Effective Date: 05th May 2025 Doc. Ref. No. DER-GDL-009-01

Review Date: 04th May 2030

National Agency for Food & Drug Administration & Control (NAFDAC)

GUIDELINES ON THE INVESTIGATION OF BIOEQUIVALENCE 2025

TABLE OF CONTENT

Executive Summary

This guideline specifies the requirements for the design, conduct and evaluation of bioequivalence studies for immediate release dosage forms with systemic action for supporting NAFDAC registration in Nigeria.

An international reference guideline for Bioequivalence (ICH M13) should be included in the early part of the Regulation.

This guideline should be read in conjunction with the ICH guidelines for conducting clinical trials, including those on:

- General Considerations for Clinical Trials (ICH E8)
- Guideline for Good Clinical Practice (ICH E6 (R3))
- Statistical Principles for Clinical Trials (ICH E9)
- Structure and Content of Clinical Study Reports (ICH E3)
- Bioanalytical Method Validation and Study Sample Analysis (ICH M10)
- Biopharmaceutics Classification System-Based Biowaivers (ICH M9

- **1.1 Introduction**

The investigation of bioavailability and bioequivalence of drugs is an essential component of the regulatory process for the approval of generic pharmaceutical products. Bioavailability refers to the degree and rate at which the active ingredient of a drug becomes available to the target site of action in the body, while bioequivalence is a measure of the equivalence of two or more products in terms of their rate and extent of active ingredient absorption. The guidelines on the investigation of bioavailability and bioequivalence of drugs provide the necessary framework for assuring the quality, safety, and efficacy of generic pharmaceutical products.

This national guidance document aligns with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) to provide the appropriate invitro and invivo requirements for ensuring that generic pharmaceutical products are interchangeable with the innovator's product without compromising safety, quality, and efficacy.

The guidelines are mainly applicable to orally administered immediate-release (IR) solid oral dosage forms and some non-oral products like transdermal systems and certain parenteral, rectal, and nasal pharmaceuticals. However, it is important to note that the concept of interchangeability raises issues for other classes of products, such as many biologicals and biotechnology-manufactured products, which are beyond the scope of this document. However, the Agency has several other guidance that can provide understanding to applicant for the registration of such products.

The investigation of bioequivalence of drugs involves the comparison of the rate and extent of active ingredient absorption between the generic product and the innovator's brands. This comparison is usually performed through clinical trials in human subjects or through in vitro studies using simulated biological fluids. The results of these studies provide the basis for determining the therapeutic equivalence and interchangeability of the generic product with the innovator's product.

To ensure the validity and reliability of the results, the guidelines on the investigation of bioavailability and bioequivalence of drugs prescribe strict scientific and ethical standards for the design, conduct, analysis, and reporting of bioavailability and bioequivalence studies. The guidelines also provide recommendations for the selection of appropriate study populations, dosage regimens, and statistical

methods for the analysis of the data. It is important to note that alternative approaches to the principles and practices described in the guidelines may be acceptable as long as they are supported by adequate scientific justification. In addition, these guidelines should be interpreted and applied without prejudice to obligations incurred through the existing international Agreement on Trade-Related Aspects of Intellectual Property Rights and the laws governing intellectual property rights in Nigeria.

2.0 In vivo equivalence studies in humans

2.1 General Considerations for Bioequivalence (BE) and Bioavailability (BA)

***Study Population*:** The selection of subjects for bioequivalence (BE) studies should be conducted with the primary objective of enabling the detection of disparities in the in vivo release characteristics among pharmaceutical products. In order to minimize variability unrelated to product differences, it is typically recommended that these studies be carried out on individuals who are in good health, unless ethical concerns arise due to safety issues associated with the drug. Conducting BE studies on healthy subjects is generally considered sufficient for identifying formulation variances and allowing for the extrapolation of results to the target population.

To ensure transparency and clarity, the study protocol should clearly outline the criteria for subject inclusion and exclusion. Prospective subjects should be at least 18 years of age and preferably possess a Body Mass Index ranging from 18.5 to 30.0 kg/m2. In the case of drug products intended for use by both males and females, it is advisable to include subjects from both genders in the study. Prior to enrolment, subjects should undergo a thorough evaluation, including clinical laboratory tests, an assessment of medical history, and a comprehensive physical examination. Depending on the therapeutic class and safety profile of the drug, additional medical investigations and precautions may need to be undertaken before, during, and after the completion of the BE study.

The well-being of women of childbearing potential must be taken into account, and it is essential for investigators to ensure that female subjects are not pregnant or breastfeeding throughout the duration of the BE study and subsequent follow-up. Preferably, subjects should be non-nicotine users and should not have a history of alcohol or substance abuse. Phenotyping and/or genotyping of subjects may be considered for reasons pertaining to safety or pharmacokinetics. In situations where the investigated active substance is known to produce adverse effects, or when the pharmacological effects or risks are deemed unacceptable for healthy subjects, the study may instead be conducted on a specific patient population under suitable precautions and supervision.

Healthy volunteers are preferred for BE studies, while patients may be required for APIs that are too potent or toxic for healthy volunteers. The number of subjects required for a BE study is determined by factors such as error variance, significance level, statistical power, mean deviation, and confidence interval.

***Purpose*:** Bioequivalence studies are conducted to determine if a generic product is equivalent to a reference product in terms of the rate and extent of active ingredient absorption, while bioavailability studies determine the extent and rate of active ingredient absorption from a product.

***Study Design*:** The preferred study design for a BE study is a randomized, two-period, two-sequence, single-dose, cross-over study conducted with healthy volunteers. Alternative study designs may be used for APIs that are too potent or toxic for healthy volunteers, or for APIs with long elimination half-lives. For BA studies, the study design may vary based on the product being studied.

***Sample Size for Bioequivalence Studies*:** The number of subjects to be included in the BE study should be based on an appropriate sample size calculation to achieve a pre-specified power and to avert a prespecified type 1 error. A sufficient number of subjects should be enrolled in the BE study to account for possible dropouts and/or withdrawals. The use of "spare" subjects is not acceptable. Additional cohort(s) of subjects may be added to the study, e.g., if the number of evaluable subjects falls below the calculated sample size; however, this should be specified in the study protocol and done prior to any bioanalysis. The number of evaluable subjects in a pivotal BE study should not be less than 12 per treatment for a crossover design or 12 per treatment group for a parallel design

Comparator and Test Products

A comparator product is the drug product accepted by regulatory agencies that an applicant can use to compare against the test product in conducting a BE study. The selection of the batch of the comparator product used in the BE study should be based on assay content. It is advisable to investigate more than one batch of the comparator product when selecting the batch of comparator product for use in the BE study. The test product used in the BE study should be representative of the product to be marketed and this should be discussed and justified by the applicant and as determined by NAFDAC.

***Dosage Regimen*:** The dosage regimen used in BE studies should follow the usual recommendations, while the dosage regimen used in BA studies should be clearly defined in the study protocol. ***Sample Collection*:** Blood samples should be collected for up to 72 hours following administration for oral products. Sample collection time should be adequate to ensure completion of gastrointestinal transit and API absorption.

Study Report: The study report should be submitted using the NAFDAC BTIF format and any other template approved by NAFDAC for purposes of registration of a generic drug product or its equivalent. The proposed statistical plan must be clearly defined in the study protocol which shall be submitted alongside the duly filled BTIF Form at relevant section of the CTD Dossier format.

2.1.1 Provisions for studies in humans

Provisions for Bioequivalence (BE) and Bioavailability (BA) studies in humans may include the following considerations:

Design of the study: For a bioequivalence study involving a generic product and a comparator product, a randomized, two-period, two-sequence, single-dose, cross-over study shall be conducted with healthy volunteers is the preferred study design. An adequate wash-out period should follow the administration of each product. For APIs that are very potent or too toxic to administer in the highest strength to healthy volunteers, it is recommended that the study be conducted using the API at a lower strength in healthy volunteers. For APIs that show unacceptable pharmacological effects in healthy volunteers, even at lower strengths, a study conducted in patients may be required.

***Subjects*:** The number of subjects required for a bioequivalence study is determined by the error variance (coefficient of variation) associated with the primary parameters to be studied, as estimated from a pilot experiment, from previous studies or from published data. The proposed statistical plan must be clearly defined in the study protocol, including the sample size calculation provided in the study protocol.

Fasting and Fed Study Conditions

BE studies should be conducted under standardised conditions that minimise variability to better detect potential PK differences between drug products. For Immediate Release solid oral dosage forms, single-dose BE studies conducted under fasting conditions typically provide greater discrimination between the PK profiles of two products. Therefore, for the majority of these drug products, BE may be demonstrated in a single study conducted under fasting conditions.

However, food can have a differential, formulation-dependent impact on the absorption of drug substances from drug products that are of high risk (see “High-risk products” section below), which would preclude the extrapolation of BE under fasting conditions to fed conditions. In such cases, BE under fed conditions also needs to be demonstrated. The design of a BE study with regard to the use of fasting and/or fed conditions depends on the dosing instructions of the comparator product as well

as the properties of the drug substance and product formulation. A rationale should be provided for the selection of the type of BE study(ies) (fasting or fed or both) and meal type, e.g., fat and calorie content, based on the understanding of the comparator product and the test product (high or non-high risk) as described below. The rationale can be supported by modelling, e.g., appropriately validated/qualified physiologically based pharmacokinetic (PBPK) modelling or semi-mechanistic absorption models.

In addition, safety-related aspects need to be considered when selecting the appropriate condition for a BE study regarding food intake. If safety concerns make it unethical to administer a single dose of the drug product under either fed or fasted conditions, the BE study should be conducted under the condition with less safety concerns.

***For non-high-risk products, the following is recommended*:**

- For a product that is labelled to be taken only under fasting conditions or can be taken under fasting or fed conditions i.e., without regard to food, a single BE study conducted under fasting conditions is recommended to demonstrate bioequivalence.
- For a product that is labelled to be taken only with food due to PK reasons, e.g., enhancing absorption or reducing variability, a single BE study conducted under fed conditions is recommended to demonstrate bioequivalence.
- For a product that is labelled to be taken only with food due to tolerability reasons, e.g., stomach irritation, a single BE study conducted under either fasting or fed conditions is acceptable
- ***High-risk products*:**

High-risk products are those where the complexity of the formulation design or manufacturing process leads to an increased likelihood that in vivo performance will be impacted differently by varying gastrointestinal (GI) conditions between the fasted and fed states. For these products, performance differences related to differences in formulation and/or manufacturing process may not be detected with a single BE study, i.e., results from a fasting BE study may not be extrapolated to predict fed BE study outcome or vice versa, thus both fasting and fed BE studies should be conducted. For example, some drug products containing low solubility drug substances (as defined by the BCS low solubility criterion described in ICH M9) have complex formulation and/or manufacturing methods (such as solid dispersions, microemulsions, lipid-based formulations, nanotechnologies, or other specialised technologies) to ensure sufficient solubility of the drug substance and dissolution of the drug products or to manage the impact of food. For these high risk products, BE studies should be conducted under both fasting and fed conditions, irrespective of the product labelling with regard to food intake, except when safety concerns make it unethical to administer a single dose of the drug product under either fed

or fasted conditions. Then the BE study should be conducted under the condition with less safety concerns.

Especially for low solubility drug substances, the comparator product may be the result of an extensive formulation and/or manufacturing process development program, obtaining for instance a specific formulation without a food effect. If the test product uses a substantially different manufacturing technology or particle size control method from the comparator, or if substantially different excipients are used in the test and comparator that are likely to impact dissolution, solubility, or permeability, this may warrant the need for BE studies under fasting and fed conditions.

The above principles with regard to fasting and fed study conditions also apply when BE studies are deemed necessary to bridge formulation and/or manufacturing process changes during pre- or postmarketing phases.

Standardisation with regard to meals and water

For studies conducted under fasting conditions, subjects should be fasted for at least 10 hours before drug administration. Subjects should be allowed water as desired, except for 1 hour before and 1 hour after drug administration. The dose should be administered with a standardised volume of water, in the range of 150 to 250 millilitres (ml). No food should be allowed for at least 4 hours post-dose on each day of drug administration and meals taken should be standardised with respect to composition and timing.

In the case of studies conducted under fed conditions, the same controls should be employed with the exception that a pre-dose meal should be provided. For a fed BE study, it is recommended that subjects start the meal 30 minutes before administration of the drug product and consume the meal within 30 minutes. If BE studies are conducted under both fasting and fed conditions, i.e., for high-risk products, the BE study conducted under fed conditions should be conducted using a meal that has the potential to cause the greatest effect on GI physiology. The meal should be a high-fat (approximately 50% of total caloric content of the meal) and high-calorie (approximately 800 to 1000 kcal) meal, which should derive approximately 150, 250, and 500-600 kcal from protein, carbohydrate, and fat, respectively. It is recognised that there may be situations where it is appropriate to administer a pre-dose meal with a different caloric/fat content from these recommendations, e.g., for studies performed in patient populations who cannot tolerate the recommended meal composition. If, however, only one BE study conducted under fed conditions is needed for a non-high-risk product, either a high-fat, high-calorie meal or a low-fat, low-calorie meal, e.g., a meal of approximately 500 kcal with approximately 25%

of calories from fat, may be administered. If the type of meal to be consumed at the time of drug product administration is clearly specified in the comparator product labelling, then this meal should be employed in the BE study.

The composition of the meal to be administered should be described with regard to protein, carbohydrate, and fat content (specified in grams, kcal, and relative caloric content (%)) in the study protocol. In all situations, subjects should abstain from foods and drinks that may interact with circulatory, GI transporter, GI enzymatic, hepatic, or renal function, e.g., alcoholic or caffeinated drinks, or certain fruit juices such as grapefruit juice, during a suitable period before and during the study.

2.1.2 Conduct of the study: The signed and dated study protocol should be approved by the relevant regulatory authority before commencing the study. For studies to be conducted in Nigeria, the signed and dated study protocol should be approved by NAFDAC. The study report should be submitted using the NAFDAC BTIF format which can be obtained from the NAFDAC website.

Considerations for active pharmaceutical ingredients with long elimination half-lives: Ideally the interval between administration of the products should not be less than five terminal elimination halflives of the active compound or metabolite, if the latter is measured. If the cross-over study is problematic owing to a very long elimination half-life, a bioequivalence study with a parallel design may be more appropriate. For both cross-over and parallel design studies of oral products, sample collection time should be adequate to ensure completion of gastrointestinal (GI) transit of the pharmaceutical product and absorption of the API.

Considerations for multiple-dose studies: Multiple dose studies in patients are most useful in cases where the API being studied is considered to be too potent and/or too toxic to be administered to healthy volunteers, even in single doses. In this case, the study is performed without interrupting therapy even for a cross-over study. The dosage regimen used in multiple dose studies should follow the usual dosage recommendations. In steady-state studies, the wash-out of the last dose of the previous treatment can overlap with the approach to steady state of the second treatment, provided the approach period is sufficiently long (at least five times the terminal half-life). Appropriate dosage administration and sampling should be carried out to document the attainment of a steady state.

2.1.3 Study Protocol

Guideline for Study Protocol for Bioequivalence and Bioavailability Studies in Humans ***Study Protocol***

The study protocol should be signed and dated and approved by the National Agency for Food and Drug Administration and Control (NAFDAC) before commencing the study. The study report should be submitted using the NAFDAC BTIF format. The study protocol should include the following details:

Objective

Clearly state the objective of the study, which should be based on scientific principles and ethical considerations.

Study Design

Describe the study design, including the number of subjects, the inclusion and exclusion criteria, the randomization procedure, the dose regimen, the sample collection times, and the statistical analysis plan.

Drug Products

Provide a detailed description of the test and reference drug products, including their composition, strength, and route of administration.

Pharmacokinetic Assessments

Describe the methods for measuring plasma drug concentrations, the analytical procedures, the validation criteria, and the quality control measures.

Safety and Tolerability Assessments

Describe the methods for assessing adverse events, the criteria for discontinuation of the study, and the measures to be taken in case

3.0 Pharmacokinetic comparative bioavailability (bioequivalence) studies in humans Participants

Guideline on Pharmacokinetic Comparative Bioavailability (Bioequivalence) Studies in Humans

3.1.1 Introduction

Pharmacokinetic comparative bioavailability (bioequivalence) studies in humans are conducted to compare the pharmacokinetic parameters of a test product with a reference product to establish that the

test product is therapeutically equivalent to the reference product. This guideline provides recommendations for the design, conduct, and reporting of these studies.

3.1.2 Screening

Subjects should be appropriately screened and selected based on the inclusion and exclusion criteria defined in the study protocol. The number of subjects should be determined by the error variance associated with the primary parameters to be studied, the significance level desired, the statistical power desired, the mean deviation from the comparator product compatible with bioequivalence and with safety and efficacy, and the need for the 90% confidence interval around the geometric mean ratio to be within bioequivalence limits.

In a cross-over study, each subject should be given the test product and the reference product in randomized order, and an adequate wash-out period should follow the administration of each product. In a parallel design study, the interval between the administration of the test product and the reference product should not be less than five terminal elimination half-lives of the active compound or metabolite, if the latter is measured.

3.1.3 Sample Collection and Analysis

Blood sampling should be conducted for up to 72 hours following administration, and sampling beyond this time is generally not necessary for immediate-release products. The dosage regimen used in multiple dose studies should follow the usual dosage recommendation. The analytical sensitivity should be adequate to adequately characterize the pharmacokinetic profile after a single dose, and appropriate dosage administration and sampling should be carried out to document the attainment of a steady state.

3.1.4 Study Reporting

The study report should be submitted using the National Agency for Food and Drug Administration and Control (NAFDAC) Bioavailability and Bioequivalence Trial Information Format (BTIF). The report should include the study protocol, the statistical analysis plan, the results of the study, and a discussion of the results.

Pharmacokinetic comparative bioavailability (bioequivalence) studies in humans play a critical role in establishing the therapeutic equivalence of a test product with a reference product. By following the

guidelines outlined in this document, researchers can design, conduct, and report these studies in a manner that is scientifically rigorous and meets the regulatory requirements of NAFDAC.

Guideline on Alternative Study Designs for Studies in Patients

In some cases, it may not be possible or ethical to conduct a bioequivalence study using healthy volunteers. In such situations, alternative study designs involving patients may be necessary. Here are some guidelines for alternative study designs for studies in patients:

For APIs that are very potent or too toxic to administer in the highest strength to healthy volunteers, it is recommended that the study be conducted using the API at a lower strength in healthy volunteers.

For APIs that show unacceptable pharmacological effects in healthy volunteers, even at lower strengths, a study conducted in patients may be required.

For active pharmaceutical ingredients with long elimination half-lives, a bioequivalence study with a parallel design may be more appropriate if the cross-over study is problematic. A parallel design may also be necessary when comparing some depot formulations.

For both cross-over and parallel design studies of oral products, sample collection time should be adequate to ensure completion of gastrointestinal (GI) transit of the pharmaceutical product and absorption of the API.

3.1.5 Considerations for active pharmaceutical ingredients with long elimination half-Lives

For drugs with half-lives greater than 24 hours, bioequivalence is measured using the AUC to 72 hours post-dose (AUC0-72h). It is recommended to use a single-dose, cross-over study design, where the drug is administered twice with a washout period in between. The interval between administrations should be long enough to allow the previous dose to be eliminated from the body, typically at least five terminal elimination half-lives of the active compound or metabolite. If this study design is not feasible, a bioequivalence study with a parallel design may be more appropriate. In either case, it is

important to collect blood samples for up to 72 hours following administration to ensure complete absorption and elimination.

3.2 Considerations for multiple-dose studies

When conducting clinical trials for a medication, sometimes it's necessary to administer the medication multiple times to patients. This is known as a multiple-dose study.

There are a few situations where multiple-dose studies are appropriate. For example, if the medication being tested is considered too strong or dangerous to give to healthy volunteers, then it may be necessary to give it to patients instead. It's important to follow the recommended dosage for the medication in a multiple-dose study, just like you would for a single-dose study.

Other reasons for doing multiple-dose studies include cases where the analytical sensitivity is too low to accurately measure the effects of a single dose, or when testing extended-release medications that accumulate in the body over time. In some cases, it's possible to overlap the "wash-out" period between the last dose of one medication and the beginning of a new medication, as long as there is a sufficient amount of time in between (at least five times the terminal half-life). When conducting multiple-dose studies, it's important to carefully document the results to ensure that the medication has reached a steady state.

3.3 Participants

3.3.1 Number of participants

The number of participants required for a bioequivalence study, and the factors that determine this number include:

- The error variance (coefficient of variation) associated with the primary parameters to be studied, as estimated from a pilot experiment, from previous studies, or from published data.
- The desired significance level, which is usually 5%.
- The desired statistical power.
- The mean deviation from the comparator product that is compatible with bioequivalence, safety, and efficacy.

The need for the 90% confidence interval around the geometric mean ratio to be within bioequivalence limits, which is normally 80-125% for log-transformed data.

The number of participants recruited for the study should be estimated using an appropriate method, and extra participants should also be recruited to account for expected drop-outs and withdrawals based

on the safety and tolerability profile of the API. The number of participants should always be justified by the sample size calculation provided in the study protocol, and a minimum of 12 participants per treatment is required.

In situations where reliable information about the expected variability in the parameters to be estimated is not available, a two-stage sequential study design can be used as an alternative to conducting a pilot study. However, adjustments must be made to protect the overall Type 1 error rate and maintain it at 5%. The proposed statistical plan should also be clearly defined in the study protocol, including the adjusted significance level to be used during each analysis.

3.3.2 Dropouts and withdrawals

It is important for sponsors to recruit a sufficient number of study participants to account for possible drop-outs or withdrawals. Replacing participants who drop out during the study could complicate the statistical model and analysis, so it's generally not recommended. All reasons for withdrawal, such as adverse reactions or personal reasons, must be reported in the study documentation. If a participant is withdrawn due to an adverse event after receiving at least one dose of the study medication, the participant's plasma/serum concentration data should be provided.

The concentration-time profiles of participants who exhibit pre-dose concentrations higher than 5% of the corresponding Cmax should be excluded from the statistical analysis.
The concentration-time profiles of participants who exhibit pre-dose concentrations equal to or less than 5% of the corresponding Cmax should be included in the statistical analysis without correction.

3.3.3 Exclusion of participant data

In a bioequivalence study, extreme values can have a significant impact on the study data due to the relatively small number of participants involved. However, it is generally not acceptable to exclude data unless there is a justifiable reason to do so. The study protocol should include potential reasons for excluding participant data and the procedure to be followed. Exclusion of data for statistical or pharmacokinetic reasons alone is not acceptable, and retesting of participants is not recommended.

3.3.4 Selection of participants

Clear criteria for the inclusion and exclusion of participants should be clearly defined in the study protocol for bioequivalence studies. The use of healthy volunteers is generally recommended, and both

male and female participants should be included if the product is intended for use in both sexes. Female volunteers should be assessed for potential risks, and they should be warned of any possible dangers to the fetus if they become pregnant. Urine tests should be conducted before the first and last doses of the study medication to confirm that female volunteers are not pregnant or likely to become pregnant during the study.

The age of the participants should generally fall between 18 and 55 years, and their weight should be within the normal range with a body mass index (BMI) between 18 and 30 kg/m2. Participants should not have any history of alcohol or drug abuse problems and should preferably be non-smokers. Standard laboratory tests, a medical history, and a physical examination should be used to screen participants for their suitability. Additional medical investigations may be necessary depending on the pharmacology of the active pharmaceutical ingredient (API), such as an electrocardiogram if the API has a cardiac effect. Participants' ability to understand and comply with the study protocol should be assessed.

Participants who have been treated for GI problems or convulsive, depressive, or hepatic disorders and who are at risk of recurrence during the study period should be excluded. If a parallel design study is planned, standardization of the two groups of participants is important to minimize variation not attributable to the investigational products. Special considerations for genetic phenotyping should be made for products with phenotype-linked metabolism. If the bioequivalence study aims to address specific questions, such as bioequivalence in a special population, the selection criteria should be adjusted accordingly.

3.3.5 Monitoring the health of participants during the study

To ensure the safety of participants, it is essential to monitor their health during the study and record any adverse effects, toxicity or intercurrent diseases that may arise. The investigator should judge the probability that any adverse event is due to the pharmaceutical product being tested, and all observed events must be reported, including their incidence, severity, seriousness and duration. A qualified medical practitioner licensed in the jurisdiction where the study is conducted must supervise the health monitoring before, during and after the study in accordance with GCP guidelines.

3.4 Investigational product

3.4.1 Generic pharmaceutical product

The product being tested in bioequivalence studies for registration purposes should be the same as the planned commercial product in terms of composition, quality characteristics (including stability), and manufacturing methods (including equipment and procedures). The test products should be manufactured under GMP regulations and their batch control results, lot number, manufacturing date, and expiry date (if possible) should be stated. Ideally, samples should be taken from industrial-scale batches, but if not feasible, pilot or small-scale batches may be used, provided that they are produced with the same formulation and similar equipment and process as those planned for commercial production batches. If a biobatch is less than 100,000 units, it may be accepted if it is the proposed production batch size. However, any future scale-up for production batches will require in vitro and/or in vivo data to support it.

3.4.2 Choice of comparator product

The innovator pharmaceutical product sourced from an SRA region or a secured supply chain within the country is the preferred comparator product for a generic pharmaceutical product. If the innovator product is not available, the WHO guidance for the selection of comparator pharmaceutical products for equivalence assessment of interchangeable generic products (9) can be consulted for guidance on alternative comparator products. The final determination of the suitability of the comparator product will be made by the regulatory agency, and it is recommended to consult with the agency for the choice of comparator product for a bioequivalence study that has not yet been conducted.

Before performing an equivalence study, it is recommended to determine the potency and in vitro dissolution characteristics of both the generic and the comparator pharmaceutical products. The API(s) content of the comparator product should be close to the label claim, and the difference between the two products being compared should not be more than ± 5%. If it is not possible to study batches with potencies within ± 5% due to the lack of availability of different batches of the comparator product, potency correction may be required on the statistical results from the bioequivalence study.

3.5 Selection of strength

In bioequivalence studies, the molar equivalent dose of the generic and comparator products should be used. If a series of strengths can be considered proportionally formulated, the strength with the highest sensitivity for bioequivalence assessment should be administered as a single unit, which is usually the highest marketed strength. In cases where analytical difficulties exist, a higher dose may be used, but

the total single dose should not exceed the maximum daily dose of the dosage regimen. A lower strength may be used if it is chosen for reasons of safety or if the API is highly soluble and its pharmacokinetics are linear over the therapeutic range.

3.5.1 Non-linear pharmacokinetics

When the active pharmaceutical ingredient (API) in a series of proportionally formulated drug strengths exhibits non-linear pharmacokinetics over the range of strengths, special consideration is required in selecting the appropriate strength for the bioequivalence study. For APIs with more than proportional increases in AUC with increasing dose, the highest marketed strength should be studied. For APIs with less than proportional increases in AUC due to saturable absorption, the bioequivalence study should be conducted on at least the lowest strength (or a strength in the linear range). For APIs with less than proportional increases in AUC due to limited solubility, bioequivalence studies should be conducted on both the lowest and highest strength (or a strength in the linear range).

In the case of drugs with non-linear pharmacokinetics characterized by more than proportional increases in AUC with increasing dose, the bioequivalence study should generally be conducted at the highest strength, unless safety/tolerability issues prevent the administration of the highest strength to healthy volunteers. Similarly, a higher dose may be used in case of analytical sensitivity problems. For drugs with less than proportional increases in AUC with increasing dose, bioequivalence should be established at both the highest and lowest strength (or a strength in the linear range). If non-linearity is due to saturation of uptake transporters, and the test and reference products do not contain any excipients that may affect gastrointestinal motility or transport proteins, demonstrating bioequivalence at the lowest strength (or a strength in the linear range) is sufficient. Selection of other strengths may be justified if there are analytical sensitivity problems preventing a study at the lowest strength or if the highest strength cannot be administered to healthy volunteers for safety/tolerability reasons.

3.5.2 Study standardization

To ensure consistent results and minimize variability in pharmaceutical studies, standardization of study conditions is essential. This includes controlling factors such as exercise, diet, fluid intake, and posture, as well as limiting the intake of certain substances such as alcohol, caffeine, and some fruit juices, as well as medications that may interact with the study product. Participants should also refrain from taking any non-study medications, supplements, or alcoholic beverages for a specified period before and during the study, and any emergency use of non-study medicine should be reported.

Additionally, physical activity and posture should be standardized to minimize their effects on gastrointestinal blood flow and motility. This involves maintaining a consistent pattern of activity and posture for each day of the study, and specifying the time of day when the study product is to be administered. By implementing these standardization measures, studies can ensure accurate and reliable results.

3.5.3 Co-administration of food and fluid with the dose

To ensure consistency in bioavailability studies, it is recommended that participants fast for at least 10 hours prior to FPP administration, though they may drink water during this time. One hour before administration, water consumption is not permitted. The recommended volume of water for administration with the FPP is usually between 150-250mL. Two hours after administration, participants are free to drink water as often as they wish. Four hours after administration, a standard meal is provided, with its composition specified in the study protocol and report. Note that in some cases, the investigational product may need to be administered after a meal, rather than on an empty stomach.

3.5.3.1 Immediate-release formulations

When conducting studies on investigational products, it is generally preferred to administer them to participants in a fasted state. However, if the product causes gastrointestinal disturbances in a fasted state or if the comparator product is labelled for administration in the fed state, then a fed-state study is the preferred approach. For certain types of products, such as microemulsions and solid dispersions, bioequivalence studies are required to be performed under both fasted and fed conditions, unless the product is only taken in a specific state. For immediate-release products, there may be instances where a pre-dose meal with a different caloric/fat content from the recommended meal is appropriate, and the test meal should be consumed 30 minutes before the administration of the investigational product.

3.5.3.2 Modified-release formulations

Modified release dosage forms are formulations where the rate and/or site of release of the active ingredient(s) are different from that of the immediate release dosage form administered by the same route. This deliberate modification is achieved by special formulation design and/or manufacturing methods amongs others. Modified-release products include extended-release products and delayed-release products. Extended-release products are also known as controlled-release, prolonged-release

and sustained-release products. To establish the bioequivalence of modified-release products, a single-dose, non-replicate cross-over, fasting study comparing the highest strength of the generic and the reference listed product should be performed. Single dose studies are preferred to multiple-dose studies as single-dose studies are considered to provide more sensitive measurements of the release of APIs from the pharmaceutical product into the systemic circulation. Multiple dose studies may need to be considered (in addition to a single dose study) for extended-release dosage forms with a tendency to accumulate. The reference listed product in this study should be a pharmaceutically equivalent modified release product. The pharmacokinetic bioequivalence criteria for modified-release products are basically the same as for conventional-release dosage forms.

Further clarity on complex formulations like modified release formulations; fixed dose combinations, reference products; applicant should contact the Agency.

3.5.4 Wash-out interval

The period between doses of each formulation should be long enough to allow the previous dose to be eliminated from the body. This period, known as the wash-out interval, should be consistent across all subjects and usually be more than five times the median terminal half-life of the active pharmaceutical ingredient (API). In situations where active metabolites with longer half-lives are produced, or if the elimination rate of the API has high variability between subjects, consideration should be given to extending the wash-out interval. Just prior to administration of the treatment during the second study period, blood samples should be collected and assayed to determine the concentration of the API or metabolites. The minimum wash-out interval should be at least seven days, unless a shorter period is justified by a short half-life. The adequacy of the wash-out interval can be estimated from the pre-dose concentrations of the API in the second study period, and it should be less than 5% of the observed Cmax.

3.5.5 Sampling times

Blood samples should be collected at a frequency that is sufficient for evaluating Cmax, AUC, and other relevant parameters. The sampling schedule should include a pre-dose sample, at least one to two points prior to Cmax, two points around Cmax, and three to four points during the elimination phase. For accurate determination of the maximum concentration of the API in the blood (Cmax) and terminal elimination rate constant in all subjects, at least seven sampling points will be necessary. However, for most APIs, a higher number of samples will be required to account for between-subject differences in absorption and elimination rates. Generally, sampling should continue for a duration that ensures at

least 80% of the AUC0-∞ is accrued, but should not exceed 72 hours. The duration of sample collection should be based on the nature of the API and the input function from the administered dosage form.

3.5.6 Sample fluids and their collection

Typically, blood is the primary biological fluid sampled to determine the concentrations of the API under normal circumstances. Serum or plasma is usually used to measure the API or its metabolites. If blood samples are not feasible, urine can be sampled since the API is excreted unchanged and there is a proportional relationship between plasma and urine concentrations. The volume of each urine sample must be measured at the study centre, where possible immediately after collection, and the measurements included in the report. However, in most cases, the exclusive use of urine excretion data should be avoided since this does not allow estimation of the tmax and the maximum concentration. The number of samples should be sufficient to allow the estimation of pharmacokinetic parameters. Blood, plasma, serum, and urine samples should be processed and stored under conditions that have been shown not to cause degradation of the analytes.

3.5.7 Studies of metabolites

When evaluating bioequivalence, the measured concentrations of the active pharmaceutical ingredient (API) are typically used instead of the metabolite. This is because the API's concentration-time profile is more sensitive to changes in formulation performance. However, in some rare cases, the API cannot be measured reliably due to low concentrations or instability in the biological matrix. In these situations, concentrations of the primary active metabolite may be measured instead. Adjustments to the wash-out period and sampling times may be required to adequately characterize the pharmacokinetic profile of the metabolite. It is essential to decide in advance which chemical entity (API or metabolite) will be analyzed in the samples and identify the analyte to be used in assessing bioequivalence. Choosing only one analyte carries the risk of making a type 1 error, so it is necessary to select the analyte that provides the most reliable data for the assessment. Once chosen, the analyte whose data will be used for the assessment of bioequivalence cannot be changed retrospectively.

3.5.8 Measurement of individual enantiomers

For most bioequivalence studies, a non-stereoselective assay is adequate. However, when the enantiomers exhibit different pharmacokinetic or pharmacodynamic properties, and changes in the rate of absorption alter the exposure of the enantiomers as estimated by their AUC ratio or Cmax ratio, a stereospecific assay measuring the individual enantiomers should be used.

3.6 Quantification of active pharmaceutical ingredient

Measuring the amount of active pharmaceutical ingredient and/or metabolites in biological matrices such as serum, plasma, blood, and urine requires a well-characterized, fully validated, and well documented bioanalytical method to ensure reliable results. For clinical trials on humans, bioanalytical methods and subject sample analyses should follow the principles of good clinical practice (GCP) and good laboratory practice (GLP), and adhere to current regulatory guidelines for bioanalytical method validation, such as the EMA, ICH guidelines on bioanalytical method validation.

The bioanalytical method should use state-of-the-art principles and procedures for validation and analysis of study samples. Key characteristics that must be ensured for acceptable performance and reliable analytical results include selectivity, lower limit of quantification, response function and calibration range (calibration curve performance), accuracy, precision, matrix effects, stability of the analyte(s) in the biological matrix, and stability of the analyte(s) and the internal standard throughout the entire period of storage and processing.

The analytical protocol and/or the standard operating procedure (SOP) should specify the validation procedures, methodology, and acceptance criteria. The method validation report must describe all experiments used to support the validity of the method. The analytical report should include the results of subject sample determination, calibration and QC sample results, repeat analyses, reinjections and reintegrations (if any), and a representative number of sample chromatograms.

In general, the analytical method must be able to differentiate the analyte(s) of interest and, if used, the internal standard from other components in the sample, including endogenous components in the matrix. The LLOQ should be estimated to prove that the analyte(s) can be quantified reliably with acceptable accuracy and precision. The calibration curve must be prepared in the same matrix as the subject samples, and should consist of a blank sample, a zero sample, and 6-8 non-zero samples covering the expected range. The within-run and between-run accuracy and precision should be assessed on QC samples spiked with known amounts of the analyte at a minimum of three different concentrations.

When using mass spectrometric methods, matrix effects should be investigated. The stability of the analyte in the stock solution and the matrix should be verified for every step taken during sample preparation and analysis, as well as the storage conditions used. In the case of multiple analytes, stability of the analytes in the presence of each other should be demonstrated under standard conditions. Partial validation may be acceptable if changes are made to a previously validated analytical method. Cross-validation is necessary when data are obtained from different methods within and across studies,

or when data are obtained from different laboratories applying the same method. Analysis of subject samples should be carried out only after verifying the performance of the bioanalytical method, and calibration and QC standards should be processed in the same manner and at the same time as the subject samples. Reasons for reanalysis, reinjection, and reintegration of subject samples should be predefined in the protocol, study plan, or SOP. Reinjection without any identified analytical cause is not acceptable for bioequivalence studies.

3.7 Statistical analysis

Bioequivalence assessment aims to minimize the risk of falsely declaring equivalence between multisource and comparator products. To demonstrate this, statistical analysis should be conducted during the bioequivalence trial. The statistical methods for testing bioequivalence should be specified in the protocol before data collection. The 90% confidence interval around the ratio of the log-transformed population means of the pharmacokinetic parameters under consideration should be determined, with two one-sided tests carried out at the 5% level of significance. The calculated confidence interval should fall within a predetermined bioequivalence limit to establish bioequivalence. The decision scheme should be symmetrical, regardless of which formulation is being compared.

Concentration-dependent pharmacokinetic parameters, including AUC and Cmax, should be log-transformed using consistent logarithmic bases, and the ANOVA model should include formulation, period, sequence, and subject factors. Parametric methods, based on normal distribution theory, are recommended for analyzing log-transformed bioequivalence measures.

A 90% confidence interval for μT-μR should be constructed, and pharmacokinetic equivalence can be established if the confidence interval falls within the stated limits. The antilogs of the confidence limits obtained constitute the 90% confidence interval for the ratio of the geometric means between multisource and comparator products. Descriptive statistics should be provided for tmax, with a comparison of the median and range between the test and comparator to exclude clinically relevant numerical differences. A formal statistical comparison is rarely necessary. However, if required, nonparametric methods should be used to analyze untransformed data. Only descriptive statistics should be given for parameters describing the elimination phase (t1/2).

3.8 Acceptance ranges

The acceptance ranges for measures of bioequivalence are critical for determining the comparability of a test drug product with a reference drug product. Here are some examples of acceptance ranges for bioequivalence measures:

AUC0-t Ratio:

The 90% confidence interval for this measure of relative bioavailability should fall within a bioequivalence range of 80.00–125.00%. However, if the active pharmaceutical ingredient (API) is identified as possessing a narrow therapeutic index (NTI), the bioequivalence acceptance range should be restricted to 90.00–111.11%. This same criterion applies to AUCτ in multiple-dose studies and to partial AUCs when comparative testing of a modified-release product is necessary.

Cmax Ratio:

For maximal concentration data, the acceptance limit of 80.00–125.00% should be applied to the 90% confidence interval for the mean Cmax ratio. However, since this measure of relative bioavailability is inherently more variable than AUC ratio, proving bioequivalence can be challenging in certain cases. Section 7.9.3 provides information on an approach to proving bioequivalence when the intra-subject variability for the Cmax parameter is high. If the API is determined to possess an NTI, the bioequivalence acceptance range may need to be restricted to 90.00–111.11%, if appropriate. This same criterion applies to Cmax and Ctau parameters in multiple-dose studies.

Tmax Difference:

Statistical evaluation of tmax only makes sense if there is a clinically relevant claim for rapid onset of action or concerns about adverse effects. If that's the case, then comparison of the median and range data for each product should be undertaken. For other pharmacokinetic parameters, the same considerations as outlined above apply

3.9 Reporting of results

A bioequivalence study report should provide comprehensive documentation of its protocol, conduct, and evaluation in compliance with Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) rules. The report should be prepared using the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline (13) or a similar guide. The responsible investigator(s) should sign the relevant sections of the report. The report should include

the names and affiliations of the responsible investigator(s), site of the study, and period of its execution.

The report should also include the names and batch numbers of the pharmaceutical products used in the study, as well as the composition(s) of the test product(s). Results of in vitro dissolution tests conducted in media with pHs of 1.2, 4.5, and 6.8, and the QC media (if different) should be provided. The applicant should also submit a signed statement confirming that the test product is identical to the pharmaceutical product submitted for registration.
The bioanalytical validation report, including the information recommended in the relevant regulatory agency's guidance for the bioanalytical portion of the study (e.g., Section 7.5 of the NAFDAC BTIF template), should be attached.

All results should be presented clearly. The report should include tabulated results showing all measured and calculated pharmacokinetic parameters for each subject-formulation combination, along with descriptive statistics. The concentrations measured in each subject and the sampling time should be tabulated for each formulation. The tabulated results should present the date of the run, subject, study period, product administered (multisource or comparator), and time elapsed between FPP administration and blood sampling in a clear format. The procedure for calculating the parameters used (e.g. AUC) from the raw data should be stated. Any deletion of data should be documented and justified.

Individual blood concentration/time curves should be plotted on a linear/linear and log/linear scale. All individual data and results should be given, including information on subjects who dropped out. The drop-outs and/or withdrawn subjects should be reported and accounted for. All adverse events that occurred during the study should be reported together with the study physician’s classification of the events. Further, any treatments given to address adverse events should be reported. The statistical report should be sufficiently detailed to enable the statistical analyses to be repeated if necessary. If the statistical methods applied deviate from those specified in the study protocol, the reasons for the deviations should be stated.

3.9.1 Data Integrity

BE studies should be conducted according to the principles and recommendations in ICH E6, *Good Clinical Practice*. In conducting BE studies, sponsors, study investigators, and service providers, *e.g.*, contract research organisations or laboratories, should ensure that the data generated are attributable, legible, contemporaneously documented, original (or a certified copy), accurate, complete, and traceable. The ultimate responsibility for the quality and integrity of the study data submitted to a regulatory authority lies with the applicant.

4.0 Other Immediate Release Dosage Forms

4.0.1 Orally Disintegrating Tablets

Orally disintegrating tablets (ODTs) should be administered in BE studies according to the comparator product labelling with regard to intake of water.

If the comparator product labelling states that the ODT can be taken with or without water, the test and comparator products should be administered in the BE study without water, as this is considered to be the more discriminating scenario. BE of the test and comparator ODT products taken with water can then be inferred.

For new intended label use/instructions, *e.g.*, ODT as an extension to another orally administered IR drug product, BE studies may be conducted to determine whether the ODT is BE to the comparator product. In this scenario, the ODT product should be administered according to its intended labelling and compared with the comparator product administered as per its labelling.

If the new intended label use/instructions state that the ODT can be taken with and without water, a 3-arm BE study is recommended to demonstrate BE of the ODT administered with and without water compared to the comparator product administered as per its labelling.

In studies evaluating ODTs without water, it is recommended to wet the mouth by swallowing a small amount of water, *e.g.*, 20 ml, directly before applying the ODT on the tongue. It is recommended not to allow fluid intake earlier than 1 hour after administration.

Other oral formulations such as orodispersible films, buccal tablets or films, and sublingual tablets may be handled in a similar way to that described above for ODTs.

4.0.2 Chewable Tablets

Chewable tablets should be administered in BE studies according to the comparator product labelling with regard to intake of water.

If the comparator product labelling states that the chewable tablets can be taken with or without water, the test and comparator products should be administered in the BE study without water, as this is considered to be the more discriminating scenario. BE of the test and comparator chewable tablet products taken with water can then be inferred.

For new intended label use/instructions, *e.g.*, chewable tablets as an extension to another orally administered IR drug product, BE studies may be conducted to determine whether the chewable tablet is BE to the comparator product. In this scenario, the chewable tablet product should be administered according to its intended labelling and compared with the comparator product administered as per its labelling.

If the new intended label use/instructions state that the chewable tablets can be taken with and without water, a 3-arm BE study is recommended to demonstrate BE of the chewable tablets administered with and without water compared to the comparator product administered as per its labelling.

4.0.3 Oral Suspensions

For tablets, granules, and powders labelled as being only intended to be dispersed in a liquid before administration as an oral suspension, BE studies should be conducted according to the comparator product labelling.

For new intended label use/instructions, *e.g.*, oral suspensions as an extension to another orally administered IR drug product, BE studies may be conducted to determine whether the oral suspension is BE to the comparator product. In this scenario, the oral suspension product should be administered according to its intended labelling and compared with the comparator product administered as per its labelling.

4.1 Special considerations

4.1.1 Fixed dose combination products

When assessing the bioequivalence of fixed-dose combination (FDC) products through in vivo studies, the study design should adhere to the principles outlined in previous sections. The generic FDC product should be compared to the pharmaceutically equivalent comparator FDC product, and in cases where the latter is not available on the market, separate products administered in free combination can be

used as a comparator. Sampling times should be selected to ensure that the pharmacokinetic parameters of all active pharmaceutical ingredients (APIs) can be adequately assessed. The bioanalytical method should be validated to account for all analytes measured in the presence of the other analytes. The statistical analysis should be performed using pharmacokinetic data collected on all active ingredients, and the 90% confidence intervals of the test/comparator ratio for all active ingredients should be within the acceptance limits.

4.1.2 EndogenousCompounds

In some cases, endogenous compounds are identical to the drug that is being administered. For these drugs, it can be challenging to determine the amount of drug released from the dosage form and absorbed for BE assessment. Therefore, in most cases, it is important to measure the baseline endogenous concentrations in biological matrices, *e.g.*, blood, plasma, or urine, and subtract these concentrations from the total concentrations measured from each subject after the drug product is administered.

When the endogenous concentrations are influenced by diet, restricting or standardising the dietary intake of the substance before and during the study should be considered.

The exact method for baseline correction should be pre-specified and justified in the study protocol. Multiple baseline endogenous concentrations should be measured from each subject in the time period before administration of the study drug. The time-averaged baseline or time-matched baseline concentrations are subtracted from post-dose concentrations for those subjects in an appropriate manner consistent with the PK properties of the drug. For the time-averaged method, either the mean or median value may be used.

Baseline concentrations should be determined for each period and baseline correction should be period specific. It should be ensured that the washout period is of an adequate duration because carry-over effects cannot be readily detected. If a baseline correction results in a negative concentration value, the value should be set to zero.

PK and statistical analyses should be performed on both baseline uncorrected and baseline corrected data. In general, determination of BE should be based on the baseline corrected data.

When considered necessary to ensure adequate separation of treatment-induced concentrations over baseline, a high dose may be administered in BE studies of endogenous compounds if the dose is well tolerated and dose proportionality in PK is maintained. Alternatively, the need for baseline correction may be avoided by enrolling study subjects with low or no production of the endogenous compounds.

4.2 Clinically important variations in bioavailability

Effective pharmaceutical formulations should maintain acceptable bioavailability characteristics to ensure interchangeability of the products. If an innovator develops a new formulation with favorable bioavailability, it should be used as the comparator product. However, a newly developed formulation that does not fall within the acceptable range of bioavailability of an existing pharmaceutical product is not interchangeable by definition. Innovators should prioritize developing formulations with optimal bioavailability to ensure the effectiveness and interchangeability of their products.

3.10.3 “Highly variable active pharmaceutical ingredients”

A "highly variable API" refers to an API with intrasubject variability of more than 30% in terms of ANOVA CV. Bioequivalence studies involving highly variable APIs can be challenging as larger subject groups are needed to achieve adequate statistical power because the wider the 90% confidence interval, the higher the ANOVA CV. Although regulatory authorities have different approaches to dealing with highly variable APIs, the most rigorous of the current methods involves scaling bioequivalence acceptance criteria based on the intrasubject standard deviation observed in the relevant parameters for the comparator product.

For highly variable FPPs, it is recommended to conduct a three-way partial replicate or a four-way fully replicated cross-over bioequivalence study and use reference-scaled average bioequivalence to widen the acceptance interval for the Cmax parameter if the intrasubject variability for Cmax following replicate administrations of the comparator product is greater than 30%. The acceptance criteria for Cmax can be widened to a maximum of 69.84-143.19% in this case, and the applicant must justify that the calculated intrasubject variability is a reliable estimate and not the result of outliers.

The extent of the widening of the acceptance interval for Cmax is determined by the intrasubject variability seen in the bioequivalence study using scaled average bioequivalence according to [U, L] = exp [± k·sWR], where U is the upper limit of the acceptance range, L is the lower limit of the acceptance range, k is the regulatory constant set to 0.760, and sWR is the intrasubject standard deviation of the log-transformed values of Cmax of the reference product. The geometric mean ratio (GMR) for Cmax should lie within the conventional acceptance range of 80.00-125.00%. The standard bioequivalence acceptance criterion for AUC should be maintained without scaling.

If the intrasubject variability for Cmax following replicate administration of the comparator is less than 30%, standard bioequivalence acceptance criteria should be applied to both AUC and Cmax without scaling. For multiple-dose studies, a similar approach can be applied to the Cmax, Ctau, and partial AUCs if required, if the intrasubject variability for the parameter is greater than 30%. The standard bioequivalence acceptance criterion will apply to AUCτ without scaling. The study protocol should clearly define the approach to be employed prospectively.

5.0 In vitro equivalence testing

5.1 In vitro equivalence testing in the context of the Biopharmaceutics Classification System

All claims for a Biowaiver must be supported by scientific data and/or by initro testing. The results and procedures must be submitted alongside other regulatory requirements for review. This does not preclude the submission of a Biowaiver application form (BAF).

5.1 .1 Biopharmaceutics Classification System

The Biopharmaceutics Classification System (BCS) is a system used to classify active pharmaceutical ingredients (APIs) based on their aqueous solubility and intestinal permeability. The BCS categorizes APIs into one of four classes:

- Class 1: APIs with high solubility and high permeability
- Class 2: APIs with low solubility and high permeability
- Class 3: APIs with high solubility and low permeability
- Class 4: APIs with low solubility and low permeability

Based on the solubility and permeability of the API, excipient nature, excipient content, and dissolution characteristics of the dosage form, the BCS approach provides an opportunity to waive in vivo bioequivalence testing for certain categories of immediate-release finished pharmaceutical products (FPPs). Immediate-release dosage forms can be categorized as having "very rapid," "rapid," or "not rapid" dissolution characteristics.

However, it's important to note that oral FPPs containing an API with a narrow therapeutic index are not eligible for a biowaiver based on the BCS approach. Understanding the BCS is important for pharmaceutical companies and regulatory agencies as it can help streamline the drug development process and regulatory approval of generic drugs.

5.1.2 High solubility

A substance is classified as having high solubility if the highest single therapeutic dose, as defined by the labeling for the innovator product, is soluble in 250 mL or less of aqueous media over the pH range of 1.2-6.8. To determine the pH solubility profile of the substance, it should be tested in aqueous media at 37 ± 1°C, with a minimum of three replicate determinations of solubility at each pH condition recommended.

5.1.3 High permeability

An API is considered highly permeable when the extent of its absorption in humans is 85% or more, as determined by mass balance or in comparison with an intravenous comparator dose. Ideally, this study should be conducted at the same dose used for solubility classification, and dose linearity should be established to justify the use of other doses.

In vivo intestinal perfusion in humans is an acceptable alternative test method for permeation studies. The methodology's suitability should be demonstrated, including determining permeability relative to that of a reference compound whose absorbed fraction is at least 85%, and a negative control. Supportive data may be provided by in vivo or in situ intestinal perfusion using animal models or in vitro permeation across a monolayer of cultured epithelial cells, although neither method would be considered acceptable on a stand-alone basis.

An API's high permeability can also be evaluated relative to a series of reference compounds with documented permeabilities and absorbed fractions, including some with at least 85% fraction of dose absorbed. Absolute bioavailability or mass balance data obtained from published literature may be accepted as evidence, provided that the studies were appropriately designed and conducted.

5.2 Determination of dissolution characteristics of generic products in consideration of a biowaiver based on the Biopharmaceutics

The determination of the dissolution characteristics of a generic product, with consideration of a biowaiver based on the biopharmaceutics classification system (BCS), is required to exempt the product from an in vivo bioequivalence study. For an immediate release, generic product to be exempted, it should exhibit very rapid or rapid in vitro dissolution characteristics, depending on the BCS properties of the active pharmaceutical ingredient (API).

In addition to rapid dissolution, the in vitro data should demonstrate the similarity of dissolution profiles between the generic and comparator products. Dissolution data obtained from published

literature may be accepted as evidence if it is clearly established that the data were derived from appropriately designed studies.

5.2.1 Very rapidly dissolving

A product is considered to be very rapidly dissolving when at least 85% of the labeled amount of the API dissolves within 15 minutes under the following conditions: at 37 ± 1°C using either a paddle apparatus at 75 rpm or a basket apparatus at 100 rpm in a volume of 900 mL or less in each of the following media:

- pH 1.2 HCl solution or buffer
- pH 4.5 acetate buffer
- pH 6.8 phosphate buffer

It is recommended to use pharmacopoeial buffers (e.g., Ph. Int.) at these three pH values. The dissolution media should not contain surfactants, and enzymes (pepsin at pH 1.2 and pancreatin at pH 6.8) may be used if the pharmaceutical product contains gelatin (e.g., capsules or caplets) due to the possibility of cross-linking.

5.2.2 Rapidly dissolving

When a product is described as "rapidly dissolving," it means that at least 85% of the labeled amount of the active pharmaceutical ingredient (API) dissolves within 15 minutes under specific conditions. These conditions include using a paddle or basket apparatus at 75-100 rpm in a volume of 900 mL or less in each of the following media: pH 1.2 HCl solution or buffer, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer. It is recommended to use pharmacopoeial buffers at these three pH values, and surfactants should not be present in the dissolution media. In cases where the pharmaceutical product contains gelatin (e.g. capsules or caplets), enzymes such as pepsin at pH 1.2 and pancreatin at pH 6.8 may be used due to the possibility of cross-linking.

For a generic product, the definition of "rapidly dissolving" remains the same, but the dissolution time is extended to 30 minutes under the same conditions.

Here are the guides rephrased in a new format:

- A product is considered "rapidly dissolving" when at least 85% of the labeled amount of the API dissolves within 15 minutes under specific conditions using a paddle or basket apparatus and specific buffers, without surfactants present in the dissolution media. Enzymes may be used if the product contains gelatin.

For multisource products, the definition of "rapidly dissolving" is the same as above but extends to 30 minutes.

6.0 Qualification for a biowaiver based on the Biopharmaceutics Classification

System

The Biopharmaceutics Classification System (BCS) can be used to determine if a biowaiver is appropriate for a drug product. The decision to waive bioequivalence testing in favor of in vitro methods depends on a risk-benefit analysis of the drug's solubility and intestinal permeability, dissolution profiles in various media, excipients used in the formulation, and potential risks to public health and individual patients. In cases where there is an acceptable risk-benefit balance, in vitro methods can be used as a test of product equivalence.

6.1 In vitro equivalence testing based on dose-proportionality of formulations

The approval of different strengths of a generic or multisource product can be considered on the basis of dissolution profiles if the formulations have proportionally similar compositions. In certain circumstances, in vivo bioavailability or bioequivalence data requirements may be waived, and in vitro data may be accepted in lieu of in vivo data. For example, an in vivo data requirement may be waived for different strengths of an immediate-release drug product when the new strength is proportionally similar in its active and inactive ingredients to another drug product for which the same manufacturer has obtained approval and meets an appropriate in vitro test as outlined in the regulation. Additionally, linearity of the pharmacokinetics over the therapeutic dose range should be demonstrated for waiving higher strengths.

6.2 Proportional formulations

Proportional formulations can be defined in two ways based on the strength of dosage forms: (i) all active and inactive ingredients are in exactly the same proportion in different strengths, and
(ii) for high-potency drug substances, the total weight of the dosage form remains nearly the same for all strengths, the same inactive ingredients are used for all strengths, and the change in any strength is obtained by altering the amount of the active ingredients and one or more of the inactive ingredients.

For bilayer tablets, both layers should be proportionally similar. Exceptions to the above definitions may be possible if adequate justification is provided and discussed with the appropriate review division. It is important to note that if only one layer is proportionally similar and the other is not, the products (whole tablet) are not considered proportionally similar, as there can be interactions between the different tablet layers.

6.2.1 Qualification for a biowaiver based on dose-proportionality of Formulations

6.2.1.1 Immediate-release tablets

Biowaivers for multisource immediate-release tablet products may be granted based on dose proportionality if certain criteria are met. These include conducting an in vivo equivalence study on at least one strength of the product, ensuring all strengths have proportionally similar formulations to the strength studied, and demonstrating similar dissolution profiles for different strengths at pH 1.2, 4.5, 6.8, and for the QC media. If both strengths release 85% or more of the API label amount in 15 minutes, a profile comparison with an f_2 test is unnecessary. A bracketing approach can be used for immediaterelease dosage forms with several strengths that deviate from proportionality, and if one strength is approved based on a BCS-based biowaiver, other strengths in the series should also be assessed based on BCS-based biowaivers.

6.2.1.2 Delayed-release tablets and capsules

The following guide pertains to delayed-release tablets and capsules in generic products. In the case of delayed-release tablets, a lower strength may be granted a biowaiver if it exhibits similar dissolution profiles to the studied strength, with an f_2 score of ≥ 50, and the gastro-resistant coating proportion is the same. The recommended test condition for delayed-release products is a dissolution test in pH 1.2 acid medium for 2 hours followed by dissolution in pH 6.8. For delayed-release capsules, if different strengths are achieved by adjusting the number of API-containing beads, a biowaiver can be granted if the dissolution profile of the lower strength is similar to that of the approved strength with an f_2 score of >50 under the same test conditions recommended for delayed release products.

6 .2.1.3 Extended-release tablets and capsules

For extended-release tablets and capsules, a biowaiver can be granted for lower strengths if they exhibit similar dissolution profiles to the highest strength or approved strength, $f_2 \geq 50$, in the recommended test conditions for the specific product. In the case of extended-release tablets with an osmotic pump

release mechanism and extended-release beaded capsules where different strengths are achieved by adjusting the number of API-containing beads, a dissolution profile comparison under one recommended test condition is sufficient for a biowaiver based on dose proportionality of formulation. It is also recommended to conduct in vivo bioequivalence studies with the highest proposed strength for a series of strengths of a multisource product that are proportionally similar in active and inactive ingredients and have the same API release mechanism.

6.2.4 Reporting of biowaiver request

To request a biowaiver based on the BCS, a comparison of dissolution profiles between two products can be conducted using a model-independent mathematical approach, such as the f_2 test. The dissolution profiles of the reference strength and the additional strength should be measured under the same test conditions and sampling times. For example, immediate release products should be measured at 5, 10, 15, 20, 30, 45, and 60 minutes, while 12-hour and 24-hour extended-release products should be measured at 1, 2, 4, 6, 8, 12, and 16 hours for the 24-hour product. To make a biowaiver request, use the appropriate reporting format: Biowaiver Application Form: BCS for BCS-based requests and Biowaiver Application Form: Additional Strength for additional strength-based requests.

Appendix 1: Recommendations for conducting and assessing comparative dissolution profiles

The guide provides recommendations for conducting and assessing comparative dissolution profiles of finished pharmaceutical products. To ensure a scientifically sound comparison, dissolution measurements of test and comparator products, or two different strengths of the same product, should be made under the same test conditions. A minimum of three time points should be included, with the sampling intervals being short for immediate-release dosage forms, and longer for extended-release products. At least three media covering the physiological range, including pH 1.2 hydrochloric acid, pH 4.5 buffer, and pH 6.8 buffer, should be used.

If both the test and comparator products dissolve more than 85% of API in 15 minutes, they are considered similar, and no calculations are required. Otherwise, the similarity of the profiles should be calculated using the f_2 equation. An f_2 value between 50 and 100 suggests similarity between the two dissolution profiles. Mean dissolution values can be used to estimate the similarity factor, f_2, with the percentage coefficient of variation being not more than 20% at time points up to 10 minutes, and not more than 10% at other time points. F_2 equation should be included.

Surfactants should be avoided in comparative dissolution testing, and when the API is not soluble in any of the media, profiles in the absence of surfactant should be provided, with the rationale for the choice and concentration of surfactant being provided for other cases. The concentration of the surfactant should be such that the discriminatory power of the test is not compromised.

In summary, to assess comparative dissolution profiles of finished pharmaceutical products, it is recommended to use the same test conditions for both the test and comparator products, with a minimum of three time points and short sampling intervals for immediate-release dosage forms, and longer intervals for extended-release products. At least three media should be used, and surfactants should be avoided. Similarity between the two dissolution profiles is determined by the f_2 equation, with a value between 50 and 100 indicating similarity. When the API is not soluble in any of the media, profiles in the absence of surfactant should be provided, with the rationale for the choice and concentration of surfactant being provided for other cases.

Appendix 2: Equilibrium solubility experiments for the purpose of classification of active pharmaceutical ingredients according to the biopharmaceutics classification system

The Biopharmaceutics Classification System (BCS) categorizes active pharmaceutical ingredients (APIs) based on their solubility and permeability. The recommended method for determining solubility is described below. The BCS classification and qualification of multisource products for a biowaiver based on the BCS is further explained in the Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. This information is based on the Proposal to waive in vivo bioequivalence requirements for WHO Model List of Essential Medicines immediaterelease, solid oral dosage forms, the Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability, and the Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system.

Recommendations for conducting experiments for assessing solubility of APIs

- To ensure accurate results, a detailed protocol for a solubility study should be prepared before the experiment. The protocol should describe the equipment and procedures in detail, including sample preparation, experimental conditions such as temperature, method and rate of agitation, solid/solution separation of the API, and sample analysis. Record the source and purity of the API, as well as the methods used to characterize the material.
- Prior to the experiment, characterize the solid API as depth of the characterization depends on existing knowledge of the solid-state properties. It may be necessary to characterize the solid starting material and the solid residue remaining after equilibrium has been reached and sampling has been completed.
- The preferred method for determining equilibrium solubility is the shake-flask method, although other methods are acceptable if justified. Conditions employed should be fully described in the study protocol. pH-solubility profiles of the API should be determined over the pH range of 1.2–6.8 at 37 ± 1 °C. Measurements should be made in triplicate under at least three pH conditions, pH 1.2, 4.5, and 6.8, as well as at the pH of any known solubility minima in aqueous media within that pH range. The use of pharmacopoeial buffer solutions is recommended.

- The method of solid/solution separation employed, including details such as filter type and pore size or centrifugation speed, should be provided in the study protocol. Sedimentation, centrifugation, and filtration are the standard methods of separation.
- A validated, stability-indicating analytical method should be used for determining the solubility of APIs, such as high-performance liquid chromatographic analysis or an alternative, validated stability-indicating assay.
- A study report should be prepared detailing the actual experimental conditions, results (raw data plus mean values with standard deviations), and observations such as the degradation of an API as a result of pH or buffer composition. The section describing the experimental conditions should include initial and equilibrium pH of solutions and de facto buffer concentrations. If applicable, filter adsorption studies should be documented.
- Finally, the dose/solubility ratio should be calculated as follows: highest single therapeutic dose (mg) divided by solubility (mg/mL). An API is considered highly soluble when the highest single therapeutic dose is soluble in 250 mL or less of aqueous media over the pH range of 1.2–6.8, i.e. the dose/solubility ratio is ≤ 250.

ANNEX I

PRESENTATION OF BIOEQUIVALENCE TRIAL INFORMATION

BIOEQUIVALENCE TRIAL INFORMATION

General Instructions:

Please review all the instructions thoroughly and carefully prior to completing the bioequivalence trial information form (BTIF). Neither the format nor the content of the document (text and tables) should be changed. New tables can be inserted but it should maintain the format of the tables in the form.

Provide as much detailed, accurate and final information as possible. Note that the greyed areas are NOT to be completed in by the applicant but are for NAFDAC use only.

Please state the exact Common Technical Document (CTD) location (Modular number, Annex number, Page number) of appended documents in the relevant sections of the BTIF. This procedure must be followed throughout the entire document where location of annexed documents is requested.

Before submitting the completed BTIF, kindly check that you have provided all requested information and enclosed all requested documents.

Should you have any questions regarding this Form, please contact us at registration@nafdac.gov.ng with the subject prefix "BTIF-...".

A properly filled out BTIF in MSWord format along with all its annexes must be placed in Module One of the Common Technical Document (CTD) and submitted as CD-ROM to Registration and Regulatory Affairs Directorate, NAFDAC (detail of address provided below). A signed cover letter affirming the authenticity of the information provided in the BTIF should be submitted along with the CD-ROM.

CONFIDENTIAL

Director General (NAFDAC)

Attention: The Director, Registration and Regulatory Affairs

Product Name:

NAFDAC

Plot 1 Isolo Industrial Area

Apapa-Oshodi Expressway
Isolo, Lagos

BIOEQUIVALENCE TRIAL INFORMATION

1 SUMMARY

1.1 Summary of bioequivalence studies performed

(Provide a brief description of each comparative bioavailability study included in the submission)

1.2 Tabulation of the composition of the formulation(s) proposed for marketing and those used for bioequivalence studies

(State the location of the master formulae in the quality part of the submission)

(Tabulate the composition of the biobatch using the table below. For solid oral dosage forms the table should contain only the ingredients in tablet core /contents of a capsule. A copy of the table should be filled in for the film coating / hard capsule, if any.

***Important**: If the formulation proposed for marketing and those used for bioequivalence studies are not identical, copies of this table should be filled in for each formulation with clear identification in which bioequivalence study the respective formulation was used.)*

Composition of the batches used for bioequivalence studies					
Batch number					
Batch size (number of unit doses)[1]					
Comments, if any					
Comparison of unit dose compositions and of clinical FPP batches (duplicate this table for each strength, if compositions are different)					
Ingredients (and quality standard)	Function	Unit dose (mg)	Unit dose (%)	Biobatch (kg)	Biobatch (%)
Total					
Equivalence of the compositions or justified differences					
Maximum intended commercial batch size					

[1] Bioequivalence batches should be at least of pilot scale (10% of production scale or 100,000 capsules/tablets whichever is the greater) and manufacturing method should be the same as for production scale.

2 CLINICAL STUDY REPORT

a) Study number:

b) Study title:

c) Location of study protocol:

d) Start and stop dates for each phase of the clinical study:

e) Dates of product administration:

2.1 ETHICS

a) State the name of review committee, date of approval of protocol and consent form and the location of approval letter in the submission

b) State location of a reference copy of the informed consent form

2.2 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

a) Name of principal investigator(s) (State location of c.v. in the submission)

b) Clinical Facility (Name and full mailing address)

c) Clinical Laboratories (Name and full mailing address)

d) Analytical Laboratories (Name and full mailing address)

e) Company performing pharmacokinetic/statistical analysis (Name and full mailing address)

2.3 STUDY OBJECTIVES

Briefly state the study objectives.

2.4 INVESTIGATIONAL PLAN

2.4.1 Overall study design and plan —description

(Describe the type of study design employed in 1-2 sentences)

2.4.2 Selection of study population

2.4.2.1 Inclusion Criteria

(List the inclusion criteria applied to subjects)

2.4.2.2 Exclusion Criteria

(List the exclusion criteria applied to subjects)

2.4.2.3 Health Verification

(State location of the individual data included in the submission)

a) List criteria used and all tests performed in order to judge health status

b) Indicate when tests were performed

c) Study site normal values

d) (State location in submission of study site normal values for blood clinical chemistry, haematology, and urinalysis clinical screen)

e) Report any results that were outside of study site normal values

f) (State location in submission of the summary of anomalous values)

2.4.2.4 Removal of Trial subjects from Trial or Assessment

a) Number of subjects enrolled in the study
(All subjects including alternates, withdrawals, and dropouts)

b) Alternates
(Please note: Generally all subjects enrolled in the study should be included in the data set i.e., alternate subjects are strongly discouraged. However, in cases where there are alternate subjects, describe the procedure of including/excluding the alternates and whether alternates have been included in the study)

c) Withdrawals/dropouts
(Identify each withdrawal/dropout by subject and provide the reason for withdrawal/dropout and at what point in the study the withdrawal/dropout occurred)

2.4.3 Products Administered

2.4.3.1 Test Product

a) Batch number, size, date of manufacture and expiry date for the test product

b) Potency (measured content) of test product as a percentage of label claim as per validated assay method

c) *(This information should be cross-referenced to the location of the certificate of analysis in the submission)*

2.4.3.2 Comparator (Reference) Product

(Append to this template a copy of product labelling (snap shot of the box, on which the name of the product, name and address of the manufacturer, batch number, and expiry date are clearly visible on the labelling)

a) Name and manufacturer of the comparator product and market where the comparator product was purchased

b) Batch number and expiry date for the comparator product

c) Purchase, shipment, storage of the comparator product
(Indicate from which company/pharmaceutical distributor the comparator product has been obtained. Clearly indicate in chronological order the steps and dates of shipment/transport from company of purchase to the study site. In addition, the storage conditions should be given. This information should be cross-referenced to location in submission of documents (e.g. receipts) proving conditions)

d) Potency (measured content) of the comparator product as a percentage of label claim, as measured by the same laboratory and under the same conditions as the test product

e) *(This information should be cross-referenced to the location of the certificate of analysis in the submission)*

f) Justification of choice of comparator product

g) *(Provide short summary here and cross-reference to location of comprehensive justification in* study protocol)

2.4.4 Selection of doses in the study

a) State dose administered
(Indicate the number of dosage units comprising a single dose, e.g., 400 mg as 1 x 400 mg or 2 x 200 mg tablets)

2.4.5 Selection and Timing of Dose for Each Subject

a) State volume and type of fluid consumed with dose

b) Interval between doses*(i.e., length of washout)*

c) Protocol for the administration of food and fluid

d) Restrictions on posture and physical activity during the study

2.4.6 Blinding

2.4.6.1 Identify which of the following were blinded. If any of the groups were not blinded, provide a justification for not doing so

a) study monitors: Yes ☐ / No ☐ If No, justify:

b) subjects: Yes ☐ / No ☐ If No, justify:

c) analysts: Yes ☐ / No ☐ If No, justify:

2.4.6.2 Identify who held the study code and when the code was broken

2.4.7 Drug Concentration Measurements

2.4.7.1 Biological fluid(s) sampled

2.4.7.2 Sampling protocol

a) Number of samples collected per subject

b) Volume of fluid collected per sample

c) Total volume of fluid collected per subject per phase of the study

d) List the study sampling times

e) Identify any deviations from the sampling protocol

f) (State location of summary in the submission)

g) (Describe and explain reasons for deviations from sampling protocol. Comment on impact on study. Indicate whether the deviations were accounted for in the pharmacokinetic analysis)

2.4.7.3 Sample Handling

a) Describe the method of sample collection

b) Describe sample handling and storage procedures

2.5 Comments from review of Section 2 – NAFDAC use only

3 TRIAL SUBJECTS

3.1 Demographic and other baseline characteristics

a) Identify study population (i.e., normal, healthy adult volunteers or patients)

b) Summary of ethnic origin and gender of subjects

c) Identify subjects noted to have special characteristics and state notable characteristics

d) Range and mean age ± SD of subjects

e) Range and mean height and weight ± SD of subjects

f) Identify subjects whose ratio is not within 15% of the values given on a standard height/weight table

3.2 Subjects who smoke

a) Number of smokers included in the study

b) Indicate how many cigarettes smoked per day per subject

c) Comment on the impact on study

3.3 Comments from review of Section 3 – NAFDAC use only

4 PROTOCOL DEVIATIONS

4.1 Protocol deviations during the clinical study

(Describe any such deviations and discuss their implications with respect to bioequivalence)

4.2 Comments from review of Section 4 – NAFDAC use only

5 SAFETY EVALUATION

5.1 Identify adverse events observed

(List any adverse events by subject number. State whether a reaction occurred following administration of the test or reference product, identify any causal relationships, and note any treatments required. State location of this summary in the submission.)

(Discuss the implications of the observed adverse events with respect to bioequivalence.)

5.2 Comments from review of Section 5 – NAFDAC use only

6 EFFICACY EVALUATION

EFFICACY RESULTS AND TABULATIONS OF INDIVIDUAL TRIAL SUBJECTS DATA

6.1 Presentation of data

a) State location in submission of tables of mean and individual subject concentrations

b) State location in submission of (mean and individual) linear and semi-logarithmic subject drug concentration vs. time plots

6.2 Pharmacokinetic (PK) parameters

a) State how the pharmacokineticparameters where calculated/obtained for AUC_{0-inf}, AUC_{0-t}, C_{max}, tmax, the elimination rate constant, and $t_{½}$ (indicate location of description in protocol)

b) State whether actual sampling time points were used for estimation of the pharmacokinetic parameters

c) Complete the table below

	Test			Reference		
Parameter	Arithmetic mean	Standard deviation	Interindividual coefficient of variation (%)	Arithmetic mean	Standard deviation	Interindividual coefficient of variation (%)
AUC0-t (units)						
AUC0-inf (units)						
Cmax (units)						
tmax (units)						
t½ (units)						

d) Ratio of AUC_{0-t} to AUC_{0-inf}

(State mean ratio for both test and reference, state location in submission where individual ratios can be found)

6.3 Statistical analysis

(State the method of calculation of the 90% confidence intervals for the ratio of test formulation over the reference formulation and indicate how treatment, period, sequence and subjects within sequence were included as factors in the ANOVA. Provide the following results from the ANOVA (parametric) on the logarithmically transformed $AUC_{0-t}$and C_{MAX} and other relevant parameters. State software used for computing ANOVA.)

a) Geometric means, results from ANOVA, Degrees of Freedom (DF) and derived CV (intra-subject)

Parameter	Test	Reference	% Ratio of geometric means	90 % Confidence interval	DF	CV (%)
AUC0-t (units)						
AUC0-inf (units)						
Cmax (units)						

b) Comparison of the results

(Compare the results, including mean values, inter- and intra-individual variability, of this study with published results (literature, product information of reference product (innovator), WHOPARs, etc), and copies of the references used should be appended to this document)

6.4 Discussion of results

(State location of the discussion of the results in the submission)

6.5 Comments from review of Section 6 – NAFDAC use only

7 ANALYTICAL VALIDATION REPORT

7.1 Analytical technique

7.1.1 Validation protocol

(State the location of the validation protocol)

7.1.2 Identify analyte(s) monitored

7.1.3 Comment on source and validity of reference standard

7.1.4 Identify internal standard

7.1.5 Comment on source and validity of internal standard

7.1.6 Identify method of extraction

7.1.7 Identify analytical technique or method of separation employed

7.1.8 Identify method of detection

7.1.9 Identify anticoagulant used ***(if applicable)***

7.1.10 If based on a published procedure, state reference citation

7.1.11 Identify any deviations from protocol

7.2 Selectivity

(Address the methods to verify selectivity against endogenous/exogenous compounds & results)

7.3 Sensitivity

(Address the methods to verify sensitivity & results)

7.4 Carry-over

(Summarize the method to verify carry-over & results)

7.5 Standard curves

(State location in submission of tabulated raw data and back calculated data with descriptive statistics)

a) List number and concentration of calibration standards used

b) Describe the regression model used including any weighting

c) List the back-calculated concentrations of the calibration standards of the validation runs *(highlight the values outside of the acceptance range, e.g., 15%, except 20% for LLOQ)*

7.6 Quality control samples

a) Identify the concentrations of the QC samples and the storage conditions employed prior to their analysis

7.7 Precision and accuracy during validation

a) Summarize inter-day/inter-run accuracy and precision of the calibration standards during assay validation

b) Summarize inter-day/inter-run accuracy and precision of the calibration standards during assay re-validation

(If applicable)

c) Summarize inter-day/inter-run and intra-day/intra-run accuracy and precision of the QC samples during assay validation

d) Summarize inter-day/inter-run and intra-day/intra-run accuracy and precision of the QC samples during assay re-validation

e) (If applicable)

7.8 Dilution integrity

(Summarize the method to verify dilution integrity& results)

7.9 Matrix effect (in case of MS detection)

(Summarize methods to verify the matrix effect & results)

7.10 Stability

(For each section provide the location of the raw data, a description of the methodology employed and a summary of the data.)

a) Summarize data on long-term storage stability

b) Summarize data on freeze-thaw stability

c) Summarize data on bench top stability

d) Summarize data on auto-sampler storage stability

e) Summarize data from any other stability studies conducted

f) (e.g. long-term stock solution and working solution stability, short-term stock solution and working solution stability, dry-extract stability, wet-extract stability, stability in blood before sample processing)

7.11 Re-injection reproducibility

(Summarize the method to verify re-injection reproducibility& results)

7.12 Comments from review of Section 7 – NAFDAC use only

8 BIOANALYTICAL STUDY REPORT

(State the location of the bioanalytical report for the analysis of the study subject samples)

8.1 Analytical technique

(Confirm whether the method is the same as the validated methodand whether the same equipment was employed. Identify any differences between the validated method described above in Section 7 and the method employed for subject sample analyses)

8.1.1 Analytical protocol

(State the location of the analytical protocol)

8.1.2 Identify any deviations from protocol

8.1.3 Dates of subject sample analysis

8.1.4 Longest period of subject sample storage

(Identify the time elapsed between the first day of sample collection and the last day of subject sample analysis)

8.1.5 State whether all samples for a given subject were analysed together in a single analysis run

8.2 Standard curves

(State location in submission of tabulated raw data and back calculated data with descriptive statistics)

a) List number and concentration of calibration standards used

b) State number of curves run during the study *(valid and failed runs, including reasons of failure)*.

c) Summarize descriptive data including slope, intercept, correlation coefficients

d) List the back-calculated concentrations of the calibration standards of the study runs *(highlight the values outside of the acceptance range, e.g., 15%, except 20% for LLOQ)*

8.3 Quality control samples

a) Identify the concentrations of the QC samples, their date of preparation and the storage conditions employed prior to their analysis

b) State the number of QC samples in each analytical run per concentration

c) List the back-calculated concentrations of the QC samples of the study runs *(highlight the values outside of the acceptance range, e.g., 15%)*

d) Discuss whether the concentrations of the QC sample concentrations are similar to the concentrations observed in the study samples

e) State the percentage of QC samples per run with respect to the total number samples assayed in each run

8.4 Precision and accuracy

a) Summarize inter-day precision of back-calculated standards and inter-day and intra-day precision and accuracy of QC samples analysed during subject sample analysis

8.5 Repeat analysis (re-analysis, re-injection and re-integration)

a) List re-analysed samples by sample identification and include the following information for each re-analysis: initial value; reason for re-analysis; re-analysed value(s); accepted value; and reason for acceptance

b) Report the number of re-analysis as a percentage of the total number samples assayed

c) List re-injected samples by sample identification and include the following information for each re-injection: initial value; reason for re-injection; re-injected value; accepted value; and reason for acceptance

d) Report the number of re-injections as a percentage of the total number samples assayed

e) List re-integrated chromatograms by sample identification and include the following information for each re-integration: initial value; reason for re-integration; re-integrated value(s); accepted value; and reason for acceptance

f) Report the number of re-integrated chromatograms as a percentage of the total number of samples assayed

8.6 Incurred sample reanalysis

(State location in the submission and summarize the results of incurred sample reanalysis, including the number of subject samples included in ISR and the total number of samples analysed in the study)

8.7 Chromatograms

(State the location in the submission where the sample chromatograms can be found. The chromatograms should be obtained from a minimum of two analytical batches and include at least 20% of the subjects, up to a maximum of five. A complete set includes standards, QC samples, pre-dose and post-dose subject samples for both phases. Each chromatogram should be clearly labelled with respect to the following: date of analysis; subject ID number; study period; sampling time; analyte; standard or QC, with concentration; analyte and internal standard peaks; peak heights and/or areas)

8.8 Comments from review of Section 9 – NAFDAC use only

9 QUALITY ASSURANCE

9.1 Internal quality assurance methods

(State locations in the submission where internal quality assurance methods and results are described for each of study sites (see 3.2 b-d.)

9.2 Monitoring, auditing, inspections

(Provide a list of all monitoring and auditing reports of the study, and of recent inspections of study sites by regulatory agencies. State locations in the submission of the respective reports for each study site (see 3.2 b-d.)

9.3 Comments from review of Section 10 – NAFDAC use only

10.0 CONCLUSIONS AND RECOMMENDATIONS – NAFDAC use only

DOWNLOAD LINK: BTIF-NAFDACDER-GDL-009-01 ANNEX1.docx

ANNEX 2

Application for a Biowaiver:
Biopharmaceutics Classification System (BCS)

This application form is designed to facilitate information exchange between the applicant and the NAFDAC if the applicant seeks to waive bioequivalence studies based on the Biopharmaceutics Classification System (BCS). This form is not to be used if a biowaiver is requested for additional strength(s) of a submitted product(s), in which case a separate *Biowaiver Additional Strength: Application Form* should be used.

Some active pharmaceutical ingredients (APIs) have been identified as eligible for a BCS-based biowaiver application by Stringent Regulatory Authority (SRA) or WHO Prequalification Team (PQTm). For those APIs, you are only required to provide evidence of eligibility, it may not necessary to provide data to support the BCS classification of the respective API(s) in the application i.e., data supporting the drug substance solubility or absorption/permeability class.

General instructions:

Please review all the instructions thoroughly and carefully prior to completing the current Application Form.
Provide as much detailed, accurate, and final information as possible. Note that the greyed areas are NOT to be completed in by the applicant but are for NAFDAC use only.
Please enclose the required documentation in full and state in the relevant sections of the application form the exact location (annex number) of the appended documents.
Please provide the document as an MS Word file.
Please do not paste snapshots into the document.
The appended electronic documents should be clearly identifiable by their file names, which should include the product name and annex number.
Before submitting the completed application form, kindly check that you have provided all requested information and enclosed all requested documents.
Should you have any questions regarding this procedure, please contact us at registration@nafdac.gov.ng with the subject prefix "BCS Form-..."

This form should be properly filled out in MSWord format and be placed in Module One of the Common Technical Document (CTD) along with all its annexes and submitted as CD-ROM to Registration and Regulatory Affairs Directorate, NAFDAC (detail of address provided below). A signed cover letter affirming the authenticity of the information provided in this form should be submitted along with the CD-ROM:

CONFIDENTIAL
Director General (NAFDAC)
Attention: The Director, Registration and Regulatory Affairs
Product Name:
NAFDAC
Plot 1 Isolo Industrial Area
Apapa-Oshodi Expressway
Isolo, Lagos

Administrative data

1. International Non-proprietary Name of active ingredient(s)
<< Please enter information here >>

2. Dosage form and strength
<< Please enter information here >>

3. Product NAFDAC Reference number *(if product dossier has been accepted for assessment)*
<< Please enter information here >>

4. Name of applicant and official address
<< Please enter information here >>

5. Name of manufacturer of finished product and official address
<< Please enter information here >>

6. Name and address of the laboratory or contract research organization(s) where the BCS-based biowaiver solubility and dissolution studies were conducted
<< Please enter information here >>

I, the undersigned, certify, that the information provided in this application and the attached documents is correct and true
Signed on behalf of
<***Company***>

_____ (Date)

_____ (Name and title)

JUSTIFICATION FOR A BCS BIOWAIVER

Active pharmaceutical ingredient (API)

Please confirm that the proposed product contains the same active substance (e.g. salt, ester, ether, isomer) as the comparator.

<< Please enter information here >>

Therapeutic index of the API

Please enclose a copy of the comparator product labelling and literature references employed to support that the drug does not exhibit a narrow therapeutic index for all authorized indications

<< Please enter information here >>

Pharmacokinetic properties of the API

Please enclose a copy of the literature references employed to document the pharmacokinetic (PK) properties (PK linearity or reasons for non-linearity).

<< Please enter information here >>

Dosage form

Please confirm that:

the dosage form is an immediate release product for systemic action
the posology is limited to oral administration
the administration without water is not included in the proposed posology

<< Please enter information here >>

1.0 COMMENTS FROM REVIEW OF SECTION 1 – *NAFDAC USE ONLY*

SOLUBILITY (COMPLETION OF THIS SECTION IS NOT NECESSARY IF THE API(S) ARE INCLUDED ON THE LIST OF BIOWAIVER-ELIGIBLE APIS BY AN SRA OR IN THE PQTM DOCUMENT *GENERAL NOTES ON BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS)-BASED BIOWAIVER APPLICATIONS*.)

Maximum therapeutic dose of the API

Please enclose a copy of the labelling of the comparator product to document the maximum single dose that can be administered in a single administration (e.g. two tablets together).

<< Please enter information here >>

Stability of the drug in the physiological pH range

Please discuss stability of the API in the pH range from 1.2 to 6.8 and in the gastrointestinal tract.
Please discuss the ability of the analytical method to distinguish the API from its degradation products.

<< Please enter information here >>

Method of solubility determination

Please describe method and conditions (e.g. shake flask method at 37±1ºC)
Please indicate also location of the solubility study protocol.

<< Please enter information here >>

Solubility study dates

Please indicate dates of study protocol, study conductance and study report

<< Please enter information here >>

Analytical method validation

Please summarize the results and indicate location in the documentation.

<< Please enter information here >>

Results

Please indicate location of the solubility study report.

Please fill in the following table for the necessary pH values. Add as many rows as necessary to create a solubility – pH profile

Theoretical pH	Observed pH	Adjusted pH	Individual concentration at saturation (Cs) values	Cs (mean and CV (%))	Amount that can be dissolved in 250 ml
pH 1.2	Experiment 1 Experiment 2 Experiment 3	Experiment 1 Experiment 2 Experiment 3	Experiment 1 Experiment 2 Experiment 3		
Intermediate pHs	Experiment 1 Experiment 2 Experiment 3	Experiment 1 Experiment 2 Experiment 3	Experiment 1 Experiment 2 Experiment 3		
pH 4.5	Experiment 1 Experiment 2 Experiment 3	Experiment 1 Experiment 2 Experiment 3	Experiment 1 Experiment 2 Experiment 3		
Intermediate pHs	Experiment 1 Experiment 2 Experiment 3	Experiment 1 Experiment 2 Experiment 3	Experiment 1 Experiment 2 Experiment 3		
pH 6.8	Experiment 1 Experiment 2 Experiment 3	Experiment 1 Experiment 2 Experiment 3	Experiment 1 Experiment 2 Experiment 3		
Other intermediate pH values (e.g. pKa, pKa-1, pKa+1)	Experiment 1 Experiment 2 Experiment 3	Experiment 1 Experiment 2 Experiment 3	Experiment 1 Experiment 2 Experiment 3		

Plot the solubility – pH profile

Please attach the plot of the pH-solubility profile based on the above data

<< Please enter information here >>

2.0 COMMENTS FROM REVIEW OF SECTION 2 – *NAFDAC USE ONLY*

ABSORPTION / PERMEABILITY(COMPLETION OF THIS SECTION IS NOT NECESSARY IF THE API(S) ARE INCLUDED ON THE LIST OF BIOWAIVER-ELIGIBLE APIS IN THE PQTM DOCUMENT *GENERAL NOTES ON BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS)-BASED BIOWAIVER APPLICATIONS*.)

Human mass balance studies

Summarize results of all studies found in the literature.
Please enclose a copy of the references describing human mass balance studies of the API.

<< Please enter information here >>

Human absolute bioavailability studies

Summarize results of all studies found in the literature.
Please enclose a copy of the references describing human absolute bioavailability of the API.

<< Please enter information here >>

Supportive studies

Summarize results of all studies found in the literature regarding in vivo or in situ intestinal perfusion animal models or in vitro permeation across a monolayer of cultured epithelial cells (e.g. Caco-2) with a positive and negative control.
Please enclose a copy of the references.

<< Please enter information here >>

3.0 COMMENTS FROM REVIEW OF SECTION 3 – *NAFDAC USE ONLY*

TEST PRODUCT

Tabulation of the composition of the formulation(s) proposed for marketing and those used for comparative dissolution studies

Please state the location of the master formulae in the quality part of the submission.
Tabulate the composition of each product strength using the table below.
For solid oral dosage forms the table should contain only the ingredients in tablet core or contents of a capsule. A copy of the table should be filled in for the film coating/hard capsule, if any.
Biowaiver batches should be at least of pilot scale (10% of production scale or 100,000 capsules or tablets whichever is greater) and manufacturing method should be the same as for production scale.
Please note: If the formulation proposed for marketing and those used for comparative dissolution studies are not identical, copies of this table should be filled in for each formulation with clear identification in which study the respective formulation was used

Composition of the batches used for comparative dissolution studies				
Batch number				
Batch size (number of unit doses)				
Date of manufacture				
Comments, if any				
Comparison of unit dose compositions and of clinical FPP batches (duplicate this table for each strength, if compositions are different)				
Ingredients (Quality standard)	Unit dose (mg)	Unit dose (%)	Biobatch (kg)	Biobatch (%)
Equivalence of the compositions or justified differences				

Potency (measured content) of test product as a percentage of label claim as per validated assay method

This information should be cross-referenced to the location of the Certificate of Analysis in this biowaiver submission.

<< Please enter information here >>

4.0 COMMENTS FROM REVIEW OF SECTION 4 – *NAFDAC USE ONLY*

COMPARATOR PRODUCT

Comparator product

Please indicate location in the documentation of the following documents that should be enclosed:
A copy of product labelling (summary of product characteristics), as authorized in country of purchase, and translation into English, if appropriate.
A copy of the comparator product carton outer box. The name of the product, name and address of the manufacturer, batch number, and expiry date should be clearly visible on the labelling.
This information should be cross-referenced to the location of the Certificate of Analysis in this biowaiver submission.

<< Please enter information here >>

Name and manufacturer of the comparator product and official address

<< Please enter information here >>

Qualitative (and quantitative, if available) information on the composition of the comparator product

Please tabulate the composition of the comparator product based on available information and state the source of this information.

Composition of the comparator product used in dissolution studies		
Batch number		
Expiry date		
Comments, if any		
Ingredients	Unit dose (mg)	Unit dose (%)

Identify the source of the comparator product (where it was purchased), the method of shipment, and storage conditions of the comparator product from the time of purchase until completion of the comparative dissolution studies

Please attach relevant copies of the following documents proving the stated conditions:
A copy of the invoice from the distributor or company from which the comparator product was purchased. The address of the distributor must be clearly visible on the invoice.
Documentation verifying the method of shipment and storage conditions of the comparator product from the time of purchase to the time of study initiation.

<< Please enter information here >>

Potency (measured content) of the comparator product as a percentage of label claim, as measured by the same laboratory under the same conditions as the test product.

This information should be cross-referenced to the location of the Certificate of Analysis in this biowaiver submission.

<< Please enter information here >>

5.0 COMMENTS FROM REVIEW OF SECTION 5 – *NAFDAC USE ONLY*

COMPARISON OF TEST AND COMPARATOR FORMULATIONS

Identify any excipients present in either product that are known to impact in vivo absorption processes

A literature-based summary of the mechanism by which these effects are known to occur should be included and relevant full discussion enclosed, if applicable.

<< Please enter information here >>

Identify all qualitative (and quantitative, if available) differences between the compositions of the test and comparator products

The data obtained and methods used for the determination of the quantitative composition of the comparator product as required by the guidance documents should be summarized here for assessment.

<< Please enter information here >>

Provide a detailed comment on the impact of any differences between the compositions of the test and comparator products with respect to drug release and in vivo absorption

<< Please enter information here >>

6.0 COMMENTS FROM REVIEW OF SECTION 6 – *NAFDAC USE ONLY*

COMPARATIVE IN VITRO DISSOLUTION

Comparative in vitro dissolution

Information regarding the comparative dissolution studies should be included below to provide adequate evidence supporting the biowaiver request. Comparative dissolution data will be reviewed during the assessment of the Quality part of the dossier.

Please state the location of:

the dissolution study protocol(s) in this biowaiver application

the dissolution study report(s) in this biowaiver application

the analytical method validation report in this biowaiver application

<< Please enter information here >>

Dissolution study dates

Please indicate dates of study protocol, study conductance and study report

<< Please enter information here >>

Summary of the dissolution conditions and method described in the study report(s)

Summary provided below should include the composition, temperature, volume, and method of de-aeration of the dissolution media, the type of apparatus employed, the agitation speed(s) employed, the number of units employed, the method of sample collection including sampling times, sample handling, filtration and storage. Deviations from the sampling protocol should also be reported.

Dissolution media: Composition, temperature, volume, and method of de-aeration

<< Please enter information here >>

Type of apparatus and agitation speed(s) employed

<< Please enter information here >>

Number of units employed

<< Please enter information here >>

Sample collection: method of collection, sampling times, sample handling, filtration and storage

<< Please enter information here >>

Deviations from sampling protocol

<< Please enter information here >>

Summarize the results of the dissolution study(s)
Please provide a tabulated summary of individual and mean results with %CV, graphic summary, and any calculations used to determine the similarity of profiles **for each set of experimental conditions**.

<< Please enter information here >>

Summarize conclusions taken from dissolution study(s)
Please provide a summary statement of the studies performed.

<< Please enter information here >>

Dissolution specifications
Please provide proposed dissolution specifications and discuss them in relation to the results obtained in the BCS biowaiver

<< Please enter information here >>

7.0 COMMENTS FROM REVIEW OF SECTION 7 – *NAFDAC USE ONLY*

QUALITY ASSURANCE

Internal quality assurance methods

Please state location in this biowaiver application where internal quality assurance methods and results are described for each of the study sites.

<< Please enter information here >>

Auditing and inspections

Provide a list of all auditing reports of the study, and of recent inspections of study sites by regulatory agencies. State locations in this biowaiver application of the respective reports for each of the study sites e.g. analytical laboratory, laboratory where dissolution studies were performed.

<< Please enter information here >>

8.0 COMMENTS FROM REVIEW OF SECTION 8 – *NAFDAC USE ONLY*

CONCLUSIONS AND RECOMMENDATIONS – *NAFDAC USE ONLY*

DOWNLOAD LINK: BCS-Biowaiver-Form-NAFDACDER-GDL-009-01 ANNEX 2.docx

References.

1. ICH M13A Guidelines on Bioequivalence for Immediate release oral solid dosage forms (July 2024.
2. WHO TRS 1052, WHO Expert Committee On Specifications For Pharmaceutical Preparations(Annex 11) (April, 2024)
3. The International Pharmacopoeia. World Health Organization (March, 2023).
4. NAFDAC Good Clinical Practice Guideline (2025)
5. WHO TRS 929, Guidelines for registration of fixed-dose combination medicinal products (Annex 5) (2005)

Abbreviations

ANOVA: Analysis of variance

API: Active Pharmaceutical Ingredient

AUC: Area under the Curve

BCS: Biopharmaceutics Classification System

BTIF: Bioequivalence Trial Information Form

Cmax: maximum concentration

Ctau: last quantifiable concentration at steady state

CV: Coefficient of Variation

EMA: European Medicines Agency

FDA: United States Food and Drug Administration

FDC: Fixed Dose Combination

FPP: Finished Pharmaceutical Product

GCP: Good Clinical Practice

GI: gastrointestinal

GLP: Good Laboratory Practice

GMP: Good Manufacturing Practice

GMR: Geometric Mean Ratio

Ke: elimination rate constant

NAFDAC: National Agency for Food and Drug Administration and Control

QC: Quality Control

SOP: Standard Operating Procedure SRA:

Stringent Regulatory Authority

sWR: within-subject standard deviation of reference product

Tmax: Time at maximum concentration

WHO: World Health Organisation