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GUIDANCE FOR INDUSTRY

Conduct and Analysis of Bioavailability and Bioequivalence Studies - Part B: Oral Modified Release Formulations

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FOREWORD

Guidance documents are meant to provide assistance to industry and health care professionals on **how** to comply with the policies and governing statutes and regulations. They also serve to provide review and compliance guidance to staff, thereby ensuring that mandates are implemented in a fair, consistent and effective manner.

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 scientific 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 guidance, 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 guidances.

TABLE OF CONTENTS

1 INTRODUCTION

This guidance document provides information about how to establish and conduct bioavailability studies for modified-release (MR) dosage forms of oral drugs that are used for systemic effects. The information contained in this guidance document deals with drugs that have *uncomplicated characteristics* (as described in *Conduct and Analysis of Bioavailability and Bioequivalence Studies: Part A—Oral Dosage Formulations Used for Systemic Effects*).

Separate guidances and standards are required for each of the circumstances in which an MR formulation might be developed. These circumstances fall into three groups:

- **Group I**—includes original MR dosage forms when the drug is not marketed in either a conventional-release or MR dosage form.
- **Group II**—includes first market entry MR dosage forms that are developed after a conventional-release or a different kind of MR formulation is marketed.
- **Group III**—includes second and subsequent market entry MR dosage forms that are developed to be bioequivalent to marketed MR dosage forms. This guidance mainly addresses Groups II and III.

Each of the remaining sections of this guidance document deals with a particular aspect of a bioavailability study:

- Section 2, “Types of Studies and Data Required for Approval”, covers the types of studies and data required for approval.
- Section 3, “Standards for Comparable Bioavailability”, describes the standards used for determining bioavailability.
- Section 4, “Standards for Bioequivalence”, describes the standards used for determining bioequivalence.
- Section 5, “Planning a Bioavailability Study”, presents the procedures to be followed during the planning stage of the study.
- Section 6, “Selection of Subjects for a Study”, discusses methods for choosing subjects for a study.
- Section 7, “Study Design and Environment”, describes the study design and the environment in which a study should be conducted.
- Section 8, “Test and Reference Drug Products”, discusses the dosages and strengths of test and reference drug products used in a study.
- Section 9, “Measurement Methodology”, explains the measuring of active substances, or metabolites, or both over time, as well as the validation requirements for analytical methods.
- Section 10, “Analysis of Data”, describes the analysis of recorded data.

- Section 11, "Sample Analysis for a Single-Dose Comparative Bioavailability Study", contains sample analyses and calculations of data from a single-dose study of comparable bioavailabilities for Group II formulations.
- Section 12, "Sample Analysis for a Multiple-Dose Comparative Bioavailability Study", contains sample analyses and calculations of data from a multiple-dose study of comparable bioavailabilities for Group II formulations.
- Section 13, "Sample Analysis for a Single-Dose Bioequivalence Study", contains sample analyses and calculations of data from a single-dose study of bioequivalence for Group III formulations.
- Section 14, "Sample Analysis for a Multi-Dose Bioequivalence Study", contains sample analyses and calculations of data from a multiple-dose study of bioequivalence for Group III formulations.
- Section 15, "Glossary of Terms", provides definitions of important terms.

Information about establishing and conducting bioavailability studies for *conventional* formulations of oral drugs is provided in the guidance document *Conduct and Analysis of Bioavailability and Bioequivalence Studies: Part A-Oral Dosage Formulations Used for Systemic Effects.*

1.1 Definition of Modified-Release 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, MR 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.

MR formulations require guidances and standards that differ from those for conventional drug formulations because:

- a greater likelihood exists that increased inter-subject variability in bioavailability will occur, including the possibility of dose-dumping;
- an increased risk of adverse effects such as gastrointestinal irritation may exist; depending on the site of drug release, or absorption, or both; and
- a possibility exists that accumulation may occur when the drug is given in repeated doses at the recommended dose intervals.

2 TYPES OF STUDIES AND DATA REQUIRED FOR APPROVAL

Bioavailability data must be obtained for all modified-release forms. However, the types of studies required and the pharmacokinetic parameters to be evaluated differ depending on the product. Factors to be considered include:

- Whether the drug product is the original entry of the chemical entity, or the first or second market entry of the modified-release formulation.
- The extent to which the drug accumulates after repeated dosing.
- The potential for adverse reactions-especially from drugs with a steep dose-response curve and those with potential organ toxicity.

Appropriate clinical studies must support claims for the effectiveness and safety of original and first market entry MR formulations (Groups I and II). For second and subsequent market entry MR preparations (Group III) that have been shown to be bioequivalent with the original or first market entry MR form, plasma concentrations alone may form the basis of an approval for the new product. Exemption from clinical trials may also be given for first market entry (Group II) MR products composed of certain non-prescription drugs; however, manufacturers should clear this approach with Health Canada (HC).

2.1 Delayed-Release Drug Products

In the case of enteric-coated drugs, comparative bioavailability can usually be demonstrated using the AUC and C_{max} requirements for uncomplicated drug formulations, provided that the only difference between the enteric-coated formulation and the corresponding immediate-release product is a time shift in the concentration-time curve (i.e., no other modification of release occurs). Studies must be carried out using both subjects that have fasted and those that have eaten an appropriate meal at a specified time

before taking the drug. The reference product for Group III is to be the innovator's enteric-coated product (or the market leader's enteric-coated product if there is no recognized innovator).

2.2 Market Entry

If the MR formulation is the original market entry of the chemical substance (Group I), selected pharmacokinetic parameters must be determined as part of demonstrating the product's efficacy and safety.

If the MR formulation is a first-entry product, and a conventional-release formulation is already marketed (Group II), then comparable bioavailability should be demonstrated. The studies should be generally pursued in the context of demonstrating the efficacy and safety of the recently-developed drug product and should use an appropriate reference formulation. The investigations should show that the product meets the standards outlined in (Section 3, "Standards for Comparable Bioavailability").

If the MR formulation is a second or subsequent market entry product (Group III), developed while marketed MR product(s) are already available, then bioequivalence studies using an appropriate reference product must be performed. The studies should show that the product meets the standards outlined in (Section 4, "Standards for Bioequivalence"). When the standards in (Section 4) are not met, full clinical studies are required to support claims as prescribed for Group II products.

2.3 Single-Dose Studies

For the assessment of first market entry and second market entry MR formulations, studies must be performed using single-dose administration, both in subjects that have fasted and in subjects that have eaten a meal standardized to challenge the formulation. The following pharmacokinetic parameters should be calculated from the concentrations in plasma (or blood or serum):

- AUC_X
- AUC_T
- AUC_I
- C_{max}
- T_{max}
- λ

2.4 Multiple-Dose Studies for Formulations Likely to Accumulate

For formulations that are likely to accumulate (i.e., $AUC_X/AUC_I < 0.8$), safety requires that steady-state studies be performed in addition to the single-dose studies. The following pharmacokinetic parameters should be calculated from the concentrations in plasma (or blood or serum):

- AUC_τ
- C_{max}
- T_{max}
- C_{pd}
- C_{min}
- fluctuation

Where the AUC_X/AUC_I ratio cannot be reliably determined, accumulation must be assumed to occur.

3 STANDARDS FOR COMPARABLE BIOAVAILABILITY (GROUP II DRUG PRODUCTS)

3.1 General Requirements

The standards described below must be met for parameters calculated from the observed concentrations, as well as those corrected for measured drug content.

The pharmacokinetic characteristics should support the claims of the manufacturer that appear on the label.

3.2 Single-Dose Studies

3.2.1 Design of Studies

A single dose study should be a comparison between a single dose of the first market entry MR formulation and the doses of the innovator's conventional formulation that the MR formulation is intended to replace. (The doses of the conventional formulation are administered according to the conventional dosing regimen.) When identical doses of conventional and MR formulations cannot be administered, a proportionality correction must be made for the calculation of relevant parameters.

One objective of these studies is to evaluate comparable bioavailability under fasting conditions. However, safety of the subjects may require that an investigation be conducted after the administration of a standardized meal to challenge the formulation. In this case, manufacturers should consult with Health Canada before undertaking the study.

Another objective of these studies is to compare the bioavailability of the test drug product observed under fed and fasting conditions. Such a comparison contributes to the appropriate labelling of the MR drug product.

These objectives can be achieved by applying one of the following design schemes:

a) A four-period cross-over trial with four complementary sequences of four treatment conditions. In the four treatment conditions, both the test and reference drug products should be assessed in the fasting state as well as following the administration of a standardized meal to challenge the formulation.

b) A three-period cross-over trial with three complementary sequences of treatment conditions. Both test and reference drug products should be assessed in the fasting state. In addition, the test formulation should be evaluated following the administration of a standardized meal to challenge the formulation.

c) Two cross-over trials, both with two periods and two sequences of drug product administration. One trial should compare the test and reference formulations in the fasting state. The other trial should evaluate the test drug product, both in the fasting state and following the administration of a standardized meal to challenge the formulation.

3.2.2. Relative Mean AUC

The relative mean measured AUC of the modified-release formulation to the conventional formulation should be between 80% and 125% in the fasting state.

The AUC may be evaluated by determining AUC_T, provided that AUC_T obtained by the linear trapezoidal rule is at least 80% of the extrapolated AUC_I (i.e., $AUC_T/AUC_I \geq 0.80$).

3.2.3 Relative Mean C_{max}

The relative mean measured C_{max} of a single dose of the modified-release formulation to the conventional formulation should not exceed 125% in the fasting state. The maximum concentration measured with a single dose of the MR formulation should be compared with the largest peak concentration recorded following repeated administration of the conventional formulation. For the conventional formulation, the same peak must be selected for all subjects.

In some cases, the intended use of the MR formulation may call for a modification of the above C_{max} criterion. In such cases, manufacturers should consult with Health Canada before undertaking the study.

3.3 Steady-State Studies for Formulations Used at a Dose Interval Likely to Lead to Accumulation ($AUC_X/AUC_I < 0.8$ for the MR Product)

3.3.1 Design of Studies

In addition to the single-dose studies described in the Section 3.2, a comparison should be made between the first market entry MR formulation and equivalent doses of the conventional formulation over the dosing interval (claimed for the MR product) at steady state.

Generally, steady-state studies should be performed under fasting conditions. However, safety of the subjects may require that an investigation be conducted after the administration of an appropriate meal at a specified time before taking the drug. In this case, manufacturers should consult with Health Canada before undertaking a study.

3.3.2 Relative Mean AUC_τ

The relative mean AUC_τ at steady state of the modified-release formulation to the conventional formulation should be between 80% and 125%.

3.3.3 Relative Mean C_{max}

The relative mean measured C_{max} at steady state of the modified-release formulation to the largest peak concentration of the conventional formulation should not exceed 125%.

4 STANDARDS FOR BIOEQUIVALENCE (GROUP III DRUG PRODUCTS)

4.1 General Requirements

The standards described below must be met for parameters calculated from the observed concentrations, as well as those corrected for measured drug content.

The pharmacokinetic characteristics should support the claims of the manufacturer that appear on the label.

Other standards may be required to compare the shapes of the plasma concentration versus time curves.

4.2 Single-Dose Studies

4.2.1 Design of Studies

A second or subsequent market entry MR formulation should be compared with the Group I or II MR product with which bioequivalence is claimed. Both formulations should be administered as single doses.

The objective of these studies is to evaluate the bioequivalence of the test and reference drug products under both fasting and fed conditions. However, safety of the subjects may require that an investigation be conducted after the administration of an appropriate meal at a specified time before taking the drug. In this case, manufacturers should consult with Health Canada before undertaking the study.

This objective can be achieved by applying one of the following design schemes (A is the most informative, C the least):

a) A four-period cross-over trial with four complementary sequences of four treatment conditions. Both the test and reference drug products should be assessed in the fasting state as well as after the administration of an appropriate meal at a specified time before taking the drug.

b) Two cross-over trials. The first trial should compare the test and reference drug products under fasting conditions. The drugs should be administered during two periods and with two sequences of treatment conditions. The second trial should compare the test and reference formulations following the administration of an appropriate meal at a specified time before taking

the drug, as well as the test formulation under fasting conditions. The trial should be conducted with three periods and three complementary sequences of drug administrations.

c) Two cross-over trials, both with two periods and two sequences of test and reference drug product administration. One trial should be conducted in the fasting state. The other trial should be conducted after the administration of an appropriate meal at a specified time before taking the drug.

4.2.2 Relative Mean AUC

The 90% confidence interval of the relative mean AUC of the test to reference formulation should be within 80% to 125% in the fasting state and after the administration of an appropriate meal at a specified time before taking the drug.

AUC may be evaluated by determining AUC_T, provided that AUC_T obtained by the linear trapezoidal rule is at least 80% of the extrapolated AUC_I (i.e., $AUC_T/AUC_I \geq 0.80$).

4.2.3 Relative Mean C_{max}

The relative mean measured C_{max} of the test to reference formulation should be between 80% and 125% in the fasting state and after the administration of a standardized meal to challenge the formulation.

4.3 Steady-State Studies for Formulations Used at a Dose Interval Likely to Lead to Accumulation ($AUC_X/AUC_I < 0.8$ for the MR Product)

4.3.1 Design of Studies

A second or subsequent market entry MR formulation should be compared with the Group I or II MR product with which bioequivalence is claimed. Both formulations should be administered at steady state. This comparison should be conducted in addition to the single- dose studies described in (Section 4.2).

Generally, steady-state studies should be performed under fasting conditions. However, safety of the subjects may require that an investigation be conducted after the administration of an appropriate meal at a specified time before taking the drug. In this case, manufacturers should consult with Health Canada before undertaking the study.

4.3.2 Relative Mean AUC_τ

The 90% confidence interval of the relative mean AUC_τ of the test to reference formulation should be within 80% to 125%.

4.3.3 Relative Mean C_{max} and C_{min}

The relative mean measured C_{max} at steady state of the test to reference formulation should be within 80% to 125%.

The relative mean measured C_{min} at steady state of the test to reference formulation should not be less than 80%.

5 PLANNING A BIOAVAILABILITY STUDY

This section identifies the documentation that must be prepared when a bioavailability study is planned. Descriptions of the study objectives, principal and other investigators, facilities, and ethical review boards must be included in the reports that follow or accompany each bioavailability study.

5.1 Study Objectives

The objectives of the bioavailability study should be clearly stated, together with the therapeutic rationale for, and the pharmacokinetic objectives of, the modified-release formulation. Information should be provided to justify why the drug is included in the category of modified-release drugs without complicated characteristics.

5.2 Principal and Other Investigators

The identity and duties of the individuals who are responsible for the study, and for the safety of the subjects participating in the study, must be provided. * Co-investigators, including those responsible for the clinical component of the study, the drug measurements, and the statistical analyses, must be identified. A *curriculum vitae* for each investigator must be obtained and appended to the study documentation.

*Guidelines on Research Involving Human Subjects, Medical Research Council of Canada, 1987.

5.3 Clinical, Laboratory, and Analytical Facilities

The location of all facilities should be identified and their suitability demonstrated. "Suitability" is determined with respect to the physical plant and the capability of a facility involved in experiments using human subjects or in the analysis of biological samples. These aspects of the facility should conform to current requirements for *Good Clinical Practice* or *Good Laboratory Practice*.*

5.4 Institutional (Ethical) Review

Documentary evidence that the study has been approved by an appropriate institutional ethical review board must be provided. The current guidelines of the Medical Research Council of Canada (MRC)** or a comparable agency should be used for such a review.

The guideline used for the ethical review should be identified in the documentary evidence for the study.

The reimbursement policy should be specified before initiating the study, and should be in agreement with MRC or similar guidelines.

6 SELECTION OF SUBJECTS FOR A STUDY

This section describes the selection criteria for including subjects in a bioavailability study. This section also identifies how the characteristics of the subjects may affect the study.

6.1 Choice of Subjects

Modified-release dosage forms of drugs with uncomplicated characteristics can usually be tested in normal, healthy volunteers. Investigators should ensure that female volunteers are not pregnant or likely to become pregnant during the study. Confirmation should be obtained by pregnancy tests conducted just before the first and last doses of the study.

Bioavailability studies of a modified-release dosage form in healthy control volunteers may not apply to all circumstances. Where substantial differences in the absorption or

*Source: Proposed Rule Obligations of Clinical Investigators, Federal Register, 43, 35210, (1978) and Code of Federal Regulations, 21, Part 58 (revised April 1988).

**Guidelines on Research Involving Human Subjects, Medical Research Council of Canada, 1987.

disposition of a drug owing to a disease process are known, the more appropriate approach may be to study the product in patients. Examples include:

- Preparations indicated for use in young children, where rapid clearance may invalidate the label claim of less frequent administration.
- MR products whose sustained-release effect is dependent on pH changes in the gastrointestinal tract, where the drug is designed for use in patients likely to have achlorhydria.

Generally, such patients will have to be studied under controlled conditions during regular treatment with the drug. The number of samples or level of control of study conditions may therefore be limited. Study design and other important issues should be discussed with Health Canada before initiating studies undertaken in patients.

6.1.1 Blood Volume

In studies of patients where the total volume of blood samples is an issue, the normally stringent criteria governing blood sampling frequency for AUC calculations may have to be modified. Consultation with Health Canada is encouraged if such a modification is made.

6.2 Considerations of Subject Characteristics

An important objective in the selection of subjects is the reduction of intra-subject variability in pharmacokinetics that may be attributed to certain characteristics of the subjects. Individuals should be assigned in such a way that the study design is balanced for factors that are suspected to contribute to variability:

a) **Age** - Subjects should be between the ages of legal majority and the 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% of the normal range, (e.g., the range given in current Ciba-Geigy or Metropolitan Life Insurance tables).

An electrocardiogram should be included in the study documentation if the drug has a cardiac effect. Aberrant laboratory values should be double-checked, and a summary must be presented together with the physician's opinion.

Psychological characteristics should also be assessed by the physician so that patients unlikely to comply with study restrictions or unlikely to complete the study can be excluded.

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.

6.3 Number of Subjects

A minimum of 12 subjects should be used and, if a subject withdraws or must be removed from the study, an explanation for the withdrawal or removal must be included in the study documentation.

6.3.1 Estimating the Number of Subjects

The number of subjects to be used in the cross-over study should be estimated by considering the standards that must be passed (see Sections 3 and 4) and the drug products being compared. The probability that a study of a given size will pass the standards depends on:

- the expected mean difference between the test and reference formulations for the parameters of interest; and
- the anticipated intra-subject coefficient of variation (CV) of these parameters.

For many drugs with uncomplicated characteristics, the residual CV in the analyses of variance (see Sections 11 through 14) is generally less than 20%. However, as a result of sampling, or if the study is poorly run, the residual CV can be higher.

The minimum number of subjects is 12, but a larger number is often required.

This sample size calculation must be provided in the study protocol. To estimate the number of subjects required, refer to *Conduct and Analysis of Bioavailability and Bioequivalence Studies: Part A*, (Section 3.3).

6.3.2 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 drop-outs and withdrawals.

Two basic methods are used to account for drop-outs and withdrawals. First, a fixed number (one or two for each sequence) of subjects are added to the sample-size number. Second, a fixed number of subjects are added into the study. These subjects are designated as extras. Only if a subject drops out will the appropriate extra subject's blood samples be assayed. The method of accounting for drop-outs and withdrawals must be outlined in the protocol.

6.3.3 Additional Trials

Because of the possibility of random variation or larger-than-expected relative difference, or both, there is no guarantee that a calculated sample size will pass the standards. If the study is run with the appropriate size and the standards are not met, the sponsor may conduct an additional trial with a minimum of 12 subjects. This option, if chosen, must be stated in the study protocol. Two criteria must be met before the combination of results from trials is acceptable (see *Conduct and Analysis of Bioavailability and Bioequivalence Studies: Part A*):

- The same protocol must be used (i.e., same formulations, same blood sampling times, a minimum number of 12 subjects, etc.).
- Consistency tests must be met at an ALPHA error rate of 5%.

6.4 Drop-outs and Withdrawal of Subjects from a Study

The subjects must be available, without coercion, for all legs of the study. It is recommended that the number of subjects should be sufficient to allow for possible drop-outs or withdrawals. (See Section 6.3, “Number of Subjects”.)

Reasons for withdrawal (e.g., adverse drug reactions) must be reported, and the subject's plasma (or blood or serum) levels provided. The results of all samples that were measured in subjects who were withdrawn from the study must be included in the report. If a subject drops out of the study for personal reasons, the individual's blood samples do not have to be assayed.

7 STUDY DESIGN AND ENVIRONMENT

The design of a bioavailability or bioequivalence study should minimize variability that is not attributable to the drug itself and should eliminate bias to any possible extent. The guidances in this section can be used for the usual cases. Other designs may be permissible after consultation with the Health Canada before the study is initiated.

7.1 Standardization

Every effort should be made to standardize the study conditions (e.g., exercise, diet, smoking, and alcohol use) in all phases of the study. It is preferable to use non-smokers; where smokers are included, they must be so identified.

Volunteers should take no other drug, including alcoholic beverages and over-the-counter (OTC) drugs, for an appropriate interval before or during the study. In an emergency, the use of another drug must be reported (i.e., dose and time of administration). The decision to include or exclude the results from a subject who has varied from the established protocol should be made before statistical analysis commences.

7.2 Blinding

If possible, bioequivalence trials should be conducted in such a way that the subjects do not know which product (test or reference) is being administered. Individuals involved in the administration of the drugs, the surveillance of the patients, and checking for adverse reactions should not know which product was administered. Furthermore, in both bioequivalence and comparable bioavailability studies, the person conducting the analysis of samples must not know which product was administered.

7.3 Administration of Food and Fluid

The administration of food and fluid should be carefully controlled. Subjects should fast for at least 10 hours before drug administration. A fast means that no food or fluids are to be consumed, although alcohol-free and xanthine-free clear fluids are permissible the night before the study. On the morning of the study, up to 250 mL of water may be permitted up to two hours before administration of the drug. The dose should be taken with a standard volume of water (e.g., 150 mL) and at a standard temperature. Two hours after administration of the drug, 250 mL of xanthine-free fluids are permitted. Four hours after administration of the drug, a standard meal may be taken. All meals should be standardized and repeated on each study day.

Safety of the subjects may require that an investigation be conducted after the administration of an appropriate meal at a specified time before taking the drug, rather than under fasting conditions. In this case, manufacturers should consult with Health Canada before undertaking the study.

If steady-state studies are required, the food and fluid restrictions noted above should apply on the day the plasma profiles are to be obtained, as well as on the preceding evening. If the oral preparation is being compared with an intravenous (IV) dose, the food and fluid restrictions noted above should also apply to the IV dose.

The nature of the test meal-in the part of the study where the formulation is given in the presence of food-should be determined based on the physicochemical and pharmacokinetic characteristics of the drug and its formulation. The purpose is to select a test meal that can challenge the formulation (i.e., the meal has the greatest potential to demonstrate altered bioavailability). The meal should be given within a pre-determined, constant time of administration of the drug. The timing and contents of the meal should be chosen carefully.

7.4 Posture and Physical Activity

For most drugs, subjects should not be allowed to recline until at least two hours after ingestion of the drug. 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 activity should be maintained for each study day.

7.5 Interval Between Study Days

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, should normally be not less than 10 times the mean terminal half-life of the drug (generally, the interval between study days should not exceed four weeks). In addition, the drugs must be administered at approximately the same time on each study day and, where possible, the same day of the week.

7.6 Sampling Times

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

To permit calculation of the relevant pharmacokinetic parameters, 12 to 18 samples should be collected per subject per dose. The inter-subject variability should be taken into account in the placement and number of samples. The exact times at which the samples are taken must be recorded and spaced such that the following information can be estimated accurately and precisely:

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

b) when appropriate, the AUC_T should be at least 80% of the calculated AUC_I; and

c) the terminal disposition rate constant of the drug.

7.6.1 Estimating the Terminal Disposition Rate Constant

The estimated terminal disposition rate constant may not be precise if only a few points are used for its estimation by linear regression. To reduce this imprecision, four or more points should be determined during the terminal log-linear phase of the curve. If urine is used as the biological sampling fluid (see Section 7.7), sufficient samples must be obtained to permit an estimate of the rate and extent of renal excretion.

7.6.2 Evidence of Steady State

For steady-state studies of drugs with uncomplicated characteristics, at least three consecutive pre-dose concentration levels (C_{pd}) are required to provide evidence of steady state. Generally, observations of C_{pd} for the test and reference products should be recorded at the same time of the day. One of these measurements could be taken based on the first sample of the study day in which a profile over the dosing interval is being established. Steady state is usually achieved when repeated doses of a formulation are administered over a period that exceeds five disposition half-lives of the modified-release form.

The number and timing of samples required during steady-state studies are dependent on:

- the length of the dose interval;
- timing of meals;
- the pharmacology and the pharmacokinetics of the drug; and
- whether the conventional or modified-release form is being studied.

7.6.3 Other Considerations

The levels at the beginning of the dosing interval and at the end are both required. The number of samples must be sufficient to provide the information required to sustain label claims, to identify C_{max} and C_{min}, and to calculate AUC over the dosing interval (AUC_{τ}).

For studies involving food consumption, additional sampling may be required during the period when modifications of the serum concentration versus time curve can be anticipated.

7.7 Sampling of Blood or Urine

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 (more than 40%) of the drug is eliminated unchanged in the urine, then urine may serve as the biological fluid to be sampled.

The volume of each urine sample must be measured immediately after collection and be included in the report. Urine should be collected over 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.

7.8 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 there is evidence that metabolites are likely to interconvert to the parent drug.

7.9 Identification of Adverse Reactions and Side Effects

In some cases, adverse drug reactions result from factors other than the active ingredient in a formulation. The rate of dissolution or absorption, and excipients within formulations, may affect the frequency, onset, and severity of adverse drug reactions. The incidence, severity, and duration of adverse reactions and side effects observed during the study must be reported. The probability that an adverse effect is drug-induced is to be judged by the investigator.

As much as possible, the same observer and format should be used for eliciting and recording information on adverse drug reactions for all subjects. Questions concerning adverse reactions and side effects should be asked on each study day by the "blinded" observer.

For drugs with known adverse reactions and side effects-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 reactions 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 reactions are expected or unexpected. The subject should be questioned in private.

8 TEST AND REFERENCE DRUG PRODUCTS

8.1 Chemistry

The products must meet a Schedule B or other applicable standard of identity, quality, purity, and potency 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.08.002(2) and C.08.005(1), respectively.

8.2 Dosage and Strength

In bioavailability studies, the dose that is administered for both the modified-release and conventional-release products should be identical. When identical doses cannot be administered, a proportionality correction must be made for the calculation of relevant parameters.

In bioequivalence studies, the molar equivalent dose of each product should be used. The lots for bioavailability and bioequivalence testing should be taken from a batch that is comparable in size and is produced using the same type of equipment and procedures that are proposed for market. In other words, the lots for bioavailability and bioequivalence testing should be representative of proposed production batches.

8.2.1 Strength

For an uncomplicated drug in which the proportions of excipients to the drug and the dissolution characteristics are the same across all strengths, it is sufficient to establish the bioavailability of one strength. Whether all of the strengths of other products should be tested will depend on the extent to which the formulation differs among the various strengths.

8.2.2 Proportional Dose Claims

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.

8.3 Selection of Reference Product

8.3.1 New Active Substance

For a first market entry new active substance (Group I), an oral solution should be used as the reference product, when possible. The oral solution can be prepared from an intravenous solution, if one is available. In the case of insoluble drugs, the reference product should be as agreed with Health Canada.

8.3.2 Marketed Products

The first modified-release (Group II) product must be compared with the marketed conventional product.

For bioequivalence studies (Group III), the reference product must be a drug product marketed in Canada by the innovator, the licensee, or the market leader (if there is no recognized innovator). For bioequivalence studies, the reference product must be the product with which bioequivalence is claimed.

9 MEASUREMENT METHODOLOGY

Bioavailability determinations rely on the adequacy of the analytical methods used for the parent drugs and, when appropriate, the metabolites of those drugs. This section describes the attributes of such methods and the validation procedures required in reports to establish and maintain selectivity, range, precision, and accuracy.

9.1 Drug and Drug Metabolites

The determination of bioavailability is dependent on the reliable, precise, and accurate measurement of the active ingredient, or its metabolites, as a function of time. Normally, measurement of the parent compound or active ingredient will be adequate; however, in certain circumstances, the measurement of metabolites may be required. When a pro-drug is administered, the active component should be measured.

9.2 Assay Methodology

The analytical methods used to measure the drug, or its metabolites, in plasma, blood, serum, or urine must 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) 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, according to the principles of Good Laboratory Practice (GLP).

9.3 Stability

For samples to maintain their stability (avoiding degradation of analytes), they should be handled according to the directions outlined in (Section 7.8, "Handling of Samples"). Validation must be included.

9.4 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 (LOQ) should be established, based on an intra-day and inter-day coefficient of variation (CV) not greater than 20%. 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".

9.5 Recovery

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

9.6 Specificity

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

9.7 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 run on each day of the analysis. The within-day and between-day variability in the standard curves must be reported, together with the coefficients of variation (CVs) obtained during measurement of the samples. 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.

9.8 Precision and Accuracy

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

9.8.1 Replicate Samples

In general, single-sample analysis will suffice. When single assays are performed, 15% of the incurred samples must be randomly selected and re-assayed. (Studies in which the sample of blood is insufficient for duplicate analysis should include a pre-study verification with incurred samples.)

The second measurement is not to be averaged with the first; only the variation between samples is to be summarized and then reported separately. The purpose of re-assaying is to establish that the degree of precision obtained with incurred samples is similar to that obtained for spiked standard or quality control (QC) samples.

If the proposed method does not have the potential to give the required precision, all samples may be replicated. If this is done, the replicates would be averaged. The variation between samples should be reported separately.

9.9 Quality Control for Spiked Samples

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

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

A minimum of six QC samples, composed of three concentrations in duplicate, must be blinded and analyzed with each batch of study samples for each analytical day or run.

9.10 Aberrant Values (Repeat Assays)

In most studies, some blood/plasma/serum or urine samples will require re-assay. Criteria for identifying these samples should be pre-established.

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

- processing errors,
- equipment failure,
- obviously poor chromatography, or
- quality control samples outside pre-defined tolerances.

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

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

10 ANALYSIS OF DATA

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

10.1 Presentation of Data

The concentrations of the drug in plasma (or blood, or serum) for each subject, sampling time, and formulation should be tabulated. Unadjusted, measured concentrations should be provided. All concentrations and sample times should be supplied, for each combination of subjects and formulations, in computer-readable form.*

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. Two graphs should also be drawn for the mean values of all subjects. In each case, one of the graphs should be linear while the other semilogarithmic. On these graphs, the drug concentrations from the reference and 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.

10.2 Pharmacokinetic Parameters

The estimated pharmacokinetic parameters should be supplied, for each combination of subjects and formulations, in computer-readable form. * All parameters should be estimated from measured drug concentrations.

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*See the *Guidelines on the Preparation of Human Abbreviated Drug Submissions: Bioequivalence Studies* Drugs Directorate, 1996

10.2.1 Single-Dose Studies

For single-dose studies, estimates of the following pharmacokinetic parameters must be tabulated for each combination of subjects and formulations:

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

b) **AUC_X** - Area under the concentration versus time curve over the usual dosing interval, 0-X.

c) **AUC_I** - AUC measurement up to the last quantifiable concentration, plus an additional area extrapolated to infinity, calculated using λ.

d) **AUC_X/AUC_I** - Ratio of AUC over the dosing interval to the total AUC extrapolated to infinity.

e) **AUC_T/AUC_I** - Ratio of AUC, measured to the last quantifiable concentration, to the total AUC extrapolated to infinity.

f) **C_{max}** - Maximum observed concentration.

g) **T_{max}** - Time at which the observed C_{max} occurred.

h) λ - Terminal disposition rate constant.

10.2.2 Steady-State Studies

For steady-state studies, estimates of the following pharmacokinetic parameters must be tabulated for each combination of subjects and formulations:

a) **C_{pd}** - Pre-dose concentrations determined immediately before a dose at steady state. (Note: to assess whether steady state has been achieved, at least three consecutive pre-dose concentrations, generally at the same time of day, must be determined.)

b) **AUC_τ** - Area under the concentration versus time curve measured throughout the dosing interval, using the linear trapezoidal rule.

c) **C_{max}** - Maximum observed concentration.

d) **C_{min}** - Minimum observed concentration.

e) **T_{max}** - Time at which the observed C_{max} occurred.

f) **$(C_{max} - C_{min})/(AUC_{\tau}/\tau) \times 100$** - Fluctuation (expressed as a percentage) determined as the range of concentrations divided by the average steady-state concentration.

10.2.3 Additional Parameters

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.

10.3 Statistical Analysis

The analysis of variance (ANOVA) tables submitted with the study documentation (report) should include the appropriate statistical tests of all effects in the model. The output from ANOVAs appropriate to the study design and execution must be expressed with enough significant figures to permit further calculations.

Analysis of T_{max}, λ, and fluctuation should be carried out on the raw scale, while calculations for AUC_X, AUC_T, AUC_{τ}, AUC_I, C_{min}, C_{pd}, and C_{max} should use the logarithmic (ln) scale.

The analyses should include all data for all subjects. Supplementary analyses may also be carried out with selected points, or subjects, or both, excluded from the analyses. Such exclusions must be justified, and the reasons documented. It is rarely acceptable to exclude more than 5% of the subjects or more than 10% of the data for a single subject-formulation combination.

A summary of results should be reported on a separate page for each parameter as detailed in the samples provided in (Sections 11 and 14). The report should contain:

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

b) the ANOVA;

c) in single-dose studies: AUC_X, AUC_T, and C_{max} ratios, T_{max}, and λ or in steady-state studies: AUC_{τ}, C_{min}, and C_{max} ratios and fluctuation differences for test products versus reference products;

d) the 90% confidence interval about the mean AUC_T, AUC_τ, C_{min}, C_{max}, and fluctuation comparisons; and

e) a summary of comparisons for AUC_X, AUC_T, AUC_τ, λ, C_{pd}, C_{min}, C_{max}, and fluctuation uncorrected and corrected for measured content of the dosage forms.

11 SAMPLE ANALYSIS FOR A SINGLE-DOSE COMPARATIVE BIOAVAILABILITY STUDY

The following tables and figures illustrate data collected and used in a sample single-dose comparative 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 reference formulation. The analysis of a single-dose comparative bioavailability study must 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 11.1.)

b) A summary of drug concentrations (visual and quantitative) at each sampling time for each subject for both test and reference formulations. (See Section 11.2.)

c) A summary of the estimates of the parameters as defined in Section 11.3 for both test and reference formulations, including the means, standard deviations, and CVs. (See Section 11.4.)

d) A formal statistical analysis of the relevant parameters with comparisons of the test formulations to the reference formulations. (See Sections 11.5 through 11.9.)

e) A summary of corrections for potency (measured content) in estimates. (See Section 11.10.)

f) Sample concentration-time profiles that should be given for each subject. (See Section 11.11.)

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

11.1 Randomization Scheme of the Design

Shown in Table 11-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 must be included. Even those subjects that did not complete the study must be included and identified accordingly. Subject numbers that appear on informed consent forms and reporting forms must 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 must be given.

11.2 Summary of Drug Concentrations

Tables 11-B and 11-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 limit of quantitation of the analytical method is given in this table.

TABLE 11-A
Randomization Scheme of the Cross-over Design for the Comparison of Test (T) Versus Reference (R) Formulations

Subject			Period	
Number	ID	Sequence	Feb. 1, 1989	Feb. 8, 1989
001	A	RT	R	T
002	B	TR	T	R
003	C	TR	T	R
004	D	RT	R	T
005*	E	TR	T	-
006	F	TR	T	R
007	G	RT	R	T
008	H	RT	R	T
009	I	RT	R	T
010	J	TR	T	R
011**	K	TR	-	-
012	L	RT	R	T
013	M	TR	T	R
014	N	RT	R	T

* Subject did not appear for second period.
** Subject did not appear for either period.

TABLE 11- B
Drug Concentrations* (μg/mL) for the test Formulations

			Sampling Times (hours)																			
ID	Seq.	Per.	0.0	0.4	1.0	2.0	4.0	6.0	8.0	10.0	12.0	13.0	14.0	16.0	18.0	20.0	22.0	24.0	36.0	48.0
A	RT	8 Feb.		0.4	5.2	24.4	45.8	42.0	40.1	41.5	42.0	40.7	45.1	42.6	45.5	44.7	42.5	42.3	27.1	18.1
B	TR	1 Feb.		0.0	12.8	61.8	65.5	58.3	46.4	49.2	40.1	46.7	47.5	40.1	34.9	32.2	29.2	29.0	13.1	8.3
C	TR	1 Feb.		0.0	21.7	49.1	57.0	53.1	45.0	41.6	44.2	47.9	53.6	56.7	61.3	60.3	60.8	66.8	36.7	22.4
D	RT	8 Feb.		0.0	19.4	46.9	56.4	57.8	55.5	60.9	45.7	46.1	40.2	38.4	34.2	28.6	24.1	27.0	12.8	7.3
F	TR	1 Feb.		0.0	23.7	37.0	39.2	41.9	41.4	51.3	46.1	40.2	38.8	30.3	26.7	24.5	22.2	20.4	11.8	6.5
G	RT	8 Feb.		0.3	31.2	54.8	65.7	61.1	57.3	55.8	48.0	48.2	50.6	44.4	43.3	45.7	45.8	46.2	22.9	13.6
H	RT	8 Feb.		0.0	23.2	29.4	32.4	43.8	41.9	42.5	38.0	36.0	31.9	24.9	24.0	23.8	22.3	21.1	10.1	6.4
I	RT	8 Feb.		0.0	25.0	58.5	63.3	51.4	42.0	47.8	43.6	43.8	36.2	31.9	26.8	35.8	31.8	29.4	25.3	13.7
J	TR	1 Feb.		0.0	17.1	28.0	38.1	47.9	42.1	44.8	44.7	41.6	48.2	44.3	39.4	39.3	42.0	43.6	26.3	18.0
L	RT	8 Feb.		0.0	17.2	15.0	12.8	20.0	27.5	30.3	28.7	27.0	23.7	25.9	22.7	19.8	19.0	18.2	9.4	6.1
M	TR	1 Feb.		0.0	4.9	1.7	36.7	62.6	54.4	47.2	43.1	37.5	37.6	34.0	30.5	27.4	22.8	23.1	11.1	6.9
N	RT	8 Feb.		0.5	34.6	52.1	56.3	52.5	47.2	52.7	71.8	76.3	68.9	55.8	53.8	54.8	53.1	51.2	25.2	18.8
.	.	.		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MEAN	—	—		0.1	19.7	38.1	47.3	49.4	45.1	47.1	44.7	44.3	43.5	39.1	37.8	36.4	34.6	34.9	19.3	12.2
STD	—	—		0.2	9.1	18.6	16.2	11.7	8.1	7.9	9.9	11.7	11.6	10.4	11.7	12.8	13.8	15.0	9.0	6.0
CV	—	—		185 g	46.1	48.8	34.2	23.6	18.0	46.8	22.2	11.9	26.7	26.6	31.1	35.2	40.0	43.1	46.4	49.0

* Limit of quantitation is 0.2μg/mL. Any concentration below this limit is reported as Below Quantitation Limit (BQL) except at time 0 and times before first observed concentration. However, in the calculation of summary statistics a zero is used.

TABLE 11-C
Drug Concentrations* (μg/mL) for the Reference Formulation

			Sampling Times (hours)																			
ID	Seq.	Per.	0.0	1.0	2.0	4.0	6.0	8.0	10.0	12.0	13.0	14.0	16.0	18.0	20.0	22.0	24.0	36.0	48.0			
A	RT	1 Feb.	0.0	11.9	25.4	60.1	54.1	41.0	34.9	31.7	30.6	31.0	58.7	68.1	62.2	57.1	51.9	28.1	17.4			
B	TR	8 Feb.	0.0	78.4	53.7	40.4	31.9	29.8	24.6	20.8	27.6	55.8	53.3	46.8	39.4	36.9	36.9	16.9	12.1			
C	TR	8 Feb.	0.8	89.2	70.5	53.6	43.4	36.8	30.7	27.1	63.3	66.0	64.6	57.5	52.8	47.2	46.8	27.6	17.6			
D	RT	1 Feb.	0.0	110.7	71.6	52.0	47.2	36.8	31.9	27.7	88.5	89.7	70.5	64.4	52.4	44.4	40.8	18.8	10.5			
F	TR	8 Feb.	0.0	54.5	54.4	39.2	32.9	29.9	25.6	22.2	75.1	69.8	51.3	42.2	34.4	29.8	29.9	15.3	10.8			
G	RT	1 Feb.	0.0	33.2	72.6	64.7	47.3	40.4	33.5	28.9	26.2	24.2	67.4	63.2	60.3	51.4	50.5	21.7	12.2			
H	RT	1 Feb.	0.0	69.3	66.9	46.3	35.2	31.2	25.1	24.4	44.2	73.5	57.7	49.6	40.8	36.0	34.5	15.0	9.1			
I	RT	1 Feb.	0.0	49.3	65.1	74.6	56.8	44.6	37.2	32.1	29.2	46.7	63.4	38.0	69.7	59.2	54.6	25.1	14.6			
J	TR	8 Feb.	0.3	5.9	15.9	61.0	44.0	33.3	32.7	27.4	25.8	73.6	63.8	53.3	49.3	41.2	40.2	18.4	13.7			
L	RT	1 Feb.	0.0	44.3	83.0	57.8	47.9	39.2	33.2	29.1	27.3	31.9	64.3	59.6	56.3	48.0	46.6	20.9	13.6			
M	TR	8 Feb.	0.0	66.5	54.5	41.2	36.1	28.5	23.8	19.4	76.7	68.6	49.6	48.8	37.8	31.6	31.4	12.9	12.1			
N	RT	1 Feb.	0.0	79.1	66.5	51.4	43.5	36.1	30.7	24.8	84.6	79.0	63.9	55.8	49.1	45.7	44.0	22.5	13.6			
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
MEAN	—	—	0.1	57.7	58.3	53.5	43.4	35.6	30.3	26.3	49.9	59.1	60.7	53.9	50.4	44.0	42.3	20.3	13.1			
STD	—	—	0.2	31.0	19.7	10.8	8.0	5.1	4.5	4.1	25.6	21.1	6.6	9.2	10.8	9.4	8.1	5.0	2.6			
CV	—	—	260 g	53.7	33.7	20.1	18.5	14.4	14.7	15.5	51.3	35.7	10.8	17.1	21.5	21.3	19.1	24.4	19.6			

* Limit of quantitation is 0.2μg/mL. Any concentration below this limit is reported as Below Quantitation Limit (BQL) except at time 0 and times before first observed concentration. However, in the calculation of summary statistics a zero is used.

11.3 List of Parameters and Definitions

Table 11-D shows a list of the parameters used in the analysis and their definitions. If any other parameters are used, they must also be clearly defined.

TABLE 11-D
Parameter Definitions

Parameter	Definition
C_{max}	Maximum observed concentration (µg/mL)
T_{max}	Sampling time at which C_{max} occurred (h).
AUC_X	Area under the raw concentration versus time curve, over the dosing interval of the test formulation, calculated using the linear trapezoidal rule (µg•h/mL).
AUC_T	Area under the raw concentration versus time curve calculated using the linear trapezoidal rule from time 0 to LQCT (µg•h/mL).
AUC_I	Area to infinity = $AUC_T + C_T/\lambda$, where C_T is the estimated concentration at LQCT (µg•h/mL).
$\frac{AUC_T}{AUC_I} \times 100$	Percent of the area measured by AUC_T relative to the extrapolated total AUC (%).
$\frac{AUC_X}{AUC_I} \times 100$	Percent of the area measured by AUC_X 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 limit of quantitation (h).
$T_{1/2}$	Drug half-life = $In2/\lambda = 0.693/\lambda$ (h).

11.4 Summaries of Parameter Estimates

Table 11-E and 11-F list, for each subject, the estimates of the parameters defined in Table 11-D for the test and reference formulations respectively. Summary statistics (arithmetic means or medians, standard deviations, and CVs) should be given for each formulation.

The AUC_X/AUC_I ratio is used to determine whether the drug accumulates. For this example, the mean ratio of less than 80 percent indicates that the drug accumulates. Therefore, a multiple-dose study must be run.

The AUC_T/AUC_I ratio is used to determine whether the subjects were sampled for a sufficient length of time. For this example, the mean ratio is greater than 80 percent, indicating that the subjects were sufficiently sampled. If the reference is an immediate-release formulation, then C_{max} and T_{max} are taken from the dosing interval that shows the largest peak (C_{max}).

TABLE 11-E
Summary of Parameters for Each Subject Given the Test Formulation

			Parameters										
ID	Seq.	Per.	C_{max} (µg/mL)	T_{max} (h)	AUC_X (µg•h/mL)	AUC_T (µg•h/mL)	AUC_I (µg•h/mL)	$\frac{AUC_X}{AUC_I}$ %	$\frac{AUC_T}{AUC_I}$ %	λ (h^{-1})	TLIN (h)	LQCT (h)	$T_{1/2}$ (h)
A	RT	8 Feb.	46	4	945	1633	2166	44	75	0.0339	24	48	20.4
B	TR	1 Feb.	66	4	1024	1405	1559	66	90	0.0511	24	48	13.6
C	TR	1 Feb.	67	24	1228	2204	2746	45	80	0.0415	24	48	16.7
D	RT	8 Feb.	61	10	1004	1363	1508	67	90	0.0499	24	48	13.9
F	TR	1 Feb.	51	10	822	1125	1264	65	89	0.0472	24	48	14.7
G	RT	8 Feb.	66	4	1194	1828	2100	57	87	0.0490	24	48	14.1
H	RT	8 Feb.	44	6	740	1027	1149	64	89	0.0498	24	48	13.9
I	RT	8 Feb.	63	4	984	1546	2038	48	76	0.0303	24	48	22.9
J	TR	1 Feb.	48	14	959	1645	2158	44	76	0.0347	24	48	20.0
L	RT	8 Feb.	30	10	520	778	908	57	86	0.0453	24	48	15.3
M	TR	1 Feb.	63	6	818	1132	1269	65	89	0.0486	16	48	14.3
N	RT	8 Feb.	76	13	1304	2026	2440	53	83	0.0423	22	48	16.4
.	.	.	.	.	.	.	.	.	.	.	.	.	
.	.	.	.	.	.	.	.	.	.	.	.	.	
MEAN*	—	—	57	8	962	1476	1776	56	84	0.0436	24	48	16.3
STD	—	—	13	5.9	220	422	575	9	6	0.0071	2.3	0	3.1
CV	—	—	23	65	23	29	32	16	7	16.354	10	0	18.9

* For T_{max}, TLIN, and LQCT, these are medians.

TABLE 11-F
Summary of Parameters for Each Subject Given the Reference Formulation

				Parameters									
ID	Seq.	Per.	C_{max} (µg/mL)	T_{max} (h)	AUC_X (µg•h/mL)	AUC_T (µg•h/mL)	AUC_I (µg•h/mL)	$\frac{AUC_X}{AUC_I}$ %	$\frac{AUC_T}{AUC_I}$ %	λ (h^{-1})	TLIN (h)	LQCT (h)	$T_{1/2}$ (h)
A	RT	1 Feb.	60	4	1099	1852	2219	50	83	0.0462	12	48	15.0
B	TR	8 Feb.	78	1	945	1441	1686	56	85	0.0460	12	48	15.1
C	TR	8 Feb.	89	1	1218	1936	2381	51	81	0.0393	12	48	17.6
D	RT	1 Feb.	111	1	1323	1855	2035	65	91	0.0565	12	48	12.3
F	TR	8 Feb.	55	1	950	1378	1625	58	85	0.0416	12	48	16.7
G	RT	1 Feb.	73	2	1155	1791	1995	58	90	0.0578	12	48	12.0
H	RT	1 Feb.	69	1	1039	1481	1637	63	90	0.0552	12	48	12.6
I	RT	1 Feb.	75	4	1236	1952	2206	56	88	0.0552	12	48	12.6
J	TR	8 Feb.	61	4	1005	1549	1827	55	85	0.0452	12	48	15.3
L	RT	1 Feb.	83	2	1147	1759	2010	57	88	0.0510	12	48	13.6
M	TR	8 Feb.	67	1	983	1399	1652	60	85	0.0413	12	48	16.8
N	RT	1 Feb.	79	1	1215	1831	2107	58	87	0.0481	12	48	14.4
.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.
MEAN*	—	—	75	1	1109	1685	1948	57	87	0.0486	12	48	14.5
STD	—	—	15	1	125	219	259	4	3	0.0064	2.5	0	1.9
CV	—	—	20	68	11	13	13	8	3	13.222	23	0	13.3

* For T_{max}, TLIN, and LQCT, these are medians.

11.5 AUC_T Analysis

The necessary information and summary for the analyses of AUC_T are shown in Tables 11-G, 11-H, and 11-I.

In this example, the AUC_T ratio passes the bioavailability criterion.

TABLE 11-G
AUC_T (μg•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	1633	1852	88	7.40	7.52
B	1405	1441	97	7.25	7.27
C	2204	1936	114	7.70	7.57
D	1363	1855	73	7.22	7.53
F	1125	1378	82	7.03	7.23
G	1828	1791	102	7.51	7.49
H	1027	1481	69	6.93	7.3
I	1546	1952	79	7.34	7.58
J	1645	1549	106	7.41	7.35
L	778	1759	44	6.66	7.47
M	1132	1399	81	7.03	7.24
N	2026	1831	111	7.61	7.51
. . .	. . .	. . .	. . .	. . .	. . .
MEAN	1476	1685	87	7.26	7.42
STD	422	219	20	0.3	0.13
CV	29	13	23	-	-

TABLE 11-H
AUC_T (μg•h/mL) Analysis—ANOVA for ln(AUC_T)

Source	df	SS	MS	F	PR > F
Seq	1	0.0183	0.0183	0.23	0.644
Subject (Seq)	10	0.8042	0.0804	2.50	0.082
Period	1	0.0564	0.0564	1.76	0.215
Form	1	0.1285	0.1285	3.99	0.074
Residual	10	0.3216	0.0322	-	-

Intra-subject CV = 100 x $(\text{MS Residual})^{0.5}$ = 100 x $(0.0322)^{0.5}$ = 18 percent

TABLE 11-I
AUC_T (μg•h/mL) Analysis—Calculations

Difference = Text $\bar{x}$ – Reference $\bar{x}$ = 7.2604 – 7.4089 = -0.1485

$SE_{Difference}$ = 0.0743

AUC Ratio = 100 x $e^{Difference}$ = 100 x $e^{-0.1485}$ = 86%

90% Confidence Limits

Lower, Upper = 100 x $e^{(Difference \pm t_{0.05, 10} \times SE_{Difference})}$

Lower = 100 x $e^{(-0.1485 - 1.812 \times 0.0743)}$ = 75%

Upper = 100 x $e^{(-0.1485 + 1.812 \times 0.0743)}$ = 99%

11.6 C_{max} Analysis

The necessary information and summary for the analyses of C_{max} are shown in Tables 11-J, 11-K, and 11-L.

In this example, the C_{max} ratio passes the bioavailability criterion.

TABLE 11-J
C_{max} (µg/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	46	60	76	3.82	4.10
B	66	78	84	4.18	4.36
C	67	89	75	4.2	4.49
D	61	111	55	4.11	4.71
F	51	55	94	3.94	4.00
G	66	73	90	4.19	4.28
H	44	69	63	3.78	4.24
I	63	75	85	4.15	4.31
J	48	61	79	3.88	4.11
L	30	83	37	3.41	4.42
M	63	67	94	4.14	4.20
N	76	79	96	4.33	4.37
. . .	. . .	. . .	. . .	. . .	. . .
MEAN	57	75	77	4.01	4.30
STD	13	15	18	0.26	0.19
CV	23	20	23	-	-

TABLE 11-K
C_{max} (µg/mL) Analysis—ANOVA for ln(C_{max})

Source	df	SS	MS	F	PR > F
Seq	1	0.0005	0.0005	0.01	0.933
Subject (Seq)	10	0.6867	0.0687	1.84	0.175
Period	1	0.0652	0.0652	1.75	0.215
Form	1	0.4279	0.4279	11.49	0.007
Residual	10	0.3275	0.037	-	-
Intra-subject CV = 19 percent					

TABLE 11-L
C_{max} (µg/mL) Analysis—Calculations

Difference = Text $\bar{x}$ – Reference $\bar{x}$ = 4.0185 – 4.2893 = -0.2708

$SE_{Difference}$ = 0.0799

C_{max} Ratio = 100 x $e^{Difference}$ = 100 x $e^{-0.2708}$ = 76%

90% Confidence Limits

Lower, Upper = 100 x $e^{(Difference \pm t_{0.05, 10} \times SE_{Difference})}$

Lower = 100 x $e^{(-0.2708 - 1.812 \times 0.0799)}$ = 66%

Upper = 100 x $e^{(-0.2708 + 1.812 \times 0.0799)}$ = 88%

11.7 AUC_I Analysis

The necessary information and summary for the analyses of AUC_I are shown in Tables 11-M and 11-N.

TABLE 11-M
AUC_I (μg•h/mL) Analysis—Data

ID	Raw Scale			Log Scale	
	Test AUC_I	Reference AUC_I	Relative AUC_I (%)	Test $\ln(AUC_I)$	Reference $\ln(AUC_I)$
A	2166	2219	98	7.68	7.7
B	1559	1686	92	7.35	7.43
C	2746	2381	115	7.92	7.78
D	1508	2035	74	7.32	7.62
F	1264	1625	78	7.14	7.39
G	2100	1995	105	7.65	7.6
H	1149	1637	70	7.05	7.4
I	2038	2206	92	7.62	7.7
J	2158	1827	118	7.68	7.51
L	908	2010	45	6.81	7.61
M	1269	1652	77	7.15	7.41
N	2440	2107	116	7.80	7.65
. . .	. . .	. . .	. . .	. . .	. . .
MEAN	1776	1948	90	7.43	7.57
STD	575	259	22	0.34	0.13
CV	32	13	25	-	-

TABLE 11-N
AUC_I (μg•h/mL) Analysis—ANOVA for ln(AUC_I)

Source	df	SS	MS	F	PR > F
Seq	1	0.0090	0.0090	0.08	0.777
Subject (Seq)	10	1.0623	0.1062	2.7	0.066
Period	1	0.0271	0.0271	0.69	0.426
Form	1	0.0912	0.0912	2.32	0.159
Residual	10	0.3931	0.0393	-	-
Intra-subject CV = 20 percent					

11.8 T_{max} Analysis

The necessary information and summary for the analyses of T_{max} are shown in Tables 11-O and 11-P.

TABLE 11-O
T_{max} (h) Analysis—Data

ID	Test T_{max}	Reference T_{max}
A	4	4
B	4	1
C	24	1
D	10	1
F	10	1
G	4	2
H	6	1
I	4	4
J	14	4
L	10	2
M	6	1
N	13	1
. . .	. . .	. . .
MEDIAN	8	1
STD	5.9	1.3

TABLE 11-P
T_{max} (h) Analysis—ANOVA

Source	df	SS	MS	F	PR > F
Seq	1	20.74	20.74	1.28	0.285
Subject (Seq)	10	162.26	16.23	0.86	0.591
Period	1	34.40	34.40	1.83	0.206
Form	1	334.40	334.40	17.75	0.002
Residual	10	188.43	18.84	-	-
Intra-subject CV = 77 percent					

11.9 λ Analysis

The necessary information and summary for the analyses of $\lambda(h^{-1})$ are shown in Tables 11-Q and 11-R.

TABLE 11-Q
$\lambda(h^{-1})$ Analysis—Data

ID	Test λ	Reference λ
A	0.0339	0.0462
B	0.0511	0.046
C	0.0415	0.0393
D	0.0499	0.0565
F	0.0472	0.0416
G	0.0490	0.0578
H	0.0498	0.0552
I	0.0303	0.0552
J	0.0347	0.0452
L	0.0453	0.051
M	0.0486	0.0413
N	0.0423	0.0481
. . .	. . .	. . .
MEDIAN	0.0436	0.0486
STD	0.0071	0.0064
CV	16.354	13.222

TABLE 11-R
$\lambda(h^{-1})$ Analysis—ANOVA

Source	df	SS	MS	F	PR > F
Seq	1	0.0001	0.0001	2.33	0.158
Subject (Seq)	10	0.0005	0.0001	1.79	0.186
Period	1	0.0002	0.0002	8.19	0.017
Form	1	0.0001	0.0001	3.72	0.083
Residual	10	0.0003	0.0003	-	-
Intra-subject CV = 11 percent					

11.10 Calculations for AUC Ratio and C_{max} Ratio Estimates Corrected for Measured Content

Tables 11-S and 11-T show the method of calculating the AUC ratio and C_{max} ratio estimates and their confidence limits, based on correction for measured content. Each formulation is adjusted to 100 percent of the label claim. The whole analysis need not be repeated; only the corrected estimates need be given.

TABLE 11-S
Estimates Based on Correction for Measured Content

	Test Formulation	Reference Formulation
Lot number	EX110	40905
Expiry date	06/90	05/90
Date of analysis	06/14/88	06/14/88
Measured content (% of label claim)	95.5	99.0
Correction factors		
! raw scale-multiply	1.0471	1.0101
! log scale-add	0.0460	0.0100

TABLE 11-T
AUC Ratio and C_{max} Ratio—Calculations

Based on measured contents in Table 11-S, the following factor is obtained:

$$\ln\left(\frac{99.0}{95.5}\right) = 0.0360$$

which is to be added to the estimates on log scale.

Therefore:

$$AUC_x \text{ Ratio} = 100 \times e^{(-0.1486 + 0.0360)} = 89\%$$

$$\text{Lower Limit} = 100 \times e^{(-0.1486 + 0.0360 - 1.812 \times 0.0681)} = 79\%$$

$$\text{Upper Limit} = 100 \times e^{(-0.1486 + 0.0360 + 1.812 \times 0.0681)} = 101\%$$

$$AUC_T \text{ Ratio} = e^{(-0.1485 + 0.0360)} \times 100\% = 89\%$$

$$\text{Lower Limit} = 100 \times e^{(-0.1485 + 0.0360 - 1.812 \times 0.0743)} = 78\%$$

$$\text{Upper Limit} = 100 \times e^{(-0.1485 + 0.0360 + 1.812 \times 0.0743)} = 102\%$$

$$C_{max} \text{ Ratio} = 100 \times e^{(-0.2708 + 0.0360)} = 79\%$$

$$\text{Lower Limit} = 100 \times e^{(-0.2708 + 0.0360 - 1.812 \times 0.0799)} = 68\%$$

$$\text{Upper Limit} = 100 \times e^{(-0.2708 + 0.0360 + 1.812 \times 0.0799)} = 99\%$$

11.11 Concentration - Time Profiles (Subject A)

Figure 11.1 gives a plot of the concentration–time profile for subject A. Each plot must include profiles for all formulations given to that subject. Similar profiles should be given for each subject.

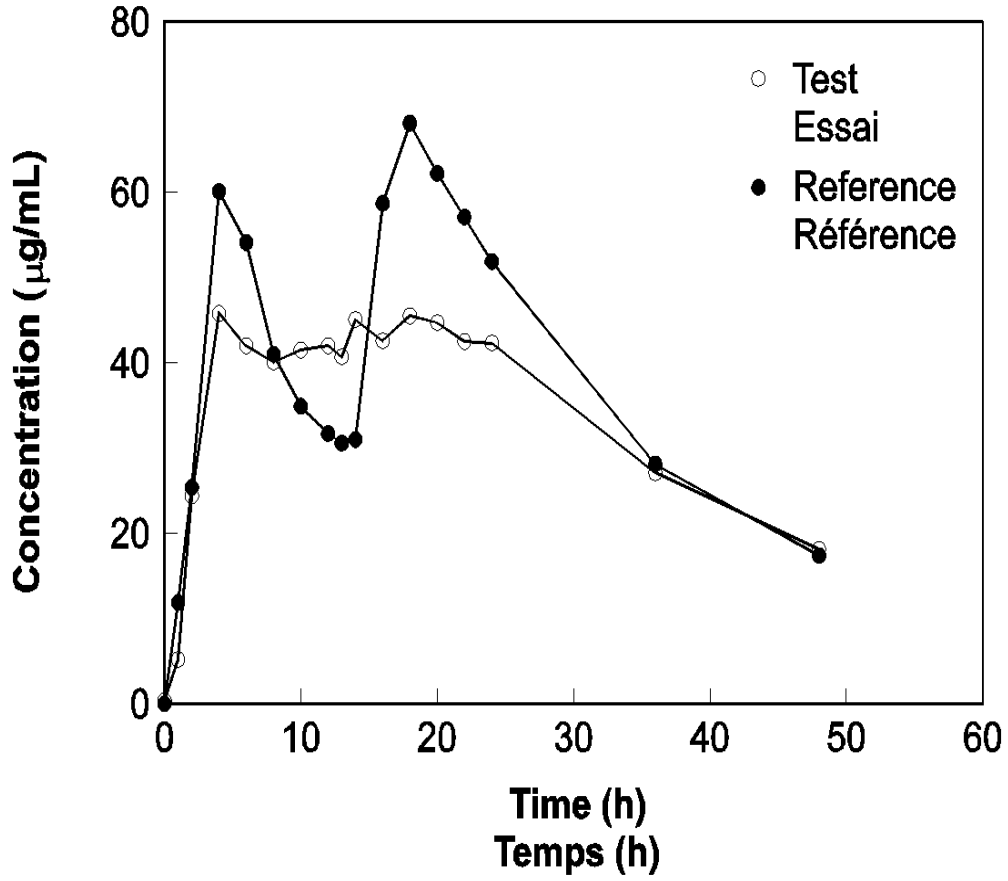

Figure 11.1: Concentration–Time Profile for Subject A

Figure 11.2 gives a plot of the ln(concentration)-time profile for subject A. This plot must contain the regression lines from which the terminal disposition rate constants (λ) were estimated. This line must 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 must be identified.

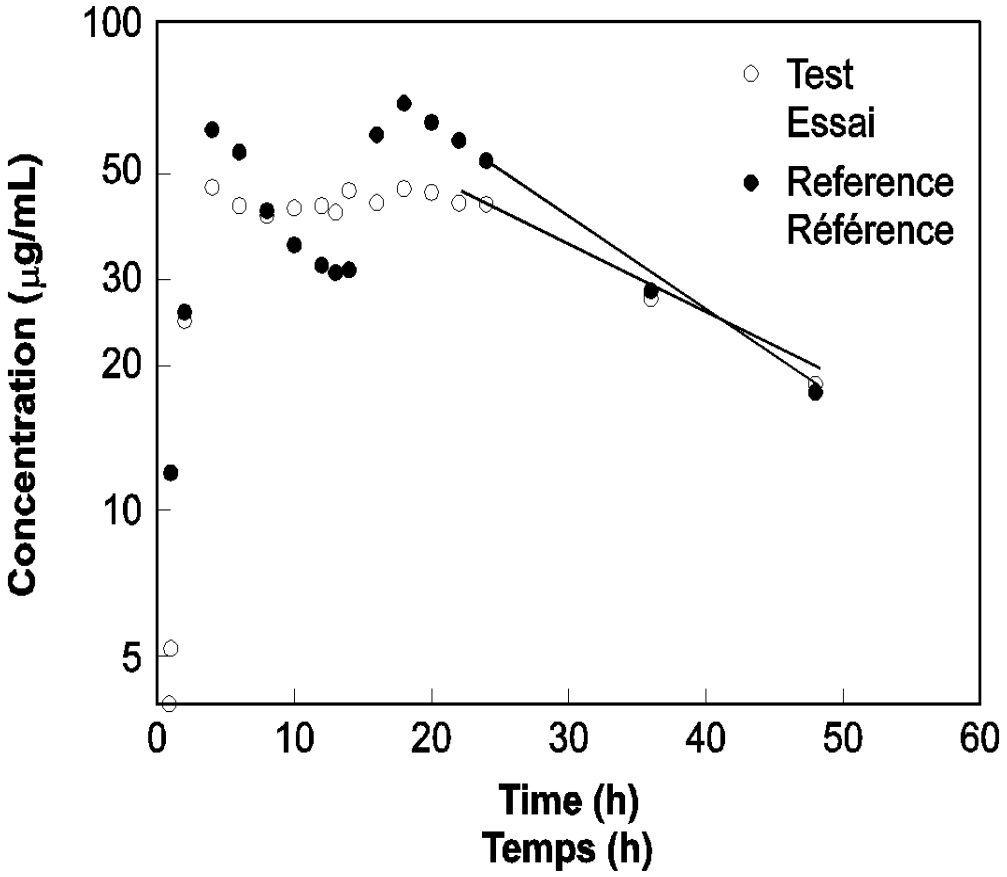

Figure 11.2: Ln(concentration)–Time Profile for Subject A

Figure 11.3 shows a profile of the arithmetic means over all subjects for each sampling time.

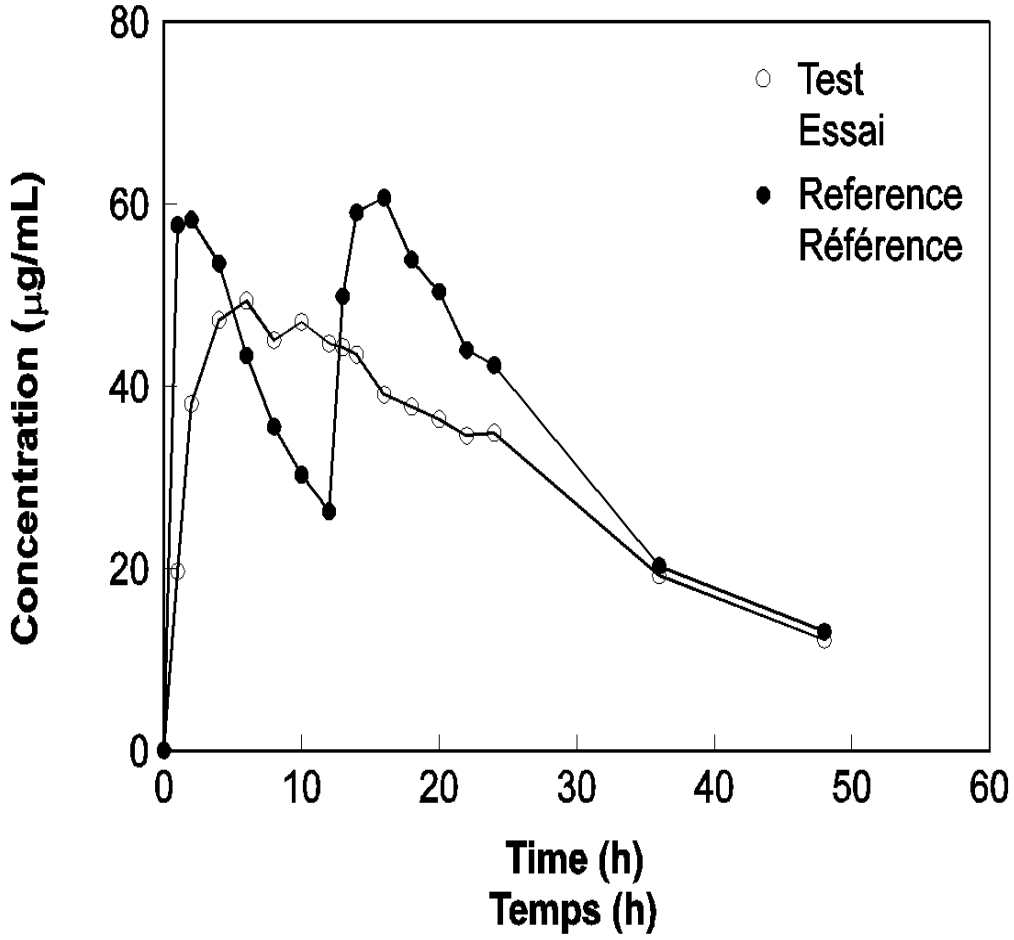

Figure 11.3: Average Concentration–Time Profile for All Subjects

Figure 11.4 shows a profile of the ln(arithmetic means) over all subjects for each sampling time.

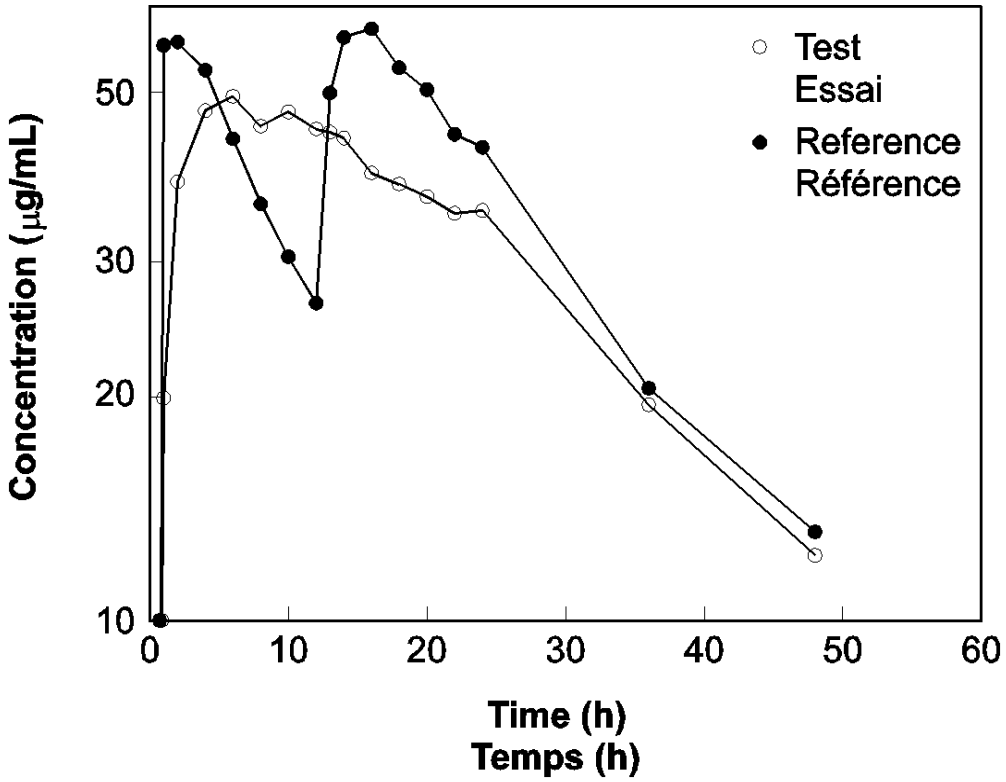

Figure 11.4: Ln(average concentration)–Time Profile for All Subjects

12 SAMPLE ANALYSIS FOR A MULTIPLE-DOSE COMPARATIVE BIOAVAILABILITY STUDY

The following tables and figures illustrate data collected and used in a sample multiple-dose comparative 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 reference formulation.

The analysis of a multiple-dose comparative bioavailability study must 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 12.1.)

b) A summary of drug concentrations (visual and quantitative) at each sampling time for each subject for both test and reference formulations. (See Section 12.2.)

c) A summary of the estimates of the parameters as defined in Section 12.3 for both test and reference formulations, including the means, standard deviations, and CVs. (See Section 12.4.)

d) A summary of pre-dose concentrations of the test and reference formulations. (See Section 12.5.)

e) A formal statistical analysis of the relevant parameters with comparisons of the test formulations to the reference formulations. (See Sections 12.6 through 12.9.)

f) A summary of corrections for potency (measured content) in estimates. (See Section 12.10.)

g) Sample concentration–time profiles that should be provided for each subject. (See Section 12.11.)

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

12.1 Randomization Scheme of the Design

Shown in Table 12-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 must be included. Even those subjects that did not complete the study must be included and identified accordingly. Subject numbers that appear on informed consent forms and reporting forms must 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 must be given.

12.2 Summary of Drug Concentrations

Tables 12-B and 12-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 limit of quantitation of the analytical method is given at this table.

TABLE 12-A
Randomization Scheme of the Cross-over Design for the Comparison of Test (T) Versus Reference (R) Formulations

Subject			Period	
Number	ID	Sequence	Feb. 14, 1989	Feb. 28, 1989
001	A	RT	R	T
002	B	TR	T	R
003	C	TR	T	R
004	D	RT	R	T
005*	E	TR	T	-
006	F	TR	T	R
007	G	RT	R	T
008	H	RT	R	T
009	I	RT	R	T
010	J	TR	T	R
011**	K	TR	-	-
012	L	RT	R	T
013	M	TR	T	R
014	N	RT	R	T

* Subject did not appear for second period.
** Subject did not appear for either period.

TABLE 12-B
Drug Concentrations* (μg/mL) for the Test Formulation

			Sampling Times (hours)																	
ID	Seq	Per.	0.0	1.0	2	4.0	6.0	8.0	10.0	12.0	13.0	14.0	16.0	18.0	20.0	22.0	24	36.0	48
A	RT	28 Feb	46.4	56.5	69.5	86.3	73.7	76.0	68.8	64.9	64.9	65.5	65.8	54.7	52.7	53.5	50.1	24.4	15.9
B	TR	14 Feb	31.0	40.9	50.1	56.1	50.4	47.2	39.2	35.8	32.8	29.2	25.5	22.6	21.4	19.3	17.6	9.6	6.6
C	TR	14 Feb	51.0	57.0	54.7	55.0	67.8	70.8	81.7	71.9	72.8	69.9	58.4	69.2	65.7	51.1	53.0	30.0	19.1
D	RT	28 Feb	31.3	43.6	64.5	62.5	67.5	58.9	59.9	50.4	58.7	59.6	46.2	39.8	40.5	36.4	45.0	23.0	14.1
F	TR	14 Feb	43.2	58.6	69.1	76.2	64.5	57.5	49.9	57.8	55.2	56.1	51.2	49.6	46.6	42.9	47.6	29.5	16.2
G	RT	28 Feb	48.7	52.6	60.5	99.2	79.1	74.0	82.2	82.3	67.7	70.4	55.1	55.5	47.8	47.0	59.7	25.7	14.3
H	RT	28 Feb	35.8	46.3	65.0	69.6	68.4	59.7	50.0	43.1	39.9	43.5	36.5	33.3	37.6	33.9	38.0	24.6	12.9
I	RT	28 Feb	47.0	44.9	52.1	88.0	75.7	60.4	52.2	46.6	4.05	38.6	32.9	35.0	36.6	35.8	31.9	17.3	9.3
J	TR	14 Feb	46.7	48.8	48.4	74.9	63.9	57.8	46.0	41.0	39.6	37.1	32.4	31.3	28.6	29.7	30.8	17.1	12.0
L	RT	28 Feb	60.9	61.0	60.2	82.2	67.0	62.0	53.9	46.2	43.8	41.2	30.6	30.6	29.0	29.0	27.6	13.7	7.7
M	TR	14 Feb	37.8	50.2	56.8	63.6	55.8	43.9	42.5	37.5	35.3	33.2	31.1	33.9	28.9	25.8	24.8	20.1	7.7
N	RT	28 Feb	38.9	34.4	49.1	79.9	65.7	70.2	82.1	71.6	67.0	73.4	65.1	61.4	55.7	53.0	56.2	29.2	17.0
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MEAN	—	—	43.2	49.6	58.3	74.5	66.6	61.5	59.0	54.1	51.5	51.5	44.2	43.1	40.9	38.1	40.2	22.0	12.7
STD	—	—	8.7	8.0	7.6	13.6	7.9	9.9	15.8	15.3	14.4	16.1	14.5	14.5	13.1	11.3	13.6	6.5	4.1
CV	—	—	20.1	16.1	13.0	18.2	11.9	16.1	26.8	28.3	27.9	31.2	32.8	33.7	32.1	29.7	33.9	29.7	32.3

* Limit of quantitation is 0.2μg/mL. Any concentration below this limit is reported as Below Quantitation Limit (BQL) except at time 0 and times before first observed concentration. However, in the calculation of summary statistics a zero is used.

TABLE 12-C
Drug Concentrations* (μg/mL) for the Reference Formulation

			Sampling Times (hours)																			
ID	Seq	Per.	0	1.0	2	4	6.0	8.0	10.0	12.0	13.0	14.0	16.0	18.0	20.0	22	24.0	36	48
A	RT	14 Feb	42.8	63.9	90.5	66.4	62.2	54.7	46.2	41.4	90.8	81.4	65.1	60.8	53.1	50.1	42.4	25.3	17.6
B	TR	28 Feb	46.0	101.2	80.9	67.1	53.8	44.7	40.2	34.8	56.3	77.9	61.6	52.2	45.9	45.1	40.4	19.4	10.1
C	TR	28 Feb	55.8	115.9	95.0	87.1	68.7	59.5	55.7	48.6	46.2	57.6	77.5	74.7	67.6	61.5	60.5	33.0	22.1
D	RT	14 Feb	36.2	48.3	96.5	67.9	59.3	50.4	39.7	33.3	81.6	92.4	67.9	50.6	47.8	41.5	38.0	18.2	10.8
F	TR	28 Feb	36.8	89.3	77.2	60.0	50.0	41.2	41.8	33.2	49.5	74.5	69.5	50.8	45.6	40.0	39.6	17.9	10.9
G	RT	14 Feb	52.1	77.8	103.2	78.7	64.1	51.3	43.2	38.5	35.6	52.5	64.3	70.7	65.0	53.7	49.2	23.3	12.8
H	RT	14 Feb	43.3	68.1	83.7	67.4	59.5	48.0	39.3	35.2	71.3	89.8	71.4	56.2	43.8	37.7	35.2	19.9	10.1
I	RT	14 Feb	46.6	87.7	102.1	89.2	64.2	53.8	47.9	40.4	36.4	48.9	67.8	69.3	66.8	57.9	54.7	27.4	13.7
J	TR	28 Feb	44.2	95.1	74.5	75.0	59.8	44.4	45.2	37.2	76.8	93.5	67.5	55.4	53.4	43.0	42.7	21.7	13.6
L	RT	14 Feb	48.5	90.1	95.8	71.4	62.4	49.4	49.7	41.9	39.4	65.1	75.5	72.5	57.0	49.5	46.6	13.9	12.3
M	TR	28 Feb	48.7	74.1	87.9	71.4	61.7	53.7	43.0	37.4	92.6	87.3	69.6	59.4	50.3	43.9	41.8	19.7	11.7
N	RT	14 Feb	43.9	102.1	93.0	73.4	58.0	50.5	42.7	40.1	44.5	57.4	66.7	68.9	63.4	57.1	55.9	36.4	17.3
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MEAN	—	—	45.4	84.5	90.0	72.9	60.3	50.1	44.6	38.5	60.1	73.2	68.7	61.8	55.0	48.4	45.6	23.0	13.6
STD	—	—	5.6	18.8	9.4	8.6	4.9	5.1	4.8	4.4	21.4	16.3	4.5	9.0	8.8	7.8	7.9	6.5	3.7
CV	—	—	12.6	22.3	10.4	11.8	8.1	10.1	10.7	11.4	35.6	22.3	6.5	14.5	16.0	16.0	17.3	28.4	27.0

* Limit of quantitation is 0.2μg/mL. Any concentration below this limit is reported as Below Quantitation Limit (BQL) except at time 0 and times before first observed concentration. However, in the calculation of summary statistics a zero is used.

12.3 List of Parameters and Definitions

Table 12-D shows a list of the parameters used in the analysis and their definitions. If any other parameters are used, they must also be clearly defined.

TABLE 12-D
Parameter Definitions

Parameter	Definition
C_{max}	Maximum observed concentration (µg/mL).
C_{min}	Minimum observed concentration (µg/mL).
C_{pd}	Pre-dose concentration from same time of each day (µg/mL).
T_{max}	Sampling time at which C_{max} occurred (h).
AUC_{τ}	Area under the concentration versus time curve, over the dosing interval of the test formulation, calculated using the linear trapezoidal rule (µg•h/mL).
Fluctuation	$(C_{max} - C_{min}) / (AUC_{\tau}/\tau) \times 100$.

12.4 Summaries of Parameters Estimates

Tables 12-E and 12-F list, for each subject, the estimates of the parameters defined in Table 12-D for the test and reference formulations respectively. Summary statistics (arithmetic means or medians, standard deviations, and CVs) should be given for each formulation. If the reference is an immediate-release formulation, C_{max} and T_{max} are taken from the dosing interval of largest peak (largest C_{max}).

TABLE 12-E
Summary of Parameters for Each Subject Given the Test Formulation

ID	Seq.	Period	Parameters				
			C_{max} (µg/mL)	C_{min} (µg/mL)	T_{max} (h)	AUC_{τ} (µg•h/mL)	FL** (%)
A	RT	28 Feb	86	46	4	1558	61
B	TR	14 Feb	56	18	4	843	110
C	TR	14 Feb	82	51	10	1542	48
D	RT	28 Feb	68	31	6	1248	70
F	TR	14 Feb.	76	43	4	1334	60
G	RT	28 Feb	99	47	4	1604	78
H	RT	28 Feb	70	33	4	1148	76
I	RT	28 Feb	88	32	4	1180	114
J	TR	14 Feb	75	29	4	1062	105
L	RT	28 Feb	82	28	4	1153	114
M	TR	14 Feb	64	25	4	971	96
N	RT	28 Feb	82	34	10	1534	75
. .	. .	. .	. .	. .	. .	. .	. .
MEAN*	-	-	77	35	4	1265	84
STD	-	-	12	10	2	251	23
CV	-	-	15	29	45	20	27

* For T_{max}, this is the median.
** Fluctuation.

TABLE 12-F
Summary of Parameters for Each Subject Given the Reference Formulation

ID	Seq.	Period	Parameters				
			C_{max} (µg/mL)	C_{min} (µg/mL)	T_{max} (h)	AUC_{τ} (µg•h/mL)	FL** (%)
A	RE	14 Feb	91	41	2	1456	81
B	ER	28 Feb	101	35	1	1333	120
C	ER	28 Feb	116	46	1	1657	101
D	RE	14 Feb	97	33	2	1370	111
F	ER	28 Feb.	89	33	1	1272	106
G	RE	14 Feb	103	36	2	1462	111
H	RE	14 Feb	84	34	2	1351	88
I	RE	14 Feb	102	36	2	1523	104
J	ER	28 Feb	95	37	1	1432	97
L	RE	14 Feb	96	39	2	1479	91
M	ER	28 Feb	88	37	2	1458	83
N	RE	14 Feb	102	40	1	1472	101
. .	. .	. .	. .	. .	. .	. .	. .
MEAN*	-	-	97	37	2	1439	99
STD	-	-	9	4	1	100	12
CV	-	-	9	10	33	7	12

* For T_{max}, this is the median.
** Fluctuation.

12.5 Pre-dose Concentrations

Tables 12-G and 12-H list the pre-dose concentrations for each subject on each day of the study for the test and reference formulations respectively.

These tables are used to check for both compliance and whether steady-state concentrations had been reached during the study. Intra-subject statistics should be calculated for the last three pre-dose concentrations.

TABLE 12-G
Pre-dose Concentrations (μg/mL) for Each Subject Given the Test Formulation

	Day						Intra-Subject Statistics*		
ID	3	4	5	6	7	8	MEAN	STD	CV
A	18	37	37	41	46	50	46	5	10
B	8	33	54	44	31	18	31	13	42
C	22	60	43	45	51	53	50	4	8
D	7	31	31	48	31	45	41	9	22
F	7	6	14	41	43	48	44	4	8
G	14	49	44	36	49	60	48	12	25
H	6	28	31	34	36	38	36	2	5
I	14	20	68	24	47	32	34	12	34
J	18	35	48	37	47	31	38	8	21
L	6	31	34	35	61	28	41	18	43
M	7	20	49	31	38	25	31	6	21
N	19	61	42	39	39	56	45	10	22
. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
MEAN	12	34	41	38	43	40	-	-	-
STD	6	16	14	6	9	14	-	-	-
CV	49	47	33	17	20	34	-	-	-

* Based on concentrations from days 6, 7, and 8.

TABLE 12-H
Pre-dose Concentrations (μg/mL) for Each Subject Given the Reference Formulation

	Day						Intra-Subject Statistics*		
ID	3	4	5	6	7	8	MEAN	STD	CV
A	17	45	53	47	43	42	44	2	5
B	12	41	43	38	46	40	42	4	10
C	18	54	55	63	56	61	60	4	6
D	11	36	39	32	36	38	35	3	9
F	11	31	32	35	37	40	37	3	7
G	12	43	47	47	52	49	49	3	5
H	9	36	40	31	34	35	34	2	6
I	15	49	67	46	47	55	49	5	10
J	14	39	45	39	44	43	42	2	6
L	14	48	45	46	49	47	47	1	3
M	12	33	42	39	49	42	43	5	12
N	14	44	40	41	44	56	47	8	17
. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
MEAN	13	42	46	42	45	46	-	-	-
STD	3	7	9	9	6	8	-	-	-
CV	20	17	20	21	14	17	-	-	-

* Based on concentrations from days 6, 7, and 8.

A repeated measures analysis of the last three pre-dose concentrations should be provided. An example of this analysis is shown in Table 12-I. Of main importance in this analysis is the time and time*form interaction. The error term to test time is the time*ID (Seq). The error term to test time*form is time*form*ID (Seq). Should either the time or time*form effects be significant, the study may not have been at steady state for one or both formulations.

TABLE 12-I
Repeated Measures Analysis of $\ln(C_{pd})$

Source	df	SS	MS	F	PR > F
Seq	1	0.01500416	0.01500416	0.12	0.7313
ID (Seq)	10	1.20256303	0.12025630	2.11	0.1278
Period	1	0.04556284	0.04556284	0.80	0.3926
Form	1	0.22620312	0.22620312	3.96	0.0745
ID*Form (Seq)	10	0.57066567	0.0570666	-	-
Time	2	0.11182757	0.05591378	1.60	0.2274
Time*Seq	2	0.13927056	0.06963528	1.99	0.1631
Time*ID (Seq)	20	0.70058566	0.03502928	-	-
Time*Period	2	0.07672565	0.03836282	1.24	0.3110
Time*Form	2	0.06390201	0.03195101	1.03	0.3746
Time*Form*ID (Seq)	20	0.61937447	0.03096872	-	-
Corrected Total	71	3.74093818	-	-	-

12.6 AUC_τ Analysis

Tables 12-J, 12-K, and 12-L provide the complete analysis required for AUC_τ. Table 12-J lists the AUC_τ estimates on the raw scale and the log scale. Also given is the test AUC_τ as a percentage of the reference AUC_τ. Summary statistics are calculated for each variable.

TABLE 12-J
AUC_τ (μg•h/mL) Analysis—Data

ID	Raw Scale			Log Scale	
	Test AUC_τ	Reference AUC_τ	Relative AUC_τ (%)	Test $\ln(AUC_\tau)$	Reference $\ln(AUC_\tau)$
A	1558	1456	107	7.35	7.28
B	843	1333	63	6.74	7.19
C	1542	1657	93	7.34	7.41
D	1248	1370	91	7.13	7.22
F	1334	1272	105	7.20	7.15
G	1604	1462	110	7.38	7.29
H	1148	1351	85	7.05	7.21
I	1180	1523	77	7.07	7.33
J	1062	1432	74	6.97	7.27
L	1153	1479	78	7.05	7.30
M	971	1458	67	6.88	7.28
N	1534	1472	104	7.34	7.29
. . .	. . .	. . .	. . .	. . .	. . .
MEAN	1265	1439	88	7.12	7.27
STD	251	100	16	0.21	0.07
CV	20	7	18	-	-

Table 12-K gives the analysis of variance (ANOVA) for the cross-over design model for ln(AUC_τ). This analysis gives the appropriate intra-subject 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 intra-subject and inter-subject CVs should also be calculated.

TABLE 12-K
AUC_τ (μg•h/mL) Analysis—ANOVA for ln(AUC_τ)

Source	df	SS	MS	F	PR > F
Seq	1	0.0495	0.0495	1.85	0.204
Subject (Seq)	10	0.2685	0.0269	1.66	0.217
Period	1	0.0362	0.0362	2.24	0.165
Form	1	0.1467	0.1467	9.09	0.013
Residual	10	0.1614	0.0161	-	-
Intra–subject CV = 100 x (MS Residual)$^{0.5}$ = 100 x (0.0161)$^{0.5}$ = 13 percent					

The AUC_τ ratio estimate and its 90% confidence interval are derived in the calculations shown in Table 12-L. If this study had a balanced design (i.e., an equal number of subjects per sequence) the difference would simply be the difference in the arithmetic means of the ln(AUC)s. Since the study was not balanced, the least-squares mean estimate for each formulation is used to form this difference, together with the appropriate standard error.

TABLE 12-L
AUC_τ (μg•h/mL) Analysis—Calculations

$$\text{Difference} = \text{Test } \bar{x} - \text{Reference } \bar{x} = 7.1095 - 7.2681 = -0.1586$$

$$SE_{Difference} = 0.0526$$

$$AUC_T \text{ Ratio} = 100 \times e^{Difference} = 100 \times e^{-0.1586} = 85\%$$

90% Confidence Limits

$$\text{Lower, Upper} = 100 \times e^{(Difference \pm t_{0.025,\ 10} \times SE_{Difference})}$$

$$\text{Lower} = 100 \times e^{(-0.1586 - 1.812 \times 0.0526)} = 78\%$$

$$\text{Upper} = 100 \times e^{(-0.1586 + 1.812 \times 0.0526)} = 94\%$$

12.7 C_{max} Analysis

The necessary information and summary for the analyses of C_{max} are shown in Tables 12-M, 12-N, and 12-O.

For this example, the C_{max} ratio passes the bioavailability criterion.

TABLE 12-M
C_{max} (µg/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	86	91	95	4.46	4.51
B	56	101	55	4.03	4.62
C	82	116	70	4.40	4.75
D	68	97	70	4.21	4.57
F	76	89	85	4.33	4.49
G	99	103	96	4.60	4.64
H	70	84	83	4.24	4.43
I	88	102	86	4.48	4.63
J	75	95	79	4.32	4.55
L	82	96	86	4.41	4.56
M	64	88	72	4.15	4.48
N	82	102	80	4.41	4.63
. . .	. . .	. . .	. . .	. . .	. . .
MEAN	77	97	80	4.34	4.57
STD	12	9	12	0.16	0.09
CV	15	9	14	-	-

TABLE 12-N

C_{max} (μg/mL) Analysis—ANOVA for ln(C_{max})

Source	df	SS	MS	F	PR > F
Seq	1	0.0287	0.0287	1.44	0.258
Subject (Seq)	10	0.1198	0.0200	2.27	0.106
Period	1	0.0412	0.412	4.68	0.056
Form	1	0.3591	0.3591	4.09	<0.001
Residual	10	0.0879	0.0088	-	-
Intra–subject CV = 9 percent					

TABLE 12-O

C_{max} Analysis—Calculations

$$C_{max}\ \text{Ratio} = 100 \times e^{\text{Difference}} = 100 \times e^{(4.3235 - 4.5717)} = 78\%$$

12.8 C_{min} Analysis

The necessary information and summary for the analyses of C_{min} are shown in Tables 12-P, 12-Q, and 12-R.

TABLE 12-P
C_{min} (μg/mL) Analysis—Data

ID	Raw Scale			Log Scale	
	Test C_{min}	Reference C_{min}	Relative C_{min} (%)	Test $\ln(C_{min})$	Reference $\ln(C_{min})$
A	46	41	112	3.84	3.72
B	18	35	51	2.87	3.55
C	51	46	110	3.93	3.83
D	31	33	94	3.44	3.51
F	43	33	129	3.76	3.50
G	47	36	132	3.85	3.57
H	33	35	97	3.51	3.54
I	32	36	88	3.46	3.59
J	29	37	77	3.35	3.62
L	28	39	70	3.32	3.67
M	25	37	66	3.21	3.62
N	34	40	86	3.54	3.69
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
MEAN	35	37	93	3.51	3.62
STD	10	4	25	0.31	0.10
CV	29	10	27	-	-

TABLE 12-Q

C_{min} (μg/mL) Analysis—ANOVA for ln(C_{min})

Source	df	SS	MS	F	PR > F
Seq	1	0.0245	0.0245	0.37	0.559
ID (Seq)	0	0.6694	0.0669	1.62	0.228
Period	1	0.0334	0.0334	0.81	0.389
Form	1	0.0902	0.0902	2.19	0.17
Residual	0	0.4120	0.0412	-	-
Intra–subject CV = 20 percent					

TABLE 12-R

C_{min} Analysis—Calculations

$$C_{min}\ \text{Ratio} = 100\ X\ e^{\text{Difference}} = 100\ x\ e^{(3.4948\ -\ 3.6192)} = 88\%$$

12.9 Fluctuation Analysis

The necessary information and summary for the analyses of Fluctuation are provided in Tables 12-S and 12-T.

TABLE 12-S

Fluctuation (%) Analysis—Data

ID	Test Fluctuation	Reference Fluctuation
A	61	81
B	110	120
C	48	101
D	70	111
F	60	106
G	78	111
H	76	88
I	114	103
J	105	97
L	114	91
M	96	83
N	75	101
. . .	. . .	. . .
MEAN	84	99
STD	23	12
CV	27	12

TABLE 12-T

Fluctuation Analysis—ANOVA for Fluctuation

Source	df	SS	MS	F	PR > F
Seq	1	12.8	12.8	0.03	0.863
Subject (Seq)	10	3879.3	388.0	1.13	0.427
Period	1	18.9	18.9	0.05	0.819
Form	1	1484.5	1484.5	4.31	0.065
Residual	10	3440.5	344.1	-	-
Intra–subject CV = 20 percent					

12.10 Calculations for AUC_{τ} Ratio, C_{max} Ratio, and C_{min} Ratio Estimates Corrected for Measured Content

The whole analysis need not be repeated; only the corrected estimates need be given.

TABLE 12-U

Estimates Based on Correction for Measured Content

	Test Formulation	Reference Formulation
Lot number	EX110	40905
Expiry date	06/90	05/90
Date of analysis	06/14/88	06/14/88
Measured content (% of label claim)	95.5	99
Correction factors		
! raw scale-multiply	1.0471	1.0101
! log scale-add	0.0460	0.0100

TABLE 12-V

AUC_τ Ratio, C_{max}, Ratio and C_{min} Ratio—Calculations

Based on measured contents in Table 12-U, the following factor is obtained:

$$\ln\left(\frac{99.0}{95.5}\right) = 0.0360$$

which is to be added to the estimates on the log scale.

Therefore:

$$AUC_\tau \text{ Ratio} = e^{(-0.1586 + 0.0360)} \times 100 = 93\%$$

90% Confidence Limits

$$\text{Lower Limit} = 100 \times e^{(-0.1586 + 0.0360 - 1.812 \times 0.0526)} = 80\%$$

$$\text{Upper Limit} = 100 \times e^{(-0.1586 + 0.0360 + 1.812 \times 0.0526)} = 97\%$$

$$C_{max} \text{ Ratio} = 100 \times e^{(-0.2482 + 0.0360)} = 81\%$$

$$C_{min} \text{ Ratio} = 100 \times e^{(-0.1244 + 0.0360)} = 92\%$$

12.11 Concentration–Time Profiles (Subject A)

Figure 12.1 shows a plot of the concentration–time profile for subject A. Each plot must include profiles for all formulations given to that subject. Similar profiles should be given for each subject.

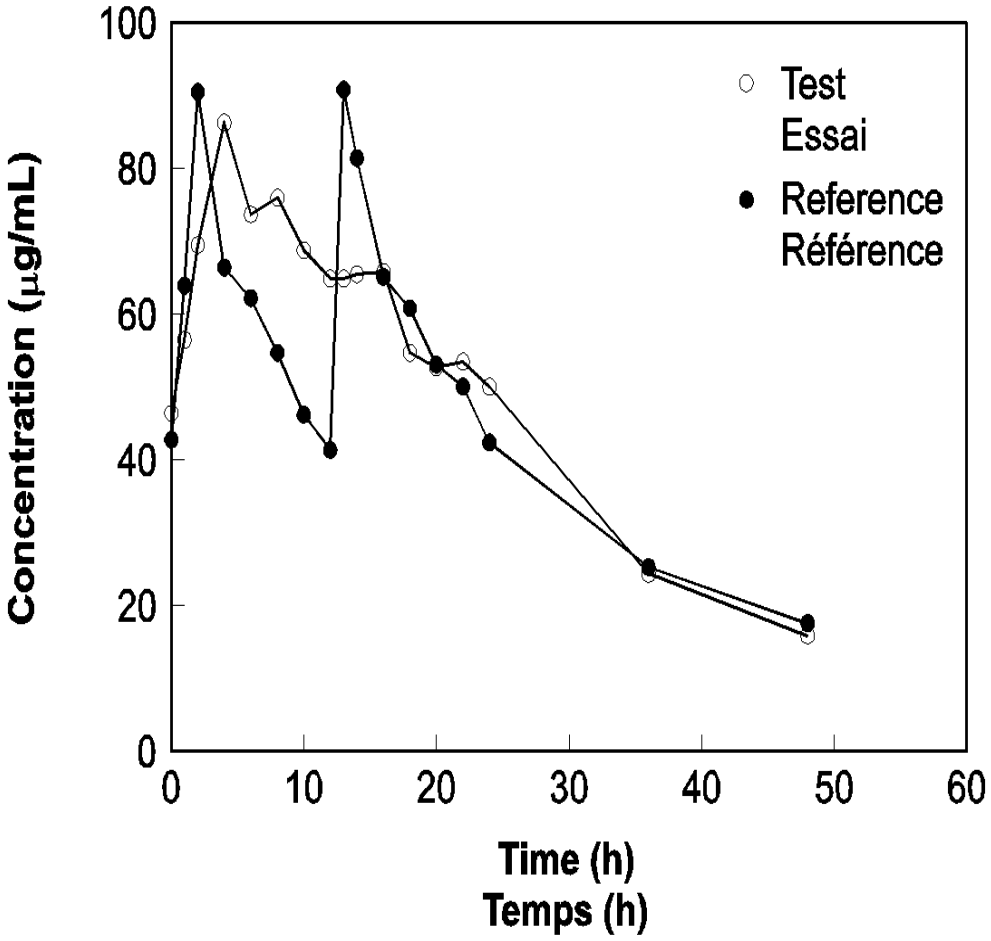

Figure 12.1: Concentration–Time Profile for Subject A

Figure 12.2 gives a plot of the ln(concentration)–time profile for subject A. This plot must contain the regression lines from which the terminal disposition rate constants (λ) were estimated. This line must 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 must be identified.

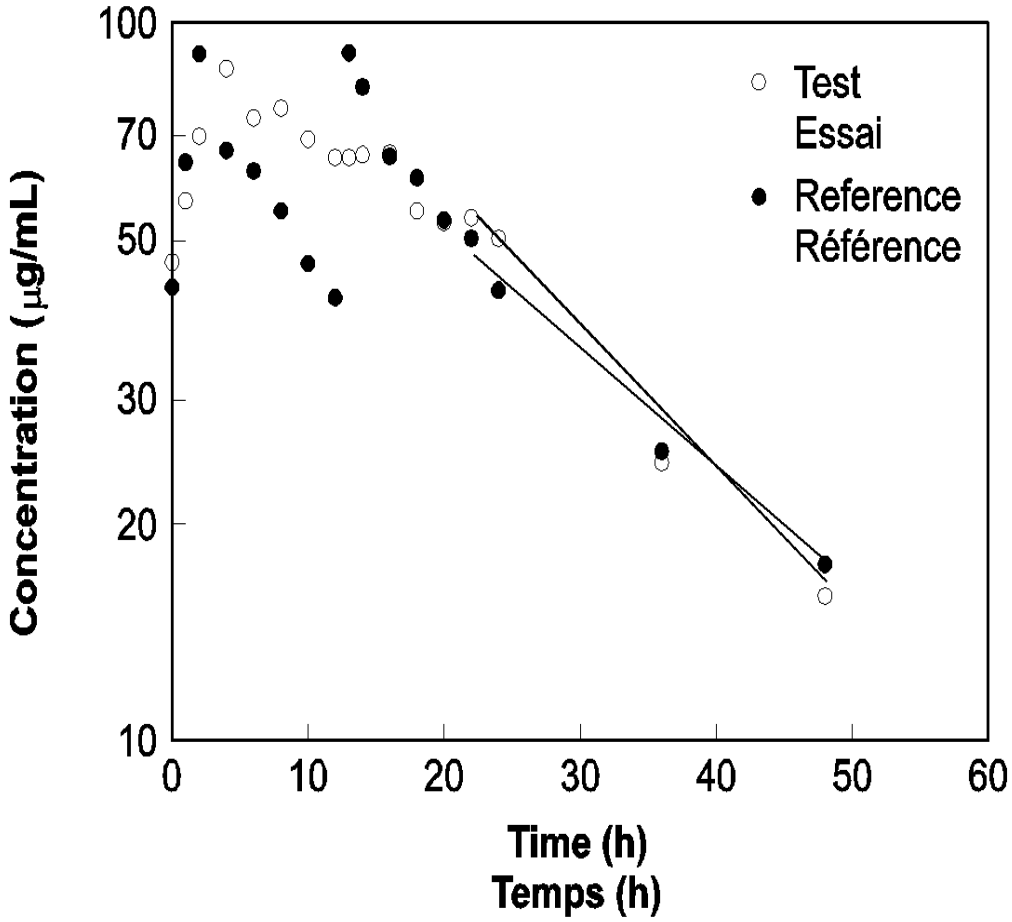

Figure 12.2: Ln(concentration)–Time Profile for Subject A

Figure 12.3 shows a profile of the arithmetic means over all subjects for each sampling time.

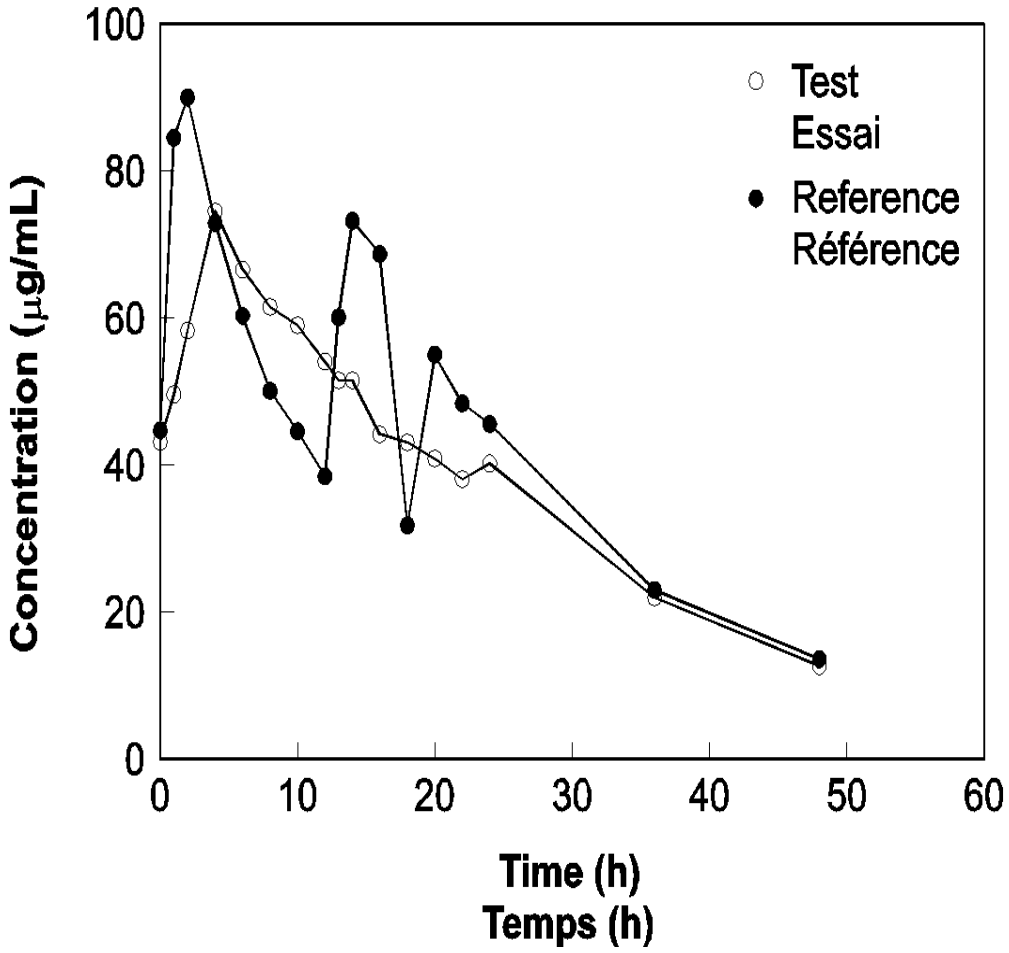

Figure 12.3: Average Concentration–Time Profile for All Subjects

Figure 12.4 shows a profile of the ln(arithmetic means) over all subjects for each sampling time.

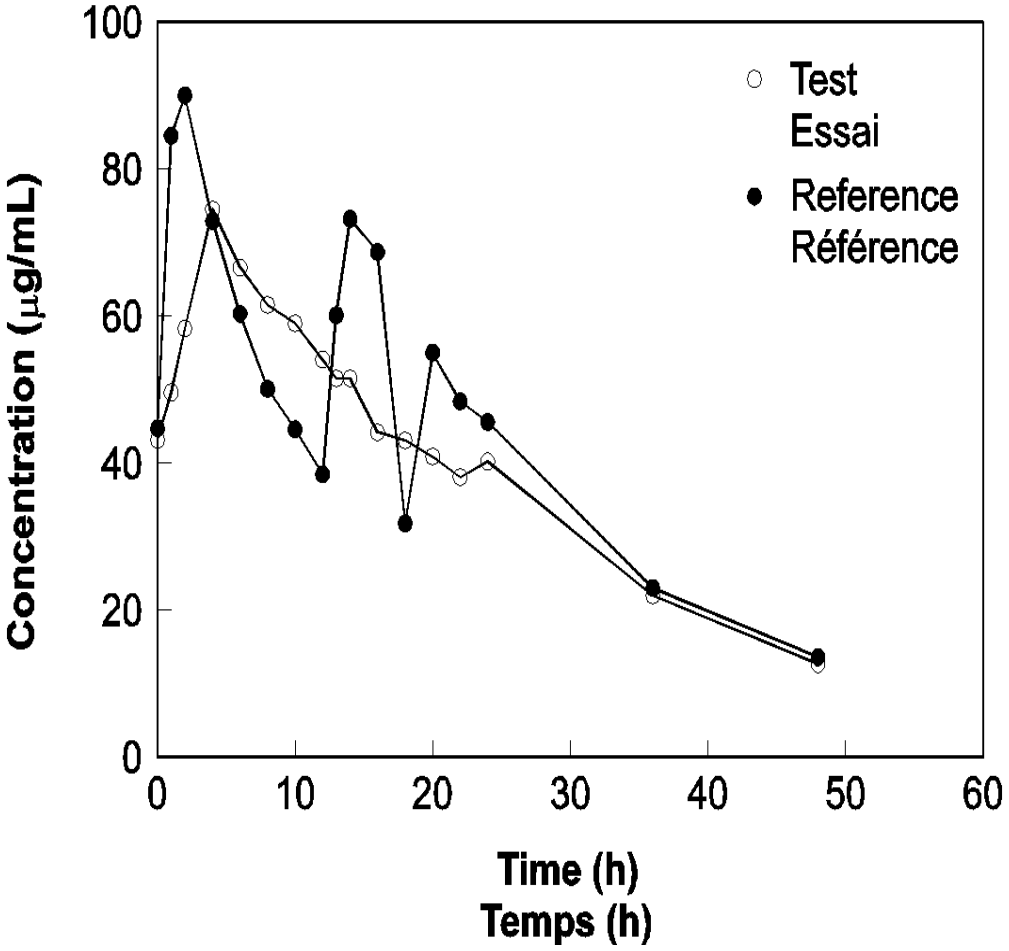

Figure 12.4: Ln(average concentration)–Time Profile for All Subjects

13 SAMPLE ANALYSIS FOR A SINGLE-DOSE BIOEQUIVALENCE STUDY

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

Although a bioequivalence study may include many formulations, the basic analysis is the same–each test formulation is compared to a reference formulation.

The analysis of a single-dose comparative bioequivalence study must 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. (See Section 13.1.)

b) A summary of drug concentrations (visual and quantitative) at each sampling time for each subject for the test and reference formulations being compared. (See Section 13.2.)

c) A summary of the estimates of the parameters as defined in Section 13.3 for the test and reference formulations being compared, including the means, standard deviations, and CVs. (See Section 13.4.)

d) A formal statistical analysis of the relevant parameters with comparisons of the test formulations to the reference formulations. (See Sections 13.5 through 13.9.)

e) A summary of corrections for potency (measured content) in estimates. (See Section 13.10.)

f) Sample concentration–time profiles that should be given for each subject. (See Section 13.11.)

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

13.1 Randomization Scheme of the Design

Shown in Table 13-A is the randomization scheme of the cross-over design used in the study. In any study, all subjects who were randomized into the study must be included. Even those subjects that did not complete the study must be included and identified accordingly. Subject numbers that appear on informed consent forms and reporting forms must 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 must be given.

13.2 Summary of Drug Concentrations

Tables 13-B and 13-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 limit of quantitation of the analytical method is given in this table, along with the measured potency for the formulation.

TABLE 13-A

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

Subject			Period	
Number	ID	Sequence	Aug. 23, 1995	Aug. 30, 1995
001	1	TR	T	R
002	2	RT	R	T
003	3	RT	R	T
004	4	TR	T	R
005*	5	TR	T	-
006	6	RT	R	T
007	7	TR	T	R
008	8	RT	R	T
009	9	RT	R	T
010	10	TR	T	R
011**	11	TR	-	-
012	12	TR	T	R
013	13	TR	T	R
014	14	RT	R	T

* Subject did not appear for second period.

** Subject did not appear for either period.

TABLE 13-B

Drug Concentrations* (μg/mL) for the Test Formulation

			Sampling Times (hours)																					
ID	Seq	Per.	0.0	1.0	2.0	3.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	20.0	24.0	28.0	32.0	36.0	40.0	48.0
1	TR	23 Aug	0.0	0.0	0.0	16.9	4.8	13.8	13.6	10.6	11.0	14.1	14.6	10.0	6.52	4.61	27.7	13.6	8.2	9.37
2	RT	30 Aug	0.0	0.0	0.0	7.87	31.6	63.6	63.9	63.3	99.3	118	111	82	4.93	34.6	13.9	8.64	0.0	6.89
3	RT	30 Aug	0.0	0.0	20.1	11.4	16.3	11.8	7.3	67.8	99.1	13.4	14.8	10.6	10.7	72.7	31.5	14.3	9.95	0.0
4	TR	23 Aug	0.0	0.0	8.4	67.4	10.2	14.0	88.6	10.6	95.4	94.5	92.3	72.5	66.5	28.1	12.1	6.45	0.0	0.0
5	RT	30 Aug	0.0	0.0	0.0	24.1	6.9	96.7	94.8	76.5	9.7	10.9	11.6	89.5	99.3	61.4	31.7	13	7.14	0.0
7	TR	23 Aug	0.0	0.0	0.0	13.8	33.4	66.4	60.5	62	88.1	90.7	7.1	67.5	61.7	36.8	24.8	13.2	8.93	0.0
8	RT	30 Aug	0.0	0.0	0.0	3.6	62.2	60.7	45.8	39.4	41.2	3.7	36.9	26.5	22.6	16.3	10.5	6.88	0.0	0.0
9	RT	30 Aug	0.0	0.0	9.87	36.1	84.7	12.9	12.8	13.0	18.0	17.2	16.3	91.7	71.1	48.4	21	11.8	6.88	0.0
10	TR	23 Aug	0.0	0.0	5.71	32.5	63.9	72.4	69.5	41.5	43.1	9.5	86.3	82.4	69	62	41.2	22.8	16	7.84
12	TR	23 Aug	0.0	0.0	30.7	12.1	43.6	11.0	89.7	91.5	10.4	90	82.6	60	67	60.8	29	24.5	36.9	17.7
13	TR	23 Aug	0.0	0.0	1.4	76.6	92.5	94.9	63.9	60.1	7.0	73.2	61.3	48.1	39.9	29.1	16.6	9.03	0.0	0.0
14	RT	30 Aug	0.0	0.0	0.0	23.7	47.9	4.7	31.7	19.6	39.2	6.1	63.4	42.1	39.6	34.7	17.9	10.2	7.6	0.0
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MEAN	.	.	0	0	7.4	47.3	68.5	93.8	76.3	71.1	88.9	98.8	97.4	71.5	63.2	43.3	23.1	12.7	8.43	3.4
STD	.	.	0	0	9.83	38.4	34.9	33.5	32.2	32.3	38.8	38.6	38.1	24.9	25.4	16.5	9.47	6.92	10.3	6.71
CV	.	.	.	.	134	81.2	50.9	35.7	42.2	45.4	43.6	39	39.1	34.9	40.2	38.1	41	46.7	122	188

* Limit of quantitation is 0.2μg/mL. Any concentration below this limit is reported as Below Quantitation Limit (BQL) except at time 0 and times before first observed concentration. However, in the calculation of summary statistics a zero is used. Measured content is 98%.

TABLE 13-C

Drug Concentrations* (μg/mL) for the Reference Formulation

			Sampling Times (hours)																					
ID	Seq	Per.	0.0	1.0	2.0	3.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	20.0	24.0	28.0	32.0	36.0	40.0	48.0
1	TR	30 Apr	0.0	0.0	0.0	0.0	64.2	133	862	87.7	131	143	104	83.6	63.4	40.9	19.6	9.3	0.0	0.0
2	RT	23 Apr	0.0	0.0	0.0	0.0	18	83.7	632	35	49.2	71.8	117	81.1	66.4	36.8	18.4	7.84	14.2	7.88
3	RT	23 Apr	0.0	0.0	0.0	16.9	112	210	100	93	178	211	243	145	107	64.1	30.5	16.5	11	0.0
4	TR	30 Apr	0.0	0.0	0.0	0.0	8.32	130	94	63.8	66.5	63.5	37	20.8	18.7	8.14	0.0	0.0	0.0	0.0
5	RT	23 Apr	0.0	0.0	0.0	0.0	42	104	65.7	48.7	92.3	129	160	125	116	66.5	26.7	13.3	8.8	0.0
7	TR	30 Apr	0.0	0.0	0.0	0.0	31.6	111	89.6	69	107	130	118	79.8	85	55.7	30.5	12.3	7.48	0.0
8	RT	23 Apr	0.0	0.0	0.0	0.0	0.0	46.5	32.4	19	27.2	35.9	37	33	28.4	19.6	11.6	8.43	5.68	0.0
9	RT	23 Apr	0.0	0.0	0.0	9.83	158	149	112	147	22.7	198	151	98.6	63.9	38.1	19.7	8.84	6.12	5.68
10	TR	30 Apr	0.0	0.0	0.0	0.0	13.4	124	90.4	47.9	41.4	63	89.5	114	143	105	53.7	30.1	19	9.83
12	TR	30 Apr	0.0	36.3	11.8	13.3	131	113	82.4	68.7	80.6	101	85.6	76.4	70.5	63.2	19.5	8.05	14.2	11.5
13	TR	30 Apr	0.0	0.0	0.0	0.0	0.0	69.3	49.3	34	66.7	81.1	83.4	66	71.6	63.3	26.8	17.4	10.4	8.23
14	RT	23 Apr	0.0	0.0	0.0	0.0	14.4	106	68.1	36.1	71.8	76.5	74.6	67.4	66.5	47.1	25.9	16.1	8.69	0.0
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MEAN	.	.	D	2.94	9.81	13.2	48.6	111	76.9	62.4	94.8	107	108	82.7	73.3	49.8	23.6	12.3	8.78	6.07
STD	.	.	D	10.2	34	37.9	54.7	42.4	23.4	36	68.6	66.6	66.8	36.6	36.4	24.9	12.8	7.36	6.69	6.74
CV	.	.	.	346	346	287	113	37.1	30.5	58	61.8	62.9	62.5	43	49.2	50.1	54.3	59.9	83.6	141

* Limit of quantitation is 0.2 μg/mL. Any concentration below this limit is reported as Below Quantitation Limit (BQL) except at time 0 and times before first observed concentration. However, in the calculation of summary statistics a zero is used. Measured content is 95%.

13.3 List of Parameters and Definitions

Table 13-D shows a list of the parameters used in the analysis and their definitions. If any other parameters are used, they must also be clearly defined.

TABLE 13-D

Parameter Definitions

Parameter	Definition
C_{max}	Maximum observed concentration (µg/mL).
T_{max}	Sampling time at which C_{max} occurred (h).
AUC_X	Area under the raw concentration versus time curve, over the dosing interval of the test formulation, calculated using the linear trapezoidal rule (µg•h/mL).
AUC_T	Area under the raw concentration versus time curve calculated using the linear trapezoidal rule from time 0 to LQCT (µg•h/mL).
AUC_I	Area to infinity = $AUC_T + C_T/\lambda$, where C_T is the estimated concentration at LQCT (λgh/mL).
$\frac{AUC_T}{AUC_I} \times 100$	Percent of the area measured by AUC_T relative to the extrapolated total AUC (%).
$\frac{AUC_X}{AUC_I} \times 100$	Percent of the area measured by AUC_X 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 limit of quantitation (h).
$T_{½}$	Drug half-life = $In2/\lambda = 0.693/\lambda$ (h).

13.4 Summaries of Parameter Estimates

Tables 13-E and 13-F list, for each subject, the estimates of the parameters defined in Table 13-D for the test and reference formulations respectively. Summary statistics (arithmetic means or medians, standard deviations, and CVs) should be given for each formulation.

The AUC_X/AUC_I ratio is used to determine whether the drug accumulates. For this example, the mean ratio of less than 80 percent indicates that the drug accumulates. Therefore, a multiple-dose study must be run.

The AUC_T/AUC_I ratio is used to determine whether the subjects were sampled for a sufficient length of time. For this example the mean ratio is greater than 80 percent, indicating that the subjects were sufficiently sampled.

TABLE 13-E

Summary of Parameters for Each Subject Given the Test Formulation

ID	Seq	Per.	Parameters										
			C_{max} (µg/mL)	T_{max} (h)	AUC_X (µg•h/mL)	AUC_T (µg•h/mL)	AUC_I (µg•h/mL)	$\frac{AUC_X}{AUC_I}$ (%)	$\frac{AUC_T}{AUC_I}$ (%)	λ (h^{-1})	TLIN (h)	LQCT (h)	$T_{1/2}$ (h)
1	TR	23 Aug	146	16.00	2321	2884	3001	77	96	0.0631	32	48.00	10.98
2	RT	30 Aug	118	14.00	1612	1922	1977	82	97	0.1544	24.00	36	4.49
3	RT	30 Aug	153	4.00	2433	3204	3257	75	98	0.0991	32.00	48	6.99
4	TR	23 Aug	140	6.00	1958	2422	2242	86	99	0.1967	24.00	36.00	3.52
6	RT	30 Aug	116	16.00	1967	2604	2641	74	99	0.1837	28.00	40.00	5.77
7	TR	30 Aug	91	14.00	1303	1720	1793	73	96	0.1201	28.00	40.00	5.77
8	RT	30 Aug	62	4.00	832	995	1047	79	95	0.1157	24.00	36.00	5.99
9	RT	30 Aug	180	12.00	2619	3100	3138	83	99	0.1640	28.00	40.00	4.23
10	TR	23 Aug	89	24.00	1484	2293	2367	63	97	0.1007	32.00	48.00	6.88
12	TR	23 Aug	121	3.00	1809	2653	3431	53	77	0.0264	32.00	48.00	26.21
13	TR	23 Aug	95	6.00	1388	1665	1739	80	96	0.1270	24.00	40.00	5.37
14	RT	30 Aug	63	16.00	910	1256	1309	70	96	0.1290	28.00	40.00	5.37
. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
MEAN*	—	—	115	13.00	1720	2211	2331	75	95	0.1233	28.00	40.00	7.47
STD	—	—	36	6.57	572	713	782	9	6	0.0485	3.41	5.21	6.23
CV	—	—	32	58.37	33	32	34	13	6	39.32	12.18	12.61	83.3

* For T_{max}, TLIN, and LQCT, these are the medians.

TABLE 13-F

Summary of Parameters for Each Subject Given the Reference Formulation

ID	Seq.	Per.	Parameters										
			C_{max} (µg/mL)	T_{max} (h)	AUC_X (µg•h/mL)	AUC_T (µg•h/mL)	AUC_I (µg•h/mL)	$\frac{AUC_X}{AUC_I}$ (%)	$\frac{AUC_T}{AUC_I}$ (%)	λ (h^{-1})	TLIN (h)	LQCT (h)	$T_{1/2}$ (h)
1	TR	30 Aug	143	14.00	1993	2361	2429	82	97	0.1495	24.00	36.00	4.64
2	RT	23 Aug	117	16.00	1420	1917	2113	67	91	0.0398	32.00	48.00	17.40
3	RT	23 Aug	243	16.00	3292	3969	4035	82	98	0.1490	28.00	40.00	4.65
4	TR	30 Aug	130	6.00	1060	1114	1193	89	93	0.1162	16.00	28.00	5.96
6	RT	23 Aug	160	16.00	2159	2833	2879	75	98	0.1692	28.00	40.00	4.10
7	TR	30 Aug	130	14.00	1911	2490	2530	76	98	0.1732	28.00	40.00	4.00
8	RT	23 Aug	47	6.00	622	848	902	69	94	0.1022	28.00	40.00	6.78
9	RT	23 Aug	227	12.00	2889	3342	3406	85	98	0.0722	32.00	48.00	9.59
10	TR	30 Aug	143	24.00	1746	2941	3029	58	97	0.1042	32.00	48.00	6.65
12	TR	30 Aug	133	3.00	2078	2737	1153	180	238	-0.010	32.00	48.00	-71.6
13	TR	30 Aug	83	16.00	1236	1865	1965	63	95	0.0734	32.00	48.00	9.44
14	RT	23 Aug	106	6.00	1341	1827	1890	71	97	0.1394	28.00	40.00	4.97
. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
MEAN*	—	—	138	14.00	1812	2354	2294	83	108	0.1066	28.00	40.00	0.55
STD	—	—	54	6.05	755	896	953	32	41	0.0551	4.66	6.27	23.04
CV	—	—	39	48.74	42	38	52	39	38	51.75	16.44	14.92	4220

***** For T_{max}, TLIN, and LQCT, these are the medians.

13.5 AUC_T Analysis

Tables 13-G, 13-H, and 13-I provide the complete analysis required for AUC_T. Table 13-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 provided for each variable.

TABLE 13-G
AUC_T (µg•h/mL) Analysis—Data

ID	Raw Scale			Log Scale	
	Test AUC_T	Reference AUC_T	Relative AUC_T (%)	Test $\ln(AUC_T)$	Reference $\ln(AUC_T)$
1	2884.06	2360.72	122.17	7.97	7.77
2	1922.05	1916.80	100.27	7.56	7.56
3	3204.37	3968.85	80.74	8.07	8.29
4	2242.25	1113.89	201.30	7.72	7.02
6	2604.06	2832.96	91.92	7.86	7.95
7	1720.14	2489.54	69.09	7.45	7.82
8	995.12	848.23	117.32	6.90	6.74
9	3099.54	3342.32	92.74	8.04	8.11
10	2292.55	2941.48	77.94	7.74	7.99
12	2652.71	2737.45	96.90	7.88	7.91
13	1665.04	1865.01	89.28	7.42	7.53
14	1255.93	1827.23	68.73	7.14	7.51
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
MEAN	962	1109	87	6.84	7.01
STD	220	125	18	0.25	0.11
CV	23	11	21	-	-

Table 13-H gives the analysis of variance (ANOVA) for the cross-over design model for $\ln(AUC_T)$. This analysis gives the appropriate intra-subject 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 intra-subject CV should also be calculated.

TABLE 13-H

AUC_T (µg•h/mL) Analysis—ANOVA for $\ln(AUC_T)$

Source	df	SS	MS	F	PR > F
Seq	1	0.00912	0.00912	0.02874	0.86877
Subject (Seq)	10	3.17243	0.31724	6.99908	0.00248
Period	1	0.02173	0.02173	0.47941	0.50445
Form	1	0.00844	0.00844	0.18618	0.67527
Residual	10	0.45326	0.04533	.	.

Intra-subject CV - 100 x $(\text{MS Residual})^{0.5}$ = 100 x $(0.0453)^{0.5}$ = 21 percent

The AUC_T ratio estimate and its 90% confidence interval are derived in the calculations shown in Table 13-I. Since this study is a balanced design (i.e., equal number of subjects per sequence) then the difference is simply the difference in the arithmetic means of the ln(AUC)s. When the study is unbalanced, the lease-squares means estimate for each formulation and the standard error of the difference in least-squares means are used. For this example, the AUC_T ratio passes the bioequivalence standard since the lower and upper limits fall within 80% to 125%.

TABLE 13-I

AUC_T (μg•h/mL) Analysis—Calculations

$$\text{Difference} = \text{Test } \bar{x} - \text{Reference } \bar{x} = 7.6455 - 7.6830 = -0.0375$$

$$SE_{\text{Difference}} = 0.0869$$

$$\text{AUC Ratio} = 100 \times e^{\text{Difference}} = 100 \times e^{-0.0375} = 96\%$$

90% Confidence Limits

$$\text{Lower, Upper} = 100 \times e^{(\text{Difference} \pm t_{0.05,\ 10} \times SE_{\text{Difference}})}$$

$$\text{Lower} = 100 \times e^{(-0.0375 - 1.812 \times 0.0869)} = 82\%$$

$$\text{Upper} = 100 \times e^{(-0.0375 + 1.812 \times 0.0869)} = 113\%$$

13.6 C_{max} Analysis

The necessary information and summary for the analyses of C_{max} are shown in Tables 13-J, 13-K, and 13-L.

TABLE 13-J
C_{max} (µg/mL) Analysis—Data

ID	Raw Scale			Log Scale	
	Test C_{max}	Reference C_{max}	Relative C_{max} (%)	Test $\ln(C_{max})$	Reference $\ln(C_{max})$
1	146.26	143.1	102.21	4.99	4.96
2	118.45	116.60	101.59	4.77	4.76
3	153.47	242.74	63.22	5.03	5.49
4	140.08	130.38	107.44	4.94	4.87
6	116.39	160.06	72.72	4.76	5.08
7	90.74	130.38	69.60	4.51	4.87
8	62.21	46.53	133.69	4.13	3.84
9	180.25	226.84	79.46	5.19	5.42
10	88.99	143.10	62.19	4.49	4.96
12	120.51	132.50	90.95	4.79	4.89
13	94.86	83.42	113.71	4.55	4.42
14	63.45	106.00	59.86	4.15	4.66
. . .	. . .	. . .	. . .	. . .	. . .
MEAN	114.64	138.47	88.05	4.69	4.85
STD	36.31	54.25	23.82	0.34	0.43
CV	31.67	39.18	27.05	-	-

TABLE 13-K

C_{max} (µg/mL) Analysis—ANOVA for ln(C_{max})

Source	df	SS	MS	F	PR>F
Seq	1	0.00009	0.00009	0.00032	0.98605
Subject (Seq)	10	2.91103	0.29110	7.55217	0.00183
Period	1	0.01058	0.00158	0.27454	0.61172
Form	1	0.15428	0.15428	4.00253	0.07331
Residual	10	0.38546	0.03855	.	.
Intra-subject CV = 20 percent					

TABLE 13-L

C_{max} (µg/mL) Analysis—Calculations

Difference = Test $\bar{x}$ – Reference $\bar{x}$ = 4.6924 – 4.8527 = -0.1604

$SE_{Difference}$ = 0.0802

C_{max} Ratio = 100 x $e^{Difference}$ = 100 x $e^{-0.1604}$ = 85%

90% Confidence Limits

Lower, Upper = 100 x $e^{(Difference \pm t_{0.05,\ 10}\ x\ SE_{Difference})}$

Lower = 100 x $e^{(-0.1604 - 1.812 \times 0.0802)}$ = 74%

Upper = 100 x $e^{(-0.1604 + 1.812 \times 0.0802)}$ = 99%

13.7 AUC_I Analysis

The necessary information and summary for the analyses of AUC_I are shown in Tables 13-M and 13-N.

TABLE 13-M

AUC_I (μg•h/mL) Analysis—Data

ID	Raw Scale			Log Scale	
	Test AUC_I	Reference AUC_I	Relative AUC_I(%)	Test $\ln(AUC_I)$	Reference $\ln(AUC_I)$
1	3001.16	2429.22	123.54	8.01	7.80
2	1976.50	2113.50	93.52	7.59	7.66
3	3257.08	4034.81	80.72	8.09	8.30
4	2270.34	1193.06	190.29	7.73	7.08
6	2641.31	2878.64	91.76	7.88	7.97
7	1793.06	2530.14	70.87	7.49	7.84
8	1047.44	902.05	116.12	6.95	6.80
9	3137.79	3405.78	92.13	8.05	8.13
10	2366.76	3029.45	78.13	7.77	8.02
12	3431.12	1152.57	297.69	8.14	7.05
13	1738.86	1964.50	88.51	7.46	7.58
14	1308.74	1890.16	69.24	7.18	7.54
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
MEAN	2330.85	2293.66	116.04	7.69	7.65
STD	782.04	952.50	66.00	0.37	0.46
CV	33.55	41.53	56.87	-	-

TABLE 13-N

AUC_I (μg•h/mL) Analysis—ANOVA for ln(AUC_I)

Source	df	SS	MS	F	PR > F
Seq	1	0.00140	0.00140	0.00487	0.94572
Subject (Seq)	10	2.88134	0.28813	3.31638	0.03602
Period	1	0.15039	0.15039	1.73094	0.21765
Form	1	0.01331	0.01331	0.15321	0.70370
Residual	10	0.86882	0.08688	.	.
Intra-subject CV = 20 percent					

13.8 T_{max} Analysis

The necessary information and summary for the analyses of T_{max} are shown in Tables 13-O and 13-P.

TABLE 13-O
T_{max} (h) Analysis—Data

ID	Test T_{max}	Reference T_{max}	Difference T_{max}
1	16.00	15.00	2.00
2	14.00	16.00	-2.00
3	4.00	16.00	-12.00
4	6.00	6.00	0.00
6	16.00	16.00	0.00
7	14.00	14.00	0.00
8	4.00	6.00	-2.00
9	12.00	12.00	0.00
10	24.00	24.00	0.00
12	3.00	3.00	0.00
13	6.00	16.00	-10.00
14	16.00	6.00	10.00
. . .	. . .		. . .
MEAN	11.25	12.42	-1.17
STD	6.57	6.05	5.56

TABLE 13-P

T_{max} (h) Analysis—ANOVA

Source	df	SS	MS	F	PR > F
Seq	1	2.667	2.6667	0.03784	0.84965
ID (Seq)	10	704.667	70.4667	4.15324	0.01723
Period	1	0.167	0.1667	0.00982	0.92301
Form	1	8.167	8.1667	0.48134	0.50361
Residual	10	169.667	16.9667	.	.

Intra–subject CV = 35 percent

$100 \times (\text{MS residual})^{0.5}/\text{MEAN } T_{max} = 100 \times \frac{(16.9667)^{0.5}}{(11.25 + 12.43)/2} = 35$

13.9 λ Analysis

The necessary information and summary for analyses of $\lambda(h^{-1})$ are shown in Tables 13-Q and 13-R.

TABLE 13-Q
$\lambda(h^{-1})$ Analysis—Data

ID	Test λ	Reference λ	Difference λ
1	0.0631	0.1495	-0.0864
2	0.1544	0.0398	0.1145
3	0.0991	0.1490	-0.0499
4	0.1967	0.1162	0.0804
6	0.1837	0.1692	0.0145
7	0.1201	0.1732	-0.0532
8	0.1157	0.1022	0.0135
9	0.1640	0.0722	0.0917
10	0.1007	0.1042	-0.0035
12	0.0264	-0.0097	0.0361
13	0.1270	0.0734	0.0536
14	0.1290	0.1394	-0.0104
.	.	.	.
.	.	.	.
.	.	.	.
MEAN	0.1233	0.1066	0.0167
STD	0.0485	0.0551	0.0619
CV	39.3183	51.7452	369.4071

TABLE 13-R
$\lambda(h^{-1})$ Analysis—ANOVA

Source	df	SS	MS	F	PR > F
Seq	1	0.003192	0.0031924	0.91030	0.36253
ID (Seq)	10	0.035070	0.0035070	1.73974	0.19802
Period	1	0.000898	0.0008985	0.44572	0.51948
Residual	10	0.020158	0.0020158	.	.
Intra–subject CV = 39 percent					

13.10 Calculations for AUC Ratio and C_{max} Ratio Estimates Corrected for Measured Content

Please refer to Section 11.10.

13.11 Concentration–Time Profiles (Subject 1)

Figure 13.1 gives a plot of the concentration–time profile for subject 1. Each plot must include profiles for all formulations given to that subject. Similar profiles should be given for each subject.

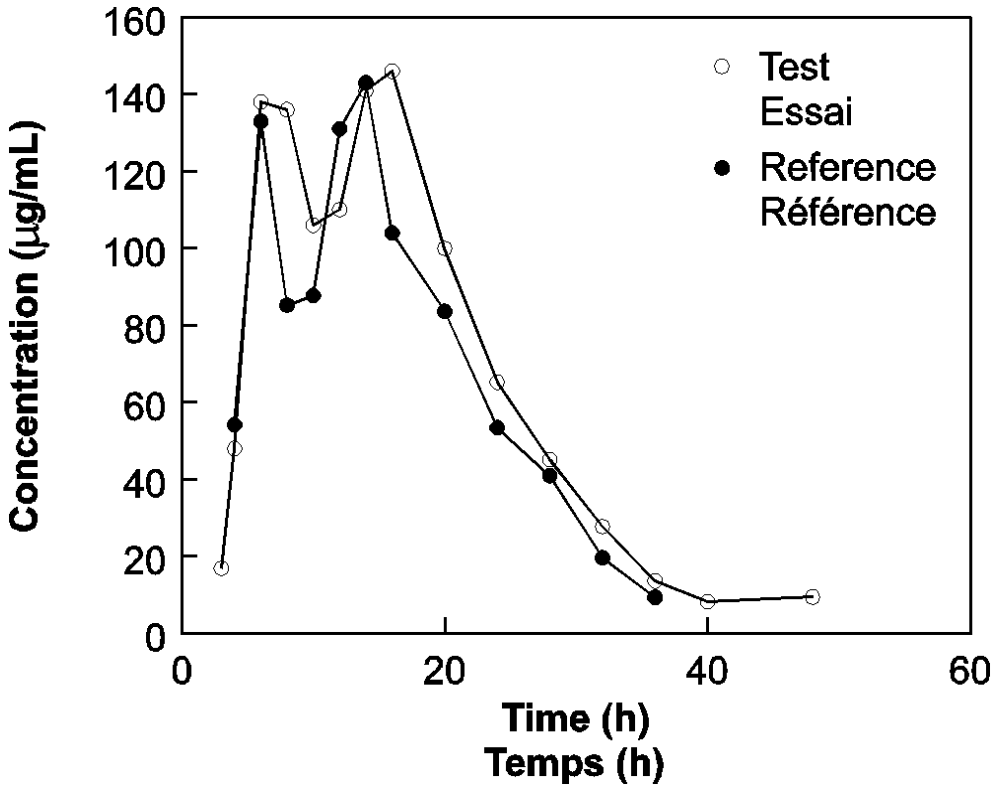

Figure 13.1: Concentration–Time Profile for Subject 1

Figure 13.2 gives a plot of the ln(concentration)–time profile for subject 1. This plot must contain the regression lines from which the terminal disposition rate constants (λ) were estimated. This line must 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 must be identified.

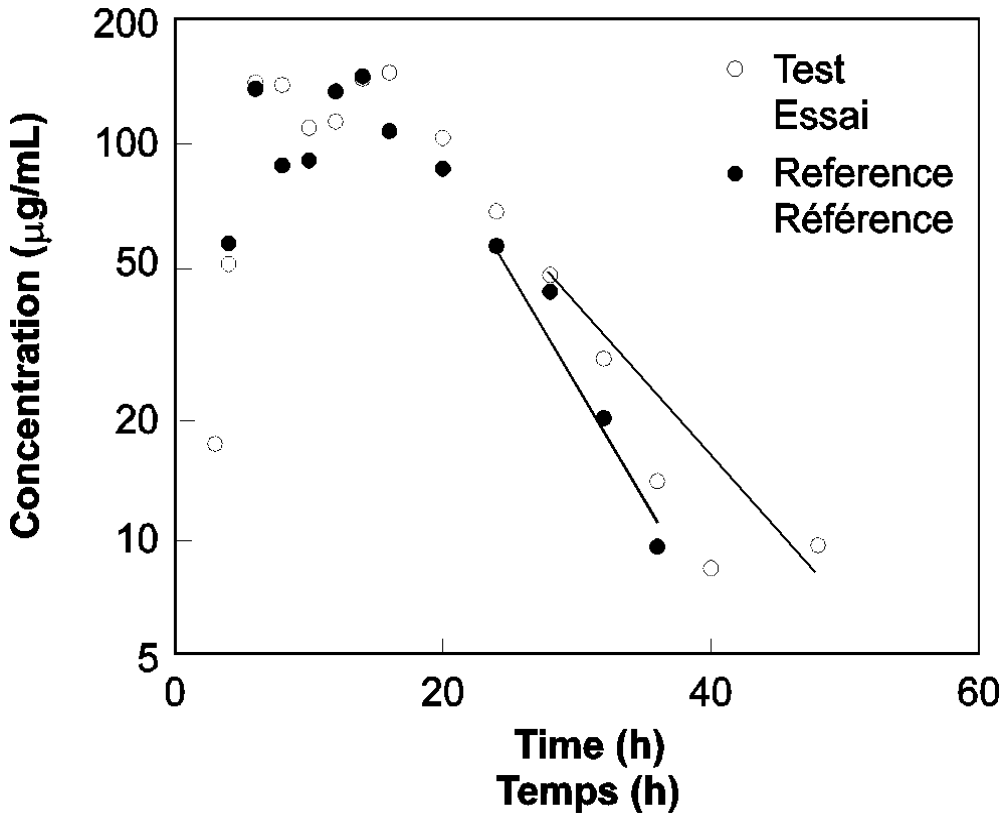

Figure 13.2: Ln(concentration)–Time Profile for Subject 1

Figure 13.3 shows a profile of the arithmetic means over all subjects for each sampling time.

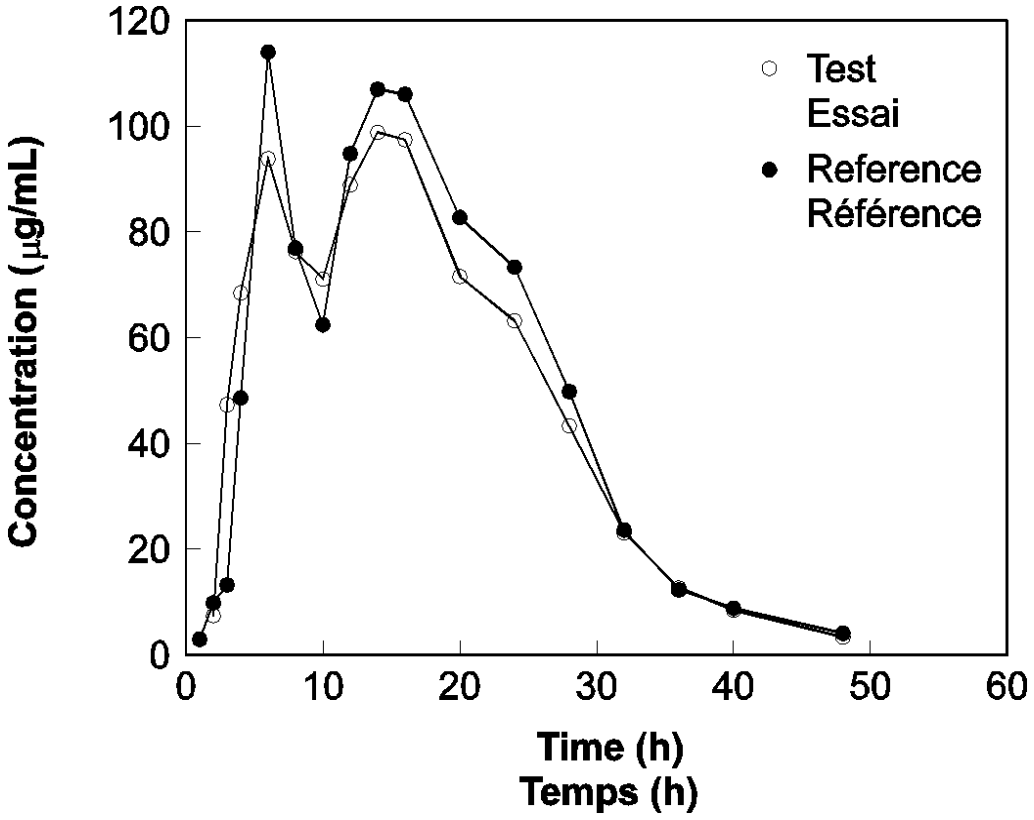

Figure 13.3: Average Concentration–Time Profile for All Subjects

Figure 13.4 shows a profile of the ln(arithmetic means) over all subjects for each sampling time.

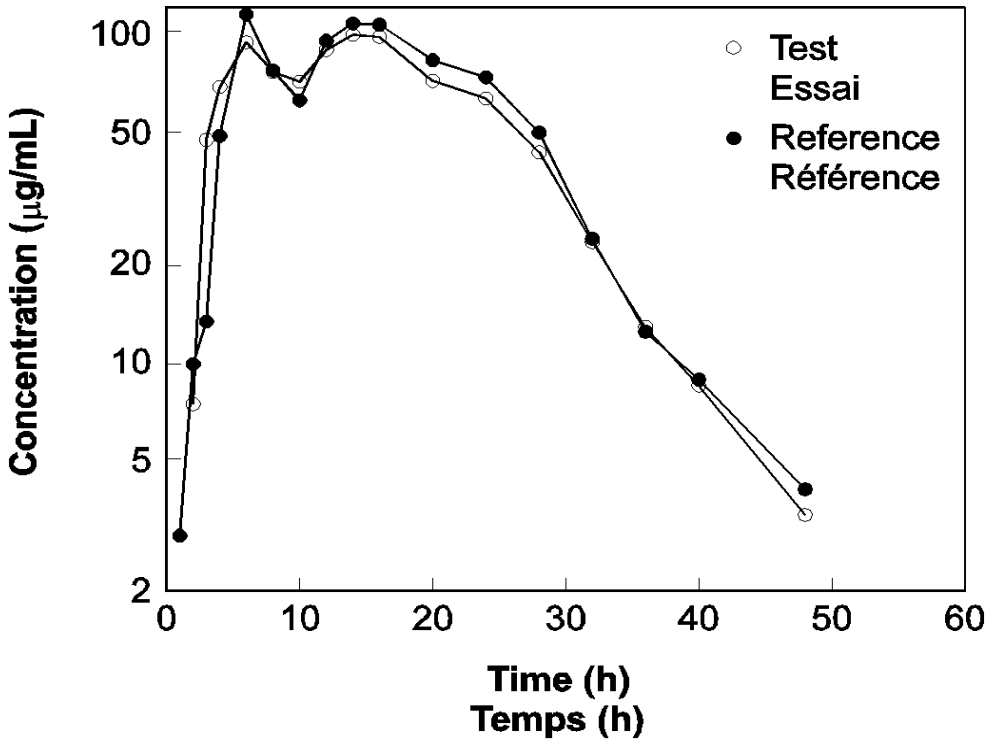

Figure 13.4: Ln(average concentration)–Time Profile for All Subjects

14 SAMPLE ANALYSIS FOR A MULTIPLE-DOSE BIOEQUIVALENCE STUDY

The following tables and figures illustrate data collected and used in a sample multi-dose comparative 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 reference formulation.

The analysis of a multi-dose comparative bioavailability study must 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 14.1.)

b) A summary of drug concentrations (visual and quantitative) at each sampling time for each subject for both the test and reference formulations. (See Section 14.2.)

c) A summary of the estimates of the parameters as defined in Section 14.3 for both test and reference formulations, including the means, standard deviations, and CVs. (See Section 14.4.)

d) A summary of pre-dose concentrations of the test and reference formulations. (See Section 14.5.)

e) A formal statistical analysis of the relevant parameters with comparisons of the test formulations to the reference formulations. (See Sections 14.6 through 14.9.)

f) A summary of corrections for potency (measured content) in estimates. (See Section 14.10.)

g) Sample concentration–time profiles that should be provided for each subject. (See Section 14.11.)

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

14.1 Randomization Scheme of the Design

Shown in Table 14-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 must be included. Even those subjects that did not complete the study must be included and identified accordingly. Subject numbers that appear on informed consent forms and reporting forms must 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 must be given.

14.2 Summary of Drug Concentrations

Tables 14-B and 14-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 limit of quantitation of the analytical method is given at this table.

TABLE 14-A

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

Subject			Period	
Number	ID	Sequence	Sept. 3, 1995	Sept. 10, 1995
001	1	TR	T	R
002*	2	RT	R	-
003**	3	RT	-	-
004	4	RT	R	T
005	5	RT	R	T
006	6	TR	T	R
007	7	RT	R	T
008	8	TR	T	R
009**	9	TR	-	-
010	10	RT	R	T
011*	11	TR	T	-
012	12	TR	T	R
013	13	TR	T	R
014	14	RT	R	T
015	15	TR	T	R
016	16	RT	R	T
017	17	RT	R	T
018	18	TR	T	R
019	19	TR	T	R
020	20	RT	R	T

* Subject did not appear for second period.

** Subject did not appear for either period.

TABLE 14-B

Drug Concentrations* (μg/mL) for the Test Formulation

			Sampling Times (hours)												
ID	Seq	Per.	0	1.0	2.0	3.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	20.0	24.0
1	TR	03 Sep	162.0	177.0	163.0	162.0	178.0	267.0	227.0	207.0	219.0	255.0	272.0	194.0	169.0
4	RT	10 Sep	245.0	258.0	225.0	227.0	214.0	258.0	276.0	243.0	188.0	184.0	179.0	142.0	192.0
5	RT	10 Sep	128.0	123.0	126.0	126.0	135.0	283.0	217.0	146.0	219.0	170.0	160.0	145.0	86.3
6	TR	03 Sep	113.0	81.6	92.0	111.0	135.0	225.0	231.0	144.0	132.0	105.0	110.0	98.2	85.8
7	RT	10 Sep	105.0	124.0	109.0	124.0	124.0	245.0	182.0	135.0	117.0	137.0	161.0	114.0	129.0
8	TR	03 Sep	49.6	49.5	81.6	106.0	125.0	188.0	138.0	102.0	113.0	123.0	98.0	81.4	65.2
10	RT	10 Sep	121	148.0	173.0	239.0	242.0	327.0	235.0	166.0	193.0	239.0	260.0	144.0	130.0
12	TR	03 Sep	81.9	82.2	84.5	91.3	112.0	162.0	147.0	110.0	113.0	119.0	117.0	81.2	75.8
13	TR	03 Sep	45.7	51.2	73.0	73.7	99.1	128.0	105.0	71.1	59.9	69.2	65.7	55.8	42.4
14	RT	10 Sep	202.0	323.0	240.0	285.0	386.0	420.0	316.0	244.0	258.0	198.0	188.0	134.0	142.0
15	TR	03 Sep	101.0	99.1	96.3	108.0	117.0	218.0	222.0	163.0	161.0	175.0	173.0	122.0	92.1
16	RT	10 Sep	135.0	124.0	125.0	142.0	160.0	316.0	216.0	175.0	202.0	236.0	212.0	163.0	94.2
17	RT	10 Sep	74.9	72.8	69.6	65.1	133.0	76.9	118.0	101.0	112.0	128.0	119.0	104.0	99.4
18	TR	03 Sep	62.8	55.9	56.8	67.8	82.5	144.0	115.0	97.4	102.0	99.4	84.0	52.6	30.6
19	TR	03 Sep	117	119.0	97.2	108.0	112.0	160.0	125.0	127.0	162.0	151.0	123.0	67.0	46.4
20	RT	10 Sep	40.9	35.1	55.1	64.7	90.4	119.0	95.6	75.3	68.8	71.4	75.1	64.2	46.8
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MEAN	-	-	112	120.0	117.0	131.0	153.0	221.0	185.0	144.0	151.0	154.0	150.0	110.0	95.4
STD	-	-	56.2	77.9	56.3	66.0	75.7	90.1	66.4	53.5	57.7	57.9	62.1	41.6	46.5
CV	-	-	50.4	64.8	48.3	50.3	49.5	40.8	35.8	37.1	38.1	37.7	41.5	37.8	48.7

* Limit of quantitation is 0.2μg/mL. Any concentration below this limit is reported as Below Quantitation Limit (BQL) except at time 0 and times before first observed concentration. However, in the calculation of summary statistics a zero is used.

TABLE 14-C

Drug Concentrations* (μg/mL) for the Reference Formulation

			Sampling Times (hours)												
ID	Seq	Per.	0.0	1.0	2.0	3.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	20.0	24.0
1	TR	10 Sep	200.0	199.0	172.0	169.0	153.0	139.0	153.0	250.0	167.0	189.0	181.0	163.0	194.0
4	RT	03 Sep	295.0	302.0	289.0	263.0	232.0	199.0	216.0	251.0	246.0	226.0	193.0	137.0	235.0
5	RT	03 Sep	125.0	124.0	111.0	101.0	100.0	306.0	201.0	131.0	96.5	99.6	93.1	109.0	163.0
6	TR	10 Sep	162.0	155.0	145.0	124.0	124.0	296.0	318.0	212.0	175.0	216.0	334.0	172.0	152.0
7	RT	03 Sep	190.0	144.0	183.0	155.0	156.0	233.0	208.0	171.0	188.0	145.0	172.0	123.0	138.0
8	TR	10 Sep	109.0	102.0	73.1	57.20	44.1	50.6	78.4	77.0	88.8	154.0	108.0	111.0	87.5
10	RT	03 Sep	128.0	141.0	118.0	95.3	96.5	357.0	245.0	162.0	162.0	217.0	189.0	166.0	122.0
12	TR	10 Sep	144.0	237.0	131.0	127.0	182.0	217.0	170.0	133.0	102.0	102.0	119.0	107.0	112.0
13	TR	10 Sep	72.1	82.6	60.8	60.4	41.4	75.3	116.0	81.8	93.1	97.4	98.4	84.3	62.4
14	RT	03 Sep	211.0	209.0	208.0	217.0	246.0	328.0	258.0	212.0	282.0	258.0	288.0	191.0	209.0
15	TR	10 Sep	231.0	230.0	205.0	186.0	151.0	198.0	271.0	188.0	161.0	169.0	144.0	121.0	124.0
16	RT	03 Sep	79.3	74.8	63.9	60.6	50.4	204.0	122.0	190.0	175.0	156.0	176.0	113.0	80.8
17	RT	03 Sep	138.0	126.0	112.0	101.0	93.7	149.0	131.0	107.0	111.0	136.0	122.0	145.0	136.0
18	TR	10 Sep	74.6	76.2	71.0	58.3	51.0	98.5	131.0	91.2	72.3	63.1	64.4	51.3	83.7
19	TR	10 Sep	111.0	99.9	87.7	80.8	68.2	127.0	162.0	117.0	160.0	182.0	159.0	91.4	71.3
20	RT	03 Sep	74.2	69.2	62.5	63.0	43.2	122.0	83.6	61.4	61.3	70.3	68.7	77.6	64.4
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MEAN	-	-	147.0	148.0	131.0	120.0	114.0	194.0	179.0	149.0	146.0	155.0	157.0	123.0	127.0
STD	-	-	64.4	69.0	65.7	62.6	66.8	92.4	69.8	57.0	61.8	58.0	73.5	37.9	52.9
CV	-	-	43.9	46.5	50.2	52.2	58.3	47.7	39.0	38.3	42.3	37.5	46.9	30.9	41.6

* Limit of quantitation is 0.2μg/mL. Any concentration below this limit is reported as Below Quantitation Limit (BQL) except at time 0 and times before first observed concentration. However, in the calculation of summary statistics a zero is used.

14.3 List of Parameters and Definitions

Table 14-D shows a list of the parameters used in the analysis and their definitions. If any other parameters are used, they must also be clearly defined.

TABLE 14-D
Parameter Definitions

Parameter	Definition
C_{max}	Maximum observed concentration (µg/mL).
C_{min}	Minimum observed concentration (µg/mL).
C_{pd}	Pre-dose concentration from same time of each day (µg/mL).
T_{max}	Sampling time at which C_{max} occurred (h).
AUC_{τ}	Area under the concentration versus time curve, over the dosing interval of the test formulation, calculated using the linear trapezoidal rule (µg•h/mL).
Fluctuation	$(C_{max} - C_{min}) / (AUC_{\tau}/\tau) \times 100$.

14.4 Summaries of Parameter Estimates

Tables 14-E and 14-F list, for each subject, the estimates of the parameters defined in Table 14-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 14-E

Summary of Parameters for Each Subject Given the Test Formulation

ID	Seq.	Period	Parameters				
			C_{max} (µg/mL)	C_{min} (µg/mL)	T_{max} (h)	AUC_{τ} (µg•h/mL)	FL** (%)
1	TR	03 Sep	272	16.00	162	5128	52
4	RT	10 Sep	276	8.00	142	4941	65
5	RT	10 Sep	283	6.00	86	3945	120
6	TR	03 Sep	231	8.00	82	3111	115
7	RT	10 Sep	245	6.00	105	3425	98
8	TR	03 Sep	188	6.00	50	2527	132
10	RT	10 Sep	327	6.00	121	4916	101
12	TR	03 Sep	162	6.00	76	2600	79
13	TR	03 Sep	128	6.00	42	1741	118
14	RT	10 Sep	420	6.00	134	5786	119
15	TR	03 Sep	222	8.00	92	3598	87
16	RT	10 Sep	316	6.00	94	4464	119
17	RT	10 Sep	133	4.00	65	2491	65
18	TR	03 Sep	144	6.00	31	1976	138
19	TR	03 Sep	162	12.00	46	2728	101
20	RT	10 Sep	119	6.00	35	1748	116
. .	. .	. .	. .	. .	. .	. .	. .
MEAN*	-	-	227	6.00	85	3445	102
STD	-	-	86	291	40	1293	25
CV	-	-	38	40.13	47	38	25

* For T_{max}, this is the median.

** Fluctuation.

TABLE 14-F

Summary of Parameters for Each Subject Given the Reference Formulation

ID	Seq.	Period	Parameters				
			C_{max} (µg/mL)	C_{min} (µg/mL)	T_{max} (h)	AUC_{τ} (µg•h/mL)	FL** (%)
1	TR	10 Sep	250	10.00	139	4249	63
4	RT	03 Sep	302	1.00	137	5223	76
5	RT	03 Sep	306	6.00	93	3262	157
6	TR	10 Sep	334	16.00	124	5117	98
7	RT	03 Sep	233	6.00	123	3982	66
8	TR	10 Sep	154	14.00	44	2195	120
10	RT	10 Sep	357	6	95	4326	145
12	TR	10 Sep	237	1.00	102	3296	99
13	TR	10 Sep	116	8.00	41	1985	90
14	RT	03 Sep	328	6.00	191	5827	56
15	TR	10 Sep	271	8.00	121	4099	88
16	RT	03 Sep	204	6.00	50	3149	117
17	RT	03 Sep	149	6.00	94	3036	44
18	TR	10 Sep	131	8.00	51	1798	107
19	TR	10 Sep	182	14.00	68	2908	94
20	RT	03 Sep	122	6.00	43	1739	109
. .	. .	. .	. .	. .	. .	. .	. .
MEAN*	-	-	230	7.63	95	3512	96
STD	-	-	81	4.19	43	1255	31
CV	-	-	35	54.99	46	36	32

* For T_{max}, this is the median.

** Fluctuation.

14.5 Pre-dose Concentrations

Tables 14-G and 14-H list the pre-dose concentrations for each subject on each day of the study for the test and reference formulations respectively.

These tables are used to check for both compliance and whether steady-state concentrations had been reached during the study. Intra-subject statistics should be calculated for the last three pre-dose concentrations.

TABLE 14-G

Pre-dose Concentrations (μg/mL) for Each Subject Given the Test Formulation

	Day					Intra-Subject Statistics*		
ID	4	5	6	7	8	MEAN	STD	CV
1	175	156	147	157	164	156	9	5
4	195	234	181	238	186	202	32	16
5	93	118	142	124	84	117	30	26
6	119	103	91	110	83	95	14	15
7	121	110	102	102	125	110	13	12
8	76	56	50	48	63	54	8	16
10	89	106	89	117	126	111	19	17
12	83	81	75	80	74	76	3	4
13	47	61	42	44	41	43	2	4
14	247	193	144	196	138	159	32	20
15	69	69	63	98	89	84	18	22
16	220	84	112	131	92	112	20	18
17	90	89	76	73	97	82	13	16
18	38	25	38	61	30	43	16	38
19	96	99	101	114	45	87	37	42
20	44	46	47	40	45	44	4	9
. .	. .	. .	. .	. .	. .	. .	. .	. .
MEAN	113	102	94	108	93	-	-	-
STD	64	54	43	55	45	-	-	-
CV	57	53	45	50	49	-	-	-

* Based on concentrations from days 6, 7, and 8.

TABLE 14-H

Pre-dose Concentrations (μg/mL) for Each Subject Given the Reference Formulation

	Day					Intra-Subject Statistics*		
ID	4	5	6	7	8	MEAN	STD	CV
1	172	147	154	189	183	175	19	11
4	217	230	240	278	222	247	29	12
5	157	215	162	118	154	145	23	16
6	252	180	163	153	143	153	10	7
7	181	195	173	179	130	161	27	17
8	116	74	57	103	83	81	23	29
10	160	115	82	121	115	106	21	20
12	112	134	117	136	106	120	15	13
13	74	50	56	68	59	61	6	10
14	145	155	141	199	197	179	33	18
15	172	70	101	218	117	145	63	44
16	137	61	85	75	76	79	6	7
17	114	127	145	130	128	134	9	7
18	29	41	41	70	79	63	20	32
19	122	71	86	105	67	86	19	22
20	45	50	49	70	61	60	11	18
. .	. .	. .	. .	. .	. .	. .	. .	. .
MEAN	138	120	116	138	120	-	-	-
STD	58	63	56	61	50	-	-	-
CV	42	53	48	44	42	-	-	-

* Based on concentrations from days 6, 7, and 8.

A repeated measures analysis of the last three pre-dose concentrations should be provided. An example of this analysis is shown in Table 14-I. Of main importance in this analysis is the time and time*form interaction. Should either the time or time*form effects be significant, the study may not have been at steady state for one or both formulations. For this example, although the time term is significant, the significance is due to a higher value for seventh day C_{pd}. Since there is no increase in the means for the three C_{pd}, steady state is assumed.

TABLE 14-I

Repeated Measures Analysis of ln(C_{pd})

Source	df	SS	MS	F	PR > F
Seq	1	2.347	2.347	2.14	0.166
ID (Seq)	14	15.348	1.096	-	-
Period	1	0.186	0.186	2.53	0.134
Form	1	1.573	1.573	21.41	< 0.001
ID*Form (Seq)	14	1.029	0.073	2.28	0.031
Time	2	0.529	0.265	6.16	0.006
Time*Seq	2	0.130	0.065	1.51	0.238
Time*ID (Seq)	28	1.201	0.043	1.34	0.216
Time*Period	2	0.029	0.014	0.45	0.643
Time*Form	2	0.047	0.023	0.74	0.488
Time*Form*ID (Seq)	28	0.889	0.032	-	-
Corrected Total	95	-	-	-	-

14.6 AUC_τ Analysis

Tables 14-J, 14-K and 14-L provide the complete analysis required for AUC_τ. Table 14-J lists the AUC_τ estimates on the raw scale and the log scale. Also given is the test AUC_τ as a percentage of the reference AUC_τ. Summary statistics are calculated for each variable.

TABLE 14-J

AUC_τ (μg•h/mL) Analysis—Data

ID	Raw Scale			Log Scale	
	Test AUC_τ	Reference AUC_τ	Relative AUC_τ (%)	Test $\ln(AUC_\tau)$	Reference $\ln(AUC_\tau)$
1	5214.31	4248.69	122.73	8.56	8.35
4	4940.91	5223.15	94.60	8.51	8.56
5	3945	3262.36	120.92	8.28	8.09
6	3111.42	5116.62	60.81	8.04	8.54
7	3424.75	3982.42	86.00	8.14	8.29
8	2526.8	2194.62	115.14	7.83	7.69
10	4916.19	4325.75	113.65	8.50	8.37
12	2599.77	3296.18	78.87	7.86	8.10
13	1740.8	1985.33	87.68	7.46	7.59
14	5786.03	5827.35	99.29	8.66	8.67
15	3598.41	4099.02	87.79	8.19	8.32
16	4464.02	3149.42	141.74	8.40	8.05
17	2490.9	3036.37	82.04	7.82	8.02
18	1976	1798.24	109.89	7.59	7.49
19	2728.37	2908.06	93.82	7.91	7.98
20	1747.55	1738.93	100.50	7.47	7.46
. . .	. . .	. . .	. . .	. . .	. . .
MEAN	3450.7	3512.03	99.72	8.08	8.10
STD	1300.28	1254.67	20.13	0.39	0.38
CV	37.68	35.19	20.19	-	-

Table 14-K gives the analysis of variance (ANOVA) for the cross-over design model for $\ln(AUC_\tau)$. This analysis gives the appropriate intra-subject 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 intra-subject and inter-subject CVs should also be calculated.

TABLE 14-K

AUC_τ (μg•h/mL) Analysis—ANOVA for $\ln(AUC_\tau)$

Source	df	SS	MS	F	PR > F
Seq	1	0.44521	0.44521	1.6849	0.21525
ID (Seq)	14	3.69921	0.26423	12.4240	0
Period	1	0.02422	0.02422	1.1389	0.30394
Form	1	0.00405	0.00405	0.1905	0.66917
Residual	14	0.29775	0.02127	-	-
Intra–subject CV = 100 x $(\text{MS Residual})^{0.5}$ = 100 x $(0.0213)^{0.5}$ = 15 percent					

The AUC_τ ratio estimate and its 90% confidence interval are derived in the calculations shown in Table 14-L. If this study had a balanced design (i.e., an equal number of subjects per sequence) the difference would simply be the difference in the arithmetic means of the ln(AUC)s. Since the study was not balanced, the least-squares mean estimate for each formulation is used to form this difference, together with the appropriate standard error.

TABLE 14-L

AUC_τ (μg•h/mL) Analysis—Calculations

$$\text{Difference} = \text{Test} - \text{Reference} = 8.0768 - 8.0992 = -0.0225$$

$$SE_{\text{Difference}} = 0.0516$$

$$AUC_\tau \text{ Ratio} = 100 \times e^{\text{Difference}} = 100 \times e^{-0.235} = 98\%$$

90% Confidence Limits

$$\text{Lower, Upper} = 100 \times e^{(\text{Difference} \pm t_{0.025,\ 14} \times SE_{\text{Difference}})}$$

$$\text{Lower} = 100 \times e^{(-0.0225 - 1.761 \times 0.0516)} = 89\%$$

$$\text{Upper} = 100 \times e^{(-0.0225 + 1.761 \times 0.0516)} = 107\%$$

14.7 C_{max} Analysis

The necessary information and summary for the analyses of C_{max} are shown in Tables 14-M, 14-N, and 14-O.

For this example, the C_{max} ratio passes the bioequivalence criterion.

TABLE 14-M

C_{max} (µg/mL) Analysis—Data

ID	Raw Scale			Log Scale	
	Test C_{max}	Reference C_{max}	Relative C_{max} (%)	Test $\ln(C_{max})$	Reference $\ln(C_{max})$
1	271.92	250.00	108.77	5.61	5.52
4	276.04	302.10	91.37	5.62	5.71
5	283.25	306.34	92.46	5.65	5.72
6	230.72	333.90	69.10	5.44	5.81
7	245.14	233.20	105.12	5.50	5.45
8	188.49	153.70	122.64	5.24	5.04
10	326.51	357.22	91.40	5.79	5.88
12	161.71	237.44	68.11	5.09	5.47
13	127.72	115.54	110.54	4.85	4.75
14	420.24	327.54	128.30	6.04	5.79
15	222.48	271.36	81.99	5.40	5.60
16	316.21	203.52	155.37	5.76	5.32
17	132.87	149.46	88.90	4.89	5.01
18	144.20	131.44	109.71	4.97	4.88
19	161.71	182.32	88.70	5.09	5.21
20	119.48	121.90	98.01	4.78	4.80
. . .	. . .	. . .	. . .	. . .	. . .
MEAN	226.79	229.81	100.66	5.36	5.37
STD	85.75	81.44	22.27	0.38	0.38
CV	37.81	35.44	22.12	-	-

TABLE 14-N

C_{max} (µg/mL) Analysis—ANOVA for ln(C_{max})

Source	df	SS	MS	F	PR > F
Seq	1	0.0287	0.0287	1.44	0.258
Subject (Seq)	10	0.1198	0.200	2.27	0.106
Period	1	0.412	0.12	4.68	0.056
Form	1	0.3591	0.3591	4.09	<0.001
Residual	10	0.0879	0.0088	-	-
Intra–subject CV = 15 percent					

TABLE 14-O

C_{max} Analysis—Calculations

$$C_{max}\ \text{Ratio} = 100 \times e^{\text{Difference}} = 100 \times e^{(5.3564 - 5.3724)} = 98\%$$

14.8 C_{min} Analysis

The necessary information and summary for the analyses of C_{min} are shown in Tables 14-P, 14-Q, and 14-R.

For this example, the C_{min} ratio meets the bioequivalence criterion.

TABLE 14-P
C_{min} (µg•h/mL) Analysis—Data

ID	Raw Scale			Log Scale	
	Test C_{min}	Reference C_{min}	Relative C_{min} (%)	Test $\ln(C_{min})$	Reference $\ln(C_{min})$
1	161.71	138.86	116.46	5.09	4.93
4	142.14	136.74	103.95	4.96	4.92
5	86.31	93.07	92.74	4.46	4.53
6	81.58	124.02	65.78	4.40	4.82
7	105.06	122.96	85.44	4.65	4.81
8	49.54	44.10	112.35	3.90	3.79
10	120.51	95.29	126.46	4.79	4.56
12	75.81	101.55	74.65	4.33	4.62
13	42.44	41.45	102.39	3.75	3.72
14	133.90	190.80	70.18	4.90	5.25
15	92.08	120.84	76.20	4.52	4.79
16	94.25	50.35	187.18	4.55	3.92
17	65.10	93.70	69.47	4.18	4.54
18	30.59	50.99	60.00	3.42	3.93
19	46.35	68.16	68.00	3.84	4.22
20	35.12	43.25	81.21	3.56	3.77
. . .	. . .	. . .	. . .	. . .	. . .
MEAN	85.16	94.76	93.28	4.33	4.45
STD	39.87	43.18	32.11	.051	0.49
CV	46.82	45.57	34.42	-	-

TABLE 14-Q

C_{min} (μg/mL) Analysis—ANOVA for ln(C_{min})

Source	df	SS	MS	F	PR > F
Seq	1	0.56639	0.56639	1.2684	0.279
ID (Seq)	14	6.25139	0.44653	10.0826	0.00005
Period	1	0.05514	0.05514	1.2450	0.2833
Form	1	0.10647	0.10647	2.4041	0.14332
Residual	14	0.62002	0.04429	-	-
Intra–subject CV = 21 percent					

TABLE 14-R

C_{min} Analysis—Calculations

$$C_{min}\ \text{Ratio} = 100\ X\ e^{\text{Difference}} = 100\ x\ e^{(4.3303\ -\ 4.4457)} = 89\%$$

14.9 Fluctuation Analysis

The necessary information and summary for the analyses of Fluctuation are provided in Tables 14-S and 14-T.

TABLE 14-S
Fluctuation (%) Analysis—Data

ID	Test Fluctuation	Reference Fluctuation
1	50.7266	62.7808
4	65.0407	75.9817
5	119.8089	156.8964
6	115.0424	98.4462
7	98.1654	66.436
8	131.9746	119.8609
10	100.5657	145.3209
12	79.3001	98.9452
13	117.5788	89.5699
14	118.7717	56.3165
15	86.9705	88.1303
16	119.3355	116.7225
17	65.3007	44.0705
18	137.9864	107.3772
19	101.4761	94.2171
20	115.8518	108.5523
.	.	.
.	.	.
.	.	.
MEAN	101.4935	95.6015
STD	25.5512	30.7625
CV	25.1752	32.1779

TABLE 14-T

Fluctuation Analysis—ANOVA for Fluctuation

Source	df	SS	MS	F	PR > F
Seq	1	1.64	1.64	0.00125	0.97234
ID (Seq)	14	18441.42	1317.24	3.34187	0.01553
Period	1	26.62	26.62	0.06753	0.79875
Form	1	277.73	277.73	0.70460	0.41535
Residual	14	5518.3	394.16	-	-
Intra–subject CV = 20 percent					

14.10 Calculations for AUC_{τ} Ratio, C_{max} Ratio, and C_{min} Ratio Estimates Corrected for Measured Content

Please refer to Section 12.10.

14.11 Concentration–Time Profiles (Subject 1)

Please refer to Section 12.11.

Figure 14.1 shows a plot of the concentration–time profile for subject 1. Each plot must include profiles for all formulations given to that subject. Similar profiles should be given for each subject.

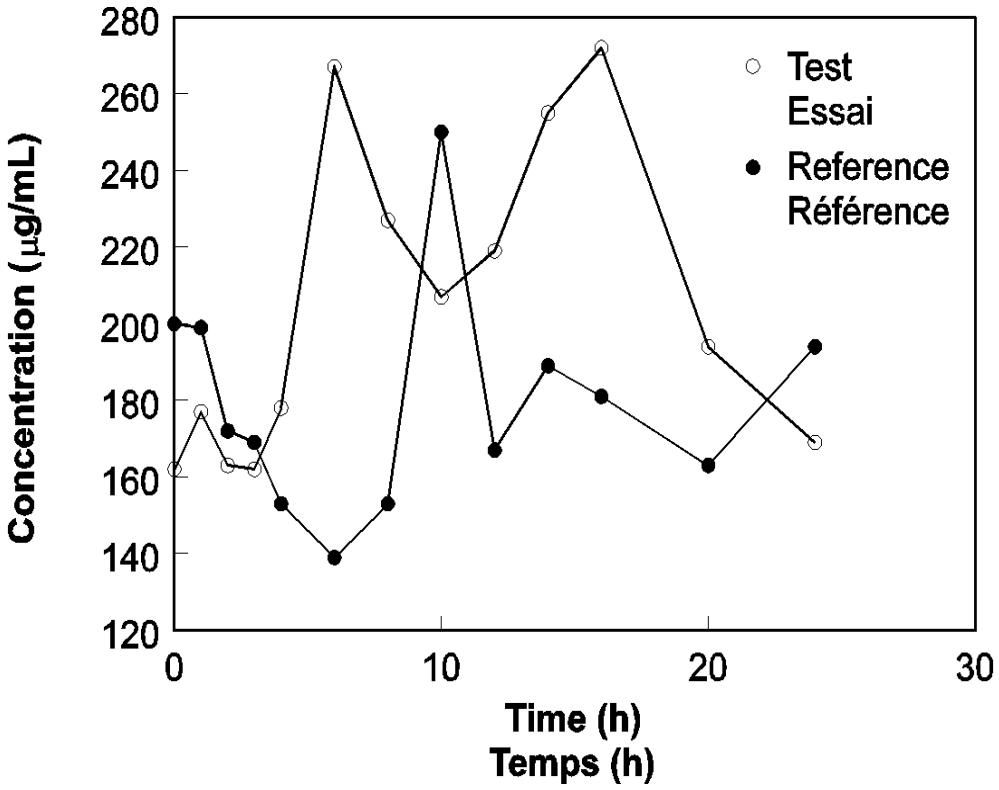

Figure 14.1 Concentration–Time Profile for Subject 1

Figure 14.2 gives a plot of the ln(concentration)-time profile for subject 1. This plot must contain the regression lines from which the terminal disposition rate constants (λ) were estimated. This line must 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 must be identified.

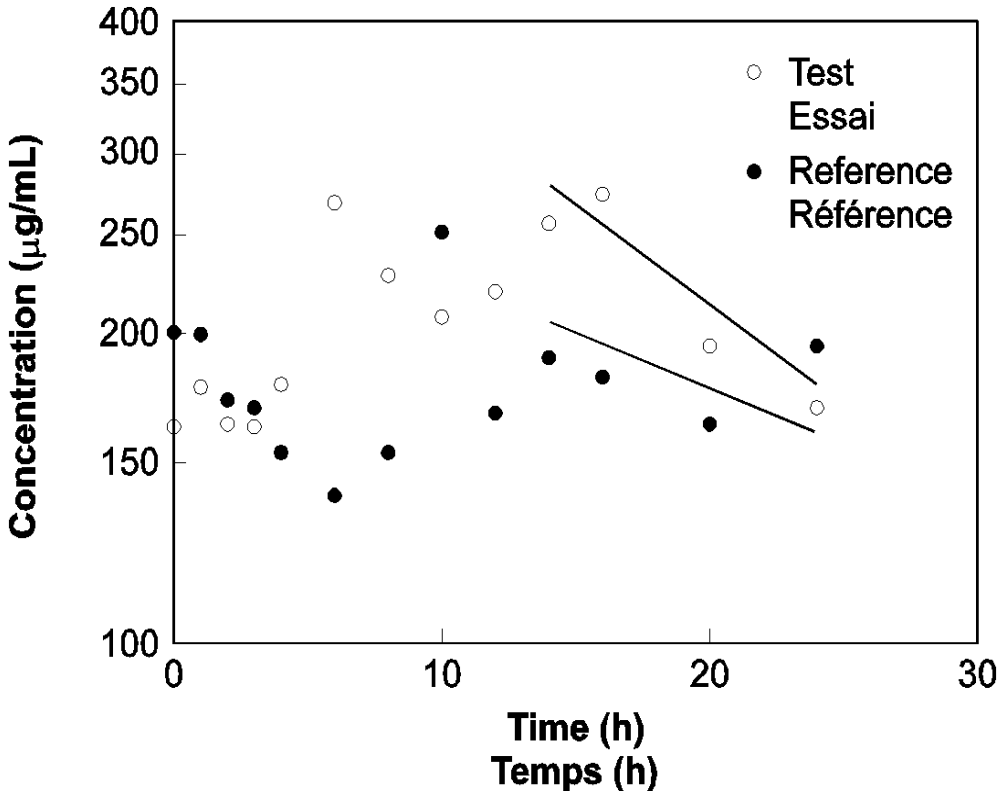

Figure 14.2: Ln(concentration)–Time Profile for Subject 1

Figure 14.3 shows a profile of the arithmetic means over all subjects for each sampling time.

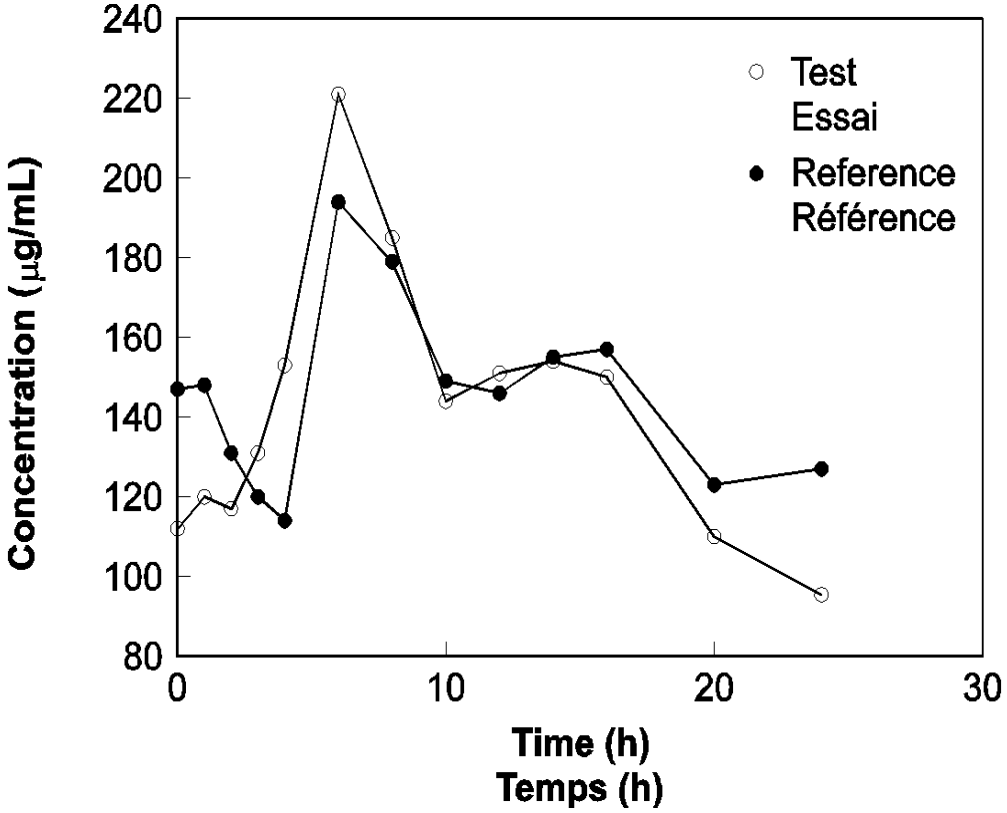

Figure 14.3: Average Concentration–Time Profile for All Subjects

Figure 14.4 shows a profile of the ln(arithmetic means) over all subjects for each sampling time.

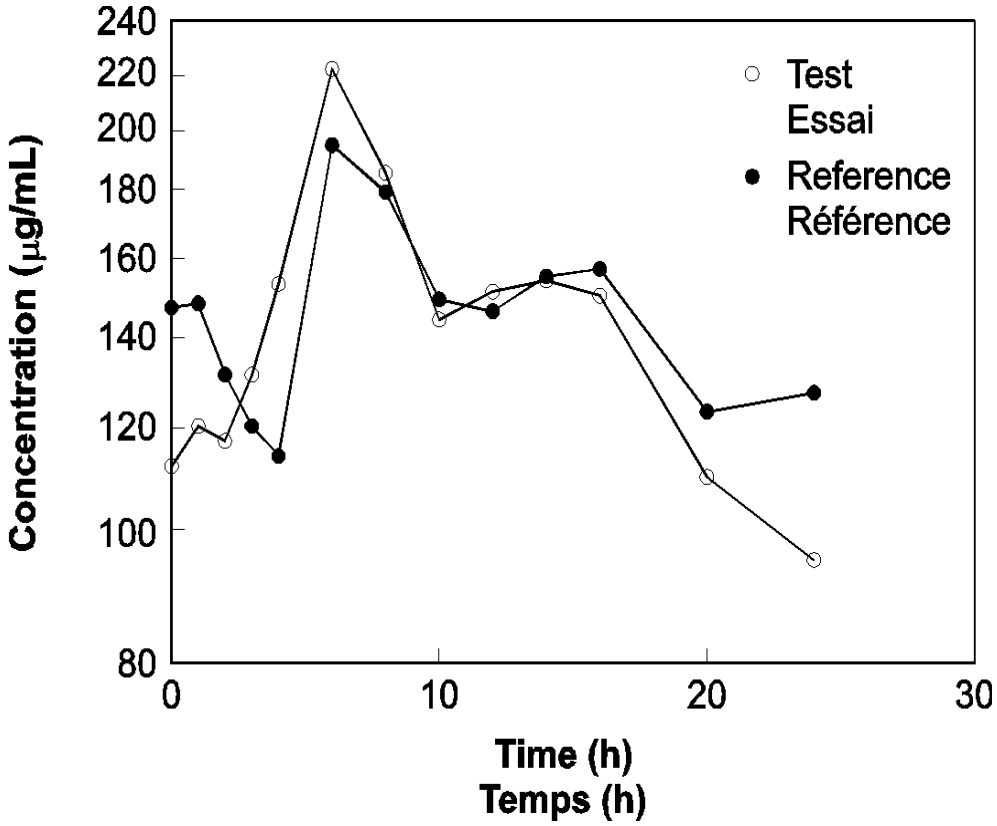

Figure 14.4: Ln(average concentration)–Time Profile for All Subjects

15 GLOSSARY OF TERMS

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

Adverse Drug Reaction - An undesirable effect that is suspected to be associated with the use of a drug by a subject.

ANOVA Appropriate To the Design - The ANOVA (analysis of variance) selected to reflect the effects of the way in which the study was executed. For the two-period cross-over design these effects include: sequence, subject (within sequence), period, formulation and the residual error term which is at times referred to (as an approximation) as within-subject or intra-subject error.

AUC (Area Under the Curve) - The area under the drug (or metabolite) concentration in plasma (or serum, or whole blood) 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 is calculated from observed data at specific time points.

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

AUC_T (AUC To the Time of the Last Quantifiable Concentration) - AUC_T is calculated from the data observed at specific time points by the linear trapezoidal rule.

AUC_τ (AUC Over the Steady-State Dosage Interval) - Area under the curve for one dosing interval (τ) at steady state. AUC_τ is evaluated by the linear trapezoidal rule.

AUC_X (AUC Over the Normal Dosing Interval) - Area under the curve during the usual dosing interval (0-X) following a single dose of the MR product. AUC_X is evaluated by the linear trapezoidal rule.

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} and C_{min} ratios should be similarly calculated. (See Sections 11, 12, 13 and 14.)

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 to which a drug reaches 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 these guidances.

Chiral Effects - Stereoselective effects on the disposition of drugs.

C_{max} (Maximum Observed Concentration) - The observed maximum or peak concentration of drug (or metabolite) in plasma (or serum, or whole blood).

C_{min} (Minimum Observed Concentration) - The observed minimum concentration at steady state. This observation is distinguished from the concentration measured immediately pre-dose at steady state (C_{pd}), although it may be quantitatively identical.

C_{pd} (Pre-dose Observed Concentration) - The concentration observed immediately before administering a dose at or near steady state.

C_T (Last Quantifiable Concentration) - The last concentration of the drug (or metabolite) profile in plasma (or serum, or blood) that can be quantified and is equal to or larger than the limit of quantitation.

Comparable Bioavailability (for Group II Drug Products) - The similarity between the rate and extent to which the same molar doses of two drug products reach the systemic circulation. The comparison is between the first-market entry modified-release formulation and the innovator's conventional-release product that the MR formulation is intended to replace.

Conventional Formulation - A product formulated in a conventional manner for rapid disintegration or dissolution and systemic absorption.

Dose-Dumping - The unintended sudden release of large amounts of drug into the systemic circulation.

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

First-Pass (or Pre-Systemic) Metabolism - The metabolism of an orally administered drug occurring during its first pass through certain metabolizing organs, before reaching the systemic circulation. Although the major site of first-pass metabolism is the liver, first-pass metabolism may also occur in the intestine or portal blood owing to the presence of enzymes (e.g., esterases). First-pass metabolism is usually assessed by comparing areas under the curve obtained following oral and intravenous doses of the drug.

Fluctuation $(C_{max}-C_{min})/(AUC_{\tau}/\tau) \times 100$ - The range of steady-state concentrations divided by the average concentration (in %).

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

Genetic Phenotype - A category or group to which a person may be assigned on the basis of differences in drug metabolism attributable to genetic characteristics (e.g., slow or fast metabolism).

HC - Health Canada.

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.

Limit Of Detection (LOD) - The lowest concentration that can be differentiated from background levels.

Limit Of Quantitation (LOQ) - The lowest measured concentration on the standard curve having an acceptable degree of precision and accuracy. 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 content of representative samples (i.e., the lots used in the bioavailability/bioequivalence study) of the test and reference drug products established as percent of label claim by an appropriate assay, such as USP.

Minimum Observed Concentration (C_{min}) - See C_{min}.

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.*

MRC - Medical Research Council of Canada.

90% Confidence Interval - An interval about the estimated value that provides 90% assurance that it contains the true value. The method of constructing the interval is described in Sections 11, 12, 13 and 14.

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).

Pre-dose Observed Concentration (C_{pd}) - See C_{pd}.

*Adapted from USP XXI (1985).

*Source: Ludden T.M. Non-Linear Pharmocokinetics. Clin Pharmacokinet, 1991, 20(6) 429-446

Pro-Drug - An inactive (or much less active) precursor that is transformed *in vivo* to the active drug.

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

Side-Effects - Drug-related effects not usually associated with the primary purpose of the therapy.

Standard Meal - A meal of known and fixed caloric content and carbohydrate, protein, fat, and fluid composition.

Suitability of Facilities - The physical plant and capability of a facility involved in experiments with human subjects or in the analysis of biological samples. Facilities should conform to requirements for Good Clinical Practice or Good Laboratory Practice.*

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 ($T_{1/2}$) is calculated from this constant ($T_{1/2}=\ln 2/\lambda$). (Also known as Terminal Elimination Rate Constant.)

Terminal Elimination Rate Constant - See Terminal Disposition Rate Constant (λ).

Terminal Half-Life - See Terminal Disposition Rate Constant (λ).

Therapeutic Equivalence - Therapeutic equivalence means that a chemical equivalent of a drug product (i.e., containing the same amount of the same drug in the same dosage form) when administered to the same individuals in the same dosage regimen will provide essentially the same efficacy and toxicity.**

Time of Maximum Observed Concentration (T_{max}) - The time after administration of the drug at which C_{max} is observed.

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*Source: Drug Bioequivalence. A Report of the Office of Technology AssessmentDrug Bioquivalence Study Panel Washington. 1974.

**Source: Proposed rule. Obligations of Clinical Investigators,. Federal Register, 43, 35210, 1978, and Code of Federal Regulations, 21, Part 58 revised April 1988.