January 25, 2010

Notice

Our file number: 10-100644-454

Health Canada is pleased to announce the release of two draft guidance documents, entitled *Conduct and Analysis of Comparative Bioavailability Studies* and *Comparative Bioavailability Standards: Formulations used for Systemic Effects*, for stakeholder comment.

The purpose of these documents is to update and consolidate eleven existing Health Canada documents related to the conduct and analysis of comparative bioavailability studies and the standards to be met in those studies in order to comply with Sections C.08.002(2)(h), C.08.002.1(2)(c)(ii) and C.08.003(3) of the *Food and Drug Regulations*. Please note, however, until such time as these guidances are finalized and published, current bioequivalence requirements remain unchanged and proposals in the draft guidances are not to be implemented.

The existing documents which will be superseded, once the two draft documents are finalized, are as follows:

1. Guidance for Industry: Conduct and Analysis of Bioavailability and Bioequivalence Studies - Part A: Oral Dosage Formulations Used for Systemic Effects (1992).

2. Report C (of the Expert Advisory Committee on Bioavailability and Bioequivalence): Report on Bioavailability of Oral Dosage Formulations, Not in Modified Release Form, of Drugs Used for Systemic Effects, Having Complicated or Variable Pharmacokinetics (1992).

3. Guidance for Industry: Conduct and Analysis of Bioavailability and Bioequivalence Studies - Part B: Oral Modified Release Formulations (1996).

4. Draft Policy: Bioequivalence Requirements: Drugs Exhibiting Non-Linear Pharmacokinetics (2003).

5. Notice to industry: Removal of Requirement for 15% Random Replicate Samples (2003).

6. Draft Guidance for Industry: Use of Metabolite Data in Comparative Bioavailability Studies (2004).

7. Notice to industry: Bioequivalence requirements for combination drug products (2004).

8. Guidance for Industry: Bioequivalence Requirements: Comparative Bioavailability Studies Conducted in the Fed State (2005).

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9. Notice to Industry: Bioequivalence Requirements for Drugs for Which an Early Time of Onset or Rapid Rate of Absorption Is Important (rapid onset drugs) (2005).

10. Notice to Industry: Bioequivalence Requirements for Long Half-life Drugs (2005).

11. Guidance for Industry: Bioequivalence Requirements: Critical Dose Drugs (2006).

Please note, however, that Section 2.6: *Analytical Methodology* in the draft document *Conduct and Analysis of Comparative Bioavailability Studies*, is currently still under revision and further consultation will be undertaken, as appropriate. We invite stakeholders to provide advance recommendations on analytical methodology, particularly assay validation. These recommendations will be taken into consideration in revising this section.

Comments should be provided to Health Canada, preferably in electronic format using the attached template, within 60 days of the publication of this Notice.

Comments or requests for an electronic copy of the guidances should be directed to:

Bureau of Policy, Science and International Programs
Therapeutic Products Directorate
Health Canada
1600 Scott Street
Holland Cross, Tower B
2nd Floor, Address Locator 3102C5
Ottawa, Ontario
K1A 0K9

Telephone: 613-948-4623
Facsimile: 613-941-1812
E-mail: Policy_Bureau_Enquiries@hc-sc.gc.ca

Stakeholder Feedback on
Draft Guidance Documents
Conduct and Analysis of Comparative Bioavailability Studies
Published for External Consultation on January 25, 2010

Comments submitted by: <full name>, <company/association name (if applicable)>
Telephone number: <telephone number>
Address: <full mailing address>
Email: <email address>
Date: <date of comment submission>

Comment #	Section / Line #*	Comment and Rationale	Proposed Revised Text
1			
2			
3			
4			
5			
etc.			

*Please refer to the Adobe (PDF) version of the document to ensure accuracy in line numbers.

Health Canada Santé Canada

DRAFT GUIDANCE DOCUMENT

Conduct and Analysis of Comparative Bioavailability Studies

This guidance document is being distributed for comment purposes only.

Published by authority of the
Minister of Health

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Health Products and Food Branch

Canada

Our mission is to help the people of Canada maintain and improve their health. *Health Canada*	The Health Products and Food Branch's mandate is to take an integrated approach to management of the risks and benefits to health related products and food by: • minimizing health risk factors to Canadians while maximizing the safety provided by the regulatory system for health products and food; and, • promoting conditions that enable Canadians to make healthy choices and providing information so that they can make informed decisions about their health. *Health Products and Food Branch*

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

FOREWORD

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

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

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

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

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1 INTRODUCTION

1.1 Policy Objectives

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

1.2 Policy Statements

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

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

1.3 Scope and Application

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

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

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

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

1.4 Background

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

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

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

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

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

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

2 GUIDANCE FOR IMPLEMENTATION

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

2.1 Planning a Bioavailability Study

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

2.1.1 Study Objectives

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

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

2.2 Selection of Subjects for a Study

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

2.2.1 Choice of Subjects

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

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

2.2.2 Inclusion / Exclusion Criteria

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

a) Age

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

b) Height/weight ratio

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

c) Health

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

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

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

d) Safety

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

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

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

2.3 Study Design

2.3.1 Parallel versus Cross-over

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

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

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

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

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

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

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

2.3.1.1 Number of Subjects

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

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

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

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

2.3.2 Other Strategies for Collecting Data

2.3.2.1 Add-ons

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

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

2.3.2.2 Sequential designs

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

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

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

2.3.2.3 Adaptive designs

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

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

2.3.3 Accounting for Drop-outs and Withdrawals

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

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

2.3.4 Outlier consideration

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

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

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

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

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

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

2.4 Study Conduct

2.4.1 Standardization

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

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

2.4.2 Blinding

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

2.4.3 Administration of Food and Fluid

2.4.3.1 Fasted study

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

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

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

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

2.4.3.2 Fed Study

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

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

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

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

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

2.4.3.3 Steady-state Studies

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

2.4.4 Posture and Physical Activity

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

2.4.5 Interval Between Doses

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

2.4.6 Sampling Times

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

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

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

a) peak concentration of the drug in the blood (C_{max});

b) the area under the concentration time curve (AUC_T) is at least 80 percent of the known AUC_I; and

c) the terminal disposition rate constant of the drug.

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

2.4.7 Sample Collection

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

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

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

2.4.8 Handling of Samples

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

2.4.9 Identification of Adverse Events

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

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

2.5 Test and Reference Drug Products

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

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

2.5.1 Chemistry

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

2.5.2 Dosage and Strength

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

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

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

2.5.3 Selection of Reference Product

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

In bioequivalence studies, the Canadian reference product is:

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

2.6 Analytical Methodology

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

2.6.1 Drug and Drug Metabolites

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

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

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

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

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

2.6.2 Assay Methodology

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

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

2.6.3 Stability

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

2.6.4 Limit of Quantitation (LOQ)

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

2.6.5 Specificity

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

2.6.6 Recovery

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

2.6.7 Standard Curves

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

2.6.8 Precision and Accuracy

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

2.6.9 Quality Control for Spiked Samples

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

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

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

2.6.10 Aberrant Values (Repeat Assays)

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

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

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

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

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

2.7 Analysis of Data

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

2.7.1 Presentation of Data

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

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

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

2.7.2 Pharmacokinetic Parameters

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

a) AUC_T
Area under the concentration-time curve measured to the last quantifiable concentration, using the trapezoidal rule.

b) AUC_I
AUC_T plus additional area extrapolated to infinity, calculated using λ.

c) AUC_T/AUC_I
The ratio of AUC_T to AUC_I.

d) C_{max}
Maximum observed concentration.

e) t_{max}
Observed time after dosing, at which C_{max} occurred.

f) λ
Terminal disposition rate constant.

g) $T_{1/2}$
Terminal elimination half-life.

Where the time to onset of action is important the following parameter should also be reported:

h) $AUC_{RefTmax}$
Area under the curve to the time of the maximum concentration of the reference product, calculated for each study subject.

Where multiple dose studies are conducted, the following parameters should also be reported:

i) C_{min}
Minimum observed concentration.

j) C_{pd}
Pre-dose concentrations determined immediately before a dose at steady state.

k) AUC_{τ}
Area under the concentration versus time curve, over the dosing interval of the test formulation, calculated using the linear trapezoidal rule.

l) Fluctuation
$(C_{max} - C_{min}) / (AUC_{\tau}/\tau) \times 100$.

Where comparative bioavailability is based upon urine data, the following parameters should be reported:

m) Ae_{0-T}
Cumulative amount of drug excreted to last sampling time.

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

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

2.7.3 Data Collection

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

2.7.4 Statistical Analysis

2.7.4.1 Outlier analysis

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

2.7.4.2 Model Fitting

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

2.7.4.3 Testing of fixed effects

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

2.7.4.4 Estimation of random effects

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.

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.

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:

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

b) testing and estimates for fixed and random effects;

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

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

Appendices

Appendix 1 Number of Subjects

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

To use the table:

a) Obtain an estimate of the intrasubject CV from the literature.

b) Choose table A1-A or A1-B depending on the bioequivalence interval required.

c) Choose the power required (80% or 90%).

d) Choose an expected true ratio of test over reference means (usually 100%, but consider potency differences between the test and reference products).

e) Go down the column until you arrive at the rounded CV. The number is the sample size.

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

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

Table A1-A. Sample Sizes for 2x2 Crossover Design for Interval Hypotheses for 80-125% Rule to Attain a Power of 80 and 90%, Respectively in the Case of the Multiplicative Model (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	40	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								

Table A1-B. Sample Sizes for 2x2 Crossover Design for Interval Hypotheses for 90-112% Rule to Attain a Power of 80 and 90%, Respectively in the Case of the Multiplicative Model (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	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

Table A1-C. Sample Sizes for Parallel Design for Interval Hypotheses for 80-125% Rule to Attain a Power of 80 and 90%, Respectively in the Case of the Multiplicative Model (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	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								

Table A1-D. Sample Sizes for Parallel Design for Interval Hypotheses for 90-112% Rule to Attain a Power of 80 and 90%, Respectively in the Case of the Multiplicative Model (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

Appendix 2 Sample Analysis for a Comparative Bioavailability Study

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

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

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

a) A randomization scheme for the design, where all subjects randomized into the study are included and identified by code, sequence, and dates of the dosing periods for both test and reference formulations (see Section A2.1.).

b) A summary of drug concentrations (graphic and quantitative) at each sampling time for each subject for both test and reference formulations (see Section A2.2.).

c) A summary of the estimates of the parameters as defined in Section A2.3 for both test and reference formulations, including the means, standard deviations, and CVs (see Section A2.4.).

d) A formal statistical analysis of the relevant parameters with comparisons of the test formulations to the reference formulations (see Sections A2.5 through A2.9.).

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

A2.1 Randomization Scheme of the Design

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

A2.2 Summary of Drug Concentrations

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

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

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

Subject			Period	
Number	ID	Sequence	May 14, 2008	May 21, 2008
001	A	TR	T	R
002	B	RT	R	T
003	C	RT	R	T
004*	D	TR	T	-
005	E	TR	T	R
006	F	RT	R	T
007	G	TR	T	R
008	H	RT	R	T
009	T	TR	T	R
010**	I	RT	-	-
011	K	RT	R	T
012	L	TR	T	R
013	M	TR	T	R
014	N	RT	R	T
015	O	RT	R	T
016	P	TR	T	R
017	Q	RT	R	T
018	R	TR	T	R
*	Subject did not appear for second period.			
**	Subject did not appear for either period.			

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
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*
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*
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*
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*
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*
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*
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*
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*
K	RT	21 May	0.00	BQL*	79.34	99.41	154.80	58.60	57.12	32.57	19.82	BQL*	BQL*	BQL*
L	TR	14 May	0.00	14.78	55.54	56.88	46.87	37.29	28.75	25.20	BQL*	BQL*	BQL*	BQL*
M	TR	14 May	0.00	BQL*	BQL*	BQL*	BQL*	BQL*	8.37	23.15	19.74	16.49	5.74	5.18
N	RT	21 May	0.00	BQL*	37.76	28.58	21.56	19.02	13.25	12.44	6.38	BQL*	BQL*	BQL*
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*
P	TR	14 May	0.00	BQL*	68.25	52.57	51.97	28.64	23.70	12.74	BQL*	BQL*	BQL*	BQL*
Q	RT	21 May	0.00	BQL*	5.90	13.00	27.54	13.32	12.34	9.81	9.73	BQL*	BQL*	BQL*
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*
. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
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
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
CV	-	-	-	228.66	89.09	70.72	70.22	65.66	57.79	48.51	57.18	84.94	169.84	400
*	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.													

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
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*
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*
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*
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*
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*
G	TR	21 May	0.00	BQL*	6.89	50.04	55.27	51.68	38.58	26.19	7.79	BQL*	BQL*	BQL*
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*
I	TR	21 May	0.00	BQL*	181.90	135.80	96.51	90.50	62.58	30.43	18.50	BQL*	BQL*	BQL*
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*
L	TR	21 May	0.00	BQL*	14.29	21.32	24.32	25.56	25.51	10.49	5.49	BQL*	BQL*	BQL*
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*
N	RT	14 May	0.00	BQL*	47.20	34.90	34.90	24.19	20.11	8.08	7.27	BQL*	BQL*	BQL*
O	RT	14 May	0.00	BQL*	BQL*	20.35	70.88	70.60	70.38	40.51	26.93	8.20	BQL*	BQL*
P	TR	21 May	0.00	BQL*	39.23	86.29	97.46	52.26	40.53	26.74	12.54	BQL*	BQL*	BQL*
Q	RT	14 May	0.00	BQL*	BQL*	30.86	88.38	37.67	29.28	14.99	6.38	BQL*	BQL*	BQL*
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*
. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
MEAN	-	-	0.00	-	45.33	73.28	82.85	70.94	49.62	29.09	17.19	6.49	1.64	-
STD	-	-	0.00	-	53.30	54.49	46.24	39.78	22.51	12.88	8.83	5.98	2.96	-
CV	-	-	-	-	117.59	74.37	55.82	56.08	45.37	44.28	51.38	92.23	180.73	-
*	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.													

A2.3 List of Parameters and Definitions

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

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_I	Area to infinity = $AUC_T + C_T/\lambda$ where C_T is the estimated concentration at LQCT (ng·h/mL).
$\frac{AUC_T}{AUC_I} \times 100$	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).
$t_{½}$	Drug half-life = $ln_2/\lambda = 0.693/\lambda$ (h).

A2.4 Summaries of Parameter Estimates

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

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

ID	Seq	Period	TEST FORMULATIONS								
			C_{max} (ng/mL)	t_{max} (h)	AUC_T (ng·h/mL)	AUC_I (ng·h/mL)	AUC_T (%)	λ (h^{-1})	TLIN (h)	LQCT (h)	$t_{½}$ (h)
A	TR	14 May	122	1.50	365	409	89	0.3002	2.0	8.0	2.3
B	RT	21 May	102	1.50	405	432	94	0.2384	3.0	12.0	2.9
C	RT	21 May	202	0.66	703	774	91	0.1776	4.0	12.0	3.9
E	TR	14 May	59	3.00	233	256	91	0.3680	3.0	8.0	1.9
F	RT	21 May	66	1.00	247	265	93	0.3902	3.0	8.0	1.8
G	TR	14 May	54	1.50	178	205	87	0.2768	3.0	8.0	2.5
H	RT	21 May	101	1.00	246	263	94	0.3437	2.0	8.0	2.0
I	TR	14 May	90	1.50	408	433	94	0.2486	3.0	12.0	2.8
K	RT	21 May	155	1.50	315	372	85	0.3379	3.0	6.0	2.1
L	TR	14 May	57	1.00	140	331	42	0.1318	3.0	4.0	5.3
M	TR	14 May	23	4.00	165	195	85	0.1485	6.0	16.0	4.7
N	RT	21 May	38	0.66	88	113	78	0.2620	2.0	6.0	2.6
O	RT	21 May	43	1.00	183	215	85	0.2671	3.0	8.0	2.6
P	TR	14 May	68	0.66	122	148	83	0.5031	1.5	4.0	1.4
Q	RT	21 May	28	1.50	68	113	60	0.1833	1.5	6.0	3.8
R	TR	14 May	60	2.00	275	292	94	0.2546	3.0	12.0	2.7
. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
MEAN*	-	-	79	1.50	259	301	84	0.2770	3.0	8.0	2.8
STD	-	-	48	0.89	158	164	14	0.0967	1.1	3.3	1.1
CV	-	-	61	59.35	61	54	17	34.92	37.3	38.5	37.9
*	for t_{max}, TLIN, and LQCT, these are medians.										

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)	$t_{½}$ (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
.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.
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

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

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

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

ID	Raw Scale			Log Scale	
	Test AUCT	Reference AUCT	Relative AUCT (%)	Test ln(AUCT)	Reference ln(AUCT)
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
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
MEAN	259	281	94	5.39	5.52
STD	158	136	35	0.61	0.52
CV	61	48	37	-	-

Table A2-H gives the analysis of variance (ANOVA) for the cross-over design model for ln(AUC_T). This analysis gives the appropriate intrasubject variance estimate, MS (Residual), for the calculation of the 90% confidence interval. Any significant effects in the model, other than Subject(Seq), should be investigated. The intrasubject and intersubject CVs should also be calculated.

Table A2-H: AUC_T (ng·h/mL) Analysis - Type3 Tests of Fixed Effects for ln(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

* p-value

Table A2-I: AUC_T (ng·h/mL) Analysis - Variance Estimates for ln(AUC_T)

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

Intrasubject CV = 100 x $(MSResidual)^{0.5}$ = 100 x $(0.0729)^{0.5}$ = 27 percent
Intersubject CV = 100 x $(MSSubject\ (Seq)\ )^{0.5}$ = 100 x $(0.2648)^{0.5}$ = 51.45 percent

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

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

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

$SE_{Difference} = (2MSResidual/n)^{0.5} = (2 \times 0.0729/16)^{0.5} = 0.0955$

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

90% Confidence Limits

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

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

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

A2.6 Maximum Observed Concentration (C_{max}) Analysis

The necessary information and summary for the analyses of C_{max} is shown in Table A2-J.

Table A2-K: C_{max} (ng/mL) Analysis - Data

ID	Raw Scale			Log Scale	
	Test Cmax	Reference Cmax	Relative Cmax (%)	Test ln(Cmax)	Reference ln(Cmax)
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
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
MEAN	79	99	98	4.21	4.42
STD	48	59	68	0.59	0.61
CV	61	60	69	-	-

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

* p-value

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

Intrasubject CV = 100 x $(MSResidual)^{0.5}$ = 100 x $(0.2048)^{0.5}$ = 45.25 percent
Intersubject CV = 100 x $(MSSubject\ (Seq)\ )^{0.5}$ = 100 x $(0.1610)^{0.5}$ = 40.12 percent

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

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

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

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

90% Confidence Limits

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

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

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

A2.7 Concentration versus Time Profiles (Subject A)

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

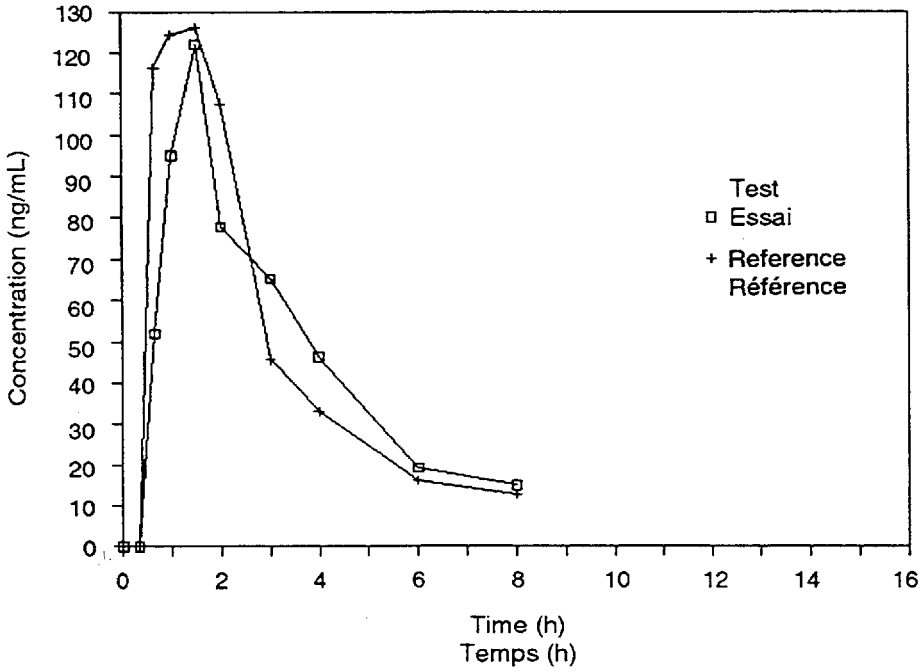

Figure 1: Concentration-Time Profile for Subject A

Figure 2 gives a plot of the ln (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.

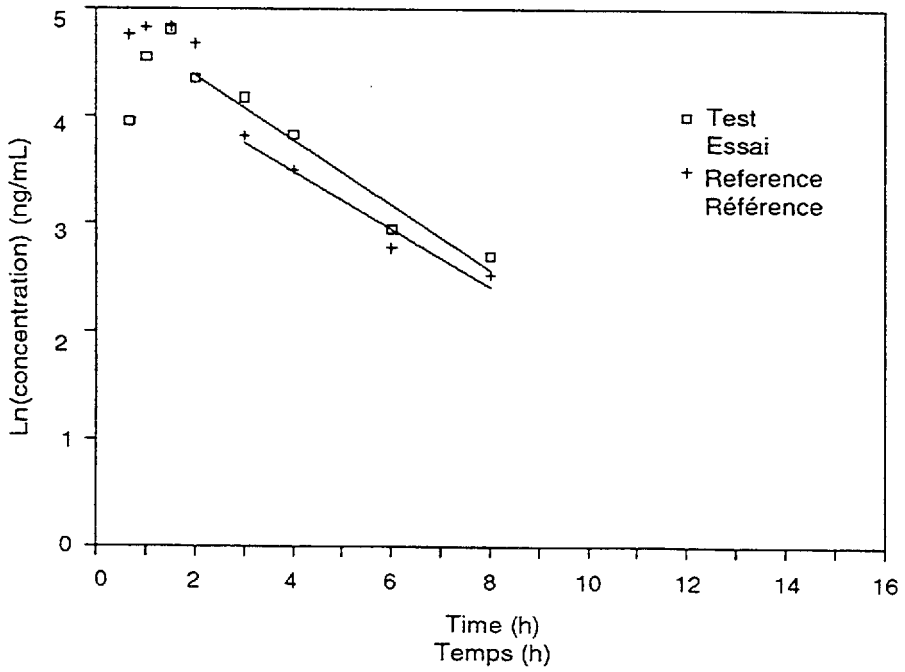

Figure .2: Ln (concentration) - Time Profile for Subject A

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

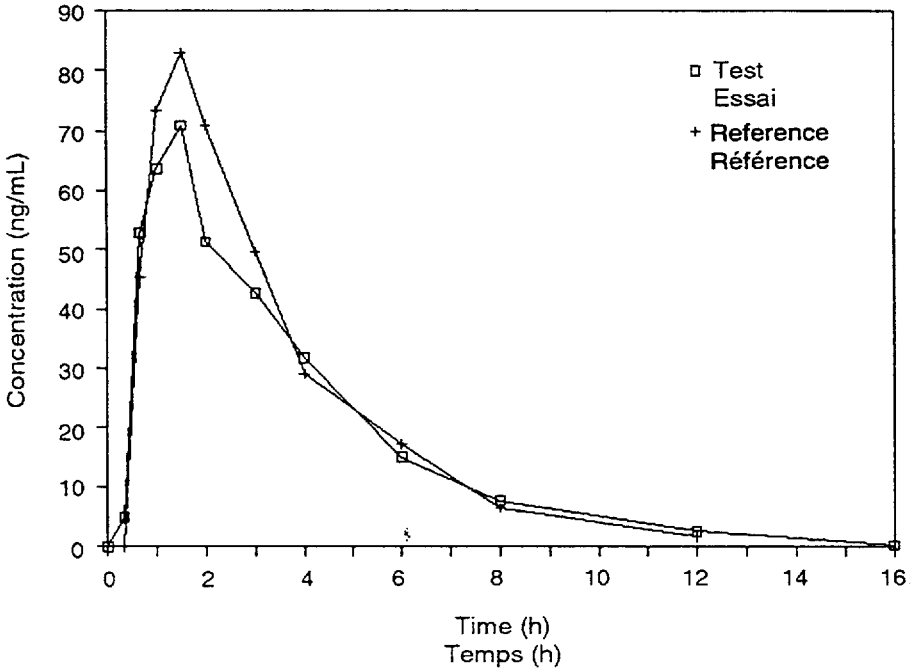

Figure 3: Average Concentration-Time Profile for All Subjects

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

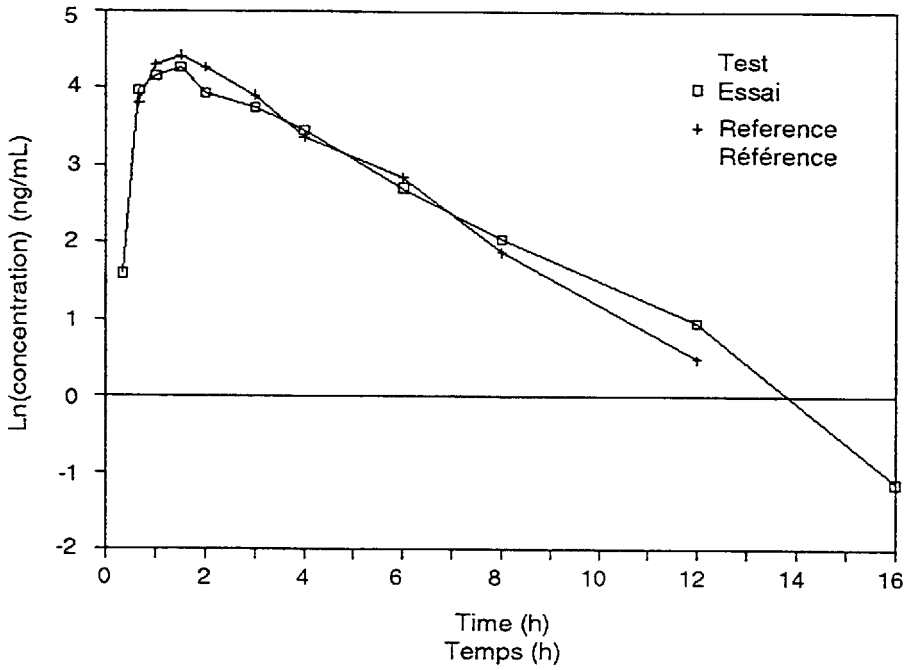

Figure 4: Ln(average concentration)-Time Profile for All Subjects

Appendix 3 Glossary of Terms

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

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

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

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

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

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

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

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

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

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

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

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

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

C_{PD} (pre-dose concentration) - Pre-dose concentration from same time of each day.

C_T (last quantifiable concentration) - The last concentration that can be quantified and is equal to or greater than the lowest limit of quantitation.

Dropout - A subject in a clinical trial who for any reason fails to continue in the trial until the last visit required of him/her by the study protocol.

Excipient - Any ingredient, excluding the drug substances, incorporated in a formulation for the purpose of enhancing stability, usefulness or elegance, or facilitating preparation; for example, base, carrier, coating, colour, flavour, preservative, stabilizer, and vehicle.

Fluctuation - Fluctuation between maximum and minimum concentrations within a dosing interval in a multiple-dose study, calculated as $(C_{max} - C_{min}) / (AUC_\tau/\tau) \times 100$.

Formulation - An ingredient or mixture of specific ingredients; that is, drug substances and excipients in specific amounts, defining a given product.

Label - Includes any legend, word, or mark attached to, included in, belonging to, or accompanying any drug or package. (Section 2 of the *Food and Drugs Act*.)

Last quantifiable concentration (C_T) - See C_T.

Lowest limit of detection(LOD) - The lowest concentration that can be differentiated from background levels.

Lowest limit of quantitation (LOQ) - The lowest measured concentration on the standard curve having an acceptable degree of precision. The LOQ cannot be below the lowest nominal concentration on the same standard curve.

Maximum observed concentration (C_{max}) - See C_{max}.

Measured content of the drug product - The drug contents of representative samples (i.e., the lots used in the bioavailability/bioequivalence study) of the test and reference drug products established as percent label claim by an appropriate assay, such as USP.

Modified-release dosage form - A dosage form for which the drug-release characteristics of time-course or drug-release location are chosen to accomplish therapeutic or convenience objectives not offered by conventional dosage forms.

Modified-release dosage forms are drug formulations that differ from conventional formulations in the rate at which the drug is released. For the purpose of these guidances, modified-release forms include formulations designed to meet one or more of the following objectives:

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

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

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

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

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

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

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

Terminal disposition rate constant (λ) - The rate constant estimated from the slope of the terminal portion of the ln (drug concentration) versus time curve. The terminal half-life ($t_{½}$) is calculated from this constant ($t_{½}=\ln 2/\lambda$). (Also known as Terminal Elimination Rate Constant.)

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

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