CHAPTER 48 – BIORESEARCH MONITORING

SUBJECT: Procedures for FDA Staff: In Vivo Bioavailability/Bioequivalence Studies (Analytical)	IMPLEMENTATION DATE: 05/01/2018
DATA REPORTING	
PRODUCT CODES	PRODUCT/ASSIGNMENT CODES
Product coding not required for biopharmaceutical establishments	48004A BIOANALYTICAL IN-VIVO BA/BE (ANDAS) 48004N BIOANALYTICAL IN-VIVO BA/BE (NDAS AND BLAS) 48004P BIOANALYTICAL PEPFAR ANDA BA/BE 48004Q BIOANALYTICAL PEPFAR NDA BA/BE 48004B BIOANALYTICAL BA/BE - BIOSIMILARS

FIELD REPORTING REQUIREMENTS:

To meet the established deadline(s) issued by the Center, notify the Center via the e-mail address identified in the assignment when Establishment Inspection Report (EIR), EIR attachments, exhibits, or any related correspondence is available in OSAR.

All EIRs should be completed in accordance with Field Management Directive (FMD) No. 86, Establishment Inspection Report – Inspection Conclusions and District Decisions (http://www.fda.gov/downloads/ICECI/Inspections/FieldManagementDirectives/UCM382035.pdf).

When Form FDA 483, “Inspectional Observations” (483) is issued, a copy should be sent to the Center contact, and the Center’s email mailbox, generally no later than 3 business days following the close of the inspection, or upon return from an international inspection.

Contents

PART I – BACKGROUND

1. Scope of Document

This Compliance Program (CP)[1] outlines procedures for FDA investigators who inspect domestic and international sites to ensure that the analytical portions of *in vivo* bioavailability (BA), bioequivalence (BE), pharmacokinetic (PK), or pharmacodynamic (PD) studies[2] submitted to the Center for Drug Evaluation and Research (CDER) are conducted using the highest laboratory standards and in accordance with applicable regulations. As stated, this CP is designed to ensure studies are conducted using the highest laboratory standards and in accordance with applicable regulations. The acceptability of any study mentioned herein, including any repeat study, will be determined during application review.

This CP replaces the analytical portions of the prior CP, 7348.001, Chapter 48 – Bioresearch Monitoring Human Drugs titled, "In Vivo Bioequivalence," issued in September 1999.

2. Introduction

New Drug Applications (NDAs) may rely on comparative BA studies or BE studies to demonstrate that a new drug formulation or a new route of administration of a drug has the same pharmacokinetic properties[3] as a reference, marketed product. Abbreviated New Drug Applications (ANDAs) rely on BE studies to demonstrate that a generic version of a drug has the same circulatory properties as an approved, reference listed drug (RLD). Regulations pertaining to these studies are principally found under 21 CFR 320.

In vivo BA/BE studies with PK endpoints generally consist of two distinct components, clinical and analytical. The clinical component involves administration of drug products to subjects, monitoring of subject safety during the study, and collection of biological samples (usually plasma samples) for safety and PK assessments. The analytical component involves processing the biological samples collected during the clinical phase of the study to measure drug concentrations present in those samples. Subsequently, the measured drug concentrations are used to generate PK parameters, which serve as the basis for determining BA or BE between test and reference formulations.

In addition to BA and BE studies with PK endpoints, this program covers inspections of BE studies with PD endpoints, as these studies rely on PD measurements other than systemic drug or metabolite concentrations. PD endpoint assignments will usually contain a clinical and analytical inspection. Examples of PD endpoint studies are clinical studies that measure drug effects on surrogate markers (e.g., skin blanching, forced expiratory volume (FEV1)), or immunogenic responses.

This CP covers the analytical component of BA and BE studies only. History and Application of Bioavailability (BA) and Bioequivalence (BE) Regulations

[1] This document represents procedures for the Compliance Program.
[2] For the purpose of this CP, all study types listed fall under the general term BA/BE studies.
[3] Pharmacokinetic properties in the context of this CP are defined as the area under the concentration curve (AUC) and the peak or maximum concentration (Cmax).

On January 7, 1977, FDA issued final regulations in part 320 (21 CFR 320) establishing definitions and requirements for BA and BE studies (42 FR 1624). The regulations outline the requirements for the submission of *in vivo* BA and BE data as a condition of marketing a new formulation for an NDA (e.g., for a new drug under regulatory review or an already marketed drug product[s]), or a generic drug formulation submitted under an ANDA. 21 CFR 320 also provides information concerning the design and conduct of BA and BE studies. Additionally, the FDA has published draft guidance for NDAs/Investigational New Drugs (INDs) (*Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs -General Considerations* (March 2014)[4] and ANDAs *(Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an Abbreviated New Drug Application,* (December 2013)[5].

FDA also has published a guidance for industry on *Bioanalytical Method Validation* (May 2001)[6], which informs sponsors on bioanalytical method validation. A draft update was published in September 2013 and it is currently undergoing revisions[7].

[4] http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm389370.pdf
[5] http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm377465.pdf
[6] http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070107.pdf
[7] http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm368107.pdf

PART II – IMPLEMENTATION

1. Objectives

The objectives of the *in vivo* BA/BE Bioresearch Monitoring (BIMO) Program are:

- To ensure the protection of the rights, safety, and welfare of human subjects participating in studies;
- To ensure the quality, integrity and validity of clinical, analytical, and statistical data from BA/BE studies; and
- To ensure compliance with applicable FDA regulations and to identify significant deviations.

2. Regulated Industry: Analytical Sites

This section describes the regulated industry as it applies to individuals, laboratories, organizations, and corporations who conduct bioanalytical operations to generate data from BA/BE studies used in support of drug applications.

A. Sponsors

Organizations or corporations that initiate BA or BE studies and have been identified by FDA through receipt of an investigational exemption or marketing application. Sponsors can use their own facilities to analyze biological samples and generate pharmacodynamic or pharmacokinetic data in support of their own BA/BE studies or use an alternative, such as the ones listed in section B.

B. Contract Research Organizations (CROs)

Individuals, organizations or corporations that have a contractual agreement with a sponsor to perform one or more obligations of a sponsor including, but not limited to, analysis of biological samples, generation of pharmacodynamic/pharmacokinetic data, statistical assessment of BA/BE, and drafting of the study report. Examples of CROs include academic laboratories, clinical sites, and analytical sites.

3. Inspection Assignments

The CDER Office of Translational Sciences (OTS)/Office of Study Integrity and Surveillance (OSIS) receives requests for BA/BE study inspections directly from CDER offices that review drug applications. Directed inspections may also originate from complainants and informants (self-identified or anonymously) from the public and private sectors, who may report potential fraud at a site, or a site's deviations from practices that protect subject safety and data integrity. Additionally, OSIS monitors incoming drug applications that include BA or BE studies to identify sites for surveillance inspections. All assignments will be generated by the Center and issued to Office of

Regulatory Affairs (ORA) for completion. Center staff may accompany ORA investigators during inspections as subject matter experts.

A. Scheduling an Inspection

ORA and OSIS staff together or OSIS staff alone[8]may conduct BA/BE analytical inspections. FDA staff may also participate in BA/BE inspections with non-FDA international regulators, domestically and internationally. For domestic and international inspections, Center staff should collaborate with ORA field investigators to schedule inspections of analytical sites.

The primary objective of the inspection is to evaluate the overall quality, integrity and validity of analytical data at the site. Except when inspections are otherwise directed, routine BA/BE analytical inspections require approximately one week of on-site inspection time. An amended assignment may be issued from the Center to add studies to the original assignment prior to the start of the inspection. Extension of the inspection is not anticipated, rather, the time spent conducting the inspection will be apportioned across all studies to assess compliance. When multiple studies are included in the assignment, there is no expectation that all aspects of study records for all studies identified in the assignment will be examined. A thorough reconciliation of all study records is not necessary, except in rare instances when those directions are indicated in the assignment.

In the event that serious deficiencies are noted, the ORA investigator's supervisor and/or Center point of contact (POC) should be informed to determine to what extent the scope of the inspection should be expanded.

B. Announced vs. Unannounced Inspections

Domestic inspections are usually not announced prior to arriving at the site. Any instructions for announcing the inspection will be in the inspection assignment. Should ORA investigators have questions on whether or not to announce the inspection, the center POC should be contacted.

International inspections will normally be announced prior to arriving at the site, due to the logistics involved in conducting these inspections. If an unannounced international inspection is required, the Center will contact ORA Office of Bioresearch Monitoring Operations (OBIMO) headquarters (HQ) at ORAHQ BIMO Inspection POC to discuss.

Announcement of inspection to the site should not include application numbers, identity of test article, study numbers, and/or study sponsors.

[8] Memorandum of Understanding between the Center for Drug Evaluation and Research and the Office of Regulatory Affairs, effective February 6, 2017 to February 6, 2018.

C. Roles and Responsibilities While Conducting the Inspection

On inspections that include both Center and ORA staff, the ORA investigator is the Team Lead in accordance with the Investigations Operation Manual (IOM) Section 5.1.2.5.

On inspections that include both the Center and the ORA staff, the Center staff will:

- Provide on-site support to the ORA investigator, including coordinating or conducting parts of the inspection, as needed.
- Provide expert technical guidance, advice, information, and support to ORA investigators prior to, during, and after inspection, including contacting the ORA investigator when new information becomes available.
- Attend daily wrap up meetings held by the inspection Team Lead to discuss findings and status of the inspection and ensure appropriate evidence is collected to document observed violations when warranted.
- Draft appropriate sections of the EIR and provide to the ORA investigator within agreed upon timeframes.

The Center may arrange for a consultative teleconference immediately prior to an inspection if, for example, there is new information concerning the site or the studies assigned, or there are data concerns not previously conveyed to ORA in the assignment.

PART III – INSPECTIONAL

This section outlines the minimum components to be included in an analytical BA/BE inspection[6]. This is not meant to be an all-inclusive list of components that may be covered during an inspection. Deviations from the minimum components should be documented with appropriate explanation in the EIR.

1. Organization

A. Responsible Persons

- Identify the most responsible persons at the site and those who had leading roles at the time the studies to be inspected were conducted.
- Document the names, titles, duties, roles and responsibilities of these individuals in the EIR as per IOM 5.10.4.3.7.
- Issue the Form FDA 482 and Form FDA 483 to the most responsible person at the site.

B. Personnel

- Obtain a copy of the organizational chart that details the most responsible person at the site and displays the reporting relationship of all staff. If no organizational chart is available, obtain full details/information regarding reporting lines of management up to the Chief Executive Officer (CEO)/President and document in the EIR.
- Identify the person who should receive official correspondence.
- For inspections that involve a CRO, determine if there is a written agreement between the sponsor and the CRO that describes the study responsibilities the CRO will perform, and collect a copy if available.
- Obtain the name, address, and email of third party entities, if any, who might be responsible for any aspect of the study, such as storage of the test article and reference product reserve samples.

C. Analytical Site and Equipment

- Evaluate the site layout and consider whether there are logistical issues that could impede site activities.
- Evaluate whether the analytical site has adequate space to enable work flow, conduct of operations, and separation of functions.
- Inspect and evaluate the area where test articles and biological samples are stored.

- Document in the EIR conditions in areas where sample processing and storage occur that may compromise study integrity, and/or contribute to the potential for sample loss, mix-up, contamination/degradation, etc, during storage, processing, and transit.

- Determine whether the site has a standard operating procedure (SOP) index and whether the current versions of SOPs are readily available in locations where relevant processes are conducted.

- Determine whether calibration and maintenance of equipment are performed in accordance with SOPs on scales, centrifuges, refrigerators, and other critical equipment used during analysis.

2. Study Administration and Responsibility

A. Master List of BA/BE or Other Studies

Although sites are not required to maintain a list of BA/BE studies, FDA investigators should request a list when possible. Most sites maintain a master list of previously completed and ongoing BA/BE or other studies. The master list may contain information about these studies including, but not limited to:

- Study titles and number
- Dates of study conduct
- Dates of sample analysis
- Drug products tested
- Number of subjects analyzed
- Methodology and sample processing techniques, amendments, SOPs, etc.
- Analytical staff involved
- Study Sponsors
- Information as to whether the study was conducted to support a drug application submitted to a regulatory agency (e.g., FDA, European Medicine Agency (EMA), etc.).
- Drug expiration dating period (shelf-life)

B. Organizing Information from Selected Studies for Audit

To facilitate the inspection, FDA investigators may request analytical run summary tables. The following information may be included in the tables:

- Master stock solution used for preparing quality control (QCs) samples and calibrators
- Expiration dates of reference material
- Analytical run number
- Subjects included
- Nominal calibrators included (single or duplicates)
- Failed calibrators
- Nominal QCs included (replicates)

- Failed QCs
- Analysts involved
- Date of extraction
- Date of instrumental acquisition
- Identification of instruments
- Run results (Pass/Fail)
- Comments (Reinjections if any, reason for failure, etc.)
- Incurred sample reanalysis (ISR) outcome
- General stability considerations

3. **Stability**

During, or after a clinical study concludes, the analytical site will receive biological samples for analysis. Shipping may occur via courier, or by hand delivery if the clinical unit and analytical site are located in the same building. The analytical site should store the study samples from sample receipt through sample analysis under stability conditions established during method validation.

A. Sample Receipt

- Evaluate the sample receiving area for adequate space, workflow patterns, and separation of operations. Determine if the area has sufficient space for receiving, processing, and logging samples.

- Review the sample shipment records and determine the condition of the samples upon arrival to the analytical site. Check for temperature excursions during sample shipment and determine if the site has an SOP for handling shipping temperature excursions. Identify if any thawed samples were documented, and verify if the site conducted an assessment to evaluate if thawing affected the validity of data originating from these particular samples.

- Review any documentation showing sample tracking. Sample tracking documentation may include, but it is not limited to, logbooks, Laboratory Information Management System (LIMS), temperature logs, and shipping receipts.

- Request the SOP for sample receipt and assess if the analytical site has designated a receiving area and person(s) responsible for receiving the samples. Interview the sample custodian, if possible. Ask him or her to describe the process of sample receipt, visual inspection, and sample reconciliation.

- Request the sample accountability log.

- Verify the number of samples received by the analytical site against the list of samples shipped.

- Verify the number of samples analyzed by the analytical site against the list of samples shipped.

- Inquire if the analytical site had queries regarding specific study samples. If so, determine how those queries were resolved between the analytical and clinical sites after receiving the samples.

- Request a copy of written SOPs for receipt of study samples during the hours of normal operation, and outside of those hours, such as on weekends and holidays.

B. Sample Handling

- Determine if the analytical site handled samples according to the specifications listed in the study protocol. Request the study protocol to review this information, if needed.

- If the analytes are light sensitive, verify that the site implemented appropriate measures (e.g., vapor lamps, amber vials, etc.) to protect study samples from light exposure. Verify if similar procedures are also implemented for reference standards, if appropriate.

- Request documentation that shows the number of instances when samples were retrieved from and stored again in the storage chambers. Compare this documentation with the number of freeze thaw cycles allowable in accordance to the method development elements shown below in Section 5 F.

C. Storage Chambers – Freezers and Cold Rooms

- Determine if access to the storage chamber used for study samples is controlled.

- Sites typically maintain a sample logging sheet for every storage chamber which documents personnel ingress and egress, and removing and restoring of the study samples. If available, request the sample logging sheet.

- Note the individuals accessing the storage chamber and the individuals requesting the study samples. Determine if these individuals have a legitimate reason to handle the study samples.

- Note the date and time when the custodian removed and returned study samples.

- Request the site's SOP that outlines the necessary steps to handle storage temperature excursions.

- Document whether the storage chambers (cold rooms and refrigerators) are equipped with alarm systems that alert custodians when the power supply fails, equipment malfunctions, or when temperature excursions exceed the storage temperature range.

- Request and review the temperature records for each storage chamber used for storing study samples. Determine if the records cover the entire storage period during sample analysis, including ISR. Scrutinize temperature excursions exceeding the demonstrated storage stability of study samples.

- Document if the analytical site has a designated contact in the event of a temperature excursion, or planned equipment servicing or malfunctions, including the name of the individual who responded to the temperature excursion or who serviced the equipment (contact person); the action taken in response to the temperature excursion or equipment servicing, including an assessment of the impact on sample integrity; and adequate coverage for weekends, holidays, and after hours.

- Verify that samples were stored at temperatures previously validated and for the demonstrated stability period.

D. General Considerations for Stability Experiments (Where Applicable)

- Determine if the stability of the stock solution was assessed and confirm that stock solutions used in experiments did not exceed the period of validated stability.

- If the stability of the stock solution has not been assessed, determine if the stability assessment used a set of samples prepared from a freshly prepared stock solution of the analyte in the appropriate analyte-free, interference-free biological matrix.

- Determine if the stability samples were compared to freshly prepared calibrators and/or QCs.

- Determine if the stability experiments included at least three replicates (e.g., at each of the low and the high concentration of QCs).

- Determine if the deviation from nominal concentrations for the stability results were within those specified in the current FDA Guidance on *Bioanalytical Method Validation* (May 2001).

E. Long-term Stability

- Determine if the sample storage time in a long-term stability evaluation met or exceeded the time between the date of sample collection at the clinical site and the date of last sample analysis at the analytical site.

- Determine if the stability samples used for the long-term stability experiments were stored at the same temperature as subject samples.

- Document whether the stability samples were prepared in the same matrix with the same additive as the subject sample matrix.

- Document whether stability sample aliquots were taken from multiple containers or from a single container.

F. Freeze and Thaw Stability

Determine if any of the study samples exceeded the number of freeze and thaw evaluations validated during method development.

G. Benchtop Matrix Stability

Determine if the sample preparation and storage time on the benchtop for study samples exceeded the validated benchtop stability period.

H. Processed Sample Stability and Autosampler Stability

Determine if the validated stability period includes the storage conditions prior to sample analysis, including benchtop, refrigerator, and autosampler.

I. Stock Solution Stability

If the stock solution is in a different state or in a different solvent than the reference standard, determine if the stability data for the stock solution supports the duration of storage for the stock solutions used during sample analysis. Note, stock solution stability is usually not demonstrated in matrix. The assessment typically involves a comparison of peak areas to a freshly prepared stock solution. Document if stability was determined for intermediate or working stock solutions.

J. Reference Standard Storage and Procurement

Analysis of drugs and their metabolites in a biological matrix is performed using calibration standards and QC samples spiked with the reference standard. The purity of the reference standard used to prepare spiked samples can affect accuracy and precision. For this reason, FDA's Guidance on *Bioanalytical Method Validation* (May 2001) recommends using authenticated analytical reference standards of known identity and purity to prepare solutions of known concentrations (see below). When possible, FDA recommends using a reference standard that is identical to the analyte. When it is not possible, an established chemical form (freebase or acid, salt or ester) of known purity can be used with appropriate correction factors.

- If the reference standard is light sensitive, document whether the site uses appropriate handling conditions including amber vials and/or vapor lamps to prevent product degradation.

- According to the certificate of analysis (COA) for the reference standard or other appropriate documentation, determine if expired reference standards were used at any point during the period of method validation and sample analysis.

- If the reference standard is hygroscopic (i.e, absorbs water), document whether the raw analyte used in creating calibration standards and QC samples was stored under dry conditions.

- Verify whether concentrations were adjusted for salts, counter ions, water, carriers, and impurities.

4. Methodology

Determine if the site has a general and/or study-specific SOP on method validation, and whether the site followed it.

A. Precision and Accuracy

- Document how the site validated the within-run (intra-batch) and between-run (inter-batch) accuracy and precision of the analytical method. Determine whether the site assessed an intra- and inter-batch assessment with data from all accuracy and precision analytical runs.

- Document if any of the demonstrations of precision and accuracy used calibrators and QCs prepared from separate stock solutions.

- Document how many precision and accuracy runs were conducted and how many different concentration values were evaluated in each run. Also, document how many times each concentration value was measured within each run. For example, a precision and accuracy evaluation might include three independent runs, each run evaluates a minimum of four concentrations (including the lower limit of quantification [LLOQ]) covering the range of expected concentrations in subject samples, and each concentration is measured at least five times within each run.

- Determine if all valid runs (i.e., acceptable calibration curve) were included in the accuracy and precision assessment.

B. Recovery

The recovery of an analyte in an assay is the detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true (prepared) concentration of the analyte in pure solvent.

Determine if recovery experiments were performed by comparing the analytical results for extracted samples at three concentrations (low, medium, and high) with unextracted standards (analyte in solvent) that represent 100% recovery. Recovery of the analyte need not be 100%, but the extent of recovery of an analyte and of the internal standard (IS) should be consistent, precise and reproducible.

C. Calibration Curve

The calibration (standard) curve is the relationship between instrument response and known concentrations of the analyte.

- Calibration standards can contain more than one analyte. However, one calibration curve should be generated for each analyte in the sample.

- Determine if the calibration curve was prepared in the same biological matrix as the samples in the intended study by spiking the matrix with known concentrations of the analyte.

- Document, and collect evidence, if the site provided justification when surrogate matrices different from the study sample matrix are used.

- Determine if the calibration curve is representative of the concentration observed in study samples. Any concentrations below (LLOQ) or above the upper limit of quantification (ULOQ) should not be extrapolated. Samples with concentrations above the ULOQ should be diluted with the appropriate matrix. The dilution factor for the samples should be supported by the dilution experiments. Refer to the evaluation of dilution integrity in Section 6 H. The concentration range should include a blank sample, a zero sample, and at least six non-zero samples covering the expected concentration range in study samples.

D. Lower Limit of Quantification

Determine if the analyte response at the LLOQ is at least five times the response compared to background noise. If not, collect relevant documentation demonstrating that the analytical method was sufficiently sensitive to measure the analyte of interest.

Determine if the analyte peak (response) is identifiable, discrete, and reproducible, and the accuracy and precision of the back-calculated concentration meets acceptance criteria.

E. Upper Limit of Quantification

Document the precision and accuracy of the ULOQ. The highest calibration standard defines the ULOQ of an analytical method.

F. Selectivity/Specificity

Document whether the site assessed the selectivity/specificity of each analyte of interest using at least six sources of the appropriate matrix (e.g., plasma, urine, or other matrix).

Determine whether each blank matrix sample was tested for interference and selectivity/specificity at the LLOQ.

G. Matrix Effect

Matrix effect is the direct or indirect alteration or interference in response due to the presence of unintended analytes (for analysis) or other interfering substances in the sample. Matrix effect can cause inaccuracy in quantitation of the analyte of interest.

- Document whether the site evaluated matrix effect on the analyte and IS.
- If matrix effect was evaluated, determine if the site evaluated the matrix effect using at least six separate sources of blank matrix.
- Document if the site evaluated the matrix factor when a matrix effect was observed.

H. Dilutions

- Determine if the concentration of any study sample exceeded the ULOQ. Check if the site diluted and re-analyzed these sample(s).
- If study samples were diluted and re-analyzed, determine if the site conducted a dilution integrity experiment during method validation or sample analysis.
- If a dilution integrity experiment was conducted during method validation, determine if the dilution factor used on study samples was less than or equal to the dilution factor (≤ DF) evaluated in the dilution integrity experiment.
    - If the dilution factor used on study samples exceeded the dilution factor (> DF) evaluated in method validation experiment, see (6) below.
    - Determine if the study sample(s) were diluted with like matrix including same additives.
- If a dilution integrity experiment was not conducted during method validation, but study samples were diluted during sample analysis, determine if the site included dilution QCs in study sample runs.
    - Determine if the run acceptance criteria were based on run QCs (low, mid, high) and not on dilution QCs.
    - Determine if the dilution QCs were diluted with like matrix including same additives.
- Determine if the unadjusted concentrations of diluted study samples (prior to applying a dilution factor) fell within the calibration curve range.
- Document if the site has an SOP that discusses procedures for diluting study samples and reporting concentrations of diluted samples.

I. Carryover

Determine if the site assessed carryover during method validation.

J. Partial Validation

A partial validation evaluates changes in a bioanalytical method that is already validated. The evaluation can range from a minimum of one intra-assay accuracy and precision experiment to nearly all the experiments that are conducted for a full validation.

For specific examples where a partial validation is warranted, please refer to the current FDA Guidance on *Bioanalytical Method Validation* (May 2001). Also, refer to Section 4 A for instructions on assessing precision and accuracy.

5. Documentation

The FDA's current Guidance on *Bioanalytical Method Validation* (May 2001) encourages sites to maintain sufficient documentation throughout study conduct to allow reconstruction and verification of all critical activities during method validation and sample handling and analysis.

Determine if documentation is attributable, legible, contemporaneous, original, accurate, complete and consistent. The determination typically includes auditing the site's analytical procedure (AP) sheets, sample preparation records, raw chromatograms, records for sample receipt and handling, deviation/investigation reports, institutional SOPs, audit trails, and correspondence with sponsors or clinical sites.

Records may exist electronically or in a paper format. For electronic records, determine if the site has processes and quality systems to secure and protect network and computerized systems from inappropriate internal and external access. Document whether all electronic data and computer systems are validated for their 'intended uses.' Refer to 21 CFR part 11 Electronic Records and Electronic Signatures.

A. Laboratory Instruments

- Determine if calibration and maintenance records for major instruments used in the study are complete and up-to-date. Examples of major instruments may include, but are not limited to, balances, freezers, spectrometers, and pipettes.

- Document whether written SOPs exist that specify how frequently calibration and maintenance activities are performed, and the person(s) responsible for instrument calibration and maintenance as well as emergency contacts.

- Examine instrument logs and evaluate how instrument malfunctions are reported and documented.

- Document whether the site has appropriate procedures for handling study samples if instrument failure occurs during the sample analysis.

B. Source Data

- Determine if source records (paper or electronic format) allow reconstruction of the study. Examples of source records include chromatograms, instrument-generated readings or spectra, or any other records from automated instruments.

- Evaluate the source data and ensure that changes are appropriately traceable and documented. Determine if there are adequate systems and/or administrative controls to prevent deletion or alteration of source data.

- Document whether the site has an SOP to ensure that source data are consistently organized and retrievable, and whether the site follows the SOP.

C. Re-integration/Re-analysis

- Determine if the site implements automatic integration of chromatographic peaks and applies consistent integration parameters within a run.

- Document whether the site has an SOP for re-integration of chromatograms and re-analysis of samples.

- Determine if the procedure provides clear and objective criteria for re-integrating chromatograms and re-analysis of study samples.

- Determine whether staff follow the site's SOP during chromatogram re-integration and sample re-analysis including documentation, justification, and supervisory approval.

- If chromatograms for QCs and calibrators were re-integrated, determine if run acceptance was affected.

D. Audit Trails

- Determine if the software implemented for acquiring, integrating, and regressing study data has audit trail capabilities.

- Determine if the audit trail option was enabled during the sample analysis.

- Evaluate the site's administrative procedures and/or access rights to determine if adequate controls exist over audit trail functionality.

- Determine whether the audit trail records reconstruct the course of events during the analysis of study samples.

- Review the audit trails from selected studies and determine if there are any indications of potential alterations of study data. Document any changes captured in the audit trail without adequate justification.

E. Data Security

- Document if the site has an SOP for controlling access to operating systems and instrument software and that it is followed.

- Determine if the operating system and/or the instrument software require unique login for individual staff.

- Determine if a computer lock mechanism is in use to prevent unauthorized access to open workstations and/or data.

- Review the access rights regarding the ability to create, integrate, modify, regress, remove, transfer, and report data.

- Review the site's SOP and requirements for data storage and protection. Evaluate if storage, back-up, transfer, and archive of study data are adequate to ensure data security.

- Document whether the site has an SOP to prevent deletion of electronic data.

F. Data Reporting

- Determine if data found at the site are consistent with those in the study reports included in the FDA submission, when applicable.

- Determine if all method validation experiments, whether passed or failed, were reported in the method validation report. Evaluate whether the site conducted any investigation to identify the reasons for such failures, if any.

- Determine if rejected runs during the study sample analysis were documented and the reason for rejecting the run was reported in the study report.

- If study samples were re-analyzed, determine if the re-analysis is justifiable and whether final concentrations were reported according to established SOPs.

- Determine how the site documents and handles protocol/SOP violations. If violations occurred, describe in the EIR and collect information to support the Center's evaluation of the potential impact of these events on data quality and integrity.

- Determine if reports are accurate and complete by randomly selecting representative runs.

- Document whether all failed BE studies were reported to the study Sponsor.

G. Communications with Internal and External Parties

- Request and review communications between the analytical site, the sponsor, clinical sites, or study monitors regarding the study and data analysis.

- Evaluate any requests from the sponsor or study monitors that resulted in changes to the final study report. Describe in the EIR and collect information to facilitate the Center's evaluation of the impact of those changes on study data and conclusions.

- Inquire whether the site reported any issues with the analytical method or study data to the study sponsor, clinical sites, or study monitors. If so, determine how these issues were documented, addressed, and whether they affected data quality and integrity.

6. Analysis of Study Samples

A. System Suitability

- Document whether the site has an SOP on system suitability.

- Determine if the system suitability samples were labeled and identified as such, prior to conducting an analytical run containing study samples.

- Determine if the system suitability samples were independent from the analytical run containing study samples.

- Determine if system suitability samples were injected prior to the analytical run containing study samples.

- Determine if system suitability samples were injected after instrument malfunctions, and prior to resuming an analytical run containing study samples.

B. Run Acceptance

For each analytical run audited, document if:

- At least three concentrations of QCs in duplicate were incorporated into each study sample run. One concentration should be within three times the LLOQ (low QC), one in the midrange (middle QC), and one approaching the high end (high QC) of the range of the expected study concentrations. See Part III.4.H. regarding dilution QCs.

- At least 67% of the QC concentration results are within 15% of their respective nominal concentrations.

- At least 50% of the QCs at each concentration level are within 15% of their nominal concentrations.[7]

- The number of QCs in a run meets or exceeds 5% of the total number of unknown samples or six total QCs, whichever number is greater.

Determine whether the QCs represent the analyte concentrations observed in study samples.

C. Improper Processing

- Document whether the site has a procedure to handle samples that were processed improperly and whether it is followed.

- If the site determined that study samples were processed improperly, was the incident documented contemporaneously and did the site conduct an investigation to determine the cause?

- Was this incident isolated or does it affect multiple samples in the same run or across runs?

- Did the site re-inject or re-process the study samples? If samples were re-injected, was the processed sample stability timeframe exceeded?

D. Shift in Retention Time of Analyte/IS

- Document whether the site has an SOP for investigating shifts in retention time and whether the site followed it.

- Determine if any runs or samples were rejected due to a substantial shift in retention time, and whether the site was justified to reject those runs and/or samples.

- Determine if the shift in retention time is isolated to a single study run or across multiple runs.

E. Re-injection of Study Samples

- Document whether the site has an SOP that discusses procedures for re-injecting study samples. If so, did the site follow this SOP?

- Determine if the site re-injected study samples in any of the audited studies. Did the site document the reason for re-injection?

- Does the processed sample/autosampler stability support the time of re-injection of study samples?

- Are the final concentrations that are reported for reinjected samples acceptable?

- Were all re-injected samples identified in the study report?

F. IS Variability/Drift

- Document whether the site has an SOP describing the acceptable variability in the peak area response of IS. In addition, determine if the SOP specifies when a run should be rejected based on unacceptable IS variability.

- Document whether the site identified all QCs and study samples with IS variability outside the specified limits. Were study samples repeated if the IS variability exceeded the specified limits? Was the SOP followed?

- Determine if IS variability affected run acceptability or study samples.

G. PK Anomalies

- Document whether the site has an SOP outlining the procedures for repeating samples with PK anomalies.

- Were study samples repeated based on PK anomalies? Was the SOP followed?

- Determine if the site investigated the potential reasons for the anomalous concentration(s).

- Determine who requested the sample repeat. Is there adequate documentation regarding the request?

- Determine if the bioanalytical report includes both the anomalous and final reported concentrations, including the rationale for selecting the final concentration.

H. Incurred sample reanalysis (ISR)

- Document whether the firm conducted the ISR. Check the number of samples analyzed.

- Did the ISR assessment include study samples representative of the analyte(s) Cmax and elimination half-life, where applicable?

- Determine and document if the percentage difference for the original and ISR values are within pre-defined acceptance criteria.

- Document if the site has written procedures to investigate ISR failures.

PART IV – ANALYTICAL

Not applicable to this Compliance Program.

PART V – REGULATORY/ADMINISTRATIVE STRATEGY

If significant violations are observed during the inspection, prepare a Form FDA 483, Inspectional Observations, to issue to the most responsible individual. Form FDA 483 observations should be factual statements. Avoid recording unsupported opinions on a Form FDA 483. Observations recorded on a Form FDA 483 should include supporting documentation attached to and discussed within the EIR whenever possible.

Analytical sites should conduct bioanalysis using best practices to ensure data quality and integrity. Significant inspectional observations may not have specific, corresponding CFRs that support them. When observations are made with a potential to have significant impact on data quality or reliability, but there are no corresponding CFRs, collect the evidence related to these observations, discuss the observations with the most responsible individual and document the observations, discussion and evidence in the EIR.

Center reviewers will evaluate Form FDA 483 observations, as well as any discussion items and evidence contained in the EIR, and consider the impact of the site's actions (frequency, scope, and severity) on acceptability and reliability of study data. There are often varying gradations in the severity among similar Form FDA 483 examples. The specific observation(s) and information collected should support the Center's evaluation of the reliability and acceptability of data for FDA decision-making purposes.

PART VI – REFERENCES, ATTACHMENTS, AND PROGRAM CONTACTS

1. FDA Guidance Documents and References

A. FDA Laws

Federal Food, Drug, and Cosmetic Act (FFDCA)
(https://www.fda.gov/RegulatoryInformation/LawsEnforcedbyFDA/FederalFoodDrugandCosmeticActFDCAct/FDCActChapterVDrugsandDevices/default.htm)

B. Relevant 21 CFRs

- Part 320 – Bioavailability and Bioequivalence Requirements
(http://www.ecfr.gov/cgi-bin/text-idx?SID=93111e2feefbb29bcaba7cb37a13aa9b&mc=true&node=pt21.5.320&rgn=div5)

- Part 11 – Electronic Records; Electronic Signatures
(http://www.ecfr.gov/cgi-bin/text-idx?SID=93111e2feefbb29bcaba7cb37a13aa9b&mc=true&node=pt21.1.11&rgn=div5)

C. Relevant FDA Guidelines, Guidance Documents, and Inspection Guides

For an overview of bioavailability, bioequivalence, and other clinical pharmacology studies, please refer to the available FDA Guidance Documents referenced below.

- Guidance for Industry: Bioanalytical Method Validation, 2001
(http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf)

- Draft Guidance for Industry: Bioanalytical Method Validation, 2013
(http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM368107.pdf)

- Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations
(http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm389370.pdf)

- Guidance for Industry: Food-Effect Bioavailability and Fed Bioequivalence Studies, 2002
(https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070241.pdf)

- Draft Guidance for Industry: General Considerations for Pediatric Pharmacokinetic Studies for Drugs and Biological Products, 1998
(http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm425885.pdf)

- Draft Guidance for Industry: Circumstances that Constitute Delaying, Denying, Limiting, or Refusing a Drug Inspection, 2013 (http://www.fda.gov/downloads/regulatoryinformation/guidances/ucm360484.pdf)

- Draft Guidance for Industry: Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action, 2003 (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070111.pdf)

- Draft Guidance for Industry: Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs — General Considerations, 2014 (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM389370.pdf)

- Draft Guidance for Industry: Bioequivalence Studies With Pharmacokinetic Endpoints for Drugs Submitted Under an Abbreviated New Drug Application, 2013 (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM377465.pdf)

- List of FDA Guidance Documents: (http://www.fda.gov/RegulatoryInformation/Guidances/)

- Investigations Operations Manual: (http://www.fda.gov/ICECI/Inspections/IOM/)

- FMD-86: (http://www.fda.gov/downloads/ICECI/Inspections/FieldManagementDirectives/UCM382035.pd)

- Form FDA 483: (http://www.fda.gov/downloads/ICECI/Inspections/IOM/UCM127434.pdf)

- Form FDA 482: (http://www.fda.gov/downloads/ICECI/Inspections/IOM/UCM127428.pdf)

2. **Program Contacts**

When technical or scientific questions or issues arise from a specific assignment, or if additional information is required about a specific assignment, consult the Center POC identified in the assignment.

For operational questions, contact:

- Office of Regulatory Affairs (ORA)/Office of Medical Products and Tobacco Operations (OMPTO)/Office of Bioresearch Monitoring Operations (OBIMO) - ORAHQ BIMO Inspection POC

- Center for Drug Evaluation and Research (CDER)/Office of Translational Sciences (OTS)/Office of Study Integrity and Surveillance (OSIS) - CDER-OSIS-BEQ@fda.hhs.gov

For questions about the BA/BE compliance program, contact:

- Center for Drug Evaluation and Research (CDER)/Office of Translational Sciences (OTS)/Office of Study Integrity and Surveillance (OSIS) - CDER-OSIS-BEQ@fda.hhs.gov

PART VII – CENTER AND ORA HQ RESPONSIBILITIES

1. **CDER Review Divisions – OND, OGD, OPQ, and OCP**

- Request directed audits of selected studies, including BA/BE studies.

- Send consults for directed audits to OSIS. The inspection consults contain the site's name, study(ies) to be inspected, application number(s), and specific details about the Agency's concern with the site's operations or study data.

- Answer technical questions and provide technical guidance related to the application to the OSIS subject matter expert (SME) prior to, during, and after the inspection/audit.

- Review Divisions receive EIR reviews from OSIS, and have the ultimate authority to accept or reject study data based on inspection findings.

2. **OSIS**

- For directed inspections/audits, receives an inspection consult and drafts assignments.

- For surveillance inspections/audits, identifies the sites to be inspected/audited based on the current OSIS surveillance model.

- Drafts and issues assignments and includes contact information for the OSIS SMEs and relevant FDA reviewer.

- Assigns the audit for the inspection to staff.

- Provides expert technical guidance, advice, information, and support to OSIS staff and ORA investigator, if applicable, prior to, during, and after inspection.

- Accompanies the ORA investigator on the analytical inspection or, after contacting ORA, may also perform an independent audit without ORA.[8]

- May conduct analytical audits and communicate findings and observations with appropriate site personnel during the course of the audit, as appropriate. Conclusions regarding data reliability or acceptability are not discussed during the audit.

- If OSIS and ORA will perform a directed or surveillance inspection as a team inspection, OSIS staff contacts ORA prior to the inspection. OSIS also provides additional details to ORA investigators, as needed.

- OSIS staff draft EIRs and submit them within appropriate timeframes.

- Reviews an EIR and provides recommendations to Review Divisions regarding the reliability and/or acceptability of study data.

- Enters the final classification into Field Accomplishments and Compliance Tracking System (FACTS), or other appropriate electronic system, and notifies ORA.

- May issue untitled letters to inspected sites following voluntary action indicated (VAI) or no action indicated (NAI) final classifications.

- Promptly forwards to the ORA field investigator and any appropriate district personnel copies of post-inspection correspondence issued to the inspected party.

- Contacts the assigned ORA investigator at least two weeks prior to the start date to provide clarification on details of the inspection or scientific inquiries that may assist the ORA field investigator when conducting the assignment or that may change the focus of the inspection.

3. **OSI**

- Receives potential official action indicated (OAI) cases from OSIS.

- Reviews the EIR for potential OAI cases to determine if the inspection observation(s) warrants compliance/enforcement actions for a site. Takes compliance/enforcement action as warranted.

- In collaboration with OSIS and ORA, may determine that a follow-up inspection of an inspected site is necessary to verify whether the site has implemented any proposed corrective plans, and that the site is in compliance with applicable regulations.

4. **Office of Regulatory Affairs (ORA)**

- ORA OBIMO HQ receives assignment from OSIS via ORAHQ BIMO Inspection POC email. OBIMO assigns to the appropriate division (OBIMO Division I (East) or OBIMO Division II (West)). Management assigns the inspection to an ORA field investigator.

- International assignments are addressed to OBIMO and sent via ORAHQ BIMO Inspection POC email. OBIMO HQ issues the assignment to the ORA field investigator selected to conduct the foreign inspection.

ATTACHMENT A – Abbreviations and Acronyms List

A

ANDA	Abbreviated new drug application
AP	Analytical procedure

B

BA	Bioavailability
BE	Bioequivalence
BIMO	Bioresearch monitoring

C

CDER	Center for Drug Evaluation and Research
CEO	Chief Executive Officer
CFR	Code of Federal Regulations
Cmax	Maximum concentration
COA	Certificate of analysis
CP	Compliance program
CRO	Contract research organization

D

DF	Dilution factor

E

EIR	Establishment Inspection Report
EMA	European Medicines Agency
etc.	Et cetera

F

FACTS	Field Accomplishments and Compliance Tracking System
FMD	Field management directive
FDA	Food and Drug Administration

H

HQ	Headquarters

I

IND	Investigational new drug application
IS	Internal standard
IOM	Investigations operations manual
ISR	Incurred sample reanalysis

L

LIMS	Laboratory Information Management System
LLOQ	Lower limit of quantification

N

NAI	No action indicated
NDA	New drug application

O

OAI	Official action indicated
OCP	Office of Clinical Pharmacology
OGD	Office of Generic Drugs
OMPTO	Office of Medical Products and Tobacco Operations
OND	Office of New Drugs
OPQ	Office of Pharmaceutical Quality
ORA	Office of Regulatory Affairs
OSI	Office of Scientific Investigations
OSIS	Office of Study Integrity and Surveillance
OTS	Office of Translational Science

P

PD	Pharmacodynamic
PK	Pharmacokinetic
POC	Point of contact

Q

QC	Quality control

R

RLD	Reference listed drug

S

SME	Subject matter expert
SOP	Standard operating procedure

U

ULOQ	Upper limit of quantification

V

VAI	Voluntary action indicated

Change History

Item	Change	Date
Document	New Document	05/01/2018