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~Modeling and simulation for the optimization of sampling schedule in Bioequivalence Studies~

ΕΥΑΓΓΕΛΙΑ ΡΟΥΜΑΝΑ

ΕΘΝΙΚΟ & ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ

ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ

«ΑΝΑΠΤΥΞΗ ΝΕΩΝ ΦΑΡΜΑΚΩΝ : ΕΡΕΥΝΑ, ΚΥΚΛΟΦΟΡΙΑ ΚΑΙ ΠΡΟΣΒΑΣΗ»

ΤΡΙΜΕΛΗΣ ΕΠΙΤΡΟΠΗ ΔΙΠΛΩΜΑΤΙΚΗΣ ΕΡΓΑΣΙΑΣ

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ΠΕΡΙΛΗΨΗ

Οι μελέτες βιοϊσοδυναμίας είναι μελέτες συγκριτικής βιοδιαθεσιμότητας που βασίζονται στο γεγονός ότι το θεραπευτικό προφίλ ενός φαρμακευτικού προϊόντος είναι συνάρτηση της συγκέντρωσης του δραστικού συστατικού στη θέση δράσης του. Οι μελέτες Βιοϊσοδυναμίας συνιστούν νομοθετική απαίτηση για την έγκριση γενόσημων προϊόντων. Η αξιοπιστία των αποτελεσμάτων μιας μελέτης Βιοϊσοδυναμίας εξαρτάται από τον σχεδιασμό της. Μεταξύ άλλων παραγόντων, η σημασία του σχήματος δειγματοληψίας είναι ευρέως αναγνωρισμένη καθώς ένα ανεπαρκές σχήμα δειγματοληψίας μπορεί να αυξήσει την ανακρίβεια και την αβεβαιότητα των αποτελεσμάτων ενώ ένα υπερβολικά πυκνό σχήμα δειγματοληψίας μπορεί να αυξήσει την απαιτούμενη εργασία, το κόστος της μελέτης και κυριότερα την επιβάρυνση των συμμετεχόντων. Τις τελευταίες δεκαετίες, έχει παρατηρηθεί μία αυξανόμενη εφαρμογή μοντελοποίησης και προσομοιώσεων στον τομέα των μελετών βιοϊσοδυναμίας. Οι in silico κλινικές μελέτες έχουν αποδειχτεί χρήσιμο εργαλείο σε ποικίλα επίπεδα του σχεδιασμού των μελετών βιοϊσοδυναμίας συμπεριλαμβανομένου του προσδιορισμού του σχήματος δειγματοληψίας. Ο σκοπός της παρούσας εργασίας είναι η αξιολόγηση της επίδρασης μεταβολών του δειγματοληπτικού σχήματος στο αποτέλεσμα της μελέτης βιοϊσοδυναμίας, με τη χρήση μοντελοποίησης και προσομοιώσεων. Για το σκοπό αυτό, χρησιμοποιήθηκε ο σχεδιασμός μιας in vivo μελέτης βιοϊσοδυναμίας και δημιουργήθηκαν διαφορετικά σενάρια με αλλαγές στο μέγεθος του δείγματος, στο σχήμα δειγματοληψίας, στην δια-ατομική μεταβλητότητα και στην διαφορά στον ρυθμό απορρόφησης του υπό έλεγχο προϊόντος και του προϊόντος αναφοράς. Στην συνέχεια πραγματοποιήθηκαν

προσομοιώσεις σε υπολογιστικό εργαλείο που έχει αναπτυχθεί σε γλώσσα Matlab® 2018b. Έπειτα, αξιολογήθηκε η επίδραση των αλλαγών στην ισχύ της μελέτης και στο GMR για τις φαρμακοκινητικές παραμέτρους C_{max} και AUC. Τα αποτελέσματα των προσομοιώσεων έδειξαν ότι λιγότερο πυκνά και μικρότερης διάρκειας σχήματα δειγματοληψίας δεν επηρεάζουν το αποτέλεσμα της μελέτης βιοϊσοδυναμίας. Επιπλέον οι in silico κλινικές μελέτες αποδείχθηκαν χρήσιμο εργαλείο στην αξιολόγηση και στον προσδιορισμό των παραμέτρων του κλινικού σχεδιασμού των μελετών βιοϊσοδυναμίας.

ABSTRACT

Bioequivalence (BE) studies are comparative bioavailability studies based on the fact that the therapeutic profile of a product is a function of the concentration of the active pharmaceutical ingredient at the site of action. BE studies form the required basis for regulatory approval of generic drugs. The reliability of the results of a BE study is influenced by the design of the study. Among other factors, the importance of the sampling design of a BE study is widely recognized, as inadequate sampling designs can lead to study inaccuracies and uncertain results, while a very dense sampling design can increase the workload, the cost of the study, and most importantly, the inconvenience to participants. In recent decades, there has been an increasing use of modeling and simulation in the field of bioequivalence. In Silico Clinical Trials (ISCT) have proven to be very useful for various aspects of bioequivalence assessment, including determining the sampling design. The aim of the present work is to evaluate the impact of differences in sampling schedule on bioequivalence outcome using modeling and simulation, i.e., in silico clinical trials. For this purpose, the design of a bioequivalence study performed in vivo is used as a target scenario. Several scenarios are created by changing the sample size, sampling design, Between-Subject Variability (BSV) and similarity of absorption rate of test and reference product. The simulations are performed in a computer program developed in Matlab® 2018b and the power of the study and GMR for the pharmacokinetic parameters C_{max} and AUC are evaluated for each scenario. The results of the simulations showed that less dense and shorter sampling schedules did not significantly affect the power of the study and the GMR for

the two pharmacokinetic parameters. In addition, the ISCTs proved to be very helpful in investigating and determining the clinical design parameters in a BE study.

A. INTRODUCTION

A.1 Bioequivalence studies

A.1.1 General Information

According to European Medicines Agency (EMA) a medicinal product is a substance or a combination of substances that are used in order to treat, prevent or diagnose a disease. A necessary prerequisite for a medicinal product to be placed on the market is the regulatory approval and the issue of a marketing authorization by a regulatory authority. A medicinal product can be approved by a full application, generic application, hybrid medicinal product application, similar biologic, well established use, fixed dose combination or informed consent application. (Directive 2001/83/EC). Each type of application has several regulatory demands in order to be accepted and approved. In all cases, the quality, safety and efficacy of the new medicinal product have to be proven.

The efficacy of a new drug product is demonstrated through clinical trials.

According to the US Department of Health and Human services, a clinical trial is a research performed on human subjects aiming at the evaluation of a medical intervention. In the European Regulation on clinical trials on medicinal products for human use, a clinical trial is described as a study that meets one or more of the following criteria:

a) The subjects' assignment to a specific therapeutic strategy is predetermined and does not fall within normal clinical practice of the Member State concerned.

(b) The decision to prescribe investigational medicinal products is made in conjunction with the decision to enrol (REGULATION (EU) No 536/14).

In the case of a generic application, the proof of efficacy and safety is easier than in the case of a full application. Clinical trials can be replaced by bioequivalence (BE) studies, which demonstrate pharmaceutical equivalence between the test product to be registered and an already authorized product as a reference product.

Before explaining the content of BE studies, some definitions must be given.

a. Generic drug products

The aim of a bioequivalence study is to provide the necessary efficacy and safety data for the registration and authorization of a generic medicinal product.

According to Directive 2001/83/ EC of the European Parliament and of the Council of 6 November 2001, the term "generic medicinal product" means a product with the same (qualitative and quantitative) composition in active pharmaceutical ingredients and the same pharmaceutical form as the reference product. In addition, bioequivalence between the two products must have been demonstrated.

If the various salts, esters, ethers, isomers, mixtures of isomers, complexes or derivatives of an active substance do not differ significantly in terms of safety and/or efficacy, they may be considered to be the same active substance. Where significant differences in safety or efficacy are identified, additional information demonstrating the safety and/or efficacy of the different salts, esters or derivatives of an approved active substance shall be provided. The different immediate-release oral dosage forms shall be considered as the same dosage form. Bioequivalence studies are not required if the applicant can demonstrate that the generic product meets the criteria defined in the relevant guidelines.

A generic medicinal product contains the same active substance(s) as the reference medicinal product and is used at the same dose(s) to treat the same condition(s). However, the excipients, appearance and container closure system of the product may differ from those of the reference product.

b. Pharmacokinetics

The evaluation of bioequivalence is based on the fact that the therapeutic profile of a product is a function of the concentration of the active ingredient at the site of action. This concentration is related to the concentration of the active ingredient in the general circulation. Thus, two drugs (test product T and reference product R) are considered bioequivalent if their plasma concentration-time profiles are similar enough to ensure comparable therapeutic outcomes. (Nazi, 2007).

Pharmacokinetics is the science that studies the pathway of a drug substance in the human body from the time of its administration to its excretion. The processes that make up the subject of pharmacokinetics and occur in the human body after the administration of a drug can be described by the acronym ADME, which stands for: Administration, Distribution, Metabolism and Excretion (Figure A.1). These processes, which affect the concentration of the drug at the site of action, depend not only on the physiology of the human body and the pharmacokinetic properties of the drug, but also on the properties of the pharmaceutical product, which may affect the release and absorption of the drug. (Niopas, 1997). Consequently, two pharmaceutical products containing the same active ingredient do not necessarily have the same pharmaceutical effect. Since the pharmaceutical effect depends on the concentration of the drug at the site of action, which in turn depends on its plasma concentration as a function of time, it goes without saying that pharmacokinetic studies are required to demonstrate therapeutic equivalence between two products containing the same active ingredient.

Thus, to compare two products with the same active ingredient, the rate and extent of absorption must be determined for each of the two products. Consequently, the term "bioavailability" (BA) was created. Bioavailability is a pharmacokinetic parameter defined as the rate at which the drug is available to the body, at the site of action and the extent to which the dose is absorbed after administration of the drug. (J.V. Turner, et al, 2004) The extent of absorption is relevant to the potency of the drug and the rate of absorption is an indicator of the onset of action of the drug in the human body. The bioavailability of a drug can be measured by plasma concentration-time curves such as the one described in Figure A.2. The pharmacokinetic parameter AUCt, which is the area under the concentration-time curve for the period t after administration of the drug, is used to measure the extent of absorption. One might assume that the best way to assess the rate of absorption is T_{max}, which represents the time at which the maximum drug concentration is reached in the blood. However, time is a continuous variable and its use in a bioavailability study would increase the difficulty of statistical analysis. Therefore, the parameter C_{max}, which represents the maximum concentration of the drug in the general circulation, is used to describe the rate of absorption.

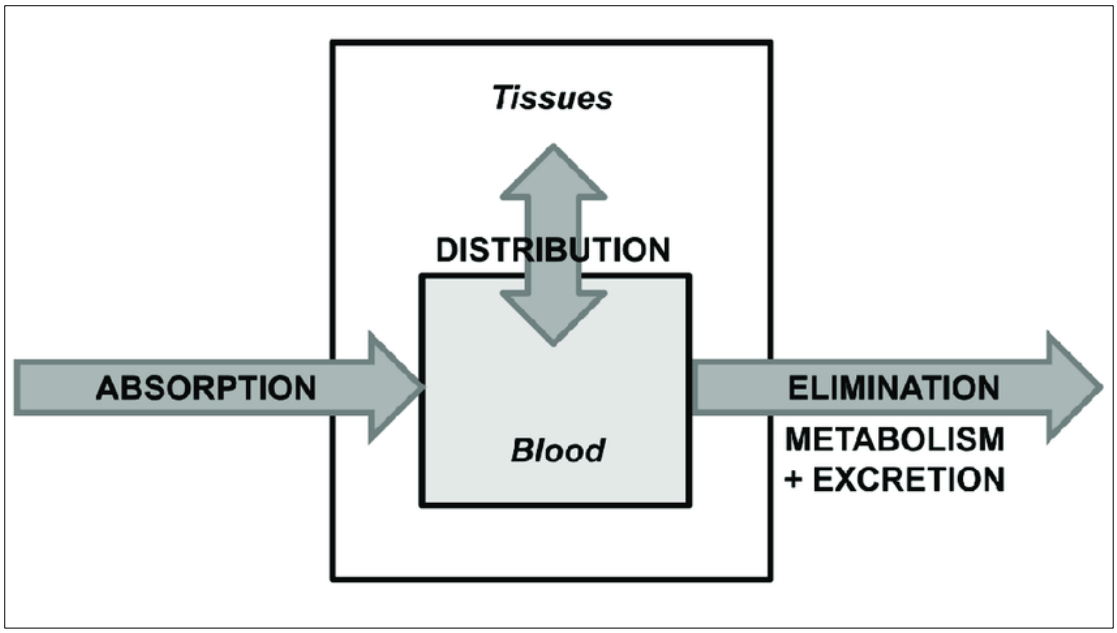


Figure A.1: *Administration, Distribution, Metabolism, Excretion (Del Amo, 2015)*

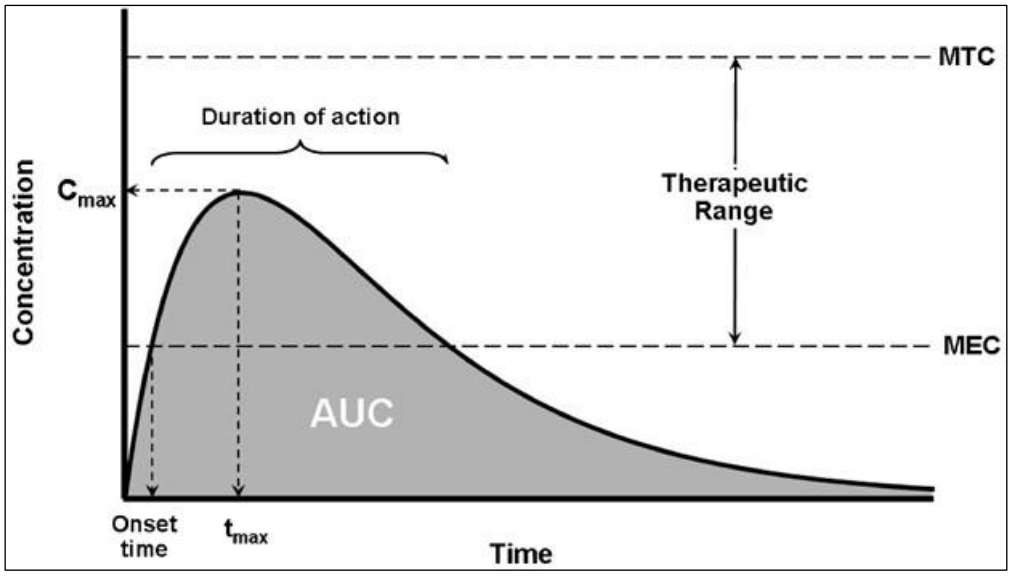


Figure A.2: *Concentration-time curve (Mehrotra N, et al, 2006)*

c. Bioequivalence studies

Bioequivalence studies (BE) are comparative bioavailability studies between two products containing the same active pharmaceutical ingredient: the test product (T) and the reference product (R).

Bioequivalence studies are the required basis for granting marketing authorization for a generic drug. These are clinical studies conducted in accordance with the EU Directive 2021/20/ EC (Baumgaertel, 2012). The aim of BE studies is to assess the in vivo equivalence between two medicines with the same active ingredient. (CHMP, 2010; CDER 2003). Because they play such a significant role for the pharmaceutical industry, regulatory authorities have published several definitions to explain the purpose and content of bioequivalence studies.

According to European Medicines Agency (EMA), two pharmaceutical products containing the same active ingredient are considered bioequivalent if they are pharmaceutically equivalent or pharmaceutically alternative and their bioavailabilities (rate and extent) after administration in the same molar dose are within acceptable predefined limits. These limits are set to ensure comparable in vivo performance, i.e., similarity in safety and efficacy. (CHMP, 2010).

Bioequivalence, as described by the U.S. Food and Drug Administration (FDA), is the absence of a substantial difference in the rate and extent to which the active pharmaceutical ingredient or active ingredient portion in pharmaceutical equivalents or alternatives becomes available at the site of action when administered at the same molar dose under comparable conditions in an appropriately designed study. (CFR 21-320.1, 2016).

According to Ted Sherwood, head of the Office of Generic Drugs at the FDA, generic drug manufacturers must submit several data to the FDA for review and evaluation. First, they must submit data showing the manufacturing process and the quality of the product. Information showing that the product behaves the same in patients as the brand-name product is also urgently needed. (CDER Talks, 11/20/2017). This information is provided by conducting a bioequivalence study.

Therefore, if bioequivalence can be demonstrated after administration of the same molar dose of a reference and a test drug, inferences or reasonable assumptions can be made about their pharmaceutical equivalence. (Baumgaertel, 2012). It can be stated that bioequivalence studies are established to demonstrate that a generic medicinal product is equivalent to a reference product so that preclinical and clinical studies can be bridged (Directive 2001/83/ EC, Article 10(2)(b)).

In a bioequivalence study, for immediate release formulations, the parameter C_{max} is used to assess the rate of absorption. The extent of absorption is described by the AUC (AUC(0-t)) from time zero to the last sampling point or the last observable concentration - whichever comes first (Bois et al, Res.11(5),1994; Bois et al, Res.11(7),1994; Jackson, 2002; Chen et al,2001). There are some other parameters that may be useful in a bioequivalence study, such as the area under the concentration-time curve extrapolated to infinity AUC∞, the time T_{max}, and the terminal slope of the concentration-time curve. The AUC truncated at 72 h (AUC(0-72h)) can be used as an alternative to the AUC∞ to compare the extent of exposure

because the absorption phase is covered by 72 h for immediate-release formulations.

For modified release products, other pharmacokinetic parameters may be used. For example, the maximum and minimum drug concentrations at steady state, AUC between administration intervals, etc. (Endrenyi and Tothfalusi, 2012; Stier et al, 2012).

d. Bioequivalence studies; an historical overview

For many years, it was assumed in the United States that the most important parameter for the pharmaceutical effect of a tablet was the disintegration of the tablet. Consequently, many drugs were approved as what are now called "generics" only if they contained the right amount of active ingredient and disintegrated in a reasonable time frame. As a result, there were many products on the market that were mistakenly approved as generics, while other products were mistakenly denied approval.

In 1984, the U.S. Congress passed the Drug price competition and patent term restoration act of 1984, also known as the Hatch Waxman act, which recognized bioequivalence studies as a requirement for approval of generic pharmaceutical products. Brazilian Law 9.787/99 also introduced the idea of conducting bioequivalence studies with reference products in order to accept a new drug as a generic. In this way, the first interchangeable generic drugs were introduced in Brazil in February 2000 (Dr. Malcom Ross, 2018). Since then, numerous guidance documents on bioequivalence studies have been published and the number of approved generics has increased, resulting in more than 65% of the global market being captured by generics (Midha and McKay 2009).

The EMA adopted the first "Note for guidance on the investigation of bioavailability and bioequivalence" in July 2001. Subsequently, in 2010, the "Guideline on the investigation of bioequivalence" was published. The latter introduced new aspects and specified important points regarding bioequivalence studies. In the years after 2010, some brand new issues emerged, such as the crucial role of modeling and simulation in

bioequivalence assessment, which is discussed later in this paper (Daousani and Karalis, 2014).

A.1.2 Performance of a Bioequivalence study

A.1.2.1. Clinical designs

Bioequivalence studies compare the bioavailabilities of the test product and the reference product. For the outcome of the study to be reliable, the effects of differences in drug formulation must be distinguished from the effects of other factors that may influence the final outcome. The clinical design of a bioequivalence study is critical to ensuring that the study achieves its goal of distinguishing the effect of the formulation from other factors that may also affect the outcome. There are several types of clinical designs that can be used in a bioequivalence study. The choice between them depends mainly on the pharmacokinetic properties of the pharmaceutical products.

Standard design

The most common clinical design for a bioequivalence study is the randomized crossover design with two sequences and two time periods (2 × 2). In this study design, each subject is administered both products (test T and reference R) in two different time periods separated by a time frame known as the washout period. The reason for this washout period is the necessary elimination of the drug administered first prior to the administration of the second drug to avoid detection of the drug at the beginning of the second period of the study. An appropriate duration for the washout period is at least five times the elimination half-life of the drug substance. A schematic representation of the standardized randomized, crossover, two sequences, two periods (2 × 2) design is shown in Figure A.3.

Note that in the randomized, crossover, two-sequence, two-period (2 × 2) design, it is not straightforward to estimate the Within-Subject Variability (WSV) of either product. Within-subject variability represents the variability that exists within a study participant. This is due to the nature of the design and the fact that a given subject only takes each product in one time period. After statistical analysis of the results, the estimated residual error is generally considered to reflect the WSV of the active substance. (CHMP, 2010; CDER, 2003; Karalis V. 2016)

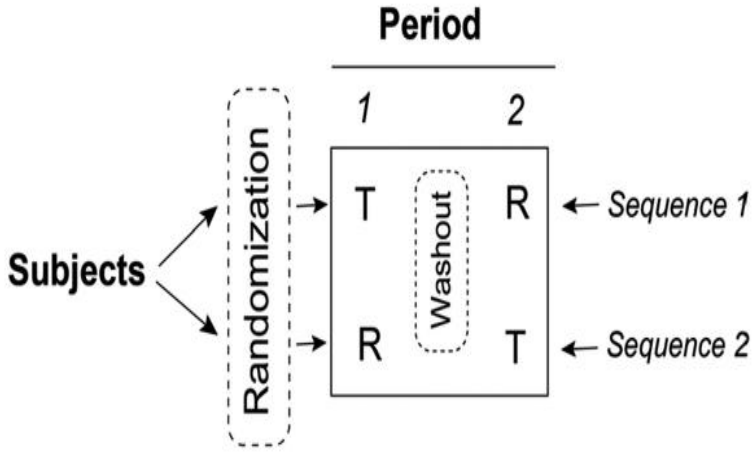


Figure A.3: *The classic two-treatment, two-period, two-sequence, crossover design. (Karalis V., 2016)*

Alternative designs

Aside from the classic crossover design, there are several other clinical designs that can be applied in a bioequivalence study.

Replicate designs

In replicate designs, each subject receives each formulation more than once. In this way, it is possible to calculate the WSV of a product since each volunteer is administered each product more than one time. This is beneficial in the case of highly variable drugs where the use of three- or four- period studies is proposed. A common advantage of replicate designs is the opportunity to reduce the required number of subjects. For example, a four-period design requires half the subjects of a standard 2×2 crossover design. (CDER, 2003; Blume et al, 1995; Shah et al, 1996). The replicate administration can be applied to either both formulations or to just one of them. It should be mentioned that the appropriate washout period should be also taken into consideration in the case of replicate designs in line with the standard crossover design. Examples of replicate designs are provided in Figure A.4.

Two-stage designs

Two-stage designs are a recent clinical design approach used in bioequivalence studies. The general concept of two-stage designs is the following: in the first stage, a group of volunteers is recruited based on some specific and predefined in the study protocol criteria, and their pharmacokinetic data are analyzed. If the predetermined standards for the study are satisfied in this stage (e.g., bioequivalence is proven), the study stops and there is no need to proceed into next stage. If there is a particular

reason also pre-specified in the protocol, the study needs to proceed to stage 2, a sample re-estimation takes place, additional subjects are included and the study proceeds. Each stage consists of a single 2×2 crossover design or a parallel design. Finally, data from both stages are appropriately combined, and bioequivalence is evaluated using specific statistical procedures.

In two-stage design, it is crucial to ensure that false approval of bioequivalence is avoided. For that reason, the design is firstly verified towards this. (CHMP, 2010; Lachun, 1998, Demets, 1989, Shih, 2006; Pocock, 1978;).

A simplified, schematic presentation of a two-stage design is presented in Figure A.5.

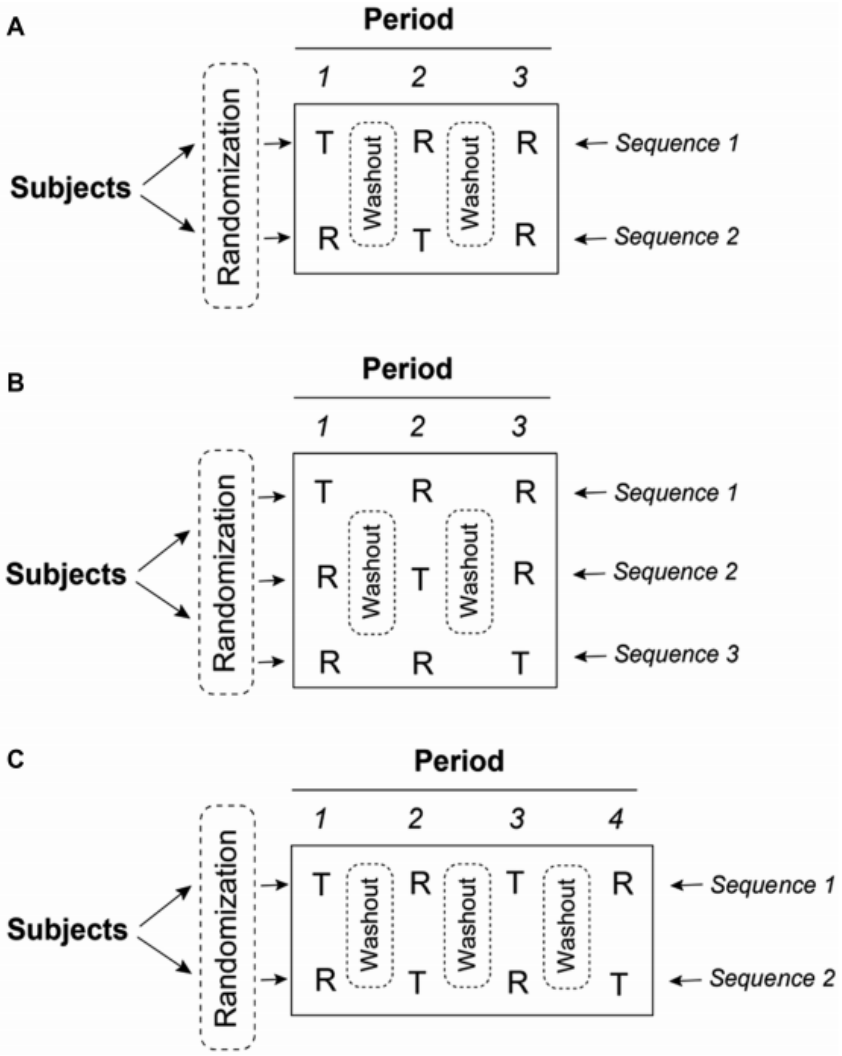


Figure A.4: *Representative examples of replicate crossover clinical designs applied in bioequivalence assessment. (A): two sequence, three-period design (B): three period-three sequence design (C): a two-sequence, four period design*

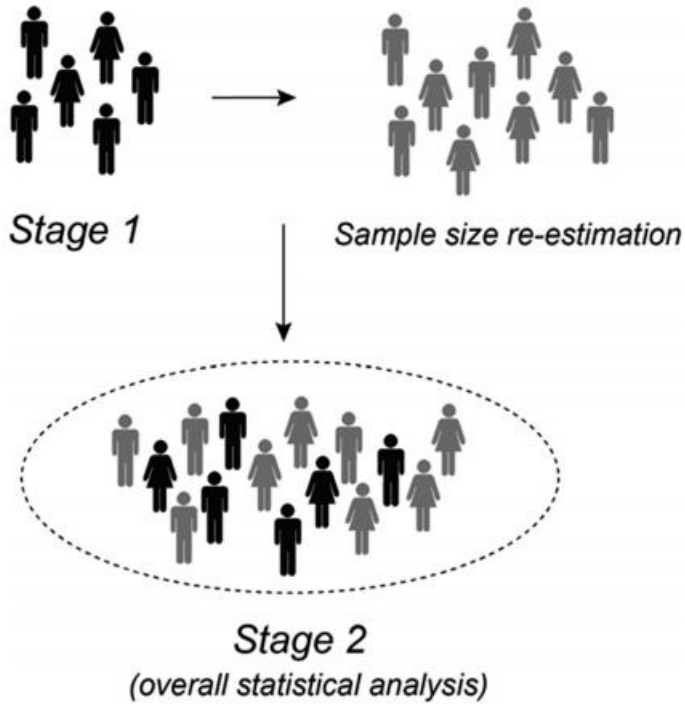


Figure A.5: *Two-stage clinical design*

Parallel design

The most traditional type of clinical trial design, used nowadays mainly for confirmatory studies, is the parallel group design. In the parallel group design, subjects are randomly assigned to one of two or more arms. A different treatment is administered in each arm. The assigned treatments may be the investigational drug in one or more doses and one or more control treatments. The assumptions underlying this design are less complex than most other designs. The parallel design often works as an alternative to other clinical designs and is particularly useful when the drug under study has a very long half-life.

A.1.2.2. Subjects and standardization of a Bioequivalence study

A bioequivalence study is a clinical trial conducted on healthy volunteers rather than patients. The reason for this is to try to minimize variability in the study. As mentioned earlier, the main purpose of the bioequivalence study is to compare the bioavailability of the two formulations (Test T and Reference R) and to investigate a statistically significant difference between the two drugs. In any scientific study, there is a "background noise" that makes it difficult to detect the true effect. In the case of bioequivalence studies, this noise, also known as "bionoise", is caused by the variability of biological measurements. Thus, it is entirely possible that differences in bioavailability between the two formulations are masked or caused by this bionoise. The cause of the bionoise may be the Within-Subject Variability (WSV, i.e., physiological variability within an individual) or the Between-Subject Variability (BSV, i.e., variability between different subjects). Patients have higher

variability due to their different constitution, comorbidities and co-medication and this heterogeneity leads to greater bionoise and complicates the comparison between the two drugs. The participants in the bioequivalence study are therefore healthy subjects who are standardized as much as possible to minimize variability and to be able to detect any difference, no matter how small, between the formulations being compared. (Baumgaertel, 2012). An exception to the selection of healthy subjects is the bioequivalence studies of drugs that are very potent or too toxic to administer to the healthy population. In this case, the study may be conducted in healthy subjects using a lower strength, and if unacceptable pharmacological effects are observed, a bioequivalence study conducted in patients may be required. (WHO, 2017).

The selection criteria for healthy subjects are strict and should be clearly stated in the study protocol. An example of these criteria is to be healthy, 18-55 years old with a body mass index (BMI) between 18.5 and 30 kg/m2. Subjects can be either men or women, but the possible risk for women of childbearing age should be considered. Subjects should be non-smokers and have no history of alcohol or drug abuse. Phenotyping and/or genotyping of subjects may be considered for safety or pharmacokinetic reasons. Clinical laboratory tests, a medical history, and a physical examination should be used to determine the eligibility of potential participants.

Throughout the study, diet, fluid intake, and exercise are standardized, concurrent use of medications or alcohol is not allowed, and subjects are closely monitored throughout the study period. All these conditions aim to ensure the reliability of the results and to allow the identification of possible differences between the two pharmaceutical products. (Christoph

Baumgaertel, 2012). The health status of the subjects is also monitored during the study so that the onset of side effects, toxicity or underlying disease can be tracked and appropriate action taken. The occurrence, severity, and duration of any observed adverse event must be reported, as well as the likelihood that it is related to the pharmaceutical product under study. (WHO, 2017; Guideline on the investigation of BE, CHMP, EMA).

In general, a bioequivalence study should be conducted under fasting conditions, unless the summary of Product Characteristics (SmPC) recommends that the product be taken after eating. For products with specific formulation characteristics, bioequivalence studies should be conducted under both fasting and fed conditions, unless the products only need to be taken in a specific condition. For modified-release products, bioequivalence must be demonstrated in both fasting and fed states to account for the influence of diet. Water intake is allowed as desired, except for one hour before and one hour after drug administration. For bioequivalence studies conducted under fasting conditions, no food intake is allowed for at least 4 hours after drug administration, and meals taken after administration should be of standard composition. For studies conducted under feeding conditions, it is recommended that the timing of the administration of the medicinal product be chosen in relation to food intake, in accordance with the SmPC of the originator product. If no specific recommendation is made in the SmPC of the originator product, it is recommended that subjects begin eating 30 minutes prior to administration of the drug product and consume the meal within 30 minutes. Because the bioavailability of a drug product from a dosage form could depend on gastrointestinal transit times and regional blood flows,

posture and physical activity must also be standardized. Subjects should avoid foods and beverages that may affect circulatory, gastrointestinal, hepatic, or renal function (e.g., alcoholic beverages or certain fruit juices such as grapefruit juice) for a reasonable period of time before and during the study. In addition, subjects should refrain from taking other concomitant medications (including herbal remedies) for an appropriate period of time before and during the study. However, contraceptives are permitted. If concomitant medication is unavoidable, e.g., adverse events such as headache, the intake must be reported (dose and timing of intake) and possible effects on study outcome must be addressed. (CHMP, 2010; Dr Malcom Ross, 2018).

A.1.2.3. Sample size

Estimating the sample size is an essential step in the bioequivalence study. The goal is to find the happy medium between having a sufficient number of subjects to demonstrate bioequivalence and avoiding unnecessary exposure of humans to drugs. The possibility of dropouts or statistically insignificant results should be considered when calculating sample size. The US FDA and EMA have set the lowest limit of a bioequivalence study at 12. (CHMP, 2010). Other authorities set different minimum sample sizes for bioequivalence studies. For example, in Russia the minimum sample size is 18 subjects and in Saudi Arabia 24, although a reduction to 12 subjects is acceptable if statistically justifiable. In Japan, the guidelines refer to a "sufficient number", while in India, the minimum sample size must be "adequate". (Dr. Malcom Ross, 2018). No maximum number of samples is mentioned in any guideline. The adequate sample size for a bioequivalence study depends on the clinical design of the study, possibility of failure of the study, expected difference between the two products, residual variability encountered limitations of the bioequivalence assumption, etc., among other factors. Estimating the sample size of a bioequivalence study can be done using mathematical formulas, asymptotic methods, or Monte Carlo simulations. Sample size issues are of great importance because studies that use samples that are too large or too small may be judged unethical: A study that uses a larger sample than necessary could have achieved the goal with fewer subjects, so some of them would have participated in the study unnecessarily. On the other hand, a study that uses fewer patients than necessary has a low chance of success,

so subjects may have been exposed to potential trauma without benefit (Altman, 1980; Julious, 2004).

A.1.2.4 Sampling schedule & Duration of the study.

Another important aspect of the bioequivalence study is the sampling schedule and more specifically the frequency with which samples are taken from the subjects. In general, the number of samples collected should be sufficient to adequately describe the concentration-time profile of the plasma. Primarily, the sampling plan should include frequent sampling around the predicted T_{max} to provide a reliable estimate of peak exposure. In addition, the sampling plan should ensure that the C_{max} is not the first point on a concentration-time curve. In addition, the sampling plan should cover the plasma concentration-time curve long enough to provide a reliable estimate of the magnitude of exposure, which is achieved when the AUC (0-t) covers at least 80 % of the AUC(0-∞). In addition, according to the relevant guidelines, at least three to four samples are required during the terminal log-linear phase. To ensure accurate determination of AUC(0-τ), in multiple-dose studies, the pre-dose sample should be collected immediately before (within 5 min) dosing and the final sample should be collected within 10 min after the nominal time for the dosing interval. If urine is used as the biological sampling fluid, it should normally be collected over not less than three times the terminal elimination half-life. However, in accordance with plasma sampling recommendations, urine should be collected for no longer than 72 hours. Thus, it is evident that sampling points should include one sample before dose, at least one or two samples before C_{max}, two samples around C_{max}, and

three to four samples during the elimination phase, resulting in a total of at least seven sampling points for the entire bioequivalence study. For the majority of compounds, the number of samples needs to be higher to compensate for differences between subjects in absorption and elimination rates. (WHO, 2014).

Another important aspect of the bioequivalence study design is the time interval between sample collection from the first subject and the last subject. A certain amount of time is required to collect a sample from each subject, and the total time difference between the first and last sample collection can range from 10 to 20 minutes depending on the number of subjects and technicians participating in the study. Finally, it is very important to strictly adhere to the sampling schedule regarding the order of subjects. Otherwise, there may be significant differences between the actual time the drug remains in the body and the specified sampling time for each subject. (Nagadugra, 2019)

A.1.3 Statistical evaluation

Bioequivalence evaluation is a comparison of the pharmacokinetic performances of two products (test product T and reference product R). Thus, the first step in evaluating the results is to establish a specific and complete statistical framework for this comparison. Therefore, a full statistical analysis is used to evaluate the potential bioequivalence between the two products.

The way in which pharmacokinetic parameters are treated in the evaluation of bioequivalence has changed considerably in recent years. Initially, a comparison of the average pharmacokinetic parameters of the test and reference products took place. In this comparison, the parameters of the test product should not differ by more than 20% from the respective parameters of the reference product. Another methodology was the 75/75 rule. According to this rule, the ratio of the pharmacokinetic parameters of the test product and the reference product had to be within the acceptance limits of 75% to 125% for 75% of the subjects. In this case, the comparison of the parameters had to be done separately for each subject. Finally, when the fact that each drug has a different degree of variability became apparent, Confidence Interval (CI) was introduced into the statistical evaluation of bioequivalence. Since then, the statistical comparison between the two products has been performed by measuring a 90% CI and evaluating whether it falls between the acceptance limits of 80.00%-125.00%. More specifically, the calculations are performed in the logarithmic scale and the 90% CI of the geometric mean of the ratio of the pharmacokinetic parameters of the two products should be within ln(0.8) to ln(1.25) to demonstrate bioequivalence.

It should be emphasized that the comparison refers to the ratio of the parameters and not to their difference. Moreover, the pharmacokinetic parameters follow the log-normal distribution and thus the log-modified parameters follow the normal distribution. These are the reasons why regulatory authorities suggest the modification of parameters in logarithmic scale before their statistical evaluation (CDER, 2001).

The limits 80.00%-125.00% result from a fixed limit ±20% set by the EMA (CHMP, 2010). Although not strictly justified in the literature, it is accepted that a difference of 20% is not considered significant in terms of mean clinical effect (Tucker et al, 1995). On the other hand, a difference in bioavailability of the two products beyond this could pose safety problems for the patient. The 80.00%-125.00% limit is the general rule that applies to most drugs. However, exceptions exist for Highly-Variable Drugs (HVD) and for Narrow Therapeutic Index Drugs (NTID), which are discussed later in this section.

A.1.3.1 Confidence Interval

After conducting the bioequivalence study and recording the results for the two drugs, plasma concentration-time curves are generated for each subject in the test and reference product groups. From these concentration-time profiles, AUC and C_{max} are measured for each individual. The acceptance criteria for either C_{max} or AUC are described by the following equation:

$$(\mu_T - \mu_R)^2 \leq \theta_A^2$$

Equation A.1

This is equal to:

$$-\theta_A \leq (\mu_T - \mu_R) \leq \theta_A$$

Equation A.2

Where:

μ_T : The average of the logarithmically modified values of a pharmacokinetic parameter for the test product

μ_R : The average of the logarithmically modified values of a pharmacokinetic parameter for the reference product

θ_A : The logarithmically modified bioequivalence limit

The average of logarithmically modified parameters is calculated as follows:

$$\frac{1}{n}\sum_{i=1}^{n} \ln Y_i$$

Equation A.3

And the geometrical mean value:

$$e^{\left(\frac{1}{n}\sum_{i=1}^{n}\ln Y_i\right)}$$

Equation A.4

As a result the geometric mean ratio (GMR) is:

$$e^{(\mu_T-\mu_R)}$$

Equation A.5

As mentioned before, the bioequivalence assessment is based on the 90% Confidence interval. This equals to two 5% one-sided hypothesis controls.

The first hypothesis control includes:

Null Hypothesis: H_{01}: $\mu_T-\mu_R < \theta_1$

Alternative Hypothesis: $H_{11:}$ $\mu_T-\mu_R \geq \theta_1$

The second hypothesis control includes:

Null Hypothesis: H_{02}: $\mu_T-\mu_R > \theta_2$

Alternative Hypothesis: $H_{12:}$ $\mu_T-\mu_R \leq \theta_2$

In these equations, μ_T and μ_R represent the mean of the log-modified parameters and θ_1, θ_2 represent the log-modified lower and upper bioequivalence limits, respectively.

The null hypothesis H_0 represents the lack of bioequivalence between the two drugs or, in other words, the inability of the study to demonstrate bioequivalence. The alternative hypothesis H_1, on the other hand, represents the rejection of H_0 and the demonstration of bioequivalence. It is clear that in a bioequivalence study, the goal is to prove the alternative hypothesis or else reject the null hypothesis.

Based on these two types of hypotheses, there are two types of possible errors in a bioequivalence study.

Error type I (or α): The probability of falsely rejecting Ho and proving that the two products are bioequivalent while they are not. This is the most important risk, the so called “patient’s risk’’ because it sets the patient’s health at danger. Since the result of a BE study comes as a two sided 90% CI, and since it is not possible for the GMR ratio to be <80% and > 125% at the same time, the error type I or α in a Bioequivalence study is 5% or 0.05.

Error type II or β: The probability of falsely accepting H_0 and rejecting bioequivalence between two drugs that in reality are pharmaceutically equivalent. This is the manufacturer’s risk. The manufacturer’s risk is in close relation with the power of the design. More specifically the power of the design is defined as 1-β.

Type II error is fixed in the design planning as β ≤0.10-0.20 where the power of the design is ≥80-90%.

The statistical power of a clinical trial, and thus of a bioequivalence study, depends in part on the sample size and clinical design of the study. The larger the sample size of a study, the higher the statistical power and the lower the risk to the manufacturer. However, it is clear that a very large number of subjects cannot be included in the BE study for the sole purpose of increasing power, due to increased costs and, more importantly, for ethical reasons (Dr. Malcom Ross, 2018).

The 90% Confidence Interval is calculated with the following equation:

$$90\%\ \mathrm{CI} = \mu_T - \mu_R \pm t_{0.95\,(v)}\,\mathrm{MSE}\sqrt{\frac{1}{N1}+\frac{1}{N2}}$$

Equation A.6

where: μ_T and μ_R represent the average of the logarithmically modified parameters for the test and the reference product.

$t_{0.95(v)}$ is the point of Student's distribution that isolates a probability of 0.05 at the upper end for v degrees of freedom.

N1, N2 are the number of volunteers in each one of the two groups.

v are the degrees of freedom that depend on the total number of volunteers (sample size) and the number of participants on each group.

$$v = N1 + N2 - 2$$

Equation A.7

MSE is the Mean Square Error, the average value of the square of the calculated error.

To calculate the MSE, ANOVA (ANalysis Of VAriance) is applied with the values of pharmacokinetic parameters and the sample size of the study. In this way, the overall variability is calculated, which is partly due to known sources of variability such as the pharmaceutical products, the subjects, the time periods and the administration arms. After subtracting the known sources of variability from the total variability measured by ANOVA, the residual variability remains, which is represented by the MSE and may be attributable to the Within-subject variability. Finally, the coefficient of variation, CV, can also be calculated using the following equation:

$$\mathrm{CV} = 100\sqrt{e^{\mathrm{MSE}} - 1}$$

Equation A.8

A.1.3.2 Special acceptance limits cases

A.1.3.2.1. Highly Variable Drugs

As mentioned earlier, the generally accepted bioequivalence limits are 80.00%-125.00%. This results from the 20% difference in the ratio of pharmacokinetic parameters accepted as safe by regulatory authorities worldwide. However, questions are raised about the bioequivalence limits of Highly Variable Drugs (HVD).

The high value of variability of a drug may be due to various pathological or physiological conditions or physicochemical properties of the drug. Due to this variability, the chances of a highly variable drug product meeting the bioequivalence criteria of 80.00% - 125.00% are low and thus the demonstration of bioequivalence is more difficult.

Consequently, for the drugs with a value of Within-Subject Variability for the pharmacokinetic parameter C_{max} greater than 30% (Highly Variable Drugs - HVD), the bioequivalence limits are extended. It should be emphasized that the widening of bioequivalence limits refers only to the limits of C_{max} and not to the limits of AUC. The EMA proposes scaled bioequivalence limits depending on the WSV. The limits are up to 69.84%-143.19% for drugs with WSV 50% (Karalis et al., 2011). The FDA also proposes scaled limits for HVD, but in this case there is no upper limit in the broadening of Confidence Interval.

A.1.3.2.2. Narrow Therapeutic Index Drugs

The Therapeutic Index is the established relationship between the therapeutic and the toxic dose of a drug substance. The equation used to calculate the therapeutic index is:

$$Therapeutic\ Index = \frac{TD_{50}}{ED_{50}}$$

Equation A.9

where :

TD50 is the dose required to cause a toxic effect to 50% of the population

ED50 is the dose required to heal the 50% of the population.

A better explanation of the Therapeutic Index is given in Figure A.6

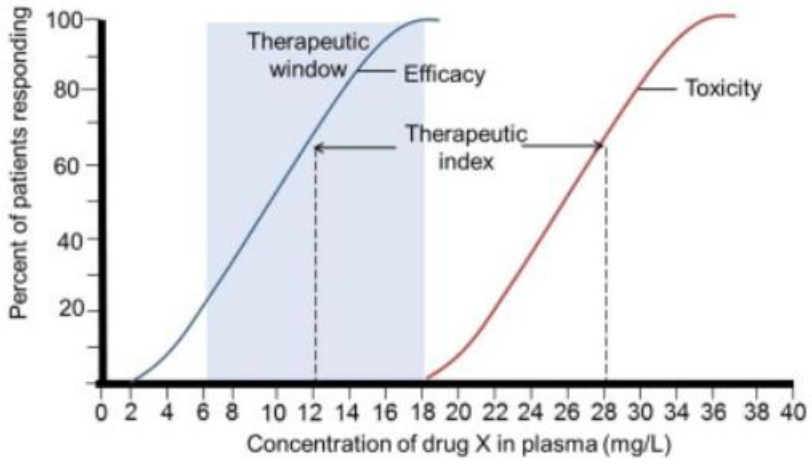


Figure A.6. *Percentage of responding patients-plasma concentration curve*

Narrow Therapeutic Index Drugs are those drugs for which TD50 and ED50 are close to each other and thus the therapeutic window is small. In these cases, minor changes in dosage or blood concentration can lead to significant changes including toxic effects or treatment failure. This is the reason why the EMA proposes to make the bioequivalence limits for NTID more stringent. According to the EMA, the accepted difference between the ratio of the test product and the reference product is 10% and the bioequivalence limits are 90.00% to 110.00%. These limits refer to the two pharmacokinetic parameters C_{max} and AUC. However, there is no clear statement on which agents are considered NTIDs, this is a case-by-case decision based on clinical data. On the other hand, FDA does not propose different BE thresholds for Narrow Therapeutic Index Drugs. FDA proposes to conduct replicate, four-period, and cross-over studies to administer each product twice to each subject to calculate the WSV. For drugs with a WSV of up to 10%, narrower limits than 90.00-110.00% are proposed. As the measured value of WSV increases, the proposed limits become wider, but always up to 80.00-125.00%.

A.2 In silico clinical trials

The term "in silico" refers to the widespread use of silicon in computer chips. It is an expression meaning "performed on a computer" or "by computer simulation" and refers to biological experiments. It was first used in the 1990s as a reference to the Latin expressions in vivo, in vitro, and in situ, which were commonly used in biology.

The term "in silico clinical trial" (ISCT) refers to the use of computer simulations in the development or regulatory evaluation of a pharmaceutical product or medical device. The primary goal of ISCT is to generate pharmacokinetic or pharmacodynamic models.

In 1961, a computer was first used to measure the pharmacokinetics of a drug substance, and by the mid-1970s, the use of pharmacokinetic models was well established. The models at this time were simple: the body was described as a "black box" and the equations simulating the pharmacokinetics of the substance were selected based on which better matched the experimental observations. Around 1980, computer stimulations were also used in the field of pharmacodynamics. In 2005, the concept of a Virtual Physiological Human was described for the first time. (White Paper, 2005). Finally, in 2011, the Virtual Physiological Human Institute introduced the use of ISCT as an alternative use for patient-specific models. Since then, many papers on modeling and simulations have been published and ISCT are being used more extensively in pharmacy. The importance of ISCT has also been recognized by the EMA and a Modeling and Simulation Working party was established in January 2013 to support the scientific committees and working

groups of European Medicines Agency in modeling and simulation related to medicines (Karalis, 2016).

An in silico clinical trial allows the outcome of the clinical trial to be predicted and any conditions that could potentially affect the outcome to be tested without actually conducting the trial. ISCT can be used to evaluate the safety and efficacy of new pharmaceutical products in accordance with clinical trials conducted in living humans. In addition, the outcome of an ISCT can be used to corroborate the outcome of a study conducted in vivo and to support clinical decisions made on that basis. (Pappalardo et al, 2019). Last but not least, ISCT plays an important role in bioequivalence assessment, as will be discussed later.

An in silico clinical trial includes:

1) Generation of virtual subjects: Simulation of different pharmacokinetic models for substitution of live subjects, using Monte Carlo simulations. Between-Subject Variability and Within-Subject Variability are also considered when generating these models.

2) Virtual conditions: Simulation of different pathological conditions.

3) Virtual drugs described by different pharmacokinetic parameters

4) Clinical designs.

At this point, it should be mentioned that the modeler can determine all parameters of the virtual population and virtual drug products in the simulation (Karalis, 2016). A visualized description of the parts of an ISCT can be found in Figure 7.

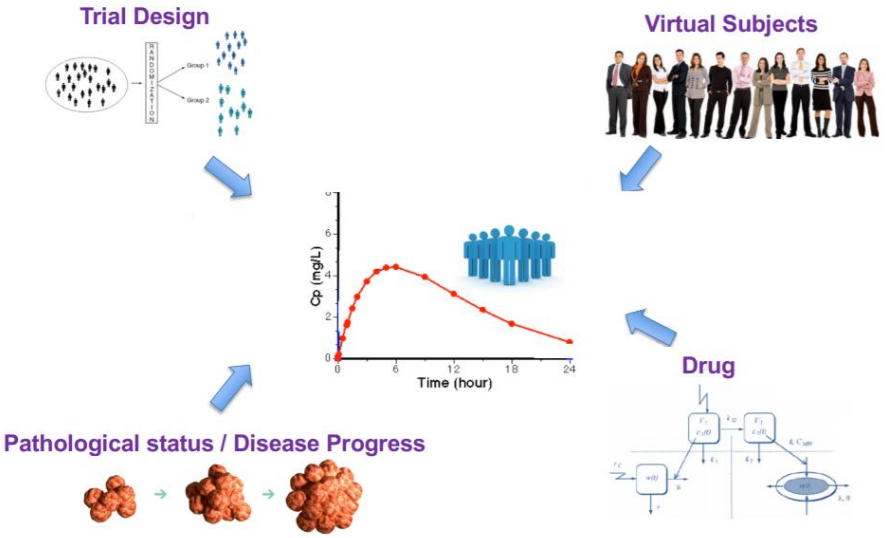


Figure A.7 : *In Silico Clinical Trials (ISCT) Description*

In silico clinical trials have many advantages. First of all, there is no ethical restriction on the number of subjects who participate in the study. The sample size can be as large as the modeler desires without the risk of harming human subjects.

Furthermore, in an ISCT, the dose range can be expanded as desired without the need to consider potential toxic effects, as is the case when dealing with live subjects.

In addition, the study can be performed as many times as desired. Monte Carlo simulations can be used. A Monte Carlo simulation is a type of simulation in which the outcome is calculated using repeated random sampling and statistical analysis. In this way, the influence of chance on the study outcome is minimized. This is explained by the Law of Large Numbers (LLN). The LLN is a theorem that describes the result of repeating an experiment a large number of times. According to this law, the average of the results from a large number of trials should be close to the true value and will tend to get closer and closer to reality as more trials are performed. (Dekking, 2005). Therefore, the ability to run a simulation many times as part of an ISCT can help increase the accuracy of the results.

In addition, as mentioned earlier, ISCTs give the modeler the ability to control the parameters of the study. Consequently, the effect of several parameters on the study outcome can be examined by changing their values and repeating the study. (Karalis, 2016)

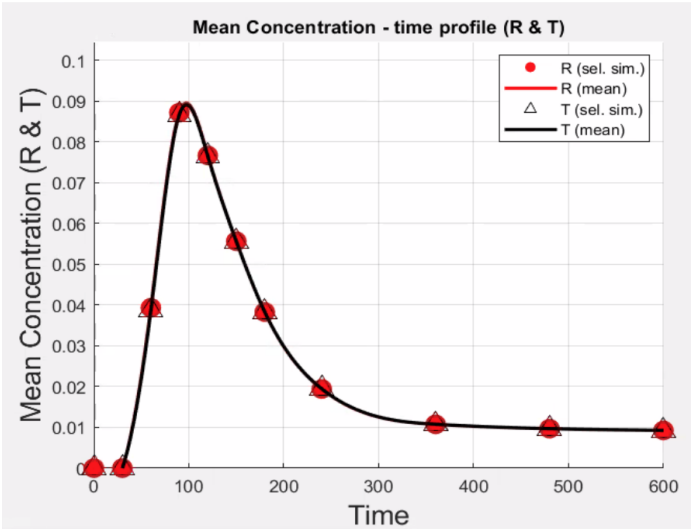
A.2.1. In silico clinical trials in bioequivalence assessment

As mentioned in the previous sections of this paper, statistics plays a very important role in bioequivalence studies. Also, computational approaches are a crucial component for in-depth bioequivalence analysis. This is the reason why pharmacokinetic models are highly associated with bioequivalence assessment. The use of modeling and simulation in bioequivalence studies can be useful in various aspects such as: the choice of pharmacokinetic parameters to describe the rate and extent of absorption, statistical framework, choice of clinical design, sample size estimation, bioequivalence assessment of highly variable drugs, etc. (Karalis, 2016). In addition, in silico models can help in planning blood sampling in bioequivalence by allowing repeated simulations to be performed at short time intervals, changing parameters and operating conditions, and then evaluating these changes in study results. In addition, modeling and simulation are now being formally used to justify biowaivers, extrapolate bioequivalence results to the pediatric population, clarify methodology for evaluating similar biopharmaceuticals (biosimilars), and incorporate genotypes and phenotypes in the field of bioequivalence. Another important area where modeling and simulations can be applied in the field of bioequivalence is the direct comparison of two generic products to address interchangeability. (Karalis, 2016).

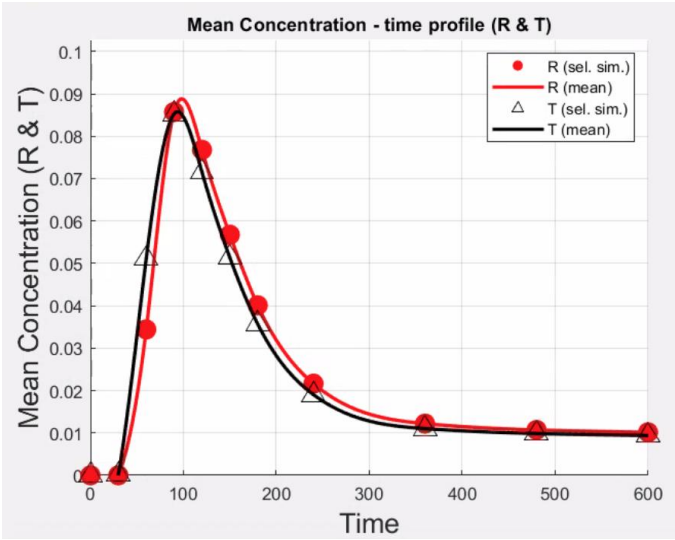
A.2.1.1 In silico clinical trials in bioequivalence assessment for the determination of sampling scheme

In silico clinical trials can be applied in various aspects of bioequivalence assessment. In particular, when trying to determine the ideal sampling plan for a bioequivalence study, in silico clinical trials are a very useful tool. The issue of sampling schedule was addressed by Eunice Kano et al. who used both in silico and in vivo clinical studies with different sampling schemes to determine the influence of sampling frequency as well as density of sampling schedule on the outcome of a bioequivalence study. In Kano's study, Monte Carlo simulations were used to obtain two in silico models for the bioequivalence studies. The comparison between the in silico and in vivo studies revealed that both studies lead to the same conclusions. (Kano et al, 2017).

Some examples of the use of in silico clinical studies in bioequivalence assessment are presented below.



A

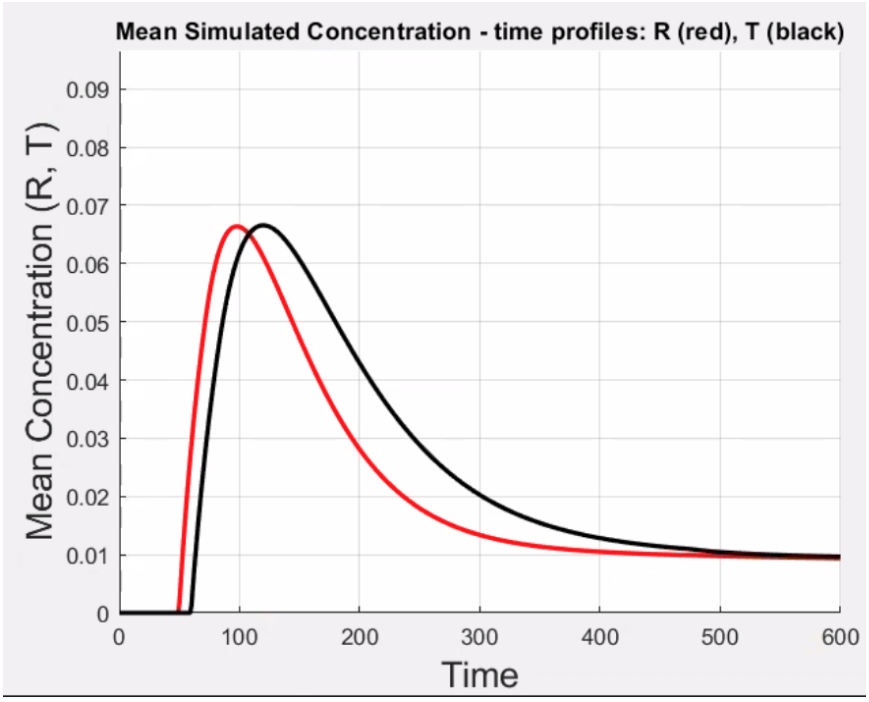


B

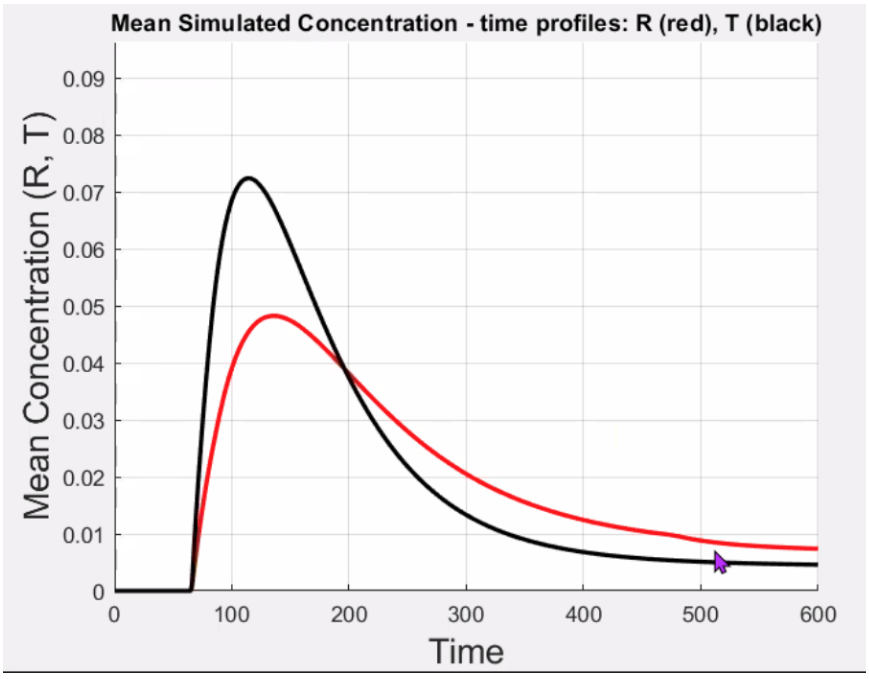
Figure A.8: *Mean concentration-time curves of test and reference product as a result of an In Silico Bioequivalence trial*

Figures A.8.A and A.8.B represent the mean concentration-time curves of the test (black curve) and reference (red curve) products as a result of an in silico bioequivalence study, with simulations performed in a computer program developed in Matlab® 2018b. (See section "B.2 Simulation Methodology" of this paper.) In these figures, the dots and the triangles represent the time points at which sampling occurs in the subjects ingesting the test and reference products, respectively. If the same experiment were performed in vivo, the results obtained would be exclusively the concentration-time pairs defined by the dots and the triangles. The continuous shape of the curves is the result of the simulations and the high number of replicates of the study that led to the figures shown.

In Figure A.8.A, the performances of the test and reference products are identical. For this reason, sampling at each time point gives the same mean concentration for the test and reference products and the two curves look as if they are a single curve. In Figure A.8.B, the case is different. In this case, the performance of the two products is different, with the test product having a slightly lower absorption rate and lower absorption range.



A



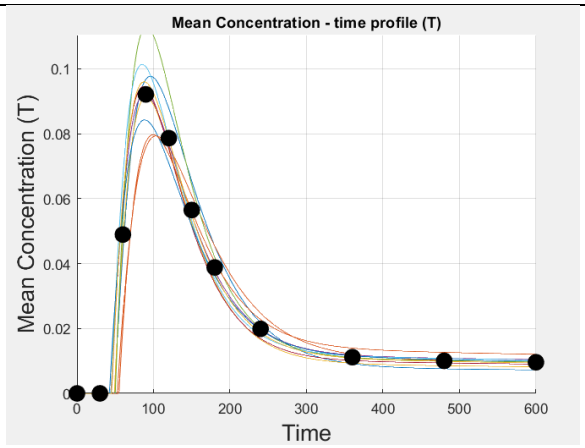
B

Figure A.9: *Mean simulated concentration-time curves of test and reference product as a result of an In Silico Bioequivalence trial*

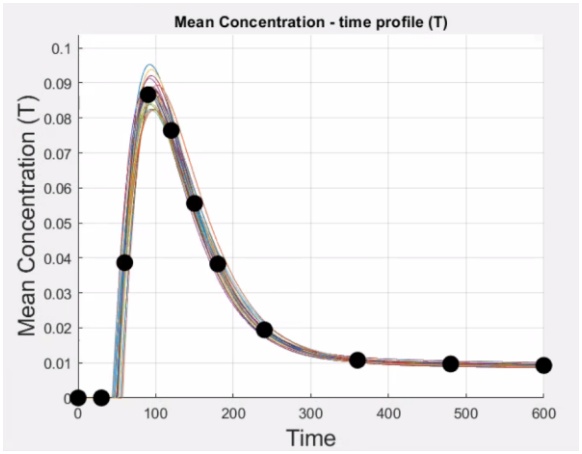
The ISCT trials are also shown in Figures A.9.A and A.9.B. These figures show the mean simulated concentration for the test (black curve) and reference (red curve) products as a function of time. These figures are the average result of the large number of replicates of the experiment and the different population data regenerated by the program for each run.

Both figures represent test and reference products that differ from each other. In Figure A.9.A, the reference product (red curve) reaches C_{max} before the test product (black curve). This means that the absorption rate of the reference product is higher.

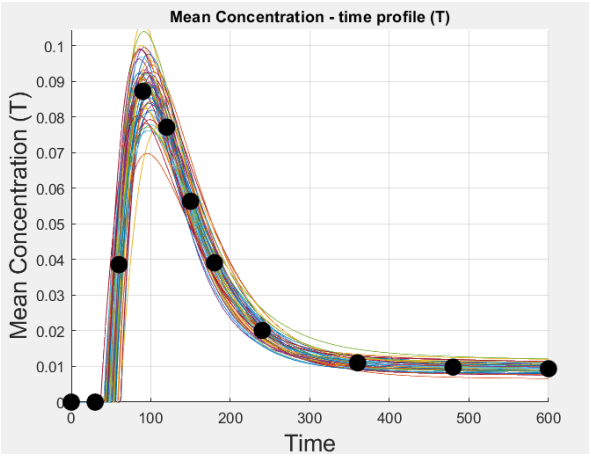
In Figure A.9.B, the test product (black curve) is the one that reaches C_{max} before the reference product. In addition, the area under the curve (AUC) for the test product is obviously larger than that of the reference product. This is a case where the test product has a higher rate and extent of absorption than the reference product.



A



B



C

Figure A.10: *Mean concentration-time curve as a result of three In Silico Bioequivalence trials with three different sample sizes*

Figures A.10.A, A.9.B, 10 A.10.C are also a plot of the concentration-time curve for the test product in an In Silico bioequivalence study.

In these figures, the simulated concentration-time curves are shown for all subjects administered the test product. Each different colored curve corresponds to a different subject.

As mentioned earlier, a major advantage of in silico clinical trials is the ability to control the study parameters. Figures A.10A, A.10.B, and A.10.C are an example of this. These figures are results of three different simulations of the same ISCT of a drug substance, with low values of Within-Subject Variability and Between-Subject Variability. The only difference is the sample size. Figure A.10.A corresponds to 5 subjects, Figure A.10.B corresponds to 24 subjects, and Figure A.10.C corresponds to 50 subjects. This can be observed in the figures because each curve corresponds to a different subject and thus the larger the sample size, the denser each figure is.

The black dots are the sample points for each of the subjects. All simulations shown were run with the same sampling plan. The average of the presented concentrations at each time point gives the mean concentration-time curve of the test product and a type of figure like Figure A.9.A.

Figures A.8, A.9, and A.10 are just a few examples of the large number of applications of ISCTs in bioequivalence assessment.

A.3 Scope of the present thesis

As mentioned earlier, when designing a bioequivalence study, there are several parameters that must be determined in an appropriate manner to obtain reliable results. Proper determination of study parameters is not only beneficial but also necessary for ethical and economic reasons. The aim is to obtain a study design with high power that causes as little inconvenience as possible for the subjects.

An important parameter of bioequivalence study design is the determination of the sampling plan or sampling design. The importance of sampling design is widely recognized as inadequate sampling designs may lead to inaccuracy of the study and uncertain results, while a very dense sampling design may unnecessarily increase the workload of the clinical center, the cost of the study, and most importantly, the inconvenience to the subjects. (Kano et al, 2017). Considering that it is unethical to use so many samples per subject in an in vivo study, the sampling points should be chosen wisely to ensure that the recorded concentration-time curve, corresponds to the actual drug concentration in the human body. Also, there is no clear statement in the EMA and FDA guidelines about an optimal number of sampling points for a bioequivalence study.

The aim of the present work is to evaluate the influence of different sampling schemes on the bioequivalence study outcome using modeling and simulation, i.e., in silico clinical studies. More specifically, different sampling schemes that differ in terms of duration and density are applied in simulations of bioequivalence studies. The power of each study and the GMR for the pharmacokinetic parameters C_{max} and AUC are evaluated in each case. At

the same time, the influence of parameters such as sample size, Within-Subject Variability and Between-Subject Variability on the bioequivalence result is also investigated.

B. METHODS

As mentioned in the previous section, the purpose of this study is to evaluate the impact of various changes in bioequivalence study design on study outcome. More specifically, changes in sampling scheme are evaluated by conducting an in silico bioequivalence study.

For this purpose, it was necessary to use information from the design of a bioequivalence study conducted in vivo. The design of the study conducted in vivo was used as a target scenario, modified according to the scope of the research. The number of subjects (sample size), density, and duration of the sampling design are the study design parameters that were modified across experiments.

After several modified scenarios were created, simulations were conducted for each scenario. Then, the power of the study and the GMR for the pharmacokinetic parameters C_{max} and AUC were measured. Finally, the impact of the modifications made on the study outcome was evaluated.

B.1 In vivo bioequivalence study

The in vivo bioequivalence study used as the Nominal Design Scenario was a study between a test product and a reference product containing the active pharmaceutical ingredient donepezil.

Donepezil is used for dementia due to Alzheimer's disease. Alzheimer's disease is the most common cause of dementia (Harper et al., 2019), which affects memory, thinking, and behavior. It is a neurodegenerative disease that can cause impaired short-term memory, function, language, and visuospatial perception. (Douglas et al, 2019). There is no current therapy for Alzheimer's disease that can eliminate symptoms and reverse neurodegeneration of the nervous system. Currently proposed medications aim to control the mental, behavioral, and psychological symptoms of the disease while delaying its progression. (Politis et al., 2016). Acetylcholinesterase (AchE) inhibitors (donepezil, galantamine, rivastigmine), the NMDA glutamine receptor antagonist memantine, and herbal medicines such as gingko biloba are used to treat mental symptoms. Reversible acetylcholinesterase (AchE) inhibitors prevent the breakdown of acetylcholine, thereby increasing its levels and enhancing decreased cholinergic transmission in the central nervous system. They exhibit a selective effect in the central nervous system relative to the rest of the body. They also have a moderate effect and are therefore used for mild to moderate illness. Side effects include diarrhea, nausea, anorexia, weight loss, and syncope. They should not be used if no benefit is observed. Donepezil has the advantage of causing fewer side effects than the other drugs in the group, and it only needs to be administered once a day. The starting dose is recommended at 5 mg, and the target dose is 10 mg per day.

The selected bioequivalence study was a crossover (2×2), randomized trial. The test product (T) was donepezil/Verisfield and the reference product was Aricept®/Pfizer. The administered dose was 10 mg for both products. The sample size of the study was 30 volunteers, both men and women, Caucasian, aged 21 to 45 years. They were non-smokers and were in good physical condition. Since this was a bioequivalence study, the efficacy and safety of the drugs were not measured directly, but a routine clinical examination (cardiac test, measurement of weight, height, blood pressure, pulse, etc.) was performed before and after the administration of each drug in all participants.

Subjects were randomly allocated to the two groups (test, reference). On day 1, a dose of 10 mg of either the test or reference product was administered to all subjects. Blood samples were collected from the subjects from day 1 to day 8. The first study period was followed by a washout period until day 29. Considering the half-life (t1/2) of donepezil of 59.7 ±16.1 hours (Ohnishi et al., 1993), a period of 20 days (480 hours) is considered an adequate washout period. This was followed by the second period in which 10 mg of the other product was administered, so that at the end of the two periods each subject would have taken both products. Blood samples were collected from the subjects by day 36.

As for the sampling scheme, the samples were collected at predetermined time intervals. More specifically, samples were collected at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 96, and 192 hours after drug administration. The sampling scheme was the same for both periods of the study. A total of 14 blood samples were collected from each subject in each period.

The volume of the subject blood sample for measuring donepezil concentration was 4 ml and for routine medical examination was 10 ml. A total of 132 ml of blood was collected from each subject (2×10 + 2×14×4 ml).

The drug was extracted from the samples by liquid-liquid extraction using hexane as organic solvent. High performance liquid chromatography with MS detector (HPLC-MS/ MS) was used for quantitative determination of donepezil in the blood samples. The analytical method was validated and proved to be linear, accurate, precise and specific.

B.2 Simulation Methodology

All computer simulations for the in silico clinical studies were performed in a computer program developed in Matlab® 2018b.

The dissolution model used followed first-order kinetics, regenerating separate release rates for the reference product R and the test product T for each scenario. The dose D (mg) of the product, the dissolution coefficient of the reference product k_{DR}, measured in min-1 for first-order kinetics models and in mg/min for zero-order models, were set in the program. Moreover, after setting the absorption coefficient ka (min^{-1}), elimination coefficient kel (min^{-1}), phenomenal volume of distribution V (L), and percentage of absorbed dose F, drug distribution simulations were performed and in vivo parameters were calculated.

Simultaneously, the Between-Subject Variability and the Within-Subject Variability were also determined. BSV stands for the variability between the different dissolution vessels for the in vitro test and for the variability between the different subjects participating in the study for the in vivo test. WSV for the in vitro test is represented by the variability of dissolution in the same vessel when the test is performed more than once with different tablets of the same product. WSV for the in vivo test is the physiological variability within a subject of the study. BSV and WSV were set for the resolution coefficients for the reference and test products. This program also allows the variability for in vivo absorption to be changed.

Prior to running each simulation, the number of trials, sample size, and percent difference in absorption rate between the test and reference products were determined. Finally, different scenarios for the sampling scheme were

also entered to account for the scope of the research and to investigate the influence of the sampling scheme on the study results.

The in vivo pharmacokinetic parameters of donepezil were kept constant in all simulations. Two levels of BSV were used (5%, 20%), while WSV was always set at 10%. In addition, different degrees of difference in the absorption rate of the two products were applied. In addition, the clinical design of the study was the standard 2 × 2 crossover design. (See Figure A.3). Finally, Monte Carlo simulations were applied and each run was repeated 1000 times, with each run regenerating the data based on the values set in the program. An image is provided to visually describe the program that was used for the simulations.

Figure B.1.A shows the parameters that can be defined in the program. Figure B.1.B shows the ability of the program to select a different sample scheme from a computer folder for each simulation.

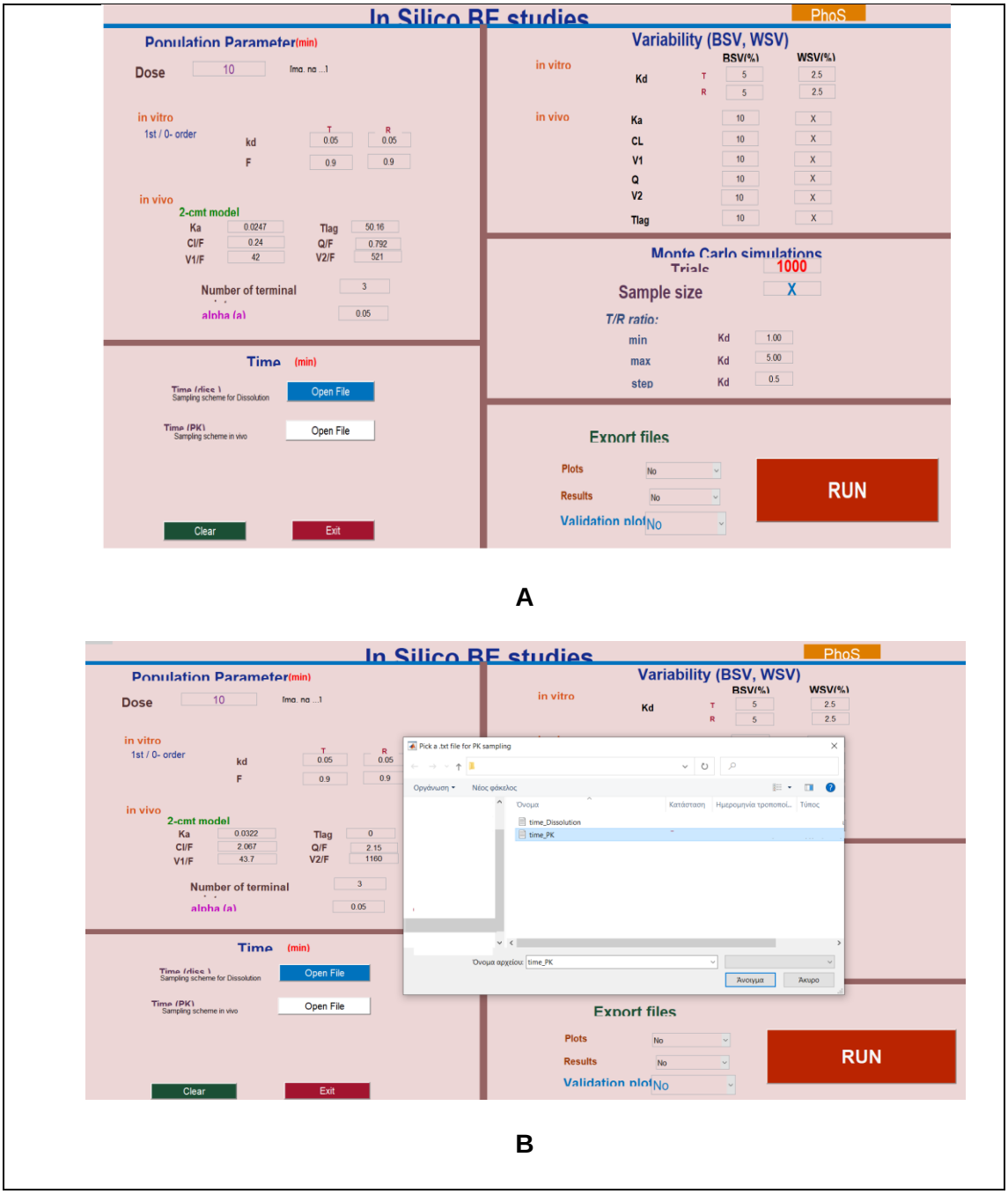


Figure B.1: *Computer program developed in MatLab® 2018b and used for simulations in terms of In Silico Clinical Trials.*

Regarding the in vivo pharmacokinetic parameters of donepezil the following values were found in literature (Tsyplakova A., 2021) and used in the performed simulations.

Ka: 0.0247 min^{-1}	T_{lag}: 50.16 min
Cl/F: 0.24 L/min	Q/F : 0.792 L/min
V1/F: 42 L	V2/F: 521 L

***Table B.1**: In vivo pharmacokinetic values of donepezil (Tsyplakova A., 2021)*

where:

Ka: The absorption rate constant

T_{lag}: The time until the first concentration of drug is detected in plasma

Cl: The clearance of the drug substance

F: The per os bioavailability of the drug

Q: The inter-compartmental clearance of the drug substance

V1: The distribution volume of the central compartment

V2: The distribution volume of the secondary compartment

The different scenarios were developed by changing the sample size of the study, the applied value of the BSV, and the timing of the sampling design.

Two sample sizes were studied: 12 and 24 subjects.

The C_{max} of donepezil exhibits a WSV of 15%. (Rojanasthien N et al, 2012, Yewon Choi et al, 2015, Gadiko C. et al, 2013). The study was conducted with a WSV of 10%, which is slightly lower than the actual variability of the drug to reduce the impact of variability and to facilitate the study of the impact of the sampling design. Two different values of BSV were applied: 5% and 10%. The value 10% represents the usual BSV of a bioequivalence study. The value 5% was applied in accordance with the lower value of WSV to minimize variability and facilitate the detection of the impact of the sampling design.

In order to develop the different scenarios of the sampling design, the T_{max} of donepezil had to be considered. According to the in vivo bioequivalence study, the T_{max} of donepezil is almost evenly distributed between 2, 3 and 4 hours after administration in 29 out of 30 subjects in both time periods (Figure B.2). This is a good case because if the majority of T_{max} observations coincided with a particular sampling time point, removal of this point is almost certain to significantly affect the results.

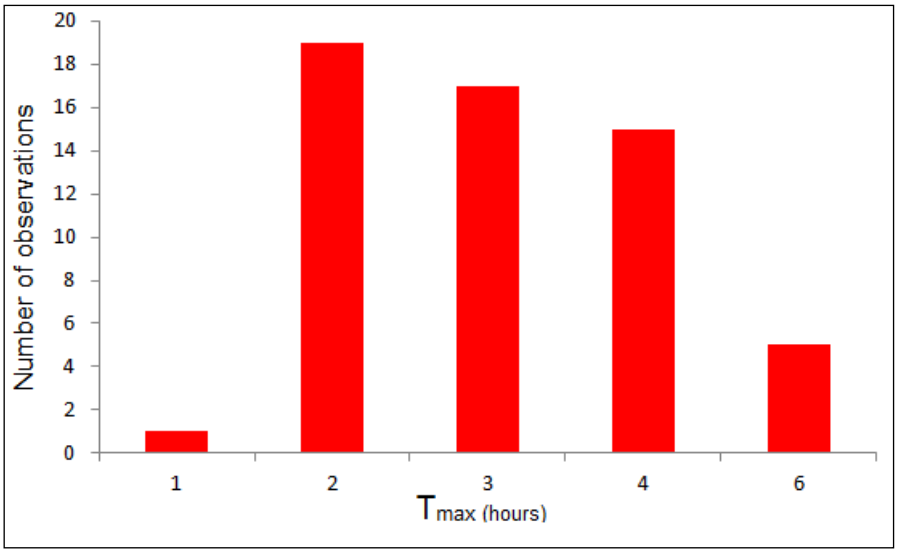


Figure B.2: *T_{max} observations in the in vivo bioequivalence study*

Regarding the methodology for subtracting sampling points, three tactics were followed. First, sampling points were subtracted at the beginning, then in the middle, and then at the end of the sampling plan. The number of points subtracted varied from two to six. Where possible, care was taken not to delete two sampling points at once, as this would involve obvious gaps in the sampling plan and would not be initially considered for a study design. In addition, the first sampling point (0 hours) was always included in the sampling plan scenario. It was expected that as the number of time points subtracted increased, the three tactics would eventually result in a sampling plan with evenly spaced time points. See Figure B.3 for a schematic representation of the sampling point subtraction methodology.

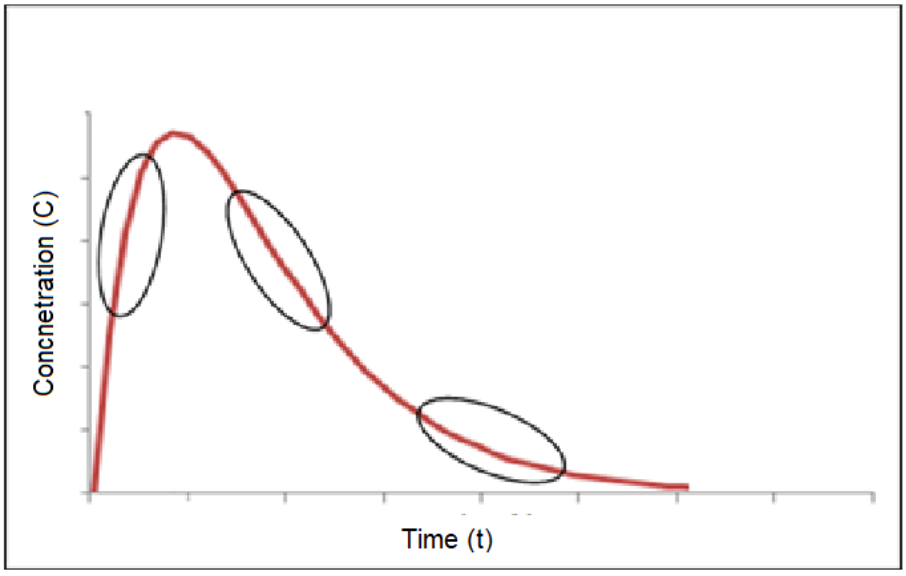


Figure B.3: *Methodology for the deletion of sampling points from the nominal sampling scheme*

In addition to examining the effects of a less dense sampling schedule, the effects of a shorter sampling schedule were also examined. Sampling points were deleted at the end of the nominal sampling schedule. First, the last time point corresponding to 192 hours or the 8th day of the study was subtracted. Day of the study was subtracted. Then, two points, three points, and so on up to six points were dropped from the sampling scheme, resulting in the worst-case scenario of an 8-hour sampling scheme where the last time point was only two hours after the last T_{max} observed in the in vivo study.

Finally, uniform deletions of sampling points were made throughout the nominal sampling scheme by deleting one time point every two time points, deleting two time points every three time points, and randomly subtracting time points throughout the sampling scheme.

Following the procedure described above, 20 different sampling scheme scenarios were created. These scenarios, as well as the nominal scenario, are shown in the table below:

Scenario No.	Sampling scheme (hours)
1	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 96, 144, 192
2	0, 0.5, 1, 2, 3, 4, 6, 8,12, 24, 48, 96, 144
3	0, 1, 3, 4, 6, 8,12, 24, 48, 96, 144, 192
4	0, 0.5, 1, 2, 6, 8, 12, 24, 48, 96, 144, 192
5	0, 0.5, 1, 2, 3, 4, 6, 12, 48, 96, 144, 192
6	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 8, 96
7	0, 2, 4, 6, 8, 12, 24, 48, 96, 144, 192
8	0, 0.5, 1, 6, 8, 12, 24, 48, 96, 144, 192
9	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 96, 192
10	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48
11	0, 3, 6, 8, 12, 24, 48, 96, 144, 192
12	0, 0.5, 6, 8, 12, 24, 48, 96, 144, 192
13	0, 0.5, 1, 2, 3, 4, 6, 24, 144, 192
14	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24
15	0, 6, 8, 12, 24, 48, 96, 144, 192
16	0, 0.5, 1, 2, 3, 4, 6, 144, 192
17	0, 0.5, 1, 2, 3, 4, 6, 8, 12
18	0, 0.5, 1, 2, 3, 4, 6, 8
19	0, 1, 3, 6, 12, 48, 144, 192
20	0, 2, 6, 24, 144, 192
21	0, 3, 4, 6, 48, 96, 144, 192

Table B.2.: *Sampling scheme scenarios applied*

The described scenarios were combined with the two different values of BSV and simulations with different sample sizes were performed.

A schematic presentation of the simulations is depicted in the following Figure:

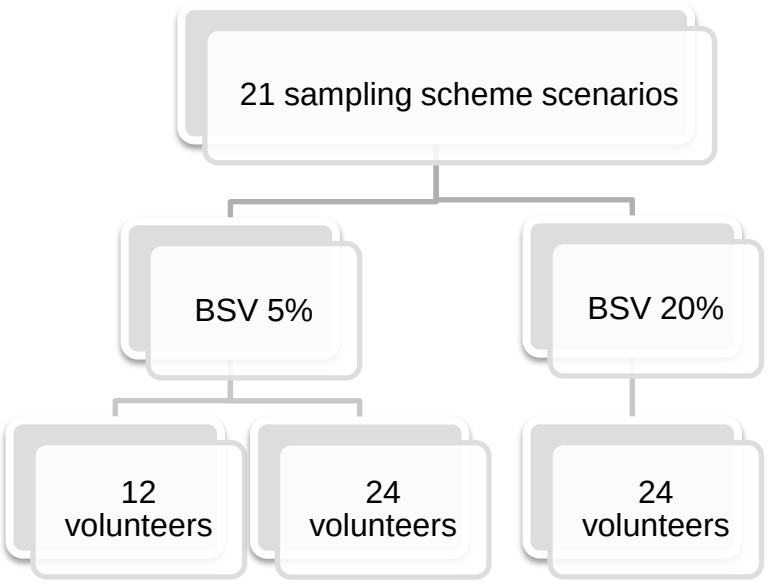


Figure B.4: *Simulations conducted for the investigation of the impact of sampling scheme*

The effect of sampling schedule was investigated as well as the effect of differences in absorption rate between the reference and the test product. Simulations were performed using the following values for the percentage difference in absorption rate: 20%, 40%, 50% and 75%. The test was performed for BSV 5% and 20% and for a sample size of 24. A schematic representation of the simulations can be seen in the following figure.

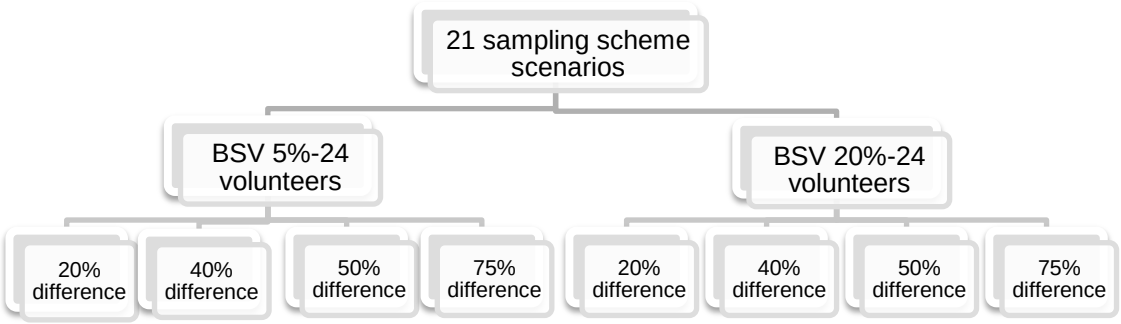


Figure B.5: *Simulations conducted for the investigation of the impact of sampling scheme combined with differences in the rate of absorption between the test and the reference product*

C. RESULTS

As mentioned above, the aim of the present work is to investigate the possibility of flexibility in the design of a bioequivalence study with respect to the duration and density of the sampling schedule. To this end, an in silico bioequivalence study is conducted based on data from the design of a bioequivalence study conducted in vivo with drugs containing the active ingredient donepezil. Different scenarios of the sampling design are applied. The influence of sample size, between-subject variability (BSV) and possible differences in pharmacokinetic properties of test and reference product are investigated.

After conducting the in silico studies, the impact of each intervention is evaluated. Valuable tools for this evaluation are the power of the study and the GMR for the pharmacokinetic parameters C_{max} and AUC. The higher the power of the study, the higher the likelihood of acceptance of the study results. Moreover as the GMR gets closer to 1.0, the test and the reference product seem to be more similar to one another.

C.1 The impact of Within-Subject Variability

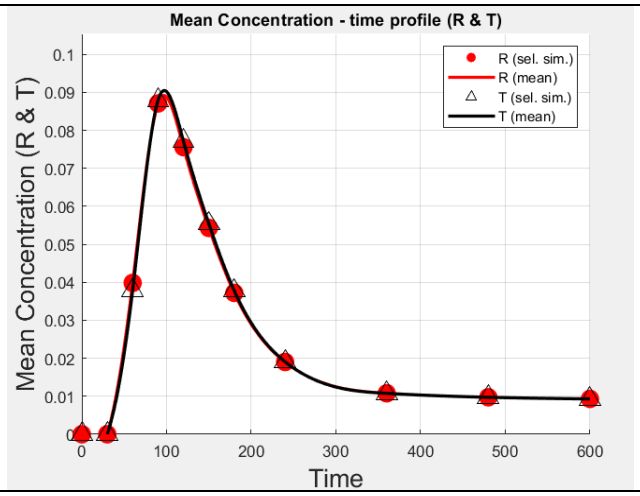
Before presenting the results of the effect of different sampling plans on the bioequivalence result, it is important to understand the importance of other factors that may affect the results of the study.

The first factor examined is Within-Subject Variability which represents the variability of a pharmacokinetic parameter within the same individual participating in the study.

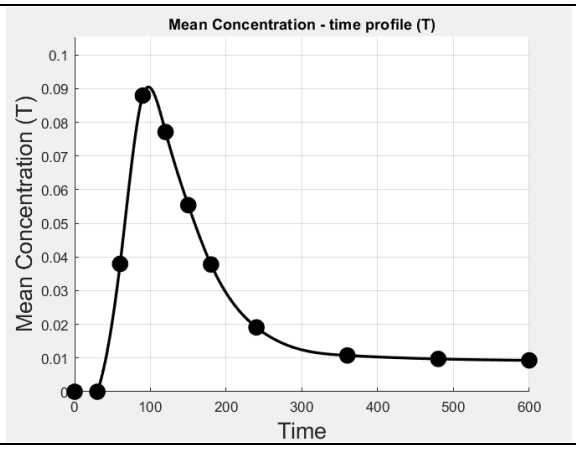
The effects of different values of WSV are shown in the following figures.

The simulations leading to the plots shown on the next pages were performed using the nominal sampling design scenario (Scenario 1, see Table B.2), with a sample size of 24 subjects and with a value of Between-Subject Variability of 5%.

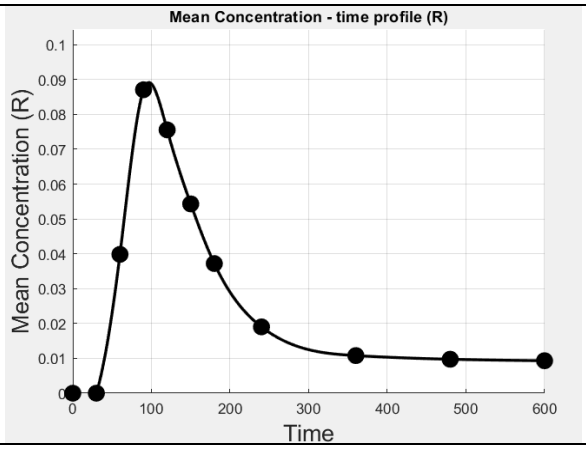
C.1.1. WSV 5%



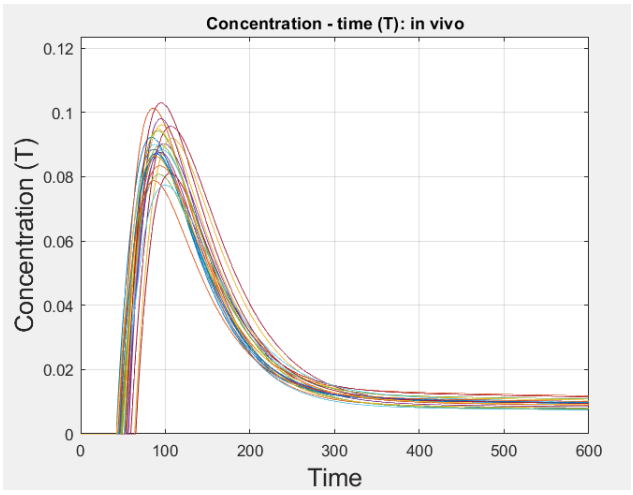
A



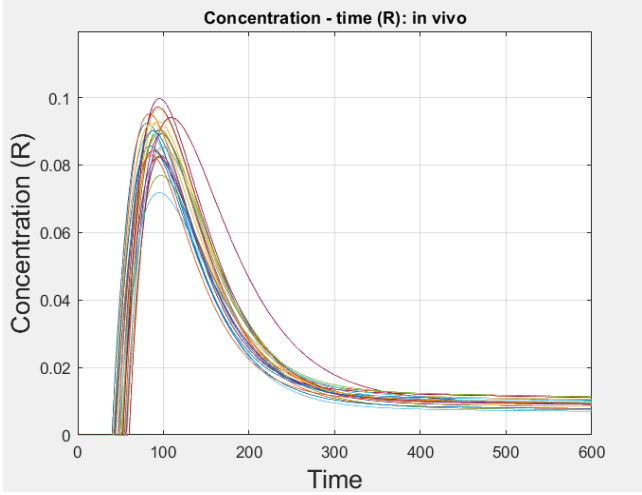
B



C



D



E

Test product	**Reference product**

Figure C.1*: Concentration-Time curves for Within-Subject Variability 5% for Test and Reference product*

Figures C.1.B and C.1.C show the mean plasma concentration of the test and reference products, respectively, as a function of time. The mean plasma concentration is the average of the plasma concentrations of all subjects taking the test and reference product, respectively. The black dots in the figures represent the time points at which sampling occurs. The simulation process contributes to the continuous shape of the curve seen in the figures. If there had been no simulation, the only result would be the black dots representing the concentration-time pairs at each time point.

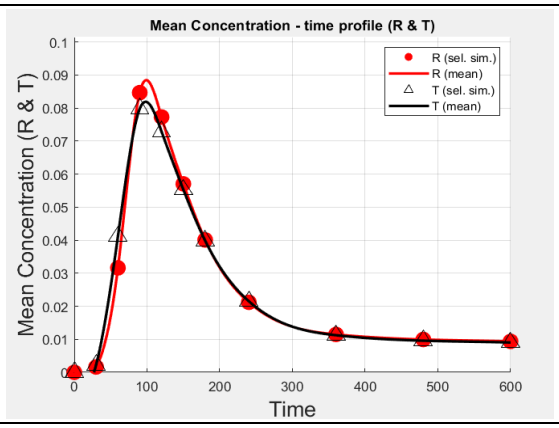
Figure C.1.A is the combined result of the mean concentration-time curves for the test and reference products. In other words, Figure C.1.A is a blend of Figures C.1.B and C.1.C. Again, the dots and triangles represent the times at which sampling occurs for the reference and test product groups, respectively. In this particular case, where the performance of the test and reference products are significantly similar and the WSV is low, the mean concentration-time curves for the two products are identical and this is the reason why they look like a single curve in Figure C.1.A.

The significance of Within-Subject Variability becomes clearer in Figures C.1.D and C.1.E. These types of graphs are called "spaghetti plots," a term derived from their shape. Figure C.1.D contains the concentration-time curves of the individual subjects administered the test product, and Figure C.1.E contains the concentration-time curves of the individual subjects ingesting the reference product. The different colored curves correspond to the different subjects.

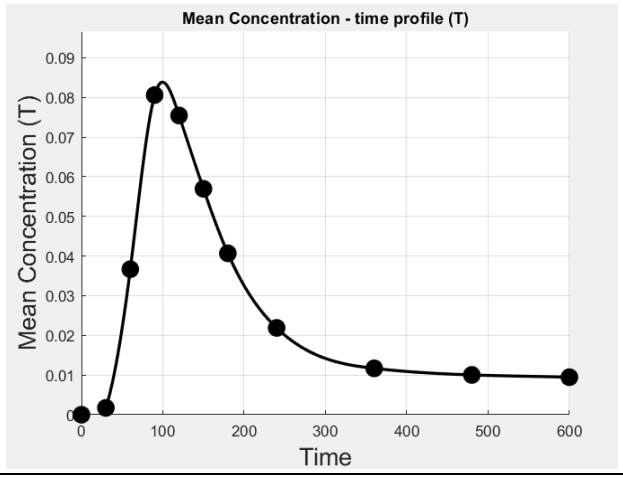
The mean values of the concentration at each time point of each of the curves of Figures C.1.D and C.1.E lead to Figures C.1.B and C.1.C, respectively.

Figures C.1.D and C.1.E are similar to each other and can also be said to both look like a single broad concentration-time curve. This is because the WSV of this run is relatively low and thus the variability of pharmacokinetic parameters within each individual is limited, resulting in similar pharmacokinetic profiles of the drugs across all participating subjects in the study.

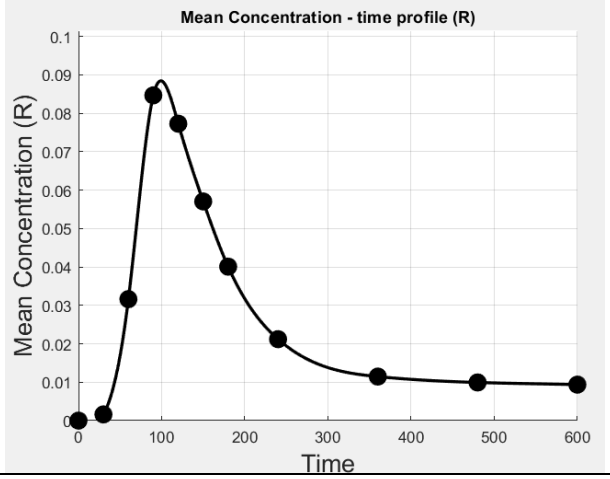
C.1.2. WSV 20 %



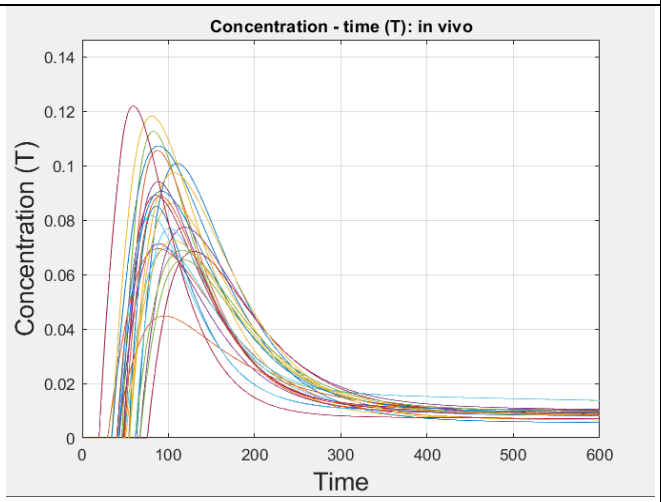
A



B

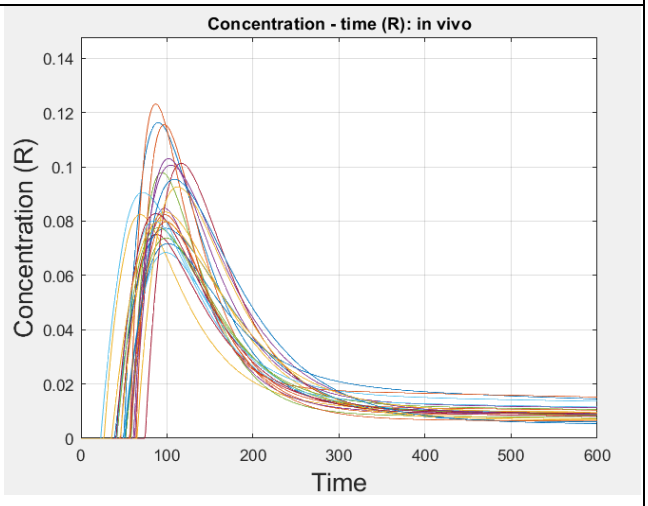


C



D

Test



E

Reference

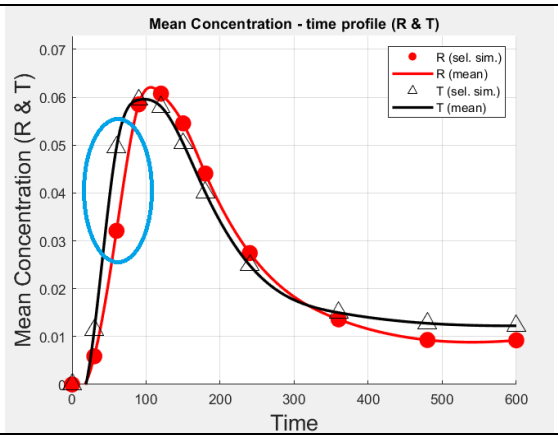
Figure C.2: *Concentration-Time curves for Within-Subject variability 20% for Test and Reference product*

In agreement with Figures C.1.B and C.1.C, Figures C.2.B and C.2.C show the mean concentration-time curve for the test and reference products, respectively, but at this stage the WSV of the simulation is 20%. Again, the black dots correspond to the sampling points.

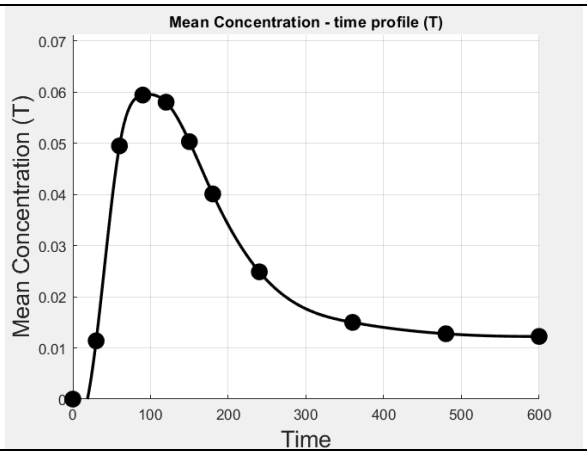
Figure C.2.A is the combined result of Figures C.2.B and C.2.C. This time the higher value of WSV has resulted in slight differences in the concentration-time curves of the two products, which can be seen more clearly in Figure C.2.A. The triangles and the points corresponding to the sampling points of the reference and test products now lead to slightly different blood concentrations and the simulated curves differ slightly.

Figures C.2.D and C.2.E, the "spaghetti plots", clearly show the influence of the WSV. Comparing Figure C.2.D with Figure C.1.D, it is clear that in the first case the individual concentration-time curves are not as similar to each other as in the second case. The same results when comparing Figure C.2.E with Figure C.1.E. The "spaghetti plots" no longer look like a single broad curve. In addition, Figures C.2.D and C.2.E are more different from each other than Figures C.1.D and C.1.E, which means that when the test and reference products with a WSV of 20% are administered to the same subject, the concentration-time curves are not similar due to within-subject variability. This fact is explained by the higher value of the WSV and justifies the differences between Figures C.2.B and C.2.C.

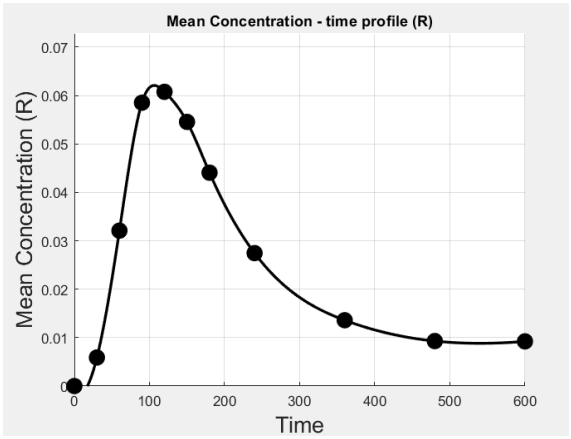
C.1.3. WSV 50%



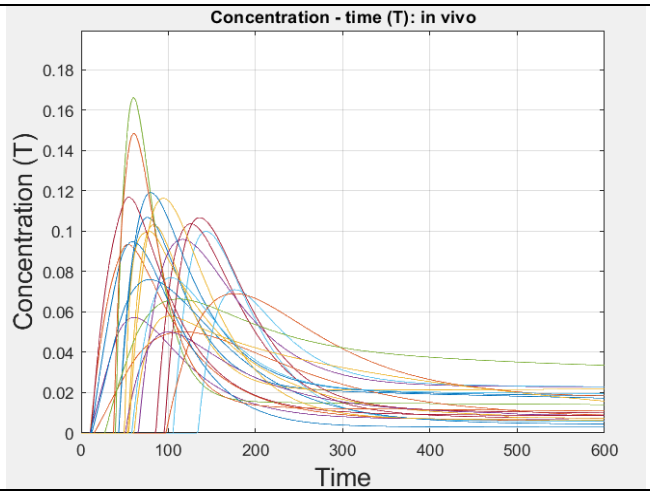
A



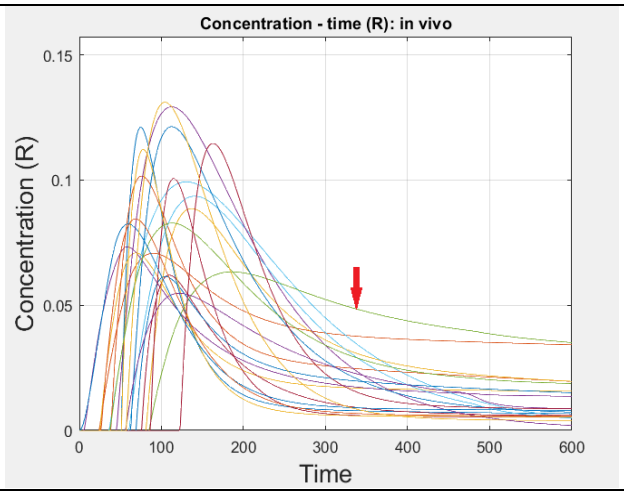
B



C



D



E

Test	**Reference**

Figure C.3: *Concentration-Time curves for Within-Subject variability 50% for Test and Reference product*

Even though this is an extreme scenario, it was appealing to examine the effects of a more variable drug with a value of WSV 50%.

A simple look at Figures C.3.A, C.3.B, C.3.C, C.3.D, and C.3.E shows the impact of a highly variable drug in the concentration-time curves.

The spaghetti plots, Figures C.3.D and C.3.E represent very different individuals with very different responses to administration of the test and reference products. The differences can be located by looking at the T_{max}, C_{max}, or T_{lag} of the curves. Some of the individuals shown in the plots differ on all of the above parameters. An example is shown with a dart in Figure C.3.E.

The high WSV of this simulation also affects the mean concentration-time curves of the test and reference products. Since the concentration-time curves of the individuals differ so much, it is expected that the mean concentrations are also affected. Figures C.3.B and C.3.C, showing the mean concentration-time relationship, are quite different from the corresponding Figures C.2.B and C.2.C and C.1.B and C.1.C.

The combined result of Figures C.3.B and C.3.C, shown in Figure C.3.A, shows two quite different mean concentration-time curves and the sampling times result in different concentration values across the sampling plan. For example, the third sampling time point corresponds to a significantly higher concentration than the reference product. It must be emphasized that this is due to a high value of the WSV of the drug and not to differences in the pharmacokinetic profiles of the two products.

C.2 The impact of Between-Subject Variability

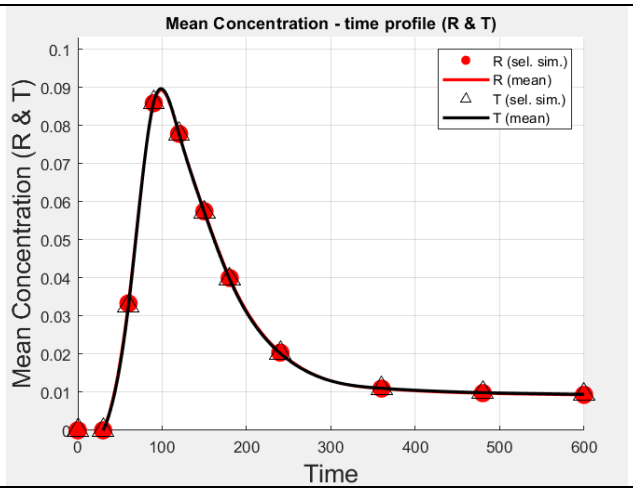
Another important factor that can affect the outcome of a bioequivalence study is the Between-Subject Variability (BSV).

The BSV is the variability in pharmacokinetic parameters between the different subjects in the study.

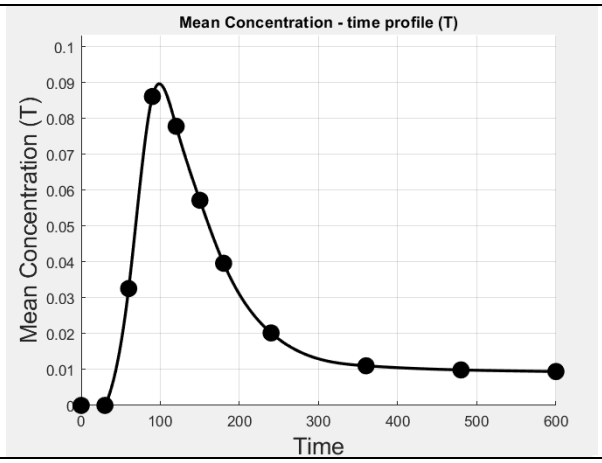
In view of investigating the influence of BSV, simulations are conducted with different values of BSV. Runs of the scenario with nominal sampling design (Scenario 1, see Table B.2), with a within-subject variability (WSV) value of 5% and with a sample size of 24 subjects are performed.

The results are shown in the following figures:

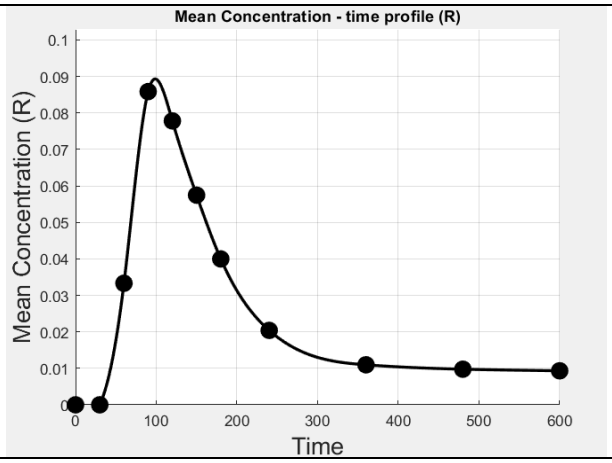
C.2.1. BSV 5 %



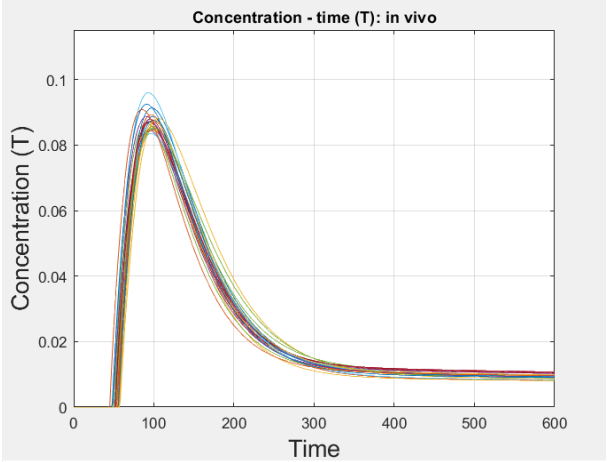
A



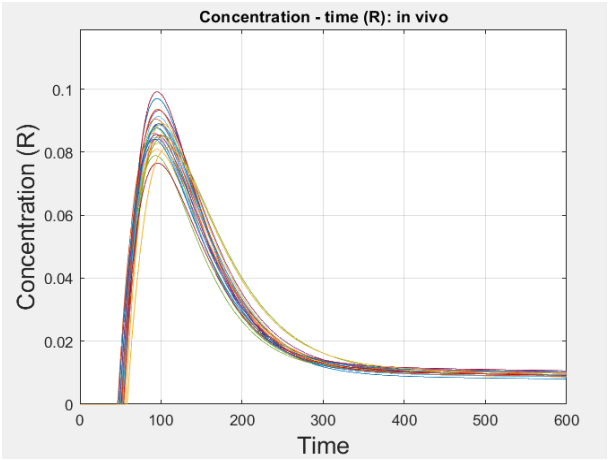
B



C



D



E

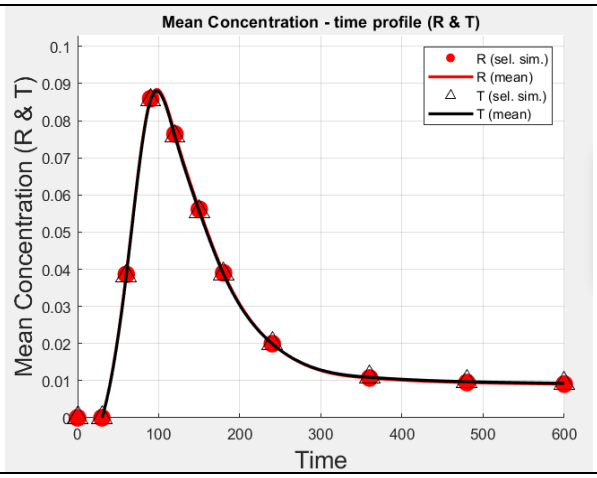
Test	**Reference**

Figure C.4: *Concentration-Time curves for Between-Subject Variability 5% for Test and Reference product*

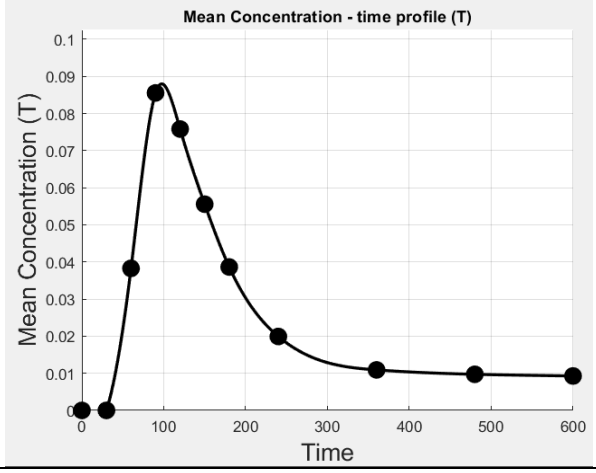
Consideration of the figures can begin by looking at Figures C.4.D and C.4.E. The spaghetti plots show the concentration-time curves for each of the subjects in the study. It is evident that the different colored curves corresponding to the different subjects in the study are nearly identical for both the test product (Figure C.4.D) and the reference product (Figure C.4.E). In other words, the performance of the drug between the different subjects of the study is almost the same. This is a consequence of the low value of the BSV. The subjects respond to the administration of the drug products almost as if they were a single person.

Figures C.4.B and C.4.C show the mean concentration-time curves of the test and reference products. The black dots are the sampling points and the continuous shape of the curve is again the result of the simulation. In Figure C.4.A, the mean concentration-time curves for both the test and reference products are shown and the dots and triangles represent the sampling points. Both WSV and BSV of the present simulation are very low, which allows the unrestricted observation of the similarity between the two products, which can be seen in Figure C.4.A; the mean concentration-time curves of the two products are so similar that only one uniform curve can be observed.

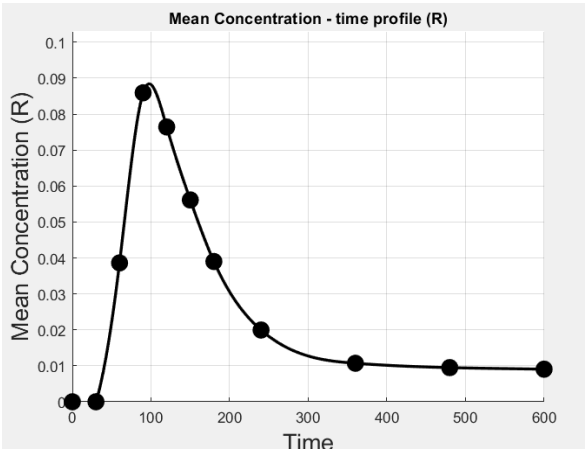
C.2.2. BSV 10 %



A



B



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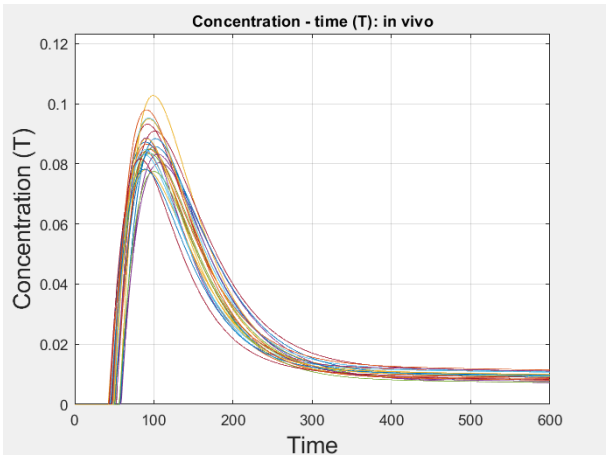

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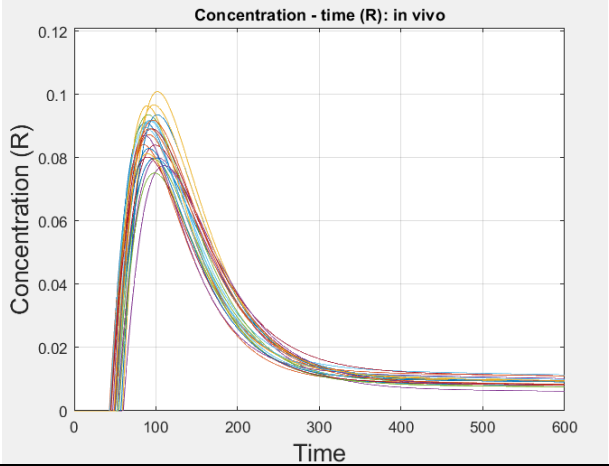


E

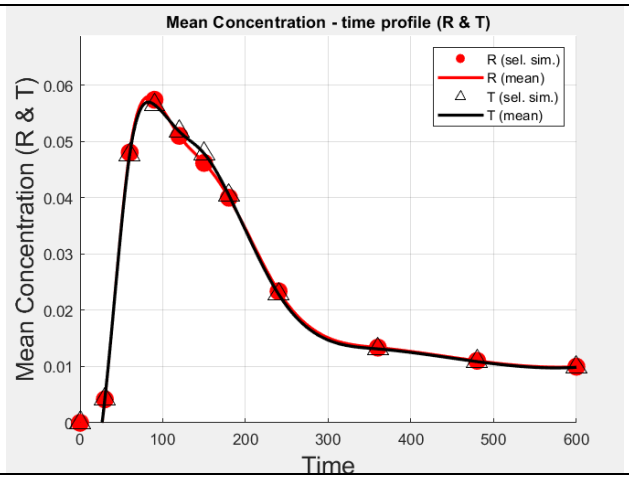
Test | **Reference**

Figure C.5: *Concentration-Time curves for Between-Subject Variability 10% for Test and Reference product*

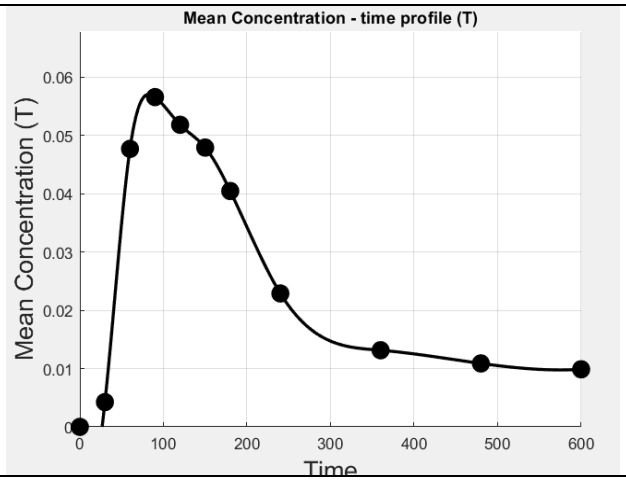
Figures C.5.A to C.5.C represent the mean concentration-time curves for a study with BSV 10%. When these figures are compared with the corresponding Figures C.4.A to C.4.E, there are not many differences.

The influence of BSV is better seen in Figures C.5.D and C.5.E, the spaghetti plots for the test and reference products, respectively. The concentration-time curves for the different subjects in the study are not exactly the same and slight differences can be observed. This is due to the fact that the BSV was set at 10%, which reflects the differences in the physiology of the human body between the subjects participating in the study. Figures C.5.D and C.5.E are closer to reality than Figures C.4.D and C.4.E, as slight variability between participants in a study is to be expected. Despite the larger value of the BSV, the mean concentration-time curves of the test (Figure C.5.B) and reference (Figure C.5.C) products are not affected.

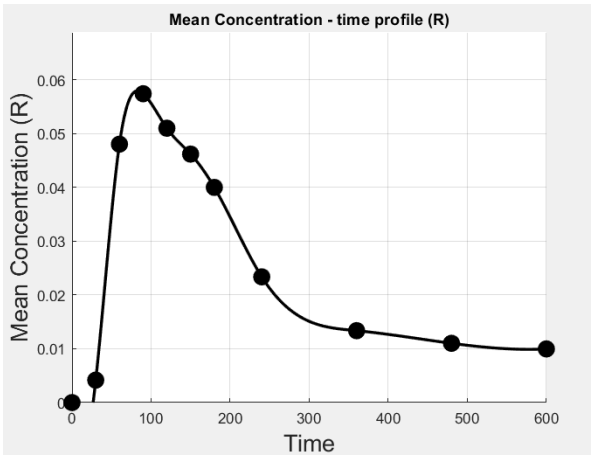
C.3.3. BSV 50 %



A



B



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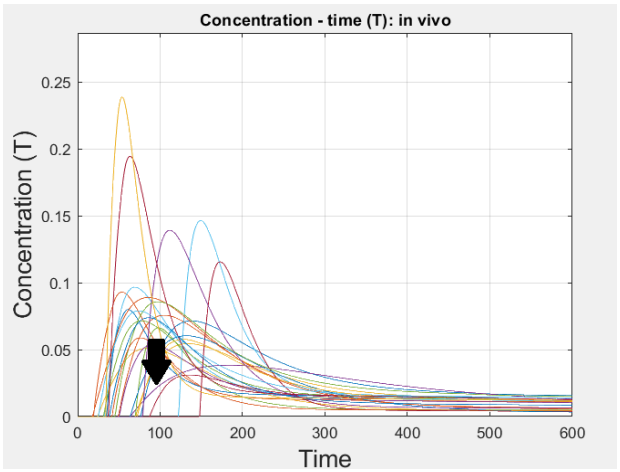

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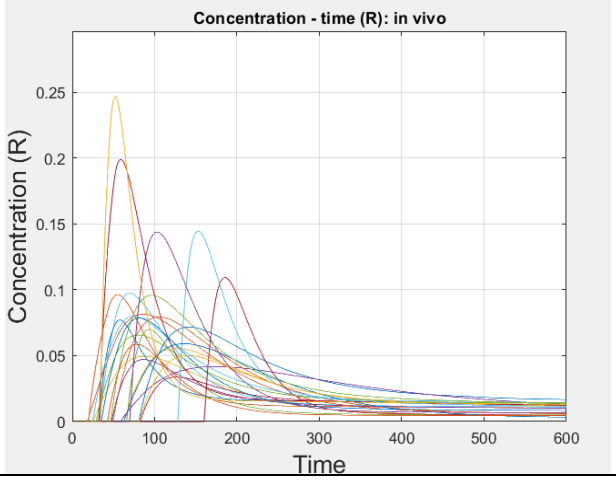


E

Test | **Reference**

Figure C.6: *Concentration-Time curves for Between-Subject Variability 50% for Test and Reference product*

When the BSV of the simulation increases further and reaches 50%, the result of the study is very different. In Figures C.6.D and C.6.E, which show the concentration-time curves of each subject in the study, it is clear that the behavior of the drug is different for each subject participating in the study. There are even examples of participants where the concentration-time curves do not even resemble those of donepezil. As an example, consider the highlighted curve in Figure C.6.D. The high variability between study subjects also affects the mean concentration-time curves. Both Figures C.6.B and C.6.C, which show the mean concentration-time curves of the test and reference products, respectively, do not correspond to the nominal shape of a concentration-time curve of donepezil. However, when these figures are blended together in Figure C.6.A, it can be seen that they do not differ from each other. This is due to the fact that despite the high level of BSV of the simulation, the WSV is low, the pharmacokinetic properties of the two products are set as identical, and most importantly, the design of the study is a randomized cross-over design (2×2 design) where both products are administered to each subject participating in the study.

At this point, it was of great interest to run the simulation multiple times with the BSV set at 50%. The results are shown in Figure C.7.

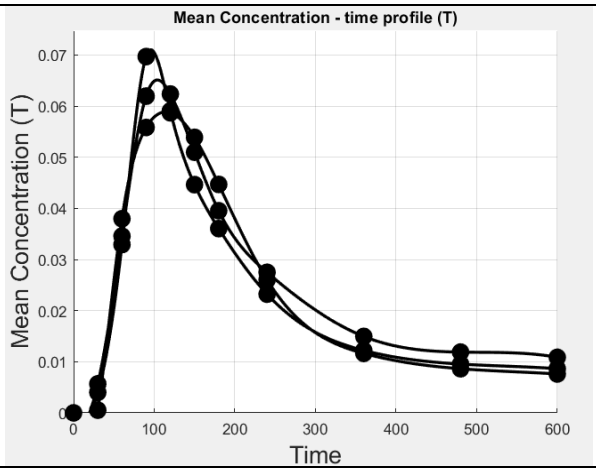
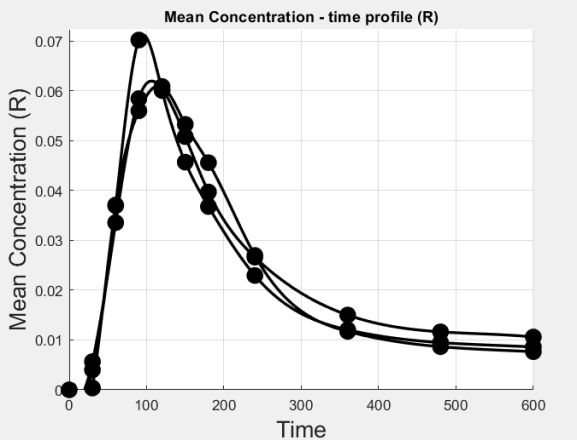
	
A	**B**
Test	**Reference**

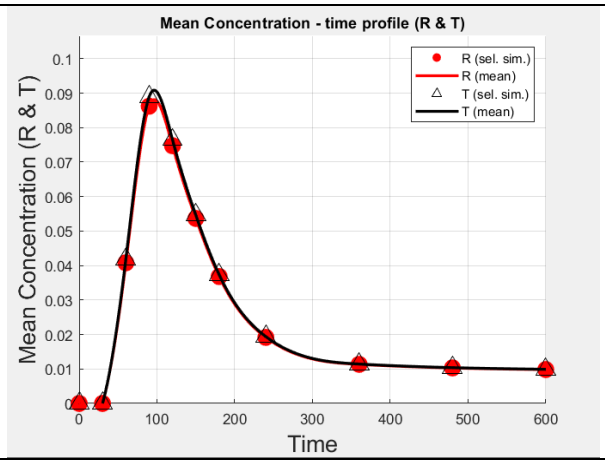
Figure C.7*: Concentration-Time curves for Between-Subject Variability 50% for Test and Reference product-multiple runs*

Figure C.7.A shows the mean concentration-time curves of the test product from three different runs of the same simulation with the BSV 50%. In agreement with Figure C.7.A, Figure C.7.B refers to the mean concentration-time curves of the reference product. It can be clearly seen that the figures contain three mean concentration-time curves that are different from each other. This is because each run of the program generates different data about the population of the study. In other words, it is as if each run was a study conducted on 24 different individuals. Furthermore, since the BSV of each simulation was set to 50%, it was as if the 24 different individuals were also very different from each other. For this reason, each time a simulation is run with a high BSV, even if all other parameters of the study remain unchanged, the concentration-time curves of the subjects and consequently the mean concentration-time curves will be different.

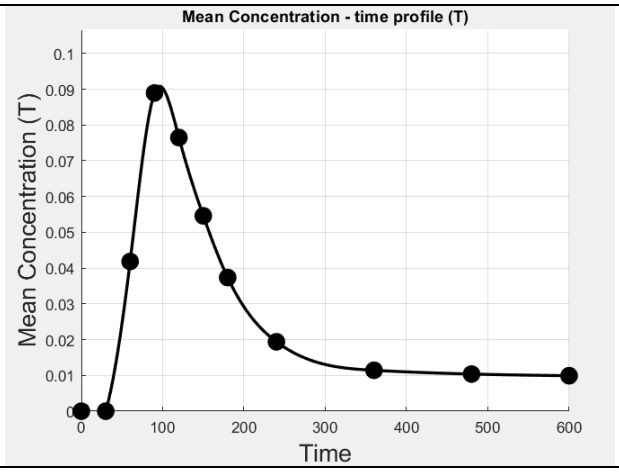
C.3 The impact of sample size

Another important parameter for a bioequivalence study is the number of participating subjects, i.e., the sample size. In the simulations presented, 3 different sample sizes are used: 12, 24 and 36 subjects. In the following figures the influence of the sample size is visualized. These figures are from simulations where the nominal sampling design scenario is applied (Scenario 1, see Table B.2), WSV is set to 5% and BSV is set to 10%.

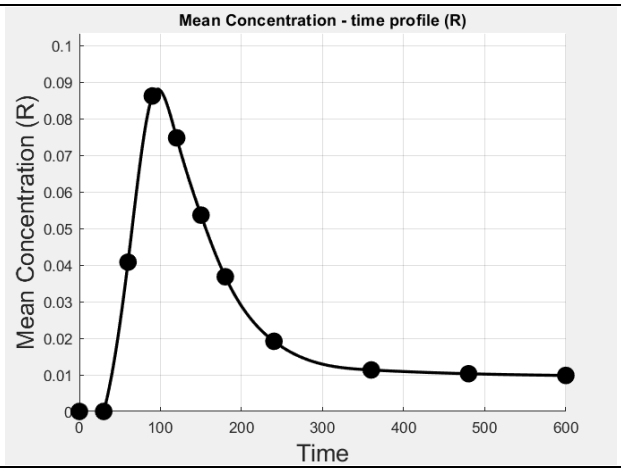
C.3.1.Sample size N=12 volunteers



A



B



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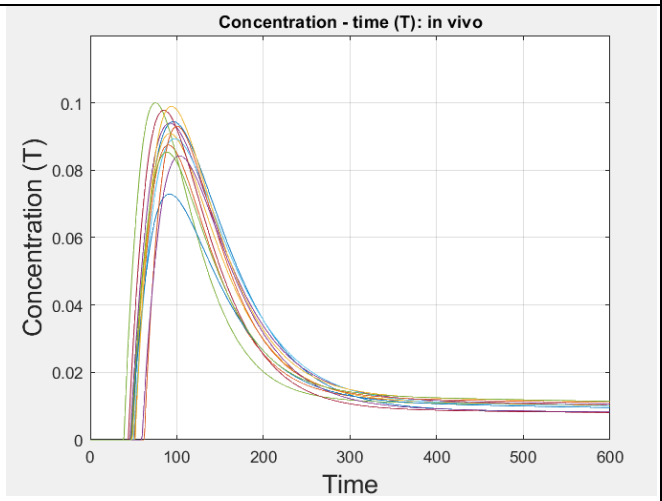
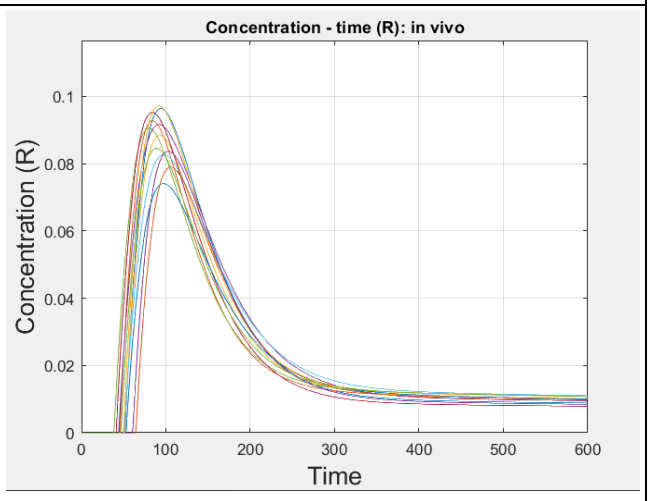


D

Test

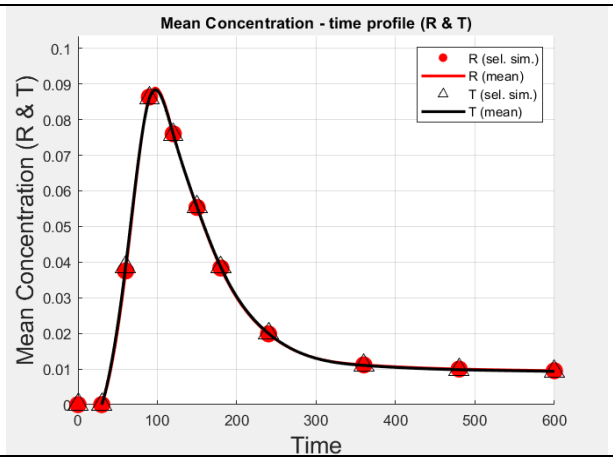


E

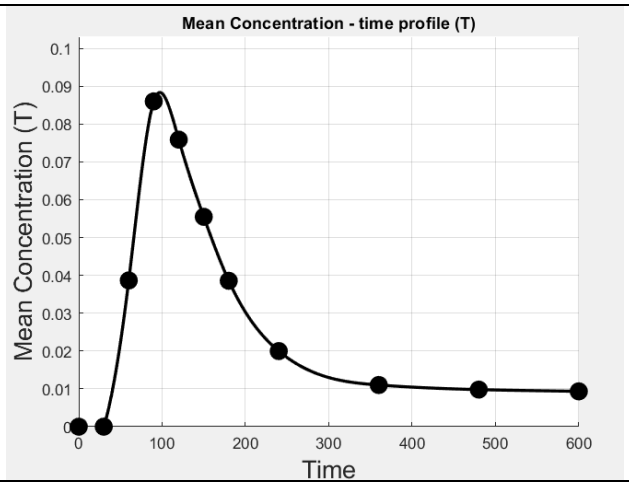
Reference

Figure C.8: *Concentration-Time curves for Test and Reference product-sample size N=12*

C.3.2.Sample size N=24 volunteers



A



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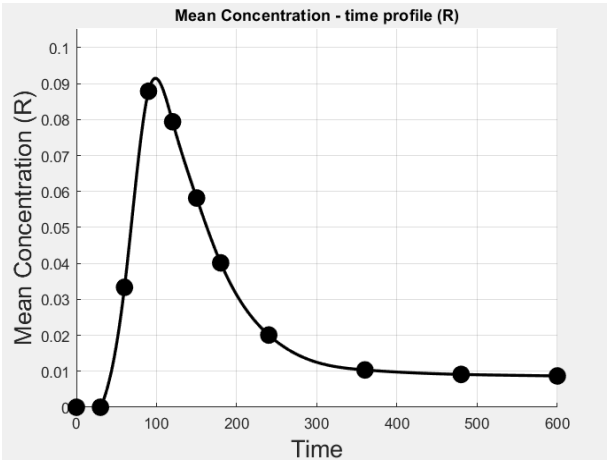

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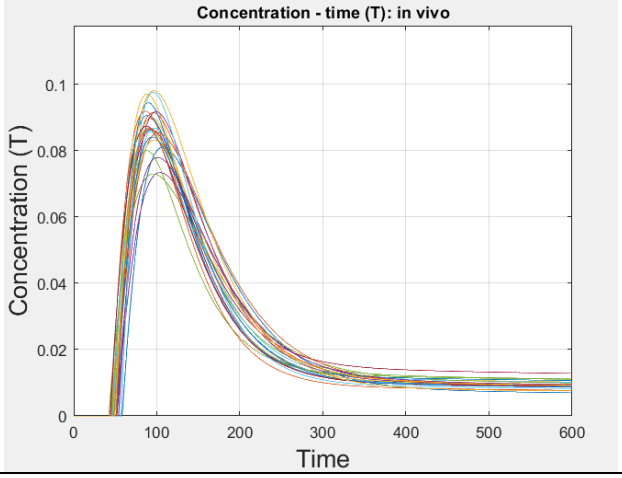

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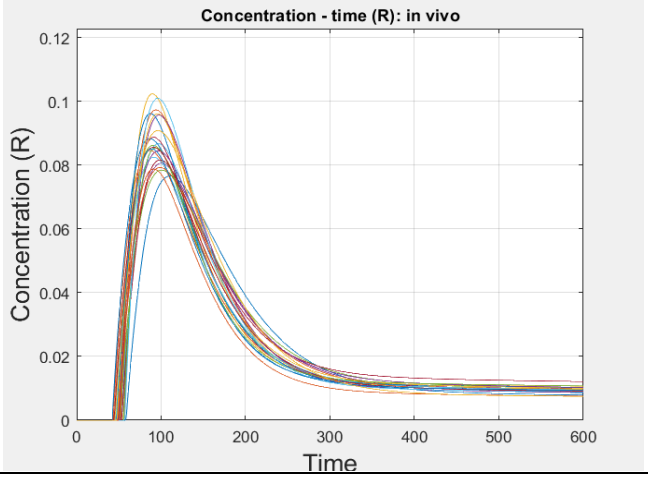
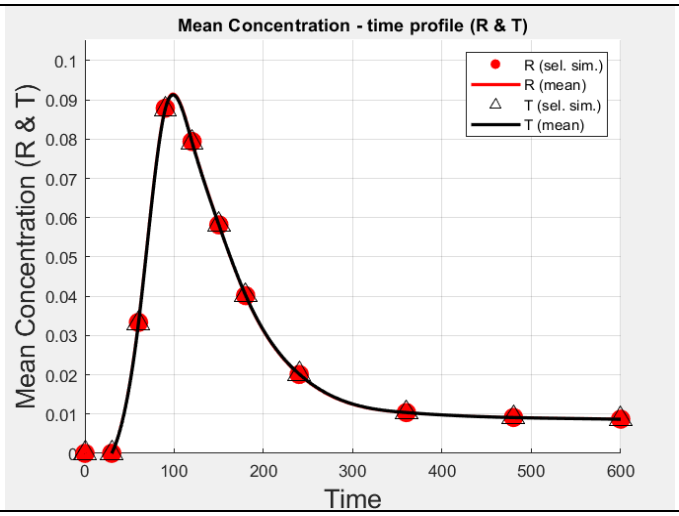


E

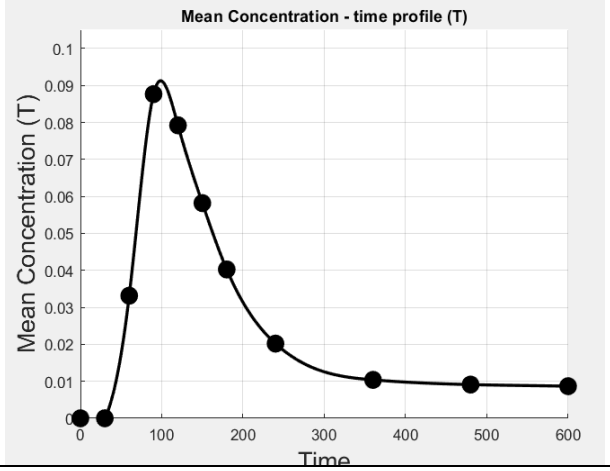
Test | **Reference**

***Figure C.9**: Concentration-Time curves for Test and Reference product-sample size N=24*

C.3.3.Sample size N=36 volunteers



A



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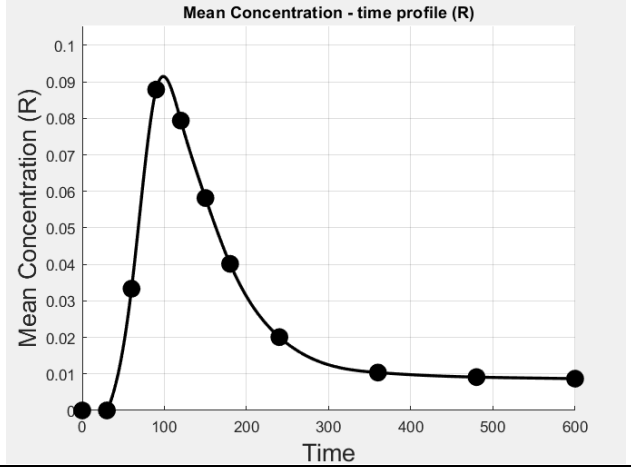

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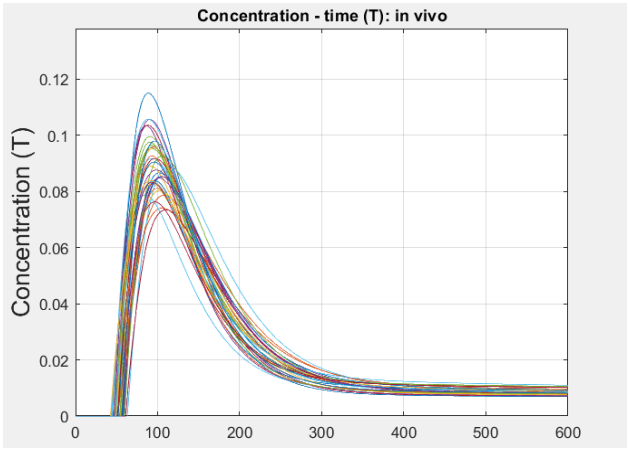
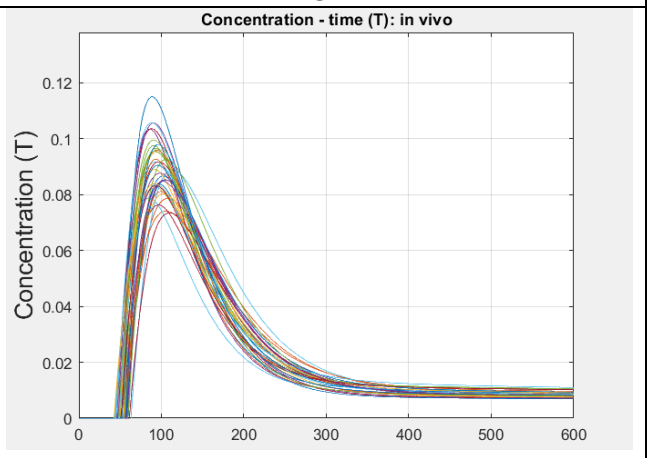


D



E

Test | **Reference**

Figure C.10: *Concentration-Time curves for Test and Reference product-sample size N=36*

Figures C.8.B, C.9.B, and C.10.B show the mean concentration-time curves for the test product in studies conducted with 12, 24, and 36 subjects, respectively. The black dots represent the sampling sites. The continuous shape of the curve is due to the simulation, as mentioned above; without it, only the concentration-time pairs for each time point would be seen. Figures C.8.C, C.9.C, and C.10.C show the corresponding results for the reference product.

Figures C.8.A, C.9.A, and C.10.A show the mean concentration-time curves for both the test and reference products in simulations run with sample sizes of 12, 24, and 36 subjects, respectively. The red dots are the sampling points for the reference product and the triangles are the sampling points for the test product.

So far, no difference can be seen between Figures 8, 9 and 10. The sample size seems to have no influence on the average plasma concentration of the drug when the pharmacokinetic profiles of the test and reference products are identical and the values of WSV and BSV are low.

To understand the influence of sample size, we need to turn to the spaghetti figures C.8.D, C.8.E, C.9.D, C.9.E, C.10.D and C.10.E. In the spaghetti figures, we can see the concentration-time curves of each participant in the study separately. Consequently, Figures C.10.D and C.10.E represent 36 concentration-time curves and are denser than Figures C.9.D and C.9.E, which represent the 24 concentration-time curves of each of the 24 subjects in the study. Accordingly, Figures C.8.D and C.8.E have only 12 concentration-time curves and are the less dense among the spaghetti figures presented in Section C.3.

C.4 The impact of sampling scheme

As mentioned above, in order to investigate the influence of the sampling plan on the bioequivalence result, several sampling plan scenarios were created based on the nominal sampling plan of the in vivo bioequivalence study. These sampling plan scenarios are presented in Table B.2. For a better understanding of these scenarios, the information from Table B.2 is also described verbally in Table C.1

As shown in Tables C.1 and B.2, 20 different sampling plan scenarios were created. These scenarios and the nominal scenario are combined with two different sample sizes and two different values of Between-Subject Variability when the two products (test and reference) have the same pharmacokinetic performances. Then, the 21 scenarios of the sampling plan are combined with two different values of BSV and with different percentages of the difference in absorption rate between the test and reference products. Simulations are performed for each combination. For each simulation, the power of the study and the GMR for the pharmacokinetic parameters C_{max} and AUC are measured.

First, the concentration-time curves of the simulations performed with the 21 different sampling schemes are presented, with a sample size of 24 subjects and the values of BSV and WSV set to 5% and 10%, respectively. Then, the influence of the differences in the absorption rate between the two products on the concentration-time curves is also shown. Finally, the measured values of GMR and the significance of the study for all the simulations performed are given.

Scenario No.	Sampling scheme (hours)	Description of each scenario
1	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 96, 144, 192	Full sampling scheme of the in vivo study-usually referred to as nominal scenario
2	0, 0.5, 1, 2, 3, 4, 6, 8,12, 24, 48, 96, 144	Delete one time point from the end of the sampling scheme
3	0, 1, 3, 4, 6, 8,12, 24, 48, 96, 144, 192	Delete two time points from the start of the sampling scheme without affecting the duration of it.
4	0, 0.5, 1, 2, 6, 8, 12, 24, 48, 96, 144, 192	Delete two time points from the middle of the sampling scheme without affecting the duration of it.
5	0, 0.5, 1, 2, 3, 4, 6, 12, 48, 96, 144, 192	Delete two time points from the end of the sampling scheme without affecting the duration of it.
6	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 8, 96	Delete two time points from the end of the sampling scheme
7	0, 2, 4, 6, 8, 12, 24, 48, 96, 144, 192	Delete three time points from the start of the sampling scheme without affecting the duration of it.
8	0, 0.5, 1, 6, 8, 12, 24, 48, 96, 144, 192	Delete three time points from the middle of the sampling scheme without affecting the duration of it.
9	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 96, 192	Delete three time points from the end of the sampling scheme without affecting the duration of it.
10	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48	Delete three time points from the end of the sampling scheme
11	0, 3, 6, 8, 12, 24, 48, 96, 144, 192	Delete four time points from the start of the sampling scheme without affecting the duration of it.
12	0, 0.5, 6, 8, 12, 24, 48, 96, 144, 192	Delete four time points from the middle of the sampling scheme without affecting the duration of it.
13	0, 0.5, 1, 2, 3, 4, 6, 24, 144, 192	Delete four time points from the end of the sampling scheme without affecting the duration of it.
14	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24	Delete four time points from the end of the sampling scheme
15	0, 6, 8, 12, 24, 48, 96, 144, 192	Delete five time points from the start of the sampling scheme without affecting the duration of it.

Table C.1: *Description of sampling scheme scenarios applied in the simulations*

Scenario No.	Sampling scheme (hours)	Description of each scenario
16	0, 0.5, 1, 2, 3, 4, 6, 144, 192	Delete five time points from the end of the sampling scheme without affecting the duration of it.
17	0, 0.5, 1, 2, 3, 4, 6, 8, 12	Delete five time points from the end of the sampling scheme
18	0, 0.5, 1, 2, 3, 4, 6, 8	Delete six time points from the end of the sampling scheme
19	0, 1, 3, 6, 12, 48, 144, 192	Uniform deletion of time points across the sampling scheme without affecting the duration of it.
20	0, 2, 6, 24, 144, 192	
21	0, 3, 4, 6, 48, 96, 144, 192	

Table C.1 (continue): *Description of sampling scheme scenarios applied in the simulations*

C.4.1 Concentration-time curves for the 21 different sampling scheme scenarios

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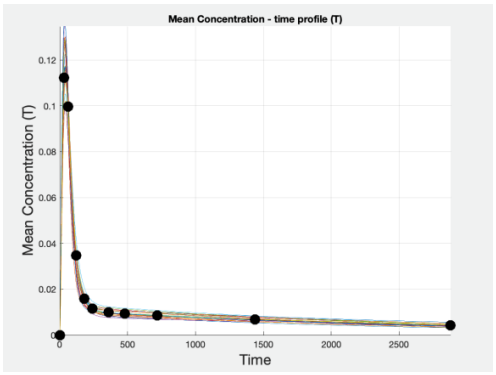
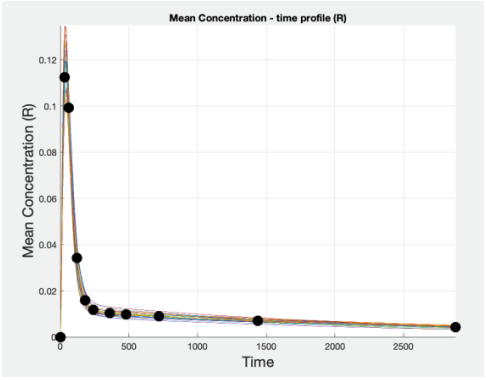
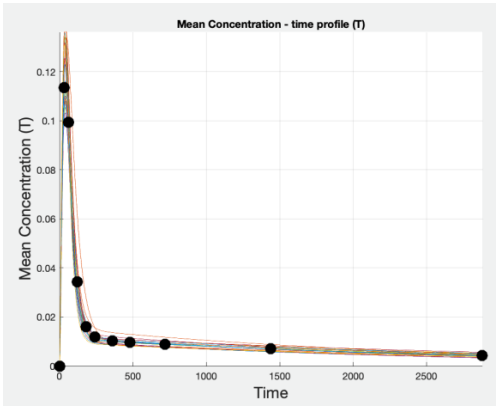
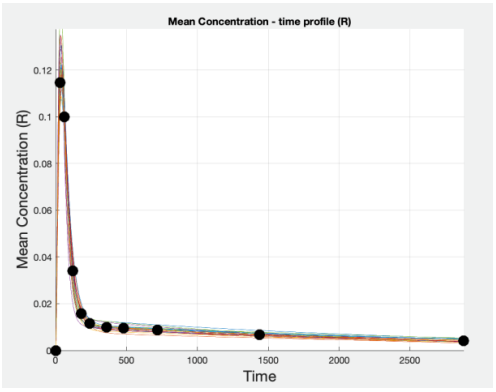
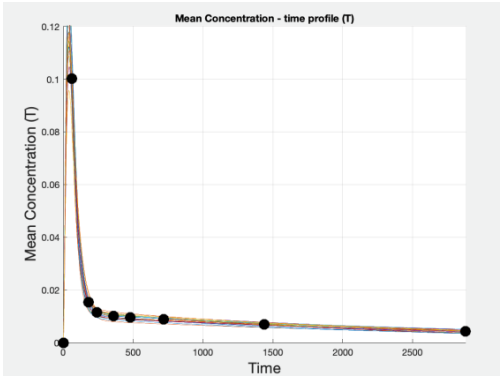
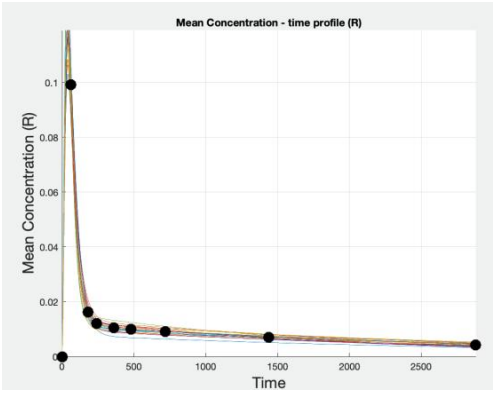
1		
2		
3		

Figure C.11: *Concentration-time curves of simulations with the 21 different sampling schemes*

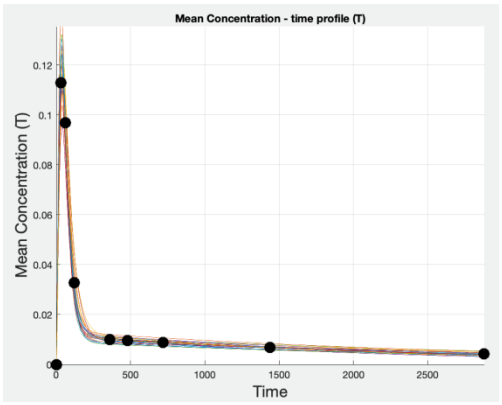
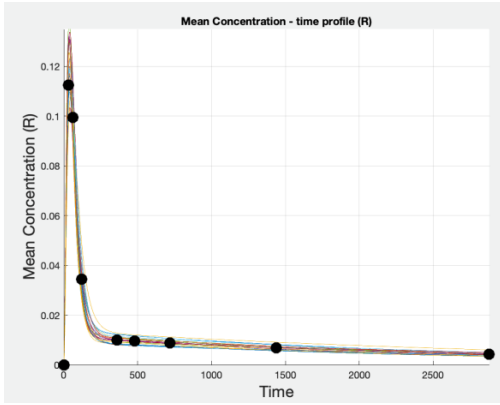
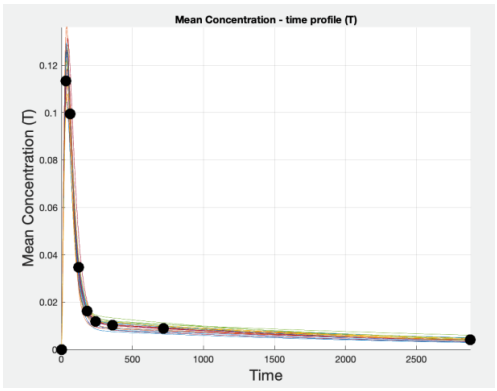
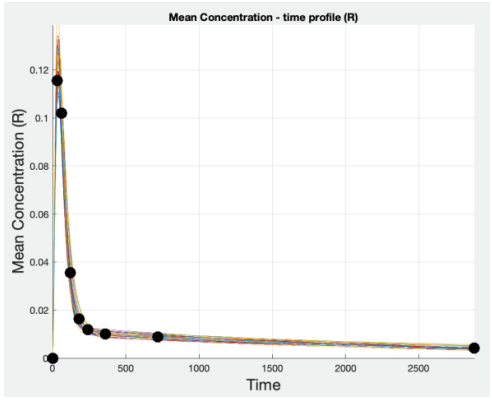
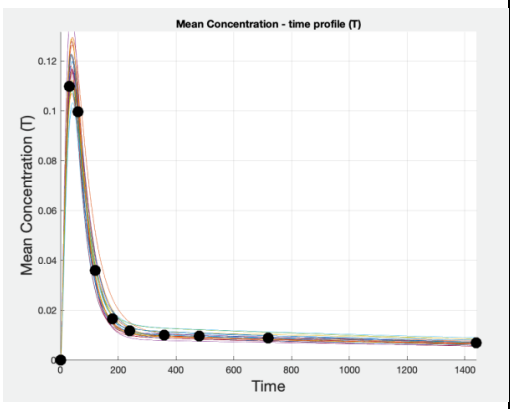
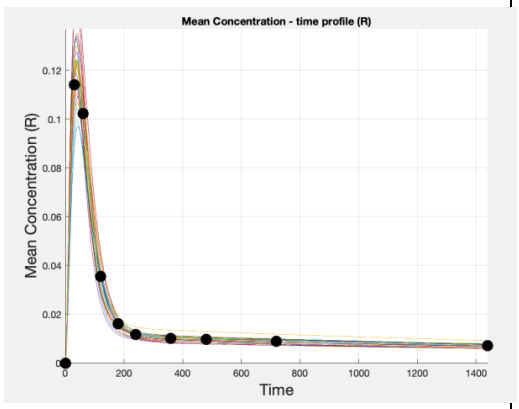
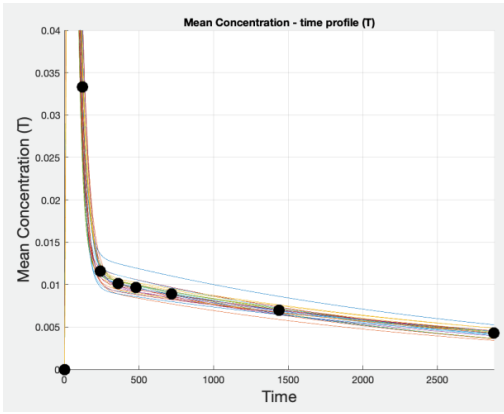
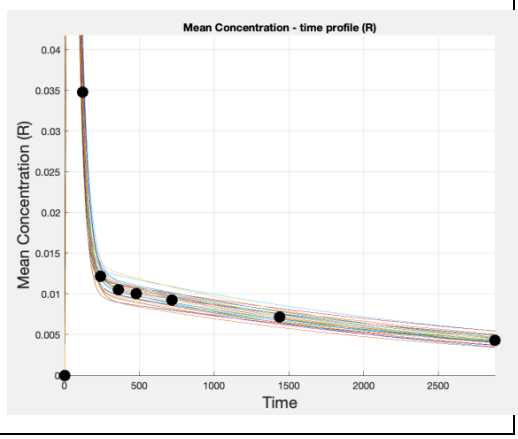
4		
5		
6		
7		

Figure C.11 (continue): *Concentration-time curves of simulations with the 21 different sampling schemes*

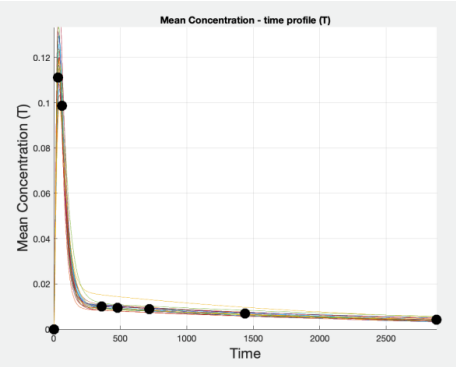
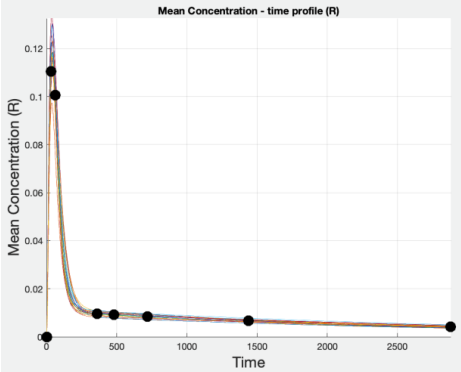
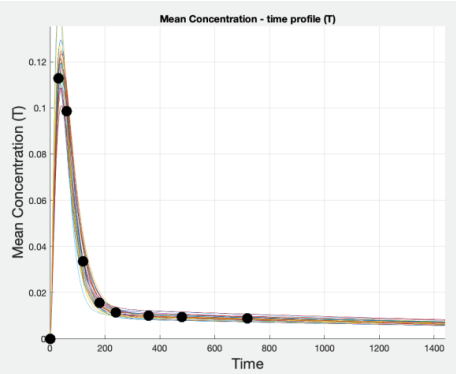
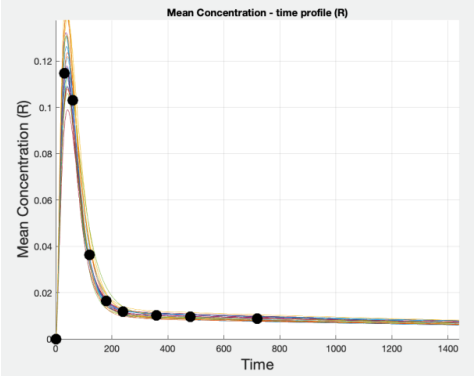
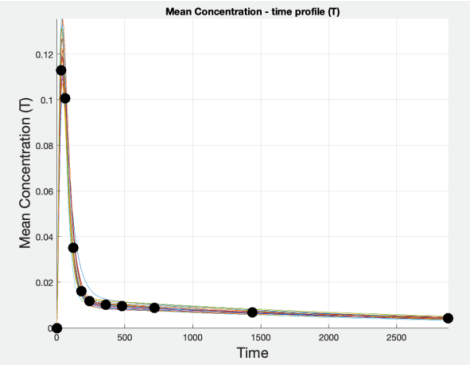
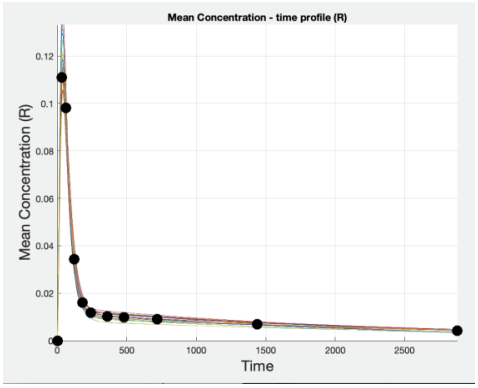
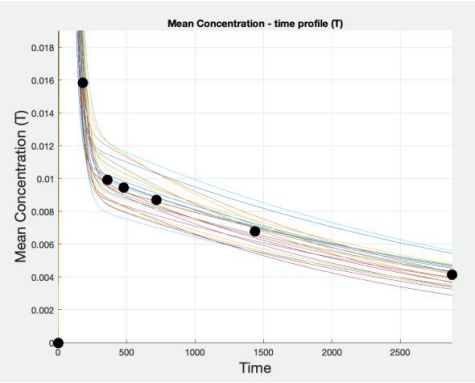
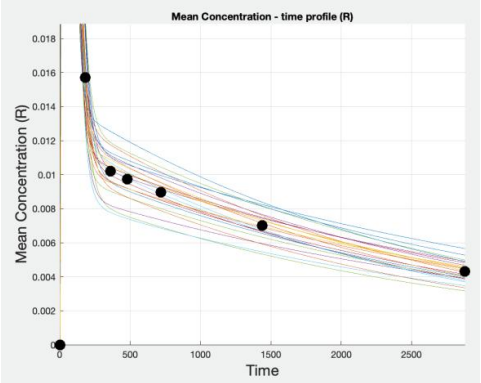
8		
9		
10		
11		

Figure C.11 (continue): *Concentration-time curves of simulations with the 21 different sampling schemes*

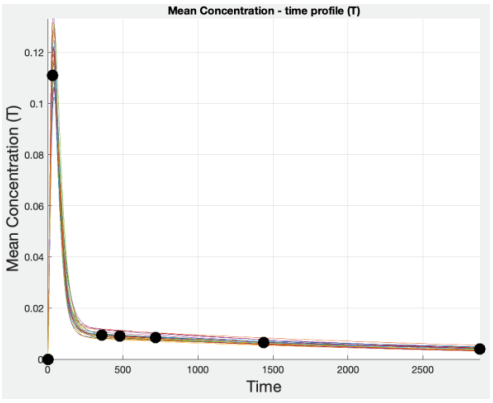
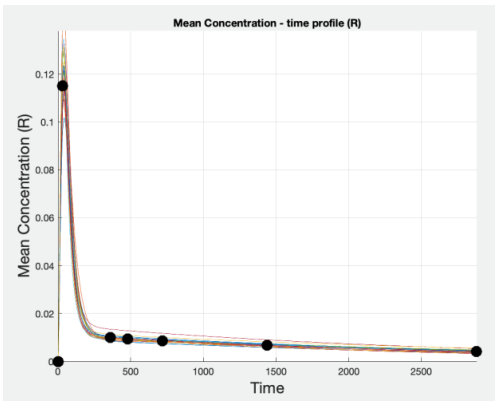
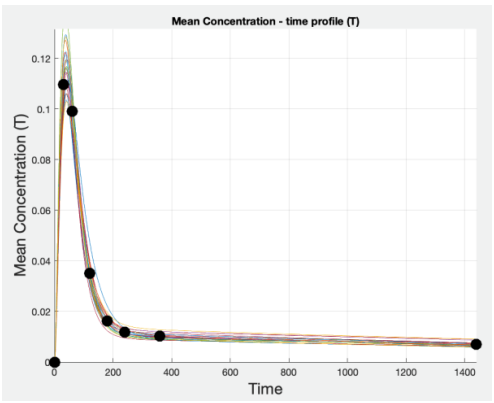
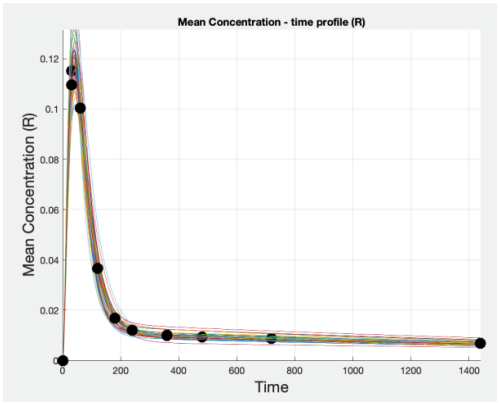
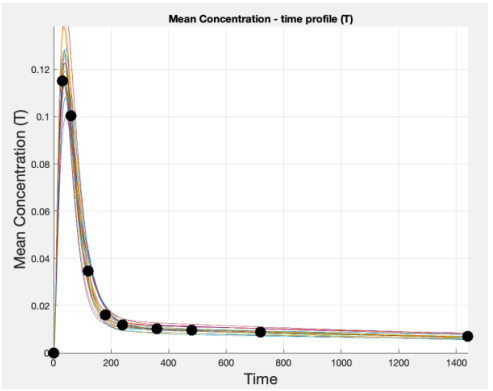
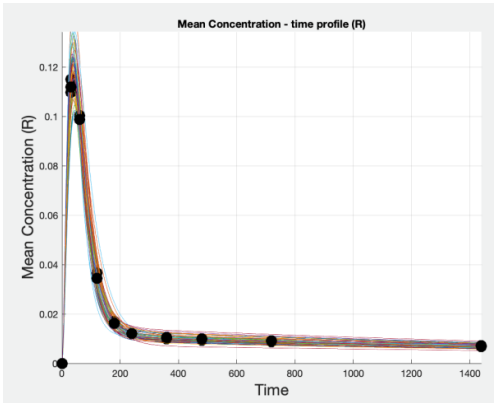
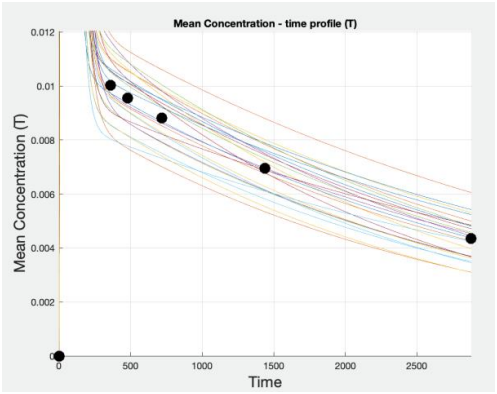
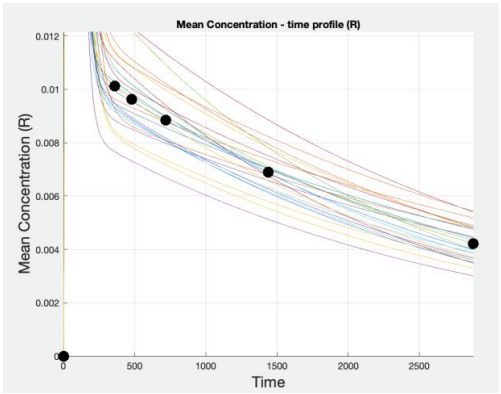
12		
13		
14		
15		

Figure C.11 (continue): *Concentration-time curves of simulations with the 21 different sampling schemes*

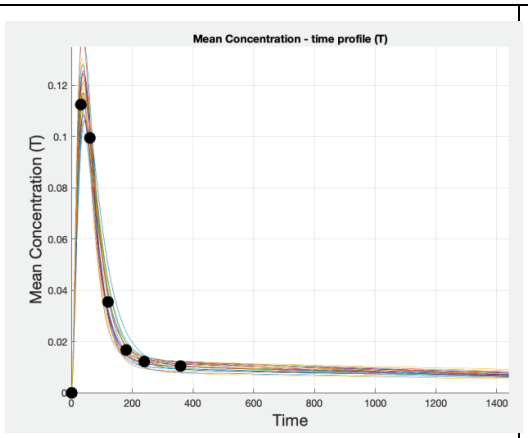
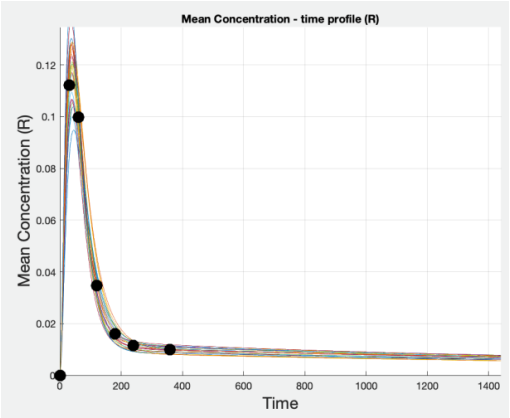
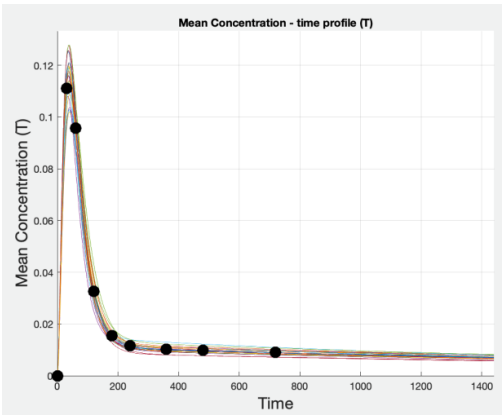
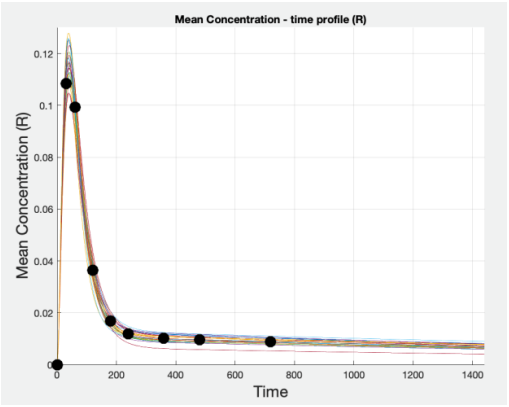
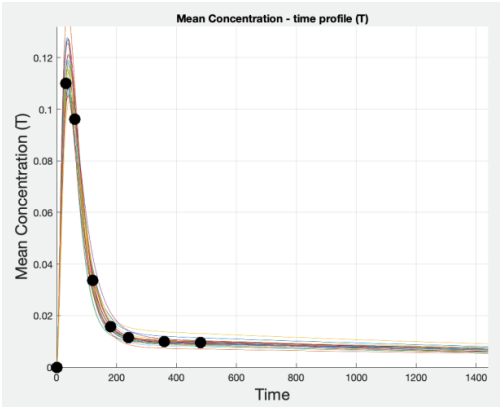
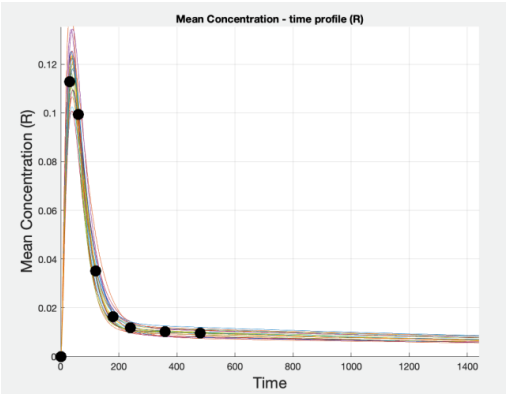
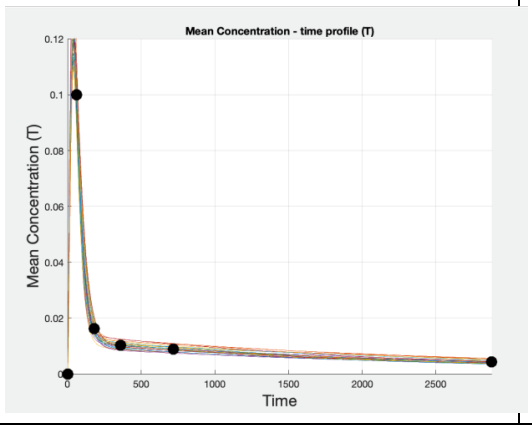
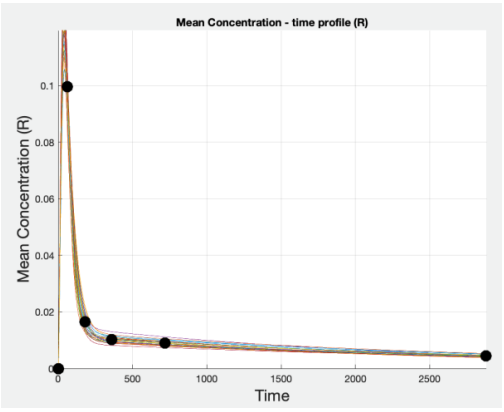
16		
17		
18		
19		

Figure C.11 (continue): *Concentration-time curves of simulations with the 21 different sampling schemes*

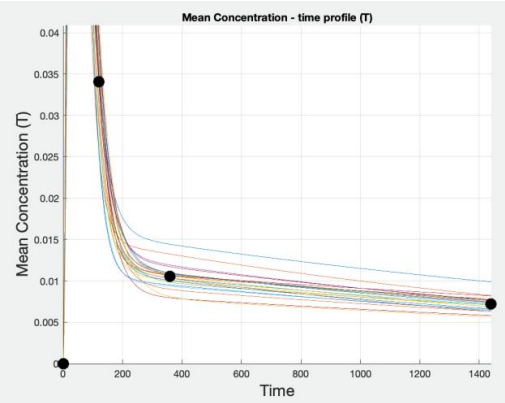
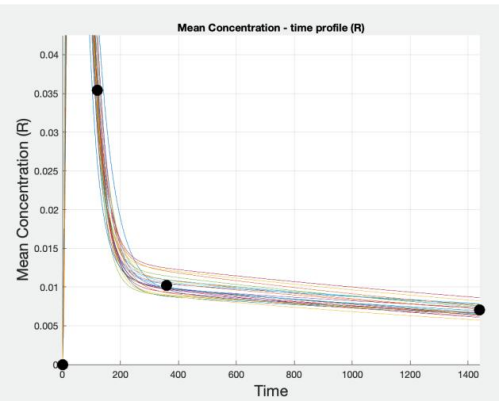
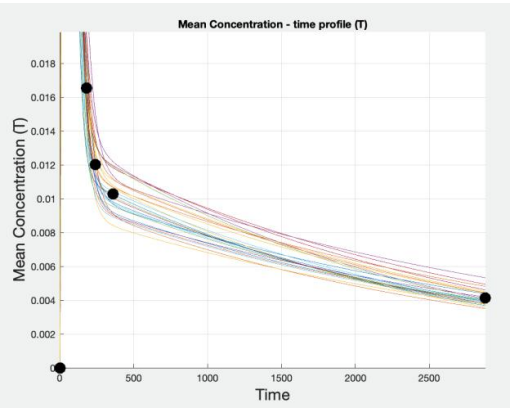
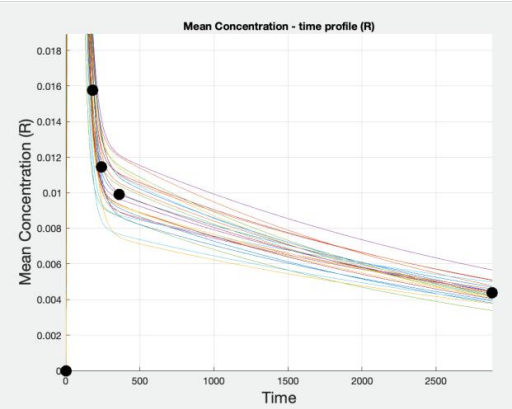
20		
21		

Figure C.11 (continue): *Concentration-time curves of simulations with the 21 different sampling schemes*

Figure C.11 shows the mean concentration-time curves of the 21 simulations with the different sampling schemes. In each plot, the simulated concentration-time curves are shown for each of the subjects administered the test product (left column) and the reference product (right column). Each curve with a different color corresponds to a different subject. In each graph, the black dots represent the collection points, i.e., the time points at which plasma samples would be collected from a human subject if this were an in vivo study, or the time points at which the substance concentration is measured in blood samples from the participants.

The various sampling schemes used fall into two categories: those involving the duration of the study (scenarios 2, 6, 10, 14, 17, and 18) and those involving the density of the sampling scheme (3, 4, 5, 7, 8, 9, 11, 12, 13, 15, 16, 19, 20, and 21). (See also Table C.1).

A comparison of the plots affecting the duration of the study with the plots corresponding to the scenario of the nominal sampling scheme (1) shows that sampling points were deleted at the end of the sampling scheme and the duration of the study is different in each case. However, no visual effect of study duration is observed in the concentration-time curves, as all curves appear to have the same shape as those of the nominal sampling plan scenario.

The situation is different for the sampling plan scenarios, which are less dense than the nominal scenario. Smaller differences are observed for scenarios 5, 9, 13, and 16, which delete time points at the end of the sampling plan without affecting the duration of the sampling plan. In addition, scenarios 4, 8, and 12, which involve deletions of time points from the middle of the

sampling plan, do not appear to significantly affect the concentration-time curves. On the other hand, the concentration-time curves of the scenarios created by deleting time points from the beginning of the sampling plan undoubtedly deviate from the plots of the nominal sampling plan scenario. In particular, the plots of scenarios 7, 11, and 15 do not even resemble concentration-time curves, the C_{max} is not plotted, and the area under the curve (AUC) is significantly different from that of scenario 1. Finally, scenarios 19, 20, and 21, which are generated by uniformly and randomly deleting sampling points, appear to result in plots that also differ from the plots of the nominal sampling plan.

C.4.2 Concentration-time curves for test and reference products with different rates of absorption

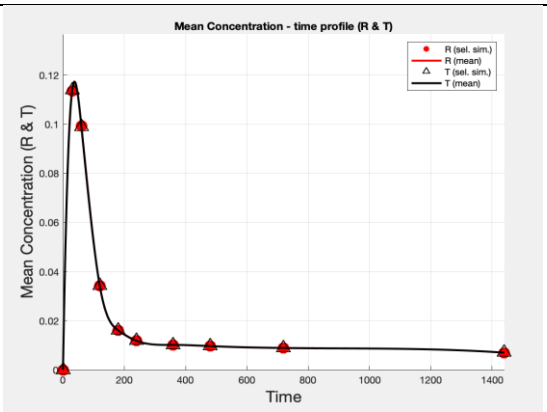
To examine the effects of differences in absorption rates between the two products, indicative concentration-time curves from simulations of the Scenario 6 sampling scheme are presented with various degrees of percent difference in the absorption rates of the test and reference products. (Figure C.12).

In the concentration-time curves of the plots, the mean plasma concentration is shown in correlation with time. The black curves correspond to the test product (T) and the red curves correspond to the reference product (R). The black dots and the red triangles represent the sampling times of the sampling plan scenario 6 for the test and the reference group, respectively.

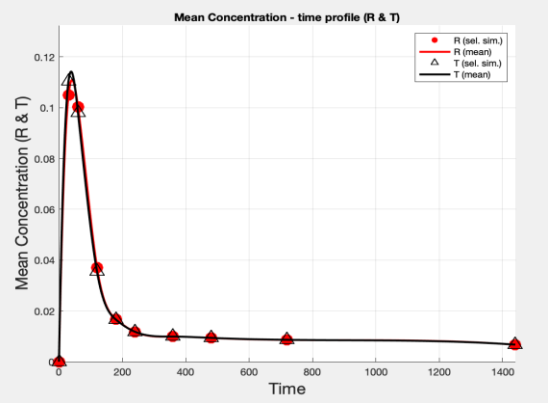
In Figure C.12.A, the absorption rate of the test product is identical to that of the reference product. This is easy to understand since both products reach C_{max} at the same time and each time point corresponds to the same concentration for both products. On the other hand, in Figure C.12.B, the absorption rate of the test product is higher than that of the reference product. In the curve, this can be understood by the fact that the test product reaches C_{max} slightly earlier than the reference. Also, in Figure C.12.C, the test product reaches C_{max} earlier than the reference product. In addition, the C_{max} of the test product is significantly higher than that of the reference product. This is the result of a 40% difference in the absorption rate of the two products. The same conclusions are drawn by looking at Figures C.12.D and C.12.E, where the percentage difference in the absorption rate of the two products is 50% and 75%, respectively. In Figures C.12.D and C.12.E, the test product reaches C_{max} at the second time point of the sampling schedule and by the

third time point the elimination phase has begun. In contrast, the reference product reaches C_{max} at the third time point of the sampling plan.

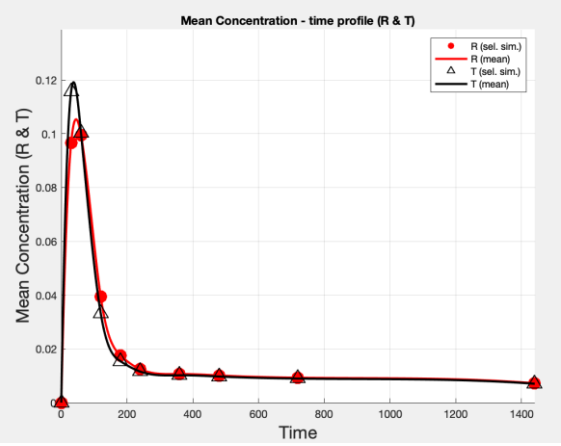
In general, Figure C.12 represents an example of concentration-time curves of test and reference product with differences in the rate of absorption. These differences result in different values of C_{max} and T_{max}. Indeed, as the absorption rate of a product increases, its T_{max} decreases and its C_{max} increases. In other words, the product with a higher absorption rate is the one that will reach a higher C_{max} earlier in the study.



A. Identical rate of absorption

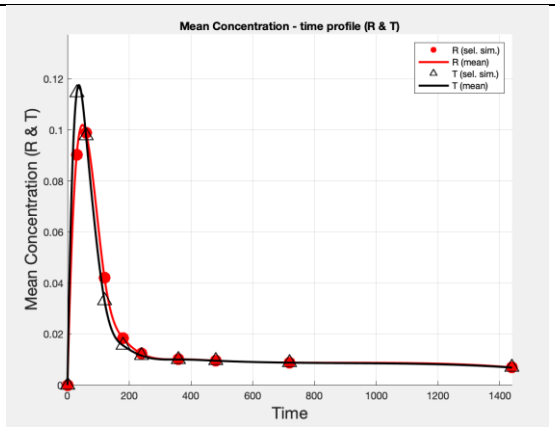


B. 20% difference in rate of absorption

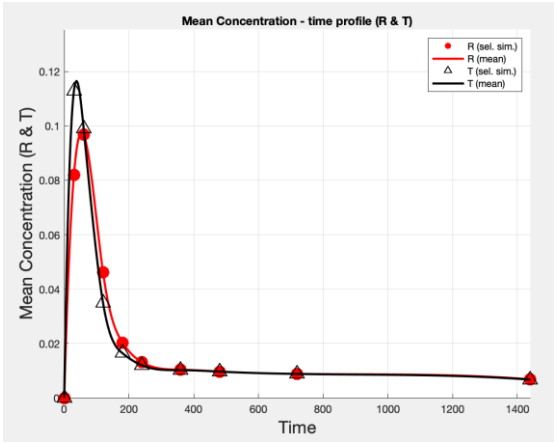


C. 40% difference in rate of absorption

Figure C.12 *Concentration-time curves of sampling scheme scenario 6 with various percentages of difference in the rate of absorption between the two products*



D. 50% difference in rate of absorption



E. 75% difference in rate of absorption

Figure C.12 (continue): *Concentration-time curves of sampling scheme scenario 6 with various percentages of difference in the rate of absorption between the two products*

C.4.3 Probability of acceptance for equal rate of absorption between the Test and the Reference product.

ID	sampling scheme	BSV	WSV	N	GMR		Power	
					C_{max}	AUC	C_{max}	AUC
Equal rate of absorption								
1	1	5	10		1.00	1.00	100	100
2	2	5	10		1.00	1.00	100	100
3	3	5	10		1.00	1.00	100	100
4	4	5	10		1.00	1.00	100	100
5	5	5	10		1.00	1.00	100	100
6	6	5	10		1.00	1.00	100	100
7	7	5	10		1.00	1.00	100	100
8	8	5	10		1.00	1.00	100	100
9	9	5	10		1.00	1.00	100	100
10	10	5	10		1.00	1.00	100	100
11	11	5	10	12	1.00	1.00	100	100
12	12	5	10		1.00	1.00	100	100
13	13	5	10		1.00	1.00	100	100
14	14	5	10		1.00	1.00	100	100
15	15	5	10		1.00	1.00	100	100
16	16	5	10		1.00	1.00	100	100
17	17	5	10		1.00	1.00	100	100
18	18	5	10		1.00	1.00	100	100
19	19	5	10		1.00	1.00	100	100
20	20	5	10		1.00	1.00	100	100
21	21	5	10		1.00	1.00	100	100
22	1	5	10		1.00	1.00	100	100
23	2	5	10		1.00	1.00	100	100
24	3	5	10		1.00	1.00	100	100
25	4	5	10		1.00	1.00	100	100
26	5	5	10	24	1.00	1.00	100	100
27	6	5	10		1.00	1.00	100	100
28	7	5	10		1.00	1.00	100	100
29	8	5	10		1.00	1.00	100	100
30	9	5	10		1.00	1.00	100	100
31	10	5	10		1.00	1.00	100	100

Table C.1: *Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with equal rate of absorption between the test and the reference product*

ID	sampling scheme	BSV	WSV	N	GMR		Power	
					C_{max}	AUC	C_{max}	AUC
Equal rate of absorption								
32	11	5	10		1.00	1.00	100	100
33	12	5	10		1.00	1.00	100	100
34	13	5	10		1.00	1.00	100	100
35	14	5	10		1.00	1.00	100	100
36	15	5	10		1.00	1.00	100	100
37	16	5	10		1.00	1.00	100	100
38	17	5	10		1.00	1.00	100	100
39	18	5	10		1.00	1.00	100	100
40	19	5	10		1.00	1.00	100	100
41	20	5	10		1.00	1.00	100	100
42	21	5	10		1.00	1.00	100	100
43	1	20	10		1.00	1.00	100	100
44	2	20	10		1.00	1.00	100	100
45	3	20	10		1.00	1.00	100	100
46	4	20	10		1.00	1.00	100	100
47	5	20	10		1.00	1.00	100	100
48	6	20	10		1.00	1.00	100	100
49	7	20	10		1.00	1.00	100	100
50	8	20	10		1.00	1.00	100	100
51	9	20	10		1.00	1.00	100	100
52	10	20	10		1.00	1.00	100	100
53	11	20	10	24	1.00	1.00	100	100
54	12	20	10		1.00	1.00	100	100
55	13	20	10		1.00	1.00	100	100
56	14	20	10		1.00	1.00	100	100
57	15	20	10		1.00	1.00	100	100
58	16	20	10		1.00	1.00	100	100
59	17	20	10		1.00	1.00	100	100
60	18	20	10		1.00	1.00	100	100
61	19	20	10		1.00	1.00	100	100
62	20	20	10		1.00	1.00	100	100
63	21	20	10		1.00	1.00	100	100

Table C.1 (continue) : *Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with equal rate of absorption between the test and the reference product*

Table C.1 shows the GMR and power of the study for the two pharmacokinetic parameters C_{max} and AUC for all scenarios applied. The value of Within-Subject Variability (WSV) is set to 10% for all simulations.

First, the GMR and power are presented for the 21 scenarios of the sampling design where the value of Between - Subject Variability (BSV) is set to 5% and the sample size is equal to 12 subjects. In this case, the power of the study and the GMR for the C_{max} and the AUC are 100% and 1, respectively, which means that the acceptance probability of the study and the similarity between the products are high regardless of the sampling plan. The same results for the GMR and power for both pharmacokinetic parameters come out when the sample size is 24 subjects.

Next, the power of the study and the GMR for the value of BSV set at 20% are demonstrated for 24 subjects. Again, the power of the study is 100% and the GMR is 1 for both C_{max} and AUC.

Overall, it is shown in Table C.1 that when the absorption rates of the comparator products are equal, the GMR and power for C_{max} and AUC of the simulations are high, regardless of the sampling design, BSV, and sample size of the study.

C.4.2 Probability of acceptance in case of Test and the Reference products with different rates of absorption

20% Difference

ID	sampling scheme	BSV	WSV	N	GMR		Power	
					C_{max}	AUC	C_{max}	AUC
20% difference								
64	1	5	10		0.93	1.00	98.30	100.00
65	2	5	10		0.93	1.00	98.30	100.00
66	3	5	10		0.93	1.00	98.30	100.00
67	4	5	10		0.93	1.00	98.30	100.00
68	5	5	10		0.93	1.00	98.30	100.00
69	6	5	10		0.93	1.00	98.30	100.00
70	7	5	10		0.93	1.00	98.30	100.00
71	8	5	10		0.93	1.00	98.30	100.00
72	9	5	10		0.93	1.00	98.30	100.00
73	10	5	10		0.93	1.00	98.30	100.00
74	11	5	10	24	0.93	1.00	98.30	100.00
75	12	5	10		0.93	1.00	98.30	100.00
76	13	5	10		0.93	1.00	98.30	100.00
77	14	5	10		0.93	1.00	98.30	100.00
78	15	5	10		0.93	1.00	98.30	100.00
79	16	5	10		0.93	1.00	98.30	100.00
80	17	5	10		0.93	1.00	98.30	100.00
81	18	5	10		0.93	1.00	98.30	100.00
82	19	5	10		0.93	1.00	98.30	100.00
83	20	5	10		0.93	1.00	98.30	100.00
84	21	5	10		0.93	1.00	98.30	100.00
85	1	20	10		0.93	1.00	98.30	100.00
86	2	20	10		0.93	1.00	98.30	100.00
87	3	20	10		0.93	1.00	98.30	100.00
88	4	20	10	24	0.93	1.00	98.30	100.00
89	5	20	10		0.93	1.00	98.30	100.00
90	6	20	10		0.93	1.00	98.30	100.00
91	7	20	10		0.93	1.00	98.30	100.00

Table C.2 : *Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with 20% difference in the rate of absorption between the Test and the Reference product*

ID	sampling scheme	BSV	WSV	N	GMR		Power	
					C_{max}	AUC	C_{max}	AUC
20% difference								
92	8	20	10		0.93	1.00	98.30	100.00
93	9	20	10		0.93	1.00	98.30	100.00
94	10	20	10		0.93	1.00	98.30	100.00
95	11	20	10		0.93	1.00	98.30	100.00
96	12	20	10		0.93	1.00	98.30	100.00
97	13	20	10		0.93	1.00	98.30	100.00
98	14	20	10		0.93	1.00	98.30	100.00
99	15	20	10		0.93	1.00	98.30	100.00
100	16	20	10		0.93	1.00	98.30	100.00
101	17	20	10		0.93	1.00	98.30	100.00
102	18	20	10		0.93	1.00	98.30	100.00
103	19	20	10		0.93	1.00	98.30	100.00
104	20	20	10		0.93	1.00	98.30	100.00
105	21	20	10		0.93	1.00	98.30	100.00

Table C.2 (continue): *Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with 20% difference in the rate of absorption between the Test and the Reference product*

Table C.2 shows the GMR and power of the study for the two pharmacokinetic parameters C_{max} and AUC for all scenarios used. The value of Within-Subject Variability is set to 10% for all simulations. In this case, the two products differ in their absorption rate by 20%. The sample size of the simulations is 24 subjects.

At the beginning, the effect of the different sampling schemes on the GMR and the significance of the study for experiments with the value of WSV set to 5% is presented. It is found that the GMR and power of the study for the pharmacokinetic parameter AUC remain at a high level despite the difference in absorption rate between the two products. The same cannot be observed for the C_{max} parameter. Both the GMR and power are slightly lower for this parameter when the difference in absorption rate between the two products is higher. The GMR for C_{max} is now 0.93 and the power is 98.3%. However, the values of GMR and potency remain the same for the different sampling schemes for both pharmacokinetic parameters. When the same simulations are performed with a BSV value of 20%, the results remain unchanged; the potency and GMR for AUC are 100% and 1, respectively, and the potency and GMR for C_{max} are 98.3% and 0.93, respectively, values that are slightly lower than those of the simulations with equal absorption rates of the two products.

40% Difference

ID	sampling scheme	BSV	WSV	N	GMR		Power	
					C_{max}	AUC	C_{max}	AUC
40% difference								
106	1	5	10		0.879	0.984	79.9	99.6
107	2	5	10		0.879	0.984	79.9	99.6
108	3	5	10		0.879	0.984	79.9	99.6
109	4	5	10		0.879	0.984	79.9	99.6
110	5	5	10		0.879	0.984	79.9	99.6
111	6	5	10		0.879	0.984	79.9	99.6
112	7	5	10		0.879	0.984	79.9	99.6
113	8	5	10		0.879	0.984	79.9	99.6
114	9	5	10		0.879	0.984	79.9	99.6
115	10	5	10		0.879	0.984	79.9	99.6
116	11	5	10	24	0.879	0.984	79.9	99.6
117	12	5	10		0.879	0.984	79.9	99.6
118	13	5	10		0.879	0.984	79.9	99.6
119	14	5	10		0.879	0.984	79.9	99.6
120	15	5	10		0.879	0.984	79.9	99.6
121	16	5	10		0.879	0.984	79.9	99.6
122	17	5	10		0.879	0.984	79.9	99.6
123	18	5	10		0.879	0.984	79.9	99.6
124	19	5	10		0.879	0.984	79.9	99.6
125	20	5	10		0.879	0.984	79.9	99.6
126	21	5	10		0.879	0.984	79.9	99.6
127	1	20	10		0.879	0.984	79.9	99.6
128	2	20	10		0.879	0.984	79.9	99.6
129	3	20	10		0.879	0.984	79.9	99.6
130	4	20	10	24	0.879	0.984	79.9	99.6
131	5	20	10		0.879	0.984	79.9	99.6
132	6	20	10		0.879	0.984	79.9	99.6
133	7	20	10		0.879	0.984	79.9	99.6

***Table C.3**: Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with 40% difference in the rate of absorption between the Test and the Reference product*

ID	sampling scheme	BSV	WSV	N	GMR		Power	
					C_{max}	AUC	C_{max}	AUC
40% difference								
134	8	20	10		0.879	0.984	79.9	99.6
135	9	20	10		0.879	0.984	79.9	99.6
136	10	20	10		0.879	0.984	79.9	99.6
137	11	20	10		0.879	0.984	79.9	99.6
138	12	20	10		0.879	0.984	79.9	99.6
139	13	20	10		0.879	0.984	79.9	99.6
140	14	20	10		0.879	0.984	79.9	99.6
141	15	20	10		0.879	0.984	79.9	99.6
142	16	20	10		0.879	0.984	79.9	99.6
143	17	20	10		0.879	0.984	79.9	99.6
144	18	20	10		0.879	0.984	79.9	99.6
145	19	20	10		0.879	0.984	79.9	99.6
146	20	20	10		0.879	0.984	79.9	99.6
147	21	20	10		0.879	0.984	79.9	99.6

Table C.3 (continue): *Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with 40% difference in the rate of absorption between the Test and the Reference product*

Table C.3 shows the GMR and power of the study for the two pharmacokinetic parameters C_{max} and AUC for all scenarios used. The value of Within-Subject Variability (WSV) is set to 10% for all simulations. The sample size of the simulations is 24 subjects. In this case, the two products differ in their absorption rate by 40%.

First, the results of the simulations are presented with the value of BSV set to 5%. Now the GMR and the significance of the study for the AUC are slightly lower than in the previous cases. The GMR of AUC is 0.984 and the power is 99.6%. The effect on C_{max} is higher; the GMR and power have decreased to 0.879 and 79.9%, respectively.

Next, the results of the same simulations with a BSV value of 20% are presented. It seems that the higher value of BSV does not affect the GMR and power for both pharmacokinetic parameters, as they both remain at the same level.

Finally, in agreement with the cases presented previously, the different sampling schemes do not seem to affect the potency and GMR for AUC and C_{max}, for both applied values of BSV.

50% Difference

ID	sampling scheme	BSV	WSV	N	GMR		Power	
					C_{max}	AUC	C_{max}	AUC
50% difference								
148	1	5	10		0.837	0.973	34	98.2
149	2	5	10		0.837	0.973	34	98.2
150	3	5	10		0.837	0.973	34	98.2
151	4	5	10		0.837	0.973	34	98.2
152	5	5	10		0.837	0.973	34	98.2
153	6	5	10		0.837	0.973	34	98.2
154	7	5	10		0.837	0.973	34	98.2
155	8	5	10		0.837	0.973	34	98.2
156	9	5	10		0.837	0.973	34	98.2
157	10	5	10		0.837	0.973	34	98.2
158	11	5	10	24	0.837	0.973	34	98.2
159	12	5	10		0.837	0.973	34	98.2
160	13	5	10		0.837	0.973	34	98.2
161	14	5	10		0.837	0.973	34	98.2
162	15	5	10		0.837	0.973	34	98.2
163	16	5	10		0.837	0.973	34	98.2
164	17	5	10		0.837	0.973	34	98.2
165	18	5	10		0.837	0.973	34	98.2
166	19	5	10		0.837	0.973	34	98.2
167	20	5	10		0.837	0.973	34	98.2
168	21	5	10		0.837	0.973	34	98.2
169	1	20	10		0.837	0.973	34	98.2
170	2	20	10		0.837	0.973	34	98.2
171	3	20	10		0.837	0.973	34	98.2
172	4	20	10	24	0.837	0.973	34	98.2
173	5	20	10		0.837	0.973	34	98.2
174	6	20	10		0.837	0.973	34	98.2
175	7	20	10		0.837	0.973	34	98.2

***Table C.4**: Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with 50% difference in the rate of absorption between the Test and the Reference product*

50	sampling scheme	BSV	WSV	N	GMR		Power	
					C_{max}	AUC	Cmax	AUC
50% difference								
176	8	20	10		0.837	0.973	34	98.2
177	9	20	10		0.837	0.973	34	98.2
178	10	20	10		0.837	0.973	34	98.2
179	11	20	10		0.837	0.973	34	98.2
180	12	20	10		0.837	0.973	34	98.2
181	13	20	10		0.837	0.973	34	98.2
182	14	20	10		0.837	0.973	34	98.2
183	15	20	10		0.837	0.973	34	98.2
184	16	20	10		0.837	0.973	34	98.2
185	17	20	10		0.837	0.973	34	98.2
186	18	20	10		0.837	0.973	34	98.2
187	19	20	10		0.837	0.973	34	98.2
188	20	20	10		0.837	0.973	34	98.2
189	21	20	10		0.837	0.973	34	98.2

***Table C.4* (*continue*):** *Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with 50% difference in the rate of absorption between the Test and the Reference product*

Next, Table C.4 presents the GMR and power of the study for the two pharmacokinetic parameters C_{max} and AUC in the case of the two products with 45% difference in absorption rate for all applied scenarios. The value of Within-Subject Variability (WSV) is again set to 10% and the sample size is 24 subjects for all simulations.

The first rows of the table show the results of the simulations where the value of BSV is set to 5%. Now the GMR and the power of the study for the AUC have decreased to 0.973 and 98.2%, respectively. However, the greatest impact is noticed in the case of the pharmacokinetic parameter C_{max}, which has a GMR of 0.837 and power decreased to 34%.

Next, the results of the same simulations are presented with the value of BSV at 20%. Again, it can be seen that the higher value of BSV does not affect the GMR and the potency for both pharmacokinetic parameters, as they both remain at the same level.

Finally, in agreement with the cases presented previously, the different sampling schedules do not seem to affect the potency and GMR for AUC and C_{max}.

75% Difference

ID	sampling scheme	BSV	WSV	N	GMR		Power	
					C_{max}	AUC	C_{max}	AUC
75% difference								
190	1	5	10	24	0.78	0.961	1.6	97.8
191	2	5	10		0.78	0.961	1.6	97.8
192	3	5	10		0.78	0.961	1.6	97.8
193	4	5	10		0.78	0.961	1.6	97.8
194	5	5	10		0.78	0.961	1.6	97.8
195	6	5	10		0.78	0.961	1.6	97.8
196	7	5	10		0.78	0.961	1.6	97.8
197	8	5	10		0.78	0.961	1.6	97.8
198	9	5	10		0.78	0.961	1.6	97.8
199	10	5	10		0.78	0.961	1.6	97.8
200	11	5	10		0.78	0.961	1.6	97.8
201	12	5	10		0.78	0.961	1.6	97.8
202	13	5	10		0.78	0.961	1.6	97.8
203	14	5	10		0.78	0.961	1.6	97.8
204	15	5	10		0.78	0.961	1.6	97.8
205	16	5	10		0.78	0.961	1.6	97.8
206	17	5	10		0.78	0.961	1.6	97.8
207	18	5	10		0.78	0.961	1.6	97.8
208	19	5	10		0.78	0.961	1.6	97.8
209	20	5	10		0.78	0.961	1.6	97.8
210	21	5	10		0.78	0.961	1.6	97.8
211	1	20	10	24	0.78	0.961	1.6	97.8
212	2	20	10		0.78	0.961	1.6	97.8
213	3	20	10		0.78	0.961	1.6	97.8
214	4	20	10		0.78	0.961	1.6	97.8
215	5	20	10		0.78	0.961	1.6	97.8
216	6	20	10		0.78	0.961	1.6	97.8

***Table C.5**: Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with 75% difference in the rate of absorption between the Test and the Reference product*

ID	sampling scheme	BSV	WSV	N	GMR		Power	
					C_{max}	AUC	C_{max}	AUC
75% difference								
217	7	20	10		0.78	0.961	1.6	97.8
218	8	20	10		0.78	0.961	1.6	97.8
219	9	20	10		0.78	0.961	1.6	97.8
220	10	20	10		0.78	0.961	1.6	97.8
221	11	20	10		0.78	0.961	1.6	97.8
222	12	20	10		0.78	0.961	1.6	97.8
223	13	20	10		0.78	0.961	1.6	97.8
224	14	20	10		0.78	0.961	1.6	97.8
225	15	20	10		0.78	0.961	1.6	97.8
226	16	20	10		0.78	0.961	1.6	97.8
227	17	20	10		0.78	0.961	1.6	97.8
228	18	20	10		0.78	0.961	1.6	97.8
229	19	20	10		0.78	0.961	1.6	97.8
230	20	20	10		0.78	0.961	1.6	97.8
231	21	20	10		0.78	0.961	1.6	97.8

Table C.5* (*continue): *Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with 75% difference in the rate of absorption between the Test and the Reference product*

Finally, Table C.5 shows the extreme scenario where a test product and a reference product differ by 75% in terms of their absorption rates. The GMR and the significance of the study for the two pharmacokinetic parameters, C_{max} and AUC, are shown for all scenarios applied. The value of Within-Subject Variability was set to 10% and the sample size of the simulations is 24 subjects.

First, the results of the simulations with the value of WSV at 5% are shown. Now the GMR and power of the study for AUC further decreased to 0.961 and 97.8 respectively. Again, the impact on C_{max} is higher: the GMR is 0.78 and the power of the study has decreased to 1.6.

Then, the results of the same simulations are presented with the value of BSV at 20%. Again, it can be seen that the higher value of BSV does not affect the GMR and the power for both pharmacokinetic parameters, as they both remain at the same level.

Finally, in agreement with all previously presented cases, the different sampling schedules do not seem to affect the potency and GMR for AUC and C_{max}.

From the results of Tables C.2 to C.5, it can be concluded that the different sampling schemes and values of BSV do not affect the significance of the study and the GMR of any of the pharmacokinetic parameters. However, the differences in absorption rate between the two products do affect the GMR and the significance for both C_{max} and AUC.

The effects of the differences in absorption rate on the potency and GMR of C_{max} and AUC are shown in Table C.6 and Figures C.13 and C.14.

Difference (%) in rate	GMR C_{max}	GMR AUC	Power C_{max}	Power AUC
0	1	1	100	100
20	0.93	1	98.3	100
40	0.879	0.984	79.9	99.6
50	0.837	0.973	34	98.2
75	0.78	0.961	1.6	97.8

***Table C.6**: Overall impact of differences in the rate of absorption in the Power and the Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters.*

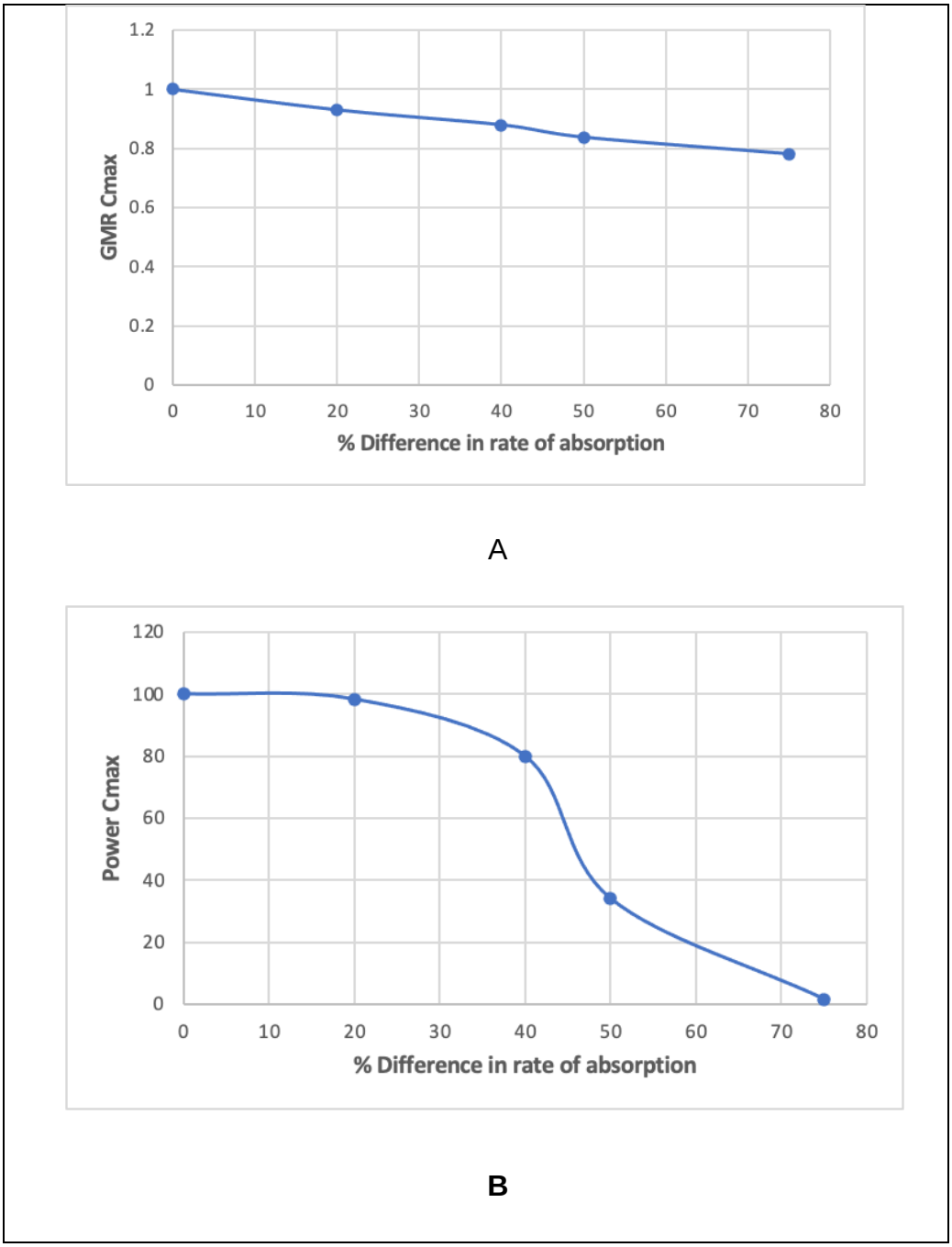


Figure C.13: *Impact of differences in the rate of absorption on the Geometrical Mean Ratio (A) and the power of the study (B) for the pharmacokinetic parameter* C_{max}

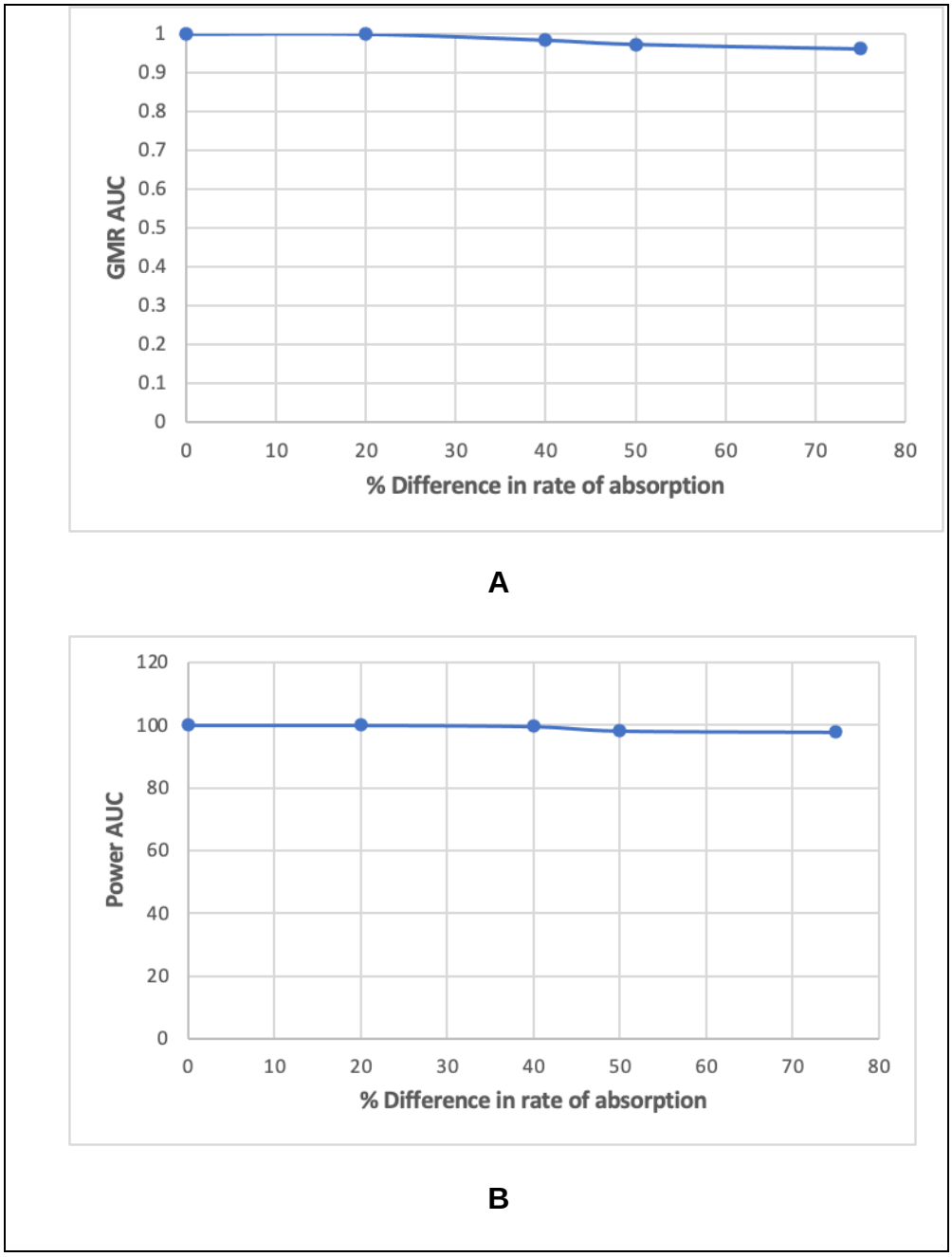


Figure C.14: *Impact of differences in the rate of absorption on the Geometrical Mean Ratio (A) and the power of the study (B) for the pharmacokinetic parameter AUC.*

The pharmacokinetic parameter C_{max} is strongly influenced by differences in the rate of absorption. More specifically, the GMR for C_{max} falls gradually as the percentage difference in absorption rate increases. The significance of the study decreases slightly when the % difference is up to 40%, but for higher percentages of difference, the significance for the pharmacokinetic parameter drops immediately, reaching 1.6% for 75% difference in absorption rate between the two products. The situation is different for the pharmacokinetic parameter AUC. The potency and GMR for this pharmacokinetic parameter are only slightly affected, and only for differences of more than 20% in the absorption rate between the two products. However, even in the worst case of a 75% difference, the GMR for the AUC is close to 1 (0.961) and the power is close to 100% (97.8%).

D. DISCUSSION

The design of a bioequivalence (BE) study is a crucial part of it, as it has an important impact not only on the reliability of the study outcome, but also on the cost of the study, the amount of work involved, and most importantly, the inconvenience to the subjects. Since the BE studies are clinical trials conducted on human participants, the ultimate scope of their design is to minimize the exposure of the subjects to the drug, to expose the smallest possible number of subjects, and to generally reduce the inconvenience to the human participants. The sampling scheme of BE studies is a part of their design that is not directly addressed by the relevant EMA or FDA guidelines, as there is no clear statement of the number of sampling sites required. Considering that as the number of samples taken from patients increases, so does the cost of the study and the inconvenience to the subjects, it is not only interesting but also beneficial to address the issue of the sampling design of BE studies.

The aim of the present work was to investigate the influence of sampling schedule on bioequivalence result through modeling and simulation. The use of in silico clinical trials in the field of bioequivalence has increased in recent decades, as they offer many advantages in various aspects of bioequivalence studies, including the study of the influence of different study parameters on the bioequivalence outcome. For this purpose, the design of a bioequivalence study conducted in vivo was used as a target scenario and changes in sampling design and sample size were combined with changes in variability and pharmacokinetic parameters of the drug products. The effects on power and GMR of AUC and C_{max} were evaluated.

Before examining the influence of sampling design, the influence of other parameters such as Within-Subject Variability (WSV), Between-Subject Variability (BSV), and sample size was examined. This was done by comparing the concentration-time curves of simulations performed with intentionally changed values of these parameters.

In the simulation performed with a high value of WSV (50%), the mean concentration-time curves of the test and reference products differed significantly, even though the pharmacokinetic parameters of the products were set as identical. This result implies that the high value of WSV of a drug substance or drug product can potentially lead to the failure of the bioequivalence study, even if the two drugs to be compared are pharmaceutically equivalent. For this reason, scaled bioequivalence limits or higher sample sizes have been proposed by regulatory authorities for the Highly-Variable Drugs. On the other hand, the high value of BSV did not seem to affect the similarity of the mean concentration-time curves of the two products when their pharmacokinetic parameters were identical. However, when the BSV was too high (50%), there were many subjects who had concentration-time curves that were significantly different from the usual concentration-time curve of donepezil, and thus the mean concentration-time curves also had an unusual shape. This deviation may reduce the reliability of the study results. For this reason, the randomized, crossover, two sequence, two period (2 × 2) design, which minimizes the influence of BSV, is the standard design for a bioequivalence study. Regarding the effect of sample size, it appeared that when the pharmacokinetic parameters of the test and

reference products are identical and the variability is low, the two mean concentration-time curves show high similarity regardless of sample size.

After examining important parameters of the bioequivalence studies, simulations were performed with the different scenarios of the sampling plan.

At the beginning, the effect of different sampling scheme scenarios was evaluated by the influence of sampling scheme on concentration-time curves. It was found that sampling schemes created by deleting time points at the beginning of the sampling schedule affected the shape of the concentration-time curves the most, did not map C_{max}, and resulted in a very different area under the curve (AUC) than the nominal sampling scheme scenario. This result is to be expected given that the T_{max} of donepezil is around 2, 3, and 4 hours, implying that the time points at the beginning of the sampling schedule are most critical for detecting C_{max}. On the other hand, sampling schedules that were shorter or did not include sampling points in the middle or end of the sampling schedule did not appear to significantly, or at least visually, affect the concentration-time curves.

Simulations were then performed combining the 21 sampling schemes with 2 sample sizes and 2 values of Between-Subject Variability (BSV) for test and reference products with equal absorption rates. The sample sizes studied were 12 and 24 subjects and the values of BSV were 5% and 20%. The sampling designs used differed in terms of density and duration. The power of the study, showing the probability of its acceptance, and the GMR, showing the degree of similarity between the two products, were assessed for both pharmacokinetic parameters. The results showed that both the power and GMR for C_{max} and AUC were high, irrespective of the sampling design,

sample size and BSV of the study. More specifically, for both C_{max} and AUC, the GMR was always 1 and the power was 100%. This means that if the two products have identical pharmacokinetic profiles, the likelihood of study acceptance is high regardless of the sampling scheme. On the other hand, when the concentration-time curves of sampling schemes with fewer time points near T_{max} were compared with those of the nominal sampling scheme, differences in the rate and extent of absorption were observed. However, these sampling schemes were applied to both the test and reference products, so they did not affect the GMR or the power of the study for any of the pharmacokinetic parameters.

In the next step of the study, the 21 different sampling schemes were combined with two values of BSV and simulations were performed with a sample size of 24 subjects. Different percentages of difference in absorption rate between the two products were applied. The results show that as the percentage difference in absorption rate increases, the potency and GMR for the pharmacokinetic parameters decrease, but to different degrees for each parameter. More specifically, the potency and GMR for the AUC decrease for a % difference greater than 20%, but always remain at a high level. On the other hand, the potency and GMR for C_{max} are more affected by differences in the absorption rate between the two products. When the % difference is 10%, the potency for C_{max} is 98.30% and the GMR is 0.93. When the % difference reaches 30%, the potency for the pharmacokinetic parameter drops to 34% and the GMR to 0.837. Finally, when the % difference in absorption rate is 45%, the potency for C_{max} is 1.6% and the GMR is 0.78. In the last two cases, bioequivalence is not demonstrated and the study fails. This represents an

expected result as the parameter C_{max} is the one that describes the absorption rate of the drug in a bioavailability study. Therefore, the GMR and potency for C_{max} are expected to be strongly influenced by the differences in absorption rate between the two products being compared in a bioequivalence study. The above conclusions apply to all sampling schemes and the applied values. Indeed, it appears that the sampling scheme and the value of BSV do not affect the potency and GMR of the pharmacokinetic parameters when the test and reference products have differences in their absorption rates. In other words, all sampling schemes were able to reveal the differences in absorption rate between the two products and they all led to the same conclusions regarding the assumption of bioequivalence.

All in all, it can be concluded that differences in the duration and density of the sampling schemes do not affect the significance and GMR for the pharmacokinetic parameters C_{max} and AUC. All sampling scheme scenarios applied were able to demonstrate bioequivalence when the pharmacokinetic parameters of the two products were identical. When the differences in absorption rate were such that the bioequivalence criteria for the parameter C_{max} could not be met, all applied sampling plans succeeded in detecting the differences and rejecting bioequivalence. Moreover, Between-Subject Variability (BSV) did not affect the power and GMR of pharmacokinetic parameters in all applied scenarios. This is attributed to the clinical design of the bioequivalence study: The randomized cross-over design (2×2) minimizes the influence of BSV. (See Figure A.3). Finally, the sample size does not influence the significance of the pharmacokinetic parameters.

The issue of bioequivalence design flexibility was recently addressed in a paper using the results of the in vivo bioequivalence study of donepezil also applied in the present paper. Concentration-time pairs were extruded over the sampling scheme, with changes in duration and density, a statistical evaluation of the results obtained was performed and the influence of the extraction of the pairs on the potency and variability of the pharmacokinetic parameters was evaluated. (Gournaris, 2020). The results of Gournaris' study proved that the power of the study for the two pharmacokinetic parameters, C_{max} and AUC, remained unchanged with all the changes made to the sampling plan. This conclusion is consistent with the results of the present work. In addition, Gournari's research concluded that less dense sampling schemes increased the residual variability for the parameter AUC. Furthermore, less dense sampling schemes were found to increase the residual variability for the pharmacokinetic parameter C_{max}, with the highest increase in variability observed when the first time points were excluded. This result can also be observed in the concentration-time curves for the different sampling schemes in the present work. This is an expected result as the T_{max} of donepezil is 2-3-4 hours. In the study of Gournaris, the duration of the sampling scheme seemed to affect only the pharmacokinetic parameter AUC. More specifically, the power of AUC decreased for sampling regimens with duration of 4 hours. This conclusion was not drawn in relation to the present study as the shortest sampling schedule studied lasted for 8 hours.

The issue of sampling schedule was also addressed by Eunice Kano et al. who used both in silico and in vivo clinical studies with different sampling schedules to determine the influence of sampling frequency as well as

sampling schedule density on the outcome of a bioequivalence study. In the in silico bioequivalence study, the results were very similar for all sampling schemes tested, proving that the exclusion of some sampling sites does not affect the results and conclusions of the bioequivalence study. The same conclusion was also drawn for the in vivo study. However, this was only the case under the condition that T_{max} or a time significantly close to it was included as sampling time. Moreover, the significance of the pharmacokinetic parameter C_{max} appeared to be affected by the density of the sampling schedule in the initial periods, whereas AUC was not. Furthermore, the results of the in vivo study showed that decreasing the sampling intervals did not lead to a more accurate study. The results of the present work are consistent with' Kano's conclusions when it comes to the power for AUC being unaffected by the sampling design. On the other hand, both the present work and Gournaris' research did not conclude that the study did not meet the bioequivalence criteria if T_{max} was not included in the sampling plan, but this is because donepezil's T_{max} is almost evenly distributed at 2, 3, and 4 hours and excluding this or a sampling point near it was not an applied scenario. Furthermore, in Kano's research, an impact on the power of the C_{max} was noted when initial time points were excluded from the sampling design. This effect was not found in the simulations performed in the present work, but this is also related to the uniform distribution of the T_{max} of donepezil at 3 time points.

In conclusion, less dense and shorter sampling designs can be used in bioequivalence studies without significantly affecting the bioequivalence result and power of the study. Moreover, after conducting the present study, it is

evident that in silico clinical trials can be very useful to investigate the influence of study design features on the results of a bioequivalence study. The above conclusions lead to the final thought that it is possible to reduce the economic burden and minimize the inconvenience to human subjects by conducting studies with less dense sampling schemes that do not include more subjects than are needed to achieve the desired power and whose study parameters have been determined or verified by modeling and simulation.

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F. SYNOPSIS

F.1 Introduction

In the world of pharmaceutical industry, bioequivalence studies (BE) have a significant role. BE Studies form the required regulatory basis for the approval of generic pharmaceutical products. According to Directive 2001/83/ EC of the European Parliament and of the Council of 6 November 2001, the term "generic medicinal product" means a product with the same (qualitative and quantitative) composition in active pharmaceutical ingredients and the same pharmaceutical form as a product authorized as a reference product. In addition, bioequivalence between the two products must have been demonstrated by appropriate bioequivalence studies.

Bioequivalence shall be evaluated with comparative bioavailability studies between the test product and the reference product. The rate and extent of absorption shall be used to compare the pharmacokinetic profiles of the two products. This is done by comparing the pharmacokinetic parameters C_{max}, which describes the rate of absorption, and AUC, which describes the extent of absorption. (Figure F.1)

If bioequivalence can be demonstrated after administration of the same molar dose of a reference and an investigational drug, their pharmaceutical equivalence can be inferred or at least reasonably assumed. (Baumgaertel, 2012)

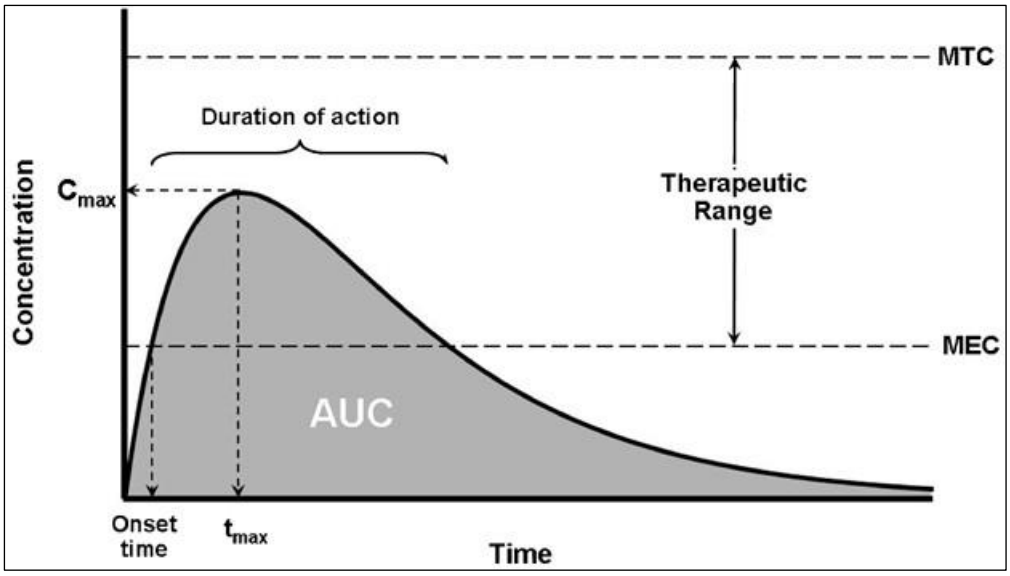

Figure F.1.: *Concentration-time curve (Mehrotra N, et al, 2006)*

For a bioequivalence study to produce a reliable result and succeed in distinguishing the influence of formulation differences from other sources of variability, it must be conducted with an appropriate clinical design. The choice of design depends primarily on the pharmacokinetic properties of the products being compared. The standard design of a bioequivalence study is the randomized, crossover, two-sequence, two-period (2 × 2) design, in which both products (Test T and Reference R) are administered to each subject at two different time periods separated by a washout period. In addition to the standard design, there are other types of clinical designs that can be used in a bioequivalence study, such as the replicate designs and the two-stage designs. The traditional parallel design is used primarily when the drug being studied has a very long half-life. Bioequivalence studies are conducted on healthy subjects that are standardized as much as possible to minimize variability and to be able to detect any difference, no matter how small, between formulations. (Baumgaertel, 2012). The selection criteria for the subjects and the administration conditions are detailed in the protocol of the

study. Another important aspect of a bioequivalence study is the sample size. The selection of the sample size is about finding the golden mean between having enough subjects to demonstrate equivalence and minimizing unnecessary human exposure to drugs. In addition, the sampling design and duration of a bioequivalence study are also important parameters of its design. It is recognized that sampling points should include one sample before dosing, at least one or two samples before C_{max}, two samples around C_{max}, and three to four samples during the elimination phase, resulting in a total of at least seven sampling points for the entire bioequivalence study.

A statistical analysis is performed to evaluate the results of the bioequivalence study. This includes the calculation of the 90% Confidence Interval (CI) of the Geometric Mean Range (GMR) of the pharmacokinetic parameters. For bioequivalence to be fulfilled, this interval must be within the acceptance limits 80.00%-125.00%.

The 90% Confidence Interval is calculated with the following equation:

$$90\%\ \mathrm{CI} = \mu_T - \mu_R \pm t_{0.95\,(v)}\ \mathrm{MSE}\sqrt{\frac{1}{N1}+\frac{1}{N2}}$$

Equation F.1

where:

μ_T and μ_R represent the average of the logarithmically modified parameters for the test and the reference product.

$t_{0.95(v)}$ is the point of Student's distribution that isolates a probability of 0.05 at the upper end for v degrees of freedom.

N1, N2 are the number of volunteers in each one of the two groups.

v are the degrees of freedom that depend on the total number of volunteers (sample size) and the number of participants on each group.

$$v = N1 + N2 - 2$$

Equation F.2

MSE is the Mean Square Error, the average value of the square of the calculated error.

For Highly Variable Drugs (HVD) and Narrow Therapeutic Index Drugs (NTID), different bioequivalence limits are proposed by regulatory authorities. In the first case, scaled bioequivalence limits based on the value of Within-Subject Variability for C_{max} are applied C_{max} and in the second case, CI limits are tightened to 90.00%-110.00%.

In recent decades, the use of modeling and simulation in bioequivalence assessment has increased. In silico clinical studies allow the outcome of a clinical trial to be predicted and any conditions that could potentially affect it to be tested without actually conducting the trial. They can be applied in various aspects of bioequivalence assessment, including determining the sampling design or investigating its impact on the bioequivalence study results.

The aim of the present work is to investigate the impact of differences in sampling scheme on bioequivalence result using modeling and simulation, i.e., in silico clinical studies. More specifically, different sampling schemes that differ in terms of duration and density are applied in simulations of bioequivalence studies. The power of each study and the GMR for the pharmacokinetic parameters C_{max} and AUC are evaluated in each case. At the same time, the influence of parameters such as sample size, Within-Subject Variability (WSV), Between-Subject Variability (BSV) and differences

in absorption rate between the two products on the bioequivalence result is investigated.

F.2 Methods

To fulfill the purpose of the study, the design of an in vivo bioequivalence study between the test product donepezil/Verisfield and the reference product Aricept®/Pfizer is used as the nominal design scenario. Based on the sampling scheme of this study, multiple sampling scheme scenarios are created by deleting sampling points throughout the sampling scheme. In this way, 20 different sampling schemes are designed. The nominal sampling scheme scenario is also used in the simulations. (Table F.1) These sampling plan scenarios are combined with two different sample sizes and two different values of Between-Subject Variability (BSV). The sample sizes used are 12 and 24 subjects. The values of BSV are 20% and 5%. In addition, the effect of the sampling design combined with the effect of differences in the absorption rate between the two products is investigated. Simulations are performed with different percentage differences in absorption rate, with 24 subjects and BSV of 5% and 20%.

All computer simulations are performed in a computer program developed in Matlab® 2018b. The above parameters as well as the in vivo pharmacokinetic parameters of donepezil found in the literature can be used in this program. For each simulation performed, the power of the study and the GMR for the pharmacokinetic parameters C_{max} and AUC are measured.

A schematic representation of the simulations is shown in Figures F.2 and F.3

Scenario No.	Sampling scheme (hours)
1	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 96, 144, 192
2	0, 0.5, 1, 2, 3, 4, 6, 8,12, 24, 48, 96, 144
3	0, 1, 3, 4, 6, 8,12, 24, 48, 96, 144, 192
4	0, 0.5, 1, 2, 6, 8, 12, 24, 48, 96, 144, 192
5	0, 0.5, 1, 2, 3, 4, 6, 12, 48, 96, 144, 192
6	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 8, 96
7	0, 2, 4, 6, 8, 12, 24, 48, 96, 144, 192
8	0, 0.5, 1, 6, 8, 12, 24, 48, 96, 144, 192
9	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 96, 192
10	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48
11	0, 3, 6, 8, 12, 24, 48, 96, 144, 192
12	0, 0.5, 6, 8, 12, 24, 48, 96, 144, 192
13	0, 0.5, 1, 2, 3, 4, 6, 24, 144, 192
14	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24
15	0, 6, 8, 12, 24, 48, 96, 144, 192
16	0, 0.5, 1, 2, 3, 4, 6, 144, 192
17	0, 0.5, 1, 2, 3, 4, 6, 8, 12
18	0, 0.5, 1, 2, 3, 4, 6, 8
19	0, 1, 3, 6, 12, 48, 144, 192
20	0, 2, 6, 24, 144, 192
21	0, 3, 4, 6, 48, 96, 144, 192

Table F.1: *Sampling scheme scenarios*

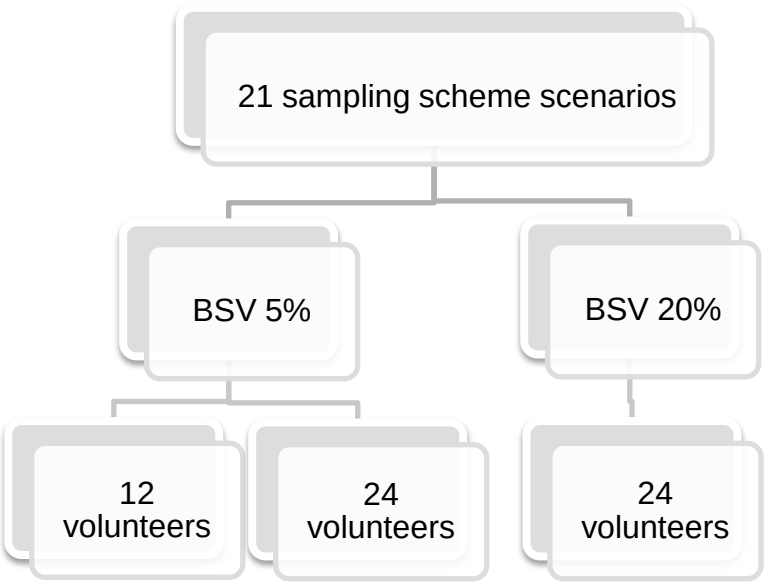

Figure F.2: *Simulations conducted for the investigation of the impact of sampling scheme*

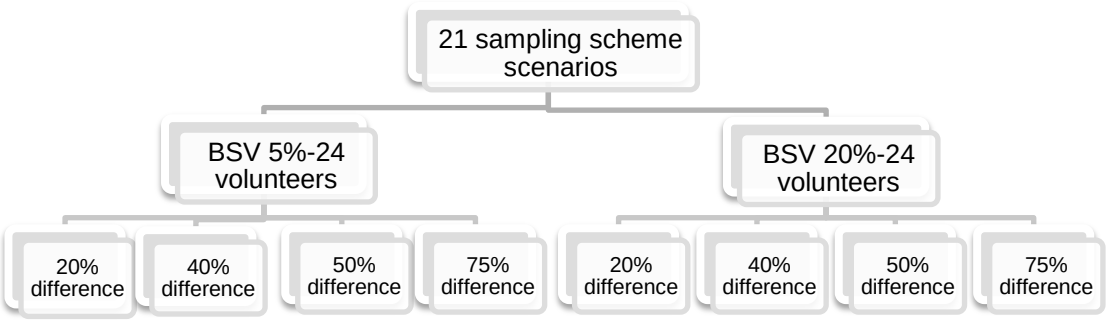

Figure F.3: *Simulations conducted for the investigation of the impact of sampling scheme combined with different pharmacokinetic properties between the test and the reference product*

Before examining the effects of different sampling schemes on the outcome of BE, the influence of other parameters such as the Within-Subject Variability, the Between-Subject Variability and the sample size is presented.

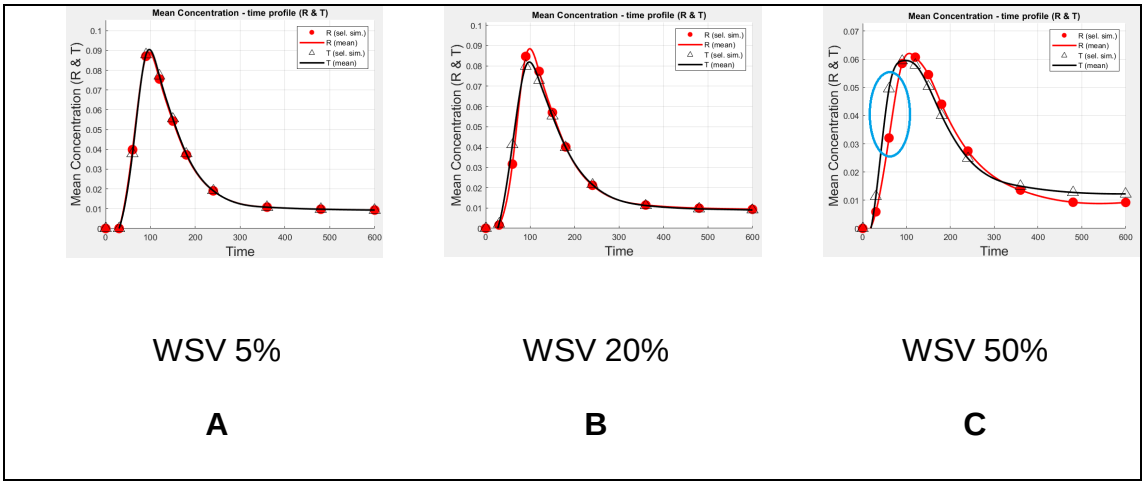

Figure F.4 : *The impact of Within-Subject Variability*

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Figure F.4 shows the influence of WSV in the mean concentration-time curves of the test and reference products. In all figures, the black curve represents the mean concentration-time curve for the test product and the red curve represents the mean concentration-time curve for the reference product. The black triangles and red dots represent the time points for the test and reference groups, respectively.

In all simulations shown, the pharmacokinetic properties of the two products are identical, and the sample size and BSV are the same. The differences between the concentration-time curves of the test and reference products, shown in Figures F.4.B and F.4.C, are the result of the WSV of the drug products. In Figure F.4.C, where the value of WSV is 50%, the differences between the two products are greater. An example is shown in the

diagram. This is the result of very different concentration-time curves between subjects in the study due to the high value of WSV.

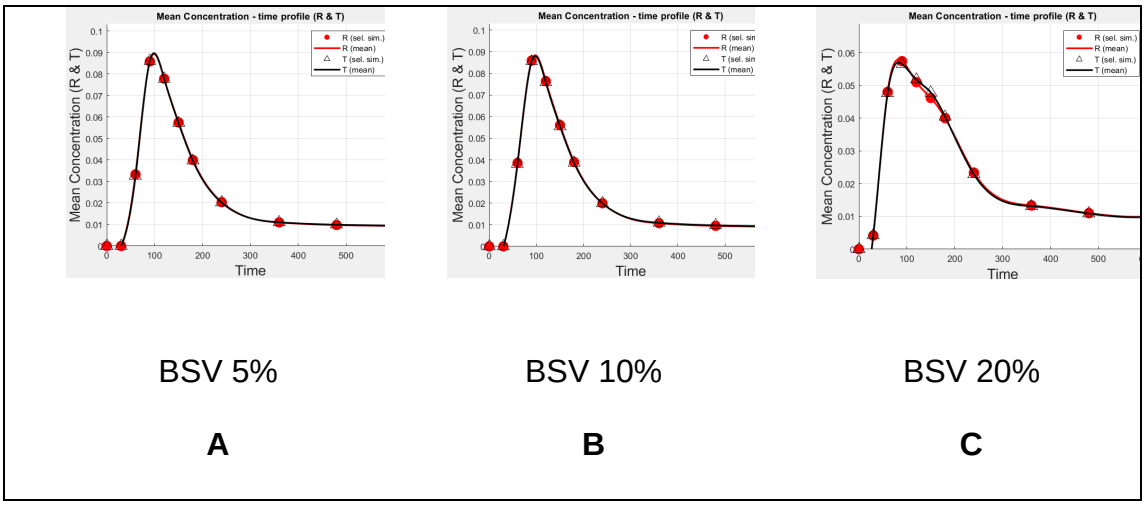


Figure F.5: *The impact of Between-Subject Variability*

By comparing Figures F.5.A., F.5.B and F.5.C, the influence of BSV can be perceived. The high value of Between-Subject variability means that there are many differences between the concentration-time curves of the subjects in the study. More precisely, the subjects differ from each other, but when the drug products have identical pharmacokinetic properties, the participants do not show differences in taking the test or reference product. For this reason, even in Figure F.5.C, the mean concentration-time curves of the test and reference products are identical. However, the high value of the BSV means that the individual concentration-time curves are not comparable with each other and thus the resulting mean concentration-time curves do not even resemble those of donepezil.

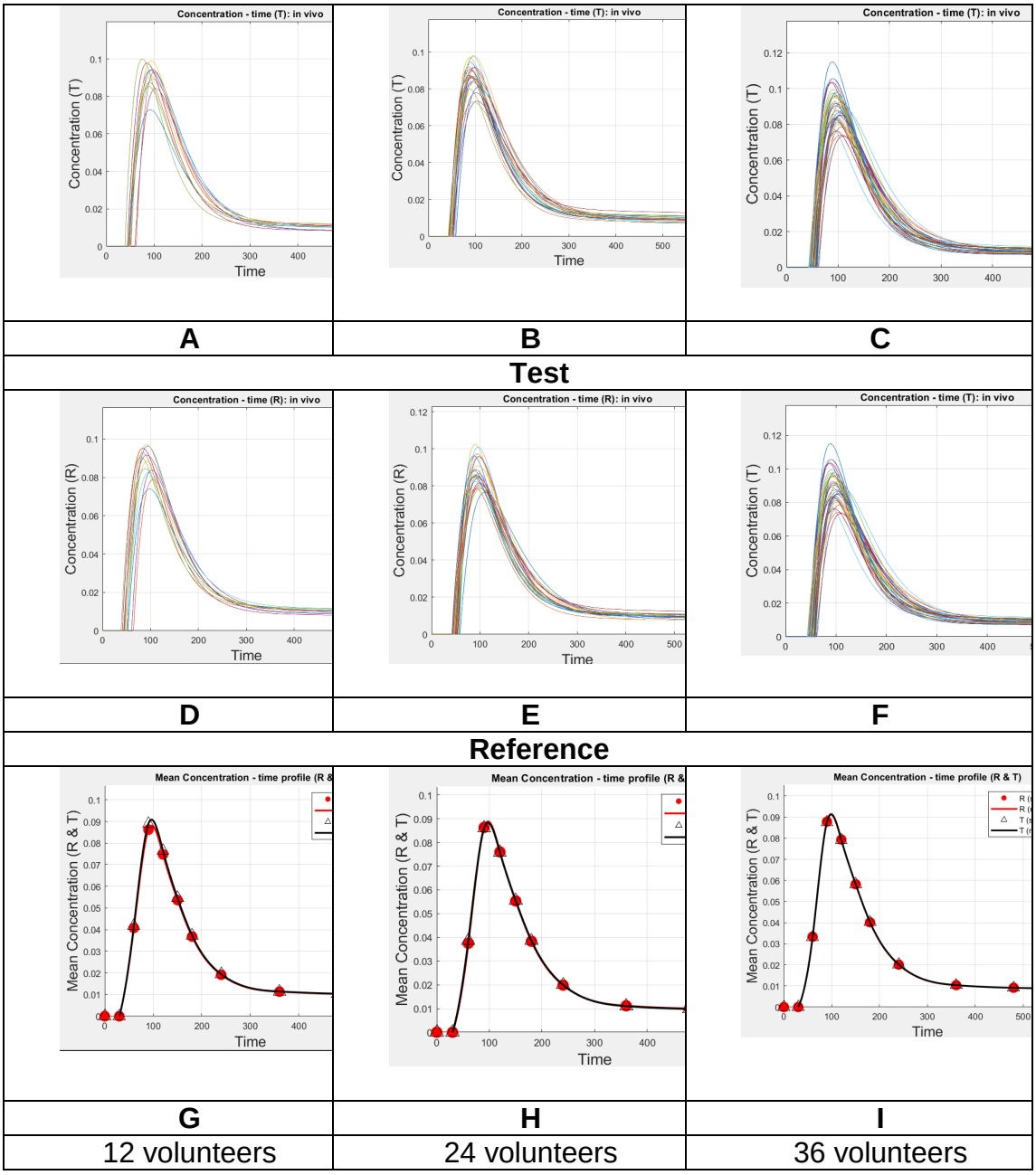
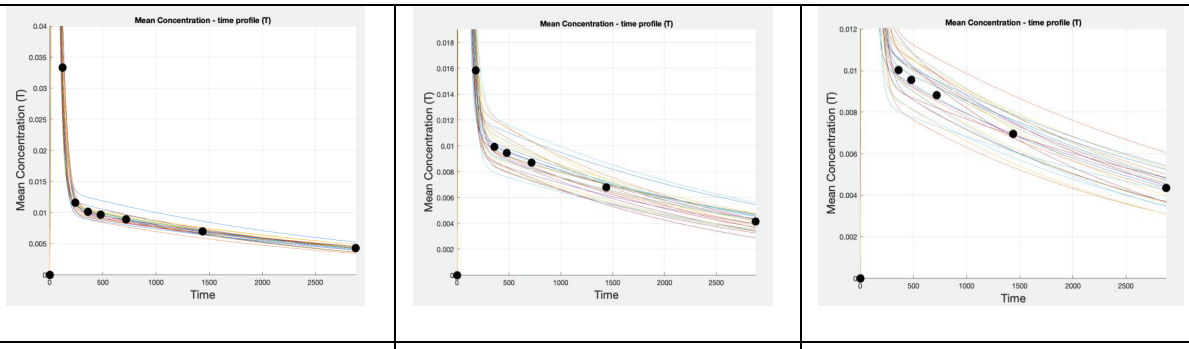


Figure F.6: *The impact of sample size*

Figure F.6 shows the concentration-time curves of each of the subjects participating in the study. Figures F.6.A, F.6.B, and F.6.C refer to the test product for sample sizes of 12, 24, and 36 subjects, respectively, and Figures F.6.D, F.6.E, and F.6.F refer to the reference product for sample sizes of 12, 24, and 36 subjects, respectively. It is evident that these figures, the so-called "spaghetti" figures, become denser with increasing sample size. However, when the pharmacokinetic properties of the two products are similar and the BSV and WSV of the study are low, all the individual concentration-time curves yield the same mean concentration-time curves, as shown in Figures F.6.G, F.6.H, and F.6.I

Then the influence of the sampling scheme is evaluated. The 21 different sampling schemes are applied in simulations with 24 subjects, where the value of WSV is set to 10% and the value of BSV is set to 5%. The concentration-time curves of these simulations are observed. The result shows that the sampling schemes with the greatest influence on the concentration-time curves are those that contain the fewest time points in the initial times. Figures F.7.A, F.7.B and F.7.C show the concentration-time curves for the test product of the simulations with sampling scheme scenarios 7, 11 and 15, respectively.



A	B	C

Figure F.7: *Concentration-time curves for the Test product (T) of sampling scheme scenarios 7 (A), 11 (B) and 15(C).*

The next step of the study is to evaluate the influence of differences in absorption rate between the test and reference products. First, indicative concentration-time curves of the sampling scheme scenario 6 with different percentages of differences in the absorption rate between the two products are considered. The corresponding figures are omitted in this synopsis. The results show that the product with the higher absorption rate reaches a higher C_{max} value in a shorter time.

Finally, simulations of the different scenarios of the sampling design are performed in combination with the two sample sizes (12 and 24 subjects), the two values of the BSV (5% and 20%) and the different percentages of the difference in the absorption rate. The GMR and power of the study for the pharmacokinetic parameters C_{max} and AUC are measured. The results are summarized in the following tables.

sampling scheme	BSV	WSV	N	GMR		Power	
				C_{max}	AUC	C_{max}	AUC
1	5	10	12 or 24	1.00	1.00	100	100
2	5	10		1.00	1.00	100	100
3	5	10		1.00	1.00	100	100
4	5	10		1.00	1.00	100	100
5	5	10		1.00	1.00	100	100
6	5	10		1.00	1.00	100	100
7	5	10		1.00	1.00	100	100
8	5	10		1.00	1.00	100	100
9	5	10		1.00	1.00	100	100
10	5	10		1.00	1.00	100	100
11	5	10		1.00	1.00	100	100
12	5	10		1.00	1.00	100	100
13	5	10		1.00	1.00	100	100
14	5	10		1.00	1.00	100	100
15	5	10		1.00	1.00	100	100
16	5	10		1.00	1.00	100	100
17	5	10		1.00	1.00	100	100
18	5	10		1.00	1.00	100	100
19	5	10		1.00	1.00	100	100
20	5	10		1.00	1.00	100	100
21	5	10		1.00	1.00	100	100
1	20	10	24	1.00	1.00	100	100
2	20	10		1.00	1.00	100	100
3	20	10		1.00	1.00	100	100
4	20	10		1.00	1.00	100	100
5	20	10		1.00	1.00	100	100
6	20	10		1.00	1.00	100	100
7	20	10		1.00	1.00	100	100
8	20	10		1.00	1.00	100	100
9	20	10		1.00	1.00	100	100
10	20	10		1.00	1.00	100	100
11	20	10		1.00	1.00	100	100
12	20	10		1.00	1.00	100	100
13	20	10		1.00	1.00	100	100
14	20	10		1.00	1.00	100	100

Table F.1: *Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with equal rate of absorption between the test and the reference product*

sampling scheme	BSV	WSV	N	GMR		Power	
				C_{max}	AUC	C_{max}	AUC
15	20	10	24	1.00	1.00	100	100
16	20	10		1.00	1.00	100	100
17	20	10		1.00	1.00	100	100
18	20	10		1.00	1.00	100	100
19	20	10		1.00	1.00	100	100
20	20	10		1.00	1.00	100	100
21	20	10		1.00	1.00	100	100

Table F.1 (continue): *Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with equal rate of absorption between the test and the reference product*

Sampling scheme scenario	Difference (%) in rate	GMR C_{max}	GMR AUC	Power C_{max}	Power AUC
Scenarios 1-21	0	1	1	100	100
	10	0.93	1	98.3	100
	20	0.879	0.984	79.9	99.6
	30	0.837	0.973	34	98.2
	40	0.78	0.961	1.6	97.8

Table F.2 *Power and Geometrical Mean Ratio (GMR) for the pharmacokinetic parameters for the 21 different sampling scheme scenarios performed with different rates of absorption between the test and the reference product*

According to the results summarized in Tables F.1 and F.2, the different sampling schemes for none of the pharmacokinetic parameters studied have an impact on the significance and GMR. This is also true for the BSV and sample size. The only factor that affects the GMR and the significance of the pharmacokinetic parameters is the percentage difference in the absorption rate of the two products. As this difference increases, the potency and GMR for the AUC and C_{max} decrease, and the most significant effect is that on the C_{max}, as seen in Table F.2.

F.4 Discussion

The design of a bioequivalence study affects its reliability, cost, and inconvenience to human participants. The sampling plan is a part of bioequivalence study design that is not directly addressed in the relevant guidelines. The aim of the present work was to investigate the influence of the sampling plan on the bioequivalence outcome through modeling and simulation. To this end, the design of a bioequivalence study conducted in vivo was used as a nominal scenario and changes in sampling design and sample size were combined with changes in variability and pharmacokinetic parameters of the drugs and simulations were performed. The effects on power and GMR of AUC and C_{max} were evaluated.

First, the influence of Within-Subject Variability, Between-Subject Variability, and sample size was examined by looking at the concentration-time curves. The results indicated that a high value of WSV of a drug substance or drug product may possibly lead to the failure of the bioequivalence study, even if the two drugs to be compared are pharmaceutically equivalent. For this reason, scaled bioequivalence limits or higher sample sizes are suggested for highly variable drugs. In addition, when the BSV was too high (50%), the mean concentration-time curves had an unusual shape that differed from that of donepezil. This discrepancy may reduce the reliability of the study results. For this reason, the randomized, crossover, two sequence, two period (2 × 2) design that minimizes the influence of BSV is the standard design for a bioequivalence study.

The effect of the different sampling scheme scenarios was then examined by observing the concentration-time curves. It was found that the sampling schemes created by deleting time points at the beginning of the sampling schedule affected the shape of the concentration-time curves the most. This result is expected since the T_{max} of donepezil is between 2 and 4 hours, which are the initial time points in the sampling schedule.

Then, simulations were performed combining the 21 sampling schedules with 2 sample sizes and 2 values of Between-Subject Variability (BSV) for test and reference products with the same absorption rates. The results showed that both the power and GMR for C_{max} and AUC were high regardless of the sampling plan, sample size and BSV of the study. This means that with identical pharmacokinetic profiles of the two products, the likelihood of study acceptability is high regardless of the sampling design. In the next step of the study, the 21 different sampling plans were combined with two values of BSV and simulations were performed with a sample size of 24 subjects. Different percentages of difference in absorption rate between the two products were applied. The results show that as the percentage difference in absorption rate increases, the potency and GMR for the pharmacokinetic parameters decrease, but to different degrees for each parameter. C_{max} is the parameter most affected by the differences in absorption rate between the two products. Again, potency and GMR for the pharmacokinetic parameters were not affected by the different sampling schedules.

Overall, it can be concluded that differences in the duration and density of the sampling schedules do not affect the potency and GMR for the pharmacokinetic parameters C_{max} and AUC. All sampling schedule scenarios

applied were able to demonstrate bioequivalence when the pharmacokinetic parameters of the two products were identical. When the differences in absorption rate were such that the bioequivalence criteria for the parameter C_{max} could not be met, all applied sampling plans succeeded in detecting the differences and rejecting bioequivalence.

It should be emphasized that the results of the present work are in agreement with the results of similar studies (Gournaris, 2020; Kano et al, 2017).

In conclusion, less dense and shorter sampling schemes can be applied in bioequivalence studies without significantly affecting the bioequivalence result and power of the study.

F.5 References

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