Guidance for Industry

Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA

DRAFT GUIDANCE

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For questions regarding this draft document, contact Teresa Ramson at 240-402-3870.

U.S. Department of Health and Human Services
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Biopharmaceutics

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Guidance for Industry[1]

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This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance provides recommendations to applicants planning to include bioequivalence (BE) information in abbreviated new drug applications (ANDAs) and ANDA supplements. The guidance describes how to meet the BE requirements set forth in the Federal Food, Drug, and Cosmetic Act (FD&C Act) and FDA regulations. The guidance is generally applicable to dosage forms intended for oral administration and to non-orally administered drug products in which reliance on systemic exposure measures is suitable for documenting BE (e.g., transdermal delivery systems and certain rectal and nasal drug products). We believe that the guidance will also be useful when planning BE studies intended to be conducted during the postapproval period for certain changes in an ANDA.

This guidance revises and replaces parts of two FDA guidances for industry,[2] relating to BE and fed BE studies to be submitted in ANDAs. This guidance does not address bioavailability (BA), BE, and food effect studies in investigational new drug applications (INDs) and new drug applications (NDAs). A separate guidance will soon be available that will address BA and BE studies for INDs, NDAs, and NDA supplements.[3] FDA has determined that separating guidances according to application type will be beneficial to applicants.

[1] This guidance was prepared by the Division of Bioequivalence in the Office of Generic Drugs, Office of Pharmaceutical Science, Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

[2] *Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs — General Considerations* and *Food-Effect Bioavailability and Fed Bioequivalence Studies.*

[3] Many guidances are referenced throughout this document, and they can be found on the Internet at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm. We update guidances periodically. To make sure you have the most recent version of a guidance, check this CDER guidance Web site.

In addition, FDA routinely publishes guidances on BE study design for specific products.[4] FDA recommends that applicants consult this general guidance in conjunction with any relevant product-specific guidance when considering the appropriate BE study for a proposed product.

FDA's guidance documents, including this guidance, 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

To receive approval for an ANDA, an applicant generally must demonstrate, among other things, that its proposed drug product is bioequivalent to the reference listed drug (RLD, or reference product).[5] The FD&C Act provides that a generic drug is bioequivalent to the listed drug if:

> The rate and extent of absorption of the drug do not show a significant difference from the rate and extent of absorption of the listed drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either a single dose or multiple doses....[6]

For most products, the focus of BE studies is on the release of the drug substance from the drug product into the systemic circulation. During such BE studies, an applicant compares the systemic exposure profile of a test drug product to that of the RLD.

III. ESTABLISHING BIOEQUIVALENCE

Under FDA regulations, an applicant must use "the most accurate, sensitive, and reproducible approach available among those set forth" in 21 CFR 320.24(b) to demonstrate BE.[7] As noted in 21 CFR 320.24, in vivo and/or in vitro methods can be used to establish BE. In general descending order of preference, these include pharmacokinetic, pharmacodynamic, clinical, and in vitro studies.[8]

[4] See guidance for industry on *Bioequivalence Recommendations for Specific Products* at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm075207.htm

[5] See section 505(j)(2)(A)(iv) of the FD&C Act; 21 CFR 314.94(a)(7).

[6] Section 505(j)(8)(B)(i) of the FD&C Act. See also section 505(j)(8)(B)(ii), (C) of the FD&C Act; 21 CFR 320.1(e), and 320.23(b).

[7] See 21 CFR 320.24(a).

[8] See 21 CFR 320.24(b).

A. Pharmacokinetic Studies

1. General Considerations

As provided above, the statutory definition of BE, expressed in terms of rate and extent of absorption of the active ingredient or moiety, emphasizes the use of pharmacokinetic endpoints in an accessible biological matrix, such as blood, plasma, and/or serum, to indicate release of the drug substance from the drug product into the systemic circulation.[9] BE frequently relies on pharmacokinetic endpoints such as C_{max} (peak plasma concentration) and AUC (area under the plasma concentration time curve) that are reflective of rate and extent of absorption, respectively.

If serial measurements of the drug or its metabolites in plasma, serum, or blood cannot be accomplished, measurement of urinary excretion can be used to demonstrate BE.

2. Pilot Study

If the applicant chooses, a pilot study in a small number of subjects can be carried out before proceeding with a full BE study. This pilot study can be used to validate analytical methodology, assess variability, optimize sample collection time intervals, and provide other information.

3. Pivotal Bioequivalence Studies

General recommendations for a standard BE study based on pharmacokinetic measurements are provided in the Attachment.

4. Study Designs

FDA recommends use of a two-period, two-sequence, two-treatment, single-dose, crossover study design, a single-dose parallel study design, or a replicate study design for BE studies. For most dosage forms that release drug intended to be systemically available, we recommend that applicants perform a two-period, two-sequence, two-treatment, single-dose, crossover study using healthy subjects. In this design, each study subject should receive each treatment (test, and RLD) in random order. The crossover design may not be practical for drugs with long pharmacokinetic half-lives (i.e., longer than 24 hours). In such cases, investigators can use a single-dose, parallel design where each treatment should be administered to a separate group of subjects with similar demographics. The general recommendations for study designs provided in the Attachment should be used in designing crossover studies as well.

A replicate crossover study may be an appropriate alternative to the parallel or nonreplicate crossover study described above, and can be conducted as either a partial (three-way) or full (four-way) replication of treatment. In this design, one or both treatments should be administered to the same subject on two separate occasions. The replicate design has the advantage of using fewer subjects although each subject should receive more treatments than in

[9] See section 505(j)(8)(B) of the FD&C Act.

the two-treatment, crossover design. The replicate design is especially useful for highly variable drugs.

We recommend that applicants use the average BE method of analysis with these study designs for establishing BE. In limited cases, applicants may use a scaled-average BE analysis approach for highly variable drugs.[10] This analysis approach is typically used with a replicate study design. Recommendations for replicate study designs and the average BE approach method can be found in the guidance for industry on *Statistical Approaches to Establishing Bioequivalence*.[11]

For applicants wishing to use variations of these study designs or analysis methods (e.g., a sequential design or scaled-average BE), we recommend that you submit a complete protocol for review and comment before starting the study.

5. Study Population

In general, unless otherwise recommended in a specific guidance:

- Subjects recruited for in vivo BE studies should be 18 years of age or older.
- In vivo BE study subjects should be representative of the general population, taking into account age, sex, and race.
- If a drug product is intended for use in both sexes, the applicant should include similar proportions of males and females in the study.
- If the drug product is predominantly intended for use in the elderly, the applicant should include as many subjects as possible at or above age 60.
- The total number of subjects in a study should be sufficient to provide adequate statistical power for BE demonstration, but we do not expect that there will be sufficient power upon which to draw conclusions for each subgroup.

In most cases, we do not recommend statistical analysis of subgroups.

We also recommend that any restrictions on admission into a study be based primarily on safety considerations. Sometimes, safety considerations preclude the use of healthy volunteers. In such

[10] For highly variable drugs (intrasubject variability $\geq$ 30%), applicants can conduct BE studies using a replicate design approach. Alternatively, a single-dose, randomized, three-period reference-scaled, average BE approach is also appropriate. The reference-scaled average BE approach adjusts the BE limits of highly variable drugs by scaling to the within-subject variability of the RLD in the study and imposes a limit of 0.8 to 1.25 on the geometric mean ratio. The within-subject variability of RLD should be determined using a three-way modified replicate-design study in which the RLD is given twice and the test product is given once. For general information on the reference-scaled approach, investigators should refer to the published book chapter, *Davit B, Conner D*. Reference-scaled average bioequivalence approach. In: Kanfer I, Shargel L, eds. Generic Drug Product Development – International Regulatory Requirements for Bioequivalence. New York, NY: Informa Healthcare, 2010:271-272.

[11] See footnote 3.

situations, applicants should attempt to enroll patients that the drug is intended to treat and whose disease process and treatments are stable for the duration of the BE study. An IND for certain BE studies may be required, for example, for cytotoxic products.[12]

6. Single-Dose Studies

We usually recommend single-dose pharmacokinetic studies for both immediate and modified release drug products to demonstrate BE because these studies are generally more sensitive than steady-state studies in assessing differences in the release of the drug substance from the drug product into the systemic circulation.

7. Steady-State Studies

When safety considerations suggest using patients who are already receiving the medication, often the only way to establish BE without disrupting a patient's ongoing treatment is in a steady-state study. We recommend that if a steady-state study is recommended, applicants carry out appropriate dosage administration and sampling to document the attainment of steady-state.

8. Bioanalytical Methodology

We recommend applicants ensure that bioanalytical methods for BE studies are accurate, precise, selective, sensitive, and reproducible. A separate draft guidance for industry on *Bioanalytical Method Validation* is available to assist applicants in validating bioanalytical methods.[13]

9. Pharmacokinetic Measures of Rate and Extent of Exposure

a. Rate of Absorption (Peak Exposure)

For both single-dose and steady-state studies, we recommend that you assess the rate of absorption by measuring the peak drug concentration (C_{max}) obtained directly from the data without interpolation. The time-to-peak drug plasma concentrations (T_{max}) can also provide important information regarding the rate of absorption.

b. Partial Exposure

For orally administered immediate release drug products, BE can generally be demonstrated by measurements of peak and total exposure. We recommend the use of partial AUC as an early exposure measure under certain circumstances. The time to truncate the partial area should be related to a clinically relevant pharmacodynamic (PD) measure. We recommend that sufficient quantifiable samples be collected to allow adequate estimation of the partial area. For further information on specific products, applicants should consult our website to determine whether a product-specific guidance for the proposed product is available.[14]

[12] See 21 CFR 312.2(c) and 320.31.
[13] See footnote 3.
[14] See footnote 3.

c. Extent of Absorption (Total Exposure)

For single-dose studies, we recommend that the indicators for extent of absorption be both of the following:

- Area under the plasma/serum/blood concentration-time curve from time zero to time t (AUC_{0-t}), where:
  t is the last time point with a measurable concentration.

- Area under the plasma/serum/blood concentration-time curve from time zero to time infinity (AUC_{0-inf}), where:
  $AUC_{0-inf} = AUC_{0-t} + C_t/\lambda_z$
  - C_t is the last measurable drug concentration
  - λ_z is the terminal or elimination rate constant calculated according to an appropriate method.

For steady-state studies, we recommend that the indicator for extent of absorption be the area under the plasma, serum, or blood concentration-time curve over a dosing interval at steady-state (AUC_{0-tau}), where tau is the length of the dosing interval.

10. Fed Bioequivalence Studies

Co-administration of food with oral drug products can influence BE. Therefore, fed BE studies can determine whether test and RLD products are bioequivalent when co-administered with meals. We usually recommend a single-dose, two-period, two-treatment, two-sequence, crossover study for fed BE studies. See Attachment for details on study design.

When a fasting in vivo BE study is recommended for an orally administered, immediate release product, we recommend that applicants conduct a fed study, except when the *dosage and administration* section of the RLD labeling states that the product should be taken only on an empty stomach (e.g., the labeling states that the product should be administered 1 hour before or 2 hours after a meal).

For orally administered, immediate release products labeled to be taken only with food, fasting and fed studies are recommended, except when serious adverse events are anticipated with fasting administration. In these latter cases, we recommend that applicants conduct only a fed study; a fasting study is not recommended.

For all orally administered, modified-release drug products, we recommend that applicants conduct a fed BE study in addition to a fasting BE study. These studies should usually be conducted on the highest strength of the drug product, unless safety considerations preclude the use of that dose in study subjects.

11. Sprinkle Bioequivalence Studies

If the label of a modified release RLD product states that the product can be administered sprinkled in soft foods, we recommend applicants conduct an additional BE study. For each treatment arm, the product should be sprinkled on one of the soft foods mentioned in the labeling of the RLD, normally applesauce. Aside from administration in the soft food, this additional study should follow the recommendations for the fasting BE study described in the Appendix.

12. Bioequivalence Studies of Products Administered in Specific Beverages

There are certain products with labeling that specifies that the product must be administered in a specific beverage. BE studies for these products should be administered mixed with one of the beverages mentioned in the labeling. If additional beverages are listed, applicants should provide evidence that using these additional beverages would not result in BE differences.

If there are questions about the use of other vehicles, or the design or analysis of such BE studies, applicants should contact the appropriate staff in the Agency's Office of Generic Drugs (OGD).

B. General Considerations on Other Bioequivalence Studies

In certain circumstances other BE studies are recommended to support a demonstration of BE. Below are some general considerations regarding these other BE studies. Sponsors should consult FDA's guidances for industry for additional information on these methods as well.[15]

1. *In Vitro Tests Predictive of Human In Vivo Bioavailability (In Vitro-In Vivo Correlation Studies)*

In vitro-in vivo correlation (IVIVC) is a scientific 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 response (e.g., plasma drug concentration or amount of drug absorbed). This model relationship facilitates the rational development and evaluation of extended-release dosage forms as a surrogate for bioavailability and/or BE testing, as well as a tool for formulation screening and setting of the dissolution/drug release acceptance criteria.

Additional information specifically on the development and validation of an IVIVC can be found in the guidance for industry on *Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations.*

2. Pharmacodynamic

A suitably validated pharmacodynamic method can be used to demonstrate BE. However, we do not recommend pharmacodynamic studies for drug products that are intended to be absorbed into the systemic circulation and for which a pharmacokinetic approach can be used to establish BE.

[15] See footnote 3.

3. Comparative Clinical Studies

When it is not possible to use the previously described methods, well-controlled BE studies with clinical endpoints in patients can be used to establish BE.

4. In Vitro Studies

Under certain circumstances, BE can be evaluated using in vitro approaches (e.g., dissolution/drug release testing) under 21 CFR 320.24(b). FDA does not recommend in vitro approaches for drug products that are intended to be systemically absorbed. Such approaches would be appropriate; however, in other circumstances (e.g., for drug products that bind bile acids in the gastrointestinal tract).

IV. ESTABLISHING BIOEQUIVALENCE FOR DIFFERENT DOSAGE FORMS

The following sections provide recommendations for establishing BE for specific dosage forms. As explained below, in certain cases BE testing may be waived.

A. Oral Solutions

For oral solutions, elixirs, syrups, tinctures, or other solubilized forms, an in vivo BE testing requirement may be waived for certain products on the ground that in vivo BE is self-evident. In such instances, the applicant would be deemed to have complied with and fulfilled any requirement for in vivo BE data.[16] For example, BE can be waived for an oral solution if the formulation has the same active ingredient in the same concentration and dosage form as the RLD, and does not contain any excipient that significantly affects drug absorption or availability.[17]

B. Immediate Release Products: Capsules and Tablets

1. Preapproval

For immediate release capsule and tablet products, we recommend the following studies: (1) a single-dose, fasting study comparing the highest strength of the test and RLD products and (2) a single-dose, fed BE study comparing the highest strength of the test and RLD products (see section III.A.10).

Conducting an in vivo study on a strength other than the highest may be appropriate for reasons of safety, with concurrence by the Division of Bioequivalence, OGD, if the following conditions are met:

- Linear elimination kinetics has been documented over the therapeutic dose range.

[16] See 21 CFR 320.22(b)(3).
[17] Ibid.

- The higher strengths of the test and RLD products are proportionally similar to their corresponding lower strength.
- Comparative dissolution testing on the higher strength of the test and RLD products has been submitted and found to be acceptable.

An in vivo BE requirement for one or more strength(s) can be waived based on (i) acceptable BE study on the designated strength, (ii) acceptable in vitro dissolution testing of all the strengths, and (iii) proportional similarity of the formulations across all strengths.[18]

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

- All active and inactive ingredients are in similar proportion between different strengths (e.g., a tablet of 50-mg strength has all the inactive ingredients—almost exactly half that of a tablet of 100-mg strength, and almost twice that of a tablet of 25-mg strength).

- For high-potency drug substances (where the amount of active drug substance in the dosage form is relatively low): (1) the total weight of the dosage form remains nearly the same for all strengths (within ± 10 % of the total weight of the strength on which a biostudy was performed), (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.

- Active and inactive ingredients that are not in similar proportion between different strengths can be considered proportionally similar with adequate justification (such as dosage form proportionality studies that demonstrate equivalent in vivo bioavailability).

Under any of these scenarios, we recommend that in vivo BE studies be accompanied by in vitro dissolution profiles on all strengths of each product. We also recommend that applicants conduct the BE study comparing the test product and the RLD using the strength(s) specified in *Approved Drug Products with Therapeutic Equivalence Evaluations* (commonly referred to as the *Orange Book*).[19]

In addition, for highly soluble, highly permeable, rapidly dissolving, and orally administered immediate release drug products, in vitro data may be acceptable to demonstrate BE based on the biopharmaceutics classification system as described in the guidance for industry on *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*.[20]

[18] See 21 CFR 320.22(d)(2).
[19] See http://www.fda.gov/cder/orange/default.htm.
[20] See footnote 3.

For additional information on BE study design for a specific product, we recommend that applicants consult our website to determine whether a product-specific guidance for your proposed product is available.[21]

2. Postapproval

Please refer to the guidance for industry *Immediate Release Solid Oral Dosage Forms, Scale-Up and Postapproval Chemistry, Manufacturing, and Controls; In Vitro Dissolution Testing and In vivo Bioequivalence Documentation* for information regarding BE testing recommended for specified types of postapproval changes.[22]

For postapproval changes, we recommend that applicants make the in vitro comparison between the prechange and postchange products. When in vivo BE studies are recommended to support a postapproval change for an ANDA product, FDA recommends that applicants compare the postchange ANDA drug product to the RLD and not to the prechange ANDA product.

C. Suspensions

We generally recommend that you establish BE for a suspension in the same manner as for other solid oral dosage forms. In vivo studies and dissolution testing should be performed as described in section B (above) on immediate release products, or in section D (below) on modified release products.

D. Modified Release Products

Modified release products include delayed release products and extended release (controlled release or sustained release) products.

1. Delayed Release Products

A *delayed release* drug product is a dosage form that releases a drug at a time later than immediately after administration (e.g., the drug product exhibits a lag time in quantifiable plasma concentrations). Typically, the coatings (e.g., enteric coatings) have been designed to delay the release of medication until the dosage form has passed through the acidic medium of the stomach. In vivo tests for delayed release drug products are similar to those for extended release drug products. We recommend that in vitro dissolution tests for these products document that they are stable under acidic conditions and that they release the drug only in a neutral medium (e.g., pH 6.8).

2. Extended Release Products

An extended release drug product is a dosage form that allows a reduction in dosing frequency and reduces fluctuations in plasma concentrations when compared to an immediate release dosage form. Extended release products can be formulated as capsules, tablets, granules, pellets,

[21] Ibid.

[22] See footnote 3.

or suspensions. If any part of a drug product includes an extended release component, the product should be treated as a modified release dosage form for the purposes of establishing BE, as specified below.

3. Bioequivalence Studies

For modified release products, we recommend the following studies: (1) a single-dose, fasting study comparing the highest strength of the test with the RLD, and (2) a single-dose fed BE study comparing the highest strength of the test with the RLD product. Because single-dose studies are considered more sensitive in addressing the primary question of BE (e.g., release of the drug substance from the drug product into the systemic circulation), multiple-dose studies are generally not recommended.

4. Demonstration of Bioequivalence: Additional Strengths

Additional strengths of modified release products may be demonstrated to be bioequivalent to the corresponding reference product strengths under 21 CFR 320.24(b)(6) if all of the following conditions have been met:

- The additional strength is proportionally similar in its active and inactive ingredients to the test product strength that underwent acceptable in vivo studies.

- The additional strength has the same drug release mechanism as the strength of the test product that underwent an acceptable in vivo study.

- Dissolution testing of all strengths is acceptable. We recommend that the drug products exhibit similar dissolution profiles between the strength on which BE testing was conducted and other strengths based on the f_2 test in at least three dissolution media (e.g., pH 1.2, 4.5, and 6.8).[23]

We recommend that applicants generate dissolution profiles on the test and RLD products of all strengths.

5. Postapproval Changes

Please refer to FDA's guidance for industry *SUPAC: Modified Release Solid Oral Dosage Forms, Chemistry Manufacturing and Controls; In Vitro Dissolution Testing and In vivo Bioequivalence Documentation* for information regarding BE testing recommended for specified types of postapproval changes for modified release dosage forms.[24]

For postapproval changes, we recommend that applicants make an in vitro comparison between the approved (prechange) product and the test (postchange) product. If appropriate, we

[23] In such instances, we anticipate that such approach will be adequate to demonstrate BE. See 21 CFR 320.24(b)(6).

[24] See footnote 3.

recommend that you use an f_2 test to compare dissolution profiles. An in vivo BE study may be needed if dissolution profiles are not shown to be similar. When in vivo BE studies are recommended to support a postapproval change for an ANDA product, FDA recommends that applicants compare the postchange ANDA drug product to the RLD and not to the prechange ANDA product.

E. Chewable Tablets

Applicants should administer chewable tablets according to the directions on the label. If the label states that the tablet must be chewed before swallowing, the product should be chewed when administered in BE studies. If the label gives the option of either chewing the product or swallowing it whole, the product should be swallowed whole, with 240 mL of water, when administered in BE studies. We also recommend that you conduct in vitro dissolution testing on intact, whole tablets of the chewable drug product.

V. SPECIAL TOPICS

There are a number of topics that may call for special consideration addressed in the following sections. Additional questions should be referred to OGD.

A. Moieties to Be Measured

1. Parent Drug Versus Metabolites

The parent drug in the dosage form should always be measured in the biological fluids collected in BE studies, unless accurate assay quantitation is not possible using state-of-the-art-technology. We generally recommend that applicants measure only the parent drug, rather than metabolites, because the concentration-time profile of the parent drug is more sensitive to changes in formulation performance than a metabolite, which is more reflective of metabolite formation, distribution, and elimination. Primary metabolite(s), formed directly from the parent compound, should be measured if they are both: (1) formed substantially through presystemic metabolism (first-pass, gut wall, or gut lumen metabolism) and (2) contribute significantly to the safety and efficacy of the product. This approach should be used for all drug products, including pro-drugs. We recommend that applicants analyze the parent drug measured in these BE studies using a confidence interval (CI) approach. You can use the metabolite data to provide supportive evidence of a comparable therapeutic outcome.

If the parent drug levels are too low to allow reliable analytical measurement in blood, plasma, or serum for an adequate length of time, the metabolite data obtained from these studies should be subject to the CI approach for BE demonstration.

2. Enantiomers Versus Racemates

For BE studies, we recommend using an achiral assay to measure the *racemate*. We only recommend measuring individual enantiomers in BE studies when all of the following conditions

have been met: (1) the enantiomers exhibit different pharmacodynamic characteristics, (2) the enantiomers exhibit different pharmacokinetic characteristics, (3) primary efficacy and safety activity reside with the minor enantiomer, and (4) nonlinear absorption is present (as expressed by a change in the enantiomer concentration ratio with change in the input rate of the drug) for at least one of the enantiomers. In such cases where all of these conditions are met, we recommend that applicants apply BE analysis to the enantiomers separately.

3. Drug Products with Complex Mixtures as the Active Ingredients

Certain drug products contain complex drug substances (e.g., active moieties or active ingredients that are mixtures of multiple synthetic and/or natural source components). Some or all of the components of these complex drug substances cannot be fully characterized with regard to chemical structure and/or biological activity. We do not encourage quantification of all active or potentially active components in pharmacokinetic studies. Rather, we recommend that applicants base BE studies on a small number of markers of rate and extent of absorption. Selection of the markers should be based on the characteristics of the drug product. Criteria for marker selection can include amount of the moiety in the dosage form, plasma, or blood levels of the moiety, and biological activity of the moiety relative to other moieties in the complex mixture.

B. Long Half-Life Drugs

For an oral immediate release product with a long elimination half-life drug (>24 hrs), applicants can conduct a single-dose, crossover study, provided an adequate washout period is used. If the crossover study is problematic, applicants should use a BE study with a parallel design. For either a crossover or parallel study, sample collection time should be adequate to ensure completion of gastrointestinal transit of the drug product and absorption of the drug substance. (which usually occurs within approximately 2 to 3 days). You can use C_{max} and a suitably truncated AUC to characterize peak and total drug exposure, respectively. For drugs that demonstrate low intrasubject variability in distribution and clearance, you can use an AUC truncated at 72 hours ($AUC_{0\text{-}72\ hr}$) in place of $AUC_{0\text{-}t}$ or $AUC_{0\text{-}inf}$. For drugs demonstrating high intrasubject variability in distribution and clearance, AUC truncation should not be used.

C. First Point C_{max}

The first point of a concentration-time curve in a BE study, based on blood and/or plasma measurements, is sometimes the highest point, which raises questions of bias in the estimation of C_{max} because of insufficient early sampling times. A carefully conducted pilot study can enable an applicant to avoid this problem.

In the main BE study, collection of blood samples at an early time point, between 5 and 15 minutes after dosing, followed by additional sample collections (e.g., two to five) in the first hour after dosing is usually sufficient to assess peak drug concentrations. Failure to include early (5-15 minute) sampling times leading to first time-point C_{max} values may result in FDA not considering the data for affected subjects from the analysis.

D. Alcoholic Beverage Effects On Modified Release Drug Products

The consumption of alcoholic beverages can affect the release of a drug substance from an MR formulation. The formulation can lose its modified release characteristics, leading to more rapid drug release and altered systemic exposure. This can have deleterious effects on the drug's safety and/or efficacy.

FDA recommends applicants developing certain extended release solid oral dosage forms to conduct in vitro studies to determine the potential for dose dumping in alcohol in vivo. In vitro assessments of the drug release from the drug product using media with various alcohol concentrations may be recommended. An in vivo BE study of the drug product when administered with alcohol may be suggested in some cases. For information on specific products, we recommend that applicants consult the guidance for industry *Individual Product Bioequivalence Recommendations* and any available relevant product-specific guidance.[25]

E. Endogenous Compounds

Endogenous compounds are drugs that are already present in the body either because the body produces them or they are present in the normal diet. Because these compounds are identical to the drug that is being administered, determining the amount of drug released from the dosage form and absorbed by each subject can be difficult. We recommend that applicants measure and approximate the baseline endogenous levels in blood (plasma) and subtract these levels from the total concentrations measured from each subject after the drug product has been administered. In this way, you can achieve an estimate of the actual drug availability from the drug product. Depending on whether the endogenous compound is naturally produced by the body or is present in the diet, the recommended approaches for determining BE differ as follows:

- When the body produces the compound, we recommend that you measure multiple baseline concentrations in the time period before administration of the study drug and subtract the baseline in an appropriate manner consistent with the pharmacokinetic properties of the drug.

- When there is dietary intake of the compound, we recommend that you strictly control the intake both before and during the study. Subjects should be housed at a clinic before the study and served standardized meals containing an amount of the compound similar to that in the meals to be served on the pharmacokinetic sampling day.

For both of the approaches above, we recommend that you determine baseline concentrations for each dosing period that are period specific. If a baseline correction results in a negative plasma concentration value, the value should be set equal to 0 before calculating the baseline-corrected AUC. Pharmacokinetic and statistical analysis should be performed on both uncorrected and corrected data. Determination of BE should be based on the baseline-corrected data.

[25] See footnote 3.

F. Orally Administered Drugs Intended For Local Action

In some cases, when a drug substance produces its effects by local action in the gastrointestinal tract, it may be appropriate to determine BE using PK endpoints. In other cases, it may be appropriate to determine BE using clinical endpoints, pharmacodynamic endpoints and/or suitably designed and validated in vitro studies in addition to, or instead of, measuring drug plasma concentrations. For information on specific products, we recommend that applicants consult the guidance for industry *Bioequivalence Recommendations for Specific Products* and any available relevant product-specific guidance.[26]

G. In Vitro Dissolution Testing

The following guidances for industry provide recommendations on the development of dissolution methodology, setting specifications, and the regulatory applications of dissolution testing:[27]

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

1. Immediate Release Products

For immediate release drug products, we recommend that applicants submit the method set forth in any related official United States Pharmacopeia (USP) drug product monograph. If there is not an official monograph for your proposed product, we recommend that you use the FDA-recommended and the methods described in the USP general chapter on dissolution.[28] A dissolution methods database describing FDA-recommended and USP methods is available to the public on the following Web site at http://www.accessdata.fda.gov/scripts/cder/dissolution/index.cfm.

If you choose to develop a new dissolution method, we recommend that you include the following information in the submission:

- The pH solubility profile of the drug substance.
- Dissolution profiles generated at different agitation speeds (e.g., 100 to 150 revolutions per minute (rpm)) for USP Apparatus I (basket), or 50 to 100 rpm for USP Apparatus II (paddle).
- Dissolution profiles generated on all strengths in at least three dissolution media (e.g., pH 1.2, 4.5, and 6.8 buffer). Water can be used as an additional

[26] Ibid.
[27] See footnote 3.
[28] USP General Chapter <711> *Dissolution*.

medium. If the drug being considered is poorly soluble, we recommend using appropriate concentrations of surfactants.

2. Modified Release Products

For modified release products, dissolution profiles using the method set forth in the official USP drug product monograph for the proposed product can be submitted. If there is not a USP drug product monograph for your proposed product, we recommend that applicants use either the FDA-recommended method (see the dissolution methods database mentioned above), or develop a method that is specific for your product. In addition, we recommend that you submit profiles using the methods described in the USP general chapter on dissolution or FDA methods in addition to those three described above (e.g., pH 1.2, 4.5 buffer, and 6.8 buffer). If you are proposing a method different from the FDA-recommended or USP method, we recommend that you submit data using the FDA-recommended or USP method in addition to your proposed method for comparison.

The applicant should select the agitation speed and medium that provide adequate discriminating ability, taking into account all the available in vitro and in vivo data.

We recommend that you use dissolution data from three newly manufactured batches of test product to set dissolution specifications for modified release dosage forms.

ATTACHMENT: GENERAL DESIGN AND DATA HANDLING OF BIOEQUIVALENCE STUDIES WITH PHARMACOKINETIC ENDPOINTS

For both replicate and nonreplicate in vivo pharmacokinetic BE studies, we recommend the following general approaches. Elements can be adjusted for certain drug substances and drug products.

Study conduct:

- The test or RLD products can be administered with about 8 ounces (240 mL) of water to an appropriate number of subjects under fasting conditions, unless the study is a fed BE study.

- Fed Treatments: We recommend that subjects start the recommended meal 30 minutes before administration of the drug product following an overnight fast of at least 10 hours. Study subjects should eat this meal in 30 minutes or less and the drug product should be administered 30 minutes after start of the meal. The drug product should be administered with 8 fluid ounces (240 mL) of water.

- No food should be allowed for at least 4 hours postdose. Water will be allowed as desired except for 1 hour before and after drug administration. Subjects should receive standardized meals scheduled at the same time in each period of the study.

- Generally, the highest-marketed strength can be administered as a single unit. If warranted to achieve sufficient bioanalytical sensitivity, multiple units of the highest strength can be administered, provided 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., more than five half-lives of the moieties to be measured) should separate each treatment.

- The lot numbers of both test and RLD products and the expiration date for the RLD product should be stated. We recommend that the assayed drug content of the test product batch not differ from the RLD product by more than +/- 5 percent. The applicant 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 RLD products. In accordance with 21 CFR 320.63, study drug test article of the test and RLD products must be retained for five years. For additional information, please refer to the guidance for industry *Handling and Retention of Bioavailability and Bioequivalence Testing Samples.*[29]

- Before and during each study phase, we recommend that subjects: (1) be allowed water as desired, except for 1 hour before and after drug administration, (2) be provided

[29] See footnote 3.

standardized 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 has been collected.

Fed studies test meal composition:

We recommend that applicants conduct fed BE studies using meals that provide the greatest effects on gastrointestinal (GI) physiology and systemic drug availability. We recommend a high-fat (approximately 50 percent of total caloric content of the meal), high-calorie (approximately 800 to 1000 calories) test meal for fed BE studies. This test meal should derive approximately 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively.[30] The caloric breakdown of the test meal should be provided in the study report.

Sample collection and sampling times:

We recommend that under normal circumstances, applicants sample blood, rather than urine or tissue. In most cases, drug or metabolites are measured in serum or plasma. However, in certain cases, whole blood may be more appropriate for analysis. We recommend drawing 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 predose sample, per subject, per dose. This sampling should continue for at least three or more terminal elimination half-lives of the drug. 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 can be spaced in such a way that the maximum concentration of drug in the blood (C_{max}) and terminal elimination rate constant (K_{el}) can be estimated accurately. At least three to four samples should be obtained during the terminal log-linear phase to obtain an accurate estimate of λz from linear regression. We recommend recording the actual clock time when samples are drawn as well as the elapsed time related to drug administration.

Subjects with predose plasma drug concentrations:

If the predose concentration is ≤ 5 percent of C_{max} value in a subject with predose plasma concentration, you can include the subject's data without any adjustments in all pharmacokinetic measurements and calculations. We recommend that if the predose value is greater than 5 percent of C_{max}, you drop the subject from all BE study evaluations.

Data deletion because of vomiting:

We recommend that data from subjects who experience emesis during the course of a BE study for immediate release products be deleted from statistical analysis if vomiting occurs at or before 2 times median T_{max}. For modified release products, we recommend deleting data from the analysis if a subject vomits during a period of time less than or equal to the dosing interval stated

[30] An example test meal would be: two eggs fried in butter, two strips of bacon, two slices of toast with butter, four ounces of hash brown potatoes and eight ounces of whole milk. Substitutions in this test meal (e.g., beef or chicken instead of bacon) can be made as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume, density, and viscosity.

in the labeling of the product.

We recommend applicants provide the following pharmacokinetic information in their submissions:

- Plasma concentrations and time points
- Subject, period, sequence, treatment
- Intersubject, intrasubject, and/or total variability, if available
- For single-dose BE studies: AUC_{0-t}, AUC_{0-inf}, and C_{max}. In addition, please report the following supportive information: T_{max}, K_{el} and $t_{1/2}$.
- For steady-state BE studies: AUC_{0-tau} and C_{maxSS}. In addition, please report C_{minSS} (concentration at the end of a dosing interval), C_{avSS} (average concentration during a dosing interval), degree of fluctuation $[(C_{max}-C_{min})/C_{avSS}]$, swing $[(C_{maxSS}-C_{minSS})/C_{minSS}]$, and T_{max}.

We recommend applicants provide the following statistical information for AUC_{0-t}, AUC_{0-inf}, and C_{max}:

- Geometric means
- Arithmetic means
- Geometric mean ratios
- 90 percent Confidence intervals (CI)

We also recommend that you provide logarithmic transformation for measures used for BE demonstration.

Rounding off of CI values:

We recommend that applicants not round off CI values; therefore, to pass a CI limit of 80 to 125 percent, the value would be at least 80.00 percent and not more than 125.00 percent.

GLOSSARY

AUC_{0-t} - Area under the concentration time curve from time zero to the last measurable time point.

AUC_{0-inf} - Area under the concentration time curve extrapolated to infinity.

AUC_{0-tau} - Area under the concentration time curve for one dosing interval at steady-state.

C_{avSS} - Average plasma concentration at steady-state.

C_{max} - Peak concentration.

C_{maxSS} - Peak concentrations during the dosing interval at steady-state.

C_{minSS} - Minimum or trough concentrations at steady-state.

Enantiomers - Two stereoisomers (molecules that are identical in atomic constitution and bonding, but differ in the three-dimensional arrangement of the atoms) that are related to each other by a reflection: they are mirror images of each other, which are nonsuperimposable. Every stereocenter in one has the opposite configuration in the other. Two compounds that are enantiomers of each other have the same physical properties, except for the direction in which they rotate the polarized light and how they interact with different optical isomers of other compounds.

Racemate - A racemate is optically inactive. Because the two isomers rotate plane-polarized light in opposite directions, they cancel out; therefore, a racemic mixture does not rotate plane-polarized light. In contrast to the two separate enantiomers, which generally have identical physical properties, a racemate often has different properties compared to either one of the pure enantiomers. Different melting points and solubilities are very common, but differing boiling points are also possible. Pharmaceuticals can be available as a racemate or as a pure enantiomer, which might have different potencies.