Bioequivalence Studies With Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA

Guidance for Industry

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

May 2026
Generic Drugs

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Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or the 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 office responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance provides recommendations to applicants planning to submit bioequivalence (BE) information in abbreviated new drug applications (ANDAs) as well as amendments and supplements to ANDAs. In addition, this guidance describes how to meet the BE requirements set forth in the Federal Food, Drug, and Cosmetic Act (FD&C Act) and FDA regulations.[2] This guidance is applicable to immediate-release and modified-release oral dosage forms.[3] It is also applicable to non-orally administered drug products in which reliance on systemic exposure measures is suitable for establishing BE (e.g., transdermal delivery systems and certain rectal and nasal drug products). This guidance will also be useful to applicants planning BE studies intended to be conducted during the postapproval period for changes to a drug product approved under an ANDA.

For more information on the scientific and technical aspects of study design and data analysis to support BE assessment for immediate-release solid oral dosage forms, such as tablets, capsules, and granules and powders for oral suspension, see the guidance for industry *M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms* (October 2024).

[1] This guidance has been prepared by the Office of Generic Drugs in the Center for Drug Evaluation and Research (CDER) in cooperation with CDER's Office of Translational Sciences and the Office of Pharmaceutical Quality at the Food and Drug Administration.

[2] FDA recommends that applicants for investigational new drug applications, new drug applications, and new drug application supplements consult the guidance for industry *Bioavailability Studies Submitted in NDAs or INDs — General Considerations* (April 2022), which addresses bioavailability studies for these submission types. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/RegulatoryInformation/Guidances/default.htm.

[3] Modified-release drug products include delayed-release drug products and extended-release (controlled-release or sustained-release) drug products.

FDA also recommends that ANDA applicants consult routinely published product-specific guidances (PSGs) when considering appropriate BE studies for a proposed drug product.[4]

In general, FDA 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 guidance 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).[5] An RLD is the listed drug identified by FDA as the drug product upon which an ANDA applicant relies in seeking approval of its ANDA.[6,7] The FD&C Act provides that a generic drug is bioequivalent to the listed drug if:

> [T]he 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.[8]

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 reference standard designated in FDA's *Approved Drug Products with Therapeutic Evaluations* (the Orange Book).[9,10] A reference standard is the drug product selected by FDA that an

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[4] For more information about FDA's PSG publications and to search for the most recent version of a PSG, see the Product-Specific Guidances for Generic Drug Development web page at https://www.fda.gov/drugs/guidances-drugs/product-specific-guidances-generic-drug-development.

[5] See section 505(j)(2)(A)(iv) of the FD&C Act (21 U.S.C. 355(j)(2)(A)(iv)) and 21 CFR 314.94(a)(7). In general, to obtain approval of an ANDA for a generic drug, an ANDA applicant first must identify the previously approved drug product it seeks to duplicate, i.e., the RLD, and must show, among other things, that the generic drug is bioequivalent to the RLD.

[6] 21 CFR 314.3(b).

[7] The guidance for industry *M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms* (October 2024) uses the term *comparator product*, which, in the context of that guidance, is the drug product accepted by regulatory agencies that an applicant can use to compare against the test product in conducting a BE study. As discussed further below, a comparator product used in conducting an in vivo BE study to support an ANDA in the United States is the reference standard (21 CFR 314.3(b)).

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

[9] The Orange Book is available at https://www.accessdata.fda.gov/scripts/cder/ob/.

[10] 21 CFR 314.3(b).

applicant seeking approval of an ANDA must use in conducting an in vivo BE study for approval of the ANDA.[11,12]

III. ESTABLISHING BE

The regulations governing BE are provided at 21 CFR part 320. As noted in 21 CFR 320.24, in vivo methods, in vitro methods, or both can be used to establish BE. These methods can include in vivo BE studies with pharmacokinetic (PK) endpoints, in vitro tests predictive of human in vivo bioavailability (BA) (e.g., in vitro-in vivo correlation (IVIVC)), in vivo BE studies with pharmacodynamic (PD) endpoints, comparative clinical endpoint BE studies, and in vitro BE studies.[13]

A. In Vivo BE Studies With PK Endpoints

1. General Considerations

As provided above, the statutory definition of BE, expressed in terms of rate and extent of absorption of the drug, emphasizes the use of PK endpoints in an accessible biological matrix (such as whole blood, plasma, or serum) to indicate release of the active ingredient or active moiety from the drug product into the systemic circulation.[14] BE frequently relies on PK endpoints such as **C_{max}**[15] and **AUC** that are reflective of the rate and extent of absorption.

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

ANDA applicants are required to submit information from all BE studies, including pilot studies and failed studies, conducted with the same formulation of the proposed drug product.[16] For failed studies, ANDA applicants should provide the reason(s) for the failure.

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[11] 21 CFR 314.3(b). Ordinarily, FDA selects the RLD as the reference standard. There may be circumstances (e.g., when the RLD is no longer marketed) in which the reference standard is a drug product other than the RLD. For more information regarding the distinction between an RLD and reference standard, refer to the guidance for industry *Referencing Approved Drug Products in ANDA Submissions* (October 2020).

[12] Although the regulations do not require that an applicant use a particular product for in vitro testing, FDA recommends that applicants use the reference standard for in vitro testing.

[13] See 21 CFR 320.24(b), which lists these methods in descending order of accuracy, sensitivity, and reproducibility.

[14] Section 505(j)(8)(B) of the FD&C Act and 21 CFR 314.3(b).

[15] Terms that appear in bold type are defined in the glossary at the end of this guidance.

[16] See 21 CFR 314.94(a)(7) and FDA's guidance for industry *Submission of Summary Bioequivalence Data for ANDAs* (May 2011).

2. Pilot Studies

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

3. Pivotal BE Studies

General recommendations for a standard BE study based on PK endpoints are provided in Appendix A.

4. Study Designs

FDA recommends that applicants use (1) a single-dose, nonreplicate crossover study design, (2) a single-dose, parallel study design, or (3) a single-dose, replicate crossover study design for BE studies.

In general, BE studies should be conducted in healthy subjects (see section III.A.5, Study Population) and should be conducted on the highest to-be-marketed strength. The general recommendations for study designs provided in the Appendix should be considered in designing studies. FDA recommends that applicants use the average BE approach for analysis with these study designs.

For most dosage forms that release a drug intended to be systemically available, FDA recommends that applicants perform a single-dose, crossover study using either healthy subjects or other populations, as appropriate. In this design, each subject should receive each treatment (the test product and the reference standard) in a random order.

If the highest to-be-marketed strength cannot be administrated to healthy subjects for safety or tolerability reasons, a lower strength may be administered in a single-dose study in healthy subjects. Alternatively, applicants could consider conducting a single-dose study in patients with the disease or condition for which the product is indicated, using the highest to-be-marketed strength.

A multiple-dose, steady-state study may be conducted in patients if a single-dose study cannot be conducted in either healthy subjects for safety and/or tolerability reasons or in patients for ethical reasons. For a multiple-dose study, the study protocol should include an appropriate number of dosage administrations to reach steady-state, which can be justified using an appropriate sampling scheme, i.e., concentrations at the end of the dosing interval should be sampled sequentially until $\mathbf{C_{tau}}$ is stable.

A replicate crossover study design (either partial or fully replicate) is appropriate for highly variable drugs[17] as well as non-highly variable drugs. A replicate design can have the advantage

[17] Highly variably drugs are drugs with high intrasubject variability (within subject variability in BE measures 30 percent or greater) and that are not considered narrow therapeutic index drugs.

of using fewer subjects compared to a nonreplicate design, although each subject in a replicate design study would receive more treatments.

FDA specifically recommends that a replicate design be used under the following scenarios:

- A replicate design is advantageous over a nonreplicate design for non-narrow therapeutic index (NTI) drugs with a high intrasubject variability. Either a partial or fully replicate design may be used, but a reference-scaled BE analysis approach should only be applied to specific PK metrics that exhibit a high within-subject variability for the reference standard in the pivotal BE study. Refer to the guidance for industry *Statistical Approaches to Establishing Bioequivalence* (May 2026) for the statistical analysis for the reference-scaled average BE analysis approach for highly variable drugs.

- A fully replicate design is recommended for NTI drugs, where within-subject variability for both the reference standard and test product can be computed and a reference scaled-average BE analysis can be conducted to properly adjust the BE acceptance criteria. Refer to the guidance for industry *Statistical Approaches to Establishing Bioequivalence* (May 2026) for the statistical analysis for the reference-scaled average BE analysis approach for NTI drugs.

Additional information and recommendations for nonreplicate study designs, replicate study designs, and the average BE approach can be found in the guidance for industry *Statistical Approaches to Establishing Bioequivalence* (May 2026).

Applicants can request an evaluation of a BE study design or analysis method (e.g., a sequential design) that deviates from the recommendations in a PSG or general guidance by submitting a controlled correspondence[18] or requesting a meeting, as appropriate, with specific questions about their alternative approach before starting the study.[19]

5. Study Population

In general, unless otherwise recommended in a PSG:

- Healthy subjects[20] should be recruited.

- Subjects recruited for in vivo BE studies should be 18 years of age or older and preferably have a body mass index between 18.5 and 30.0 kg/m^2.

[18] See the guidance for industry *Controlled Correspondence Related to Generic Drug Development* (March 2024).

[19] For more information on meeting types, such as product development meetings, see the guidance of industry *Formal Meetings Between FDA and ANDA Applicants of Complex Products Under GDUFA* (October 2022).

[20] *Healthy subjects* are, in general, non-smoking adults 18 years of age or older without existing medical conditions or required medications that exert physiological effects.

- If a drug product is intended for use in both sexes, the applicant should consider including male and female subjects in the study. If a drug product is intended for use in a single sex, then FDA recommends that the study only include subjects of that sex.

- Female subjects of reproductive potential[21] should not be pregnant or lactating.

- If the drug product is intended for use in older adults, the applicant should consider including subjects at or above age 65 in the study, unless they are associated with greater susceptibility to safety risks.

- In general, a BE assessment in adults between the test product and the reference standard can be used to support a BE assessment in pediatric patients; however, if the RLD is predominantly intended for use in pediatric patients younger than 6 years of age, the applicant should justify that the BE study results obtained from adult subjects are relevant to the pediatric population. FDA also recommends that applicants include justification that the inactive ingredients in the proposed products are appropriate for use in the pediatric population.

- Genetic polymorphisms of drug metabolizing enzymes or transport proteins can affect PK, PD, or both. For drugs with intersubject PK variability due to established genetic polymorphisms, the application of a pharmacogenomic approach can identify subjects at increased risk of adverse events or PK outliers.[22] Thus, phenotyping of subjects, genotyping of subjects, or both should be considered for safety or PK reasons for certain drug products.

- The total number of subjects in a study should be sufficient to provide adequate statistical power for a BE demonstration in the proposed study design.[23]

Admission into a study may be restricted based on safety considerations. For example, safety considerations sometimes preclude conducting the BE study in healthy subjects. In such situations, applicants should attempt to enroll patients for whom the drug is intended. These patients should have stable disease process and treatments for the duration of the BE study. An investigational new drug application may be required for certain products (such as cytotoxic products).[24]

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[21] For purposes of this guidance, *females not of reproductive potential* means persons who are postmenopausal or have another reason for permanent infertility (e.g., hysterectomy, salpingo-oophorectomy). Postmenopausal is defined as 12 months of spontaneous amenorrhea or 6 months of spontaneous amenorrhea with serum follicle-stimulating hormone levels >40mIU/ml.

[22] Applicants should refer to the applicable PSG, if available, for recommendations regarding these drug products.

[23] The number of subjects with evaluable data in a pivotal BE study should not be less than 12. For highly variable drug products, a minimum of 24 subjects is recommended for BE assessment. See the guidance for industry *Statistical Approaches to Establishing Bioequivalence* (May 2026). See also the guidance for industry *M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms* (October 2024).

[24] 21 CFR 312.2(c) and 320.31.

6. Single-Dose Studies

In general, FDA recommends single-dose PK studies for both immediate- and modified-release drug products to demonstrate BE. This is because single-dose studies are generally more sensitive than steady-state studies in assessing differences in the release of the active ingredient or active moiety from the drug product into the systemic circulation.

7. Steady-State Studies

When safety considerations necessitate conducting the BE study in patients who are already receiving a medication, the study can be conducted at a steady state without disrupting a patient's ongoing treatment. If a steady-state study is used, FDA recommends that applicants carry out appropriate dosage administration and sampling to demonstrate the attainment of steady state.

8. Bioanalytical Methodology

FDA recommends applicants ensure that their bioanalytical methods for BE studies are accurate, precise, selective, sensitive, and reproducible. The guidance for industry *M10 Bioanalytical Method Validation and Study Sample Analysis* (November 2022) is available to assist applicants in validating bioanalytical methods.

9. Pharmacokinetic Measures of Rate and Extent of Absorption

a. Rate of absorption

For both single-dose and steady-state studies, FDA recommends that applicants assess the rate of absorption by measuring the C_{max} obtained directly from the data (i.e., without interpolation). **T_{max}** can also provide important information regarding the rate of absorption. Applicants should evaluate T_{max} differences between the test product and the reference standard for any clinical implications or as specified in the applicable PSG. In some situations, C_{max} together with **AUC_{0-t}** may be insufficient to adequately assess the BE between the test product and the reference standard, e.g., when the early onset of action is clinically relevant. In these cases, an additional PK parameter, such as partial AUC (**pAUC**), may be applied (see section III.A.9.c, Partial exposure).

b. Extent of absorption (total exposure)

For single-dose studies for immediate-release products, FDA recommends that the indicator for the extent of absorption be the area under the plasma, serum, or blood concentration-time curve from time zero to time t (AUC_{0-t}), where t is the last time point with a measurable concentration.

For single-dose studies for modified-release products, FDA recommends that the indicators for the extent of absorption be both of the following:

- AUC_{0-t}, where t is the last time point with a measurable concentration

- Area under the plasma, serum, or blood concentration-time curve from time zero to time infinity (**$AUC_{0\text{-}inf}$**), where:

  $AUC_{0\text{-}inf} = AUC_{0\text{-}t} + C_t/\lambda_z$

  - 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, FDA recommends that the indicator for the extent of absorption be the area under the plasma, serum, or blood concentration-time curve over a dosing interval at steady-state (**$AUC_{0\text{-}tau}$**, where *tau* is the length of the dosing interval).

c. Partial exposure

Although BE generally can be demonstrated by measurements of C_{max} and AUC, FDA recommends applicants use a pAUC as an additional exposure measure to C_{max} and AUC if specified in the applicable PSG. For instance, pAUC(s) may be used for certain products in which the different phases of release correspond to a clinical effect. The beginning and ending times for the pAUC(s) should relate to a clinically relevant measure. FDA recommends that sufficient quantifiable samples be collected to allow adequate estimation of the pAUC(s). As mentioned in section I, Introduction, for further information on specific products, applicants should consult FDA's website to determine whether a PSG for the proposed product is available.[25]

For immediate-release drug products with a long elimination half-life, a truncated AUC can be used,[26] provided that the truncated AUC covers the complete absorption phase.

10. Fasting and Fed BE Study Conditions

BE studies should be conducted under standardized conditions that minimize sources of variability outside the products being tested to better detect potential PK differences between the test product and the reference standard.

The design of a BE study with regard to the use of fasting, fed, or both conditions depends on the dosing instructions of the RLD as well as the properties of the drug substance and product formulation. Applicants should refer to the applicable PSG, if available, for recommendations on the design of the BE study or studies for that particular drug product.

Co-administration of food with certain oral drug products can have a differential, formulation-dependent impact on the absorption of active ingredients or active moieties from drug products with special characteristics that result in a higher risk of bioinequivalence due to food effects,

[25] See footnote 4.

[26] See section V.B, Long Half-Life Drugs.

and hence preclude the extrapolation of BE under fasting conditions to fed conditions. In such cases, BE under fed conditions also needs to be demonstrated.

The design of the fed BE study should generally be one of the designs described in section III.A.4, Study Designs. The fed BE study for products where variability is different (i.e., when compared to fasting conditions) may use a different design from the fasting BE study based on the considerations in section III.A.4, Study Designs. Refer to the Appendix for details on study design, including information on meal content.

For orally administered immediate-release drug products with a high-risk of bioinequivalence due to food effect,[27] FDA recommends that applicants conduct BE studies under both fasting and fed conditions, irrespective of the RLD labeling regarding food intake, if safety permits (e.g., when serious adverse events are not anticipated with administration of the drug product under both fasting and fed conditions).

For orally administered immediate-release drug products without a high risk of bioinequivalence due to food effect,[28] single-dose BE studies conducted under fasting conditions typically provide greater discrimination between the PK profiles of the test product and the reference standard than studies conducted under fed conditions. Therefore, for the majority of these drug products, BE may be demonstrated in a single study conducted under fasting conditions.

For these orally administered immediate-release drug products without a high risk of bioinequivalence due to food effect, applicants should also consider the product labeling to determine BE study conditions with regard to food. FDA recommends that applicants conduct:

- A single BE study under fasting conditions for a drug product that is labeled to be taken only under fasting conditions or can be taken under fasting or fed conditions, i.e., without regard to food.
- A single BE study under fed conditions for a drug product that is labeled to be taken only with food due to PK reasons, i.e., enhancing absorption or reducing variability.
- A single BE study under either fasting or fed conditions for a drug product that is labeled to be taken only with food due to tolerability reasons, e.g., stomach irritation or other non-PK reasons.[29]

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[27] Orally administered immediate-release drug products with a high-risk of bioinequivalence are those where the drug substance characteristics in combination with the complexity of the formulation design or manufacturing process lead to an increased likelihood that in vivo performance will be impacted differently by varying gastrointestinal conditions between the fasted and fed conditions. For these drug products, performance differences related to differences in formulation, manufacturing process, or both may not be detected with a single BE study, i.e., results from a fasting BE study may not be extrapolated to predict fed BE study outcome or vice versa, and thus both fasting and fed BE studies should be conducted. See the guidance for industry *M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms* (October 2024) and the guidance for industry *M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms – Questions and Answers* (October 2024) for more information.

[28] The guidance for industry *M13A Bioequivalence for Immediate Release Solide Oral Dosage Forms* (October 2024) uses the term *non-high-risk products*.

[29] See also the guidance for industry *M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms* (October 2024).

For all orally administered modified-release drug products, FDA recommends that applicants conduct BE studies under both fasting and fed conditions, irrespective of the RLD labeling regarding food intake.

For non-orally administered drug products in which reliance on systemic exposure measures is suitable for establishing BE, refer to the applicable PSG for recommendations on the design of a BE study with regard to the use of fasting, fed, or both conditions for that particular drug product when relevant.

Applicants should refer to the applicable PSG for recommendations for a particular drug product when safety would not permit conducting BE studies under fasting conditions, fed conditions, or both. If no PSG is available, applicants can consider alternative approaches, such as using a lower strength, conducting the studies in patients instead of healthy subjects, or using a modeling approach to justify why one study is sufficient to demonstrate BE. Applicants can request an evaluation of an alternative approach by submitting a controlled correspondence or requesting a meeting, as appropriate, with specific questions about their alternative approach before starting the study.[30]

11. Sprinkle BE Studies

If the labeling of a modified-release RLD product states that the product can be administered sprinkled in soft foods, FDA recommends that applicants conduct a sprinkle BE study. For each treatment arm of a sprinkle BE study, the product should be sprinkled on one of the soft foods mentioned in the RLD labeling, normally applesauce. A sprinkle BE study should follow the recommendations for the fasting BE study described in the Appendix, except that the drug product should be administered in the soft food. When serious adverse events are anticipated with fasting administration, a sprinkle BE study should follow the recommendations for the fed BE study described in the Appendix.

12. BE Studies of Products Administered in Specific Beverages

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

If applicants have questions not addressed in the applicable PSG about the use of other vehicles or about the design or analysis of such BE studies, they should contact the Office of Generic Drugs by submitting a controlled correspondence or requesting a meeting, as appropriate.[31]

[30] See footnotes 18 and 19.

[31] Ibid.

B. General Considerations on Other BE Approaches

In certain circumstances, other types of approaches are recommended to support BE. Some general considerations regarding these approaches are described in the following sections. Applicants should refer to FDA's guidances for industry for additional information on these approaches as well.[32]

1. In Vitro BE Studies

In general, FDA does not recommend in vitro approaches for drug products that are intended to be systemically absorbed. However, under certain circumstances, in vitro approaches may be appropriate, and BE can be demonstrated using in vitro approaches (e.g., dissolution/drug-release testing).[33]

For highly soluble and rapidly or very rapidly dissolving 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 *M9 Biopharmaceutics Classification System-Based Biowaivers* (May 2021).

The following FDA guidances for industry provide recommendations on developing dissolution methodology, setting specifications, and the regulatory applications of dissolution testing for immediate-release drug products:

- *Dissolution Testing of Immediate Release Solid Oral Dosage Forms* (August 1997)
- *Dissolution Testing and Acceptance Criteria for Immediate-Release Solid Oral Dosage Form Drug Products Containing High Solubility Drug Substances* (August 2018)

2. In Vitro Tests Predictive of Human In Vivo Bioavailability

IVIVC and physiologically based pharmacokinetic (PBPK) modeling for oral absorption are scientific approaches 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 PK parameter (e.g., plasma drug concentration or amount of drug absorbed). IVIVC facilitates the rational development and evaluation of modified-release dosage forms and, less commonly, other dosage forms. PBPK modeling can be utilized for immediate-release, modified-release, and other dosage forms. Once an IVIVC or PBPK model is validated, it can serve to establish a BE safe space.[34] This ensures

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[32] For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/RegulatoryInformation/Guidances/default.htm.

[33] 21 CFR 320.24(b)(5) and (6).

[34] A BE safe space is the boundaries defined by in vitro specifications, such as dissolution or other relevant drug product quality attributes, within which drug product variants are anticipated to be bioequivalent to one another. See the draft guidance for industry *The Use of Physiologically Based Pharmacokinetic Applications for Oral Drug Product Development, Manufacturing Changes, and Controls* (October 2020). When final, this guidance will represent FDA's current thinking on this topic.

that the in vitro testing can serve as a surrogate for in vivo BA and/or BE testing, as well as a tool for drug product development.

Additional information on the development and validation of an IVIVC can be found in the guidance for industry *Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations* (September 1997).

Additional information on PBPK analyses can be found in the guidance for industry *Physiologically Based Pharmacokinetic Analyses – Format and Content* (August 2018) and the draft guidance for industry *The Use of Physiologically Based Pharmacokinetic Analyses – Biopharmaceutics Applications for Oral Drug Product Development, Manufacturing Changes, and Controls* (September 2020).[35]

3. In Vivo BE Studies with PD Endpoints

A validated PD method can be used to demonstrate BE. However, FDA does not recommend PD studies for drug products that are intended to be absorbed into the systemic circulation and for which a PK or in vitro approach can be used to establish BE.

4. Comparative Clinical Endpoint BE Studies

When it is not possible or sufficient to use the previously described methods, well-controlled BE studies with comparative clinical endpoints in patients can be used to establish BE.[36]

IV. ESTABLISHING BE FOR DIFFERENT DOSAGE FORMS

The following subsections provide recommendations for establishing BE for specific dosage forms. As explained below, in certain cases, a requirement for in vivo BE testing may be waived[37] or an alternative approach may be more accurate, sensitive, and reproducible.[38]

A. Oral Solutions and Other Solubilized Dosage Forms

For oral solutions, elixirs, syrups, tinctures, or other solubilized forms, the in vivo BE testing requirement may be waived if in vivo BE is self-evident.[39] In such instances, the applicant

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

[36] 21 CFR 320.24(b)(4).

[37] 21 CFR 320.22.

[38] In addition to waiver of an in vivo BE requirement under 21 CFR 320.22, there are certain circumstances in which BE can be demonstrated using in vitro approaches under 21 CFR 320.24(b)(6). In such circumstances, an in vivo data requirement is not waived, but rather, FDA has determined that in vitro data are the most accurate, sensitive, and reproducible for a product, as required under 21 CFR 320.24(a).

[39] 21 CFR 320.22(b)(3).

would be deemed to have complied with and fulfilled any requirement for in vivo BE data.[40] For example, an in vivo BE data requirement 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.[41]

There are certain excipients that can alter the BA (e.g., sorbitol can reduce the BA of drugs[42]) in amounts sometimes used in oral, liquid dosage forms. If the formulation contains such an excipient, an in vivo BE study may be required.[43]

B. Immediate-Release Drug Products

This subsection provides recommendations for immediate-release capsules, tablets, powder or granules for suspension, and suspensions.

1. BE Study Designs and Dose

For immediate-release drug products without a high risk of bioinequivalence due to food effect, FDA generally recommends that applicants conduct a single-dose, fasting BE study comparing the highest strength of the test product and reference standard. For immediate-release drug products with a high risk of bioinequivalence due to food effect, FDA generally recommends that applicants conduct the following studies: (1) a single-dose, fasting BE study comparing the highest strength of the test product and reference standard; and (2) a single-dose, fed BE study comparing the highest strength of the test product and reference standard.[44]

If an applicant does not intend to submit an ANDA for the highest strength of the RLD, then FDA generally recommends using the highest to-be-marketed strength for BE studies.

Conducting an in vivo BE study on a strength other than the highest may be appropriate for reasons of safety. Use of a lower strength for reasons of safety is generally acceptable if the following conditions are met:

- Dose proportionality in PK has been documented over the therapeutic dose range.
- The recommendations in section IV.B.2, Demonstration of BE: Additional Strengths in this guidance are followed.

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[40] Ibid.

[41] Ibid.

[42] See the guidance for industry *Bioavailability Studies Submitted in NDAs or INDs — General Considerations* (April 2022).

[43] 21 CFR 320.22(b)(3)(iii).

[44] See section III.A.10, Fasting and Fed Bioequivalence Studies for more information on fasting and fed BE studies. See also the guidance for industry *M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms* (October 2024) for more information.

In other cases (such as non-dose proportionality in PK), applicants may contact the Office of Generic Drugs via a controlled correspondence if there is no applicable PSG or if the proposed strength differs from what is recommended in the applicable PSG.[45]

2. Demonstration of BE: Additional Strengths

An in vivo BE requirement for one or more strength(s) can be waived based on (1) acceptable BE study or studies on the designated strength, (2) acceptable in vitro dissolution testing of all the strengths, and (3) proportional similarity of the formulations across all strengths.[46]

In this guidance, *proportional similarity of the formulations* means any of the following:

- All active and inactive ingredients are in similar proportion between different strengths (e.g., a tablet of 50-milligram (mg) strength has for the active and inactive ingredients almost half that of a tablet of 100-mg strength, and almost twice that 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 (within +/- 10 percent 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 as stated above can be considered proportionally similar with adequate justification. To discuss adequate justification for a particular drug product, applicants can submit a controlled correspondence. FDA will evaluate proportionality during the ANDA assessment.

Under any of the above scenarios, FDA recommends that in vivo BE studies be accompanied by in vitro dissolution profiles on all strengths of each product in the selected quality control method. See section V.F, In Vitro Dissolution Testing for more information. FDA recommends that the similarity factor (f_2) test or other appropriate statistical approach (e.g., multivariate model-independent approach or a model-dependent approach) be used to compare dissolution profiles.

[45] See the guidance for industry *M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms* (October 2024) for more information on the dose to be studied under different scenarios.

[46] 21 CFR 320.22(d)(2).

For information on the designated strength for the BE study or studies and demonstration of BE for a specific product, FDA recommends that applicants refer to the applicable PSG for that particular drug product.[47]

3. Postapproval Changes

For postapproval changes, FDA generally recommends that applicants make an in vitro comparison between the prechange and postchange products. FDA recommends that the f_2 test or other appropriate statistical approach (e.g., multivariate model-independent approach or a model-dependent approach) be used to compare dissolution profiles. When in vivo BE studies are recommended to support a postapproval change for an ANDA product, FDA recommends that applicants compare the postchange ANDA product to the reference standard and not to the pre-change ANDA product. For orally administered immediate-release drug products with a high-risk of bioinequivalence due to food effect, applicants should conduct BE studies under both fasting and fed conditions.[48] For orally administered immediate-release drug products without a high risk of bioinequivalence due to food effect, applicants should conduct a single BE study under either fasting or fed conditions.[49]

For additional information regarding the BE testing recommended for specified types of postapproval changes, refer to the guidance for industry *Immediate Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation* (November 1995).

C. Modified-Release Drug Products

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

1. Delayed-Release Products

A *delayed-release drug product* is a dosage form that releases the active ingredient or active moiety 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) of delayed-release products have been designed to delay the release of the 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, described below. FDA recommends 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., a pH of 6.8). For certain delayed-release products, differences in the delayed-release coating polymer(s) between the test product and RLD can impact the PK profiles at a pH between acidic and neutral which may be clinically undesirable, thus dissolution testing in additional

[47] See footnote 4.

[48] See section III.A.10, Fasting and Fed Bioequivalence Study Conditions. See the guidance for industry *M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms* (October 2024).

[49] Ibid.

pH/media may be warranted. FDA recommends that applicants consult this guidance in conjunction with any applicable PSGs that contain product-specific recommendations for a need to conduct dissolution testing in additional pH/media.[50]

2. Extended-Release Products

An *extended-release drug product* is a dosage form that both allows a reduction in the 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, 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 to establish BE, as specified in sections IV.C.3, BE Study Designs and Dose and IV.C.4, Demonstration of BE: Additional Strengths.

3. BE Study Designs and Dose

For modified-release drug products, FDA generally recommends the following studies: (1) a single-dose, fasting BE study comparing the highest strength of the test product with the reference standard and (2) a single-dose, fed BE study comparing the highest strength of the test product with the reference standard. 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.

Conducting an in vivo BE study on a strength other than the highest may be appropriate for reasons of safety. Use of a lower strength for reasons of safety is generally acceptable if the following conditions are met:

- Dose proportionality in PK has been documented over the therapeutic dose range.
- The recommendations in section IV.C.4, Demonstration of BE: Additional Strengths are followed.

In other cases (such as non-dose proportionality in PK), applicants can contact the Office of Generic Drugs via a controlled correspondence if there is no applicable PSG or the proposed strength differs from what is recommended in the applicable PSG.

4. Demonstration of BE: Additional Strengths

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

- The RLD demonstrates dosage form equivalence among different strengths and demonstrates similar dissolution performance across different strengths.

[50] See footnote 4.

- The test product includes the same excipients for different strengths and the ratios of drug and excipients among different strengths of the test product is justified and appropriate for the drug release mechanism of the test product (e.g., drug and excipients of different strengths can be either proportional or not proportional in quantity, with appropriate justification).

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

- Dissolution testing of all strengths is acceptable. The drug products should exhibit similar dissolution profiles between the strength on which the BE testing was conducted and other strengths, based on the similarity factor (f_2) test or other appropriate statistical approaches (e.g., a multivariate model-independent approach or a model-dependent approach) in at least three dissolution media (e.g., pH of 1.2, 4.5, and 6.8) and in the quality control medium (unless the medium is identical to one of the dissolution media).[51]

FDA recommends that applicants generate dissolution profiles on all strengths of the test product and RLD to better understand the release mechanism between the test product and the RLD. See section V.F, In Vitro Dissolution Testing for more information. To note, there may be instances in which an in vivo BE study for nonproportionally formulated strengths may be necessary to demonstrate BE. The decision of the acceptability of the approach will be made during ANDA assessment based on the totality of evidence (in addition to the dissolution data).

5. Postapproval Changes

For postapproval changes, FDA recommends that applicants perform an in vitro comparison between the prechange product and the postchange product. If appropriate, FDA recommends that the f_2 test be used to compare dissolution profiles. If the f_2 test requirements are not met for the comparison of the dissolution profiles, applicants should use another appropriate statistical approach (e.g., a multivariate model-independent approach or a model-dependent approach). An in vivo BE study may be needed if dissolution profiles are not shown to be similar. When an in vivo BE study is recommended to support a postapproval change for an ANDA product, FDA recommends that applicants compare the postchange ANDA drug product to the reference standard and not to the prechange ANDA product.

Refer to FDA's guidance for industry *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* (October 1997) for information regarding BE testing recommended for specified types of postapproval changes for modified-release dosage forms.

[51] In such instances, FDA anticipates that such an approach will be adequate to demonstrate BE. See 21 CFR 320.24(b)(6).

D. Chewable Tablets

Applicants should administer chewable tablets in BE studies according to the directions in the RLD labeling.

If the RLD labeling states that the chewable tablets can be taken with or without water, the test product and reference standard should be administrated in the BE study without water, as this is considered to be the more discriminating scenario. BE of the test product and reference standard taken with water can then be inferred.

If the labeling states that the tablet must be chewed before swallowing, the product should be chewed when administered in BE studies. If the labeling gives the option of either chewing the product or swallowing it whole, the product should be swallowed whole, with 150 to 250 milliliters of water, when administered in BE studies. FDA also recommends that applicants conduct in vitro dissolution testing on intact tablets of the chewable drug product.

E. Orally Disintegrating Tablets

Applicants should administer orally disintegrating tablets (ODT) according to the directions in the RLD labeling with regard to water intake.

If the RLD labeling states that the ODT can be administered with or without water, the test product and reference standard should be administrated in the BE study without water, as this is considered to be the more discriminating scenario.

In studies evaluating ODTs without water, the subject's mouth should be wet by swallowing a small amount of water, e.g., 20 mL, directly before applying the ODT to the tongue. FDA recommends not allowing fluid intake earlier than one hour after administration.

F. Sublingual Tablets

Sublingual tablets should not be swallowed. The tablets should be placed under the tongue until they are dissolved. Follow the RLD labeling instruction or the applicable PSG for additional information on the method of administration.

G. Transdermal Drug Products

Transdermal drug products are administered to the skin and designed to deliver the drug through (rather than to) the skin. Most transdermal products are extended-release film dosage forms, more commonly known as *transdermal delivery systems*. These deliver drugs into the systemic circulation at a controlled rate for a specified duration. To demonstrate the BE of a transdermal delivery system, an in vivo single-dose, crossover BE study with PK endpoints is recommended. Refer to the draft guidances for industry *Assessing Adhesion with Transdermal and Topical Delivery Systems for ANDAs* (April 2023) and *Assessing the Irritation and Sensitization*

Potential of Transdermal and Topical Delivery Systems for ANDAs (April 2023)[52] and the applicable PSG for recommendations on noninferiority studies on adhesion and skin irritation/sensitization.

Administration of transdermal delivery systems should be to the intact skin unless the RLD labeling indicates otherwise. Transdermal delivery systems should be applied as directed in the RLD labeling unless recommended otherwise in the applicable PSGs. Reservoir transdermal delivery systems should not be cut or otherwise altered before application. Semisolid formulations such as creams, gels, ointments, lotions, or other formulations intended for a systemic effect should be applied as directed over a body surface area consistent with the labeled use or in accordance with the recommendations in the applicable PSG. Systemic BE assessments can be made for transdermal delivery systems and topical formulations. If a product can be administered interchangeably to multiple body sites, it is generally suggested that applicants use a single administration site to demonstrate BE or refer to recommendations in the applicable PSGs.

V. SPECIFIC TOPICS

A number of specific topics are addressed in the following subsections. If a PSG is available on FDA's Product-Specific Guidances for Generic Drug Development web page,[53] the recommendations in that PSG generally supersede those described within this section.

A. Moieties To Be Measured

1. Parent Drug Versus Metabolites

FDA generally recommends that applicants measure only the parent drug, rather than metabolites, because the concentration-time profile of the parent drug is usually considered more sensitive to changes in formulation performance than a metabolite, which is more reflective of metabolite formation, distribution, and elimination. The parent drug should be measured in the biological fluids collected in BE studies, unless accurate assay quantitation is not possible using state-of-the-art-technology. FDA recommends that applicants analyze the parent drug measured using a confidence interval approach.

In rare cases, demonstration of BE based on the parent drug alone may not be sufficient and BE on metabolites may be necessary. Primary active metabolite(s) should be measured if they (1) are formed substantially through presystemic metabolism (i.e., gut wall or gut lumen metabolism) and (2) contribute significantly to the safety and/or efficacy of the product. This approach should be used for all drug products, including prodrugs. Applicants can use the metabolite data to provide supportive evidence of a comparable therapeutic outcome.

[52] When final, these guidances will represent FDA's current thinking on these topics.

[53] See footnote 4.

If the parent drug concentrations are too low to allow reliable analytical measurement in blood, plasma, or serum for an adequate length of time, such as some prodrugs that are rapidly metabolized, it is acceptable to demonstrate BE based on a primary metabolite, a first-step metabolite of the parent drug, without measurement of the parent compound. The metabolite data obtained from these studies should be subject to the confidence interval approach for BE demonstration.

2. Enantiomers Versus Racemates

For BE studies, FDA recommends using an achiral bioanalytical assay to measure the **racemate**. FDA recommends measuring individual **enantiomers** in BE studies only when all the following conditions have been met: (1) the enantiomers exhibit different PD characteristics, (2) the enantiomers exhibit different PK characteristics, and (3) the exposure (AUC) ratio of enantiomers is modified by a difference in the rate of absorption. It is sufficient to demonstrate BE for only the active enantiomer in cases where one enantiomer is inactive (or makes a low contribution) with respect to both safety and efficacy.

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), where some or all the components of these complex drug substances cannot be fully characterized with regard to chemical structure and/or biological activity. For these complex drug products, FDA does not encourage quantification of all active or potentially active components in PK studies. Rather, FDA recommends 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 and mechanism of action of the drug product. Criteria for marker selection can include the biopharmaceutics of the dosage form; the amount of the moiety in the dosage form; the biological fluid concentrations of the moiety; and the biological activity of the moiety relative to other moieties in the complex mixture.

B. Long Half-Life Drugs

For a product with a long elimination half-life drug (i.e., 24 hours or longer), applicants can conduct a single-dose, crossover study, provided an adequate washout period is used. If conducting the crossover study is impractical, applicants should conduct a BE study with a parallel design. For either a crossover or parallel study, sample collection times should be adequate to ensure completion of gastrointestinal transit of the drug product and absorption of the drug substance (which usually occurs within 72 hours).

For orally administered immediate-release drug products, applicants can use C_{max} and a suitably truncated AUC (for instance, an AUC truncated at 72 hours ($AUC_{0\text{-}72\ hr}$)) to characterize peak and total drug exposure, respectively. Truncating AUC for orally administered immediate-release drug products with a long elimination half-life mitigates the clinical challenge of prolonged sampling and follow-up. However, sampling should ensure that the complete drug absorption phase is covered and characterized.

For drugs exhibiting flip-flop kinetics with reported $t_{1/2}$ >24 hours, truncation of AUC may not be appropriate.

C. First Point C_{max}

The first point of a concentration-time curve in a BE study is sometimes the highest point, which raises questions of bias in the estimation of C_{max} because of insufficient early sampling times. The sampling schedule should include frequent sampling around the anticipated T_{max} to provide a reliable estimate of C_{max}. In particular, the occurrence of C_{max} at the first postdose sampling time point should be avoided by careful consideration of the known PK properties of the drug and selection of a suitable early sampling schedule. For example, for products with rapid absorption, 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 samples) in the first hour after dosing might be appropriate to assess peak drug concentrations.

For subjects where C_{max} occurs at the first postdose sampling time, the actual C_{max} may have been missed as it could have occurred at an earlier time point. When this occurs, the robustness of the study results in relation to the potential missed C_{max} should be discussed. This could include additional analysis where data from the affected subjects are removed from the analysis.

D. Alcoholic Beverage Effects on Modified-Release Drug Products

The consumption of alcoholic beverages can affect the release of active ingredient or active moiety from a modified-release formulation. The formulation can lose its modified-release characteristics, leading to a more rapid drug release and an altered systemic exposure, which can have deleterious effects on the drug's safety, efficacy, or both.

FDA recommends that applicants developing certain modified-release solid oral drug products conduct in vitro studies to determine the potential for dose dumping in alcohol, which may occur in vivo. In vitro assessments of the drug release from the drug product using media with various alcohol concentrations may be recommended. If the in vitro testing suggests drug release differences exist when administered with alcohol, then an in vivo BE study of the drug product when administered with alcohol may be appropriate in some cases.

For information on specific products, applicants should refer to any applicable PSG.[54]

E. Endogenous Compounds

Endogenous compounds are already present in the body either because the body produces them or because they are present in a 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. FDA recommends that applicants measure and approximate the baseline endogenous concentrations in biological matrices, e.g., blood, plasma,

[54] See footnote 4.

or urine and subtract these concentrations from the total concentrations measured from each subject after the drug product is administered to 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, applicants should measure multiple baseline concentrations from each individual subject in the time period before administration of the study drug and subtract the time-averaged baseline or time-matched baseline from postdose concentrations for those subjects in an appropriate manner consistent with the PK properties of the drug.

- When there is a dietary intake of the compound, applicants should 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 PK sampling day.

For both approaches above, FDA recommends that applicants determine baseline concentrations for each dosing period and perform baseline corrections 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. PK and statistical analyses should be performed on both uncorrected and corrected data. Determination of BE should be based on the baseline-corrected data.

F. In Vitro Dissolution Testing

The following guidances for industry provide recommendations for developing a dissolution methodology and setting acceptance criteria/criterion, and describe the regulatory considerations for dissolution testing:

- *Dissolution Testing of Immediate Release Solid Oral Dosage Forms* (August 1997)

- *Dissolution Testing and Acceptance Criteria for Immediate-Release Solid Oral Dosage Form Drug Products Containing High Solubility Drug Substances* (August 2018)

- *Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations* (September 1997)

- *Tablet Scoring: Nomenclature, Labeling, and Data for Evaluation* (March 2013)

The dissolution methods and acceptance criteria are specific to each drug product. Therefore, for both immediate- and modified-release drug products, FDA recommends that applicants include a dissolution method development and validation report. The report should contain information and data that demonstrate the appropriateness of the proposed dissolution method and acceptance criteria, such as the ability to discriminate changes in critical quality attributes that could potentially impact drug product performance. If available, applicants should submit data

showing the method and acceptance criteria are stability indicating and clinically relevant (e.g., building a BE safe space using approaches including PBPK modeling, and rejecting drug products that are bioinequivalent to the RLD).[55]

Applicants can use the dissolution method set forth in the U.S. Pharmacopeia (USP) drug product monograph, FDA's dissolution database method,[56] or another discriminating dissolution method developed by the applicant.

Note that for immediate-release oral drug products containing biopharmaceutics classification system Class I or III drug substances that meet the rapidly or very rapidly dissolving criteria,[57] generally, the demonstration of discriminating ability is not necessary.

G. Enteral Feeding Tube

If the approved RLD labeling states that the product may be administered by an enteral feeding tube (e.g., a nasogastric or a gastric tube), the applicant should conduct in vitro comparative testing to compare the performance of the test product to that of the reference standard; this comparative testing supports the administration of drugs via enteral feeding tubes.[58] Refer to PSGs for individual product recommendations.[59]

H. pH-Dependency

The absorption of drug substances with pH-dependent solubility may be influenced by the gastric pH. This impact on drug absorption can be altered due to the use of, for instance, pH-modifying excipients in the formulation. Moreover, the formulation of the RLD may be the result of an extensive formulation development program, obtaining, for instance, a specific formulation without an effect on drug absorption due to gastric pH differences. Therefore, in certain situations, an additional BE assessment with concomitant treatment with a pH-modifying drug product would generally be necessary if all of the following criteria are met:

1. The drug products under comparison contain a drug substance with pH-dependent solubility in the pH range of 1.2 to 6.8.
2. The drug product is expected to be taken with acid reducing agents, e.g., proton pump inhibitors, or is going to be used in certain populations, e.g., patients with achlorhydria.
3. There are qualitative or quantitative differences in the pH-modifying excipient(s), significant differences in manufacturing process that may affect drug absorption due to

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[55] See footnote 34.

[56] FDA's dissolution database, which describes FDA's dissolution methods, is available at http://www.accessdata.fda.gov/scripts/cder/dissolution/index.cfm.

[57] See the guidance for industry *M9 Biopharmaceutics Classification System-Based Biowaivers* (May 2021).

[58] See the draft guidance for industry *Oral Drug Products Administered Via External Feed Tube: In Vitro Testing and Labeling Recommendations* (June 2021) for more information. When final, this guidance will represent FDA's current thinking on this topic.

[59] See footnote 4.

gastric pH differences, or differences in the polymorphic form that possess a different pH-dependent solubility.

For immediate-release drug products without a high risk of bioinequivalence due to food effect, the additional study with concomitant treatment with a pH-modifying drug product should be conducted under the same condition (with regard to fasting or fed) as stipulated in section III.A.10, Fasting and Fed BE Study Conditions.

For immediate-release drug products with a high-risk of bioinequivalence due to food effect, in general, a fasting BE study with concomitant treatment with a pH-modifying drug product would be necessary in addition to fasting and fed BE studies. However, for drug products labeled to be taken only with food, the additional study with concomitant treatment with a pH-modifying drug product should be conducted under fed conditions.

Applicants can provide a scientific justification to demonstrate that a BE study in a gastric pH-altered situation may not be needed. Such a justification should be based on the totality of evidence with reference to the pH-solubility profile of the drug substance, impact of excipients, formulation and manufacturing design, e.g., formulation designed to overcome pH effects, extent of the differences between the test product and RLD, and comparative dissolution testing at multiple pHs. Modeling and simulation, e.g., appropriately validated PBPK modeling or semi-mechanistic absorption models, and virtual BE simulation can be used to further assess the risk of bioinequivalence.

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

For both replicate and nonreplicate in vivo bioequivalence (BE) studies with pharmacokinetic (PK) endpoints, the Food and Drug Administration (FDA) recommends the following general approaches. However, elements can be adjusted for certain drug substances and drug products.

Study conduct:

- Fasting Study: The test product or reference standard should be administered with about 5 to 8 fluid ounces (150 to 250 milliliters) of water to subjects under fasting conditions (i.e., after an overnight fast of at least 8 hours). No food should be allowed for at least 4 hours postdose on each day of drug administration, and meals taken should be standardized with respect to composition and timing throughout the study.

- Fed Study
  - Subjects should start the recommended meal 30 minutes before administration of the test product or reference standard following an overnight fast of at least 8 hours. Study subjects should finish eating 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 about 5 to 8 fluid ounces (150 to 250 milliliters) of water.
  - In general, fed BE studies should be conducted using meals that provide the greatest effects on gastrointestinal physiology and systemic drug availability. FDA recommends a high-fat (approximately 50 percent of total caloric content of the meal), high-calorie (approximately 900 to 1000 kilocalories (kcal)) test meal for such fed BE studies. This test meal should derive approximately 150, 250, and 500 to 600 kcal from protein, carbohydrate, and fat, respectively.[60,61]
  - For immediate-release drug products without a high risk of bioinequivalence due to food effect, if only one BE study conducted under fed conditions is needed, either a high-fat, high-calorie meal or a low-fat (approximately 25 percent of total caloric content of the meal), low-calorie (approximately 500 kcal) test meal for the fed BE study may be administered. If the type of meal to be consumed at the time of product administration is clearly specified in the RLD labeling, then it is acceptable to employ that meal in the fed BE study.

[60] 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 proteins, carbohydrates, and fat and has a comparable meal volume, density, and viscosity.

[61] There may be situations where it is appropriate to administer a pre-dose meal with a different caloric/fat content from these recommendations, e.g., for studies performed in patient populations who cannot tolerate the recommended meal composition.

  - The composition of the meal to be administered should be described with regard to protein, carbohydrate, and fat content (specified in grams, kcal, and relative caloric content (percentage)) in the study protocol.

  - No food should be allowed for at least 4 hours postdose.

  - Subjects should be allowed water as desired, except for 1 hour before to 1 hour after drug administration.

  - Subjects should receive standardized meals scheduled at the same time in each period of the study.

- Subjects should abstain from foods and drinks that are known to interact with circulatory, gastrointestinal transporter, gastrointestinal enzymatic, hepatic, or renal function, e.g., alcoholic or caffeinated drinks, or certain fruit juices, such as grapefruit juice, during a suitable period before and during the study.

- Generally, the highest-marketed strength can be administered as a single unit. If the highest-marketed strength is not deemed safe for healthy subjects, then the study can be performed with individuals already prescribed and taking the drug at the highest-marketed strength, or alternatively, in healthy subjects using a lower strength, where appropriate. If warranted to achieve sufficient bioanalytical sensitivity, multiple units of the highest-marketed strength can be administered, provided that the total single dose remains within the labeled dose range or 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 product and reference standard and the expiration date for the reference standard used in the study should be stated in the study report and the applicable Bioequivalence Summary Tables. The assayed drug content of the test product batch should not differ from the reference standard 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 the test product and reference standard. Under 21 CFR 320.63, the study drug test article of the test product and reference standard must be retained for 5 years. For additional information, refer to the draft guidance for industry *Handling and Retention of BA and BE Testing Samples* (March 2024).[62]

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

Sample collection and sampling times:

Under normal circumstances, applicants should 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. Applicants should draw blood samples at appropriate times to describe the absorption, distribution, and elimination phases of the drug. For most drugs, applicants should collect 12 to 18 samples, including a pre-dose 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 factors such as 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}**[63] and **λ_z** can be estimated accurately. At least three samples should be obtained during the terminal log-linear phase to obtain an accurate estimate of λ_z from linear regression. Applicants should record the actual clock time when samples are drawn as well as the elapsed time related to drug administration.

Subjects with pre-dose concentrations:

If a subject's pre-dose concentration is ≤5 percent of the C_{max} value for the subject in that period, applicants can include the subject's data without any adjustments in all PK measurements and calculations. If the pre-dose value is >5 percent of the C_{max}, applicants should drop the subject of the affected period from BE study evaluations.

Data deletion because of vomiting:

For immediate-release products, data from subjects who experience vomiting during a BE study should be deleted from statistical analysis if that vomiting occurred at or before two times median **T_{max}**. For modified-release products, data from subjects who experience vomiting during a BE study should be deleted from statistical analysis if the vomiting occurred during a period of time less than or equal to the dosing interval stated in the labeling of the RLD. The concentration data for the subject who vomited should be reported.

Handling of outliers:

A statistical outlier in the dataset can have a large impact on the outcome of the BE study. Although statistical tests may identify statistical outliers, applicants should not remove data from the statistical analysis of BE studies solely because those data are identified as statistical outliers. Outlier data may only be removed from the BE statistical analysis if there is a real-time documentation demonstrating a protocol violation during the clinical phase, analytical phase, or both of the BE study.[64] Applicants should include a prospective plan with scientific rationale in the BE study protocol for handling outliers related to protocol deviation or violation in the BE

[63] Terms that appear in bold type are defined in the glossary at the end of this guidance.

[64] An exception can be made for a subject without measurable concentrations or only very low concentrations following either reference standard or test product administration. See the guidance for industry *M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms* (October 2024) for more information on when such data can be excluded.

statistical analysis (e.g., a clinician documents in a case report form that the subject did not swallow the tablet, based on a mouth check of the subject).

Data from redosing studies, i.e., studies where a subgroup of subjects from the original study is dosed again, are not considered evidence to support removal of outlier data from the statistical analysis.

Note that all subject data should be submitted and potential outliers flagged with appropriate documentation as part of the submission.

Pharmacokinetic information in submissions:

Applicants should provide the following PK information in their submissions:

- Plasma or other acceptable matrix concentrations and time points (both actual and nominal sampling 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}**, AUC truncated or partial AUCs if applicable, and C_{max}. In addition, report the following supportive information, that while not pivotal, can be used to assess BE: T_{max}, **K_{el}**, and **$t_{1/2}$**.

- For steady-state BE studies: **AUC_{0-tau}** and **C_{maxSS}**. In addition, report **C_{minSS}** (minimum concentration in a dosing interval), **C_{avSS}** (average concentration during a dosing interval), degree of fluctuation [(C_{maxSS}-C_{minSS})/C_{avSS}], swing [(C_{maxSS}-C_{minSS})/C_{minSS}], and T_{max}.

- Additional analysis may be recommended in certain cases to ensure that the two products are bioequivalent.

Submission of data from in vivo BE studies:

- For information about submitting electronic datasets, including concentration data in appropriate biological fluid (under PC domain), PK parameter data (under PP domain), and other applicable data domains for abbreviated new drug application (ANDA) submissions, refer to the Study Data Tabulation Model Implementation Guide web page.[65]

[65] The Study Data Tabulation Model Implementation Guide web page is available on the Clinical Interchange Standards Consortium's website at https://www.cdisc.org/standards/foundational/sdtmig.

- For the most recent version of FDA's study data guidance and technical specifications, check FDA's Study Data Standards Resources web page.[66] This page includes links to the following:

    - The guidance for industry on study data standards entitled *Providing Regulatory Submissions in Electronic Format—Standardized Study Data* (June 2021)

    - Relevant technical specifications found in the *FDA Data Standards Catalog* and the *Study Data Technical Conformance Guide*

Statistical information for AUC_{0-t}, AUC_{0-inf}, and C_{max}:

For immediate-release products, FDA recommends that applicants provide the following statistical information for AUC_{0-t} and C_{max}:

- Geometric means
- Arithmetic means
- Geometric mean ratios and their corresponding 90 percent confidence intervals and/or 95 percent upper confidence bound, as applicable

For modified-release products, FDA recommends that applicants provide the following statistical information for AUC_{0-t}, AUC_{0-inf}, and C_{max}:

- Geometric means
- Arithmetic means
- Geometric mean ratios and their corresponding 90 percent confidence intervals and/or 95 percent upper confidence bound, as applicable

FDA also recommends that in addition to original scale data, applicants provide logarithmic transformation for measures used for BE demonstration and consult the guidance for industry *Statistical Approaches to Establishing Bioequivalence* (May 2026).

Confidence interval values for unscaled average BE analyses:

For unscaled average BE analyses, to pass a confidence interval limit of 80 to 125 percent, the rounded confidence interval value should be at least 80.00 percent and not more than 125.00. FDA thus recommends that when applicants evaluate the confidence interval to assess BE using

[66] FDA's Study Data Standards Resources web page is available at http://www.fda.gov/ForIndustry/DataStandards/StudyDataStandards/default.htm.

an unscaled average BE analysis during the development program, applicants round confidence interval values to two digits after the decimal point.

Highly variable drugs:

For non-narrow therapeutic index drugs exhibiting high intra-subject variability, applicants may consider using a reference-scaled average BE approach. If using this approach, the applicant should provide evidence of high variability in the PK parameters including AUC and/or C_{max} for BE assessment. For the statistical analysis using the reference-scaled average BE approach for highly variable drugs, refer to the guidance for industry *Statistical Approaches to Establishing Bioequivalence* (May 2026) and product-specific guidances for individual product recommendations.[67]

Narrow therapeutic index drugs:

Narrow therapeutic index (NTI) drugs are defined as those drugs where small differences in dose or blood concentration may lead to serious therapeutic failures and/or adverse drug reactions that are life-threatening or result in persistent or significant disability or incapacity.[68] For BE assessment for NTI drugs, FDA recommends a reference-scaled average BE approach with a four-way, fully replicated, crossover design study that permits the simultaneous equivalence comparison of the mean and within-subject variability of the test product and reference standard.[69] For the statistical analysis using the reference-scaled average BE approach for NTI drugs, refer to the guidance for industry *Statistical Approaches to Establishing Bioequivalence* (May 2026) and product-specific guidances for individual product recommendations.[70]

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[67] See the Product-Specific Guidances for Generic Drug Development web page at https://www.fda.gov/drugs/guidances-drugs/product-specific-guidances-generic-drug-development to search for published product-specific guidances.

[68] Yu L, et. al., Novel Bioequivalence Approach for Narrow Therapeutic Index Drugs. *Clin Pharm & Ther*, 97(3), 286-291, 2015.

[69] See the guidance for industry *Statistical Approaches to Establishing Bioequivalence* (May 2026).

[70] See footnote 4.

GLOSSARY

AUC	Area under the curve
$AUC_{0\text{-}inf}$	Area under the curve extrapolated to infinity
$AUC_{0\text{-}t}$	Area under the curve from time zero to the last measurable time point
$AUC_{0\text{-}tau}$	Area under the curve for one dosing interval at steady state
C_{avSS}	Average concentration at steady state
C_{max}	Maximum concentration
C_{maxSS}	Maximum concentrations during the dosing interval at steady state
C_{minSS}	Minimum concentrations at steady state
C_{tau}	Concentration observed at end of dosing interval
Enantiomers	Two stereoisomers (molecules that are identical in atomic constitution and bonding but different 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.
K_{el}	The apparent terminal elimination rate constant
pAUC	Area under the curve between two specific time points
λ_z	Terminal or elimination rate constant
Racemate	A racemate is a mixture that contains equal amounts of two isomers. It 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 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.
T_{max}	Time to maximum observed concentration
$t_{1/2}$	The apparent terminal elimination half-life