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# Bioequivalence Studies With Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA Guidance for Industry

## *DRAFT GUIDANCE*

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

**August 2021  
Generics**

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# Bioequivalence Studies With Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA

## Guidance for Industry

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

**August 2021  
Generics**

*Contains Nonbinding Recommendations*

*Draft — Not for Implementation*

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**Bioequivalence Studies With Pharmacokinetic Endpoints for Drugs  
Submitted Under an ANDA**

**Guidance for Industry<sup>1</sup>**

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic.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

**I. INTRODUCTION**

This guidance provides recommendations to applicants planning to include bioequivalence (BE) information in abbreviated new drug applications (ANDAs) and ANDA supplements. 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. This 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 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 post-approval period for changes to a drug product approved under an ANDA.

This guidance revises the draft guidance for industry *Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA* that was issued in December 2013.<sup>2</sup>

The contents of this document do not have the force and effect of law and are not meant to bind the public in any way, unless specifically incorporated into a contract. This document is intended only to provide clarity to the public regarding existing requirements under the law.

<sup>1</sup> 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.

<sup>2</sup> FDA recommends that applicants for investigational new drug applications, new drug applications, and new drug application supplements consult the draft guidance for industry *Bioavailability Studies Submitted in NDAs or INDs — General Considerations* (February 2019), which addresses bioavailability studies for these submission types. When final, this guidance will represent the FDA's current thinking on this topic. For the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/RegulatoryInformation/Guidances/default.htm>. FDA also recommends that ANDA applicants consult routinely published product-specific guidances (PSGs) when considering the appropriate BE study and/or other studies for a proposed drug product. 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>.

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34 FDA guidance documents, including this guidance, show be viewed only as recommendations,  
35 unless specific regulatory or statutory requirements are cited. The use of the word *should* in  
36 Agency guidance means that something is suggested or recommended, but not required.

37  
38

### 39 **II. BACKGROUND**

40

41 To receive approval for an ANDA, an applicant generally must demonstrate among other things,  
42 that its proposed drug product is bioequivalent to the reference listed drug (RLD).<sup>3</sup> The FD&C  
43 Act provides that a generic drug is bioequivalent to the listed drug if:

44

45 The rate and extent of absorption of the drug do not show a significant difference  
46 from the rate and extent of absorption of the listed drug when administered at the  
47 same molar dose of the therapeutic ingredient under similar experimental  
48 conditions in either a single dose or multiple doses.<sup>4</sup>

49

50 For most products, the focus of BE studies is on the release of the drug substance from the drug  
51 product into the systemic circulation. During such BE studies, an applicant compares the  
52 systemic exposure profile of a test drug product to that of the RLD designated in FDA's  
53 *Approved Drug Products with Therapeutic Evaluations* (the Orange Book).<sup>5, 6</sup>

54

55

### 56 **III. ESTABLISHING BIOEQUIVALENCE**

57

58 Under FDA regulations, an applicant must use “the most accurate, sensitive, and reproducible  
59 approach available among those set forth” in 21 CFR 320.24(b) to demonstrate BE.<sup>7</sup> As noted in  
60 21 CFR 320.24, in vivo and/or in vitro methods can be used to establish BE. These methods  
61 include comparative pharmacokinetic (PK), in vitro tests predictive of human in vivo  
62 bioavailability (BA) (in vitro-in vivo correlation (IVIVC)), pharmacodynamic (PD), clinical  
63 endpoint, and in vitro studies.<sup>8</sup>

64

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<sup>3</sup> 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. A reference standard (RS) selected by FDA is the specific drug product that the ANDA applicant must use in conducting any in vivo BE testing required to support approval of its ANDA. The RS, selected by FDA, is ordinarily the RLD. For ease of the reader, this guidance document will only use the terms RLD or reference product when describing regulatory requirements and recommendations relating to BE. For more information regarding the distinction between an RLD and RS, refer to FDA's guidance for industry *Referencing Approved Drug Products in ANDA Submissions* (October 2020).

<sup>4</sup> 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).

<sup>5</sup> The Orange Book is available at <https://www.accessdata.fda.gov/scripts/cder/ob/>.

<sup>6</sup> 21 CFR 314.3(b) and FDA's guidance for industry *Referencing Approved Drug Products in ANDA Submissions* (October 2020).

<sup>7</sup> See 21 CFR 320.24(a).

<sup>8</sup> See 21 CFR 320.24(b).

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65           **A.     Pharmacokinetic Studies**

66  
67           **1.     General Considerations**

68  
69     As provided above, the statutory definition of BE, expressed in terms of rate and extent of  
70     absorption of the active ingredient or moiety, emphasizes the use of PK endpoints in an  
71     accessible biological matrix (such as whole blood, plasma, and/or serum) to indicate release of  
72     the drug substance from the drug product into the systemic circulation.<sup>9</sup> BE frequently relies on  
73     PK endpoints such as **C<sub>max</sub>**<sup>10</sup> and **AUC** that are reflective of the rate and extent of absorption,  
74     respectively.

75  
76     If serial measurements of the drug and/or its metabolites in plasma, serum, or whole blood  
77     cannot be accomplished, measurement of urinary excretion can be used to demonstrate BE.

78  
79           **2.     Pilot Studies**

80  
81     If the applicant chooses, a pilot study in a small number of subjects can be carried out before  
82     proceeding with a pivotal BE study. This pilot study can be used to validate analytical  
83     methodology, assess PK variability, estimate sample size to achieve adequate power, optimize  
84     sample collection time intervals, and provide other information. ANDA applicants are required  
85     to submit information from all BE studies conducted with the same formulation of the proposed  
86     drug product.<sup>11</sup>

87  
88           **3.     Pivotal Bioequivalence Studies**

89  
90     General recommendations for a standard BE study based on PK endpoints are provided in  
91     Appendix A.

92  
93           **4.     Study Designs**

94  
95     FDA recommends that applicants use (1) a two-period, two-sequence, two-treatment, single-dose  
96     crossover study design, (2) a single-dose parallel study design, or (3) a single-dose replicate  
97     study design for BE studies. The BE studies generally should be conducted on the highest  
98     strength of the drug product, unless safety considerations preclude the use of that dose in study  
99     subjects. The general recommendations for study designs provided in Appendix A should be  
100     considered in designing studies. FDA recommends that applicants use the average BE method of  
101     analysis with these study designs.

102  
103     For most dosage forms that release a drug intended to be systemically available, FDA  
104     recommends that applicants perform a two-period, two-sequence, two-treatment, single-dose,

---

<sup>9</sup> See section 505(j)(8)(B) of the FD&C Act.

<sup>10</sup> Terms that appear in bold type are defined in the glossary at the end of this guidance.

<sup>11</sup> See 21 CFR 314.94(a)(7) and FDA's guidance for industry *Submission of Summary Bioequivalence Data for ANDAs* (May 2011).

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105 crossover study using either healthy subjects or other populations, as appropriate. In this design,  
106 each subject should receive each treatment (the test and the reference product) in a random order.  
107

108 A replicate crossover study design (either partial or fully replicate) is appropriate for drugs  
109 whether the reference product is a highly variable drug or not. A replicate design can have the  
110 advantage of using fewer subjects compared to a non-replicate design, although each subject in a  
111 replicate design study would receive more treatments.  
112

113 Further, a replicate design is recommended to be used under the following scenarios:  
114

- 115 • A replicate design is advantageous over a non-replicate design for non-narrow  
116 therapeutic index (NTI) drugs with a high intrasubject variability.<sup>12</sup> Either a partial or  
117 fully replicate design may be used, but a reference-scaled BE analysis approach should  
118 only be applied to specific PK metrics that exhibit a high within-subject variability for the  
119 reference product in the pivotal BE study. Refer to Appendix B for the method of  
120 statistical analysis for the reference-scaled average BE analysis approach for highly  
121 variable drugs and to product-specific guidances (PSGs)<sup>13</sup> for detailed recommendations  
122 for particular highly variable drugs.  
123
- 124 • A fully replicate design is recommended for NTI drugs,<sup>14</sup> where within-subject  
125 variability for both the reference and test products can be computed and a reference  
126 scaled-average BE analysis can be conducted to properly adjust the BE acceptance  
127 criteria. Refer to Appendix C for the method of statistical analysis for the reference-  
128 scaled average BE analysis approach for NTI drugs and to PSGs for detailed  
129 recommendations for particular NTI drugs.  
130

131 FDA's recommendations for replicate study designs and the average BE approach method can  
132 also be found in the guidance for industry *Statistical Approaches to Establishing Bioequivalence*  
133 (February 2001).  
134

135 Applicants wishing to use variations of these study designs or analysis methods (e.g., a  
136 sequential design) may submit a controlled correspondence<sup>15</sup> with specific questions about their  
137 approach before starting the study.  
138

### 139 5. Study Population

140  
141 In general, unless otherwise recommended in a PSG:  
142

- 143 • Healthy subjects or other populations as appropriate are recruited.

---

<sup>12</sup> See Appendix B.

<sup>13</sup> See footnote 2.

<sup>14</sup> See e.g., the draft PSG on Warfarin Sodium tablets (December 2012), which is available on the Product-Specific Guidances for Generic Drug Development web page at <https://www.fda.gov/drugs/guidances-drugs/product-specific-guidances-generic-drug-development>. When final, this guidance will represent the FDA's current thinking on this topic.

<sup>15</sup> See FDA's guidance for industry *Controlled Correspondence Related to Generic Drug Development* (December 2020).



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- 166
- Subjects recruited for in vivo BE studies should be 18 years of age or older.
  - If a drug product is intended for use in both sexes, the applicant should include similar proportions of males and females in the study or provide a justification supporting the use of a single-sex population. Likewise, if a drug product is intended for use in a single sex, then the applicant should only include subjects of that sex. Females should not be pregnant or lactating, and, if applicable, should practice abstinence or contraception.
  - 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 or provide a justification if no subject at or above age 60 is included in the study.
  - In general, a BE assessment in adults between two products can be used to support a BE assessment in pediatric patients. If the drug product is predominantly intended for use in pediatric patients younger than 6 years, the applicant should justify that the BE study results obtained from adult subjects are relevant to the pediatric population. FDA recommends that this justification include information supporting that the inactive ingredients in the proposed products are appropriate for use in the pediatric population.
  - 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.

167 We also recommend that any restrictions on admission into a study be primarily based on safety  
168 considerations. Sometimes, safety considerations preclude the use of either healthy subjects or  
169 the general population.<sup>16</sup> In such situations, applicants should attempt to enroll patients for  
170 whom the drug is intended to treat and whose disease process and treatments are stable for the  
171 duration of the BE study. An investigational new drug application may be required for certain  
172 products (such as cytotoxic products).<sup>17</sup>

173

### 174 6. Single-Dose Studies

175

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

180

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<sup>16</sup> *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. However, *general population* is a broad collection of adults 18 years of age or older with or without stable, chronic medical conditions, who may or may not be treated with therapeutic drugs that will not interfere with the test medication or bioassay. Individuals in the general population may be enrolled in BE studies if they are in relatively stable condition and their medications are not considered to interfere with the test medication or bioassay. The inclusion criteria for *healthy subjects* are more restrictive than the criteria for the *general population*, and *healthy subjects* is a subset of *general population*. These two terms are not used interchangeably.

<sup>17</sup> See 21 CFR 312.2(c) and 320.31.

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### 7. Steady-State Studies

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

### 8. Bioanalytical Methodology

We recommend applicants ensure that their bioanalytical methods for BE studies are accurate, precise, selective, sensitive, and reproducible. The guidance for industry *Bioanalytical Method Validation* (May 2018) is available to assist applicants in validating bioanalytical methods.

### 9. Pharmacokinetic Measures of Rate and Extent of Absorption

#### a. Rate of absorption (peak exposure)

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 their product and the reference product for any clinical implications.

#### b. Extent of absorption (total exposure)

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

- 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
- Area under the plasma, serum, or 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
- $\lambda_z$  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 ( $AUC_{0-tau}$ , where  $tau$  is the length of the dosing interval).

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226 c. Partial exposure

227  
228 Although BE generally can be demonstrated by measurements of  $C_{max}$  and AUC, FDA  
229 recommends applicants use a partial AUC (**pAUC**) as an exposure measure if specified in the  
230 applicable PSG. For instance, pAUC may be used for certain modified-release products in which  
231 the different phases of release correspond to a clinical effect. The beginning and ending times  
232 for the pAUC should relate to a clinically relevant measure. FDA recommends that sufficient  
233 quantifiable samples be collected to allow adequate estimation of the pAUC. As mentioned in  
234 section I of this guidance, for further information on specific products, applicants should consult  
235 the FDA's website to determine whether a PSG for the proposed product is available.<sup>18</sup>

236  
237 For drugs with a long elimination half-life, a truncated AUC can be used,<sup>19</sup> provided that the  
238 truncated AUC covers the complete absorption phase.

239  
240

### 241 10. *Fed Bioequivalence Studies*

242  
243 Co-administration of food with oral drug products can impact BA. Therefore, fed BE studies can  
244 determine whether test and RLD products are bioequivalent when co-administered with meals.  
245 The design of the fed BE study should generally be one of the designs described in section  
246 III.A.4 of this guidance. The fed BE study for products where variability is different (i.e., when  
247 compared to fasting conditions) may use a different design from the fasting BE study based on  
248 the considerations in section III.A.4 of this guidance. Refer to Appendix A for details on study  
249 design.

250  
251 For an orally administered immediate-release product, FDA recommends that applicants conduct  
252 a fed BE study, in addition to a fasting BE study, except when the RLD labeling states that the  
253 product should be taken on an empty stomach or when serious adverse events are anticipated  
254 with administration of the drug product under fed conditions. Similarly, both fasting and fed BE  
255 studies are recommended for products even when the RLD labeling states that the product should  
256 be taken with food, except when serious adverse events are anticipated with fasting  
257 administration, we recommend that applicants conduct only a fed study; a fasting study is not  
258 recommended.

259  
260 For all orally administered modified-release drug products, FDA recommends that applicants  
261 conduct a fed BE study, in addition to a fasting BE study, irrespective of dosing instructions in  
262 the RLD labeling. However, a fed study is not recommended when serious adverse events are  
263 anticipated with administration of the drug product under fed conditions. Similarly, when  
264 serious adverse events are anticipated with fasting administration, we recommend that applicants  
265 conduct only a fed study; a fasting study is not recommended.

266  
267 If neither a fasting nor fed BE study can be safely conducted in healthy subjects or the general  
268 population, then a BE study in patients is recommended.

269

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<sup>18</sup> See footnote 2.

<sup>19</sup> See section V.B of this guidance.

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### 270 *11. Sprinkle Bioequivalence Studies*

271  
272 If the labeling of a modified-release RLD product states that the product can be administered  
273 sprinkled in soft foods, FDA recommends that applicants conduct a sprinkle BE study. For each  
274 treatment arm of a sprinkle BE study, the product should be sprinkled on one of the soft foods  
275 mentioned in the labeling of the RLD, normally applesauce. Aside from administration in the  
276 soft food, a sprinkle BE study should follow the recommendations for the fasting BE study  
277 described in Appendix A. When serious adverse events are anticipated with fasting  
278 administration, a sprinkle BE study should follow the recommendations for the fed BE study  
279 described in Appendix A.

### 280 281 *12. Bioequivalence Studies of Products Administered in Specific Beverages*

282  
283 If the labeling specifies that the product must be administered in a specific beverage or  
284 beverages, applicants should administer the product mixed with one of the beverages mentioned  
285 in the labeling for BE studies. If additional beverages are listed in the labeling, applicants should  
286 provide evidence that the use of these additional beverages would not result in BE differences.

287  
288 If applicants have questions not addressed in the applicable PSG about the use of other vehicles  
289 or about the design or analysis of such BE studies, they should contact the Office of Generic  
290 Drugs via a controlled correspondence.

## 291 292 **B. General Considerations on Other Bioequivalence Studies**

293  
294 In certain circumstances, other types of approaches are recommended to support BE. Some  
295 general considerations regarding these approaches are described in the following sections.  
296 Applicants should consult FDA's guidances for industry for additional information on these  
297 methods as well.<sup>20</sup>

### 298 299 *1. In Vitro Studies*

300  
301 In general, FDA does not recommend in vitro approaches for drug products that are intended to  
302 be systemically absorbed. However, under certain circumstances, BE can be evaluated using in  
303 vitro approaches (e.g., dissolution/drug-release testing).<sup>21</sup>

304  
305 For highly soluble and rapidly dissolving, orally administered immediate-release drug products,  
306 in vitro data may be acceptable to demonstrate BE based on the biopharmaceutics classification  
307 system as described in the guidance for industry *M9 Biopharmaceutics Classification System-  
308 Based Biowaivers* (May 2021).

309  
310 The following FDA guidances for industry provide recommendations on developing dissolution  
311 methodology, setting specifications, and the regulatory applications of dissolution testing for  
312 immediate-release drug products:

313

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<sup>20</sup> See footnote 2.

<sup>21</sup> See 21 CFR 320.24(b)(5) and (6).

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- 314 • *Dissolution Testing of Immediate Release Solid Oral Dosage Forms* (August 1997)  
315  
316 • *Dissolution Testing and Acceptance Criteria for Immediate-Release Solid Oral Dosage*  
317 *Form Drug Products Containing High Solubility Drug Substances* (August 2018)  
318  
319 2. *In Vitro Tests Predictive of Human In Vivo Bioavailability (In Vitro-In Vivo*  
320 *Correlation Studies or “IVIVC”)*  
321

322 IVIVC is a scientific approach to describe the relationship between an in vitro attribute of a  
323 dosage form (e.g., the rate or extent of drug release) and a relevant in vivo response (e.g., plasma  
324 drug concentration or amount of drug absorbed). This model relationship facilitates the rational  
325 development and evaluation of modified-release dosage forms and, less commonly, other dosage  
326 forms. Once an IVIVC is validated, the in vitro test serves as a surrogate for in vivo BA and/or  
327 BE testing, as well as a tool for formulation screening and for setting the dissolution/drug release  
328 acceptance criteria.  
329

330 Additional information on the development and validation of an IVIVC can be found in the  
331 guidance for industry *Extended Release Oral Dosage Forms: Development, Evaluation, and*  
332 *Application of In Vitro/In Vivo Correlations* (September 1997).  
333

### 334 3. *Pharmacodynamic Studies* 335

336 A validated PD method can be used to demonstrate BE. However, FDA does not recommend  
337 PD studies for drug products that are intended to be absorbed into the systemic circulation and  
338 for which a PK approach can be used to establish BE.  
339

### 340 4. *Comparative Clinical Endpoint Studies* 341

342 When it is not possible to use the previously described methods, well-controlled BE studies with  
343 comparative clinical endpoints in patients can be used to establish BE.  
344  
345

## 346 **IV. ESTABLISHING BIOEQUIVALENCE FOR DIFFERENT DOSAGE FORMS** 347

348 The following subsections provide recommendations for establishing BE for specific dosage  
349 forms. As explained below, in certain cases, a requirement for in vivo BE testing may be  
350 waived<sup>22</sup> or an alternative approach may be more accurate, sensitive, and reproducible.<sup>23</sup>  
351  
352  
353

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<sup>22</sup> See 21 CFR 320.22.

<sup>23</sup> In addition to waiver of an in vivo BE requirement under 21 CFR 320.22, there are certain circumstances in which BE can be evaluated 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).

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### 354 **A. Oral Solutions**

355  
356 For oral solutions, elixirs, syrups, tinctures, or other solubilized forms, the in vivo BE testing  
357 requirement may be waived if in vivo BE is self-evident.<sup>24</sup> In such instances, the applicant  
358 would be deemed to have complied with and fulfilled any requirement for in vivo BE data.<sup>25</sup> For  
359 example, an in vivo BE data requirement can be waived for an oral solution if the formulation  
360 has the same active ingredient in the same concentration and dosage form as the RLD and does  
361 not contain any excipient that significantly affects drug absorption or availability.<sup>26</sup>

### 362 **B. Immediate-Release Products: Capsules and Tablets**

#### 363 *1. Bioequivalence Study Designs and Dose*

364  
365 For immediate-release capsule and tablet products, FDA generally recommends that applicants  
366 conduct the following studies: (1) a single-dose, fasting BE study comparing the highest  
367 strength of the test and reference products and (2) a single-dose, fed BE study comparing the  
368 highest strength of the test and reference products.<sup>27</sup> If an applicant does not intend to submit an  
369 ANDA for the highest strength of the reference product, then FDA generally recommends using  
370 the highest strength included in the ANDA for BE studies.

371  
372 Conducting an in vivo BE study on a strength other than the highest may be appropriate for  
373 reasons of safety. Use of a lower strength for reasons of safety is generally acceptable if the  
374 following conditions are met:

- 375 • Linear elimination has been documented over the therapeutic dose range.
- 376 • The recommendations in section IV.B.2 in this guidance are followed.

377  
378 In other cases (such as non-linear elimination), applicants may contact the Office of Generic  
379 Drugs via a controlled correspondence if there is no applicable PSG or if the proposed strength  
380 differs from what is recommended in the applicable PSG.

#### 381 *2. Demonstration of Bioequivalence: Additional Strengths*

382  
383 An in vivo BE requirement for one or more strength(s) can be waived based on (1) acceptable  
384 BE study(ies) on the designated strength, (2) acceptable in vitro dissolution testing of all the  
385 strengths, and (3) proportional similarity of the formulations across all strengths.<sup>28</sup>

386  
387 In this guidance, *proportionally similar* means any of the following:

- 388 • All active and inactive ingredients are in similar proportion between different strengths  
389 (e.g., a tablet of 50-milligram (mg) strength has all the inactive ingredients—almost

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<sup>24</sup> See 21 CFR 320.22(b)(3).

<sup>25</sup> Ibid.

<sup>26</sup> Ibid.

<sup>27</sup> See section III.A.10 of this guidance for more information on fed BE studies.

<sup>28</sup> See 21 CFR 320.22(d)(2).

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395 exactly half that of a tablet of 100-mg strength, and almost twice that of a tablet of 25-mg  
396 strength).

- 397
- 398 • For drug products that meet the following criteria: (1) the total weight of the dosage form  
399 remains nearly the same for all strengths (within +/- 10 percent of the total weight of the  
400 strength on which a biostudy was performed), (2) the same inactive ingredients are used  
401 for all strengths, and (3) the change in any strength is obtained by altering the amount of  
402 the active ingredients and one or more of the inactive ingredients.
  - 403
  - 404 • Active and inactive ingredients that are not in similar proportion between different  
405 strengths can be considered *proportionally similar* with adequate justification. FDA's  
406 determination of proportionality will be assessed during the ANDA assessment.
  - 407

408 Under any of these scenarios, we recommend that in vivo BE studies be accompanied by in vitro  
409 dissolution profiles on all strengths of each product with the method set forth in the U. S.  
410 Pharmacopeia (USP) drug product monograph or FDA's dissolution database method.<sup>29</sup> We also  
411 recommend that applicants conduct a BE study using the strength(s) recommended in the  
412 applicable PSG.<sup>30</sup>

413

414 For additional information on the BE study design for a specific product, we recommend that  
415 applicants consult FDA's Product-Specific Guidances for Generic Drug Development web  
416 page<sup>31</sup> to determine whether a PSG for the proposed product is available.

### 417

### 418 3. *Post-Approval Changes*

419

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

424

425 For post-approval changes generally, we recommend that applicants make the in vitro  
426 comparison between the pre-change and post-change products. When in vivo BE studies are  
427 recommended to support a post-approval change for an ANDA product, FDA recommends that  
428 applicants compare the post-change ANDA product to the RLD and not to the pre-change  
429 ANDA product.

### 430

### 431 C. **Suspensions**

432

433 FDA generally recommends that applicants establish BE for a suspension in the same manner as  
434 for other solid oral dosage forms. In vivo studies and dissolution testing should be performed as  
435 described in section IV.B of this guidance on immediate-release products or in section IV.D of  
436 this guidance on modified-release products.

437

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<sup>29</sup> See section V.F of this guidance for more information on this method.

<sup>30</sup> See footnote 2.

<sup>31</sup> *Ibid.*

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### 438 **D. Modified-Release Products**

439

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

442

#### 443 1. *Delayed-Release Products*

444

445 A *delayed-release drug product* is a dosage form that releases the active pharmaceutical  
446 ingredient or active moiety at a time later than immediately after administration (e.g., the drug  
447 product exhibits a lag time in quantifiable plasma concentrations). Typically, the coatings (e.g.,  
448 enteric coatings) of delayed-release products have been designed to delay the release of the  
449 medication until the dosage form has passed through the acidic medium of the stomach. In vivo  
450 tests for delayed-release drug products are similar to those for extended-release drug products,  
451 described below. We recommend that in vitro dissolution tests for these products document  
452 that they are stable under acidic conditions and that they release the drug only in a neutral  
453 medium (e.g., a pH of 6.8). For certain delayed-release products, differences in the delayed-  
454 release coating polymer(s) between the test and reference product can impact the PK profiles at  
455 a pH between acidic and neutral which may be clinically undesirable, thus dissolution testing in  
456 additional pH/media may be warranted. FDA recommends that applicants consult this guidance  
457 in conjunction with any relevant PSGs that contain product specific recommendations for a  
458 need to conduct dissolution testing in additional pH/media.<sup>32</sup>

459

#### 460 2. *Extended-Release Products*

461

462 An *extended-release drug product* is a dosage form that both allows a reduction in the dosing  
463 frequency and reduces fluctuations in plasma concentrations when compared to an immediate-  
464 release dosage form. Extended-release products can be formulated as capsules, tablets, granules,  
465 pellets, or suspensions. If any part of a drug product includes an extended-release component,  
466 the product should be treated as a modified-release dosage form to establish BE, as specified in  
467 sections IV.D.3 and IV.D.4 of this guidance.

468

#### 469 3. *Bioequivalence Study Designs and Dose*

470

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

477

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

481

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<sup>32</sup> See footnote 2.



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- 482       • Linear elimination has been documented over the therapeutic dose range.  
483       • The recommendations in section IV.D.4 of this guidance are followed.

484  
485 In other cases (such as non-linear elimination), applicants may contact the Office of Generic  
486 Drugs via a controlled correspondence if there is no applicable PSG or the proposed strength  
487 differs from what is recommended in the applicable PSG.

### 488 489           4.       *Demonstration of Bioequivalence: Additional Strengths*

490  
491 Additional strengths of modified-release products may be demonstrated to be bioequivalent to  
492 the corresponding reference product strengths under 21 CFR 320.24(b)(6) if all the following  
493 conditions have been met:

- 494  
495       • The reference product demonstrates dosage form equivalence among different strengths  
496       and demonstrates similar dissolution performance across different strengths.  
497  
498       • The test product includes the same excipients for different strengths and the ratios of drug  
499       and excipients among different strengths of the test product is justified and appropriate  
500       for the drug release mechanism of the test product (e.g., drug and excipients of different  
501       strengths can be either proportional or not proportional in quantity).  
502  
503       • The additional strength of the test product has the same drug release mechanism as the  
504       strength of the test product that underwent an acceptable in vivo BE study compared to  
505       the reference product.  
506  
507       • Dissolution testing of all strengths is acceptable. The drug products should exhibit  
508       similar dissolution profiles between the strength on which the BE testing was conducted  
509       and other strengths, based on the similarity factor ( $f_2$ ) test or other appropriate statistical  
510       approaches (e.g., a multivariate model independent approach or a model dependent  
511       approach) in at least three dissolution media (e.g., a pH of 1.2, 4.5, and 6.8).<sup>33</sup>

512  
513 We recommend that applicants generate dissolution profiles on the test and reference products of  
514 all strengths. To note, there may be instances in which an in vivo BE study for non-  
515 proportionally formulated strengths may be necessary to demonstrate bioequivalence. The  
516 decision of the acceptability of the approach will be made during ANDA assessment based on  
517 the totality-of-evidence (in addition to the dissolution data).

### 518 519           5.       *Post-Approval Changes*

520  
521 Refer to FDA's guidance for industry *SUPAC-MR: Modified-Release Solid Oral Dosage Forms:*  
522 *Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro*  
523 *Dissolution Testing and In Vivo Bioequivalence Documentation* (October 1997) for information

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<sup>33</sup> In such instances, we anticipate that such approach will be adequate to demonstrate BE. See 21 CFR 320.24(b)(6).

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524 regarding BE testing recommended for specified types of post-approval changes for modified-  
525 release dosage forms.

526  
527 For post-approval changes, we recommend that applicants perform an in vitro comparison  
528 between the approved (pre-change) product and the test (post-change) product. If appropriate,  
529 we recommend that the  $f_2$  test be used to compare dissolution profiles. If the  $f_2$  test requirements  
530 are not met, for the comparison of the dissolution profiles, applicants should use another  
531 appropriate statistical approach (e.g., a multivariate model independent approach or a model  
532 dependent approach). An in vivo BE study may be needed if dissolution profiles are not shown  
533 to be similar. When an in vivo BE study is recommended to support a post-approval change for  
534 an ANDA product, FDA recommends that applicants compare the post-change ANDA drug  
535 product to the RLD product and not to the pre-change ANDA product.

### **E. Chewable Tablets**

537  
538  
539 Applicants should administer chewable tablets according to the directions in the RLD labeling.  
540 If the labeling states that the tablet must be chewed before swallowing, the product should be  
541 chewed when administered in BE studies. If the labeling gives the option of either chewing the  
542 product or swallowing it whole, the product should be swallowed whole, with 240 milliliters of  
543 water, when administered in BE studies. We also recommend that applicants conduct in vitro  
544 dissolution testing on intact, whole tablets of the chewable drug product.

### **F. Orally Disintegrating Tablets**

545  
546  
547 Applicants should administer orally disintegrating tablets according to the directions in the RLD  
548 labeling. If the labeling states that the tablet may be administered with or without water, BE  
549 studies should be conducted without water.

### **G. Sublingual**

550  
551  
552 Sublingual tablets should not be swallowed. The tablets should be placed under the tongue until  
553 they are dissolved. Follow the labeling instruction or the applicable PSG for additional  
554 information on the method of administration.

### **H. Transdermal**

555  
556  
557  
558 Transdermal drug products are administered to the skin and designed to deliver the drug through  
559 (rather than to) the skin. Most transdermal products are extended-release film dosage forms,  
560 more commonly known as *transdermal delivery systems*. These deliver drugs into the systemic  
561 circulation at a controlled rate for a specified duration. To demonstrate the BE of transdermal  
562 delivery system, an in vivo single-dose, two-treatment, two-period crossover BE study with PK  
563 endpoints is recommended. Studies on adhesion and skin irritation/sensitization are  
564  
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566 recommended as well to assess the noninferiority of the generic product to the reference  
567 product.<sup>34</sup>

568  
569 Administration of TDS products should be to the intact skin unless the labeling indicates  
570 otherwise. Transdermal delivery systems should be applied as directed unless recommended  
571 otherwise in the relevant PSGs. Reservoir transdermal delivery systems should not be cut or  
572 otherwise altered before application. Topically applied creams, gels, ointments, lotions, or other  
573 formulations intended for a systemic effect should be applied as directed over a body surface  
574 area consistent with the labeled use. Systemic BE assessments can be made for transdermal  
575 delivery systems and topical formulations. If a product can be administered interchangeably to  
576 multiple body sites, it is generally suggested that applicants use a single administration site to  
577 demonstrate BE or refer to recommendations in the applicable PSGs.

578  
579

### **V. SPECIAL TOPICS**

580  
581  
582 A number of topics that may warrant special consideration are addressed in the following  
583 subsections. If a PSG is available on FDA's Product-Specific Guidances for Generic Drug  
584 Development web page,<sup>35</sup> the recommendations in that PSG generally supersede those described  
585 within this section.

586

#### **A. Moieties To Be Measured**

587  
588

##### *1. Parent Drug Versus Metabolites*

589  
590

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

603

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

---

<sup>34</sup> Refer to the draft guidances for industry *Assessing Adhesion with Transdermal and Topical Delivery Systems for ANDAs* (October 2018) and *Assessing the Irritation and Sensitization Potential of Transdermal and Topical Delivery Systems for ANDAs* (October 2018) for details. When final, these guidances will represent the FDA's current thinking on these topics.

<sup>35</sup> See footnote 2.

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### 2. *Enantiomers Versus Racemates*

For BE studies, we recommend using an achiral assay to measure the **racemate**. We recommend 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, (3) the 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. When all these conditions are met, we recommend that applicants separately apply their BE analysis to the enantiomers.

### 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 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, we do not encourage quantification of all active or potentially active components in PK 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 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 plasma or blood 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 an oral immediate-release product with a long elimination half-life drug (> 24 hours), applicants can conduct a single-dose, crossover study, provided an adequate washout period is used. If the crossover study is problematic, 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 approximately 2 to 3 days). Applicants can use  $C_{\max}$  and a suitably truncated AUC (for instance, an AUC truncated at 72 hours ( $AUC_{0-72 \text{ hr}}$ )) to characterize peak and total drug exposure, respectively. 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, based on blood 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.

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653 In the BE study, collection of blood samples at an early time point, between 5 and 15 minutes  
654 after dosing, followed by additional sample collections (e.g., two to five) in the first hour after  
655 dosing is usually sufficient to assess peak drug concentrations. Failure to include early (5- to 15-  
656 minute) sampling times leading to first time point  $C_{max}$  values may result in FDA excluding the  
657 data from affected subjects from the BE analysis.  
658

### **D. Alcoholic Beverage Effects on Modified-Release Drug Products**

659  
660  
661 The consumption of alcoholic beverages can affect the release of a drug substance from a  
662 modified-release formulation. The formulation can lose its modified-release characteristics,  
663 leading to a more rapid drug release and an altered systemic exposure, which can have  
664 deleterious effects on the drug's safety and/or efficacy.  
665

666 FDA recommends that applicants developing certain modified-release solid oral dosage forms  
667 conduct in vitro studies to determine the potential for dose dumping in alcohol which may occur  
668 in vivo. In vitro assessments of the drug release from the drug product using media with various  
669 alcohol concentrations may be recommended. An in vivo BE study of the drug product when  
670 administered with alcohol may be appropriate in some cases. For information on specific  
671 products, we recommend that applicants consult any relevant PSG.<sup>36</sup>  
672

### **E. Endogenous Compounds**

673  
674  
675 *Endogenous compounds* are already present in the body either because the body produces them  
676 or because they are present in a normal diet. Because these compounds are identical to the drug  
677 that is being administered, determining the amount of drug released from the dosage form and  
678 absorbed by each subject can be difficult. We recommend that applicants measure and  
679 approximate the baseline endogenous concentrations in blood (plasma) or urine and subtract  
680 these concentrations from the total concentrations measured from each subject after the drug  
681 product is administered to achieve an estimate of the actual drug availability from the drug  
682 product. Depending on whether the endogenous compound is naturally produced by the body or  
683 is present in the diet, the recommended approaches for determining BE differ as follows:  
684

- 685 • When the body produces the compound, we recommend that applicants measure multiple  
686 baseline concentrations from each individual subject in the time period before  
687 administration of the study drug and subtract the time-averaged baseline or time-matched  
688 baseline from post-dose concentrations for those subjects in an appropriate manner  
689 consistent with the PK properties of the drug.  
690
- 691 • When there is a dietary intake of the compound, we recommend that applicants strictly  
692 control the intake both before and during the study. Subjects should be housed at a clinic  
693 before the study and served standardized meals containing an amount of the compound  
694 similar to that in the meals to be served on the PK sampling day.  
695

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<sup>36</sup> See footnote 2.

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696 For both approaches above, we recommend that applicants determine baseline concentrations for  
697 each dosing period and perform baseline corrections that are period specific. If a baseline  
698 correction results in a negative plasma concentration value, the value should be set equal to 0  
699 before calculating the baseline-corrected AUC. PK and statistical analyses should be performed  
700 on both uncorrected and corrected data. Determination of BE should be based on the baseline-  
701 corrected data.

### **F. In Vitro Dissolution Testing**

704  
705 The following guidances for industry provide recommendations for developing a dissolution  
706 methodology, setting acceptance criteria/criterion, and applying the regulatory applications of  
707 dissolution testing:

- 708 • *Dissolution Testing of Immediate Release Solid Oral Dosage Forms* (August 1997)
- 709
- 710 • *Dissolution Testing and Acceptance Criteria for Immediate-Release Solid Oral Dosage*  
711 *Form Drug Products Containing High Solubility Drug Substances* (August 2018)
- 712
- 713 • *Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In*  
714 *Vitro/In Vivo Correlations* (September 1997)
- 715
- 716 • *Tablet Scoring: Nomenclature, Labeling, and Data for Evaluation* (March 2013)
- 717

#### *1. Immediate-Release Products*

718  
719  
720  
721 The dissolution of a drug is product specific; FDA recommends that for immediate-release drug  
722 products, applicants develop optimal discriminating dissolution methods. Applicants may also  
723 use the dissolution method set forth in any related official USP drug product monograph or in  
724 FDA's dissolution database,<sup>37</sup> provided that applicants submit adequate dissolution  
725 data/information supporting the discriminating ability of the USP or FDA database method being  
726 proposed for the proposed immediate-release product.

727  
728 If a new dissolution method is developed, FDA recommends that the submission include the  
729 dissolution method development and validation report with the complete information/data  
730 supporting the proposed method.

#### *2. Modified-Release Products*

731  
732  
733  
734 For modified-release drug products, FDA recommends that applicants develop specific  
735 discriminating dissolution methods. Applicants may also use the dissolution method set forth in  
736 any related official USP drug product monograph or in FDA's dissolution database,<sup>38</sup> provided  
737 that applicants submit adequate dissolution data supporting the discriminating ability of the USP  
738 or FDA database method being proposed.

---

<sup>37</sup> FDA's dissolution database, which describes FDA's dissolution methods, is available at  
<http://www.accessdata.fda.gov/scripts/cder/dissolution/index.cfm>.

<sup>38</sup> Ibid.

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739  
740 If a new dissolution method is developed for the modified-release drug product, FDA  
741 recommends that the submission includes the dissolution method development and validation  
742 report with the complete information/data supporting the proposed method.  
743  
744 Overall, the selected dissolution method and acceptance criteria should be discriminating and  
745 sensitive enough to reject batches/lots that would perform differently from the batches/lots used  
746 in the pivotal BE studies.  
747  
748 If applicants propose a method different from the dissolution method described in the FDA  
749 dissolution database or USP, FDA recommends that they submit data using the dissolution  
750 method described in the FDA dissolution database or USP, in addition to their proposed method,  
751 for comparison.

### **G. Enteral Feeding Tube**

752  
753  
754  
755 If the approved labeling for the RLD states that the product may be administered by an enteral  
756 feeding tube (e.g., a nasogastric or a gastric tube), the applicant should conduct in vitro  
757 comparative testing to compare the performance of the test product to that of the reference  
758 product; this comparative testing supports the administration of drugs via enteral feeding tubes.  
759 Refer to PSGs for individual product recommendations.<sup>39</sup>

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<sup>39</sup> See footnote 2.

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### 760 **APPENDIX A: GENERAL DESIGN AND DATA HANDLING OF BIOEQUIVALENCE** 761 **STUDIES WITH PHARMACOKINETIC ENDPOINTS**

762  
763 For both replicate and non-replicate in vivo bioequivalence (BE) studies with pharmacokinetic  
764 (PK) endpoints, the Food and Drug Administration (FDA) recommends the following general  
765 approaches. However, elements can be adjusted for certain drug substances and drug products.  
766

#### 767 **Study conduct:**

- 768
- 769 • Fasting Study: The test or reference product should be administered with about 8 ounces  
770 (240 milliliters) of water to subjects under fasting conditions (i.e., after an overnight fast  
771 of at least 10 hours).  
772
  - 773 • Fed Study: We recommend that subjects start the recommended meal 30 minutes before  
774 administration of the test or reference product following an overnight fast of at least 10  
775 hours. Study subjects should finish eating this meal in 30 minutes or less, and the drug  
776 product should be administered 30 minutes after start of the meal. The drug product  
777 should be administered with about 8 fluid ounces (240 milliliters) of water.
  - 778 • In general, we recommend that applicants conduct fed BE studies using meals that  
779 provide the greatest effects on gastrointestinal physiology and systemic drug availability.  
780 We recommend a high-fat (approximately 50 percent of total caloric content of the meal),  
781 high-calorie (approximately 800 to 1000 kilocalories) test meal for fed BE studies. This  
782 test meal should derive approximately 150, 250, and 500 to 600 kilocalories from protein,  
783 carbohydrate, and fat, respectively.<sup>40</sup> The caloric breakdown of the test meal should be  
784 provided in the study report. No food should be allowed for at least 4 hours post-dose.  
785 Water may be allowed as desired except for 1 hour before to 1 hour after drug  
786 administration. Subjects should receive standardized meals scheduled at the same time in  
787 each period of the study.  
788
  - 789 • Before and during each study phase, we recommend that subjects abstain from alcohol  
790 for at least 24 hours before each study period and until after the last sample from each  
791 period has been collected.  
792
  - 793 • Generally, the highest-marketed strength can be administered as a single unit. If the  
794 highest strength is not deemed safe for healthy subjects or the general population, then  
795 the study can be performed with individuals already prescribed and taking the drug at the  
796 highest marketed strength, or alternatively, in healthy subjects or the general population  
797 using a lower strength, where appropriate. If warranted to achieve sufficient  
798 bioanalytical sensitivity, multiple units of the highest strength can be administered,  
799 provided that the total single dose remains within the labeled dose range and the total  
800 dose is safe for administration to the study subjects.

---

<sup>40</sup> 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.



## ***Contains Nonbinding Recommendations***

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- 814
- 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 reference products and the expiration date for the reference product used in the study should be stated in the study report and the applicable Bioequivalence Summary Tables. We recommend that the assayed drug content of the test product batch not differ from the reference 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 the test and reference products. Under 21 CFR 320.63, the study drug test article of the test and reference products must be retained for 5 years. For additional information, refer to the guidance for industry *Handling and Retention of BA and BE Testing Samples* (May 2004).

### **Sample collection and sampling times:**

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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 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  $C_{max}$ <sup>41</sup> and  $\lambda_z$  can be estimated accurately. At least three samples should be obtained during the terminal log-linear phase to obtain an accurate estimate of  $\lambda_z$  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 pre-dose plasma drug concentrations:**

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836

If the pre-dose concentration is  $\leq 5$  percent of the  $C_{max}$  value in a subject with a pre-dose plasma concentration, applicants can include the subject's data without any adjustments in all PK measurements and calculations. We recommend that if the pre-dose value is  $> 5$  percent of the  $C_{max}$ , applicants drop the subject from all BE study evaluations.

---

<sup>41</sup> Terms that appear in bold type are defined in the glossary at the end of this guidance.

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### **837 Data deletion because of vomiting:**

838  
839 We recommend that data from subjects who experience vomiting during a BE study for  
840 immediate-release products be deleted from statistical analysis if that vomiting occurred at or  
841 before 2 times median  $T_{max}$ . For modified-release products, we recommend deleting data from  
842 the analysis if a subject vomits during a period of time less than or equal to the dosing interval  
843 stated in the labeling of the product. The concentration data for the subject who vomited should  
844 be reported.

### **845 Handling of outliers:**

846  
847  
848 Applicants should not remove data from the statistical analysis of BE studies solely because that  
849 data are identified as statistical outliers. Outlier data may only be removed from the BE  
850 statistical analysis if there is a real-time documentation demonstrating a protocol violation during  
851 the clinical and/or analytical phase of the BE study. Applicants should include a prospective  
852 plan in the BE study protocol for removing subjects from the BE statistical analysis (e.g., a  
853 clinician documents in a case report form that the subject did not swallow the tablet, based on a  
854 mouth check of the subject). Data from redosing studies are not considered as evidence to  
855 support removal of outlier data from the statistical analysis. Note that all subject data should be  
856 submitted and potential outliers flagged with appropriate documentation as part of the  
857 submission.

### **858 Pharmacokinetic information in submissions:**

859  
860 We recommend that applicants provide the following PK information in their submissions:  
861

- 862  
863 • Plasma or other acceptable matrix concentrations and time points (both actual and  
864 nominal sampling time points).
- 865  
866 • Subject, period, sequence, treatment.
- 867  
868 • Intersubject, intrasubject, and/or total variability, if available.
- 869  
870 • For single-dose BE studies:  $AUC_{0-t}$ ,  $AUC_{0-inf}$ , AUC truncated or partial AUCs if  
871 applicable, and  $C_{max}$ . In addition, report the following supportive information:  $T_{max}$ ,  $K_{el}$   
872 and  $t_{1/2}$ .
- 873  
874 • For steady-state BE studies:  $AUC_{0-tau}$  and  $C_{maxSS}$ . In addition, report  $C_{minSS}$  (lowest  
875 concentration in a dosing interval),  $C_{avSS}$  (average concentration during a dosing  
876 interval), degree of fluctuation  $[(C_{maxSS}-C_{minSS})/C_{avSS}]$ , swing  $[(C_{maxSS}-C_{minSS})/C_{minSS}]$ , and  
877  $T_{max}$ .
- 878  
879 • Additional analysis may be needed in certain cases to ensure that the two products are  
880 bioequivalent.

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881

### 882 **Submission of data from in vivo bioequivalence studies:**

883

884 • For information about submitting electronic datasets, including plasma concentration data  
885 (under PC domain), PK parameter data (under PP domain), and other applicable data  
886 domains for ANDA submissions, refer to the Study Data Tabulation Model  
887 Implementation Guide web page.<sup>42</sup>

888

889 • For the most recent version of FDA’s study data guidance and technical specifications,  
890 check FDA’s Study Data Standards Resources web page.<sup>43</sup> This page includes links to  
891 the following:

892

893 ■ The guidance for industry on study data standards entitled *Providing Regulatory*  
894 *Submissions in Electronic Format—Standardized Study Data* (October 2020)

895

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

898

### 899 **Statistical information for AUC<sub>0-t</sub>, AUC<sub>0-inf</sub>, and C<sub>max</sub>:**

900

901 We recommend that applicants provide the following statistical information for AUC<sub>0-t</sub>,  
902 AUC<sub>0-inf</sub>, and C<sub>max</sub>:

903

904 • Geometric means

905

906 • Arithmetic means

907

908 • Geometric mean ratios and their corresponding 90 percent confidence intervals and/or 95  
909 percent upper confidence bound, as applicable

910

911 We also recommend that applicants provide logarithmic transformation for measures used for BE  
912 demonstration and consult the guidance for industry *Statistical Approaches to Establishing*  
913 *Bioequivalence* (February 2001).

914

### 915 **Confidence interval values for unscaled average bioequivalence analyses:**

916

917 For unscaled average bioequivalence analyses, to pass a confidence interval limit of 80 to 125  
918 percent, the rounded confidence interval value should be at least 80.00 percent and not more than  
919 125.00. We thus recommend that when applicants evaluate the confidence interval to assess  
920 bioequivalence using an unscaled average bioequivalence analysis during the development  
921 program, applicants round confidence interval values to two digits after the decimal point.

922

---

<sup>42</sup> 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>.

<sup>43</sup> FDA’s Study Data Standards Resources web page is available at <http://www.fda.gov/ForIndustry/DataStandards/StudyDataStandards/default.htm>.

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923 **Highly variable drugs:**

924  
925 For non-narrow therapeutic index (non-NTI) drugs exhibiting high intra-subject variability,  
926 applicants may consider using a reference-scaled average BE approach. If using this approach,  
927 the applicant should provide evidence of high variability in the PK parameters including AUC  
928 and/or  $C_{max}$  for BE assessment. For the method of statistical analysis using the reference-scaled  
929 average BE approach for highly variable drugs, refer to Appendix B and product-specific  
930 guidances for individual product recommendations.<sup>44</sup>

931  
932 **Narrow therapeutic index drugs:**

933  
934 Narrow therapeutic index (NTI) drugs are defined as those drugs where small differences in dose  
935 or blood concentration may lead to serious therapeutic failures and/or adverse drug reactions that  
936 are life-threatening or result in persistent or significant disability or incapacity. For BE  
937 assessment for NTI drugs, we recommend a reference-scaled average BE approach with a four-  
938 way, fully replicated, crossover design study that permits the simultaneous equivalence  
939 comparison of the mean and within-subject variability of the test and reference products.<sup>45</sup> For  
940 the method for statistical analysis using the reference-scaled average BE approach for NTI drugs,  
941 refer to Appendix C and product-specific guidances for individual product recommendations.<sup>46</sup>

942  
943

---

<sup>44</sup> 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.

<sup>45</sup> Yu L, et. al., Novel Bioequivalence Approach for Narrow Therapeutic Index Drugs. *Clin Pharm & Ther*, 97(3), 286-291, 2015.

<sup>46</sup> See footnote 2.

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### 944 945 **APPENDIX B: METHOD FOR STATISTICAL ANALYSIS USING THE REFERENCE-** 946 **SCALED AVERAGE BIOEQUIVALENCE APPROACH: HIGHLY VARIABLE DRUGS** 947

948 For highly variable drugs, a mixed scaling approach is used. Namely, the reference-scaled  
949 procedure is used for specific PK parameters that have a within subject variability of the  
950 reference product ( $s_{WR}$ )  $\geq 0.294$ , and the two one-sided tests procedure is used for PK parameters  
951 with  $s_{WR} < 0.294$ . In other words, if AUC (**AUC<sub>0-t</sub>**<sup>47</sup> and **AUC<sub>0-inf</sub>**, as applicable) and **C<sub>max</sub>** have  
952 different  $s_{WR}$  values, different BE analysis should be conducted.

953  
954 The following are the steps that can be followed to carry out the statistical analysis for the  
955 reference-scaled average bioequivalence assessment for highly variable drugs:  
956

957 **Step 1.** Determine  $s_{WR}$ , the within-subject standard deviation of the reference product, for the  
958 pharmacokinetic (PK) parameters including **AUC** and **C<sub>max</sub>**.  
959

- 960 a. If  $s_{WR} < 0.294$ , use the two one-sided tests procedure to determine bioequivalence  
961 (BE) for the individual PK parameter(s).  
962  
963 b. If  $s_{WR} \geq 0.294$ , use the reference-scaled procedure to determine BE for the  
964 individual PK parameter(s).  
965

966 Calculation for  $s_{WR}$  can be conducted as follows:

$$967 \quad s_{WR}^2 = \frac{\sum_{i=1}^m \sum_{j=1}^{n_i} (D_{ij} - \bar{D}_i)^2}{2(n-m)}$$

968  
969 Where:

- 970
- 971 •  $i$  = number of sequences  $m$  used in the study  
972  
973 [  $m=3$  for partially replicate design: TRR, RTR, and RRT;  
974  $m=2$  for fully replicate design: TRTR and RTRT ]  
975
  - 976 •  $j$  = number of subjects within each sequence  
977
  - 978 • T = Test product  
979
  - 980 • R = Reference product  
981
  - 982 •  $D_{ij} = R_{ij1} - R_{ij2}$  (where 1 and 2 represent replicate reference treatments)

---

<sup>47</sup> Terms that appear in bold type are defined in the glossary at the end of this guidance.

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$$\bar{D}_i = \frac{\sum_{j=1}^{n_i} D_{ij}}{n_i}$$

- 983 •
- 984
- 985 •  $n = \sum_{i=1}^m n_i$  (i.e., total number of subjects used in the study, while  $n_i$  is number
- 986 of subjects used in sequence  $i$ )
- 987

988 Continue with steps 2 and 3 for PK parameters that have a  $s_{WR} \geq 0.294$ .

989

990 **Step 2.** Determine the 95% upper confidence bound<sup>48</sup> for:

991

$$\left( \bar{Y}_T - \bar{Y}_R \right)^2 - \theta s_{WR}^2$$

992

993 Where:

- 994 •  $\bar{Y}_T$  and  $\bar{Y}_R$  are the means of the ln-transformed PK endpoint (AUC and/or
- 995  $C_{max}$ ) obtained from the BE study for the test and reference products,
- 996 respectively.
- 997
- 998 •  $\theta \equiv \left( \frac{\ln(1.25)}{\sigma_{w0}} \right)^2$  (scaled average BE limit).
- 999 •  $\sigma_{w0} = 0.25$  (regulatory constant).

1000

1001 **Step 3.** For the test product to be bioequivalent to the reference product, *both* of the following

1002 conditions must be satisfied for each PK parameter tested:

- 1003 a. The 95% upper confidence bound for  $\left( \bar{Y}_T - \bar{Y}_R \right)^2 - \theta s_{WR}^2$  must be  $\leq 0$  (numbers
- 1004 should be kept to a minimum of four significant figures for comparison).
- 1005 b. The point estimate of the Test/Reference geometric mean ratio must fall within
- 1006 [0.8000, 1.2500].
- 1007
- 1008

1009 Example SAS codes are presented below. It is not necessary to use SAS<sup>®</sup> if other software

1010 accomplish the same objectives.

1011

1012 If SAS<sup>®</sup> is used for statistical analysis, note the following:

---

<sup>48</sup> The method for obtaining the upper confidence bound is based on *Howe's Approximation I*, which is described in the following paper: WG Howe, 1974, Approximate Confidence Limits on the Mean of  $X+Y$  Where  $X$  and  $Y$  are Two Tabled Independent Random Variables, J Am Stat Assoc, 69(347):789–794.

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1022

- PROC GLM should be used for partially replicate (3-way) BE studies
- PROC MIXED should be used for fully replicate (4-way) BE studies

- **Example SAS Codes: Partial reference-replicate 3-way design**

For a BE study with the following sequence assignments in a partial reference-replicate 3-way crossover design:

	Period 1	Period 2	Period 3
Sequence 1	T	R	R
Sequence 2	R	T	R
Sequence 3	R	R	T

1023  
1024  
1025  
1026

1. For PK parameters with a  $s_{WR} \geq 0.294$ , use the reference-scaled procedure to determine BE.

1027  
1028  
1029

The following codes are an example of the determination of reference-scaled average BE for LAUCT with a partially replicate 3-way BE design:

**Dataset containing TEST observations:**

```
1030 data test;  
1031 set pk;  
1032 if trt='T';  
1033 latt=lauct;  
1034 run;
```

**Dataset containing REFERENCE 1 observations:**

```
1037 data refl;  
1038 set ref;  
1039 if (seq=1 and per=2) or (seq=2 and per=1) or (seq=3 and per=1);  
1040 lat1r=lauct;  
1041 run;
```

**Dataset containing REFERENCE 2 observations:**

```
1044 data ref2;  
1045 set ref;  
1046 if (seq=1 and per=3) or (seq=2 and per=3) or (seq=3 and per=2);  
1047 lat2r=lauct;  
1048 run;
```

1050  
1051  
1052

Define the following quantities:

$T_{ij}$  = the observation on T for subject j within sequence i

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$R_{ijk}$  = kth observation ( $k = 1$  or  $2$ ) on R for subject j within sequence i

1053  $I_{ij} = T_{ij} - \frac{R_{ij1} + R_{ij2}}{2}$

1054  $D_{ij} = R_{ij1} - R_{ij2}$

1055  $I_{ij}$  is the difference between a subject's (specifically, subject j within sequence i)  
1056 observation on T and the mean of the subject's two observations on R, while  $D_{ij}$  is the  
1057 difference between a subject's two observations on R.

1058

1059 **Determine  $I_{ij}$  and  $D_{ij}$**

```
1060 data scavbe;  
1061 merge test ref1 ref2;  
1062 by seq subj;  
1063 ilat=latt - 0.5*(lat1r+lat2r);  
1064 dlat=lat1r-lat2r;  
1065 run;
```

1066

1067 **Intermediate analysis - ilat**

```
1068 proc glm data=scavbe;  
1069 class seq;  
1070 model ilat=seq/clparm alpha=0.1;  
1071 estimate 'average' intercept 1 seq 0.3333333333 0.3333333333  
1072 0.3333333333;  
1073 ods output overallanova=iglm1;  
1074 ods output Estimates=iglm2;  
1075 ods output NObs=iglm3;  
1076 title1 'scaled average BE';  
1077 run;
```

1078

1079 From the dataset IGLM2, calculate the following:

1080

1081 IGLM2:

```
1082 pointest=exp(estimate);  
1083 x=estimate**2-stderr**2;  
1084 boundx=(max((abs(LowerCL)), (abs(UpperCL))))**2;
```

1085

1086 **Intermediate analysis - dlat**

```
1087 proc glm data=scavbe;  
1088 class seq;  
1089 model dlat=seq;  
1090 ods output overallanova=dglm1;  
1091 ods output NObs=dglm3;  
1092 title1 'scaled average BE';  
1093 run;
```

1094

1095 From the dataset DGLM1, calculate the following:

1096

1097 DGLM1:



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```
1098      dfd=df;
1099      s2wr=ms/2;
1100
```

1101 From the above parameters, calculate the final 95% upper confidence bound:

```
1102
1103      theta=((log(1.25))/0.25)**2;
1104      y=-theta*s2wr;
1105      boundy=y*dfd/cinv(0.95,dfd);
1106      sWR=sqrt(s2wr);
1107      critbound=(x+y)+sqrt(((boundx-x)**2)+((boundy-y)**2));
1108
1109
```

1110 2. For PK parameters with a  $s_{WR} < 0.294$ , use the unscaled average BE approach.

1111  
1112 The following codes are an example of the determination of unscaled average BE for LAUCT  
1113 with a partially replicate 3-way BE design:

```
1114      PROC MIXED
1115          data=pk;
1116          CLASSES SEQ SUBJ PER TRT;
1117          MODEL LAUCT = SEQ PER TRT/ DDFM=SATTERTH;
1118          RANDOM TRT/TYPE=FA0(2) SUB=SUBJ G;
1119          REPEATED/GRP=TRT SUB=SUBJ;
1120          ESTIMATE 'T vs. R' TRT 1 -1/CL ALPHA=0.1;
1121          ods output Estimates=unsc1;
1122          title1 'unscaled BE 90% CI - guidance version';
1123          title2 'AUCt';
1124      run;
1125
1126      data unsc1;
1127          set unsc1;
1128          unscabe_lower=exp(lower);
1129          unscabe_upper=exp(upper);
1130      run;
1131
```

1132  
1133  
1134 • **Example SAS Codes: Fully replicate 4-period, 2-sequence, 4-way crossover design**

1135  
1136 For a BE study with the following sequence assignments in a fully replicate 4-way crossover  
1137 design:

1138

	Period 1	Period 2	Period 3	Period 4
Sequence 1	T	R	T	R
Sequence 2	R	T	R	T

1139

1140 1. For PK parameters with a  $s_{WR} \geq 0.294$ , use the reference-scaled procedure to determine  
1141 BE.

1142

1143 The following codes are an example of the determination of reference-scaled average BE for  
1144 LAUCT with a fully replicate 4-way BE design:

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1145

- **Dataset containing TEST 1 observations:**

1147

```
1148 data test1;  
1149     set test;  
1150     if (seq=1 and per=1) or (seq=2 and per=2);  
1151     lat1t=lauct;  
1152 run;
```

1153

- **Dataset containing TEST 2 observations:**

1155

```
1156 data test2;  
1157     set test;  
1158     if (seq=1 and per=3) or (seq=2 and per=4);  
1159     lat2t=lauct;  
1160 run;
```

1161

- **Dataset containing REFERENCE 1 observations:**

1163

```
1164 data ref1;  
1165     set ref;  
1166     if (seq=1 and per=2) or (seq=2 and per=1);  
1167     lat1r=lauct;  
1168 run;
```

1169

- **Dataset containing REFERENCE 2 observations:**

1171

```
1172 data ref2;  
1173     set ref;  
1174     if (seq=1 and per=4) or (seq=2 and per=3);  
1175     lat2r=lauct;  
1176 run;
```

1177

1178 The number of subjects in each sequence is  $n_1$  and  $n_2$  for sequences 1 and 2, respectively.

1179

1180 Define the following quantities:

1181

1182  $T_{ijk}$  = kth observation ( $k = 1$  or  $2$ ) on T for subject  $j$  within sequence  $i$

1183

1184  $R_{ijk}$  = kth observation ( $k = 1$  or  $2$ ) on R for subject  $j$  within sequence  $i$

1185

$$1186 \quad I_{ij} = \frac{T_{ij1} + T_{ij2}}{2} - \frac{R_{ij1} + R_{ij2}}{2}$$

$$1187 \quad D_{ij} = R_{ij1} - R_{ij2}$$

1188

1189  $I_{ij}$  is the difference between the mean of two observations of a subject (specifically, subject  $j$   
1190 within sequence  $i$ ) on T and the mean of the subject's two observations on R, while  $D_{ij}$  is the  
1191 difference between a subject's two observations on R.

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1192  
1193  
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1197  
1198  
1199  
1200

### Determine $I_{ij}$ and $D_{ij}$

```
data scavbe;
  merge test1 test2 ref1 ref2;
  by seq subj;
  ilat=0.5*(lat1t+lat2t-lat1r-lat2r);
  dlat=lat1r-lat2r;
run;
```

1201  
1202

### Intermediate analysis – ilat

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1211  
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1213

```
proc mixed data=scavbe;
  class seq;
  model ilat =seq/ddfm=satterth;
  estimate 'average' intercept 1 seq 0.5 0.5/e cl alpha=0.1;
  ods output CovParms=iout1;
  ods output Estimates=iout2;
  ods output NObs=iout3;
  title1 'scaled average BE';
  title2 'intermediate analysis - ilat, mixed';
run;
```

1214

From the dataset IOU2, calculate the following:

1215

IOU2:

1216  
1217  
1218  
1219  
1220

```
pointest=exp(estimate);
x=estimate**2-stderr**2;
boundx=(max((abs(lower)),(abs(upper))))**2;
```

1221

1222

### Intermediate analysis – dlat

1223  
1224  
1225  
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1230  
1231  
1232  
1233

```
proc mixed data=scavbe;
  class seq;
  model dlat=seq/ddfm=satterth;
  estimate 'average' intercept 1 seq 0.5 0.5/e cl alpha=0.1;
  ods output CovParms=dout1;
  ods output Estimates=dout2;
  ods output NObs=dout3;
  title1 'scaled average BE';
  title2 'intermediate analysis - dlat, mixed';
run;
```

1234

From the dataset DOUT1, calculate the following:

1235

1236

DOUT1:

1237

```
s2wr=estimate/2;
```

1238  
1239

1240

From the dataset DOUT2, calculate the following:

1241

1242

DOUT2:

1243

```
dfd=df;
```

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1244  
1245  
1246  
1247  
1248  
1249  
1250  
1251  
1252  
1253

**From the above parameters, calculate the final 95% upper confidence bound:**

```
theta=((log(1.25))/0.25)**2;  
y=-theta*s2wr;  
boundy=y*dfd/cinv(0.95,dfd);  
sWR=sqrt(s2wr);  
critbound=(x+y)+sqrt(((boundx-x)**2)+((boundy-y)**2));
```

1254  
1255  
1256

2. For PK parameters with  $a < 0.294$ , use the unscaled average BE approach.

The following codes are an example of the determination of unscaled average BE for LAUCT with a fully replicate 4-way BE design:

1257  
1258  
1259  
1260  
1261  
1262  
1263  
1264  
1265  
1266  
1267  
1268  
1269  
1270  
1271  
1272  
1273  
1274  
1275  
1276

```
PROC MIXED  
  data=pk;  
  CLASSES SEQ SUBJ PER TRT;  
  MODEL LAUCT = SEQ PER TRT/ DDFM=SATTERTH;  
  RANDOM TRT/TYPE=FA0(2) SUB=SUBJ G;  
  REPEATED/GRP=TRT SUB=SUBJ;  
  ESTIMATE 'T vs. R' TRT 1 -1/CL ALPHA=0.1;  
  ods output Estimates=unsc1;  
  title1 'unscaled BE 90% CI - guidance version';  
  title2 'AUCt';  
run;  
  
data unsc1;  
  set unsc1;  
  unscabe_lower=exp(lower);  
  unscabe_upper=exp(upper);  
run;
```

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### 1277 1278 **APPENDIX C: METHOD FOR STATISTICAL ANALYSIS USING THE REFERENCE-** 1279 **SCALED AVERAGE BIOEQUIVALENCE APPROACH: NARROW THERAPEUTIC** 1280 **INDEX DRUGS**

1281  
1282 For narrow therapeutic index (NTI) drugs, the study should be a fully replicated, 4-way  
1283 crossover design to scale the bioequivalence limit to the variability of the reference product and  
1284 to simultaneously compare the mean and within-subject variability of the test and reference  
1285 products. The procedure described below includes both reference scaling and unscaled analysis  
1286 and they are combined to ensure that for NTI drugs the BE limits do not exceed 80.00%-  
1287 125.00%.

1288  
1289 The following are the steps that can be followed to carry out the statistical analysis for the  
1290 reference scaled average bioequivalence for narrow therapeutic index drugs:

1291  
1292  
1293 **Step 1.** Determine  $s_{WR}$ , the estimate of within-subject standard deviation of the reference  
1294 product, for the PK parameters including  $AUC^{49}$  and  $C_{max}$ .

1295  
1296 Calculation for  $s_{WR}$  can be conducted as follows:

$$1297 \quad s_{WR}^2 = \frac{\sum_{i=1}^m \sum_{j=1}^{n_i} (D_{ij} - \bar{D}_{i.})^2}{2(n - m)}$$

1298  
1299 Where:

- 1300
- 1301 •  $i$  = number of sequences  $m$  used in the study
  - 1302        [ $m=2$  for fully replicate design: TRTR and RTRT]
  - 1303
  - 1304
  - 1305 •  $j$  = number of subjects within each sequence
  - 1306
  - 1307 • T = Test product
  - 1308
  - 1309 • R = Reference product
  - 1310
  - 1311 •  $D_{ij} = R_{ij1} - R_{ij2}$  (where 1 and 2 represent replicate reference treatments)

$$1312 \quad \bar{D}_{i.} = \frac{\sum_{j=1}^{n_i} D_{ij}}{n_i}$$

1313

---

<sup>49</sup> Terms that appear in bold type are defined in the glossary at the end of this guidance.

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- 1314 •  $n = \sum_{i=1}^m n_i$  (i.e., total number of subjects used in the study, while  $n_i$  is number  
1315 of subjects used in sequence  $i$ )  
1316

1317 **Step 2.** Use the referenced-scaled procedure to determine BE for individual PK  
1318 parameter(s).  
1319

1320 Determine the 95% upper confidence bound<sup>50</sup> for:

$$1321 \left( \bar{Y}_T - \bar{Y}_R \right)^2 - \theta s_{WR}^2$$

1322 Where:

- 1323 •  $\bar{Y}_T$  and  $\bar{Y}_R$  are the means of the ln-transformed PK endpoint (AUC and/or  
1324  $C_{max}$ ) obtained from the BE study for the test and reference products,  
1325 respectively

- 1326 •  $\theta \equiv \left( \frac{\ln(\Delta)}{\sigma_{w0}} \right)^2$  (scaled average BE limit)

- 1327 • and  $\sigma_{w0} = 0.10$  (regulatory constant),  $\Delta = 1./0.9$  (approximately=1.11111,  
1328 the upper BE limit)

1329  
1330 **Step 3.** Use the unscaled average bioequivalence procedure to determine BE for  
1331 individual PK parameter(s).  
1332

1333 **Step 4.** Calculate the 90% confidence interval of the ratio of the within subject standard  
1334 deviation of test product to reference product  $\sigma_{WT}/\sigma_{WR}$ . The upper limit of the  
1335 90% confidence interval for  $\sigma_{WT}/\sigma_{WR}$  will be evaluated to determine if  $\sigma_{WT}$  and  
1336  $\sigma_{WR}$  are comparable.  
1337

1338 The  $(1-\alpha)100\%$  CI for  $\frac{\sigma_{WT}}{\sigma_{WR}}$  is given by

$$\left( \frac{s_{WT}/s_{WR}}{\sqrt{F_{\frac{\alpha}{2}}(v_1, v_2)}}, \frac{s_{WT}/s_{WR}}{\sqrt{F_{1-\frac{\alpha}{2}}(v_1, v_2)}} \right)$$

1339  
1340  
1341 Where:  
1342

---

<sup>50</sup> The method of obtaining the upper confidence bound is based on Howe's Approximation I, which is described in the following paper: WG Howe, 1974, Approximate Confidence Limits on the Mean of X+Y Where X and Y are Two Tabled Independent Random Variables, J Am Stat Assoc, 69 (347):789-794.

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- 1343 •  $s_{WT}$  is the estimate of  $\sigma_{WT}$  with  $\nu_1$  as the degree of freedom
- 1344 •  $s_{WR}$  is the estimate of  $\sigma_{WR}$  with  $\nu_2$  as the degree of freedom
- 1345 •  $F_{\alpha/2, \nu_1, \nu_2}$  is the value of the F-distribution with  $\nu_1$  (numerator) and  $\nu_2$
- 1346 (denominator) degrees of freedom that has probability of  $\alpha/2$  to its right.
- 1347 •  $F_{1-\alpha/2, \nu_1, \nu_2}$  is the value of the F-distribution with  $\nu_1$  (numerator) and  $\nu_2$
- 1348 (denominator) degrees of freedom that has probability of  $1-\alpha/2$  to its
- 1349 right.
- 1350 • here  $\alpha = 0.1$ .

1351  
1352 **Step 5.** For the test product to be bioequivalent to the reference product, the following  
1353 conditions must be satisfied for each PK parameter tested:

- 1354 a. The 95% upper confidence bound for  $\left(\bar{Y}_T - \bar{Y}_R\right)^2 - \theta_{WR}^2$  must be  $\leq 0$
- 1355 (numbers should be kept to a minimum of four significant figures for
- 1356 comparison).
- 1357
- 1358 b. Regular unscaled bioequivalence limits of 80.00%-125.00% should be
- 1359 passed.
- 1360
- 1361 c. The proposed requirement for the upper limit of the 90% equal-tails
- 1362 confidence interval for  $\sigma_{WT}/\sigma_{WR}$  is less than or equal to 2.500.
- 1363

1364 Example SAS codes are presented below. It is not necessary to use SAS<sup>®</sup> if other software  
1365 accomplish the same objectives.

1366  
1367 If SAS<sup>®</sup> is used for statistical analysis, PROC MIXED should be used for fully replicate 4-way  
1368 crossover BE studies.

1369  
1370 • **Example SAS Codes: Fully replicate 4-period, 2-sequence, 4-way crossover design**

1371  
1372 For a BE study with the following sequence assignments in a fully replicate 4-way crossover design:  
1373

	Period 1	Period 2	Period 3	Period 4
Sequence 1	T	R	T	R
Sequence 2	R	T	R	T

1374  
1375  
1376 The following codes are an example of the determination of reference-scaled average BE for  
1377 LAUCT. Assume that the datasets TEST and REF, have already been created, with TEST having all  
1378 the test observations and REF having all the reference observations.

1379  
1380 **Dataset containing TEST 1 observations:**  
1381

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```
1382 data test1;
1383   set test;
1384   if (seq=1 and per=1) or (seq=2 and per=2);
1385   lat1t=lauct;
1386 run;
```

1387  
1388 **Dataset containing TEST 2 observations:**

```
1389 data test2;
1390   set test;
1391   if (seq=1 and per=3) or (seq=2 and per=4);
1392   lat2t=lauct;
1393 run;
```

1394  
1395  
1396 **Dataset containing REFERENCE 1 observations:**

```
1397 data ref1;
1398   set ref;
1399   if (seq=1 and per=2) or (seq=2 and per=1);
1400   lat1r=lauct;
1401 run;
```

1402  
1403  
1404 **Dataset containing REFERENCE 2 observations:**

```
1405 data ref2;
1406   set ref;
1407   if (seq=1 and per=4) or (seq=2 and per=3);
1408   lat2r=lauct;
1409 run;
```

1410  
1411  
1412 The number of subjects in each sequence is  $n_1$  and  $n_2$  for sequences 1 and 2, respectively.

1413  
1414 Define the following quantities:

1415  
1416  $T_{ijk} = k^{\text{th}}$  observation ( $k = 1$  or  $2$ ) on  $T$  for subject  $j$  within sequence  $i$

1417  
1418  $R_{ijk} = k^{\text{th}}$  observation ( $k = 1$  or  $2$ ) on  $R$  for subject  $j$  within sequence  $i$

1419 
$$I_{ij} = \frac{T_{ij1} + T_{ij2}}{2} - \frac{R_{ij1} + R_{ij2}}{2}$$

1420  
1421 and

1422 
$$D_{ij} = R_{ij1} - R_{ij2}$$

1423  
1424  $I_{ij}$  is the difference between the mean of a subject's (specifically subject  $j$  within sequence  $i$ ) two  
1425 observations on  $T$  and the mean of the subject's two observations on  $R$ , while  $D_{ij}$  is the difference  
1426 between a subject's two observations on  $R$ .

1427  
1428 **Determine  $I_{ij}$  and  $D_{ij}$**

1429



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```
1430 data scavbe;
1431     merge test1 test2 ref1 ref2;
1432     by seq subj;
1433     ilat=0.5*(lat1t+lat2t-lat1r-lat2r);
1434     dlat=lat1r-lat2r;
1435 run;
1436
1437 Intermediate analysis - ilat
1438
1439 proc mixed data=scavbe;
1440     class seq;
1441     model ilat =seq/ddfm=satterth;
1442     estimate 'average' intercept 1 seq 0.5 0.5/e cl alpha=0.1;
1443     ods output CovParms=iout1;
1444     ods output Estimates=iout2;
1445     ods output NObs=iout3;
1446     title1 'scaled average BE';
1447     title2 'intermediate analysis - ilat, mixed';
1448 run;
1449
1450 From the dataset IOUT2, calculate the following:
1451 IOUT2:
1452 pointest=exp(estimate);
1453 x=estimate**2-stderr**2;
1454 boundx=(max((abs(lower)),(abs(upper))))**2;
1455
1456 Intermediate analysis - dlat
1457
1458 proc mixed data=scavbe;
1459     class seq;
1460     model dlat=seq/ddfm=satterth;
1461     estimate 'average' intercept 1 seq 0.5 0.5/e cl alpha=0.1;
1462     ods output CovParms=dout1;
1463     ods output Estimates=dout2;
1464     ods output NObs=dout3;
1465     title1 'scaled average BE';
1466     title2 'intermediate analysis - dlat, mixed';
1467 run;
1468
1469 From the dataset DOUT1, calculate the following:
1470 DOUT1:
1471     s2wr=estimate/2;
1472
1473 From the dataset DOUT2, calculate the following:
1474 DOUT2:
1475     dfd=df;
1476
1477 From the above parameters, calculate the final 95% upper confidence bound:
1478
1479 theta=((log(1.11111))/0.1)**2;
1480 y=-theta*s2wr;
1481 boundy=y*dfd/cinv(0.95,dfd);
1482 sWR=sqrt(s2wr);
```

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```
1483 critbound=(x+y)+sqrt(((boundx-x)**2)+((boundy-y)**2));
1484
1485 Calculation of unscaled 90% bioequivalence confidence intervals:
1486
1487 PROC MIXED data=pk;
1488 CLASSES SEQ SUBJ PER TRT;
1489 MODEL LAUCT = SEQ PER TRT/ DDFM=SATTERTH;
1490 RANDOM TRT/TYPE=FA0(2) SUB=SUBJ G;
1491 REPEATED/GRP=TRT SUB=SUBJ;
1492 ESTIMATE 'T vs. R' TRT 1 -1/CL ALPHA=0.1;
1493 ods output Estimates=unsc1;
1494 title1 'unscaled BE 90% CI - guidance version';
1495 title2 'AUCt';
1496 run;
1497
1498 data unsc1;
1499     set unsc1;
1500     unscabe_lower=exp(lower);
1501     unscabe_upper=exp(upper);
1502 run;
1503
```

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### GLOSSARY

1504  
1505

<b>AUC</b>	Area under the curve
<b>AUC<sub>0-inf</sub></b>	Area under the curve extrapolated to infinity
<b>AUC<sub>0-t</sub></b>	Area under the curve from time zero to the last measurable time point
<b>AUC<sub>0-tau</sub></b>	Area under the curve for one dosing interval at steady state
<b>C<sub>avSS</sub></b>	Average plasma concentration at steady state
<b>C<sub>max</sub></b>	Maximum plasma concentration
<b>C<sub>maxSS</sub></b>	Maximum plasma concentrations during the dosing interval at steady state
<b>C<sub>minSS</sub></b>	Minimum plasma concentrations at steady state
<b>Enantiomers</b>	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.
<b>pAUC</b>	Area under the curve between two specific time points
<b>λ<sub>z</sub></b>	Terminal or elimination rate constant
<b>Racemate</b>	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 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.
<b>T<sub>max</sub></b>	Time to maximum observed plasma concentration
<b>t<sub>1/2</sub></b>	Half-life

1506