMS

(Applicable for approved SOTPs/Protocol only)

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27.0 HISTORY

Note : It should be in tabular form describing in brief reasons for revision of SOTP/Protocol or making a new SOTP/Protocol.

SOTP/Protocol No.	Revision No.	Effective Date	Reason for change		
			Page No.	Section	Modification/ Revision
NA	Original Version	NA	NA	NA	NA