



Prequalification of Medicines Programme

WHO PUBLIC INSPECTION REPORT

Bio-Equivalence Study

Part 1: General information

WHO product numbers covered by the inspection	HA439: Emtricitabine / tenofovir 200/300mg tablet
Study number	ARL/09/404
Title of the study	A Randomized, Open Label, Balanced, Two-Treatment, Two-Period, Two-Sequence, Single Dose, Crossover, Bioequivalence Study of the Fixed Dose Combination of Emtricitabine 200 mg and Tenofovir Disoproxil Fumarate 300 mg Tablet of Cipla Ltd., India with TRUVADA [®] (Fixed Dose Combination of Emtricitabine 200 mg and Tenofovir Disoproxil Fumarate 300 mg) Tablet of Gilead Sciences, Inc. USA, in Normal, Healthy, Adult, Human Subjects Under Fasting Condition.
Clinical Part of the study: Name and address of the organization	Accutest Research Lab (I) Pvt. Ltd.(Unit-I) 4 th Floor, The Grand Monarch, Near Seema Hall, Anand Nagar Road, Satellite, Ahmedabad-380015, INDIA.

	Tel.: +91 79- 4023 1700
Bio-analytical laboratory: Name and address	Accutest Research Laboratories (I) Pvt. Ltd., A-31, M.I.D.C, T.T.C Industrial Area, Khairane, Navi Mumbai –400709, INDIA. Tel: + 91 22 2778 0718/19/21; Fax: + 91 22 2778 0720
Name of contact person (Bio-analytical part)	Dr Ashutosh Pudage
Name and address of the Sponsor	Cipla Ltd, India
Name and email address of contact person (Sponsor)	Dr SM Purandare prc@cipla.com (Present during the inspection: Milind Gole)
Date of inspection	26 and 27 September 2011

Part 2: Summary

The purpose of the inspection was to inspect the bioanalytical part of the bioequivalence study performed at Accutest, Mumbai, including the examination of related source data for HA439: Emtricitabine / tenofovir 200/300mg tablets, and to evaluate whether the study was conducted in compliance with the protocol, Good Clinical Practices (GCP) and Good Laboratory Practices (GLP) as applicable.

General information about the site

Accutest, located in Navi Mumbai was inspected on the above-mentioned dates. See previous reports for details about the site – as this was the eighth inspection of Accutest.

History of WHO and/or regulatory agency inspections

This site has been inspected by WHO PQ several times, as well as Drug Regulatory Authorities from Brazil, USA, DCGI (India) and others.

Focus of the inspection

The inspection focused on the bio-equivalence study conducted for the product HA439: Emtricitabine / tenofovir 200/300mg tablets. The inspection covered several sections of the WHO GCP and GLP texts, including the WHO guidance for organizations performing in vivo bioequivalence studies.

Inspected Areas

The inspection was started with an opening meeting. The company representatives introduced themselves. The inspector introduced himself and then he briefed the CRO about the PQ program and the purpose of the inspection. The Accutest representatives informed the inspector that there had been no changes to the laboratory on site – since the last inspection.

The following list includes some of the documents (bioanalytical part) reviewed during the inspection:

- Analytical methodology
- Method development
- Analytical method validation
- Partial analytical method validation
- Pharmacokinetic report
- Solution preparation
- Repeat analysis record
- System suitability
- Storage of study samples
- Sample dilutions

- Chromatograms for subjects for different periods
- Reference standards
- Some raw data used for the method validation (serial dilutions, overall precision and accuracy)
- Sample stability source data and report including short term, long term
- QC solution preparation for analysis and method validation (samples)
- Results of calibration curves, and quality controls were reviewed for several runs of the study.

Concerning the bio-analytical method and for study analysis - a solid phase extraction method was developed. Method development was started on 2 June 2007 for a study for another sponsor. The records for the development were reviewed briefly (records for reference standards used, instruments used, calibration records, and pipettes used. Fifteen runs were in place by 8 June 2007 after which bio-analytical method validation was started. A draft standard test procedure (STP) and validation protocol were prepared (18.06.2007). Chromatographic conditions were set.

Some changes were made for the “new” sponsor later. These were subjected to partial validation. The changes included the anticoagulant used, calibration standard range, injection volume, run time, and storage temperature. The changes were validated over a period of time until 17.03.2010).

The method validation report was signed off with statements by the Group Leader, DGM analytical and QA head as well as the analytical investigator.

Long term stability for 34 days was initially determined in July 2007 and repeated in December 2007 (for 191 days long term stability). Source data were reviewed for long term stability 191 days. The records showed that:

- This was initiated on 20 December 2007
- The IS used was Lamivudine from Cadila Health (ARL/WS/401) – COA verified
- Emtricitabine working standard was used: ARL/WS/341 (Cipla) – COA verified
- Tenofovir working standard was used: ARL/WS/410 (Matrix) – COA verified
- (Same working standards were used to prepare CC and QCs)
- Records for dilutions prepared that were checked were found to be in order
- Raw data forms and storage conditions for materials and solutions checked were found to be in order
- Chromatograms (Emtricitabine and Tenofovir at 191 days, compared to initial back calculated values) – hard copies as well as electronic data (bunching factor, smoothing factor, noise threshold, area threshold, RT window and expected RT) checked were found to be in order.
- Audit trail settings checked were found to be in order
- Result audit record checked were found to be in order
- Initial spiked matrix prepared: (Different batches of working standard) - 9 June 2007 checked were found to be in order

- Temperature record for the deep freezer: ARL/INS/2810. (It was noted that the temperature was monitored every hour, every day. The temperature was out of limit on 1 August 2007 due to cleaning of the deep freezer. The log books indicated that the samples had been moved from Deep Freezer 2810 to 2811). The records checked were found to be in order.

Validation was done 19 July 2007 at -20C (Freeze thaw: 5 cycles). Source data were reviewed for:

- LQC and HQC
- Initial spiking (Samples were kept on 12.07.2007 in the deep freezer).
- Withdrawal of samples from the Deep Freezer for 5 cycles were verified (13/7, 14/7, 14/7, 15/7, 16/7) and found to be in order
- Weighing slips checked for 16.07.2007 (Emtricitabine, Tenofovir and Lamivudine for QCs and CCs) were found to be in order.
- Chromatograms (Emtricitabine and Tenofovir after the fifth cycle as well as fresh QCs) – hard copies as well as electronic data (bunching factor, smoothing factor, noise threshold, area threshold, RT window and expected RT) checked were found to be in order.
- Audit trail settings checked were found to be in order
- Result audit record checked were found to be in order

Sample analysis

A file was maintained with the records pertaining to the study. The file included:

- Project specification
- Project information
- Certificate of analysis
- Working standard consumption record
- Analytical data sheet
- Column details
- Blank plasma details
- Plasma sample transfer record
- Plasma sample acceptance record
- Plasma sample disposal record
- Requisition sheet for repeat analysis
- Internal standard variation
- Subject analysis
- Re-integration sheet
- Chemist note book

Samples were transferred from Accutest (Ahmedabad) for analysis at Accutest (Mumbai)

Information on the analytical samples:

- Total of 44 subjects – total number of analytical samples transferred 1125 (Period I)

- Total of 44 subjects – total number of analytical samples transferred 1116 (Period II)
- 25 February 2010 (data logger activated 17:31 – monitored to 11:09 on 26 February 2010)
- Storage at -20C during transport as shown by the data logger print outs
- The samples were stored in the deep freezer: MI/01/003/0018 on 16 February at 11:49
- The temperature records were reviewed and found in order for the period of storage

Information on the control samples:

- Total of 44 subjects – total number of control samples transferred 1125 (Period I)
- Total of 44 subjects – total number of control samples transferred 1116 (Period II)
- 12 March 2010 (data logger activated 16:44 – monitored to 10:05 on 13 March 2010)
- Storage at -20C during transport as shown by the data logger print outs
- The samples were stored in the deep freezer: MI/01/003/0011 on 13 March 10:52

Sample analysis:

Start date of analysis: 19 March 2010

End date: 14 April 2010

The following documentation was reviewed and found to be in order:

- Stock solution stability: 12 days (IS was weighed and prepared at regular intervals)
- Source of plasma: Symmers PathCare
- Record of destruction of control samples (March 2011)
- Bulk spiking: 13 March 2011
- Weighing slips:
 - Emtricitabine CC Ws: ARL/WS/813 - COA
 - Tenofovir CC WS: ARL/WS/842 - COA
 - Lamivudine ARL/WS/401 – COA
- Sample analysis: 52 batches
- One run consisted of one subject samples (PI and PII)

The chromatograms and source data (electronic data) for selected subjects were reviewed during the inspection. The integration parameters and audit trail settings as well as audit reports for each batch selected in the inspection were verified, as well as peak areas (presented in documentation and obtained and stored electronically).

Integration parameters: The inspection of the source data resulted in no deficiency listed. The integration parameters in a batch were not changed for any of the batches. All samples in a batch were subjected to the same parameters.

CCs and QCs: There were some QCs that failed acceptance criteria in a few runs/batches. CCs and QCs were prepared separately (separate weighings, working solutions – and are cross-checked after spiking). Several CCs and QCs were checked for several subjects (batches/runs), together with the audit trail, audit reports, integrations, and back calculated. No deficiencies were noted.

On the second day, the inspector further reviewed the chromatograms and source data (electronic) for selected subjects, for both analytes, CCs and QCs – as well as integration parameters. The audit trail settings were verified as well as the audit report for each of the selected batches analysed. Peak areas reported were checked and back calculations were done for selected CCs, QCs and subject samples. These were all found in order. (Sample set analysis, repeat analysis and ISRA)

The sets verified included also data for selected individual sample repeat analysis.

The laboratory was briefly inspected. Areas visited included:

- Analytical balance room
- Deep freezer area
- LCMSMS
- General laboratory area for sample preparation

Documents were reviewed and included:

- Logbook for LCMSMS ARL/INS/5211
- Working standard usage records (Emtricitabine, Tenofovir and Lamivudine)
- Pipette calibration record before and after the study
- Monthly calibration record for the balances (March and April)
- Calibration of LCMSMS every six months – last record prior to sample analysis was 15 March 2010 – pumps flow rate accuracy, autosampler, autosampler temperature accuracy, column oven, PPG (positive and negative)
- Maintenance record for LCMSMS – two entries were made during the time of sample analysis – one due to leakage (tubing replaced) on 22 March 2010 and one on 23 March (leak in needle) – needle replaced
- Temperature log for the stock solution storage – MI/01/003/0019)
- Temperature log for fridge (working standard storage – MI/01/003/0001)
- Project training record
- Instrument details (to be used in analysis project)
- QA review and audit report for long term stability (191 days)
- QA review and audit report for the study
- QA audit statement

Part 3: Conclusion

Based on the areas inspected, the people met and the documents reviewed, and considering the findings of the inspection, the study ARL/09/404 - A Randomized, Open Label, Balanced, Two-Treatment, Two-Period, Two-Sequence, Single Dose, Crossover, Bioequivalence Study of the Fixed Dose Combination of Emtricitabine 200 mg and Tenofovir Disoproxil Fumarate 300 mg Tablet of Cipla Ltd., India with TRUVADA[®] (Fixed Dose Combination of Emtricitabine 200 mg and Tenofovir Disoproxil Fumarate 300 mg) Tablet of Gilead Sciences, Inc. USA, in Normal, Healthy, Adult, Human Subjects Under Fasting Condition, bio-analysis performed at Accutest Research Laboratories (I) Pvt. Ltd., A-31, M.I.D.C, T.T.C Industrial Area, Khairane, Navi Mumbai –400709, was considered to have been conducted at an acceptable level of compliance with WHO GCP and GLP.

Part 4: Reference documents

1. Guidelines for good clinical practice (GCP) for trials on pharmaceutical products. *WHO Expert Committee on the Use of Essential Drugs. Sixth Report.* Geneva, World Health Organization, 1995 (WHO Technical Report Series, No. 850), Annex 3
http://whqlibdoc.who.int/trs/WHO_TRS_850.pdf
2. OECD Principles of Good Laboratory Practice (GLP). [C(97)186/Final], 1997
http://www.oecd.org/document/63/0,2340,en_2649_34381_2346175_1_1_1_1,00.html
3. Additional guidance for organizations performing in vivo bioequivalence studies. WHO Technical Report Series, No. 937, 2006, Annex 9
www.who.int/prequal