



Prequalification of Medicines Programme

WHO PUBLIC INSPECTION REPORT

Bio-Equivalence Study

Part 1: General information

WHO product numbers covered by the inspection	<p>1. Atazanavir 300mg caps</p> <p>2. Efavirenz / Lamivudine / Tenofovir Disoproxil Fumarate 600/300/300mg tablets</p>
Study number	<p>650/08 and</p> <p>ATA/2009/421</p>
Title of the study	<p>650/08: An Open Label, Balanced, Randomized, Two-treatment, Two-period, Two-sequence, Single Dose, Crossover, Bioequivalence Study of Fixed Dose Combination of Tenofovir Disoproxil Fumarate 300mg, Lamivudine 300mg and Efavirenz 600mg Tablets of Matrix Laboratories Limited, India comparing with that of Viread[®] (containing Tenofovir Disoproxil Fumarate) 300 mg tablets of Gilead Sciences, Inc. Foster City, CA 94404, Made in Canada, EPIVIR[®] (containing Lamivudine) 300 mg tablets of GlaxoSmithKline Research Triangle Park, NC 27709, Made in England, manufactured under agreement from Shire pharmaceuticals Group Plc, Basingstoke, UK and SUSTIVA[®] (containing Efavirenz) 600 mg tablets of Bristol-Myers Squibb Company, Princeton, NJ 08543 USA in Healthy, Adult, Human Subjects under Fasting Conditions</p> <p>ATA/2009/421: Open Label, Randomized, Two-treatment, Two-Sequence, Four-period, Single-dose, Replicate Crossover</p>

	Oral Bioequivalence Study of Atazanavir Sulfate 300 mg Capsule of Matrix Laboratories Limited, India and Reyataz [®] 300mg Capsule of Bristol-Myers Squibb Company, Princeton, NJ 08543 USA, in Healthy, Adult, Human Subjects under Fed Conditions
Bio-analytical laboratory: Name and address	Matrix Laboratories Ltd Clinical Research Center Saradhi Chambers A-4, Rukminipuri, Near Poulomi Hospital, Main Road, Dr. A.S. Rao Nagar, Hyderabad - 500 062, India
Name and address of the Sponsor	Matrix Laboratories Limited 1-1-151/1, 4th Floor, Sairam Towers, Alexander Road, Secunderabad 500 003 Andhra Pradesh, India
Date of inspection	20 and 21 May 2010

Part 2: Summary

The purpose of the inspection was to inspect the bioanalytical part of the bioequivalence studies performed at Matrix Laboratories Ltd, Clinical Research Center (hereafter referred to as Matrix), including the examination of related source data for the above mentioned studies, and to evaluate whether the studies were conducted in compliance with the protocol, Good Clinical Practices (GCP) and Good Laboratory Practices (GLP) as applicable.

General information about the site

Matrix, located in Hyderabad was inspected on the above-mentioned dates. The clinical part of the studies was done at two different CROs contracted by Matrix.

History of WHO and/or regulatory agency inspections

This site was inspected by WHO Prequalification and several other DRAs previously.

Previous inspections of the site included:

- US FDA: 2007, 2008, 2008
- Thai FDA: 2007, 2010
- WHO: 2008, 2008, 2009, 2009

Focus of the inspection

The inspection focused on the bio-equivalence studies conducted for the above-mentioned products. The inspection covered the relevant sections of the WHO GCP, GLP and related texts, including the WHO guidance for organizations performing in vivo bioequivalence studies.

Inspected Areas

After arrival, there was an introduction of staff and a presentation made by the COO of this site of Matrix Labs Ltd. The inspectors were informed that 67 people were working in the center at the time - 50 on bio-analysis.

Quality Management systems covered sample chain of custody, document control systems, procedures for review and approval of data, repeat analysis, instrument qualification and investigation.

Activities of the BA lab included method validation, preparation and approval of SOPs, maintenance, analysis of bio-study samples, review and compilation of raw data and other activities.

All activities were driven by SOPs. Methods were developed and validated. Analysts were trained in techniques and specific study protocols.

Equipment and instruments on site included 16 LC MS MS, 5 centrifuges and 14 extractors, 24 deep freezers, 6 refrigerators and 3 balances. Contracts were in place with suppliers to ensure maintenance (comprehensive or service) on an annual basis for these.

Method development took about 3 to 7 days and was used for pilot studies. When pivotal studies were planned, method validation was initiated and done.

Activities in Pharmacokinetics included outsourcing of BE studies at different CROs, evaluation of in vitro data, protocol review and approval. Local DRA permission was obtained for the conduct of studies. Activities included further monitoring of studies at CROs, evaluation of pilot study data and review of final BE reports and submission to regulatory affairs.

Activities of QA included the implementation and compliance to QA systems, training and compliance with good documentation practices.

Changes that took place since the last WHO inspection included:

- Performance of Incurred sample analysis
- Monitoring of bio-analysis
- Revision of the Excel sheet for calculation (no rounding)
- Installation of two new LC MSMS systems
- Additional sample processing lab added in 1st floor
- Finalization of New Genesis 7.1 (SDBMS) for archiving of chromatograms

On average, the site performed about 8 to 9 studies per month. It was noted that the position for senior QA manager had been vacant for almost 5 months.

The inspectors briefly discussed the procedure for management of deviations and reviewed the SOP later.

The CRO then made a presentation on the study of Atazanavir 300mg capsules. The history of events included:

- Method development was started 1 September 2008
- Method validation was started on 19 September 2008
- Long term stability was determined for 82 days at -70 °C - and 166 days at -20 °C
- Analysis was done from 12 March 2009 to 1 April 2009
- 3192 samples for 42 subjects were analysed in 42 batches

- There were 3 failed batches

Method validation (MV) was then inspected. Documents inspected for MV (MV-009) included:

- List of staff involved was presented for 19092008 (job assignment)
- Roles and responsibilities were defined (VP -009 -00)
- Minutes of the meeting and training of staff
- Solution preparation:
 - Stock dilution
 - Stock preparation - calibration curve and QCs (Stock 1 and stock 2). A working standard (QC-8/ATV/WS001/08) was used. The COA was reviewed as well as the preparation record and weighing slip

Lopinovir was selected as Internal Standard (WS QC/LVR/WS001/08).

The preparation sheets for CC and QC samples (dilution) - Intermediate and Working solution preparation were inspected.

Records existed for calibrated pipettes and these were checked for performance prior to validation and sample analysis.

Other records inspected included:

- Record of Storage of CCs and QCs
- Records for bulk spiking of biological matrix: number of aliquot, LLOQ, LQC, MQC, HQC
- Matrix identification (It was noted that batches (EDTA-BM/325 - 332) were used).
- Sensitivity
- Precision and accuracy (which met requirements of concentrations (LLOQ, LQC, MQC 1 and MQC2 and HQC), within the 15% and LLOQ NMT 20%), accuracy between 85 and 115%
- Injector carryover
- Ion suppression / enhancement effect
- Ruggedness (used two different columns)
- Partial volume analysis

Records inspected for determining stability of analyte in plasma included:

- Freeze thaw study (LQC and HQC), 4 cycles
- Long term stability (at -20 and -70°C deep freezers) - 6 aliquots at each level, compared against freshly prepared QCs
- Temperature monitoring records of the deep freezers during this period

After reviewing the documentation related to the method validation, the documentation related to sample analysis was inspected. The documents reviewed for sample analysis included:

- Receiving of samples from the CRO
- Record for the verification of the condition of the samples and the storage in the deep freezer
- Placement (on 5 March 2009) of 3307 samples into the deep freezer BL/133
- Analysis starting on 12 March 2009
- Sheet for job allocation
- Training record
- Pre-study meeting and discussion
- Preparation record for stock solutions and internal standard, buffer solution and mobile phase
- Bulk spiking in biological matrix for QCs (250 aliquots)
- Pipette performance check
- Summary of sample analysis

Sample analysis

Documentation for individual batches was checked (various subjects) to verify whether the batches passed (including the calibration curve and QCs)

Focus was placed on the documentation for subjects 1 to 12, and 41 - 44

In the afternoon, the inspectors visited the laboratory and verified the electronic data including chromatograms for method validation and sample analysis. This included the following:

- Audit trail setting
- Audit trail
- Integration parameters
- IS response
- Retention times
- Injection time

The above was verified for all runs / batches for:

- Precision and accuracy in bioanalytical method validation
- Freeze thaw stability and in injector stability
- Sample analysis for selected subjects (4, 5, 9, 13, 19, 33 and 47).

Selected sample results were reviewed and back calculation was done including for selected QCs.

The chromatograms obtained in P I to IV for a selected subject were compared (overlay).

After this, the inspectors reviewed the data obtained and exported and verified the data in the tables for selected subjects.

They reviewed the QA report and the statement of compliance with QA.

On the second day of the inspection, the inspectors inspected the study for Efavirenz / Lamivudine / Tenofovir Disoproxil Fumarate 600/300/300mg tablets.

The company made a presentation on the study of the test product (3FDC) versus three individual tablets (Reference).

In summary:

- 48 subjects participated in P I
- There were 4 drop outs in P II
- 2283 samples received at the BA site
- Samples were transferred from the CRO to Matrix on 11 Oct 2008, packed in dry ice and conditions monitored with data loggers
- Shipment 1 contained aliquot 1 and 2; and shipment 2 aliquot 3
- Samples were received on ~~12~~ 11 October 2008 (aliquot-1) and 12 Nov2008 (Aliquot-2)
- Method development (Tenofovir Lamivudine) took place from ~~8-10~~ 18-30 October 2007
- Method Validation was done from 12 - 23 November 2007
- Long Term stability: 279 days at -70 °C and -20 °C was done

Summary of study plan:

- Samples were received 11 October and stored at -70 C.
- Analysis was started on 15 November and was completed on 24 November.
- 79 days were required for all samples to be analysed
- 2183 samples analysed in 25 batches
- There was no failed batch
- 16 sample repeats

Method validation: Efavirenz

- MD: 18 June to 3 July, 2008
- MV: 4 July to 11 July, 2008
- LT stability: 202 days -20 °C and -70 °C

Sample analysis

- Samples were analysed from 27 November to 8 December 2008
- 93 days were required for complete analysis
- 2183 samples were analysed for 44 subjects - in 23 batches
- There were 2 failed batches and 32 sample repeats

After the presentation of the study, the inspectors reviewed the documentation in relation to Efavirenz method validation. (*Note: The method validation for Tenofovir*

and Lamivudine was previously inspected by WHO inspectors and thus not repeated in detail in this inspection).

The documentation inspected included:

- Job assignment sheet
- Minutes of meeting and training
- Role and responsibility
- Pipette performance
- Preparation of analyte / IS stock solutions. The COAs were reviewed.
- Stock solutions for CC and QC
- Dilution of the IS stock preparation concentration
- System suitability and stock verification was done on 4 July 2008
- Stability of stock solution - 16 days was inspected and the results were verified
- Short term stock solution stability
- Specificity. (8 lots of plasma was used - documentation related to two batches (EDTA-BM/265 and 266) were checked
- Bulk spiking of matrix (CS and QC)
- Temperature monitoring log for deep freezer BL/123 (5 to 11 July 2008).

Other validation documentation inspected included:

- Precision and accuracy (PA): (5 batches), 4 levels (LLOQ, LQC, MQC and HQC)
- Deep freezer log for storage of QCs (LT) at -20 °C and -70 °C as well as temperature logs
- Dilution integrity (dilution factor 2 and 4)
- Refrigerator and bench top stability (room temperature, 18 hours)
- Freeze thaw stability at -20 °C and -70 °C was done (LQC and HQC)
- Recovery for all three analytes and IS
- Long term stability - including the preparation of fresh QCs (CC), calculations, concentrations
- Calibration curve

The inspectors then reviewed the documentation relating to the transport, receipt and storage of subject samples sent from the CRO to Matrix. Other documents inspected included:

- Sample receiving records of 11.10.2008 (aliquot 1 and 2)
- Sample check record (on receipt) - 4566 samples sent and received
- Traceability of data loggers and identification in relation to the temperature logs during shipment
- Deep freezer log for the period from receipt to analysis

Documentation relating to sample analysis was then inspected, including:

- Protocol for sample analysis
- Job assignment, training, pre-study meeting, responsibilities
- Intimation for samples for analysis

- Solution preparation (mobile phase etc)
- Bulk spiking of matrix
- Pipette performance checks 26 November 2008

Sample analysis:

Documentation reviewed included the planning and results:

- Batches were organized to allow for two subjects in one batch
- 104 samples were analysed per batch together with 12 CC and 4 QCs at each level (12)
- Data for selected subjects including CCs and QCs and back calculation of selected time points for the following subjects:
 - S1 and S2
 - S3 and S4
 - S5 and S6
 - S9 and S10
 - S11 and S12
 - S15 and S16
 - S17 and S18
 - S19 and 20 - batch failed due to QCs.
 - S21 and 22 - batch failed due to QCs.
 - S23 and 24
 - S25 and 26
 - S39 and S40

Records and data for repeat analysis were inspected for:

- S19 and 20
- S21 and 22

For the analysis of Tenofovir and Lamivudine, various documents were reviewed during the inspection including:

- Protocol for sample analysis
- Job assignment, training, pre-study meeting, responsibilities
- Intimation for samples for analysis
- Solution preparation (mobile phase etc)
- Bulk spiking of matrix
- Pipette performance checks 26 November 2008

Sample analysis:

Batches were organized to allow for samples of two subjects in one batch

No batches failed

Records inspected included sample analysis, CCs and QCs and back calculation of selected time points for the following subjects:

- S1 and 2
- S3 and 4
- S5 and 6
- S7 and 8
- S9 and 10
- S13 and 14
- S15 and 16
- S17 and 18

In the afternoon, the inspectors visited the laboratory including the sample processing area, instrumentation room and weighing (balance) room. They then proceeded to verify the electronic data for this study. This included:

- Audit trail setting
- Audit trail
- Chromatographic results including:
 - Sample name
 - Acquisition date and time
 - File name
 - Vial position
 - Plate number
 - Analyte area
 - Retention time
 - QCs
 - CCs

for bio analytical method validation and subject sample analysis. Back calculation was done for selected QCs in some batches.

The selected verification focused on, for the said study (three analytes):

- Precision and accuracy (different batches)
- Long term stability
- Freeze thaw stability
- Subject 1
- Subject 2
- Subject 9
- Subject 10
- Subject 19 (original data)
- Subject 19 (repeat analysis)
- Subject 20 (original data)
- Subject 20 (repeat analysis)

They then verified the data exported (acquired results to tabulated data) for selected subjects and time points.

The quality assurance report was reviewed and the statement for QA compliance.

The SOP for handling deviations was reviewed.

Part 3: Conclusion

Based on the areas inspected, the people met and the documents reviewed, and considering the findings of the inspection the above-mentioned two studies (650-08 and ATA/2008/421) conducted at Matrix Laboratories Ltd, India were 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
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