Bioavailability Studies Submitted in NDAs or INDs — General Considerations

Guidance for Industry

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

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TABLE OF CONTENTS

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This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance provides recommendations to sponsors submitting bioavailability (BA) information for drug products in investigational new drug applications (INDs), new drug applications (NDAs), and NDA supplements. This guidance contains recommendations on how to meet the BA requirements set forth in 21 CFR part 320 as they apply to dosage forms intended for oral administration.[2] The guidance is also applicable to non-orally administered drug products when it is appropriate to rely on systemic exposure measures to determine the BA of a drug (e.g., transdermal delivery systems and certain rectal and nasal drug products). The guidance provides recommendations on conducting relative BA studies during the IND period for a drug intended to be submitted for approval in an NDA and bioequivalence (BE) studies during the postapproval period for certain changes to drug products.[3]

This guidance does not discuss information for demonstrating BE for drug products in abbreviated new drug applications (ANDAs) and ANDA supplements. In 2013, the FDA issued a separate draft guidance on this topic entitled *Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA*.[4] Furthermore, this guidance does not provide recommendations on studies conducted in support of demonstrating comparability or biosimilarity for biological products licensed under section 351 of the Public Health Service Act

[1] This guidance has been prepared by the Office of Clinical Pharmacology with contributions from the Office of Pharmaceutical Quality in the Center for Drug Evaluation and Research at the Food and Drug Administration.

[2] These dosage forms include tablets, capsules, solutions, suspensions, conventional (e.g., immediate-release drug products) and modified-release (e.g., extended-release, delayed-release) drug products.

[3] *Bioequivalence* (BE) is a statutory term reflected in the Federal Food, Drug, and Cosmetic Act (FD&C Act) in section 505(j)(21 U.S.C.). For the full definition of BE, refer to 21 CFR 314.3.

[4] When final, this guidance will represent the FDA's current thinking on this topic. For the most recent version of a guidance, check the FDA Drugs guidance web page at https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

(see the FDA guidances for industry entitled *Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product* and *Considerations in Demonstrating Interchangeability With a Reference Product*[5] for more information).

When finalized, this guidance will revise and replace the FDA's March 2014 draft guidance for industry entitled *Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs — General Considerations*,[6] which addresses BA or BE studies for INDs, NDAs, and NDA supplements. The FDA recognizes that this guidance cannot address every issue pertaining to the assessment of BA studies for INDs and NDAs. Therefore, sponsors are encouraged to contact the appropriate review division with specific questions not addressed by this guidance.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Determining the BA of formulations is important during the life cycle of drug products and aids in the FDA's evaluation of the safety and effectiveness of a product in an IND, NDA, or NDA supplements. To determine the safety and efficacy of a drug product for the proposed indication, the FDA uses the totality of information available in the submission, which includes BE data, exposure-response evaluations, and clinical trial results.

BA is defined as the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action.[7] BA data provide an estimate of the fraction of the drug absorbed as well as information related to the pharmacokinetics of the drug, such as distribution, metabolism, excretion, the effects of food on the absorption of the drug, dose proportionality or linearity in the pharmacokinetics of the active moieties and — when appropriate — inactive moieties.

Sponsors can determine the BA for orally administered drug products by comparing a plasma exposure profile to that of a suitable reference product. A systemic exposure profile can be generated by measuring the concentration of active ingredients and/or active moieties over time and — when appropriate — active metabolites over time in samples collected from the systemic circulation. Systemic exposure profiles reflect both the release of the drug substance from the drug product as well as presystemic or systemic modifications to the drug substance after its release. Conducting a BA study with an intravenous (IV) reference product enables assessment

[5] When final, this guidance will represent the FDA's current thinking on this topic.

[6] When final, this guidance will represent the FDA's current thinking on this topic.

[7] 21 CFR 314.3

of the impact of the route of administration on BA and defines the absolute BA of the drug released from the drug product. Conducting a BA study comparing one formulation to another enables an assessment of relative BA.

For an evaluation of relative BA, a test product might result in different plasma concentration-time profiles in comparison to a reference product because of a different rate or extent of absorption. These differences can impact the FDA's assessment of the benefits and risks of the new formulation or condition of administration. For example, if the test product leads to a significantly higher systemic exposure than the reference product, the test product may result in safety concerns associated with the higher systemic concentrations. If the test product results in a significantly lower systemic exposure than the reference product, the test product may be less effective. When the variability of the test product is greater than that of the reference product, both the safety and efficacy of the test product may be affected. This increased variability may indicate that the performance of the test product is not comparable to the reference product, and the efficacy and safety of the test product are too variable for the product to be clinically useful.

A. General BA Considerations

BA studies comparing two formulations or two test conditions are usually conducted using a crossover design. For a drug with a long half-life, a parallel design may be necessary.

Determining the BA for new drug products submitted under an IND or a NDA can use the principles of BE. Demonstrating equivalent or similar BA of two products during the development of a new drug may be needed to evaluate the safety or effectiveness of a product. In general, a 90 percent confidence interval (CI) with predefined CI boundaries may be used in situations such as comparing two dosage forms during drug product development (e.g., the to-be-marketed formulation versus the clinical trial formulation) or interpreting the effect of food on a drug product.

When similarity in BA is not demonstrated, the sponsor should demonstrate that the differences in the rate and extent of absorption do not meaningfully affect the safety and efficacy of the drug product based on the available dose-response or concentration-response data. In the absence of this evidence, the sponsor should consider reformulating the test product, changing the method of manufacture for the test product, or obtaining additional safety or efficacy data for the test product.

In some cases, conclusions of similarity in BA based on the peak drug concentration (C_{max}) and area under the plasma concentration-time curve (AUC) between the test product and the reference product may be insufficient to demonstrate that there is no difference in safety or efficacy. An example of this scenario is if certain aspects of the systemic concentration-time profiles of the test product and the reference product are different (e.g., the time to reach the peak drug concentration (T_{max}) is different). In addition, differences in the shape of the systemic concentration-time profile between the test and reference products could imply that the test product may not produce the same clinical response as the reference product. In such cases, additional data analysis (e.g., partial AUCs), exposure-response evaluation, or clinical studies may be necessary to evaluate the differences in the BA of the two products.

B. Preapproval Changes

The BA of formulations used in drug development may be needed to compare: (1) the early and late clinical trial formulations; (2) the formulations used in clinical trials and stability studies, if different; (3) the clinical trial formulations and to-be-marketed drug products, if different; (4) the equivalence of product strengths; and (5) the comparison of two different products that are the subject of a 505(b)(2) submission. For each comparison, the new formulation, the formulation produced by a new method of manufacture, or the new strength is the *test product*, and the prior formulation, the product made using the prior method of manufacture, or the product with the prior strength is the *reference product*. The decision to determine the BA of a drug during development is based on the principles of relevant guidances (in this guidance, see sections II.C, Postapproval Changes and III.B, In Vitro Studies) to determine when changes in the components, the composition, or the method of manufacture suggest that further in vitro or in vivo studies should be performed.

C. Postapproval Changes

In the presence of certain major changes in the components, composition, manufacturing site, or method of manufacture of a drug after its approval, the sponsor should demonstrate the in vivo BE for the drug product after the change compared to the drug product before the change. Certain postapproval changes that require BE studies must be submitted in a supplement and approved by the FDA before distributing a drug product made with the change.[8]

Information on the types of recommended in vitro dissolution and in vivo studies for demonstrating the BE for immediate-release (IR) and modified-release (MR) drug products approved as NDAs for specified postapproval changes is provided in the following FDA guidances:

- *SUPAC-IR: Immediate Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*

- *SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*

Alternatively, a quality risk management approach may be used to support postapproval changes. Sponsors should discuss proposals for alternate approaches with the appropriate review division.

III. STUDY DESIGN CONSIDERATIONS

[8] Under section 506A(c)(2) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 356a(c)(2))

Sponsors shall use the most accurate, sensitive, and reproducible method available to demonstrate the BA or BE of a product.[9] Several in vivo and in vitro methods can be used to determine BA and establish BE. In order of preference, these methods include, but are not limited to: pharmacokinetic (PK) studies, in vitro tests that are predictive of human in vivo BA (in vitro-in vivo correlation), pharmacodynamic (PD) studies, well controlled clinical trials that establish the safety and efficacy of the drug product, and other in vitro studies as deemed appropriate by the FDA. In addition, where in vivo data are appropriate to determine the BA of a drug product, the regulations provide guidelines on the specific types of in vivo BA studies.[10] This guidance primarily focuses on the use of in vivo studies to determine the BA of a drug.

A. In Vivo Studies

1. General Considerations

For in vivo studies, the regulations allow the use of PK measures in an accessible biological matrix such as the blood, plasma, or serum to indicate the release of the drug substance from the drug product into the systemic circulation.[11] For the purpose of this guidance, the various biological matrices (i.e., blood, plasma, and serum) are used interchangeably. BA frequently relies on PK measures such as the AUC to assess the *extent* of systemic absorption and the C_{max} and T_{max} to assess the *rate* of systemic absorption. PK-based comparisons to describe relative BA assume that measuring the active moiety at the site of action is not possible and that some relationship exists between the concentration of the active moiety in the systemic circulation and the safety and efficacy of the drug. A typical PK study to determine BA is conducted as a crossover trial. The crossover design reduces variability in PK measures that are caused by subject-specific factors, thereby increasing the ability to discern differences in PK measures that are caused by different formulations.

2. Pilot Trial

If the sponsor chooses, a pilot trial with a small number of subjects can be carried out before proceeding with a full-scale BA or BE trial. The results of a pilot trial can:

- Assess the variability in PK measures
- Determine the sample size that achieves adequate power to conduct BA or BE analyses
- Optimize the time intervals for sample collection
- Determine the length of the washout period needed between treatments

For conventional IR products, careful timing of the collection of the initial PK samples can ensure that the first sample collection occurs before the C_{max}, thereby informing the optimal

[9] 21 CFR 320.24(a)

[10] 21 CFR 320.25 through 320.29

[11] 21 CFR 320.24(b)(1)(i). If serial measurements of the drug or its metabolites in plasma, serum, or blood cannot be accomplished, then measurement of urinary excretion can be used.

sample collection schedule for a full-scale trial.[12] For MR products, a pilot trial can help determine the sampling schedule needed to assess lag time and dose dumping. In some circumstances, the results of a pilot trial may be used as the sole basis to determine the BA of a drug, if the design and execution of the trial are suitable, and if enough subjects have completed the trial with evaluable PK measurements.

3. Full-Scale Study

General recommendations for a standard BA or BE study based on PK measurements are provided in appendix A. Non-replicate, crossover study designs are recommended for BA studies of IR and MR dosage forms. To determine the absolute BA, single-period studies using isotopic labeling approaches are an acceptable alternative. Sponsors have the option of using replicate designs for BA or BE studies, where the reference treatment is repeated, or both the test and the reference products are given on multiple occasions. Replicate crossover designs are used to estimate: (1) the within-subject variance for the reference product or for both the test and reference products; and (2) the subject-by-formulation interaction variance component. These designs account for the inter-occasion variability that can confound the interpretation of a BE study as compared to a non-replicate crossover approach.

In addition to the traditional approach and the use of average BE with replicate designs, the use of a reference-scaled BE approach using a replicate design can also be considered. This reference-scaled BE approach is typically used for drugs with a high intrasubject variability (greater than or equal to 30 percent) and drugs with a narrow therapeutic index.[13,14] The appropriate review division should be consulted when planning the use of the reference-scaled BE approach.

4. Study Population

In general, BA studies should be conducted in healthy subjects 18 years of age or older who are capable of giving informed consent. When safety considerations preclude the use of healthy subjects, it may be necessary to evaluate the BA of a drug in patients. In this situation, sponsors should attempt to enroll patients whose disease is expected to be stable for the duration of the study. The BA study may be conducted in the subject or patient groups most likely to provide a sensitive measure of differences in the exposure of the drug. Male and female subjects should be enrolled in BA studies unless there is a specific reason to exclude one sex (e.g., the drug product is indicated in only one sex, or there is a greater potential for adverse reactions in one sex

[12] 21 CFR 320.25 through 320.29

[13] For general principles of the reference-scaled approach, refer to Davit B, D Conner, 2010, Reference-Scaled Average Bioequivalence Approach, In: I Kanfer, L Shargel, editors, Generic Drug Product Development – International Regulatory Requirements for Bioequivalence, Informa Healthcare, 271-272.

[14] Jiang, W, F Maklouf, DJ Schuirmann, X Zhang, D Conner, LX Yu, R Lionberger, 2015, A Bioequivalence Approach for Generic Narrow Therapeutic Index Drugs: Evaluation of the Reference-Scaled Approach and Variability Comparison Criterion, AAPS J, 17(4):891-901.H

compared to the other). Female subjects enrolled in the study should not be pregnant at the beginning of the study and should not become pregnant during the study.

5. Single-Dose and Multiple-Dose (Steady-State) Testing

This guidance generally recommends single-dose, in vivo studies to assess the BA of a drug because they are generally more sensitive than steady-state studies in assessing the rate and extent of release of the drug substance from the drug product into the systemic circulation. The reader is referred to section IV.C for a discussion on the conduct of studies to determine the BA of a drug of an MR product.

The regulations provide guidelines on the design of a multiple-dose, in vivo BA study and when such studies may be required.[15] We recommend that if a multiple-dose study is performed, the sponsor should dose the product to achieve steady-state concentrations of the drug. The sponsor should provide evidence that steady-state concentrations of the drug were achieved.

6. Bioanalytical Methodology

Sponsors should use bioanalytical methods for BA studies that are accurate, precise, specific, sensitive, and reproducible. A separate FDA guidance for industry entitled *Bioanalytical Method Validation* is available to assist sponsors in validating bioanalytical methods.

7. Administration Under Fasting or Fed Conditions

The sponsor should determine the BA of the test product under fasting conditions because it is a more sensitive method to assess differences between formulations. The effect of food on the BA of the test product should also be assessed (see the FDA guidance for industry entitled, *Assessing the Effects of Food on Drugs in INDs and NDAs – Clinical Pharmacology Considerations*[16]). If BA is determined using an approved product as a reference, the reference product should be administered as described in the labeling. If tolerability issues or serious adverse events are anticipated under fasting conditions (for either the test or the reference product), we recommend that sponsors conduct the study only under fed conditions. See appendix B for additional guidance.

8. Moieties to Measure

The active ingredient that is released from the dosage form or its active moiety and, when appropriate, its active metabolites,[17] should be measured in biological fluids collected during the BA study.

[15] 21 CFR 320.27

[16] When final, this guidance will represent the FDA's current thinking on this topic.

[17] 21 CFR 320.24(b)(1)(i).

Measuring the active ingredient or the active moiety and the active metabolite(s) is generally recommended for BA studies. The concentration-time profile of the active ingredient or the active moiety is more sensitive to changes in performance of the formulation. In contrast, the metabolite is more affected by metabolite formation, distribution, and elimination. The following scenarios are instances when an active metabolite(s) should be measured and be subjected to CI analyses:

- **If the metabolite is formed by pre-systemic metabolism (e.g., gut metabolism, first-pass metabolism) and contributes to efficacy or safety (in this situation, the active ingredients or the active moiety and the active metabolite should be measured):** The CI should be computed for all moieties that are measured. However, the acceptance criteria are generally applied to the active ingredient or active moiety. Sponsors should contact the appropriate review division to determine which moieties should be measured.

- **When the active ingredient or the active moiety concentrations are too low to allow reliable bioanalytical measurements in the appropriate biological matrix:** In this case, the metabolite should be measured in lieu of the active ingredient or active moiety. The FDA recommends that the sponsor compute the CI to analyze the metabolite data obtained from these studies.

9. PK Measures of Systemic Exposure

When available, sponsors should use clinically relevant systemic exposure measures to determine BA. Exposure measures are defined relative to the peak, partial, and total exposures of the drug concentration-time profile in the appropriate biological matrix as described below.

a. Peak exposure

The sponsor should determine the peak exposure level of the drug by measuring the C_{max} obtained directly from the systemic drug concentration data without interpolation. The T_{max} can provide important information about the rate of absorption. The first point of a drug concentration-time curve based on blood or plasma measurements is sometimes the highest concentration, raising concerns that the first sampling time was too late to accurately determine the C_{max} and T_{max}. A carefully conducted pilot study can help to avoid this problem. For example, collection of a sample at an early timepoint for IR products, between 5 and 15 minutes after dosing, followed by additional sample collections (e.g., two to five) in the first hour after dosing, may be sufficient to assess early peak concentrations. If this sampling approach is followed, the FDA considers the data to be adequate, even when the highest observed concentration occurs at the first timepoint.

b. Total exposure (extent of absorption)

For single-dose studies, the sponsor should calculate the total exposure using the following:

- The area under the concentration-time curve from time zero to time t (AUC_{0-t}) from an appropriate biological matrix, where t is the last time point with a measurable concentration.

- The area under the concentration time curve from time zero to time infinity (AUC_{0-INF}) from an appropriate biological matrix, where $AUC_{0-INF} = AUC_{0-t} + Ct/\lambda z$. Ct is the last measurable drug concentration and λz is the terminal elimination rate constant calculated by an appropriate method.

- For drugs with low intrasubject variability (i.e., less than 30 percent) and a long half-life, a truncated AUC can be used (see section VI.C).

For steady-state studies, the sponsor should calculate the total exposure using the area under the concentration-time curve from time zero to time TAU in an appropriate biological matrix over a dosing interval at steady state (AUC_{0-TAU}), where TAU is the length of the dosing interval.

c. Partial exposure

In addition to peak and total exposure, for certain classes of drugs (e.g., analgesic drug products), an evaluation of the partial exposure could be required to support the determination of the relative BA of the drug products. The FDA recommends the use of partial AUC as a partial exposure measure. The time to truncate the partial AUC should be related to a clinically relevant response measure. The sponsor should collect sufficient quantifiable samples to allow an adequate estimation of the partial AUC. Sponsors should consult the appropriate review division for questions on the suitability of the PD measure or the use of partial exposure in general.

10. Comparison of Drug Exposure Measures in BA Studies

A CI approach is recommended for BA comparisons. Log-transformation of exposure measures before statistical analysis is recommended. This guidance recommends the use of an average BA criterion to compare systemic exposure measures for replicate and nonreplicate BE studies of both IR and MR products. For additional information on data analysis, refer to Appendix A and to the FDA guidance for industry entitled *Statistical Approaches to Establishing Bioequivalence*.

B. Other Approaches to Determine the BA of a Drug

In certain circumstances, other approaches are recommended to determine the BA of a drug. Below are some general considerations regarding these other approaches.

1. In Vitro Studies

Under certain circumstances, BA can be determined using in vitro approaches (e.g., dissolution, drug-release testing) during the preapproval and postapproval phases.[18] The following FDA

[18] 21 CFR 320.24(b)(5) and (6)

guidances provide recommendations on developing dissolution methodology, setting specifications, and the regulatory applications of dissolution testing:

- *Dissolution Testing of Immediate-Release Solid Oral Dosage Forms*

- *Extended-Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations*

- *Dissolution Testing and Specification Criteria for Immediate-Release Solid Oral Dosage Forms Containing Biopharmaceutics Classification System Class 1 and Class 3 Drugs Guidance for Industry*[19]

2. In Vitro Tests Predictive of Human In Vivo BA

In vitro-in vivo correlation (IVIVC) is an approach to describe the relationship between an in vitro attribute of a dosage form (e.g., the rate or extent of drug release) and a relevant in vivo measure (e.g., the plasma drug concentration or the amount of drug absorbed). Modeling of this relationship facilitates the rational development and evaluation of extended-release (ER) dosage forms, and less commonly, of other dosage forms. Once an IVIVC is validated, the in vitro test serves as a surrogate for BA testing as well as a tool to screen formulations and set the dissolution and drug-release acceptance criteria.

Specifically, in vitro dissolution and drug-release characterization are recommended for all ER product formulations (including prototype formulations), particularly when used to define the in vivo absorption characteristics for different product formulations. Such efforts can enable the establishment of an IVIVC. When an IVIVC or association is established, the in vitro test can serve not only as a quality control specification for the manufacturing process but also as an indicator of how the product will perform in vivo.[20]

Additional information on the development and validation of an IVIVC can be found in the FDA guidance for industry entitled *Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations*. Sponsors should contact the review division to seek guidance on approaches for establishing IVIVC.

3. PD Studies

PD studies are generally not recommended for orally administered drug products when the drug is absorbed into the systemic circulation and a PK approach can be used to assess the systemic exposure and determine BA. PK endpoints are preferred because they are generally the most accurate, sensitive, and reproducible. However, in instances where a PK endpoint is not possible, a well justified PD endpoint can be used to determine BA or to demonstrate BE.

[19] When final, this guidance will represent the FDA's current thinking on this topic.

[20] 21 CFR 320.24(b)(1)(ii)

4. Comparative Clinical Studies

Clinical endpoints can be used in limited circumstances, for example, for orally administered drug products when the measurement of the active ingredients or active moieties in an accessible biological fluid (i.e., the PK approach) or a PD approach is not possible. Because these circumstances do not occur very often, use of this approach is expected to be rare (section VI.D).

IV. ASSESSING BA FOR VARIOUS DOSAGE FORMS

This section summarizes the recommendations for assessing BA based on the specific dosage forms. It also describes when BA studies should occur (preapproval or postapproval).

A. Solutions and Other Solubilized Dosage Forms

For oral solutions, elixirs, syrups, tinctures, or other solubilized dosage forms, in vivo BA is generally self-evident, and a requirement of in vivo BA data for a product can be waived.[21] Although a comparative study is not necessary, characterization of the pharmacokinetics of the drug is required.[22] In addition, in vivo BA studies that compare different solution formulations are waived based on the assumptions that: (1) the release of drug substance from the drug product is self-evident; and (2) that the solutions do not contain any excipients that significantly affect drug absorption. However, there are certain excipients that may alter the BA (e.g., sorbitol may reduce the BA of drugs, and vitamin E may enhance the BA) in amounts sometimes used in oral, liquid dosage forms. In these cases, determining the in vivo BA of the drug may be required. For solutions that contain co-solvents or are buffered to maintain the drug in solution, precipitation can occur when the solution is exposed to gastric contents. Formulation changes to such products may result in drug precipitation; in such cases, the sponsor should consider an in vivo study.

B. IR Products

Included in this discussion are capsules, tablets (including conventional, buccal, chewable, orally disintegrating, and sublingual dosage forms), and suspensions.

1. Preapproval: BA Studies

For BA studies, we recommend that sponsors conduct a single-dose, fasting study. Under certain circumstances, multiple-dose BA studies (see section III.A.5) or food-effect studies may be necessary (see the FDA guidance for industry entitled *Assessing the Effects of Food on Drugs in INDs and NDAs – Clinical Pharmacology Considerations*[23]). Unconventional dosage forms

[21] 21 CFR 320.22(b)(3)

[22] 21 CFR 314.50(d)(3)

[23] When final, this guidance will represent the FDA's current thinking on this topic.

(e.g., buccal, chewable, orally disintegrating, and sublingual dosage forms) should be administered per the labeling. In addition, the sponsor may need to determine the BA of the unconventional dosage form when swallowed intact to assess the impact of accidental swallowing of the intact product. Sampling should adequately capture the T_{max} and C_{max} in addition to the total exposure.

The sponsor should evaluate in vitro dissolution for all orally administered solid, oral dosage forms, and suspensions. In vitro dissolution test conditions could be the same or different for unconventional compared to conventional dosage forms. If differences in dissolution data exist, they should be discussed with the appropriate review division.

2. Postapproval: BA Studies

An FDA guidance for industry entitled *SUPAC-IR: Immediate Release Solid Oral Dosage Forms Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation* provides recommendations on in vitro dissolution and in vivo BE studies for postapproval changes. For postapproval changes, the sponsor should compare the results of in vitro or in vivo studies between the products before and after the change.

C. MR Products

MR products include ER (e.g., controlled-release, sustained-release)[24] and delayed-release (DR) products.

ER products are dosage forms that are designed to extend or prolong the release of the active ingredient or the active moiety from the drug product. ER products may allow a reduction in dosing frequency and can reduce fluctuations in plasma concentrations when compared to an IR product. ER products can be capsules, tablets, granules, pellets, beads, or suspensions.

DR drug products are dosage forms that release the active ingredient or active moiety at a time later than immediately after administration (i.e., there is a lag between the time of administration and the first quantifiable plasma concentration). Typically, coatings (e.g., enteric coatings) are used to delay the release of the drug substance until the dosage form has passed through the acidic medium of the stomach.

Even though DR drug products are defined as MR products, many DR products behave like IR products after accounting for the delay; hence, the FDA considers the requirements for the initial BA study of a DR product to be identical to those of an IR product. In cases where the DR product has complex release characteristics, the relevant review division should be contacted for additional information. The remainder of this section focuses on recommendations for ER drug products.

[24] For the purposes of this guidance, the terms *extended, controlled,* and *sustained* are used interchangeably.

1. Preapproval: BA Studies

Regulations address the purpose and requirements of a BA study for an ER product and stipulate that "the reference material(s) for such a BA study shall be chosen to permit an appropriate scientific evaluation of the ER claims made for the drug product." [25,26] Appropriate reference products may include:

- A solution or suspension of the active drug ingredient or therapeutic moiety

- A currently marketed noncontrolled-release drug product containing the same active drug ingredient or therapeutic moiety and administered according to approved labeling of the non-controlled release drug product

- A currently marketed ER drug product containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling of the currently marketed ER product[27]

We recommend that a sponsor seeking to use as a reference product "a currently marketed extended-release drug product subject to an approved full new drug application containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling proposed for the extended release drug product" under 21 CFR 320.25(f)(2)(iii) consult with the FDA to agree on the dosing of the reference product before commencing such a study.

The FDA recommends that the following BA studies and food-effect studies be conducted for an ER drug product submitted as an NDA for the scenarios described below. In certain cases, nonequivalent doses of the ER and IR products may be evaluated. The review divisions should be contacted for additional information.

a. A new ER formulation compared to an IR product that is already approved

- For drugs with linear pharmacokinetics over the therapeutic dose range, a fasting study should compare the ER product administered as a single dose at the highest strength to the IR reference product administered over the same interval used for the ER product to achieve the same total dose as the ER product.[28] If for safety reasons the highest strength cannot be used, a lower strength is acceptable.

[25] 21 CFR 320.25(f)(1)

[26] 21 CFR 320.25(f)(2)

[27] 21 CFR 320.25(f)(2)(i), (ii), and (iv).

[28] For example, when a 150-milligram (mg) ER product administered once daily (QD) is being developed given an approved 50-mg IR reference product administered three times a day (TID) or a 75-mg product administered two times a day (BID), then for relative BA purposes, the 150-mg ER product administered as a single dose could be compared to either the 50-mg IR reference product administered TID or the 75-mg IR reference product administered BID.

- For drugs with nonlinear pharmacokinetics over the therapeutic dose range, at a minimum, a single dose of the highest and lowest strengths of the ER product should be compared to their corresponding IR reference products administered over the same time period as the ER dosing interval. If the relative BA of intermediate ER strengths cannot be inferred based on the above studies, a single-dose fasting study for the intermediate strength or strengths of the ER product should be compared to the corresponding IR reference products administered over the ER dosing interval.

- If multiple ER strengths are being developed, and the ER strengths are not proportionally similar in composition, a single-dose fasting dosage strength equivalence assessment study[29] or a dosage strength proportionality study[30] for the ER product should be conducted.

- When the ER strengths are proportionally similar in composition, and in vitro release testing demonstrates different release-rate profiles, then a single-dose, fasting, dosage-strength equivalence assessment study or a dosage-strength proportionality study for the ER product should be conducted.

- A single-dose, high-fat, food-effect study should be conducted on the highest strength of the new ER product (ER_{new}) as outlined in the FDA guidance for industry entitled Assessing the Effect of Food on Drugs in INDs and NDAs – Clinical Pharmacology Considerations.[31]

- A steady-state study should be conducted on the highest strength of the ER product compared to an approved IR reference product and dosed to achieve the equivalent total dose of the ER product.

b. New ER product (ER_{new}) comparison to an approved ER product (ER_{old}) with a different dosing interval (i.e., where ER_{new} and ER_{old} have unequal dosing intervals)

- The recommendations are the same as outlined in the previous section (i.e., development of a new ER formulation given an already approved IR product) except for the choice of the reference product. In this case, the reference product could be either the approved ER_{old} or the IR product.

[29] If three strengths, 10, 25, and 50 mg, are being developed for a new ER dosage form, the dosage strength equivalence study should be conducted using 5×10 mg strength, 2×25 mg, and 1×50 mg to achieve constancy of dose.

[30] If three strengths, 10, 25, and 50 mg, are being developed for a new ER dosage form, the dosage strength proportionality study should be conducted using 1×10 mg, 1×25 mg, and 1×50 mg.

[31] When final, this guidance will reflect the FDA's current thinking on this topic

c. New ER product (ER_{new}) comparison to an approved ER product (ER_{old}) with the same dosing interval.

- The sponsor should conduct a single-dose, fasting, BE study on the highest strength of the ER_{new} product compared to the ER_{old} product. If the ER_{new} and ER_{old} products are different strengths, then the sponsor should compare the ER_{new} versus ER_{old} products using the highest strengths of these products.

- A single-dose, high-fat food-effect study should be conducted using the highest ER_{new} strength.

- When the ER_{new} strengths are not proportionally similar in composition, a single-dose, fasting dosage-strength equivalence assessment study or a dosage-strength proportionality study for the ER_{new} product should be conducted.

- If the PK profiles of the two ER products are different (e.g., the shape of the profile is different), demonstrating BE between the new and old ER products may not be sufficient to ensure that there is no difference in safety or efficacy. Additional clinical studies could be needed to ensure that the two products are clinically equivalent.

2. Postapproval Changes

An FDA guidance for industry entitled *SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation* provides recommendations on the types of in vitro dissolution and in vivo BE studies for MR drug products approved in the presence of specific postapproval changes. For postapproval changes, the FDA recommends that the sponsor conduct in vitro or in vivo comparisons between the product made before the change and the product made after the change.

V. ADDITIONAL INFORMATION ON IN VITRO APPROACHES

A. General Considerations

The regulations indicate that if in vivo BA or BE data are required for a product, a sponsor may seek a waiver of these requirements under certain circumstances. For example, sometimes in vivo BA or BE is self-evident based on certain characteristics of the drug product,[32] and no additional in vivo data are required. In other circumstances, a requirement for in vivo BA or BE data may be waived, and in vitro data may be accepted instead.[33] For example, the requirement for in vivo data may be waived for different strengths of an IR drug product when: (1) the drug product is in the same dosage form, but in a different strength; (2) this different strength

[32] 21 CFR 320.22(b)

[33] 21 CFR 320.22(d)

formulation is *proportionally similar* in its active and inactive ingredients to another drug product for which the same manufacturer has obtained approval; and (3) the new strength formulation meets an appropriate in vitro test as outlined in the regulations.[34,35] In addition, to obtain a waiver for higher strengths, the sponsor should demonstrate that the pharmacokinetics over the therapeutic dose range are linear.

This guidance defines *proportionally similar* in the following ways:

- All active and inactive ingredients are in identical proportions between different strengths (e.g., a tablet of 50-mg strength has exactly half of the active ingredients of a tablet of 100-mg strength and twice the active ingredients of a tablet of 25-mg strength).

- For drug substances with high potency where the amount of the active drug substance in the dosage form is relatively low (i.e., the amount of the active substance is less than 5 percent of the tablet core weight or the weight of the capsule content), then: (1) the total weight of the dosage form remains nearly the same for all strengths (i.e., within plus or minus 10 percent of the total weight of the strength used in the BA study); (2) the same inactive ingredients are used for all strengths; and (3) the change in any strength is obtained by altering the amount of the active ingredients and one or more of the inactive ingredients.

- Bilayer tablets are considered a single formulation, even though they consist of two separate layers with different compositions. In assessing the proportional similarity of different strengths of bilayer tablets, all components of both layers should be proportionally similar. The fact that only one layer is proportionally similar and the other is not indicates that the products (i.e., the whole tablet) are *not* proportionally similar.

- Active and inactive ingredients are not in identical proportions between different strengths as stated above, but the ratios of the inactive ingredients to the total weight of the dosage form are within the limits defined by the FDA's SUPAC-IR and SUPAC-MR guidances for industry up to and including Level II changes.[36,37]

Exceptions to the above definitions may be possible if adequate justification is provided, and an agreement is reached with the appropriate review division.

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[34] 21 CFR 320.22(d)(2)

[35] See also 21 CFR 322.22(d)(3) and (4) for additional reasons for a waiver. Also, the FDA, for good cause, may waive or defer a requirement for the submission of evidence of in vivo BA or BE if the waiver or deferral is compatible with the protection of the public health.

[36] *SUPAC IR: Immediate-Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*

[37] *SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*

B. In Vitro Studies Conducted in Support of BA

The FDA may determine that an in vitro approach is the most accurate, sensitive, and reproducible method to determine BA.[38] Additional recommendations on the conduct of such studies is provided below.

1. IR Formulations (Capsules, Tablets, and Suspensions)

In vitro data can be used to compare formulations of drug products under certain circumstances. If a sponsor seeks to determine the BA of IR formulations for capsules, tablets, and suspensions using in vitro data, the FDA recommends that sponsors generate dissolution profiles for all strengths using an appropriate dissolution method (see III.B.2 for more information on IVIVC). If the results indicate that the dissolution characteristics of the product are not dependent on the pH or product strength, then dissolution profiles in one medium are usually sufficient to waive the need to assess the in vivo BA. If these criteria are not met, then the sponsor should collect dissolution data in at least three media (e.g., pH 1.2, 4.5, and 6.8). Similarity tests should be used to compare dissolution profiles from the different strengths of the product (see the FDA guidance for industry entitled *Dissolution Testing of Immediate Release Solid Oral Dosage Forms*). A similarity factor (f_2) value greater than or equal to 50 indicates a sufficiently similar dissolution profile to determine the drug's in vivo BA. For f_2 values less than 50, the FDA recommends discussing whether an in vivo study is needed with the appropriate review division. The f_2 approach is not suitable for drug products that dissolve very rapidly (i.e., greater than or equal to 85 percent of the drug product is dissolved in 15 minutes or less).

a. Over-encapsulation of clinical trial formulations

Blinding of drug products used in clinical trials may be done by over-encapsulation of the dosage form. The sponsor should assess the impact of this over-encapsulation on the release of the drug substance from the drug product. Dissolution may be used to assess the impact of over-encapsulation, provided that: (1) no excipients beyond those that are already in the dosage form are added to the capsule; and (2) the dissolution profiles between the over-encapsulated and non-over-encapsulated products are comparable in three media at pH 1.2, pH 4.5 and pH 6.8. However, if other excipients are added, then an in vivo study is required unless the sponsor can provide a justification as to why the excipients added do not alter the BA of the over-encapsulated product. These provisions apply equally to both the drug product under investigation as well as any product used as a comparator or reference product in the same clinical study.

b. Scale-up and postapproval changes

Following approval, drug products can undergo formulation changes for a variety of reasons. Formulation changes can occur in components and composition, and manufacturing changes can occur in scale-up, manufacturing site, manufacturing process, or equipment. Depending on the possible impact of the manufacturing change on the release of the active ingredient from the drug

[38] 21 CFR 320.24(b)(5) and (6)

product and the BA of the active ingredient, certain manufacturing changes for IR products can be approved based solely on the similarity of the dissolution profiles between the formulation after the change and the formulation before the change. Information on recommendations for using in vitro dissolution and in vivo BE studies for IR drug products in such circumstances is provided in FDA's guidance for industry entitled *SUPAC IR: Immediate-Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*. The same principles described in the above guidance can be applied to pre-approval changes such as when the to-be-marketed formulation differs from the clinical trial formulation.

2. MR Formulations

The use of in vitro data may be acceptable for MR drug products with specific postapproval changes. Specific information on the use of in vitro data for postapproval changes to MR drug products is delineated in the FDA's guidance for industry entitled *SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation.* The same principles described in the guidance may also apply to pre-approval changes. Additional considerations for the use of in vitro data in support of determining a drug's BA are described below.

a. Beaded capsules

In vivo BA studies for higher strengths of beaded capsules (e.g., a strength that is developed after initial BA studies of lower strengths) may not be necessary based on: (1) the clinical safety or efficacy data of the proposed dose and the need for the higher strength; (2) the linearity of the PK over the therapeutic dose range; and (3) whether the same dissolution procedures were used for all strengths and yielded similar dissolution results. The f_2 similarity test can be used to demonstrate similar profiles among the different strengths of the product. The sponsor can determine the in vivo BA of one or more lower strengths by comparing the dissolution profiles and conduct an in vivo BA study only on the highest strength (unless safety reasons preclude the administration of the highest strength to healthy volunteers). The dissolution profiles for each strength should be generated using the recommended dissolution method. If the dissolution method has not been finalized, dissolution profiles should be generated in at least three media (e.g., pH 1.2, 4.5, and 6.8).

b. Other MR dosage forms

For other MR dosage forms, the sponsor should conduct an in vivo BA study using the highest strength. The sponsor can determine the BA for lower strengths by comparing the dissolution profiles using f_2 evaluation when the drug product is in the same dosage form but in a different strength, and: (1) the drug exhibits linear pharmacokinetics; (2) the various strengths are proportionally similar in their active and inactive ingredients; and (3) the mechanism of the release of the drug is the same. If the formulations of all the strengths are not compositionally proportional, in vitro data can be submitted for the middle strengths if the following data are

acceptable: (1) BA or BE data, as appropriate, for both the highest and the lowest strengths; and (2) comparisons of in vitro multimedia dissolution profiles using f_2 evaluation.

The dissolution profiles for each strength should be generated using the recommended dissolution method. If the dissolution method has not been finalized, dissolution profiles should be generated in at least three media (e.g., pH 1.2, pH 4.5, and pH 6.8). The dissolution profiles should be generated on the test and reference products of all strengths using the same dissolution test conditions.

VI. SPECIAL TOPICS

A. Enantiomers Versus Racemates

During the development of a racemic drug product, the racemate should be measured in BA studies using an achiral assay. It may also be important to measure the individual enantiomers of the racemate to characterize the pharmacokinetics of the enantiomers. For the development of a specific enantiomer, chiral inversion should be assessed.

Measuring individual enantiomers in BA and BE studies is recommended only when all the following conditions are met:

- The enantiomers exhibit different PD characteristics
- The enantiomers exhibit different PK characteristics
- Primary efficacy and safety activity resides with the minor enantiomer
- At least one of the enantiomers exhibits nonlinear absorption (as expressed by a change in the enantiomer concentration ratio with change in the input rate of the drug). In such cases, the sponsor should apply BE criteria to the enantiomers separately.

B. Drug Products With Complex Mixtures as the Active Ingredients

Certain drug products may contain complex drug substances (i.e., active moieties or active ingredients that are mixtures of multiple synthetic or natural source components). The chemical structure or biological activity of some or all of the components of these complex drug substances may not be fully characterized. Quantification of all active or potentially active components in BA studies may not be possible. In such cases, sponsors should use a select number of components in BA studies. The criteria for selecting the components typically include the amount of the moiety in the dosage form, the plasma or blood levels of the moiety, and the biological activity of the moiety. When PK approaches are not feasible to assess the rate and extent of absorption of a drug substance from a drug product, the sponsor may consider PD, clinical, or in vitro approaches. In such cases, sponsors should consult the appropriate review division and seek an agreement on the approach and moieties for conducting BA studies.

C. Drugs With Long Half-Lives

In a BA or a PK study involving an IR, oral product with a long half-life (i.e., greater than or equal to 24 hours), characterization of the product's half-life should include blood sampling over an adequate period of time. To determine the BA of a drug product containing a drug with a long half-life, a nonreplicate, single-dose crossover study can be conducted if an adequate washout period is used. If the crossover study is problematic, a study with a parallel design can be used. For either a crossover or parallel study, the sample collection time should ensure that the drug product completely moves through the gastrointestinal tract so that the absorption of the drug substance (C_{max}) and a suitably truncated AUC can be used to characterize the peak and total drug exposures, respectively. In these cases, the sponsor should consult the appropriate review division to seek agreement on the duration of sampling and the choice of the PK measures for determining BA.

D. Orally Administered Drugs Intended for Local Action

Determining BA when the drug substance produces its effects by local action in the gastrointestinal tract can be achieved either by using pharmacokinetics, an acceptable PD endpoint, clinical efficacy and safety studies, or suitably designed and validated in vitro studies, as appropriate. In these cases, sponsors should consult the appropriate review division to determine the acceptable approach for assessing BA.

E. Combination and Coadministered Drug Products

Two or more active ingredients can be formulated as a single drug product, which is referred to as a fixed-dose combination product (FDC). Generally, the purpose of an in vivo BA study involving an FDC is to compare the rate and extent of absorption of each active drug ingredient or therapeutic moiety in the combination drug product to the rate and extent of absorption of each active drug ingredient or therapeutic moiety administered concurrently as separate, single-ingredient preparations.[39]

The following study designs should be considered for determining BA:

- A two-arm, single-dose, crossover, fasting study of the FDC versus the single-ingredient drug products administered concurrently or an approved combination product containing the same active ingredients. This study should use the highest strength of the FDC with matching doses of the individual drug products. Certain alternative study designs may also be acceptable depending on the specific situation. For instance, when there are no drug interactions between the components of an FDC consisting of two components, a three-arm study design comparing the combination drug product versus the single-ingredient drug products administered separately may be appropriate.

- A single-dose, high-fat, food-effect study on the FDC.

[39] 21 CFR 320.25(g)

Sponsors should consult with the review divisions to discuss their specific situation.

BA studies for the FDC product should include the measurement of systemic concentrations of each active ingredient. The CI approach should be applied to each measured entity of the FDC and its reference product.

In specific cases, drug products are given in combination (but are not co-formulated) with the objective of increasing the exposure of one of the drugs (i.e., the subject drug). The second, *booster* drug is not intended to have a therapeutic effect and is given only to increase the systemic exposure of the subject drug. When both the subject drug and booster drug are new molecular entities, the BA of each should be determined individually and when administered in combination. If there is a change in the subject drug product formulation that results in the need for a BA study, the subject drug should be administered with the booster drug for both post-change and pre-change products. The corresponding PK measures, including CIs, should be determined and reported for the subject drug. It is not necessary to measure the concentrations of the booster drug. BA studies that are needed for the booster drug should be conducted only with the booster drug; the subject drug should not be dosed with the booster drug. When the combination (which is not co-formulated) includes a new molecular entity and an approved booster drug product, only the BA of the new molecular entity should be assessed. It is assumed that the BA of the approved booster product has been previously evaluated.

F. Endogenous Substances

Drug products can contain compounds that are also endogenous to humans (e.g., testosterone). When the endogenous substances are identical to the drug that is being administered, it can be difficult to determine the amount of drug released from the dosage form and absorbed. In most cases, it is important to measure and approximate the baseline endogenous levels of the compound in the matrix of choice and subtract these levels from the total concentrations measured from each subject after the drug product is administered. Using this approach, the sponsor can estimate the true availability of the drug from the drug product and accurately determine BA and demonstrate BE. Endogenous substances may have homeostatic processes that affect their production and therefore impact their systemic concentrations. To reduce the impact of this variability in the levels of endogenous substances and to potentially avoid the need for baseline correction, an alternative approach could be to enroll patients in BA studies with low or no production of the endogenous substances instead of healthy volunteers.

Baseline concentrations of the endogenous substance produced by the body are measured in the period before administration of the study drug. Depending on the proposed indication, it may be advisable to subtract the time-averaged baseline or time-matched baseline from the post-dose concentration for each subject. When the levels of endogenous substances are influenced by diet, restricting the dietary intake of the substance before and during the study may also be appropriate. To achieve a stable baseline measurement, subjects should be housed at the study site for a sufficient period of time before the study and served standardized meals with similar content of the compound to that of the meals served on the day that PK sampling will take place.

Baseline concentrations should be determined for each dosing period, and baseline corrections should be period-specific. PK and statistical analyses should be performed on both uncorrected and corrected data. Because of the complexities associated with endogenous compounds, sponsors should contact the appropriate review division for additional information.

G. Narrow Therapeutic Index Drugs

In specific circumstances where exposure measures of drugs (AUC or C_{max}) are critical for the safe and effective use of the drug product, or where therapeutic drug monitoring is an essential tool for drug product dosing, the acceptable criteria for demonstrating BE may need to be narrowed. Because of the complexities associated with narrow therapeutic index drugs, sponsors should contact the appropriate review division for additional information.

H. Characterizing the Effects of Alcoholic Beverages on MR Drug Products

The consumption of alcoholic beverages can affect the release of a drug substance from an MR formulation, leading to a more rapid release of the drug and altered systemic exposure. The FDA recommends that sponsors who are developing certain MR, solid, oral dosage forms conduct in vitro studies to determine the potential for dose dumping from alcohol in vivo. In vitro assessments of the drug release from the drug product using media with various alcohol concentrations should be conducted. Based on these in vitro study results, an in vivo BA study co-administering the drug product with alcohol may be needed. See appendix C for details regarding designs for in vitro studies that evaluate the effect of alcohol on MR drug products.

APPENDIX A: GENERAL STUDY DESIGN AND DATA HANDLING

The following general approaches are recommended, recognizing that the elements can be adjusted for certain drug substances and drug products.

A. Study Conduct

- Generally, the BA or BE study should be conducted under fasting conditions (i.e., after an overnight fast of at least 10 hours) according to the FDA guidance for industry entitled *Assessing the Effects of Food on Drugs in INDs and NDAs — Clinical Pharmacology Considerations*.[40]

- The test and reference products should be administered with about 8 ounces (240 milliliters) of water to the study subjects.

- Generally, the highest marketed strength should be administered as a single unit. If the highest strength is not deemed safe for healthy volunteers, then the study can be performed in patients, or a lower strength may be appropriate. If bioanalytical sensitivity is a limitation, multiple units of the highest strength can be administered, if the total single dose remains within the labeled dose range, and the total dose is safe for administration to the study subjects.

- An adequate washout period (e.g., greater than or equal to five half-lives of the moieties to be measured or until the drug concentration is less than or equal to 5 percent of the C_{max} in all subjects) should separate each treatment.

- The lot numbers of both the test and reference listed products and the expiration date for the reference product should be stated. The sponsor should include a statement of the composition of the test product and, if possible, a side-by-side comparison of the compositions of test and reference listed products. Samples of the test and reference listed product should be retained in accordance with the FDA guidance for industry entitled *Handling and Retention of Bioavailability BA and Bioequivalence BE Testing Samples*.

- Before and during each study phase, we recommend that subjects: (1) are allowed water as desired except for 1 hour before and after drug administration; (2) are provided standard meals no less than 4 hours after drug administration; and (3) abstain from alcohol for 24 hours before each study period and until after the last sample from each period is collected.

B. Sample Collection and Sampling Times

[40] When final, this guidance will represent the FDA's current thinking on this topic.

Under normal circumstances, sponsors should collect blood, rather than urine or tissue. In most cases, the drug or metabolites are measured in serum or plasma. However, in certain cases, such as when an assay of sufficient sensitivity cannot be developed for plasma, whole blood may be more appropriate for analysis. We recommend that sponsors draw blood samples at appropriate times to describe the absorption, distribution, and elimination phases of the drug. For most drugs, we recommend collecting 12 to 18 samples, including a pre-dose sample, per subject, per dose. This sampling should continue for at least three terminal elimination half-lives. For multiple-dose studies, sampling must occur at steady-state across the dose interval and include the beginning and the end of the interval.[41] The exact timing for sample collection depends on the nature of the drug and the rate of input from the administered dosage form. The sample collection should be spaced in such a way that the C_{max} of the drug in the blood and terminal elimination rate constant (λ_z) can be estimated accurately. The sponsor should collect three or more samples during the terminal log-linear phase to obtain an accurate estimate of λ_z from linear regression. We recommend recording the actual clock time when the samples are drawn as well as the elapsed time after drug administration.

C. Subjects With Pre-Dose Plasma Concentrations

If the pre-dose concentration is less than or equal to 5 percent of the C_{max} value in that subject, the subject's data without any adjustments can be included in all PK measurements and calculations. We recommend that if the pre-dose value is greater than 5 percent of the C_{max}, the subject should be dropped from all PK evaluations. However, this subject's data should be flagged and reported, and the subject should be included in the safety evaluations.

D. Handling Outliers

If any data are identified as statistical outliers, sponsors should not remove the data from the statistical analysis of BA studies solely based on this fact. The only instance where outlier data can be removed from the statistical analysis of a BA study is when there is coinciding documentation demonstrating a protocol violation (e.g., real-time documentation of a sample processing error as opposed to a retrospective investigation based on the analytical results). Data from re-dosing studies are not considered as evidence to support the removal of outlier data from the statistical analysis. Data from all subjects should be submitted, and potential outliers should be flagged with appropriate documentation as part of the submission.

E. Data Deletion Because of Vomiting

- We recommend that data from subjects who experience emesis during a study for IR products be deleted from statistical analysis if vomiting occurs at, or before, two times the median T_{max}.

- For MR products, subjects who experience emesis at any time during the labeled dosing interval should not be included in the PK analysis.

[41] 21 CFR 320.27(d)(1)

- Plasma concentration data from subjects who vomited during the study should be flagged and reported even though they were excluded from the analysis.

F. Data Submission and Analysis

The sponsor should submit the following PK information:

- Drug concentrations in plasma/other acceptable matrices and their corresponding sampling time points

- Study design elements: Subject, period, sequence of drug administration, and treatment

- Measures of variability: Inter-subject, intrasubject, and total variability, if available

- PK parameters for single-dose studies: $AUC_{0\text{-}t}$, $AUC_{0\text{-}INF}$, truncated or partial AUC if applicable, C_{max}, T_{max}, t_{lag}, λz, $t_{1/2,}$ clearance, and volume of distribution

- Steady-state PK parameters for multiple-dose studies: $AUC_{0\text{-}TAU}$, C_{max}, T_{max}, the lowest concentration in a dosing interval (C_{min}), the concentration at the end of the dosing interval (C_{trough}), the average concentration during a dosing interval (C_{av}), the degree of fluctuation $[(C_{max}\text{-}C_{min})/C_{av}]$, and the swing $[(C_{max}\text{-}C_{min})/C_{min}]$. C_{trough} should be measured for at least two dosing intervals to assess whether steady-state was achieved. The drug's clearance and volume of distribution should also be reported.

- Statistical information for $AUC_{0\text{-}t,}$ $AUC_{0\text{-}INF,}$ and C_{max}: Geometric means, arithmetic means, as well as geometric mean ratios and their corresponding 90 percent CIs

For details pertaining to data analysis, consult the FDA guidance for industry entitled *Statistical Approaches to Establishing Bioequivalence*.

G. Rounding Off CI Values

Sponsors should round off CI values to two decimal places. To fall within a CI limit of 80 to 125 percent, the value would be at least 80.00 percent and not more than 125.00 percent.

APPENDIX B: GUIDELINES FOR CONDUCTING FED OR FASTED STUDIES

For new IR drug products developed via the 505(b)(1) pathway for which BA is determined using a solution, IV, or a previously developed formulation as a reference, the BA study should be conducted under fasting conditions except when tolerability issues are anticipated with fasting. The effect of food on the BA of the new drug product should be evaluated using a high-fat and high-calorie meal. If the objective is to evaluate the effect of other meal types, then other meals with different compositions may also be assessed in addition to the high-fat and high-calorie meal. For more information, consult the FDA guidance for industry entitled *Assessing the Effects of Food on Drugs in INDs and NDAs – Clinical Pharmacology Considerations*.[42]

For new drug products developed via the 505(b)(1), 505(b)(2) pathways, or postapproval changes for which BA is determined using an approved product as a reference:

- If the reference drug product is labeled to be taken under fasting conditions, then the test drug product should be compared under fasting conditions to the reference drug product. In addition, the effect of a high-fat meal on the new drug product should be evaluated. A three-way crossover study is recommended because it allows the relevant comparisons to be made directly.

- If the reference drug product is labeled to be taken without regard to meals, then the test and reference drug product should be compared under fasting conditions. In addition, the effect of a high-fat meal on the new drug product should be evaluated, or the BA of the new drug product can be compared to the reference drug product with a high fat meal.

- If the reference drug product is labeled to be taken with food, then the test drug product should be compared under fasting and fed conditions (the fed conditions in this study should be the same as described in the labeling for the reference product). A three-way crossover study is recommended because it allows the relevant comparisons to be made directly.

- If the reference drug product is labeled to be taken with food to avoid tolerability issues in the fasting state, then the BA for the test drug product should be evaluated under fed conditions according to the labeling instructions for the reference product.

For pre-approval changes in drug product formulations (e.g., bridging a to-be-marketed formulation to the clinical trial formulation), the above principles can be adopted to determine the appropriate studies needed to address issues related to BA and the impact of food intake on the BA of a new formulation. Additional food effect studies may be needed during drug development depending on the nature of formulation changes.

[42] When final, this guidance will represent the FDA's current thinking on this topic

APPENDIX C: GUIDELINES FOR CONDUCTING AN IN VITRO ALCOHOL DOSE-DUMPING STUDY

The sponsor should conduct in vitro assessments of the drug release from the drug product using media with various alcohol concentrations on the lowest and highest strengths of the MR drug product. The following points should be considered during the evaluation of the in vitro, alcohol-induced, dose dumping of MR drug products:

- Dissolution testing should be conducted using the optimal apparatus and agitation speed. Dissolution data should be generated from twelve dosage units (n=12) at multiple time points to obtain a complete dissolution profile.

- The following alcohol concentrations are recommended for the in vitro dissolution studies: 0, 5, 10, 20, and 40 percent.

- The general considerations for selecting the media are as follows:

  -If the optimal dissolution medium is 0.1N HCl: Dissolution profiles in 0.1 N HCl (pH 1.2) containing the above range of alcohol concentrations are sufficient.

  -If the optimal dissolution medium is not 0.1N HCl: Dissolution profiles using the above range of alcohol concentrations in 0.1N HCl and in the optimal dissolution medium are recommended.

  -If the optimal dissolution medium has not been identified: Dissolution profiles using the above range of alcohol concentrations in three physiologically relevant pH media (i.e. pH 1.2, 4.5, and 6.8) are recommended.

  -If the dissolution is pH-independent: Dissolution data in 0.1N HCl with the above range of alcohol concentrations are sufficient.

  -For a delayed-release (enteric-coated) product: Dissolution data in 0.1N HCl with the above range of alcohol concentrations are sufficient.

- The shape of the dissolution profiles should be compared to determine if the modified release characteristics are maintained, especially in the first 2 hours.

- The f_2 values assessing the similarity (or lack thereof) between the dissolution profiles should be estimated (using 0 percent alcohol as the reference).

- The report should include complete data (i.e., individual, mean, standard deviation, comparison plots, f_2 values, etc.) collected during the evaluation of the in vitro, alcohol-induced, dose-dumping study.

Based on the results of the in vitro assessments, an in vivo BA study of the drug product when administered with alcohol may be needed.