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Prequalification Team Inspection services
WHO INSPECTION REPORT
Bio-Equivalence Study

Part 1	**General information**
Organization details	
Company information	
Name and Address of Clinical Research Site	Azidus Laboratories Ltd., 23, School Road, Rathnamangalam, Vandalur, Chennai, 600048, India
Name and Address of Bioanalytical Research Site	Azidus Laboratories Ltd., 23, School Road, Rathnamangalam, Vandalur, Chennai, 600048, India
Name and address Statistical Site	Azidus Laboratories Ltd., 23, School Road, Rathnamangalam, Vandalur, Chennai, 600048, India
Corporate address of Organization	Azidus Laboratories Ltd., 23, School Road, Rathnamangalam, Vandalur, Chennai, 600048, India
WHO product numbers covered by the inspection/ Product names/ Study numbers/ Study titles	Study no: AZ/BE/05/16/12 **Tablets 50 mg**
Inspection details	
Dates of inspection	13-17 November 2017
Type of inspection	Routine

Introduction	
Brief summary of the activities	AZIDUS was an independent Contract Research Organization (CRO), primarily involved in catering to domestic and emerging markets, printing, commercial analytical lab and clinical research activities.
General information about the company and site	The CRO was a Unit of the ATOZ Group, sponsored by Mr. A Olaganathan since 1984. The company specialized in BA/BE studies, was located in Chennai, India on a three acre campus and built up area of 125000 sq.ft., and designed to comply with regulatory requirements. The company started with the construction of the main building in 2010 with an additional clinical facility added to the campus in 2017. In October 2016, the company acquired Lal Clinica, in Brazil and started the activities in Brazil under name of Azidus Brasil. The CRO had also plans to be operative in the USA, Wisconsin, in Q1 2018.
History	Previously the CRO was inspected by US FDA 3 times and once by EMA (Germany and Portugal) and ANVISA respectively. The most recent US FDA inspection report presented to the WHO team referred to an inspection performed 16-21 June 2014 which was covered in an US FDA EIR dated March 18, 2016. The EMA Inspection report dated 16 June 2016 performed by the team from Germany and Portugal was made available and reviewed.
Brief report of inspection activities undertaken	
Scope and limitations	
Scope of inspection	There was no previous inspection of the CRO by WHO. The current inspection covered the clinical and analytical portions of the aforementioned study. The scope of the inspection included: Inspection history and company's organization, clinical study performance, informed consent process; ethics committee approvals and correspondence, study medication accountability, dispensation and storage, processing and handling of biological (plasma) samples collected during the study, equipment calibration, employee training, computer controls, and finally a tour of the facility. Regarding the Analytical operations, coverage was provided to confirm practices, qualifications of personnel, and adherence to the CROs quality system utilized during the method validations and analytical testing. A review of the clinical study data, analytical method validation, and analytical study data was accomplished along with comparisons of the source data to the reported data.

Abbreviations	ADR	adverse drug reaction
	AE	adverse event
	ALCOA	attributable, legible, contemporaneous, original and accurate
	BE	bioequivalence
	BDL	below detection limit
	CAPA	corrective actions and preventive actions
	CC	calibration curve
	CRA	clinical research associate(e)
	CRF	(electronic) case report form
	CRO	contract research organization
	CTM	clinical trial manager
	CoA	certificate of analysis
	CSR	clinical study report
	DQ	design qualification
	ECG	electrocardiogram
	GAMP	good automated manufacturing practice
	GCP	good clinical practice
	GLP	good laboratory practice
	GMP	good manufacturing practice
	HPLC	high-performance liquid chromatograph
	HPLC-MS/MS	liquid chromatography–mass spectrometry
	IB	investigator's brochure
	ICF	informed consent form
	ICH	International Conference on Harmonization
	(I)EC	(Independent) Ethics Committee
	IMP	investigational medicinal product
	IQ	installation qualification
	LIMS	laboratory information management system
	LLOQ	lowest limit of quantification
	LOD	limit of detection
	MS	mass spectrophotometer
	MVR	monitoring visit report
	NRA	national regulatory agency
	OQ	operational qualification
	PIS	patient information sheet
	PQ	performance qualification
	PQS	pharmaceutical quality system
	QA	quality assurance
	QC	quality control
	QRM	quality risk management
	SAE	serious adverse event

	SAR	serious adverse reaction
	SOP	standard operating procedure
	SUSAR	suspected unexpected serious adverse reaction
	ULOQ	upper limit of quantification
	URS	user requirements specifications

Part 2	**Brief summary of the findings and comments (where applicable)**

General section

1. Organization and management

A presentation was provided by the President of AZIDUS explaining the organization and the application applicable to the scope of the WHO inspection. The current facility was extended to two buildings during 2017 to expand the clinical capacity to 8 CPUs with 336 beds and 13 beds in the ICU.

The CRO was a privately owned company with Dr Arjun Arumugam the Director CRO and Mr. A Olaganathan the Managing Director and founder of ATOZ Group. ATOZ Group consisted of 9 companies. The Director, Director-QA, Sr. Scientific and regulatory advisor report directly to the Managing Director. The CRO provided contract services in the area of Bioavailability/Bioequivalence studies and clinical trials phase I to phase IV.

The company employs approximately 150 staff and conducts on average 100-200 studies per year.

Recent changes in 2016-2017 were related to:

Infrastructure updates:

- Moving to 100% Solar power
- In-House Sewage treatment plant
- Improved Temperature Monitoring Systems in compliance with recent US FDA inspection

Technical Capabilities:

- In-House Data Management Capabilities as per recent CDISC requirements (Dec 2016)
- ANDA with new data system – approved by US FDA (Oct 2017)
- Implementation of Electronic Database for CRF

2. Computer systems

A list of software used in study AZ/BE/05/16/12 was provided.

The software for management of the registration of volunteers:

The Operational Qualification (OQ) protocol and the documentation, pertaining to the OQ performed in October 2015 were reviewed. The operational qualification tests were divided into 5 groups, including system Healthiness, Forms, GUI screen functions and menu bar items, power failure conditions, system securities and user privileges, reports and event viewer.

However, the performance qualification protocol did not include biometric testing (finger print). It was explained that, prior to obtaining the system, the CRO was using an Excel sheet to capture the record of volunteers. Later on, all Excel data was transferred to the system.

Documentation (dated 1 Jul 2017) was provided to validate features of the database used by the CRO. The registration database was a Windows based web application for managing volunteers. Qualification Operational tests were performed addressing the features:

- Register New Volunteer
- Record their images
- Record of their finger prints
- Search volunteer using name registration ID and finger print and other features.

Test procedures and acceptance criteria were also provided. All tests were completed and ``passed``, including Search volunteer using name, volunteer ID, study number and/or finger print. Screen shots for IQ, OQ and PQ were provided (Appendices A, B and C)

SOP for Security measures for protection of hardware, software, data and user level privileges, was reviewed. The user privilege level was provided as user specification to the vendor.

Mass-spectrometer software:

All the data provided was stored on the server.

A back-up was made automatically on the server, stored in the IT room. In addition, there were two levels of back-up:

- The data backed-up as CDs were sent to the off-site facility to be stored, once the hard-disc was filled 60 %.
- The other level of the back-up was provided when the study was completed.

Once an employee left the organization, a request was be given by HR to the IT-department to disable the respective domain which could be linked to any database. Furthermore, there was profile documentation for each user.

In the security Manager Window of the software, it was demonstrated that Administrators could fully administer the database.

Based on the system's Audit Policy the audit was enabled. The auditable events were recorded as

- Logon and Logoff
- Policy changes described as any changes to Security Manager settings
- Object access
- Other event consisted of: Security events, Attempts to compromise database security,
- Remote logging could be enabled, as well as remote alerts.

Audit events, such as File signing operations, Starting and Completing data acquisitions and creating and altering metadata or results files were reviewed and verified.

Common regulatory requirements were documented to ensure that they were implemented in the system.

The system was explicitly reviewed to confirm that, the database was provided in Full Security mode and in that mode, checksums was applied to all data and metadata files to ensure that the files created could not be modified or renamed except through security-controlled programs.

The checksum ensured that the following properties of the file were not altered:

- Filename
- Size
- Contents

It would not be possible to open the file in the database if any of these properties were changed. A warning could be displayed and an entry would be made in the Log file.

In addition, the database could be configured to send messages advising of file tampering to another machine, such as that of a senior manager who wished to be notified of attempts to breach security. It was recommended to use this option.

For raw data, a checksum file called protected was created in each raw data directory.

The software was validated on 10 Mar 2012.
The provider recommended requalifying the system 12 to 15 months after installing it and at regular periods thereafter.

According to their SOP for Installation, validation, acceptance, upgradation and maintenance of hardware and software; effective 29 Apr 2017 version 12, software should be validated, except for purchased, validated or licensed third party software.

In order to verify the access control to the database, Audit trail for the database was checked for the period of 14 Nov 2017 to 1 Sep 2017 and 1 May 2016 to 1 Jul 2016.

Annexure V of the applicable SOP defines the "user privilege levels of the software" which were divided into 4 groups: Administrator, Analyst, Method developer and Reviewer.

Each of them was designated to functions available in the Software. The functions available for the software were verified. Administrator (only IT person) had all rights, but was basically responsible to create new users / disable users. It was also verified that the rights given in the system was matching the rights defined in the SOP for analyst and Method Developers.

Audit trail set up was requested to ensure that any changes would be captured on the system.

Issues raised during the inspection were resolved in the company CAPA.

3. Quality management

The Quality management system was designed to meet the regulatory requirements.

Company's Quality management was operated by the Director-QA, according to their Quality Manual.

The QMS documentation in the CRO was in four levels:

1- Quality Manual
2- Standard Operating Procedures
3- Work Instructions
4- Log Books, Raw data Sheets, forms, records and registers

Quality Assurance department's tasks were defined in their quality manual.

QA department was, among other duties, responsible for maintaining and authorizing the quality system, scheduling and conducting independent audits of all departments, clinical study, facility and vendors as per the relevant SOP and protocols. The activities which should be audited were categorized as internal audits, project audits and vendor audits. Annual plans were provided for vendor audits, such as Pathology lab, Emergency hospitals. Two types of audits were performed by the QA personnel for activities pertaining to the projects / bioanalytical studies:

- Audit during study activities or documentation (In-process): The in-process audit was carried out by QA during the actual conduct of the study/analytical activities to confirm that the activities were carried out as per the respective study protocol and applicable SOPs.
- Audit post study activity or documentation: In the retrospective audit, the data generated and documentation were audited.

Audit plans for 2016 and 2017 were provided. Plans detailed the areas to be audited, and when the audit of each area was to be performed. It also specified who audited each activity. The plan for any type of audit-related activity was scheduled every December.

Procedures for adherence to sample processing time and temperature as specified by analytical method were included in the QA activities.

The last audit report for vendor of off-site archiving facility signed on 23 Sep 2017, was reviewed. However, it was signed to verify that it was reviewed on 21 Sep 2017 which was prior to the date of audit. An email was provided by the off-site facility confirming that the audit was done on 23 Sep 2017. The previous audit was performed on 20 Nov 2014.

In addition, each department was required to have a quality management team that reported to the head of respective department.

The following SOPs were reviewed and verified:

- SOP on SOP
- SOP for audits
- SOP for preparation and implementation of SOP documents
- SOP for subject sample analysis
- SOP for calibration and maintenance of micro balance
- SOP for operation, calibration and maintenance of piston operated micropipettes and multi pipettes
- SOP for Bioanalytical method development
- SOP for training and evaluation of staff
- SOP for Quality review assurance
- SOP for Back up, restoration of data and disaster recovery
- SOP Archival procedure
- Business Continuity Plan and Disaster Recovery Plan for all facilities, approved by QA Head.
- SOP for Disaster Management; providing the measurements taken in term of Earthquake, Floods, Lightning, cyclone and fire.
- SOP for IT disaster plan in case of data system collapse (*to note* that there are back-ups in place for power supply).
- SOP for Incurred Sample Re-analysis. The new version was updated to define the intervals between the runs.
- SOP for handling emergency medication
- SOP for preparation of study protocol
- SOP for Operation and calibration of defibrillator.

Two different defibrillators devices were available.

SOPs were maintained in hard and soft copies and historical SOPs were available and could be obtained upon request. According to the document control SOP, SOPs should be reviewed and if necessary revised every two years. A hard copy of applicable SOPs were available at each respective unit, stamped as controlled copy, signed and marked with the effective date.

All concerns raised during the inspection with respect to their quality management system were addressed adequately and respective CAPAs were provided.

4. Archive facilities

The primary archiving facility was used for storage of all paper documentation which they kept according to the applicable requirements. The facility was restricted by access-card, and accessed only by primary and secondary archivist, in addition to the electrician in case of accidents.

The entire building, including archiving facility was checked for pest, mosquito, rodent and snake regularly by MARS Pest Management systems Pvt. Ltd and each check was recorded in a respective logbook which was reviewed. The log book contained inscriptions from 7 Sep 2016 to date.

The facility was protected against fire by using chemical fire-extinguisher, and fire-extinguisher canister. The room was also equipped with rolling racks and fire proof cabinets to store record, both paper and electronical tapes, when necessary.

5. Premises

The inspection team visited the facility and its different units. The facility consisted of two main building:

1- Main building dedicated to bioanalytical activities, which comprised of
   a. Bioanalytical lab with 18 LC-MS/MS and 3 ICP-MS instruments
   b. Pharmacy
2- Clinical building which was built in 2017 and dedicated to clinical phase activities, which comprised of
   a. CPU – 8 with 336 beds; 150-180 active beds
   b. ICU – 13 beds, equipped by Fowler's Cot, ECG machine, defibrillator, multi parameter monitor, crash cart, nebulizer, oxygen cylinders
   c. Screening area
   d. In-house Diagnostic lab

The accreditation from DCGI was missing for the period between 05.04.2016 and 07.10.2016, because it was in renewal process. The correspondence with Drug Authority was provided.

Access to the facilities was both biometrics and by key card, depending on the area.

All storage facilities' temperature was monitored by digital thermometers linked to data logger, with light indicator and hooter alarm in the security room. The alarm was also sent as text message to designated personnel in case of temperature out of range fluctuations.

6. Personnel

Organizational charts, valid at the time of the inspection were provided and reviewed. The documentation was included in the CROMF.

Personnel were trained on company's manual at the time of joining to the CRO: Quality, Safety, Infection Control and Biosafety, Human Resource, ISMS and EHS.

SOP for Personnel training and evaluation was consisted of four types of trainings:

- Induction training
- Refresher training
- External training
- Protocol/Study specific training

The induction training was supervised and guided by the assigned trainer in the identified areas with relevance to the employee's job description.

The training schedule for 2016 and 2017 was provided to demonstrate which SOPs were required to be trained each month before the 15th of each month, according to the respective SOP. There were two sets of plan, one for BA and the other one for Clinical department.

The company had an overview of the training of SOPs organized by roles and department.

The refresher training record for a specific period was reviewed. All BA staff was required to be trained prior to the implementation of the SOP.

Personnel with analyst role in the study were required to be trained on any BE SOP, including SOP AZ/BA/AC/053 and /047 relating to chromatography – re-integration of chromatograms. The respective training records for two analysts for the period of our study were reviewed:

1- M. S. K:

Training record with respective test for AZ/BA/AC/053; dated 29 Aug 2013 was present.
The main training record for AZ/BA/AC/047 was missing. However, a later training was provided on 5 Feb 2015, qualified after oral test.
Refreshing training documentation was also available.

2- M. K

Training documentation was complete.

CV and JD for Research scientist, in bioanalytical belongs to F. B. signed 3 Nov 2016 was also provided and verified.

Clinical section

7. Clinical phase

The contract and Master Agreement between the sponsor (Hetero Labs Ltd, Hyderabad) and CRO (Azidus Laboratories) was reviewed and verified.

The signed ICFs were verified by using the subject specimen signature. All ICFs were signed and dated by the subjects.

The visitors' logbook was randomly checked for a number of subjects to verify the visit dates, times and signatures, time of ECG, blood sample collection times. No observation was made.

Both clinical facilities were visited. The screening area was in general well maintained.

Insurance certificate from Insurance Company Ltd covering the period of 07 October 2015 to 06 October 2016 was provided.

Approximately 300 ECG print-outs from 6 different studies were reviewed for similarity.

The medication available for medical emergencies was verified for expiration date, none of them found to be close to expiration date or expired. Medical emergencies equipment: oxygen device, defibrillator, ECG machine, suction machine, laryngoscope was verified.

The collection of blood sample was documented on the record page of the CRF, including anticoagulant used, dosing time-point, scheduled time of the sample collection, actual time of sample collection and initials /date of phlebotomist / sample collection. The process was done under hygienic conditions.

After blood samples were collected at each time point, in pre-labelled tubes, they were sent to the sample processing room for centrifuging and freezer-storage. Samples were centrifuged according to the time, speed and temperature established in the respective protocol.

The process was documented on a specific form "Centrifugation and storage Record", as well as the logbook for usage of centrifuge instrument. The documentation for running study was studied by the inspection team. If any sample was haemolysed, the sample would be identified by respective chart, coded from A to D, and samples were recorded under "Remarks". The missing samples were also documented, accordingly. There were two freezers at -70 °C, monitored by Eurotherm, in the sample processing room.

All issues raised in relation to the clinical phase of the study were addressed adequately.

8. Clinical laboratory

AZIDUS had a clinical diagnostics laboratory that was ISO 15189 accredited and held a contract with Lister Metropolis Pvt. Ltd. for additional resource and backup in the event of instrument breakdown.

9. Ethics

The registration of Independent Ethic Committee, valid from 30 August 2013 for a period of 3 years was verified. Submission of the study documentation to EC was performed on 16 Apr 2016. The independent Ethic Committee, Chennai approved the study protocol V1 dated 09 Apr 2016 and ICF (English and Tamil languages) on 19 Apr 2016.

Issue raised in context with the ICF versioning was corrected satisfactory.

Video recording for ICF process was verified for 8 random subjects from the study. The following parts of the ICF process could not be confirmed watching the video recording:

- product name,
- Protocol title
- ICF version and date
- whether the subject agreed to participate in the study.

10. Monitoring

Monitoring was performed, and the report was signed on 13 Jun 2016. Monitoring visits were verified in the visitors' log book. The monitor was present on different dates. However, there was only one monitoring visit report completed for all visits and signed on 13 Jun 2016. The report was elaborative and activities conducted were detailed properly.

11. Investigators

Training logs, Delegation & Authorisation log and CVs for the investigators involved in the study were verified.

The CAPA provided for the finding raised during inspection was adequate.

12. Receiving, storage and handling of investigational drug products

The pharmacy area, with restricted access limited to two Pharmacists, was utilized for receipt, storage, dispensation and archival retention of study medications.

Medications were stored, in cabinets, humidity chambers and refrigerator, depending on their storage condition, all monitored by digital thermometers.

All entries and exit of pharmacy were logged in a logbook.

Receipt:
Upon the receipt of the shipment, the pharmacist carried the unopened parcel to the pharmacy and completed the respective log.

The study medication accountability was performed for all subjects for both periods for Test and Reference Investigational Product. No inconsistencies were observed.

Randomization:
Prior to each study, a randomization list was provided by biostatistician using WinNolin Phoenix Version 6.4 software during of the study, approved by PI and verified by QA. At the time of inspection, the CRO was using Phoenix Version 7.0. Labels were prepared by the pharmacist in the clinical department, according to the randomization list and verified by QA.

The statistical report was prepared using SAS 9.2 during the study. At the time of the inspection, SAS program was upgraded to version 9.4, which was server-based. Correspondence with QA was verified. The randomization list was sent via email to QA password protected.
Time deviations were recorded manually, following the responsible QA responsible verification of the final results.

The randomisation list was verified against the IP labels for all subjects and for both periods. No inconsistencies were observed. The discussion was conducted with the Statistical Investigator.

Dispensing:
Dispensing of study medication took place the day prior to dosing. The pharmacist generated the labelling of study drug containers in small plastic bags. After cleaning of bench, dispensing of the test drugs was carried out first, followed by preparation of reference drugs for dispensation, according to the list of randomization. QC was carried out by QA-personnel at the time of dispensation.

Labels were in two parts, one part stayed on the dosing container and another part was placed on the Drug consumption chart of the CRF at the time of dosing, verified by QA and monitor. The labels contained all the required information.

The activities were properly documented.

Handling and storage:
The accountability was performed for all subjects,

Subject dosing
During the inspection, the dosing process was observed for a running study. The dosing was performed in the ICU unit as the subjects needed to be monitored for ECG parameters. The activities were performed according to protocol procedures.

Retention:
After dosing, the empty containers and any unused study drug / test drug was returned to the pharmacy, to be counted, logged back and documented before storing them in dedicated area separately. The retained study drugs applicable to study no AZ/BE/05/16/12 were checked and verified.

All concerns raised regarding the handling of investigational medicinal products during the inspection were addressed adequately.

13. Case report forms
CRFs for a number of subjects were verified and found to be appropriate. Protocol Deviations were noted.

14. Volunteers, recruitment methods
Volunteer management team was responsible for subject's recruitment.

The VMS (Volunteer Management System) software with the data of about 18,000 volunteers was used for registration of volunteers. In order to prevent duplicate study participation, AZIDUS had joined the inter-CRO database called OVIS "Online Volunteer Information System", where the fingerprints, as well as names and other applicable personal data of the volunteers participating in trials were captured to be used for identification purposes, by all CROs registered in the system.

According to AZIDUS presentation, there were between 150 and 200 trials, performed per year.

15. Food and fluids
Food and fluid were provided according to protocol requirements.

16. Safety, adverse events, adverse event reporting
The site had a contract signed with two emergency Hospitals:

According to the SOP for Handling of emergency medication, the hospitals should be notified prior to the subjects' enrolment in the respective study. The telephone numbers for emergency calls to the hospital were verified.

No SAE reported during the study. Only one AE was reported (Diarrhea).

Bioanalytical section

17. Method development

The method development was described in a logbook for method development. Literature used in the study was provided.

Method:
Analyte determined: Dolutegravir sodium in plasma K_3 EDTA (anticoagulant)
Internal Standard: Dolutegravir D3
Extraction: Liquid - Liquid Extraction
Aliquot volume - 100µL
Extraction solvent - Tertiary Butyl Methyl Ether
Instrument: LC-MS/MS
Calibration curve range: between 49.9 ng/ml to15044.04 ng/ml
Mobile phase: 0.1% Formic acid in Acetonitrile: Methanol: Water (30:30:40 % v/v/v)
Procedure was accomplished according to the SOP for Determination of Dolutegravir in human plasma by high performance liquid chromatography-mass spectrometry /mass spectrometry.

SOP for Method development was reviewed.

The certificate analysis for Dolutegravir was provided.

The refrigerator used to store the working standard and the pertaining record for storage of working standards was reviewed and verified.

It was documented that Dolutegravir D3 working standard and Dolutegravir was purchased on from Clearsynth labs ltd to Azidus laboratories ltd.

The temp log for refrigerator for the period of June and July 2016 was reviewed. There were excursion of temperature in the refrigerator, but they were taken care of appropriately.
Example: Temperature excursion on 28 May 2016 from 5:10 to 9:10. The monitor was notified by the alarm and the wire was changed by maintenance person in the next morning. The recorded temperature was back to the normal. Documentation was provided on 29 May 2016. This refrigerator was only used for working standards pertaining to different studies.

18. Method validation

Preparatory, the SOP for Method validation was reviewed.

During the inspection, source documentation and raw data for validation of bioanalytical method and analysis of subject plasma samples, as well as audit of the electronic data, audit trails for electronic data capture and handling of data related to the PK study were reviewed. The preparation and results from calibration standards (CC), quality control samples (QC), internal standards pertaining solutions and reagents and subject plasma samples in analytical runs were inspected, along with the chromatograms generated from analytical runs, including their respective parameters.

The method validation report was reviewed and verified by Head-Bioanalytical and Director-Quality Assurance.

Following documentation pertaining to steps taken to demonstrate the reliability of the method validation was reviewed and verified:

- Short-term stock solution and working solution stability (STSS_STWS):
- Long-term stock solution and working solution stability (STSS_STWS):
- Run ID: 15Jun2016_FTS_DES_WES_2-8 °C (Matrix)
- Run ID: 15Jun2016_BTS_DES_RT_WES_RT_IIS (Matrix)
- Documentation on Auto-sampler stability
- Long term stability in Matrix -20 °C and -80 °C
  They explained, by the time that the samples were collected, the sponsor wanted them to complete the study and sample analysis and since they already knew the stability of the analyte in the plasma through the provided literature (provided), the analysis study was conducted. Literature had concluded that the analyte was stable in human plasma stored at -80 °C for a period of at least 15 months. Storage of samples during the study periods were set up at -80 °C. Only after completion of the study, the samples were kept at -20 °C. However, the bio-analytical report version 01 was issued on 29 Aug 2016 which was after providing the result of the Long term stability.
- Selectivity of the method was established by comparing extracted blanks of 8 different lots including, one haemolysed and one Lipemic against extracted LLOQ samples.
  Matrix used, including the anticoagulant were 6bplasma lots using K3EDTA, in addition to haemolysed and lipemic plasma provided by AZIDUS laboratories. Documentation on plasma sample record, including date of collection, receipt and report generated, and required tests were verified.
- Matrix effect analysis was run, using 8 plasma log, including 2 haemolysed and 2 lipemic samples, according to the respective SOP In this particular study, since they were required to meet different regulatory' requirements, 2 haemolyzed and 2 lipemic plasma samples were used.

- Accuracy and Precision was determined by using eight calibration curve standards along with 6 replicates of LOQQC, LQC, MMQC, MQC and HQC and Precision was measured by the percentage co-efficient of variation over the concentration range. The accuracy was calculated by the absolute value of the ratio of the mean values of the quality control samples to their respective nominal values and expressed as percentage.
  Preparation of the samples was verified. Independent stock solutions were used for CC and QC:
- Number of Calibration standard sample sets stored: CC-2-100 (100 CS sets were provided each consisted of 8 samples, labelled according to their SOP; stored at -70 °C)
- Pooled matrix volume stored at Deep freezer -20: 150 mg
- Accuracy run for analyte and IS
- The carry-over test was performed using blank, LLOQ, 2xULOQ followed by two blank plasma samples.
  The results from the MassLynx; area at the retention of Analyte, was copied and pasted by Analyst to a validated Excel sheet, calculating the range of % Carry over at the RT of Analyte and % Carryover at the RT of IS, by the formula defined in their SOP section 5.2.4.
- Smooth factor was set up as 3 as default, however it could be varied from study to study and be reported. The smooth factor for this study was 3.

19. Sample collection, storage and handling of biological material

The custodian at the BE facility was interviewed to obtain the description of receipt and handling of the samples from the clinical unit. A specific form was competed documenting how many samples were sent to the BE site, along with number and identification of the missing and haemolysed samples. The data was checked and verified by the custodian on the form “for List of missing, haemolysed and sample shipment”. The temperature at the time of shipment was also read on the indicator and recorded. Samples were identified by study no., time point and dosing-period number. Number of samples was verified. The acknowledgment of receipt of the samples from clinical unit was recorded. By providing the relevant documentation, the time-points for sample storage, storage condition prior to the shipment, length of shipment time and shipment condition was verified. Although the site was operational 24 hours, no shipment would take place during the night.

All samples for each subject were arranged by time point and placed together in containers in two aliquots. The main aliquot for sample analysis purposes and the back-up aliquot to be stored separated from the main aliquot.

The freezing room with four freezers, including freezer used for the study samples was visited. The temperature alarm system was tested. The log book for the abovementioned freezer was also verified. It was tested that when the alarms were triggered when the freezer-door was kept open, a notification was sent to the custodian and Head QA’s mobiles. A new message was sent when the temperature was within the acceptable range.

Temperature freezer -70 °C log used in the study was reviewed for June and July 2016. In that period, the acceptable range was -70 ± 30, however after FDA inspection it was improved to ±15, which was the current practice.

Certificate of calibration for following equipment used in the study was reviewed and verified:

- Data logger; Euro-Therm; done by Euro Calibration Technique
- Ultra-Low temperature freezer -80; done by Euro Calibration Technique

The process of requesting the samples from Analyst was also reviewed for subject 1 and 2, along with the pertaining CC and QCs and verified. The pertaining log book from the respective freezer was also reviewed.

Documentation for freeze and thawing of the samples were recorded in the respective logbook for the freezer. Whatever was remaining would be returned to the custodian for further storage and documented.

Additionally, the following documentation was reviewed and verified:

- Freezer log book for storage of samples
- Freezer log book for storage of working solutions

According to the Master Service agreement effective 19 October 2012, with Hetero Labs limited, they should retain the samples and any other study specific documentation for a 6 month period of time. After that they had options to either keep/return or destroy it upon to the direction from the sponsor. However, they still kept the samples at the CRO in a segregated area which was visited. Additional security measurement to prevent the custodian getting trapped in the big cold storage room (-20 °C) was missing from their SOP.

Study specific agreement was signed on 6 May 2016.

20. Analysis of study samples

An analytical batch contained samples in following order: System suitability sample (aqueous AQC_MQC), reconstitution solution, CC blank, zero blank (CC blank + IS), calibration curve standards, reconstitution and samples of two subjects organized by period I and II. In addition two sets of QCs were interspersed between the plasma samples, at following levels: LQC, MMQC, MQC, HQC

There was a Sample List form prepared by MassLynx 4.1 SCN 843 consisting of all samples required for the Run.
Custodian delivered the samples according to this list to the Analyst and both had signed and dated the form.

SOP for Handling of samples in bio analytical department explained the sample storage and withdrawal of samples for analysis purposes.

SOP for Clinical sample analysis, including the applicable SOPs for the time of study were also reviewed.

The SOP required preparing, printing the sample schedule/sequence and obtaining the required samples per sample-schedule from custodian /designated personnel.
The withdrawal of samples was documented in the logbook.

The sample list report, logbooks for different freezers for storage of CC and QC working solutions sets and storage of samples were reviewed.

SOP for Quality control, together with the corresponding QC log for this study was reviewed.
QC should be performed by QC team in a manner that any conformances observed would be immediately escalated to the departmental Head for CAPA.

Activities to be controlled were defined and divided in two groups: Bio-analytical and Clinical.
Checklists were provided.

Sample analytical runs, including ISRs were randomly selected and compared with the data generated in the report for further verification of results of calibration standards and respective calibration curve, QC, IS, RT, back calculation using respective slope and intercept, preparation of CC and QC, sample processing record.

Issues raised during inspection were addressed adequately.

21. Data processing and documentation

Electronic raw data and paper raw data, including notebooks and logbooks were reviewed during the assessment of trials' conduct. Documentation regarding all trials selected for inspection was organised and labelled properly and an Index including all documentation was available for inspection.

22. Good laboratory practices

The laboratory facility consisted of laboratory, instruments and weighing room, with balances.

There was a list of instrument identification record form specified for method validation applicable to the study in the scope of inspection.

Weighing room including the refrigerator for storage of working standards was visited. Calibration ad daily check microbalance, as well as the monthly expiry control of working standards, with the respective logbooks was reviewed.

Pipette used in this MV was identified. Record documentation was reviewed and verified. Pipette performance check was done on 11 Jun 2016.

Instrument LC-MS/MS was verified, and the pertaining calibration checked. The instrument was installed 1 Mar 2012.

The lab used Type I Milli Q water purifier. The water quality was tested, once every three months by SGS lab.

During the study period, the documentation for quality control of water was provided, performed by ATOZ pharmaceuticals Pvt Ltd. The test was done to verify PH, Chlorides, resistivity at 25, conductivity, sodium silicon, microorganism, etc.

CAPA provided with regards issues raised in relation with the Good Laboratory practices were acceptable.

Pharmacokinetic, statistical calculations and reporting section

23. Pharmacokinetic, statistical calculations

The randomisation list was verified versus IP labels for all subjects and both periods, without any remarks.

Software Used for Pharmacokinetic and statistical calculation purposes were as follows:

- Phoenix Version 6.4
- SAS Version 9.2

24. Study report

Study reports were provided. No observation was made.

Miscellaneous	
Samples taken	Not applicable
Assessment of the CRO master file	The CROMF was reviewed. The company's master file provided introductory information of the organization, but it didn't cover all information required by the guideline for the preparation of a contract research organization master file (WHO Technical Report Series, No. 957, 2010, Annex 7) According to the above mentioned guideline, the CROMF should be a document prepared by the CRO containing specific and factual information about the CRO and the conduct of clinical studies, as well as the analyses of samples and related operations carried out at the named site. It was expected that a CROMF provided information on the policies, approach and general activities of a CRO. It should serve as general information by regulatory inspectors, in addition to the trail specific data and information submitted

	for assessment. It should also provide an overview of the organization's approach to GCP, GLP and other guidelines pertaining to its activities. Nevertheless, all activities pertaining clinical and bioanalytical procedures were adequately described in the company's quality management system.
Annexes attached	Not applicable

Part 3	**Conclusion**

Based on the areas inspected, the people met and the documents reviewed, and considering the findings of the inspection, including the observations listed in the Inspection Report, as well as the corrective actions taken and planned, the study was considered to have been conducted at an acceptable level of compliance with WHO GCP and GLP at CRO **Azidus Laboratories Ltd., 23, School Road, Rathnamangalam, Vandalur, Chennai, 600048, India**

All the non-compliances observed during the inspection that were listed in the complete report as well as those reflected in the WHOPIR, were addressed by the CRO, to a satisfactory level, prior to the publication of the WHOPIR.

This WHOPIR will remain valid for 3 years, provided that the outcome of any inspection conducted during this period is positive.

Part 4	**List of guidelines referenced in the inspection report**

1. Guidance for organizations performing in vivo bioequivalence studies. *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Fiftieth Report* Geneva, World Health Organization, 2016 (WHO Technical Report Series, No. 996), Annex 9
   Short name: WHO BE guidance
   http://www.who.int/medicines/publications/pharmprep/WHO_TRS_996_annex09.pdf

2. Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. In: *Expert Committee on Specifications for Pharmaceutical Preparations. Forty-ninth report*. World Health Organization, Geneva. WHO Technical Report Series, No. 992, Annex 7, 2015, pp. 347–390
   Short name: WHO multisource guidance
   http://apps.who.int/prequal/info_general/documents/TRS937/WHO_TRS_937__annex7_eng.pdf

3. Guidelines for good clinical practice for trials on pharmaceutical products. WHO Technical Report Series, No. 850, 1995 (pp. 97–137)
Short name: WHO GCP
http://apps.who.int/medicinedocs/en/d/Js5516e/19.11.html

4. WHO guidance on good data and record management practices. *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Fiftieth Report* Geneva, World Health Organization, 2016 (WHO Technical Report Series, No. 996), Annex 5
Short name: WHO TRS No. 996, Annex 5 WHO GDRMP guidance
http://www.who.int/medicines/publications/pharmprep/WHO_TRS_996_annex05.pdf

5. WHO Handbook on Good Laboratory Practice/OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1: OECD Principles on Good Laboratory Practice (as revised in 1997). Organization for Economic Co-operation and Development. ENV/MC/CHEM(98)17. 26.Jan, 1998.
Short name: WHO GLP
http://www.who.int/tdr/publications/documents/glp-handbook.pdf

6. The Good Automated Manufacturing Practice (GAMP) Guide – A risk-based approach to compliant GxP computerized systems (GAMP5). ISPE – International Society for Pharmaceutical Engineering, December 2009.
http://www.ispe.org/gamp-5

7. Guidelines on Bioanalytical Method Validation EMEA/CHMP/EWP/192217/2009 Rev.1 Corr.* Committee for Medicinal Products for Human Use (CHMP), 1 February 2012.
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf

8. WHO Operational guidelines for Ethics Committees that review biomedical research (7). WHO, TDR/PRD/ETHICS/2000.1
http://www.who.int/entity/tdr/publications/documents/ethics.pdf?ua=1

9. Good Practices for Computerised Systems in Regulated "GXP" Environments, PIC/S Guidance, Pharmaceutical Inspection Convention Pharmaceutical Inspection Co-operation Scheme, PI 011–3, 25 September 2007.
http://www.picscheme.org/pdf/27_pi-011-3-recommendation-on-computerised-systems.pdf

10. US FDA Code of Federal Regulations Part 11
http://www.accessdata.fda.gov/SCRIPTs/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=11&showFR=1

11. EU guidelines to Good Manufacturing Practice and Medicinal Products for Human and Veterinary Use Annex 11, Computerized systems
http://ec.europa.eu/health/files/eudralex/vol-4/annex11_01-2011_en.pdf

12. Handbook – Good Laboratory Practice (GLP): quality practices for regulated non-clinical research and development – Annex I: The OECD Principles on GLP, 2nd ed., 2009. This document will be referred to as "GLP".
http://www.who.int/tdr/publications/documents/glp-handbook.pdf

13. Model guidance for the storage and transport of time-and temperature-sensitive pharmaceutical products. WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-fifth Report Geneva, World Health Organization, 2011 (WHO Technical Report Series, No. 961), Annex 9.
http://apps.who.int/prequal/info_general/documents/TRS961/TRS961_Annex9.pdf

14. Guidelines for the preparation of a contract research organization master file, WHO Technical Report Series, No. 957, 2010, Annex 7
http://www.who.int/medicines/publications/TRS957_2010.pdf

15. Glove use information leaflet, Patient Safety, Save lives clean your hands, WHO, revised August 2009
http://www.who.int/gpsc/5may/Glove_Use_Information_Leaflet.pdf

16. WHO Good Clinical Laboratory Practices (GCLP)
http://www.who.int/tdr/publications/documents/gclp-web.pdf