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DRAFT GUIDANCE DOCUMENT

Conduct and Analysis of Comparative Bioavailability Studies

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Health Products and Food Branch

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13 *Également disponible en français sous le titre : Conduite et analyse des études comparatives de*
14 *biodisponibilité*

15 **FOREWORD**

16 Guidance documents are meant to provide assistance to industry and health care professionals on
17 **how** to comply with governing statutes and regulations. Guidance documents also provide
18 assistance to staff on how Health Canada mandates and objectives should be implemented in a
19 manner that is fair, consistent and effective.

20 Guidance documents are administrative instruments not having force of law and, as such, allow
21 for flexibility in approach. Alternate approaches to the principles and practices described in this
22 document *may be* acceptable provided they are supported by adequate justification. Alternate
23 approaches should be discussed in advance with the relevant program area to avoid the possible
24 finding that applicable statutory or regulatory requirements have not been met.

25 As a corollary to the above, it is equally important to note that Health Canada reserves the right
26 to request information or material, or define conditions not specifically described in this
27 document, in order to allow the Department to adequately assess the safety, efficacy or quality of
28 a therapeutic product. Health Canada is committed to ensuring that such requests are justifiable
29 and that decisions are clearly documented.

30 This document should be read in conjunction with the accompanying notice and the relevant
31 sections of other applicable guidance documents.

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102 **1 INTRODUCTION**

103 **1.1 Policy Objectives**

104 To ensure that sponsors of new drug submissions have the information necessary to
105 comply with Sections C.08.002(2)(h), C.08.002.1(2)(c)(ii) and C.08.003(3) of the *Food and*
106 *Drug Regulations* with respect to comparative bioavailability and comparative
107 pharmacodynamic studies used in support of the safety and efficacy of a drug.

108 **1.2 Policy Statements**

109 Comparative bioavailability studies should be conducted in accordance with generally
110 accepted clinical practices that are designed to ensure the protection of the rights, safety and
111 well-being of subjects and the good clinical practices referred to in Division 5 of the *Food and*
112 *Drug Regulations* and described in the International Conference on Harmonisation (ICH)
113 *Guidance (Topic E6) on Good Clinical Practice*.

114 The recommendations included in this guidance respecting study design and conduct,
115 analytical methodology and analysis of data should be followed in order to ensure compliance
116 with the *Food and Drug Regulations*.

117 **1.3 Scope and Application**

118 This guidance is intended to be applied to all comparative bioavailability studies which
119 provide pivotal evidence of the safety and efficacy of a product. Examples of cases where this
120 guidance applies are:

- 121 a) comparative bioavailability studies in support of the bioequivalence of subsequent-entry
122 products to the Canadian Reference Product;
123 b) bridging studies where the formulation to be marketed is different from the formulation
124 used in the pivotal clinical trials;
125 c) studies in support of significant post-marketing changes and line extensions;
126 d) safety studies for non-systemic drugs.

127 While this guidance is oriented toward oral dosage formulations, the principles described
128 may also be applied, as appropriate, to other non-parenteral formulations such as transdermal
129 patches, suppositories, etc. that are intended to deliver medication to the systemic circulation.

130 This guidance document should be read in conjunction with the associated Health Canada
131 draft guidance document entitled: *Comparative Bioavailability Standards: Formulations Used*
132 *for Systemic Effects*.

133 1.4 Background

134 Bioavailability is an important attribute of formulations of drugs used for systemic
135 effects. It is defined as the rate and extent of absorption of a drug into the systemic circulation.

136 Bioavailability is most frequently assessed by serial measurements of the drug in the
137 systemic circulation. These serial measurements provide a plasma concentration-time profile
138 from which a number of important pharmacokinetic parameters can be calculated, including the
139 area under the curve (AUC), the maximum observed concentration (C_{\max}) and the time when C_{\max}
140 is reached (t_{\max}). The AUC provides an estimate of the amount of drug absorbed into the
141 systemic circulation while t_{\max} reflects the rate of absorption. C_{\max} is a more complex function,
142 which, together with t_{\max} , may reflect the rate of absorption. For many drugs, AUC and C_{\max}
143 together can characterize the concentration-time profile for comparative purposes.

144 Comparison of the AUC values following oral versus intravenous administration of an
145 equivalent dose of the same active ingredient provides an estimate of *absolute bioavailability* for
146 most drugs. Comparison of the plasma concentration-time profiles of the drug between the test
147 and reference products containing the same active ingredient provides an estimate of *relative*
148 *bioavailability*.

149 If the test and reference products are comparable dosage forms and contain the identical
150 amounts of identical medicinal ingredient, they are said to be *bioequivalent* when the profiles of
151 the drug are similar. The degree of similarity between the profiles needed to establish
152 bioequivalence is determined by the appropriate statistical assessment and by meeting standards
153 established for the particular drug and formulations being compared (see Health Canada draft
154 guidance document: *Comparative bioavailability standards: Formulations used for systemic*
155 *effects*).

156 Bioequivalence implies that the test product can be expected to have the same therapeutic
157 effects and safety profile as the reference product when administered to patients under the
158 conditions specified in the labelling.

159 Bioavailability is usually established by measuring the formulated drug in plasma. If the
160 formulated drug cannot be assayed, a major primary metabolite may be used. In some situations,
161 determination of the urinary excretion of the formulated drug, but not a metabolite, may be
162 employed to measure bioavailability and establish bioequivalence. In the absence of an adequate
163 methodology for bioavailability testing, alternate approaches such as pharmacodynamic studies
164 can be used. In some instances, equivalence may have to be determined by clinical trials.

165 **2 GUIDANCE FOR IMPLEMENTATION**

166 The acceptability of data from comparative bioavailability studies will be assessed in
167 accordance with principles enunciated in Division 5 of the *Food and Drug Regulations* and the
168 ICH [Guidance \(Topic E6\) on Good Clinical Practice](#). These documents will help sponsors to
169 understand requirements for submissions to Health Canada, pursuant to the *Food and Drug*
170 *Regulations*, even if the studies or a portion of the study are conducted in other countries.

171 **2.1 Planning a Bioavailability Study**

172 This section identifies the sections of the study protocol which should be prepared before
173 the study is executed.

174 **2.1.1 Study Objectives**

175 In this section, a rationale should be provided to justify which comparative
176 bioavailability standard will be applied. Scientific justification should be provided for any
177 deviation from standard procedure, for example (e.g.), analyte upon which bioequivalence will
178 be assessed, deviation from a high fat/high calorie meal in studies conducted under fed
179 conditions.

180 Among the topics covered by the *Regulations* and the [ICH guidance on Good Clinical](#)
181 [Practice](#), and therefore not repeated in detail here are: Institutional review boards, investigators,
182 clinical, laboratory and analytical facilities.

183 **2.2 Selection of Subjects for a Study**

184 This section describes selection criteria for inclusion of subjects in a bioavailability study
185 and indicates how the characteristics of the subjects may affect the study. In general, subjects
186 should be selected so as to reduce variability that is not attributable to the drug itself.

187 **2.2.1 Choice of Subjects**

188 Bioequivalence studies can usually be conducted with normal, healthy volunteers. This
189 approach has the advantage of minimizing variability that is not due to the drug or drug product
190 *per se*. It is generally accepted that conclusions regarding relative bioavailability, drawn from
191 studies with healthy volunteers, can be expected to hold in the patient population. It is more
192 difficult to conduct cross-over comparative bioavailability studies in patients, in part due to
193 potential disease progression. In some cases, for example when the safety profile of the drug
194 being studied is such that it cannot be administered to healthy volunteers, it may be necessary to

195 conduct studies in patients who are already receiving the drug. The variability of the disease
196 states in patients in whom the studies are performed will be an important consideration in
197 deciding the size of cohort which will have to be investigated in order to satisfy the standards.

198 **2.2.2 Inclusion / Exclusion Criteria**

199 An important objective in the selection of subjects is to reduce the intrasubject variability
200 in pharmacokinetics that may be attributable to certain characteristics of the subject.

201 a) Age

202 Subjects should be between the age of legal majority and the age of onset of age-
203 associated changes in organic function. This description typically coincides with an age range of
204 18 to 55 years, inclusive.

205 b) Height/weight ratio

206 The ratio for healthy volunteer subjects should be within 15 percent of the normal range,
207 e.g., as given in current Metropolitan Life Insurance tables. Alternatively, weights within the
208 normal range according to the normal values for body mass index, are acceptable.

209 c) Health

210 The health of the volunteers should be determined by the supervising physician through
211 a medical examination and review of results of routine tests of liver, kidney, and hematological
212 functions. Aberrant laboratory values should be rechecked and a summary should be presented
213 along with the physician's opinion as to potential impact on the study's conclusions.

214 Psychological characteristics should also be assessed by the physician in order to exclude
215 patients unlikely to comply with study restrictions or unlikely to complete the study.

216 Testing for alcohol and drugs of abuse should be conducted prior to drug administration
217 in each period.

218 d) Safety

219 An electrocardiogram should be included in the study documentation if the drug has a
220 cardiac effect.

221 Subjects who have been previously treated for gastrointestinal problems (such as ulcers),
222 or convulsive, depressive, or hepatic disorders, and in whom there is a risk of a recurrence
223 during the study period, should be excluded.

224 The investigators should ensure that female volunteers are not pregnant, lactating, or
225 likely to become pregnant during the study. Confirmation regarding pregnancy should be
226 obtained by urine tests prior to drug administration in each period.

227 **2.3 Study Design**

228 **2.3.1 Parallel versus Cross-over**

229 The basic design to be used is a two-period cross-over, in which each subject is given the
230 test and reference formulations. The advantage of the cross-over design is that in the
231 construction of the confidence intervals for comparing mean differences, the intrasubject error is
232 used, which is always lower than the intersubject error used in a parallel design. The linear
233 model for the two treatment, two period, and two sequence (2x2) crossover design is given in
234 Equation 1:

$$235 Y_{ijkl} = \mu + S_i + V_{j(i)} + F_k + P_l + \epsilon_{ijkl} \quad (1)$$

236 where Y_{ijkl} = observation for subject j in sequence i given formulation k in period l ; μ = the
237 overall mean; S_i = effect of sequence i ; $V_{j(i)}$ = random effect of subjects within sequence,
238 assumed independently and identically distributed $N(0, \sigma_B^2)$, where σ_B^2 is an estimate of the
239 intersubject variability; F_k = effect of formulation k ; P_l = effect of period l ; and ϵ_{ijkl} = the residual
240 assumed to be independently identically distributed $N(0, \sigma_W^2)$, where σ_W^2 is an estimate of the
241 intrasubject variability.

242 Assumptions on this model are that observations made on different subjects are
243 independent, and that the variance of an observed Y is $\sigma_B^2 + \sigma_W^2$ and any two observations
244 have a covariance σ_B^2 .

245 In cases where more than two formulations are under study, or are studied under different
246 conditions, a higher order (that is [i.e.], more periods and sequences) should be considered. Since
247 the intrasubject error term of these designs has more degrees of freedom, smaller sample sizes
248 are often required.

249 Another type of crossover design that is sometimes used is the replicated design where
250 the formulations being tested are replicated within subjects. The main advantage of these designs
251 is that fewer subjects are required but they must appear for more periods.

252 Parallel designs are sometimes necessary to study patients where it would be unethical to
253 discontinue medication for the washout period. Such designs may also be useful when studying
254 drugs with very long elimination half-lives. The error term used is the intersubject variance.

255 **2.3.1.1 Number of Subjects**

256 The number of subjects to be used in the study should be estimated by considering the
257 objectives of the study, study design and the drug products being compared. The drug and drug
258 product determine the particular standard which needs to be met. A complete literature search
259 should be done in order to understand the drug and drug product. The standard, the expected
260 mean difference between the test and reference formulations of both AUC_T and C_{max} , the
261 anticipated intrasubject coefficient of variation (CV) of both AUC_T and C_{max} and the power
262 determine the number of subjects. The minimum number of subjects is 12, but a larger number is
263 usually required.

264 Tables A1-A and A1-B in Appendix A1 suggest sample sizes for the various scenarios of
265 CV, expected mean differences, bioequivalence limits and power for two-way crossover studies.

266 For parallel studies see Tables A1-C and A1-D, Appendix A1.

267 Higher order designs have a larger degrees of freedom and will often require slightly
268 smaller sample sizes.

269 **2.3.2 Other Strategies for Collecting Data**

270 **2.3.2.1 Add-ons**

271 As a result of random variation or a larger than expected relative difference, there is no
272 guarantee that the sample size as calculated will pass the standards. If the study is run with the
273 appropriate size and the standards are not met, the sponsor may add more subjects (a minimum
274 of 12). The same protocol should be used (i.e., same formulations, same lots, same blood
275 sampling times, a minimum number of 12 subjects, etc.). The choice to use this strategy, as with
276 all designs, should be declared and justified *a priori*.

277 The level of confidence should be adjusted using the Bonferroni procedure. The t-value
278 should be that for $p=.025$ instead of $.05$.

279 **2.3.2.2 Sequential designs**

280 In these aforementioned basic designs, a group sequential design approach (see
281 Gould A.L. Group sequential extensions of a standard bioequivalence testing procedure. *J*
282 *Pharmacokinet Biopharm* 1995 Feb;23(1):57-86) could be implemented when the best estimate
283 of the intrasubject variability is not certain.

- 284 a) Obtain an estimate of the intrasubject CV.
285 b) The total sample size should be estimated according to the procedure outlined above.
286 c) The number of subjects at which time a “peek” at the data will be determined and declared in
287 the protocol.
288 d) The overall type 1 error of the experiment should be preserved. Analysis of data from the
289 initial stage should be treated as an interim analysis and both first and second stage analyses
290 should be conducted at adjusted significance levels resulting in confidence intervals of higher
291 than 90%.
292 e) The decision rules for stopping at each stage should be provided to ensure that group
293 sequential design procedure is valid.
294 f) The choice to use a sequential design should be specified *a priori*, in the protocol, along with
295 the adjusted significance levels to be used for each of the analyses.

296 After all data is collected, the usual methods for calculating the point estimates and their
297 confidence intervals should be used.

298 **2.3.2.3 Adaptive designs**

299 An adaptive design may be used when little is known about the formulations being
300 compared, e.g., new chemical entities.

301 Sample size re-estimation is permitted when the variability in the data is larger than
302 anticipated. No penalty need to be assessed if the assessment of variability is performed blinded
303 to formulation. Increasing sample size after an unblinded assessment will be treated as add-on
304 requiring Bonferroni adjustment. All other anticipated design modifications and adaptations
305 should be specified and justified in the protocol, with attention paid to preserving type I error.

306 **2.3.3 Accounting for Drop-outs and Withdrawals**

307 More subjects than the sample size calculation requires should be recruited into the
308 study. This strategy allows for possible no-shows, drop-outs and withdrawals and
309 discontinuations. A fixed number (one or two for each sequence) of subjects should be added to
310 the sample-size number.

311 Reasons for withdrawal (e.g., adverse drug reaction) should be reported and the subject's
312 plasma level data provided. The results of all samples that were measured in subjects who were
313 withdrawn from the study should be included in the report. Data from all subjects should be
314 included in the statistical analysis, unless the subject is in a cross-over trial and does not
315 complete at least one period with the test product and one period with the reference product.

316 2.3.4 Outlier consideration

317 Comparative bioavailability studies are small studies compared to other clinical trials.
318 One or two extreme values could have a large effect on the inference to be made from these
319 small studies. The usual parametric assumptions and estimation are not robust against extreme
320 values.

321 There are three main causes of these extreme values. One cause is a possible subject by
322 formulation interaction where the two formulations act consistently differently for a
323 subpopulation of individuals. The reason is generally unknown but is more frequent with
324 modified-release formulations. Retesting the subject(s) may provide data to suggest that this
325 interaction is real, if the results of the retest on the two formulations is similar to the initial
326 results. Another potential cause is actual formulation failure. This is a more difficult cause to
327 determine since the “tablet” can only be tested once. Given current strict manufacturing
328 requirements, formulation failure is not a likely cause of the extreme values. *In vitro* testing of
329 the test biobatch should be done if outliers are declared in a data set (Section 2.7.4.1). The most
330 likely cause of a large difference between two similar formulations is the particular subject’s
331 physiology or metabolism on the specific day of testing. Again retesting of the subject on both
332 formulations may provide an explanation for the observation.

333 A strategy to identify and account for outliers should be part of the protocol. These
334 extreme values should be rare and no more than two should be identified. If a protocol for
335 handling outliers is stated it must be followed before the results of the analysis are summarised
336 into confidence intervals (i.e., regardless of whether results meet the standard the outlier protocol
337 should be followed).

338 First, in order to be considered an extreme value, the observation must be outside the
339 range of all the other observations regardless of formulation. Second it must be identified by an
340 outlier test. It is recommended that the outlier test be a simple studentised residual tested against
341 a conservative t-value at the .02 level of significance and degrees of freedom for the design. In
342 other words the test should only identify observations which are very different from all others
343 collected.

344 A declaration of how extreme values are to be dealt with, should be made *a priori*. One
345 strategy is to perform a non-parametric construction of the confidence interval. A
346 non-parametric analysis which uses the log differences is preferred. Another strategy is to retest
347 the identified subject(s). If subjects are to be retested, they should be brought back and given
348 both formulations. In addition, 3 to 5 subjects from the original study, who were not identified as
349 outliers, should be retested to serve as controls. The new results are put back into the analysis
350 and if not declared an outlier by the same procedure, the original values may be removed. The

351 retest values are not to be part of the final analysis. The subject's values, both initial and retest,
352 should be reported. Should the same values be identified as outliers, consultation with the
353 Branch is recommended.

354 **2.4 Study Conduct**

355 **2.4.1 Standardization**

356 Every effort should be made to standardize the study conditions in every phase of the
357 study—for example, exercise, diet, smoking, and alcohol use. It is preferable to use non-smokers;
358 where smokers are included, they should be so identified.

359 Volunteers should not take any other drug, including alcoholic beverages and over-the-
360 counter (OTC) drugs, for an appropriate interval before, as well as during, the study.
361 Consideration should also be given to the potential metabolic effects of dietary items, such as
362 flavonoid-containing juices, that may affect the outcome of the study. Protocol violations with
363 respect to the use of any drug should be reported (dose and time of administration). The decision
364 whether to include or exclude the results from a subject who has varied from the established
365 protocol should be made before statistical analysis commences.

366 **2.4.2 Blinding**

367 If possible, the study should be conducted in such a way that the subject is not aware of
368 which product (test or reference) is being administered. Furthermore, the person checking for
369 adverse reactions and the person conducting the analysis of samples should not know which
370 product was administered. Other individuals involved in the administration of the drugs, the
371 surveillance of the patients, or the analysis of plasma data should not know which product was
372 administered.

373 **2.4.3 Administration of Food and Fluid**

374 **2.4.3.1 Fasted study**

375 For immediate-release dosage forms, comparative bioavailability should be demonstrated
376 in single-dose studies under fasting conditions. For the majority of drugs in immediate-release
377 dosage forms, this will provide sufficient information for the assessment of bioequivalence to the
378 Canadian Reference Product.

379 The administration of food and fluid should be controlled carefully. Normally, subjects
380 should fast for 10 hours before drug administration. A fast means that no food or solids are to be
381 consumed, although alcohol-free, xanthine-free and flavonoid-free clear fluids are permissible
382 the night prior to the study. Water may be permitted up to one hour before drug administration.

383 The dose should be taken with water of a standard volume (minimum of 150 millilitre) and at a
384 standard temperature. One hour after drug administration xanthine- and flavonoid-free fluids are
385 permitted. Four hours after drug administration, a standard meal may be taken. All meals should
386 be standardized and repeated on each study day.

387 When comparing the performance of two orally disintegrating dosage forms that are
388 intended to be taken without water, the comparative bioavailability study should be designed to
389 challenge the formulation under the most discriminatory conditions. For such dosage
390 formulations, water should not be administered from one hour prior to dosing, concurrent with
391 dosing and up to one hour post dosing.

392 **2.4.3.2 Fed Study**

393 Bioequivalence should be demonstrated under both fasted and fed conditions for critical-
394 dose drugs, drugs exhibiting non-linear pharmacokinetics and drugs in modified-release dosage
395 forms (including delayed-release formulations). Requirements for modified-release formulations
396 may differ from those for conventional drug formulations because a greater likelihood exists that
397 increased intersubject variability in bioavailability will occur, including the possibility of dose-
398 dumping and there may be an increased risk of adverse effects such as gastrointestinal irritation,
399 depending on the site of drug release, or absorption, or both.

400 If, however, there is a documented serious safety risk to subjects from single-dose
401 administration of the drug or drug product in either the absence or presence of food, then an
402 appropriately designed study conducted in the indicated condition of use (fed or fasted state)
403 may be acceptable for purposes of bioequivalence assessment. This approach should be
404 scientifically justified *a priori* by the sponsor.

405 The meal used in a comparative bioavailability study under fed conditions should allow
406 maximal perturbation of systemic bioavailability of the drug from the drug product. This is
407 generally a high fat, high calorie meal. Thus, the default meal, for comparative bioavailability
408 studies under fed conditions, should be a high fat, high calorie meal.

409 Given the above, use of a meal other than a high fat, high calorie meal should only occur
410 under exceptional circumstances. Use of a meal other than a high fat, high calorie meal should
411 be scientifically justified, *a priori*, by the submission sponsor. A possible justification for use of
412 a meal other than a high fat, high calorie meal would be a documented serious safety risk to
413 subjects from single-dose administration of the drug or drug product in the presence of such a
414 meal. In any case, deviations from the default meal should be scientifically justified, *a priori*, by
415 the submission sponsor. The meal should be given within 30 minutes prior to administration of
416 the drug product.

417 A high-fat (approximately 50 percent of total caloric content of the meal) and high-
418 calorie (approximately 800 to 1000 calories) meal should derive approximately 150, 250, and
419 500-600 calories from protein, carbohydrate, and fat, respectively. One example of a test meal
420 that is expected to promote the greatest perturbation in gastrointestinal physiology so that
421 systemic drug bioavailability is maximally affected would be the following breakfast: 2 eggs
422 fried in butter, 2 strips of bacon, 2 slices of toast with butter, 120 grams of hash browns and 240
423 millilitres of whole milk.

424 **2.4.3.3 Steady-state Studies**

425 If steady-state studies are required, the food and fluid conditions and restrictions noted
426 above should apply on the preceding evening and on the day the plasma profiles are to be
427 obtained.

428 **2.4.4 Posture and Physical Activity**

429 For most drugs, subjects should not be allowed to recline until at least two hours after
430 drug ingestion. Physical activity and posture should be standardized as much as possible to limit
431 effects on gastrointestinal blood flow and motility. The same pattern of posture and physical
432 activity should be maintained for each study day.

433 **2.4.5 Interval Between Doses**

434 The interval between study days should be long enough to permit elimination of
435 essentially all of the previous dose from the body. The interval should be the same for all
436 subjects and, to account for variability in elimination rate between subjects, normally should be
437 not less than 10 times the mean terminal half-life of the drug. Normally, the interval between
438 study days should not exceed three to four weeks. Furthermore, the drugs should be administered
439 at approximately the same time on each study day.

440 **2.4.6 Sampling Times**

441 The duration of sampling in a study should be sufficient to account for at least 80 percent
442 of the known AUC to infinity (AUC_{∞}). This period is usually at least three times the terminal
443 half-life of the drug.

444 To permit calculation of the relevant pharmacokinetic parameters, a minimum of 12
445 samples should be collected per subject per dose. Intersubject variability, as well as such factors
446 as potential for erratic behaviour of some formulations under some conditions (for example, a
447 fatty environment may affect release from an enteric-coated product), should be taken into

448 consideration in the placement and number of samples. The exact times at which the samples are
449 taken should be recorded and spaced such that the following information can be estimated
450 accurately:

- 451 a) peak concentration of the drug in the blood (C_{\max});
- 452 b) the area under the concentration time curve (AUC_T) is at least 80 percent of the known
453 AUC_1 ; and
- 454 c) the terminal disposition rate constant of the drug.

455 There may be considerable inaccuracies in the estimates of the terminal disposition rate
456 constant if the constant is estimated from linear regression using only a few points. To reduce
457 these inaccuracies it is preferable that four or more points be determined during the terminal log-
458 linear phase of the curve. If urine is used as the biological sampling fluid (see below), then
459 sufficient samples should be obtained to permit an estimate of the rate and extent of renal
460 excretion.

461 **2.4.7 Sample Collection**

462 Under normal circumstances, blood should be the biological fluid sampled to measure the
463 concentrations of the drug. In most cases the drug may be measured in serum or plasma;
464 however, in some cases, whole blood may be more appropriate for analysis. If the concentrations
465 in the blood are too minute to be detected and a substantial amount (>40 percent) of the drug is
466 eliminated unchanged in the urine, then the urine may serve as the biological fluid to be
467 sampled. In those rare situations where use of drug concentrations in urine is justifiable for the
468 assessment of relative bioavailability, only parent drug concentrations may be used. That is, use
469 of metabolite concentrations in urine is not considered acceptable in the assessment of
470 bioequivalence.

471 When urine is collected at the study centre, the volume of each sample should be
472 measured immediately after collection and included in the report. Urine should be collected over
473 a period of no less than three times the terminal elimination half-life. For a 24-hour study,
474 sampling times of 0 to 2, 2 to 4, 4 to 8, 8 to 12, and 12 to 24 hours are usually appropriate.
475 Quantitative creatinine determinations on each urine sample are also required.

476 Sometimes the concentration of drug in a fluid other than blood or urine may correlate
477 better with effect. Nevertheless, the drug must first be absorbed prior to distribution to the other
478 fluids such as the cerebrospinal fluid, bronchial secretions, etc. Thus, for bioavailability
479 estimations, blood is still to be sampled and assayed.

480 **2.4.8 Handling of Samples**

481 Samples should be processed and stored under conditions that have been shown not to
482 cause significant degradation of the analytes. Appropriate storage conditions should be
483 confirmed with samples from subjects who have been given the drug under study, in case spiked
484 samples give misleading results, e.g., if there is evidence that metabolites are likely to
485 interconvert to the parent drug.

486 **2.4.9 Identification of Adverse Events**

487 In some cases, adverse events are due to factors other than the active ingredient in a
488 formulation. The rate of absorption and excipients within formulations may affect the frequency,
489 onset, and severity of adverse events. The incidence, severity, and duration of all adverse events
490 observed during the study should be reported. The probability that an adverse event is drug-
491 induced is to be judged by the investigator.

492 As much as possible, the same observer and format for eliciting and recording
493 information on adverse events should be used for all subjects. Questions concerning adverse
494 events should be asked on each study day by the "blinded" observer. For drugs with known
495 adverse events -for example, metallic taste, postural hypotension, cardiac dysrhythmia-the
496 specific questions should be raised and observations, such as blood pressure measurement and
497 electrocardiogram, should be performed and recorded at the time the events are known to occur
498 with respect to the time of administration. In asking the questions, the interviewer should avoid
499 leading the subject to believe that the events are expected or unexpected. Furthermore, the
500 subject should be questioned in private.

501 **2.5 Test and Reference Drug Products**

502 This section describes the required characteristics of the test and reference drug products
503 that should be documented, including quality, dosage, and strength.

504 The test and reference drug products should be of high quality and mention should be
505 made in the study documentation of the dosage and strength of the drug and what reference
506 product is used in the study.

507 **2.5.1 Chemistry**

508 The products should meet a Schedule B or other applicable standard acceptable to Health
509 Canada. The chemistry and manufacturing guidances for preclinical and new drug submissions
510 should be consulted for an interpretation of the general technical requirements listed in sections
511 C.08.005(1) and C.08.002(2) respectively.

512 2.5.2 Dosage and Strength

513 In bioequivalence studies, the same dose of each product should be used. The lots for
514 comparative bioavailability testing should be representative of proposed market production
515 batches. The lots for comparative bioavailability testing should be taken from a batch that is a
516 minimum of ten percent of the commercial batch size and is produced using the same type of
517 equipment and procedures, and for modified-release formulations, the same site, proposed for
518 market production.

519 For products in which the proportions of excipients and the dissolution characteristics are
520 similar, comparative bioavailability studies may not be required for all strengths. Whether all
521 strengths should be tested will depend on the extent to which the formulation differs among
522 strengths.

523 When a modified-release product in the form of a scored tablet possesses the claim that a
524 portion of the tablet may be administered to provide a proportional dose, evidence must be
525 presented to justify the claim.

526 2.5.3 Selection of Reference Product

527 For a new drug substance (i.e., the first market entry), an oral solution should be used as
528 the reference product when possible. The oral solution can be prepared from an intravenous
529 solution, if available.

530 In bioequivalence studies, the Canadian reference product is:

- 531 (a) a drug in respect of which a notice of compliance is issued pursuant to section C.08.004 of
532 the *Food and Drug Regulations* and which is marketed in Canada by the innovator of the drug;
533 (b) a drug, acceptable to the Minister, that can be used for the purpose of demonstrating
534 bioequivalence on the basis of pharmaceutical and, where applicable, bioavailability
535 characteristics, where a drug in respect of which a notice of compliance has been issued pursuant
536 to section C.08.004 cannot be used for that purpose because it is no longer marketed in Canada;
537 or
538 (c) a drug, acceptable to the Minister, that can be used for the purpose of demonstrating
539 bioequivalence on the basis of pharmaceutical and, where applicable, bioavailability
540 characteristics, in comparison to a drug referred to in paragraph (a).

541 2.6 Analytical Methodology

542 Bioavailability determinations rely on well-characterized and validated analytical
543 methods that are able to generate reliable estimates of analyte concentrations.

544 **2.6.1 Drug and Drug Metabolites**

545 Determination of bioequivalence should be based on data for the parent drug.

546 Waiver of the measurement of the parent drug will not be considered, unless
547 concentrations of the parent drug cannot be reliably measured, e.g., if the parent drug is not
548 detectable due to rapid biotransformation. In such instances, the use of metabolite data may be
549 acceptable. The measured metabolite should be a primary (first step) and major one, and
550 appropriate scientific justification for a waiver of the measurement of the parent drug and the use
551 of metabolite data should be provided. The choice of using the metabolite instead of the parent
552 drug is to be clearly stated, *a priori*, in the objective of the study in the study protocol.

553 For the purpose of this guidance, a pro-drug is to be treated as a 'parent drug'. That is, if
554 the substance released from the dosage form is absorbed intact and is reliably measurable in the
555 systemic circulation, it should be used in the assessment of bioequivalence.

556 It is not generally considered necessary to measure both parent drug and metabolite
557 levels for the purpose of bioequivalence assessment. However, quantitation of metabolite levels
558 may sometimes be helpful, e.g., to explain extreme values caused by metabolic changes within a
559 subject.

560 In those rare situations where use of drug concentrations in urine is justifiable for the
561 assessment of relative bioavailability, only parent drug concentrations may be used. That is, use
562 of metabolite concentrations in urine is not considered acceptable in the assessment of
563 bioequivalence.

564 **2.6.2 Assay Methodology**

565 The analytical methods used to measure the drug, or metabolite, in plasma, blood, serum,
566 or urine should be reproducible, specific, and sufficiently sensitive, precise, and accurate. When
567 these operating parameters have been shown to be adequate in the hands of the test laboratory,
568 the investigators can then undertake the bioavailability study.

569 The principles and procedures for analytical validation described in the summary
570 document "Analytical Methods Validation: Bioavailability, Bioequivalence, and
571 Pharmacokinetic Studies," V. P. Shah et al (1992), *Journal of Pharmaceutical Sciences* 81(3)
572 and "Workshop/Conference report - Quantitative bioanalytical methods validation and
573 implementation: Best practices for chromatographic and ligand binding assays," C.T.
574 Viswanathan et al (2007) *The AAPS Journal* 9 (1) Article 4, should be followed. In addition to
575 pre-study validation, appropriate performance characteristics (accuracy, precision, quality
576 control) should be documented for each analytical run during a study.

577 **2.6.3 Stability**

578 In order for samples to maintain their stability (degradation of analytes), they should be
579 handled according to validated handling and storage procedures (Section 2.4.8, "Handling of
580 Samples"). Validation should be included.

581 **2.6.4 Limit of Quantitation (LOQ)**

582 The analytical method chosen should be capable of assaying the analyte over the
583 expected concentration range. A reliable lowest limit of quantitation should be established based
584 on an intra- and inter-day coefficient of variation (CV) usually not greater than 20 percent. The
585 limit of detection (LOD-the lowest concentration that can be differentiated from background
586 levels) is usually lower than the LOQ. Values between LOQ and LOD should be identified as
587 "Below Quantitation Limits".

588 **2.6.5 Specificity**

589 It should be demonstrated that endogenous compounds in the biologic matrix, nutrients,
590 metabolites, and degradation products do not interfere with the assay method. In cases in which a
591 stereospecific method is used, proof of the specificity should be documented. Specificity should
592 be established using at least six independent sources of the same matrix being studied.

593 **2.6.6 Recovery**

594 The reproducibility of the absolute recovery of drug during the sample preparation
595 procedure should be demonstrated and should be established for low, medium and high
596 concentrations, based on the expected range.

597 **2.6.7 Standard Curves**

598 A standard curve demonstrates the range of concentrations over which an analyte can be
599 reliably determined in matrix, using a minimum of five concentration points. Standard curves
600 should be included with each run. The intra- and inter-run variability in the standard curves
601 should be reported together with the coefficients of variation (CVs) obtained during sample
602 measurement. These attributes will be used to determine the acceptability of the standard curve.
603 The number of standards to be used will be a function of the dynamic range and nature of the
604 concentration-detector response relationship. The standard curve should be determined using an
605 appropriate algorithm.

606 **2.6.8 Precision and Accuracy**

607 The precision and accuracy of the assay should be determined for low, medium, and high
608 drug concentrations in the biological matrix, based on the expected range. Accuracy for inter-day
609 and intra-run should be within 15 percent of the nominal value. For precision, the CV should be
610 no greater than 15 percent, except at the limit of quantitation, when a value no greater than 20
611 percent is acceptable.

612 **2.6.9 Quality Control for Spiked Samples**

613 For stable analytes, quality control (QC) samples should be prepared in the fluid of
614 interest (e.g., plasma), including concentrations at least at the low, middle, and high segments of
615 the calibration range. The quality control samples should be stored with the study samples.
616 These are accepted for stability if they exhibit similar characteristics to those taken from
617 volunteers.

618 For less stable analytes, daily or weekly quality control samples may have to be prepared.

619 A minimum of six QC samples, composed of three concentrations in duplicate, should be
620 blinded and analysed with each batch of study samples for each analytical run.

621 **2.6.10 Aberrant Values (Repeat Assays)**

622 In most studies, some plasma or urine samples will require re-assay. Criteria for
623 identifying these samples should be established ahead of time.

624 Certain aberrant values can be identified before breaking the analytical code. These
625 values may be attributed to such factors as:

- 626 a) processing errors;
627 b) equipment failure;
628 c) obviously poor chromatography; or
629 d) quality control samples outside pre-defined tolerances.

630 Other apparently aberrant values may become evident after the analytical code is broken.
631 In some such cases, the original assay value would show poor pharmacokinetic fit (but this
632 should be applied with caution). In other cases, there might be a need to confirm a double peak.
633 For aberrant values that have become evident after the analytical code is broken, the submission
634 should note the reason for the repeat assay.

635 When the results of a repeat assay differ from the original by more than 15 percent, a
636 third analysis should be performed. When three replicate analyses indicate that one is spurious,
637 then the average of the other two should be used. The criteria used in selecting among replicates
638 for inclusion in calculations should be stated.

639 **2.7 Analysis of Data**

640 When all measurements of samples have been completed, the information collected
641 should be analysed. This section discusses the data that should be recorded, the parameters of
642 that data, the statistical analyses that should be performed on the data, and the format that should
643 be used to present the results in reports.

644 **2.7.1 Presentation of Data**

645 The concentrations of the drug in plasma for each subject, the sampling time, and the
646 formulation should be tabulated. Unadjusted, measured concentrations should be provided.

647 Deviations from the protocol (e.g., missed samples or late collection of samples) should
648 be clearly identified in the tables.

649 Two graphs should be drawn for each subject and two for the mean values of all subjects,
650 one linear and the other semilogarithmic. On these graphs, the drug concentrations from the
651 reference and the test formulations should be plotted against the sampling times. Natural
652 logarithms (\ln) are to be employed. Usually, the semilogarithmic graphs should display the
653 regression lines that are employed to estimate the terminal disposition rate constant (λ) for the
654 two formulations.

655 **2.7.2 Pharmacokinetic Parameters**

656 Estimates of the following pharmacokinetic parameters should be tabulated for each
657 subject-formulation combination:

- 658 a) AUC_T
659 Area under the concentration-time curve measured to the last quantifiable concentration,
660 using the trapezoidal rule.
- 661 b) AUC_I
662 AUC_T plus additional area extrapolated to infinity, calculated using λ .
- 663 c) AUC_T/AUC_I
664 The ratio of AUC_T to AUC_I .

- 665 d) C_{\max}
666 Maximum observed concentration.
- 667 e) t_{\max}
668 Observed time after dosing, at which C_{\max} occurred.
- 669 f) λ
670 Terminal disposition rate constant.
- 671 g) $T_{1/2}$
672 Terminal elimination half-life.
- 673 Where the time to onset of action is important the following parameter should also be
674 reported:
- 675 h) $AUC_{\text{Ref}T_{\max}}$
676 Area under the curve to the time of the maximum concentration of the reference product,
677 calculated for each study subject.
- 678 Where multiple dose studies are conducted, the following parameters should also be
679 reported:
- 680 i) C_{\min}
681 Minimum observed concentration.
- 682 j) C_{pd}
683 Pre-dose concentrations determined immediately before a dose at steady state.
- 684 k) AUC_{τ}
685 Area under the concentration versus time curve, over the dosing interval of the test
686 formulation, calculated using the linear trapezoidal rule.
- 687 l) Fluctuation
688 $(C_{\max} - C_{\min}) / (AUC_{\tau} / \tau) \times 100$.
- 689 Where comparative bioavailability is based upon urine data, the following parameters
690 should be reported:
- 691 m) Ae_{0-T}
692 Cumulative amount of drug excreted to last sampling time.

693 n) R_{\max}
694 Maximum rate of urinary excretion.

695 Additional pharmacokinetic parameters may also be presented, but the methods used to
696 estimate them should be fully described. The means and coefficients of variation should be given
697 for each parameter and for each formulation.

698 **2.7.3 Data Collection**

699 If an add-on, sequential or adaptive design is used, a description of how changes were
700 made to collection of data should be provided.

701 **2.7.4 Statistical Analysis**

702 **2.7.4.1 Outlier analysis**

703 If the protocol states that outlier identification is to be performed, a summary of these
704 results should be presented before any calculation of the confidence intervals is performed. The
705 protocol test at the specified level should be performed and values identified. No more than 5
706 percent of subjects should be identified as outliers. If there are more, then the drug is more likely
707 to be a highly variable drug and appropriate action should taken (i.e., use a study design and
708 analysis appropriate for a highly variable drug). If the non-parametric analysis is to be
709 performed, the results should be presented in the analysis section below. If retesting is
710 performed, results of the retest and re-analysis of the retest values and declaration and removal
711 of original values should be shown. Uniformity of dosage units and dissolution should be re-
712 tested (as per the applicable United States Pharmacopeia (USP) or European Pharmacopeia (EP)
713 monograph) and results should be provided for the biobatches.

714 **2.7.4.2 Model Fitting**

715 By definition the crossover design is a mixed effects model with fixed and random
716 effects. The basic 2 period crossover can be analysed according to a simple fixed effects model
717 and least squares means estimation. Identical results will be obtained from a mixed effects
718 analysis such as Proc mixed in SAS. If the mixed model approach is used, parameter constraints
719 must be defined in the protocol. Higher order models must be analysed with the mixed model
720 approach in order to estimate random effects properly.

721 **2.7.4.3 Testing of fixed effects**

722 A summary of the testing of sequence, period and formulation effects and other fixed
723 effects should be presented. Explanations for significant effects should be given.

724

2.7.4.4 Estimation of random effects

725

A summary of the estimates of intersubject and intrasubject variances should be presented. For higher order designs estimates of subject by formulation and within formulation variance estimates should be given.

726

727

728

The analyses should include all data for all subjects (see Section 2.3.3, "Accounting for Drop-outs and Withdrawals") on measured data. Analysis based on less data should be justified.

729

730

Analysis should be carried out on the logarithmically transformed AUC_T and C_{max} data. The analysis and results for each parameter should be reported on a separate page as detailed in Appendix A2, "Sample Analysis for a Comparative Bioavailability Study". The reported results should include:

731

732

733

734

a) means and CVs (across subjects) for each product;

735

b) testing and estimates for fixed and random effects;

736

c) AUC_T and C_{max} ratios for test versus reference products;

737

d) the appropriate confidence interval about the parameter being analysed.

738 **Appendices**739 **Appendix 1 Number of Subjects**

740 The formula for calculating sample sizes is based on Hauschke et al., Sample size determination
741 for bioequivalence assessment using a multiplicative model. *Journal of Pharmacokinetics and*
742 *Biopharmaceutics*, 1992; **20(5)**: 557-561.

743 To use the table:

- 744 a) Obtain an estimate of the intrasubject CV from the literature.
- 745 b) Choose table A1-A or A1-B depending on the bioequivalence interval required.
- 746 c) Choose the power required (80% or 90%).
- 747 d) Choose an expected true ratio of test over reference means (usually 100%, but consider
748 potency differences between the test and reference products).
- 749 e) Go down the column until you arrive at the rounded CV. The number is the sample size.

750 This sample size algorithm should be provided in the study protocol and anticipated CV
751 declared.

752 Note: Sample size calculations, based on a standard where only the mean estimate is required to
753 fall within the bioequivalence interval, are not possible.

754 Table A1-A. Sample Sizes for 2x2 Crossover Design for Interval Hypotheses for 80-125%
 755 Rule to Attain a Power of 80 and 90%, Respectively in the Case of the Multiplicative Model
 756 (Linear interpolation can be used between stated CVs)

Power	$\theta = \mu_T/\mu_R$								
	CV (%)	0.85	0.90	0.95	1.00	1.05	1.10	1.15	1.20
80%	10	36	12	12*	12*	12*	12*	20	76
	12	50	16	12*	12*	12*	14	28	110
	14	68	20	12	12*	12	18	38	148
	16	88	24	14	12	14	22	48	192
	18	112	32	16	14	16	26	60	242
	20	138	38	20	16	18	32	74	300
	22	166	46	22	20	22	38	88	362
	24	196	54	26	22	26	46	104	430
	26	230	62	30	26	30	52	122	504
	28	266	72	34	30	34	62	142	584
	30	306	82	40	34	40	70	162	670
	32	346	94	46	38	44	80	184	762
	35	414	112	54	44	52	94	220	912
	40	540	146	70	58	68	122	286	1190
	45	684	182	88	72	84	154	362	1504
	50	842	226	108	88	104	190	448	1858
	55	1020	272	130	106	126	230	540	2246
60	1214	324	154	126	148	274	642	2674	
90%	10	50	16	12*	12*	12*	14	28	106
	12	70	20	12*	12*	12*	18	38	150
	14	94	26	14	12	14	24	50	204
	16	122	34	18	14	18	30	66	266
	18	154	42	22	18	20	38	82	336
	20	188	52	26	20	26	44	102	414
	22	228	62	30	24	30	54	122	500
	24	270	74	36	28	36	62	144	594
	26	318	86	42	32	40	74	170	698
	28	368	100	48	36	46	84	196	808
	30	422	114	54	42	54	96	224	928
	32	480	128	62	48	60	110	254	1054
	35	574	154	74	56	72	132	304	1262
	40	748	200	100	72	92	170	396	1648
	45	946	252	120	90	116	214	502	2084
	50	1168	312	148	112	144	264	618	2572
	55	1412	376	178	134	172	320	748	3112
60	1680	446	212	160	206	380	890	3702	
*	Calculated sample size < 12								

761 Table A1-B. Sample Sizes for 2x2 Crossover Design for Interval Hypotheses for 90-112% Rule
 762 to Attain a Power of 80 and 90%, Respectively in the Case of the Multiplicative Model (Linear
 763 interpolation can be used between stated CVs)

		$\theta = \mu_T/\mu_R$			
Power	CV (%)	0.95	1.00	1.05	1.10
80%	10	44	16	32	384
	12	64	22	46	550
	14	86	28	60	748
	16	110	36	78	978
	18	140	46	98	1236
	20	172	56	122	1526
	22	208	68	146	1846
	24	246	80	174	2196
	26	288	92	204	2578
	28	334	108	236	2988
	30	384	122	270	3430
	32	436	140	306	3902
	35	520	166	366	4668
	40	680	216	478	6096
	45	858	272	604	7714
	50	1060	336	744	9524
55	1282	406	900	11524	
60	1526	482	1072	13714	
90%	10	62	20	44	530
	12	88	28	62	762
	14	118	36	84	1036
	16	152	46	108	1354
	18	192	58	136	1712
	20	236	70	168	2112
	22	286	84	202	2556
	24	340	100	240	3042
	26	398	116	280	3568
	28	462	134	326	4138
	30	530	154	372	4750
	32	602	176	424	5404
	35	720	210	506	6466
	40	940	272	660	8444
	45	1190	344	836	10686
	50	1468	424	1030	13192
55	1776	512	1246	15960	
60	2112	610	1484	18994	

767 Table A1-C. Sample Sizes for Parallel Design for Interval Hypotheses for 80-125% Rule to
 768 Attain a Power of 80 and 90%, Respectively in the Case of the Multiplicative Model (Linear
 769 interpolation can be used between stated CVs)

Power	$\theta = \mu_T/\mu_R$								
	CV (%)	0.85	0.90	0.95	1.00	1.05	1.10	1.15	1.20
80%	10	70	20	12	12*	12	18	38	150
	12	100	28	14	12	14	24	54	216
	14	134	38	18	16	18	32	72	294
	16	174	48	24	20	24	42	94	382
	18	220	60	30	26	28	52	118	484
	20	272	74	36	30	36	62	144	596
	22	328	88	44	36	42	76	174	720
	24	390	106	50	42	50	90	208	858
	26	458	122	60	50	58	104	242	1006
	28	530	142	68	56	66	122	282	1166
	30	608	162	78	64	76	138	322	1338
	32	692	184	88	74	86	158	366	1522
	35	826	220	106	86	102	188	438	1820
	40	1080	288	136	112	132	244	572	2376
	45	1364	364	172	142	168	308	722	3008
	50	1684	448	212	174	206	380	892	3712
55	2038	542	256	210	248	460	1078	4492	
60	2424	644	304	250	296	548	1282	5344	
90%	10	96	28	14	12	14	24	52	208
	12	136	38	20	16	18	32	74	298
	14	186	50	26	20	24	44	100	406
	16	242	66	32	24	32	56	128	528
	18	304	82	40	32	40	70	162	668
	20	376	102	48	38	48	86	200	824
	22	454	122	58	44	58	104	242	998
	24	540	144	70	52	68	124	286	1186
	26	632	170	80	62	78	144	336	1392
	28	734	196	94	72	90	166	388	1614
	30	842	224	106	82	104	192	446	1852
	32	956	256	122	92	118	218	508	2108
	35	1144	306	144	110	140	260	606	2520
	40	1494	398	188	142	182	338	790	3292
	45	1890	502	238	178	230	428	1000	4166
	50	2332	620	292	220	284	526	1234	5142
55	2822	750	354	266	344	636	1492	6220	
60	3358	892	420	316	408	758	1776	7402	
*	Calculated sample size < 12								

774 Table A1-D. Sample Sizes for Parallel Design for Interval Hypotheses for 90-112% Rule to
 775 Attain a Power of 80 and 90%, Respectively in the Case of the Multiplicative Model
 776 (Linear interpolation can be used between stated CVs)

	$\theta = \mu_T/\mu_R$				
Power	CV (%)	0.95	1.00	1.05	1.10
80%	10	44	30	62	764
	12	64	40	88	1100
	14	86	54	118	1496
	16	110	70	154	1952
	18	140	88	194	2470
	20	172	110	240	3050
	22	208	132	290	3690
	24	246	156	344	4390
	26	288	182	404	5152
	28	334	212	468	5974
	30	384	242	536	6858
	32	436	276	610	7802
	35	520	330	730	9334
	40	680	430	952	12190
	45	860	542	1204	15428
	50	1060	670	1486	19046
	55	1282	810	1798	23044
	60	1526	962	2140	27424
90%	10	62	36	84	1058
	12	88	52	120	1522
	14	118	68	164	2070
	16	152	90	214	2704
	18	192	112	268	3422
	20	236	138	332	4222
	22	286	166	400	5110
	24	340	196	476	6080
	26	398	230	558	7136
	28	462	268	648	8274
	30	530	306	742	9498
	32	602	348	844	10806
	35	720	416	1010	12928
	40	940	542	1318	16884
	45	1190	686	1668	21368
	50	1468	846	2058	26380
	55	1776	1022	2490	31920
	60	2112	1216	2964	37986

780 **Appendix 2 Sample Analysis for a Comparative Bioavailability Study**

781 The following tables and figures illustrate data collected and used in a sample
782 bioavailability study. An analysis of this data is also shown.

783 Although a comparative bioavailability study may include many formulations, the basic
784 analysis is the same - each test formulation is compared to a standard formulation.

785 The analysis of any comparative bioavailability study should have the following sections:

- 786 a) A randomization scheme for the design, where all subjects randomized into the study are
787 included and identified by code, sequence, and dates of the dosing periods for both test
788 and reference formulations (see Section A2.1.).
- 789 b) A summary of drug concentrations (graphic and quantitative) at each sampling time for
790 each subject for both test and reference formulations (see Section A2.2.).
- 791 c) A summary of the estimates of the parameters as defined in Section A2.3 for both test
792 and reference formulations, including the means, standard deviations, and CVs (see
793 Section A2.4.).
- 794 d) A formal statistical analysis of the relevant parameters with comparisons of the test
795 formulations to the reference formulations (see Sections A2.5 through A2.9.).

796 All the sample statistical analyses that follow have the minimum two formulations (test
797 and reference) given on two dosing days or periods.

798 **A2.1 Randomization Scheme of the Design**

799 Shown in Table A2-A is the randomization scheme for the cross-over design used in the
800 study. In any study, all subjects who were randomized into the study should be included. Even
801 those subjects that did not complete the study should be included and identified accordingly.
802 Subject numbers that appear on informed consent forms and reporting forms should be given.
803 Also, if any other subject identification code was used, it should be given here. The sequence to
804 which the subject was randomized should be given. Finally, *all* dosing periods and dates should
805 be given.

806 **A2.2 Summary of Drug Concentrations**

807 Tables A2-B and A2-C show a list of the concentrations at each sampling time for each
808 subject for the test and reference formulations, respectively. If any concentration is missing, it
809 should be identified, and the reason it is missing given (e.g., lost sample; sample not collected).

810 Although no formal statistical analysis is required at each sampling time, it is
811 recommended that summary statistics be given at each sampling time for each formulation. It is
812 also helpful if the lower limit of quantitation of the analytical method is given in this table.

813 Table A2-A: Randomization Scheme of the Cross-over Design for the Comparison of Test (T)
 814 Versus Reference (R) Formulations

Subject			Period	
Number	ID	Sequence	May 14, 2008	May 21, 2008
815	001	A	TR	T
816	002	B	RT	R
817	003	C	RT	R
818	004*	D	TR	T
819	005	E	TR	T
820	006	F	RT	R
821	007	G	TR	T
822	008	H	RT	R
823	009	T	TR	T
824	010**	I	RT	-
825	011	K	RT	R
826	012	L	TR	T
827	013	M	TR	T
828	014	N	RT	R
829	015	O	RT	R
830	016	P	TR	T
831	017	Q	RT	R
832	018	R	TR	T
833	*	Subject did not appear for second period.		
834	**	Subject did not appear for either period.		

837 Table A2-B: Drug Concentrations (nanograms (ng)/millilitre (mL)) for the Test Formulation

ID	Seq	Period	Sampling Times (hours)											
			0.00	0.33	0.66	1.0	1.5	2.0	3.0	4.0	6.0	8.0	12.0	16.0
839 A	TR	14 May	0.00	BQL*	52.01	95.03	122.20	77.88	65.15	46.24	19.20	14.99	BQL*	BQL*
840 B	RT	21 May	0.00	BQL*	56.66	80.85	102.00	86.41	63.81	49.20	24.00	11.37	8.24	BQL*
841 C	RT	21 May	0.00	28.63	201.50	189.80	188.70	136.20	97.64	64.53	32.08	20.63	14.59	BQL*
842 E	TR	14 May	0.00	BQL*	9.04	34.32	47.70	52.79	59.47	32.61	17.61	8.76	BQL*	BQL*
843 F	RT	21 May	0.00	BQL*	55.33	66.40	58.97	48.29	43.19	34.23	17.30	6.15	BQL*	BQL*
844 G	TR	14 May	0.00	BQL*	33.15	45.64	54.19	34.13	32.78	21.73	10.75	8.35	BQL*	BQL*
845 H	RT	21 May	0.00	35.38	79.14	100.90	70.71	48.43	30.73	26.19	8.65	6.83	BQL*	BQL*
846 I	TR	14 May	0.00	BQL*	64.57	76.52	89.51	86.21	69.04	50.96	21.55	13.71	7.55	BQL*
847 K	RT	21 May	0.00	BQL*	79.34	99.41	154.80	58.60	57.12	32.57	19.82	BQL*	BQL*	BQL*
848 L	TR	14 May	0.00	14.78	55.54	56.88	46.87	37.29	28.75	25.20	BQL*	BQL*	BQL*	BQL*
849 M	TR	14 May	0.00	BQL*	BQL*	BQL*	BQL*	BQL*	8.37	23.15	19.74	16.49	5.74	5.18
850 N	RT	21 May	0.00	BQL*	37.76	28.58	21.56	19.02	13.25	12.44	6.38	BQL*	BQL*	BQL*
851 O	RT	21 May	0.00	BQL*	27.85	43.30	43.30	32.57	29.59	25.42	16.89	7.68	BQL*	BQL*
852 P	TR	14 May	0.00	BQL*	68.25	52.57	51.97	28.64	23.70	12.74	BQL*	BQL*	BQL*	BQL*
853 Q	RT	21 May	0.00	BQL*	5.90	13.00	27.54	13.32	12.34	9.81	9.73	BQL*	BQL*	BQL*
854 R	TR	14 May	0.00	BQL*	18.92	35.77	53.93	60.43	47.44	41.72	16.66	8.87	5.49	BQL*
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858 MEAN	-	-	0.00	4.92	52.81	63.69	70.87	51.26	42.65	31.80	15.04	7.73	2.60	0.32
859 STD	-	-	0.00	11.26	47.05	45.04	49.76	33.66	24.64	15.42	8.60	6.57	4.42	1.29
860 CV	-	-	-	228.66	89.09	70.72	70.22	65.66	57.79	48.51	57.18	84.94	169.84	400
861 *	Lower limit of quantitation is 5 ng/mL. Any concentration below this limit is reported as Below Quantification Limit (BQL) except at time 0. Zero is used in the calculation of area under the curve (AUC) for times preceding the first observed concentration and in the calculation of summary statistics.													

862 Table A2-C: Drug Concentrations (ng/mL) for the Reference Formulation

ID	Seq	Period	Sampling Times (hours)											
			0.00	0.33	0.66	1.0	1.5	2.0	3.0	4.0	6.0	8.0	12.0	16.0
864 A	TR	14 May	0.00	BQL*	116.40	124.60	126.20	107.60	45.65	33.22	16.11	12.60	BQL*	BQL*
865 B	RT	21 May	0.00	BQL*	88.45	121.40	206.90	179.00	84.53	40.02	38.01	15.12	5.39	BQL*
866 C	RT	14 May	0.00	BQL*	BQL*	95.57	122.80	103.20	101.70	57.65	23.85	14.59	6.29	BQL*
867 E	TR	21 May	0.00	BQL*	37.23	37.26	35.90	28.87	28.48	25.10	24.91	6.72	BQL*	BQL*
868 F	RT	14 May	0.00	BQL*	29.25	62.88	64.26	84.67	45.21	25.05	17.18	8.47	BQL*	BQL*
869 G	TR	21 May	0.00	BQL*	6.89	50.04	55.27	51.68	38.58	26.19	7.79	BQL*	BQL*	BQL*
870 H	RT	14 May	0.00	BQL*	113.50	218.70	125.80	69.77	45.03	32.78	18.55	5.42	BQL*	BQL*
871 I	TR	21 May	0.00	BQL*	181.90	135.80	96.51	90.50	62.58	30.43	18.50	BQL*	BQL*	BQL*
872 K	RT	14 May	0.00	BQL*	42.71	58.75	59.68	54.37	44.35	22.94	11.58	6.95	BQL*	BQL*
873 L	TR	21 May	0.00	BQL*	14.29	21.32	24.32	25.56	25.51	10.49	5.49	BQL*	BQL*	BQL*
874 M	TR	21 May	0.00	BQL*	8.21	48.87	57.05	56.32	42.08	24.79	16.54	15.81	7.60	BQL*
875 N	RT	14 May	0.00	BQL*	47.20	34.90	34.90	24.19	20.11	8.08	7.27	BQL*	BQL*	BQL*
876 O	RT	14 May	0.00	BQL*	BQL*	20.35	70.88	70.60	70.38	40.51	26.93	8.20	BQL*	BQL*
877 P	TR	21 May	0.00	BQL*	39.23	86.29	97.46	52.26	40.53	26.74	12.54	BQL*	BQL*	BQL*
878 Q	RT	14 May	0.00	BQL*	BQL*	30.86	88.38	37.67	29.28	14.99	6.38	BQL*	BQL*	BQL*
879 R	TR	21 May	0.00	BQL*	BQL*	24.84	59.27	98.82	69.98	46.50	23.46	9.91	6.96	BQL*
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883	MEAN	-	0.00	-	45.33	73.28	82.85	70.94	49.62	29.09	17.19	6.49	1.64	-
884	STD	-	0.00	-	53.30	54.49	46.24	39.78	22.51	12.88	8.83	5.98	2.96	-
885	CV	-	-	-	117.59	74.37	55.82	56.08	45.37	44.28	51.38	92.23	180.73	-
886	*	Lower limit of quantitation is 5 ng/mL. Any concentration below this limit is reported as Below Quantification Limit (BQL) except at time 0. Zero is used in the calculation of area under the curve (AUC) for times preceding the first observed concentration and in the calculation of summary statistics.												

887 **A2.3 List of Parameters and Definitions**

888 Table A2-D shows a list of the parameters used in the analysis and their definitions. If
889 any other parameters are used, they should also be clearly defined.

890 Table A2-D: Parameter Definitions

Parameter	Definition
C_{\max}	Maximum observed concentration (ng/mL).
t_{\max}	Sampling time at which C_{\max} occurred (h).
AUC_T	Area under the raw concentration versus time curve calculated using the trapezoidal rule from time 0 to LQCT (ng·h/mL).
AUC_{∞}	Area to infinity = $AUC_T + C_T/\lambda$ where C_T is the estimated concentration at LQCT (ng·h/mL).
$\frac{AUC_T \times 100}{AUC_{\infty}}$	Percent of the area measured by AUC_T relative to the extrapolated total AUC.
λ	Terminal disposition rate constant calculated from the points on the log-linear end of the concentration versus time curve (h^{-1}).
TLIN	Time point where log-linear elimination begins (h).
LQCT	Lowest Quantifiable Concentration Time. Time at which the last concentration occurred that is above the lower limit of quantitation (h).
$t_{1/2}$	Drug half-life = $\ln_2/\lambda = 0.693/\lambda$ (h).

902 **A2.4 Summaries of Parameter Estimates**

903 Tables A2-E and A2-F list, for each subject, the estimates of the parameters defined in
 904 Table A2-D for the test and reference formulations respectively. Summary statistics (arithmetic
 905 means or medians, standard deviations, and CVs) should be given for each formulation.

906 Table A2-E: Parameter Estimates for Each Subject Given the Test Formulation

ID	Seq	Period	TEST FORMULATIONS								
			C _{max} (ng/mL)	t _{max} (h)	AUCT (ng·h/mL)	AUC _I (ng·h/mL)	AUCT (%)	λ (h ⁻¹)	TLIN (h)	LQCT (h)	t _{1/2} (h)
908 A	TR	14 May	122	1.50	365	409	89	0.3002	2.0	8.0	2.3
909 B	RT	21 May	102	1.50	405	432	94	0.2384	3.0	12.0	2.9
910 C	RT	21 May	202	0.66	703	774	91	0.1776	4.0	12.0	3.9
911 E	TR	14 May	59	3.00	233	256	91	0.3680	3.0	8.0	1.9
912 F	RT	21 May	66	1.00	247	265	93	0.3902	3.0	8.0	1.8
913 G	TR	14 May	54	1.50	178	205	87	0.2768	3.0	8.0	2.5
914 H	RT	21 May	101	1.00	246	263	94	0.3437	2.0	8.0	2.0
915 I	TR	14 May	90	1.50	408	433	94	0.2486	3.0	12.0	2.8
916 K	RT	21 May	155	1.50	315	372	85	0.3379	3.0	6.0	2.1
917 L	TR	14 May	57	1.00	140	331	42	0.1318	3.0	4.0	5.3
918 M	TR	14 May	23	4.00	165	195	85	0.1485	6.0	16.0	4.7
919 N	RT	21 May	38	0.66	88	113	78	0.2620	2.0	6.0	2.6
920 O	RT	21 May	43	1.00	183	215	85	0.2671	3.0	8.0	2.6
921 P	TR	14 May	68	0.66	122	148	83	0.5031	1.5	4.0	1.4
922 Q	RT	21 May	28	1.50	68	113	60	0.1833	1.5	6.0	3.8
923 R	TR	14 May	60	2.00	275	292	94	0.2546	3.0	12.0	2.7
924
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926
927 MEAN*	-	-	79	1.50	259	301	84	0.2770	3.0	8.0	2.8
928 STD	-	-	48	0.89	158	164	14	0.0967	1.1	3.3	1.1
929 CV	-	-	61	59.35	61	54	17	34.92	37.3	38.5	37.9
930 *	for t _{max} , TLIN, and LQCT, these are medians.										

931 Table A2-F: Parameter Estimates for Each Subject Given the Reference Formulation

ID	Seq	Period	REFERENCE FORMULATION								
			C _{max} (ng/mL)	t _{max} (h)	AUC _T (ng·h/mL)	AUC _I (ng·h/mL)	AUC _I (%)	λ (h ⁻¹)	TLIN (h)	LQCT (h)	t _{1/2} (h)A
A	TR	21 May	126	1.50	375	418	90	0.2660	3.0	8.0	2.6
B	RT	14 May	207	1.50	595	613	97	0.2900	3.0	12.0	2.4
C	RT	14 May	123	1.50	471	492	96	0.2666	4.0	12.0	2.6
E	TR	21 May	37	1.00	190	224	85	0.2653	3.0	8.0	2.6
F	RT	14 May	85	2.00	257	285	90	0.3114	3.0	8.0	2.2
G	TR	21 May	55	1.50	175	190	92	0.5437	3.0	6.0	1.3
H	RT	14 May	219	1.00	382	398	96	0.4047	2.0	8.0	1.7
I	TR	21 May	182	0.66	361	406	89	0.3837	3.0	6.0	1.8
K	RT	14 May	60	1.50	218	236	93	0.3580	3.0	8.0	1.9
L	TR	21 May	26	2.00	92	105	88	0.4208	2.0	6.0	1.6
M	TR	21 May	57	1.50	269	327	82	0.1373	6.0	12.0	5.1
N	RT	14 May	47	0.66	106	125	85	0.3246	2.0	6.0	2.1
O	RT	14 May	71	1.50	290	313	93	0.4028	3.0	8.0	1.7
P	TR	21 May	97	1.50	230	266	87	0.3644	2.0	6.0	1.9
Q	RT	14 May	88	1.50	144	156	92	0.4964	3.0	6.0	1.4
R	TR	21 May	99	2.00	344	369	93	0.2370	4.0	12.0	2.9
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MEAN	-	-	99	1.50	281	308	90	0.3420	3.0	8.0	2.2
STD	-	-	59	0.41	136	138	4	0.1017	1.0	2.4	0.9
CV	-	-	60	29.05	48	45	5	29.7262	32.6	29.2	39.4

955 **A2.5 Area Under the Curve to the Last Quantifiable Concentration (AUC_T)**
 956 **analysis**

957 Tables A2-G, A2-H, and A2-I provide the complete analysis required for AUC_T. Table
 958 A2-G lists the AUC_T estimates on the raw scale and the log scale. Also given is the test AUC_T as
 959 a percentage of the reference AUC_T. Summary statistics are calculated for each variable.

960 Table A2-G: AUC_T (ng·h/mL) Analysis - Data

ID	Raw Scale			Log Scale	
	Test AUC _T	Reference AUC _T	Relative AUC _T (%)	Test ln(AUC _T)	Reference ln(AUC _T)
A	365	375	97	5.90	5.93
B	405	595	68	6.00	6.39
C	703	471	149	6.55	6.16
E	233	190	123	5.45	5.25
F	247	257	96	5.51	5.55
G	178	175	102	5.18	5.17
H	246	382	65	5.51	5.94
I	408	361	113	6.01	5.89
K	315	218	144	5.75	5.39
L	140	92	153	4.94	4.52
M	165	269	61	5.11	5.59
N	88	106	83	4.48	4.66
O	183	290	63	5.21	5.67
P	122	230	53	4.81	5.44
Q	68	144	47	4.22	4.97
R	275	344	80	5.62	5.84
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MEAN	259	281	94	5.39	5.52
STD	158	136	35	0.61	0.52
CV	61	48	37	-	-

984 Table A2-H gives the analysis of variance (ANOVA) for the cross-over design model for
 985 $\ln(\text{AUC}_T)$. This analysis gives the appropriate intrasubject variance estimate, MS (Residual), for
 986 the calculation of the 90% confidence interval. Any significant effects in the model, other than
 987 Subject(Seq), should be investigated. The intrasubject and intersubject CVs should also be
 988 calculated.

989 Table A2-H: AUC_T (ng·h/mL) Analysis - Type3 Tests of Fixed Effects for $\ln(\text{AUC}_T)$

Effects	Numerator df	Denominator df	F Value	Prob > F*
Seq	1	14	0.09	0.7699
Period	1	14	0.33	0.5751
Form	1	14	1.88	0.1916

994 * p-value

995 Table A2-I: AUC_T (ng·h/mL) Analysis - Variance Estimates for $\ln(\text{AUC}_T)$

Parameter	Variance	CV
Subject(Seq)	0.2648	55.0665
Residual	0.0729	27.5136

999 Intrasubject CV = $100 \times (\text{MSResidual})^{0.5} = 100 \times (0.0729)^{0.5} = 27$ percent

1000 Intersubject CV = $100 \times (\text{MSSubject (Seq)})^{0.5} = 100 \times (0.2648)^{0.5} = 51.45$ percent

1001 The AUC ratio estimate and its 90% confidence interval are derived in the calculations
 1002 shown in Table A2-J. Because this study had a balanced design (i.e., an equal number of subjects
 1003 per sequence) the difference is simply the difference in the arithmetic means of the $\ln(\text{AUC})$ s. If
 1004 the study was not balanced, then the least-squares mean estimate for each formulation should be
 1005 used to form this difference, together with the appropriate standard error.

1006 Table A2-J: AUC_T (ng·h/mL) Analysis - Calculations

1007
$$\text{Difference} = \text{Test } \bar{x} - \text{Reference } \bar{x} = 5.39 - 5.52 = -0.13$$

1008
$$\text{SE}_{\text{Difference}} = (2\text{MSResidual}/n)^{0.5} = (2 \times 0.0729/16)^{0.5} = 0.0955$$

1009
$$\text{AUC Ratio} = 100 \times e^{\text{Difference}} = 100 \times e^{(5.39-5.52)} = 88\%$$

1010 90% Confidence Limits

1011 Lower, Upper = $100 \times e^{(\text{Difference} \pm t_{0.05,14} \times \text{SE}_{\text{Difference}})}$

$$\text{Lower} = 100 \times e^{(-0.13 - 1.761 \times 0.0955)} = 74\%$$

$$\text{Upper} = 100 \times e^{(-0.13 + 1.761 \times 0.0955)} = 104\%$$

1012 **A2.6 Maximum Observed Concentration (C_{max}) Analysis**1013 The necessary information and summary for the analyses of C_{max} is shown in Table A2-J.1014 Table A2-K: C_{max} (ng/mL) Analysis - Data

ID	Raw Scale			Log Scale	
	Test C_{max}	Reference C_{max}	Relative C_{max} (%)	Test $\ln(C_{max})$	Reference $\ln(C_{max})$
A	122	126	97	4.81	4.84
B	102	207	49	4.62	5.33
C	202	123	164	5.31	4.81
E	59	37	160	4.09	3.62
F	66	85	78	4.20	4.44
G	54	55	98	3.99	4.01
H	101	219	46	4.61	5.39
I	90	182	49	4.49	5.20
K	155	60	259	5.04	4.09
L	57	26	223	4.04	3.24
M	23	57	41	3.14	4.04
N	38	47	80	3.63	3.85
O	43	71	61	3.77	4.26
P	68	97	70	4.22	4.58
Q	28	88	31	3.32	4.48
R	60	99	61	4.10	4.59
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MEAN	79	99	98	4.21	4.42
STD	48	59	68	0.59	0.61
CV	61	60	69	-	-

1038 Table A2-L: C_{max} (ng/mL) Analysis - Type3 Tests of Fixed Effects for $\ln(C_{max})$

Effects	Numerator df	Denominator df	F Value	Prob > F*
Seq	1	14	1.02	0.3306
Period	1	14	0.13	0.7264
Form	1	14	1.77	0.2052

1043 * p-value

1044 Table A2-M: C_{max} (ng·h/mL) Analysis - Variance Estimates for $\ln(C_{max})$

Parameter	Variance	CV
Subject(Seq)	0.161	41.7977
Residual	0.2048	47.6698

1048 Intrасubject CV = $100 \times (\text{MSResidual})^{0.5} = 100 \times (0.2048)^{0.5} = 45.25$ percent

1049 Intersubject CV = $100 \times (\text{MSSubject (Seq)})^{0.5} = 100 \times (0.1610)^{0.5} = 40.12$ percent

1050 Table A2-N: C_{max} Analysis - Calculations

1051 Difference = Test \bar{x} - Reference \bar{x} = 4.21 - 4.42 = -0.21

1052 $SE_{\text{Difference}} = (2\text{MSResidual}/N)^{0.05} = 0.1600$

1053 C_{max} Ratio = $100 \times e^{\text{Difference}} = 100 \times e^{(4.21 - 4.42)} = 81\%$

1054 90% Confidence Limits

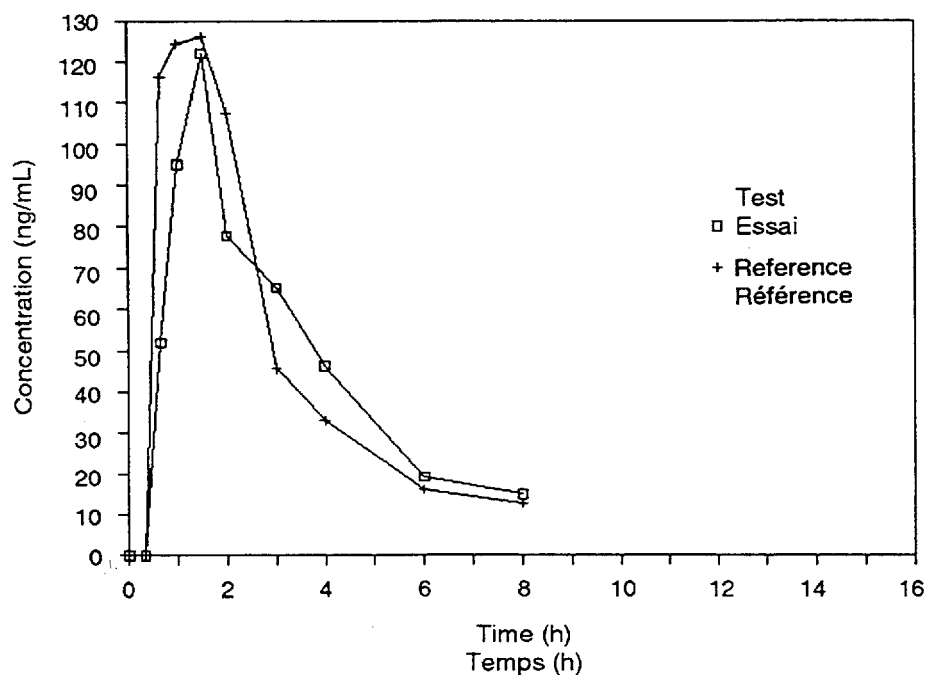
1055 Lower, Upper = $100 \times e^{(\text{Difference} \pm t_{0.05,14} \times SE_{\text{Difference}})}$

Lower = $100 \times e^{(-0.21 - 1.761 \times 0.1600)} = 61\%$

Upper = $100 \times e^{(-0.21 + 1.761 \times 0.1600)} = 107\%$

1056 **A2.7 Concentration versus Time Profiles (Subject A)**

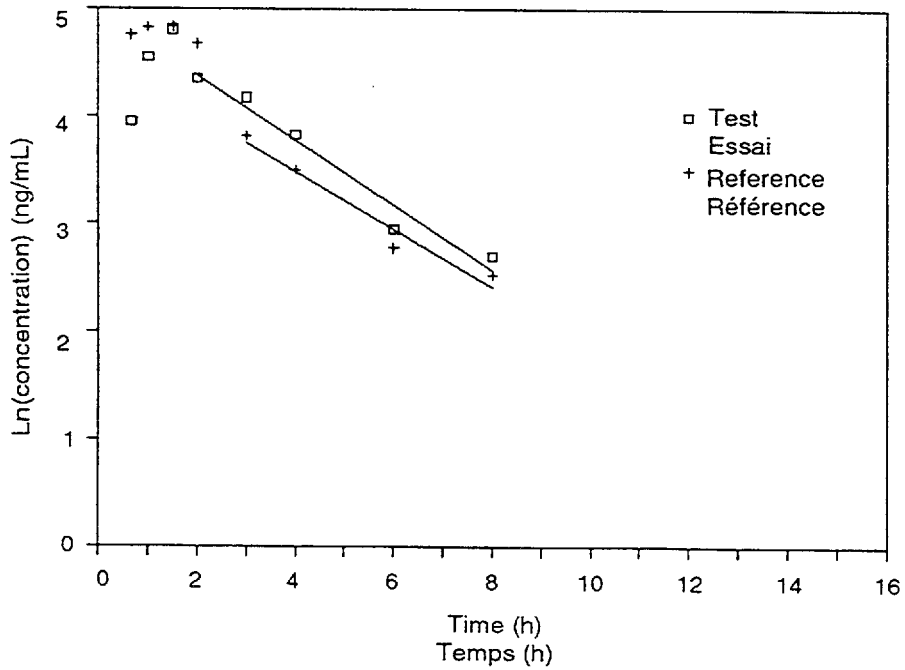
1057 Figure 1 shows a plot of the concentration versus time profile for subject A. Each plot should
1058 include profiles for all formulations given to that subject. Similar profiles should be given for
1059 each subject.



1060 Figure 1: Concentration-Time Profile for Subject A

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Figure 2 gives a plot of the $\ln(\text{concentration})$ versus time profile for subject A. This plot should contain the regression lines from which the terminal disposition rate constants (λ) were estimated. This line should start and end at the time points considered to be in the log-linear elimination phase. Any point that was not used to estimate the regression line should be identified.

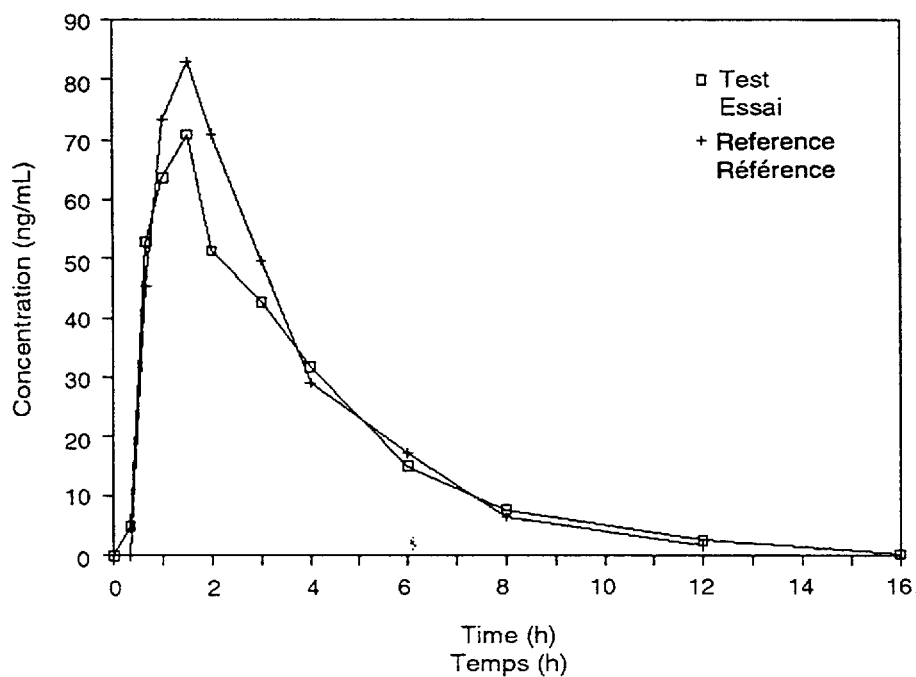


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Figure .2: Ln (concentration) - Time Profile for Subject A

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Figure 3 shows a profile of the arithmetic means over all subjects for each formulation and sampling time.

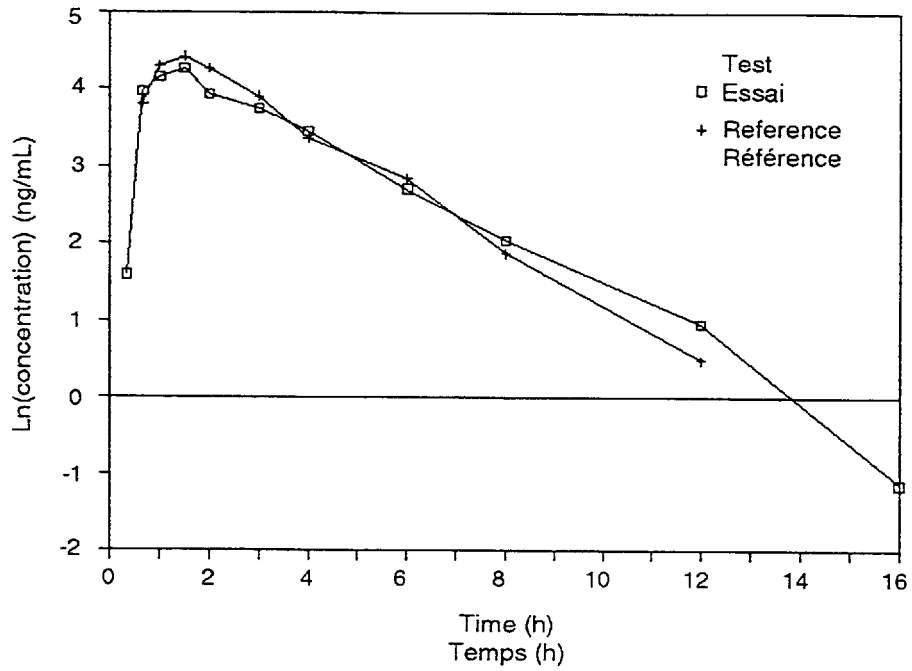


1069

Figure 3: Average Concentration-Time Profile for All Subjects

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Figure 4 shows a profile of the ln (arithmetic means) over all subjects for each formulation and sampling time.



1072

Figure 4: Ln(average concentration)-Time Profile for All Subjects

1073 **Appendix 3 Glossary of Terms**

1074 **Accuracy** - The extent to which an experimentally determined value agrees with the true or
1075 absolute value.

1076 **Adverse event** - Any untoward medical occurrence in a patient or clinical investigation subject
1077 administered a pharmaceutical product and which does not necessarily have to have a casual
1078 relationship with this treatment.

1079 **AUC (area under the curve)** - The area under the concentration versus time curve. The AUC
1080 symbol may be qualified by a specific time (e.g., 8 hours, or AUC_8), time of last quantifiable
1081 concentration (AUC_T), or infinity (AUC_I).

1082 **AUC_I (AUC to infinity)** - The area obtained by extrapolating to infinity the AUC_T . This can be
1083 calculated by adding C_T/λ to AUC_T where C_T is the estimated last quantifiable concentration and
1084 λ is the terminal disposition rate constant.

1085 **AUC ratio** - The ratio of geometric means of the test and reference AUCs. It is calculated as the
1086 antilogarithm of the difference between the means of the logarithms (ln) of the test and reference
1087 AUCs. The C_{max} ratio should be similarly calculated.

1088 **AUC_T (AUC to the last quantifiable concentration)** - This describes the AUC to the time of
1089 the last quantifiable concentration. AUC_T is calculated from observed data at specific time points
1090 by the linear trapezoidal rule.

1091 **AUC_τ (AUC over a dosing interval)** - Area under the concentration versus time curve, over the
1092 dosing interval in a multiple-dose study, calculated using the linear trapezoidal rule

1093 **Balanced cross-over design** - A cross-over design in which subjects are randomly assigned into
1094 each sequence in equal numbers.

1095 **Bioavailability** - The rate and extent of absorption of a drug into the systemic circulation.

1096 **Bioequivalence** - A high degree of similarity in the bioavailabilities of two pharmaceutical
1097 products (of the same galenic form) from the same molar dose, that are unlikely to produce
1098 clinically relevant differences in therapeutic effects, or adverse effects, or both.

1099 Bioequivalent means that test and reference products containing an identical drug or drugs, after
1100 comparison in an appropriate bioavailability study, were found to meet the standards for rate and
1101 extent of absorption specified in this guideline.

1102 **C_{max} (maximum observed concentration)** - The observed maximum or peak concentration.

1103 **C_{min} (minimum observed concentration)** - The observed minimum concentration.

- 1104 **C_{PD} (pre-dose concentration)** - Pre-dose concentration from same time of each day.
- 1105 **C_T (last quantifiable concentration)** - The last concentration that can be quantified and is equal
1106 to or greater than the lowest limit of quantitation.
- 1107 **Dropout** - A subject in a clinical trial who for any reason fails to continue in the trial until the
1108 last visit required of him/her by the study protocol.
- 1109 **Excipient** - Any ingredient, excluding the drug substances, incorporated in a formulation for the
1110 purpose of enhancing stability, usefulness or elegance, or facilitating preparation; for example,
1111 base, carrier, coating, colour, flavour, preservative, stabilizer, and vehicle.
- 1112 **Fluctuation** - Fluctuation between maximum and minimum concentrations within a dosing
1113 interval in a multiple-dose study, calculated as $(C_{\max} - C_{\min}) / (AUC_{\tau} / \tau) \times 100$.
- 1114 **Formulation** - An ingredient or mixture of specific ingredients; that is, drug substances and
1115 excipients in specific amounts, defining a given product.
- 1116 **Label** - Includes any legend, word, or mark attached to, included in, belonging to, or
1117 accompanying any drug or package. (Section 2 of the *Food and Drugs Act*.)
- 1118 **Last quantifiable concentration (C_T)** - See C_T.
- 1119 **Lowest limit of detection (LOD)** - The lowest concentration that can be differentiated from
1120 background levels.
- 1121 **Lowest limit of quantitation (LOQ)** - The lowest measured concentration on the standard curve
1122 having an acceptable degree of precision. The LOQ cannot be below the lowest nominal
1123 concentration on the same standard curve.
- 1124 **Maximum observed concentration (C_{max})** - See C_{max}.
- 1125 **Measured content of the drug product** - The drug contents of representative samples (i.e., the
1126 lots used in the bioavailability/bioequivalence study) of the test and reference drug products
1127 established as percent label claim by an appropriate assay, such as USP.
- 1128 **Modified-release dosage form** - A dosage form for which the drug-release characteristics of
1129 time-course or drug-release location are chosen to accomplish therapeutic or convenience
1130 objectives not offered by conventional dosage forms.
- 1131 Modified-release dosage forms are drug formulations that differ from conventional formulations
1132 in the rate at which the drug is released. For the purpose of these guidances, modified-release
1133 forms include formulations designed to meet one or more of the following objectives:

- 1134 - To delay disintegration, de-aggregation, or dissolution so that the drug's rate of
- 1135 degradation is altered.
- 1136 - To delay or decrease the rate of absorption so that the likelihood of gastrointestinal or
- 1137 other adverse effects is diminished (e.g., enteric-coated forms).
- 1138 - To provide effective drug concentrations for a longer period of time after a single dose.
- 1139 - To deliver the drug initially at a rate similar to that obtained with the conventional form,
- 1140 and to provide effective drug concentrations for a longer period of time.
- 1141 - To minimize fluctuations in drug concentrations during the dosing interval.
- 1142 - To provide, after single administration, multiple peaks and troughs in the serum
- 1143 concentration-time curves similar to those achieved after repeated dosing with the
- 1144 conventional formulation.

1145 **90% Confidence interval** - An interval about the estimated value that provides 90 percent
1146 assurance that it contains the true value. The method of constructing the interval is described in
1147 Appendix 2, "Sample Analysis for a Comparative Bioavailability Study").

1148 **Non-linear kinetics** - A general term referring to dose or time dependency in pharmacokinetic
1149 parameters arising from factors associated with absorption, first-pass metabolism, binding, and
1150 excretion.

1151 **Precision** - The closeness of agreement of values obtained in the analysis of replicate samples of
1152 the same specimen, usually indicated by the coefficient of variation (relative standard deviation).

1153 **Pro-drug** - An inactive (or much less active) precursor that is bio-transformed to the active drug.

1154 **Rate of absorption**- The rate at which a drug reaches the systemic circulation after oral
1155 administration.

1156 **Standard meal** - A meal of known carbohydrate, protein, fat, and fluid composition.

1157 **Terminal disposition rate constant (λ)** - The rate constant estimated from the slope of the
1158 terminal portion of the ln (drug concentration) versus time curve. The terminal half-life ($t_{1/2}$) is
1159 calculated from this constant ($t_{1/2} = \ln 2 / \lambda$). (Also known as Terminal Elimination Rate Constant.)

1160 **Terminal elimination rate constant** - See Terminal Disposition Rate
1161 Constant (λ).

1162 **Time of maximum observed concentration (t_{max})** - The time after administration of the drug at
1163 which C_{max} is observed.