STATISTICAL APPROACHES TO PERFORM DISSOLUTION PROFILE COMPARISONS

Dissertation

zur Erlangung des Grades

des Doktors der Naturwissenschaften

der Naturwissenschaftlich-Technischen Fakultät III

Chemie, Pharmazie, Bio- und Werkstoffwissenschaften

der Universität des Saarlandes

von

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Saarbrücken

2013

Tag des Kolloquiums:	16.12.2013
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Life is not a hundred meter sprint; life is an ultra-marathon with hurdles, and it is all about not losing rhythm with the obstacles.

–Vicente Casabó - (1958-2013)

May you rest in peace,

Dear professor.

“The aim of science is not to open the door to infinite wisdom, but to set a limit to infinite error.”

–Bertolt Brecht –

Life of Galileo

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1. Short Summary

Comparative dissolution testing is extensively used as a tool to evaluate equivalency between oral solid dosage forms. However the current official procedure to compare dissolution profiles, the f2 similarity factor, lacks solid statistical foundation and the statistical uncertainty is unknown. Additionally the limits to declare *in vitro* similarity with the current methodology ($f2 \geq 50$) are arbitrary and not bound to any biopharmaceutical property; therefore it cannot be considered as a good predictor of the *in vivo* performance of formulations, especially in the case of extended release formulations.

The aim of this work was to design, develop and explore two new statistical tests for comparing dissolution profiles. These tests have more statistical foundations than the current methodologies and exhibit the flexibility to be customized for a specific formulation. One test, the tolerated difference test (TDT) can be tailored to detect differences in the release profiles of extended release formulations that represent a lack of bioequivalence (or a significant difference in the performance in a combined dissolution permeability system).

Customization of the dissolution profile comparison tests for ER formulations was possible by using the TDT without sacrificing its statistical properties (known and acceptable statistical uncertainty).

In summary, a new approach to design and perform dissolution profile comparisons under typical principles of statistical experimental design is described. Four demonstrative examples of comparisons of ER formulations are presented.

2. Kurzzusammenfassung

Vergleichende Untersuchungen des Auflösungsverhaltens werden häufig zur Bestimmung der Äquivalenz von oralen, festen Arzneiformen eingesetzt. Die derzeitige offizielle Prüfung, der f2-Test, verwendet hierfür den sogenannten *similarity factor* f2, dem jedoch eine solide statistische Grundlage fehlt und dessen statistische Unsicherheit nicht bekannt ist. Darüber hinaus sind bei diesem Test die Grenzen für die *in-vitro*-Ähnlichkeit (f2 < 50) willkürlich gewählt und nicht an biopharmazeutische Eigenschaften gebunden. Daher kann diese Methode nicht als eine gute Vorhersage für das *in-vivo*-Verhalten der untersuchten Arzneiformen angesehen werden. Dies gilt insbesondere für Arzneiformen mit verlängerter Wirkstofffreigabe.

Das Ziel dieser Arbeit war die Entwicklung zweier neuer statistischer Tests zum Vergleich von Auflösungsprofilen. Im Vergleich zu momentan angewendeten Methoden besitzen diese beiden neuen Tests eine fundierte statistische Basis und verfügen über die Flexibilität, für spezifische Formulierungen angepasst werden zu können. Einer dieser Tests, der *Tolerated Difference Test* (TDT), kann so angepasst werden, dass solche Unterschiede zwischen den Dissolutionsprofilen von verlängert freisetzenden Formulierungen identifiziert werden, die auf mangelnde Bioäquivalenz hinweisen.

Im Rahmen der vorliegenden Arbeit wird ein neuer Ansatz zur Entwicklung und Durchführung von Vergleichstests für Dissolutionsprofile beschrieben, die auf Prinzipien des statistischen experimentellen Designs basieren. Die Anwendungsmöglichkeiten werden anhand von vier Beispielen für verlängert freisetzende Formulierungen dargestellt.

3. Introduction

After a drug formulation proves to be safe and effective in human trials, it is crucial to demonstrate that later formulations, following changes during drug development, post-approval stages, or production by generic manufacturers, possess the same efficacy and safety profile of the original formulation [1-3]. Because human experiments to prove efficacy and safety are highly costly, time consuming and in some cases ethically questionable, the pharmaceutical industry is constantly searching for effective surrogates for judging therapeutic equivalence of pharmaceutically equivalent drug products. One widely accepted and official procedure to ensure efficacy and safety of new formulations is the assessment of Bioequivalence (BE), in which an absence of significant differences in the rate and extent to which the active ingredient become available at the site of drug action must be demonstrated [4-9]. However, under certain conditions *in vitro* testing has been accepted as a sufficiently reliable surrogate for an *in vivo* BE study [10, 11]. When *in vitro* testing is accepted as a surrogate, the therapeutic equivalence of drug formulations is assured by comparison of their dissolution profiles.

In this chapter, the theory of *in vitro* equivalence testing and *in vivo* BE is reviewed, as well as the conditions and procedures by which *in vitro* testing is considered as a reliable surrogate for an *in vivo* BE.

3.1. Bioequivalence and Biowaiving

Testing the bioequivalence between a product, called the Test formulation, and a suitable comparator, the Reference formulation, in a pharmacokinetic (PK) study with a limited number of subjects is one way of demonstrating equivalence without having to perform a clinical trial involving many patients [8].

Two products are considered bioequivalent when the rate and extent of absorption of the Test formulation do not show a significant difference from the rate and extent of absorption of the Reference formulation when administered at the same molar dose of the active pharmaceutical ingredient (API) under similar experimental conditions [12]. As a prerequisite both formulations must have the same pharmaceutical form and the same qualitative and quantitative composition in active substances.

In bioequivalence studies, plasma concentrations obtained after administration of the Test formulation are contrasted to those of a Reference formulation. Pharmacokinetics (PK) parameters as maximum plasma concentration (Cmax) and Area Under the Curve (AUC) from both formulation are compared through 90% confidence intervals (90% CI) around the ratio of the estimated geometric means between the contrasted formulation. Acceptance criteria used by most regulators are that the 90% CI of the Test/Reference geometric mean ratios for AUC and Cmax should fall within 80-125% limits [4, 6-8, 12].

Most regulatory entities recommend that a BE study enroll at least 12 healthy volunteers to ensure reliable estimates but some jurisdictions suggest 18 or 24. Differences among guidelines to establish BE vary from one jurisdiction to another not only in the recommended number of subjects but also in: 1) use of volunteers or patients, 2)

administration of food, 3) PK parameters to be compared, 4) measurement of parent drug or metabolite, and 5) strength to be investigated [12, 13].

When the excipients of a formulation do not affect the absorption of the API, the API is not a prodrug, does not have a narrow therapeutic index and is not intended to be absorbed in the oral cavity, *in vitro* testing has been accepted as a sufficiently reliable surrogate for an *in vivo* BE study [10, 11]. The regulatory acceptance of *in vitro* testing as a reliable surrogate for an *in vivo* BE is referred as “biowaiver” [10, 11].

Requirements for granting a biowaiver of an *in vivo* BE study depend on the class of drug, type of formulation, the type of post-approval change and the information available. Drugs are normally classified according to their solubility and intestinal permeability. In 1995, Amidon *et. al.* [14] introduced the biopharmaceutical classification system (BCS) which classifies drugs as follows.

- Class I : high solubility – high permeability
- Class II : low solubility – high permeability
- Class III : high solubility – low permeability
- Class IV: low solubility – low permeability

For immediate release (IR) solid oral dosage forms of BCS class I drugs demonstration of ≥ 85% dissolution in one or several media in 15 min is normally enough for conceding a biowaiving of BE studies in the case of post-approval changes of minimal impact [11]. Debate is still open as to whether biowaiving could be accepted for IR formulations of other BCS class drugs and guidelines differ in this point [10, 13]. Likewise, biowaiving of ER is not granted in all jurisdictions and in those where it is accepted a IVIVC model must be employed to justify the decision.

3.2. Drug Dissolution Tests

In vitro dissolution studies are commonly used in drug manufacturing to monitor process control, minor formulation changes and manufacturing site changes [15]. Originally, the dissolution test was used primarily as a formulation development tool and as a quality control test for determining that the API would dissolve *in vivo* [16]. Currently, dissolution is the only test that indicates if a dosage form will dissolve in the patient and is accepted as an indicator of the ability of the dosage form to release the API and enable it to become available at its site of action [17]. For that reason dissolution tests, at first exclusively used as quality control test, have been emerging as a surrogate equivalence tests for certain categories of orally administered pharmaceutical products [8].

The most commonly employed dissolution test methods are the basket method (USP Apparatus 1) and the paddle method (USP Apparatus 2) [18]. These methods are simple, robust, well standardized regarding volumes and agitation, and used worldwide [19]. Recently a flow-through cell system (USP apparatus 4) that aims to mimic sink conditions has also received much attention because of its flexibility for research and development. This dissolution technique has been proven to be reproducible and robust, which is an important characteristic for dissolution testing [20].

Dissolution testing should be carried out under physiological conditions which normally includes temperature of 37 ± 0.5°C and aqueous medium with pH range 1.2 to 6.8. The inclusion of biorelevant media is also possible and advances in developing more biorelevant dissolution methodologies have been continuously made [21-27], and have been identified as a major priority [28, 29].

3.3. Modeling Drug Dissolution

The quantitative analysis of the data obtained from dissolution tests is deeper when mathematical formulas that express the dissolution results as a function of some characteristics of the dosage forms are used. In some cases, these mathematic models are derived from the theoretical analysis of the release process. In most of the cases a mechanistic expression is not available and some empirical equations have proven to be suitable [30]. Drug dissolution from solid dosage forms has been described by kinetic models in which the accumulated mass dissolved is a function of time.

Dissolution data is most commonly (but not always) described by the following mathematical dissolution models:

- Zero order kinetics [31]:

$$\frac{Q_t}{Q_\infty} = k_o t \qquad \text{equation 1}$$

Where Q_t is the accumulated mass is dissolved at time t and Q_∞ is the mass dissolved at infinite time, and k_0 is the zero order release constant. According to this model the drug is released is at a constant rate.

- Firs order kinetics [32]:

$$\frac{Q_t}{Q_\infty} = e^{-kt} \qquad \text{equation 2}$$

In which the rate of drug release is proportional to the remaining (not released) quantity of drug in the formulation. And k is the proportional constant.

- Higuchi Model [33] :

$$\frac{Q_t}{Q_\infty} = \mathrm{b}t^{\frac{1}{2}} \qquad \text{equation 3}$$

Where b is the Higuchi dissolution constant. This expression attempts to describe a nonlinear release kinetics with higher release rates at the beginning of the dissolution test.

- Korsmeyer model [34]:

$$\frac{Q_t}{Q_\infty} = \mathrm{k}t^n \qquad \text{equation 4}$$

This model is a semi-empirical expression in which k is a constant incorporating structural and geometric characteristics of the drug dosage form, n is the release exponent, indicative of the drug release mechanism, and the release rate is proportional to the remaining drug in the formulation. Values of $n = 0.5$ indicate Fickian release, values of $0.5 < n < 1.0$ indicate an anomalous (non-Fickian or coupled diffusion/relaxation) drug release, and values of $n = 1.0$ indicate a case II (relaxation-erosion controlled) drug release.

- Peppas model (equation 5) [35]

$$\frac{Q_t}{Q_\infty} = k_d t^m + k_r t^{2m} \qquad \text{equation 5}$$

In which k_d is the diffusional constant, k_r is the relaxation constant and m is the diffusional exponent, the latter depends on the geometrical shape of the releasing device through its aspect.

- Weibull model [36, 37] [38]:

$$\frac{Q_t}{Q_\infty} = \left(1 - e^{-(k_d t)^\beta}\right) \qquad \text{equation 6}$$

Where , k_d and β is a shape parameter that can also indicate the release mechanism [38].

- Hill model [39]:

$$\frac{Q_t}{Q_\infty} = \frac{\mathrm{t}^\mathrm{n}}{\mathrm{t}_{50}^\mathrm{n} + \mathrm{t}^\mathrm{n}} \qquad \text{equation 7}$$

In which t_{50} is the time at which 50% of the drug is released from the formulation and n is a shape parameter. Another mechanistic expressions such as Noyes-Whitney, Nernst-Brunner, and Hixson-Crowell has been widely used to describe drug dissolution of solids but have very limited applicability in describing release from complete dosage forms [40].

3.4. Dissolution Profile Comparisons

When the biowaiving of drug formulations is possible, the therapeutic equivalence of drug formulations is assured by *in vitro* comparison of dissolution profiles. The term similarity has been employed to describe the lack of difference between dissolution profiles from two different sources (formulations) and it is normally established by using a similarity factor [2, 19, 41, 42] presented in 1996 by Moore and Flanner [41] (equation 8) to compare dissolution curves. They introduced this method as especially recommended for use in stability studies and optimization during product development and scale-up. Since f2 has been proposed, several publications have explored the advantages and disadvantages of f2 [30, 43, 44] and some modifications, such as the

constructions of confidence intervals have been proposed [45, 46]. Presently, f2 is employed and recommended by regulatory authorities for scale-up and post-approval changes; however, the level of confidence of the method is uncertain and several publications have shown it to have low statistical power [30, 43].

$$f_2 = 50\ x\ Log\left\{\left[1+\frac{1}{n}\sum_{t=1}^{n} w_t(R_t - T_t)^2\right]^{-0.5} x\ 100\right\} \qquad \text{equation 8}$$

In equation 8, R_t is the mean of the dissolved drug from the Reference batch at time t, T_t is the mean of the drug dissolved from the Test batch at time t, n in the number of time points and w_t is a weight factor that can be used to enhance the influence of particular time points. If the calculation yields f2 ≥ 50, similarity of R and T is declared.

FDA guidelines [19] recommend testing 12 tablets of each batch. Theoretically, if the difference in drug dissolution between R and T is exactly 10% at every time point, the value of f2 is 50; if the differences are >10% (at every time point), f2 becomes smaller than 50, and if the differences are smaller (<10%) f2 becomes larger than 50. However, values of f2 above 50 can be obtained with differences greater than 10% at some particular time points if the differences at the other points are small enough to compensate for the larger differences; thus, the basis for choosing a value of 50 as the rejection criterion is questionable. To alleviate this problem, Moore and Flanner included the weight factor w_t in the expression; however, there is no clarity on how to employ the weight factor and it can highly favor (intentionally or unintentionally) either similarity or non-similarity. The FDA guidelines [19] also mention the weight factor but allow the researcher to decide whether to use it or not. Another disadvantage of using f2 is that the arithmetic mean is very susceptible to extreme values and this may result in large differences between individual tablets being ignored.

Apart from f2, several methodologies for comparing dissolution profiles have been described [30, 47]:

Adaptations of single value comparisons of level B parameters (area under curve, mean dissolution time, time to reach 85% of dissolution etc.) have not been well accepted because it is often not possible to properly include the information for every time point in such comparisons.

Multivariate analysis [48, 49], requires assumptions that are difficult to fulfill. Moreover, it is questionable whether comparison of dissolution profiles should be consider as multivariate problem, because the same variable is measured repeatedly over time.

Model-dependent methods have also been used, but these rely highly on fitting to a specific dissolution model, and in some cases such a model is not available. Moreover, model-dependent methods are still bound to multivariate distances with the aforementioned problems [50]. Factors as f2 which are easy to be implemented have been widely employed, but normally lack scientific justification [30] or statistical support.

3.5. *In vitro-in Vivo* Correlation Models

ER formulation are dosage forms usually designed to achieve safer and more constant *in vivo* concentrations of the administered drug or to decrease the administration frequency to improve compliance in the patient [29].

Currently, biowaiver of ER drug formulations can only be granted when an IVIVC model is available. These IVIVC models are defined as a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form and a relevant *in vivo* response [2]. The term correlation in IVIVC is due to the fact that in some cases

the strength of the relationship between parameters derived from the *in vitro* and *in vivo* studies are quantified by the Pearson correlation [15].

An acceptable IVIVC requires that the *in vitro* dissolution and *in vivo* release or dissolution behavior of a dosage form should be either similar or have a scalable relationship to each other. IVIVC could only be established when the factor controlling the appearance of the drug in the blood flow is linked with the formulation (slow release) and not with any physiological limiting factor (for example permeability) [51].

IVIVC have been divided into three groups:

Level A: The entire *in vivo* time course (normally plasma concentrations) is predicted from the *in vitro* data.

Level B: Correlation between a statistical moment of the *in vitro* dissolution (mean dissolution time MDT) and a statistical moment of the *in vivo* plasma levels (mean residence time).

Level C: Single point relationship between a dissolution parameter and a PK parameter.

Traditionally IVIVC models have been performed through the use of convolution and deconvolution processes to find mathematical functions able to connect the *in vitro* and *in vivo* mathematical functions. However, these methodologies are not based on biopharmaceutical principles or mechanistic expressions that allow a more detailed description of the in vivo situation. Recently some mechanistic IVIVC models have been introduced to overcome this weakness [52, 53].

3.6. Permeability and d/p-systems

Apart from the release kinetics the bioavailability of an orally administered drug is also determined by the intestinal permeability. However, these two properties are normally evaluated separately *in vitro* which restricts the possibility of studying the effects of drug formulations on absorption. Therefore, a tool to simultaneously study *in vitro* dissolution and permeability can be of great utility during the drug formulation development process to study the effect of drug release and excipients on oral absorption in an easy and inexpensive way, which ultimately can lead to an optimization of the drug formulation.

Several approaches have been described to study dissolution and absorption simultaneously in the same experimental apparatus [54-59], however, the only set-up that allows the evaluation of complete solid oral dosage forms in an open system using dynamic flow conditions is the one developed by Motz et al [60]. Moreover, this combined dissolution and permeation system (d/p system) has been improved to allow continuous measurements of the drug concentrations in the different compartments of the device [61] and continuous monitoring of the Caco-2 cells monolayer integrity by measuring the transepithelial electrical resistance (TEER) [62]. The d/p-system has also been recently adjusted to perform experiments with artificial membranes [63].

3.7. Aim of this Work

The current official procedure to compare dissolution profiles, the f2 similarity factor, lacks solid statistical foundation. The level of statistical uncertainty when this methodology is employed is therefore unknown. Additionally, the limits to declare *in vitro* similarity with the current methodology ($f2 \geq 50$) are arbitrary, and as they are not bound to any biopharmaceutical property, they cannot be considered as a good predictor of the *in vivo* performance of formulations, especially when ER formulations are considered. On the other hand, two major characteristics are needed in statistical tests for performing dissolution profile comparison: high statistical power and flexibility to perform in a variety of scenarios. Because these two properties are not likely to be fulfilled by the same test, two separate tests, each with one of the aforementioned properties, were designed in this thesis as a new methodology to compare dissolution profiles. The intention in this respect was to design, develop and explore new dissolution profile comparison tests (DPC-tests) with more statistical basis that the current methodologies (f2 similarity factor), and to link the limits of rejection of these DPC-tests with significant differences in relevant biopharmaceutical properties, so as to achieve a greater predictive power of the *in vivo* performance of formulations. In more detail, the major aims of this thesis were:

- To design, present and explore the statistical robustness and statistical power of two new tests based on nonparametric permutation test theory. The first test, called permutation test (PT), was designed to detect small differences in dissolution profiles and very stringent to confer similarity. The second test, called

tolerated difference test (TDT), designed with the required flexibility to be customized according to the requirements of any particular case.

- To develop drug-specific DPC-test for three ER formulations (metformin, diltiazem and pramipexole) using IVIVC models, computer simulated BE trials and permutation tests. It was intended that these customized tests should be able to detect, at a known level of certainty, differences in release profiles between ER formulations that represent a lack of BE.
- To investigate the effect of DPC-test conditions, BE-trial conditions, and drug/formulation properties in the determination of biorelevant limits of the DPC-test.
- To apply the concept of DPC-test customization in a combined dissolution permeability system (d/p-system) in order to identify as *in vitro* similar, only formulations that would not differ significantly in the permeated amount achieved in the permeability module of that system.

4. Two New, Nonparametric Test for Statistical Comparison of Dissolution Profiles

Parts of this Chapter were published in:

Gomez-Mantilla JD, Casabo VG, Schaefer UF, Lehr CM. Permutation Test (PT) and Tolerated Difference Test (TDT): Two new, robust and powerful nonparametric tests for statistical comparison of dissolution profiles. Int J Pharm. 2013;441(1-2):458-67.

The author of the thesis made the following contribution to the publication:

- Design and construction of the DCP-tests
- Design, Performance and interpretation of simulations.
- Writing the Manuscript.

4.1. Abstract

The most popular way of comparing oral solid forms of drug formulations from different batches or manufacturers is through dissolution profile comparison. Usually, a similarity factor known as (f2) is employed; However, the level of confidence associated with this method is uncertain and its statistical power is low. In addition, f2 lacks the flexibility needed to perform in special scenarios. In this study two new statistical DPC-tests based on nonparametrical permutation test theory are described, the permutation test (PT), which is very restrictive to confer similarity, and the tolerated difference test (TDT), which has flexible restrictedness to confer similarity, are described and compared to f2. The statistical power and robustness of the tests were analyzed by simulation using the Higuchi, Korsmeyer, Peppas and Weibull dissolution models. Several batches of oral solid forms were simulated while varying the velocity of dissolution (from 30 mins to 300 mins to dissolve 85% of the total content) and the variability within each batch (CV 2% to 30%). For levels of variability below 10% the new tests exhibited better statistical power than f2 and equal or better robustness than f2. TDT can also be modified to distinguish different levels of similarity and can be employed to obtain customized comparisons for specific drugs. In conclusion, two new methods, more versatile and with a stronger statistical basis than f2, are described and proposed as viable alternatives to that method. Additionally, an optimized time sampling strategy and an experimental design-driven strategy for performing dissolution profile comparisons are described.

4.2. Introduction

Comparing time profiles for dissolution data or for any other type of data is a complex statistical challenge. The highly correlated nature of this type of data, which exists in spite of its mostly unknown mechanisms, the many types of curves observed in dissolution profiles, the high variability combined with the finite nature of the variable (≤100%), and the fact that two curves may cross producing both positive and negative differences, make it difficult to determine whether two curves should be regarded as similar or different, and therefore represent a major barrier to an adequate solution to this problem [30, 41, 64]. When a variable is measured over time and compared under two or more conditions, a simple and commonly used technique is to compare the value of the variable at one or two particular time points and to test hypotheses about differences in the variable between the different conditions at these precise time points [65]. Although this approach is adequate in a broad variety of situations, it fails, when the major interest lies in the kinetic of the process, as when drug dissolution profiles are compared.

Drug dissolution assays of oral solid dosage forms are designed to predict the performance of these formulations in the gastro-intestinal tract (GIT) and ultimately provide information about the bioavailability of oral formulations. The information obtained at each time point can be crucial because the absorption of drugs varies across the GIT due to the different membrane properties of the mucosal cells, the local microclimate and, the presence or absence of transporters, enzymes and other substances ([66], [67], [68] and [69]). Because of this, comparisons using data obtained at only one or two time points are insufficient. Comparisons of areas under the curve

are also inadequate because two curves can have very similar areas under the curve but present important differences at single time points, especially if the two profiles cross [41]. To date, there is no satisfactory statistical tool, either for dissolution profiles or for other types of data that completely solves this particular problem.

In 1996, Moore and Flanner [41] described the use of an expression that they called f2 (equation 8) to compare dissolution curves. Since f2 has been proposed, several publications have explored the advantages and disadvantages of f2 [30, 43, 44] and some modifications, such as the constructions of confidence intervals have been proposed [45, 46]. Presently , f2 is employed and recommended by regulatory authorities for scale-up and post-approval changes; in addition it can be used to waive clinical bioequivalence studies (at least under certain conditions) for immediate release and modified release solid formulations [19],[2, 4]. However, the level of confidence of the method is uncertain and several publications have shown it to have low statistical power [30], [43].

Apart from f2, several methodologies for comparing dissolution profiles have been described [30] [47] like adaptations of single value comparisons of level B parameters, (area under curve, mean dissolution time, time to reach 85% of dissolution etc.), or multivariate analysis [48, 49], and model-dependent methods. However these methodologies have not been accepted by the industry because of its statistical and conceptual limitations (section 3.4). Factors as f2 which are easy to implement has been widely employed, but normally lack scientific justification [30] or statistical support.

Most available statistical tests are designed to detect differences, rather than to prove similarities, and a lack of difference does not necessarily imply similarity. However,

demonstration of a lack of difference with quantified and adequate type-I and type-II errors would provide a more solid statistically method for detecting similarities than a method based on subjective limits.

The power of a statistical test is the probability that the test will reject the null hypothesis (in this case similarity) when the null hypothesis is false (i.e. the probability of not committing a type-II error, or making a false negative decision). With the help of dissolution models (equations 1-7), scenarios when the null hypothesis is false can be generated (differences in the value of one or more parameters), and the power of the tests can be evaluated. The more powerful a test is, the smaller difference it can detect in the value of model parameters.

A more powerful DPC-test (able to detect small differences between two profiles) would be very valuable for comparing the dissolution profiles of formulations containing drugs with very narrow therapeutic windows and/or drugs classed as II, III and IV in the Biopharmaceutical Classification for which *in vivo* bioequivalence can require a more strict, almost identical *in vitro* similarity [14]. It can also be postulated that for a transporter substrate (active transport or efflux) of a transporter present in enterocytes, the effective concentration in the intestinal lumen may play a decisive role in determining the bioavailability of the compound. Very powerful statistical tests are needed, indeed, to detect small differences in dissolution profiles to assure similarity of two products from different manufacturers or from the same manufacturer after a major or minor change in production technology. In a large number of cases, the bioavailability of two different drug products with the same active molecule will be very similar if their dissolution profiles, evaluated under the relevant conditions [21], are very similar.

On the other hand, for some compounds, large differences in dissolution profiles are necessary to produce significant differences in bioavailability, and a test less strict than f2 is also needed[70] [44]. In general, a flexible DPC-test that offers variable power according to specific needs, but still retains adequate levels of robustness and statistical uncertainty, is highly desirable.

Aware of the expectations that dissolution and drug release will play an even wider role in regulating quality generic drug products in the future [71], two major characteristic are needed in statistical tests for dissolution profile comparison: High statistical power and flexibility, as these two properties are not likely to be fulfilled by the same test, two separate tests, each with one of the mentioned properties may be an adequate solution.

In this study two new statistical DPC-tests based on nonparametric permutation test theory are presented, and their ability to satisfy the above mentioned requirements (more restrictiveness and more flexibility) is assessed. The first, called permutation test (PT), is capable of detecting small differences in dissolution profiles and is very exigent to confer similarity. The second one, called tolerated difference test (TDT), the level of exigency to confer similarity can be modified to detect large or small differences according to the requirements of any particular case. Both tests were explored in terms of statistical robustness and power and were compared to f2 and bootstrap 95% Confidence Intervals of f2 (C.I.) [45, 46].

4.3. Methods

4.3.1. Dissolution Models

Dissolution data were simulated following five different mathematical dissolution models. i.e., the Higuchi model (equation 3) [33], the Korsmeyer model (equation 4) [34], the Peppas model (equation 5) [35] , the Weibull model (equation 6) [36, 37] [38] and the Hill model equation 7 [39].

4.3.2. Data Simulation

4.3.2.1. Reference and Test Formulations

Reference formulations were modeled as follows:

Higuchi Model:	$\mathrm{b} = 9\ min^{1/2}$			$t_{85} \approx 90\ min$
Korsmeyer Model:	$\mathrm{k} = 7\ min^{-n}$;	$\mathrm{n} = 0.5$		$t_{85} \approx 50\ min$
Peppas Model:	$k_d = 4.4\ min^{-2m}$;	$k_r = 0.6\ min^{-m}$	$\mathrm{m} = 0.45$;	$t_{85} \approx 30\ min$
Weibull Model:	$k_d = 0.03\ h^{-1}$;	$\beta = 0.75$;		$t_{85} \approx 50\ min$
Hilll Model:	$t_{50} = 1.605\ \mathrm{h}$;	$\mathrm{n} = 1.85$;		$t_{85} \approx 40\ min$

4.3.2.2. Intrinsic and Residual Variability

Test formulations were modeled by varying the models parameters around those of the Reference formulations to obtain a wide range of dissolution profiles (85% of labeled drug dissolved in 30 to 300 min). For each individual tablet, intrinsic (parameters of the model) and residual variability (experimental error), variability was included. Intrinsic variability was included for all parameters.

$$\theta_i = \theta_b \cdot e^{\eta}$$ equation 9

Where θ_i is the parameter for the i-th tablet, θ_b is the parameter of the batch and η is the intra-batch variability with mean value zero and variance ω^2 ($\eta \sim N(0, \omega^2)$)

The residual variability (experimental) was described by:

$$y_{ij} = y_{ijp} \cdot e^{\varepsilon}$$ equation 10

Where y_{ij} is the simulated dissolved amount (%) of drug from the i-th tablet at the j-th sample time, y_{ijp} is the predicted dissolved amount (%) of drug from tablet i at the j-th sample time and ε is the residual (experimental) variability with mean value zero and variance σ^2 ($\varepsilon \sim N(0, \sigma^2)$). In summary, for each single tablet one or more individual parameter are calculated according to equation 9, the number of parameters depends on the model and, ranges from one parameter in the Higuchi model to three parameters in the Peppas Model. Finally, a predicted value is calculated for each time point according to the models employed (equations 3-7, section 3.3) and the residual variability is also incorporated according to equation 10.

According to equation 9equation 10), the variability within the batches is due to the values of ω^2 and σ^2 employed in each simulation. For every model (equation 3equation 7) several combinations of ω^2 and σ^2 at different T_{85} were studied to address the effects of these values on variability. For each combination of T_{85}, ω^2 and σ^2, 10.000 batches were simulated and the CV at each time point for every batch was analyzed. The 95% percentile of all the measured CV's was recorded as CV_{95} as a measure of global variability.

As can be observed in Figure 4.1, no significant differences in CV_{95} were found for batches with different T_{85} for data following the Higuchi model at different T_{85}, the same

effect was observed for all the models; however, visualization is not as simple because more than one ω^2 is present in each case.

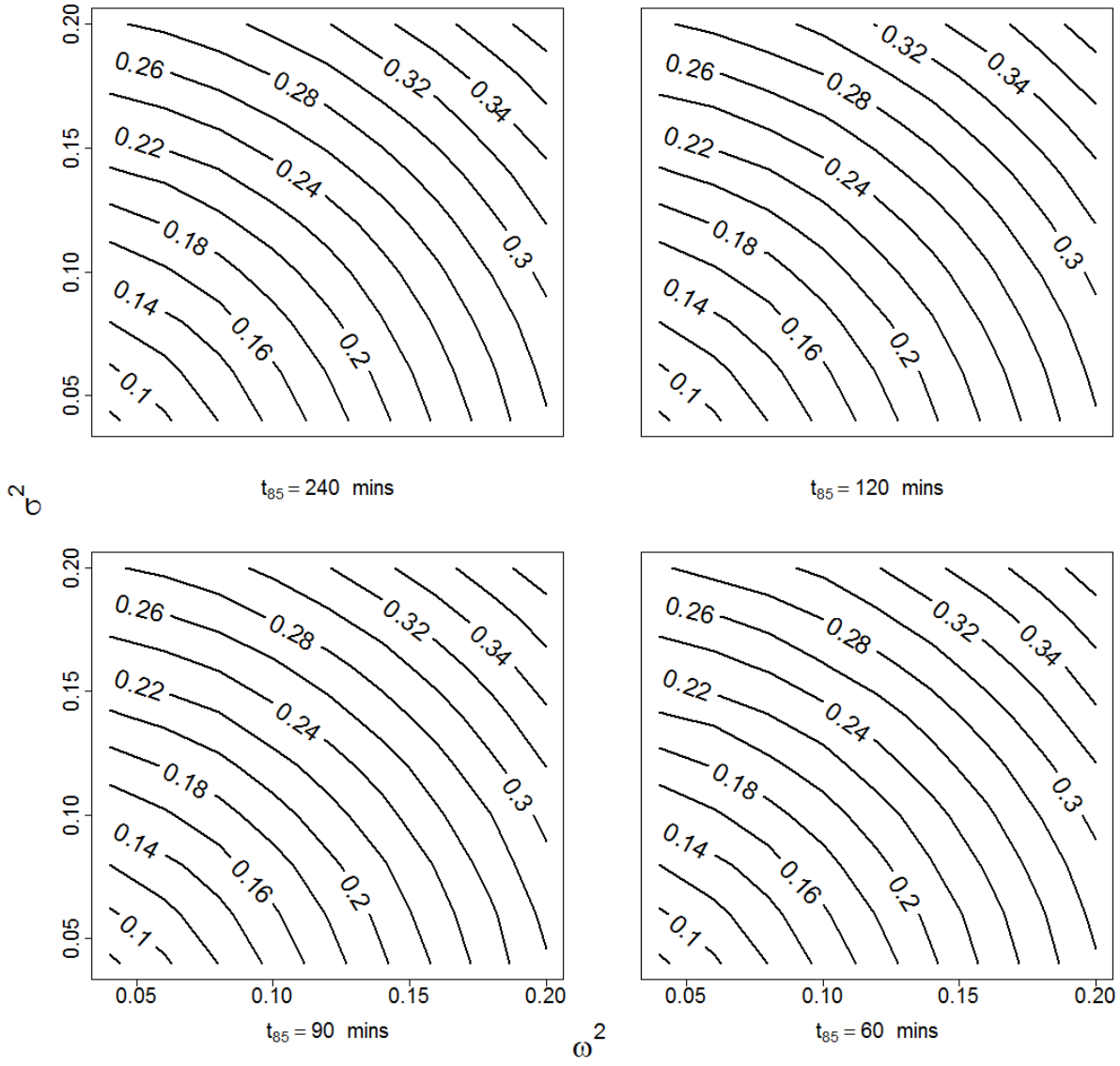


Figure 4.1. Intrinsic and residual variability of the simulated batches (Higuchi Model). Setting up of intrinsic and residual variability in the simulated tablets batches according to equation 9-10) Contour Plot of cv for different combinations of $\boldsymbol{\omega^2}$ and $\boldsymbol{\sigma^2}$. The result were almost identical for batches of tablets with different t_{85} under Higuchi model.

4.3.2.3. Sampling Time Points

For every condition, a set of values for 12 tablets was generated. Time points were established according to the following scheme:

$t_{85} \leq 40$ minutes	sampling every 5 minutes
$40 < t_{85} < 60$ minutes	sampling every 10 minutes
$60 \leq t_{85} < 90$ minutes	sampling every 15 minutes
$90 \leq t_{85} < 150$ minutes	sampling every 20 minutes
$t_{85} \geq 150$ minutes	sampling every 30 minutes

where t_{85} = time at which 85% of labeled drug is dissolved. According to the current guidelines [19] only one time point with average dissolved drug higher to 85% is considered.

4.3.3. Dissolution Profile Comparison Tests

In a typical dissolution profile comparison (Reference vs. Test) two Matrices (Reference and Test) of data points; R (m × n) and T(m × n) are evaluated, being m the number of tablets (normally 12) and n the number of time points sampled. The data for every tablet are expressed as a vector of length n which length is defined by the number of time points sampled in the dissolution profile.

4.3.3.1. f2 Similarity Factor

f2 similarity factor was calculated according to equation 8. If the calculation yielded f2 ≥ 50 similarity of R and T was declared.

4.3.3.2. f2 Bootstrap Confidence Interval (f2 CI)

Bootstrap 95% Confidence Intervals (CI) of f2 were calculated similar to ones described in the literature [45] [46]. Initial simulations were performed to establish the number of repetitions to be used. As shown in Figure 4.2, 5000 repetitions produced acceptable estimations and allowable computation time.

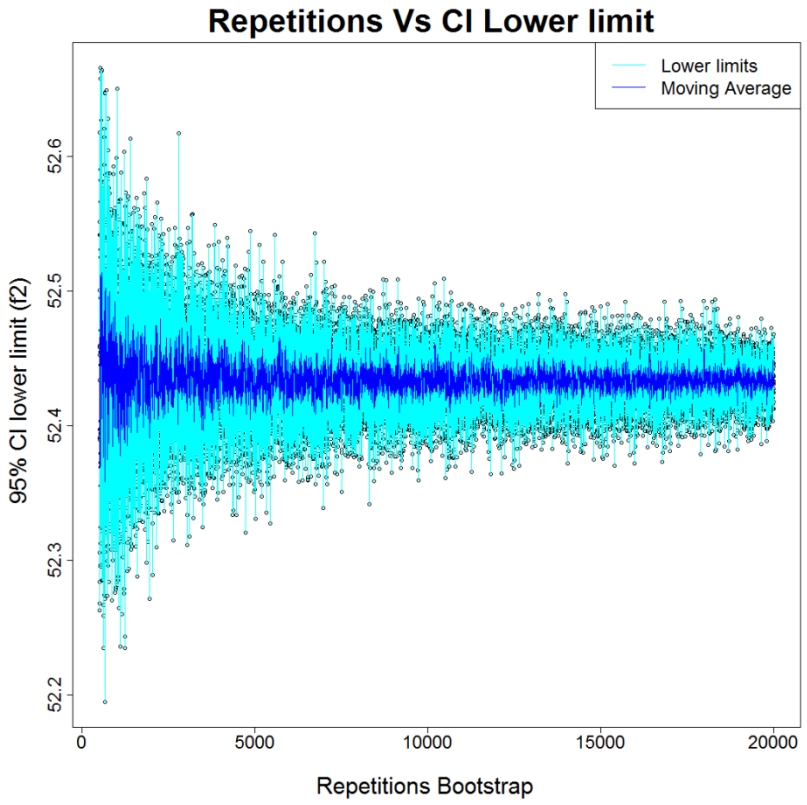


Figure 4.2. Simulations for determining the number of bootstrap repetitions to employ. The variance of the estimator is reduced increasing the number of repetitions. For a very large number of repetitions the bootstrapping estimates will converge to one value of CI-lower limit. It was observed that after 5000 repetitions, any estimation is not farther than 0.5 units from the converged value at large repetitions (200.000). To evaluate the impact of this difference the number of rejections for CI-lower limit < 50 and for CI-lower limit < 49 were recorded in all the experiments and compared. There was no significant change (less than 1%) in robustness or power by this modification.

4.3.4. Two New Nonparametric Tests for Statistical Comparison of Dissolution Profiles

4.3.4.1. Permutation Test

Figure 4.3 illustrates the procedure for the permutation test (PT). In this procedure, the mathematical distance D_0, a square difference between means at every time point is used. This value is stored as the Original Distance or D_0. Data from every tablet can be represented as a vector of length n, a set of 2 × m vectors now representing the data of the Reference and Test batches. The first subset of m vectors represents the Reference batch and the last subset of m vectors represents the Test batch. After D_0 is calculated, the vectors are randomly sampled without replacement. In this way, each vector is relocated randomly in new Reference or Test subsets, creating two new (m x n) matrices R_i and T_i. The same distance D between R_i and T_i is calculated and the value is stored as D_i. This cycle is repeated 5000 times and an empirical distribution of the D_i values (5000 values of D_i) is built. According to a predetermined type I error (typically, alpha = 0.05), a rejection value that is greater than the 1-alpha percent of all Di values in this empirical distribution is calculated. If the profiles are similar, D_0 is expected to be bellow this rejection value; D_0 above the rejection value indicates lack of similarity between the two profiles.

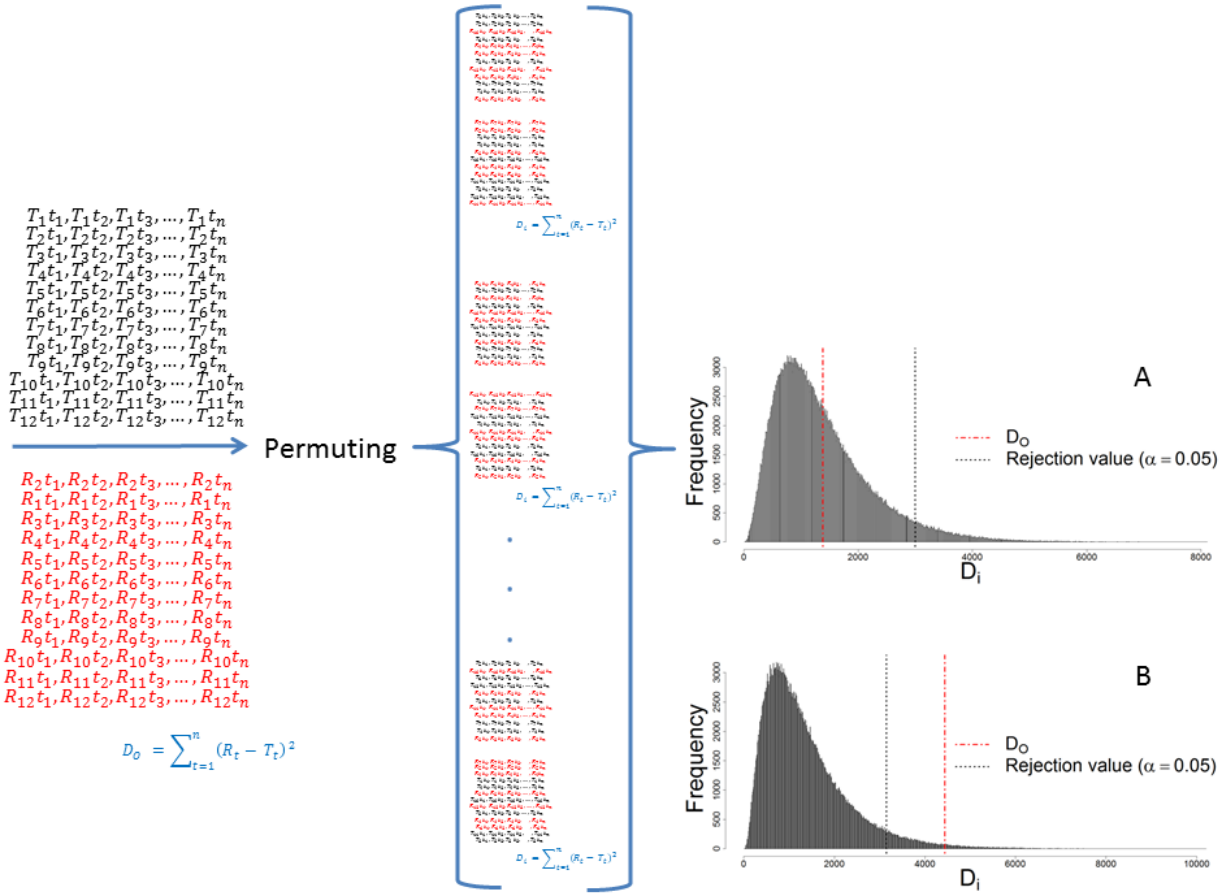


Figure 4.3. Methodology of PT. Illustrative representation of PT methodology, In this case the number of tablets m = 12, first D0, is calculated between the two profiles. Then each vector is randomly located in new reference or test subsets creating two new (m × n) matrices Ri and Ti . The same distance D is calculated between Ri and Ti and the value is stored as Di . This cycle is repeated 5000 times or more (500,000 in this example) and an empirical Distribution of the Di values (all the 500,000 values of Di) is built. According to an established type I error (alpha = 0.05), a rejection value is indicated in this empirical distribution. A and B show distribution of Di for similar and not similar profiles respectively, because it is an empirical distribution the shape is similar but not identical, it can be observed than in not similar profiles D0 is bigger than the rejection value and therefore similarity hypothesis is rejected.

4.3.4.2. Tolerated Difference Test

This test is based on a tolerated difference (δ) in dissolution between two tablets at each time point. Following the concept that there is some difference in percentage of dissolved drug that can be tolerated, this test attempts to statistically prove whether the differences between the Reference and Test samples exceed the predetermined tolerated difference or not.

Having at any time point the dissolved drug for m tablets from the Reference and m from the Test

$R_{t1}, R_{t2}, R_{t3}, \ldots, R_{tm}$; R_{ti}= dissolved drug of i-th tablet from the Reference at time t

$T_{t1}, T_{t2}, T_{t3}, \ldots, T_{tm,}$; T_{ti}= dissolved drug of i-th tablet from the Test at time t

differences between all R_{ti} and T_{ti} are evaluated, and the number of events for which this difference is greater than the established tolerated difference (δ) is counted.

For a single time point, under the null hypothesis $\mu_{Tt} - \mu_{Ft} \leq \delta$ the random variable D_d, the number of events in which difference is greater than δ, has a discrete distribution easy to calculate. For several time points the same procedure is followed but the statistic D_d is expressed as:

$$D_d = \frac{1}{n}\sum_{i=1}^{n} D_i \qquad \text{equation 11}$$

Where D_i = the sum of differences greater than δ at the i-th time point. In this work, values of δ = 5 (TDT-1) and δ = 10 (TDT-2) were analyzed. The distributions of D_d for m = 12 and n = (1,2,3, . . . ,12) are shown in Figure 4.4.

For m = 12 or less the exact discrete distribution of D_d can be calculated without difficulty for values of n ≤ 5. For any increment in the value of n the computational time

required to calculate the distribution of D_d is m^2 times longer, and not easily shortened by using parallel computing. In this work we calculated for m =12 the exact distribution of D_d for values of n = (1, 2, 3, 4 and 5). Distributions for higher values of n were built by simulation with 100.000.000 repetitions. This value was sufficient to produce a distribution that differed less than 10e-5% from the exact distribution for the case of n = 5. (Table of rejection values for TDT is in A.1)

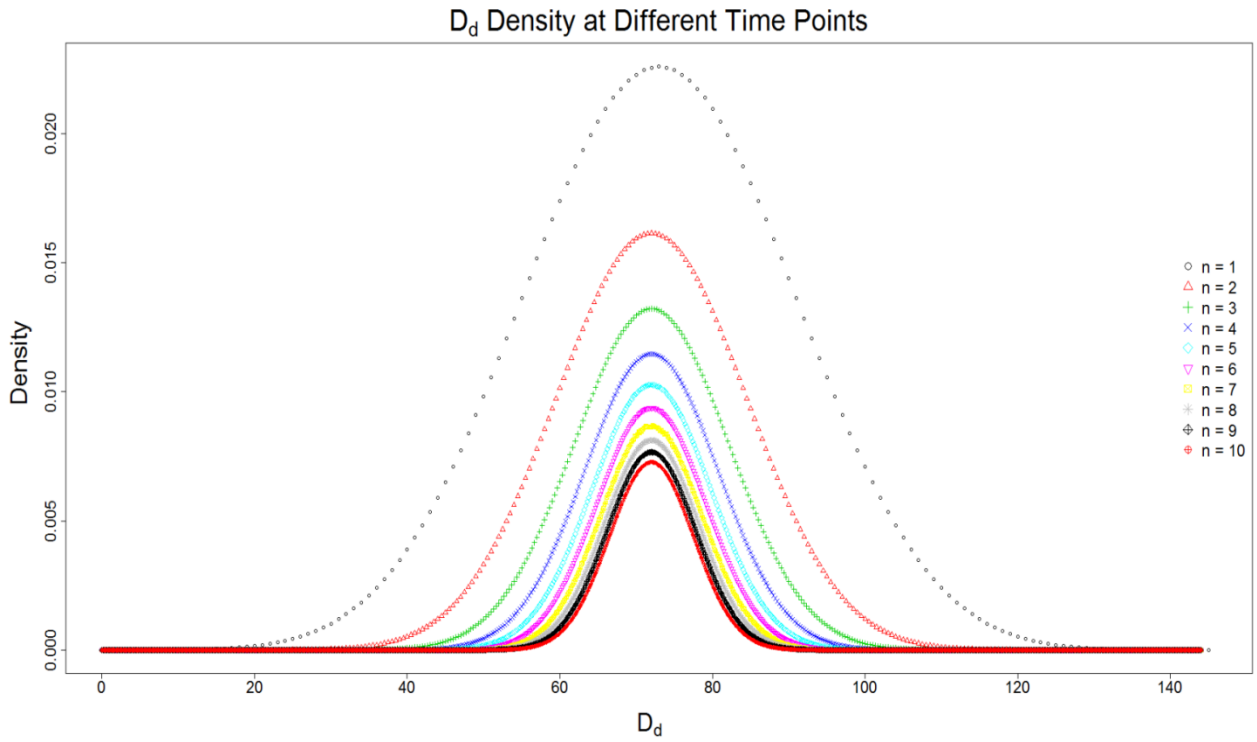


Figure 4.4. Discrete distribution of Dd for different values of n for 12 tablets. n represents the number of time points sampled in the comparison, the figure presents the probability of all the 144/n values, increasing n produces more leptokurtic shapes and narrower rejection values. For example for alpha = 0.05, rejection values are 101 for n = 1, 86.25 for n = 4 and 81.0 for n = 10. Proximity of points must not be confused with continuous distribution.

4.3.5. Robustness Explorations

Under conditions of similarity, in which the Reference and the Test formulations have equal parameters values in the dissolution models employed. Pairs of Reference-Test batches were generated at different levels of variation. Every pair of batches was compared using the four procedures described (f2, CI, TDT and PT). At every level of variation 5000 pairs of batches were generated and the percentage of rejections (no similarity) was evaluated for each method. Ideally, under conditions that satisfy the null hypothesis (in this case, similarity), a robust statistical test does not increase the level of rejections at increasing levels of variation. In the best case, the level of rejections should be constant and very similar to the set type I error of the test (normally 5%) in order to quantify uncertainty. Variation in the models were generated including intrinsic and residual variability, the 95% percentile of all the measured CV's at all-time points was recorded as CV_{95} as a measure of global variability. In preliminary experiments, stable (no difference with increment in repetitions) values of percentage of rejections were found at 2000 repetitions, internal validation with sets of 2000 from the 5000 repetitions were also made and there was no difference in the results.

4.3.6. Power Explorations

Under conditions of non-similarity (different parameters values in the model employed) pairs of Reference-Test batches were generated at different levels of variation. As in the Robustness analysis, each pair of batches was compared using the four procedures described (f2, CI, TDT and PT). Differences in parameters were designed to produce values of t85 ranging from 60 to 300 minutes. For Korsmeyer, Peppas and Weibull

models in which more than one parameter describes the kinetic of the process, differences in single parameters (keeping the others constants) and bidirectional differences (varying two parameters simultaneously) were explored. At every condition, 5000 pairs of batches were generated and the percentage of rejections (%detections of no similarity) was evaluated for each method. More powerful tests are expected to detect smaller differences in the parameters used. As for the robustness experiments, stable values of percentage of rejections for both robustness and power were found at 2000 repetitions in preliminary experiments.

4.3.7. Effect of Statistical Independence and Sample Size

It must be considered that equation 11 is completely operative only if all D_i are independent and identically distributed (iid) which may be not the case in a typical dissolution profile, because only one determination is allowed for each tablet and for each time point, this implies that for a typical case of 12 observations at 5 different time points, 60 individual tablets must be evaluated under independent conditions (iid-conditions). Although this may seem fatiguing, it could be well worth the effort in order to reduce or completely avoid *in vivo* studies. Therefore, all explorations were made under normal conditions (typical dissolution profile with questionable independence) as well as under independent conditions (iid-conditions). The options of using only 6 and 3 observations per time point (options requiring 30 or 15 tablets, respectively, for 5 time points), were also evaluated. To simulate iid-conditions, a new tablet (with the same parameters and intrinsic and residual variability) was generated to estimate the dissolved drug at each time point; n × m tablets are needed and each tablet was

evaluated just one time. Results were obtained under both conditions for all the comparison tests to evaluate the influence of independence in the comparisons. Additionally, sample size of n = 6 and n = 3 tablets were generated under iid-conditions to evaluate the influence of sample size in the comparisons.

4.3.8. Software

All the analyses, simulations and statistical tests were performed using the R software environment for statistical computing and graphics (version 2.14.2. R Development core Team 2013).

4.4. Results and Discussion

4.4.1. Statistical Robustness

All presented tests showed good robustness for standard ($CV_{95} \leq 0.1$) conditions. Figure 4.5 shows that the percentage of rejections remains under 0.05 for values of $CV_{95} \leq 0.12$ and sample size n = 12 tablets for all the tests and models. The robustness of the tests was always in the same order (from less robust to more robust) i.e., TDT<CI<f2<PT.

4.4.2. Effect of Statistical Independence and Sample Size on Robustness

Sample size and iid-conditions did not affect significantly the robustness of the tests, in all cases the rejection levels remained within the desired limits (≤0.05) for values of $CV_{95} \leq 0.1$ as shown for the Korsmeyer model in Figure 4.6 The summary of the effect of statistical independence and sample size for all models is display in Table 4.1

Robustness of f2 in not affected by iid-conditions but evidently reduced with smaller sample sizes. Robustness of CI is evidently increased under iid-conditions, and evidently reduced with smaller sample sizes.

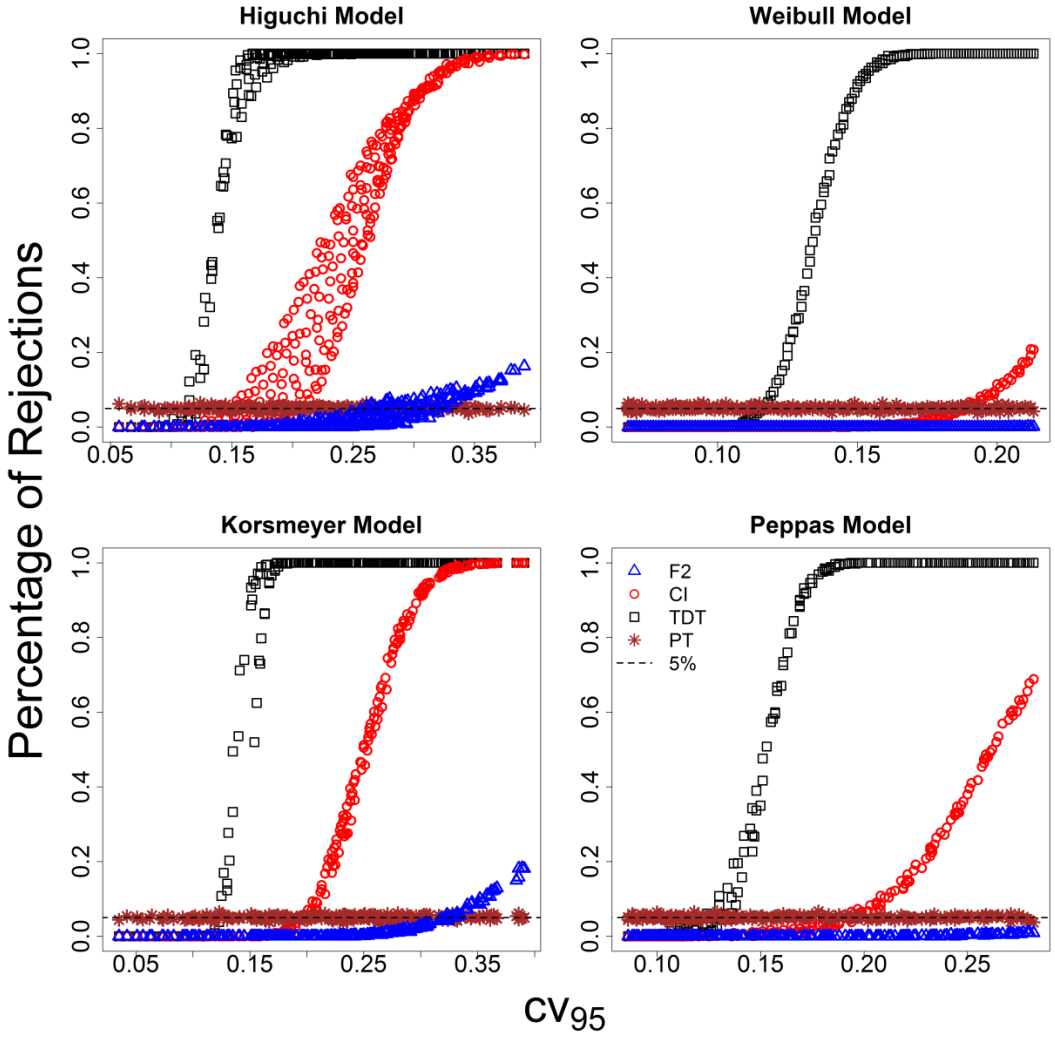


Figure 4.5. Robustness comparison of the presented tests under different dissolution models. In each model, pairs of similar batches were generated (batches with the same parameter values in equations 2-5) and the percentage of rejections is measured at different levels of variation (CV_{95}). Dotted line at 5% indicates the ideal percentage of rejections. All of the tests have acceptable levels of Rejections for values of cv ≤ 0.12. f2, and PT show ideal levels of rejection for values of cv ≤ 0.3 in all the models.

Robustness of TDT is slightly increased under iid-conditions and evidently increased with smaller sample sizes. Robustness of PT Is not affected by iid-conditions or smaller sample size except for n ≤ 3 where the total number of possible permutations does not allow useful comparisons.

For conditions of high variability (CV_{95} ≥ 0.2) just f2 and PT showed acceptable levels of rejections. PT was the only test in which the level of rejections remained under 5% for CV_{95} ≥ 0.3 in all the models and conditions studied.

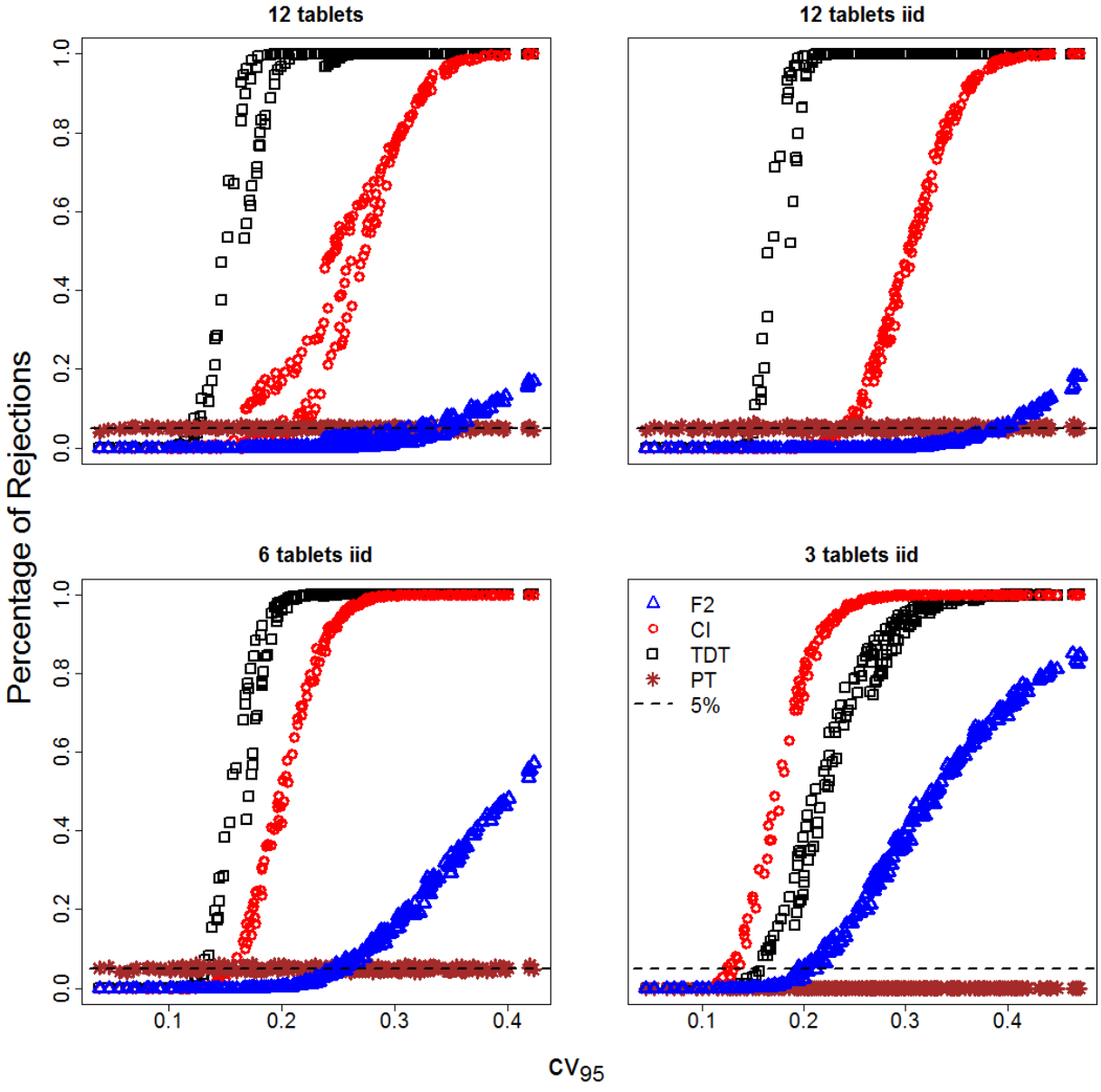


Figure 4.6. Effect of iid-conditions and sample size on test robustness (Korsmeyer model).

Table 4.1. Effect of iid conditions and batch size in Robustness for all the models and tests

Model & Test	Effect of iid conditions	Effect of smaller sample Size
Higuchi f2	+	- -
Higuchi CI	+ +	- -
Higuchi TDT	-	-
Higuchi PT	( )	( )*
Korsmeyer f2	( )	- -
Korsmeyer CI	+ +	- -
Korsmeyer TDT	+	+ +
Korsmeyer PT	( )	( )*
Peppas f2	+	- -
Peppas CI	+ +	- -
Peppas TDT	+ +	+ +
Peppas PT	( )	( )*
Weibull f2	( )	- -
Weibull CI	+ +	- -
Weibull TDT	( )	+ +
Weibull PT	( )	( )*

+ : Slight increase in Robustness
+ + : Evident increase in Robustness
- : Slight decrease in Robustness
- - : Evident decrease in Robustness
( ) : No apparent effect
( ) * For batch size = 3, level of rejections of PT remain at 0.03 at all levels of CV_{95} in all the models. For bigger batch sizes there was no effect in Robustness for batch size. See discussion for detailed explanation.

4.4.3. Statistical Power

Figure 4.7 illustrates how, under the Higuchi model, the level of rejections increases in all the tests when the difference between b_{Test} and $b_{Reference}$ becomes greater, in other words, when the simulated Reference and Test batches are more different. As shown here for the Higuchi model with sample size n = 12 and no-iid conditions, PT was the

most powerful test, followed by TDT, CI and f2. These results were very similar regardless of the T_{85} of the Reference used in the simulations (Figure 4.8. A-B). Analogous results were obtained under the other models for differences in single parameters (Figure 4.7 B-D). The magnitude of the differences detected for single parameters under all the models are summarized in Table 4.2.

Again, for conditions of high variability ($CV_{95} \geq 0.2$) PT was the only test in which the statistical power and robustness are not so severely compromised due to an increase in variability (Figure 4.8.C).

The capacity of the tests to detect simultaneous differences in more than one parameter (Power) is shown in figure 4.9, In this Power contour plots, two parameters are varied simultaneously (X and Y axis) and the combination of differences in these parameters required by each test to reach a power ≥ 0.8 is represented by a point on the contour plot. More powerful tests are able to detect smaller combination of differences with a power ≥ 0.8 (points closer to the origin on the diagram). Again, in these cases, PT was the most powerful test, detecting the smallest combination of differences (Points closer to the origin of the contour plots) of the parameters studied, followed by TDT, CI and finally f2 under all the models employed, highlighting that in the Peppas model, TDT and CI have very similar statistical power.

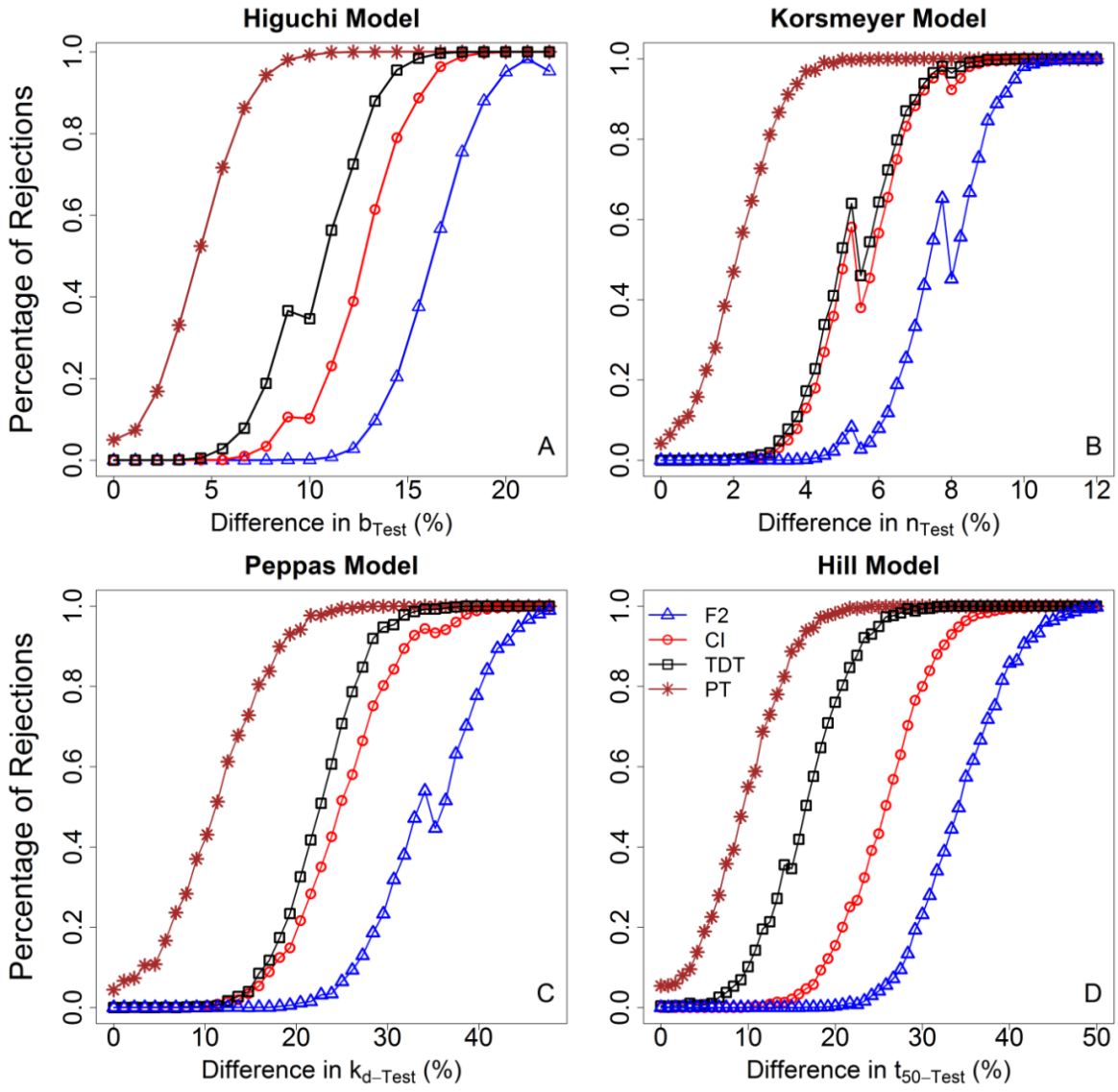


Figure 4.7. Power Comparison of the presented tests. In A (Higuchi Model), Percentage of rejections (Power) Vs Difference (%) in b_{Test} according to equation 3 $b_{Reference}$ was set at 9 (T_{85} ≈ 90 mins) and b_{Test} varying from 7(T_{85} ≈ 150 mins) to 11 (T_{85} ≈ 60 mins). In B (Hill Model), Percentage of rejections (Power) Vs Difference (%) in t_{50_test} according to equation 4 $t_{50\text{-}Reference}$ was set at 1.605 h and $n_{Reference}$ at 1.85 (T_{85} ≈ 240 mins) and $t_{50\text{-}Test}$ varying from 1.605 to 2.3554 h (T_{85} ≈ 240 mins to T_{85} ≈ 360 mins). For Peppas Model (C), Percentage of rejections (Power) Vs Difference (%) in $k_{d\text{-}Test}$, according to equation 5, $k_{r\text{-}Reference}$ was set at 0.6 and $k_{d\text{-}Reference}$ at 4.4 (T_{85} ≈ 130 mins) and $k_{d\text{-}Test}$ varying from 4.4 to 6.5 (T_{85} ≈ 130 mins to T_{85} ≈ 90 mins). For Hill Model (D), Percentage of rejections (Power) Vs Difference (%) in $t_{50\text{-}Test}$, according to equation 7, $t_{50\text{-}Reference}$ was set at 1.605 h and $n_{Reference}$ at 1.85 (T_{85} ≈ 240 mins) and $t_{50\text{-}Test}$ varying from 1.605 to 3.405 h, (T_{85} ≈ 160 to T_{85} ≈ 520 mins).

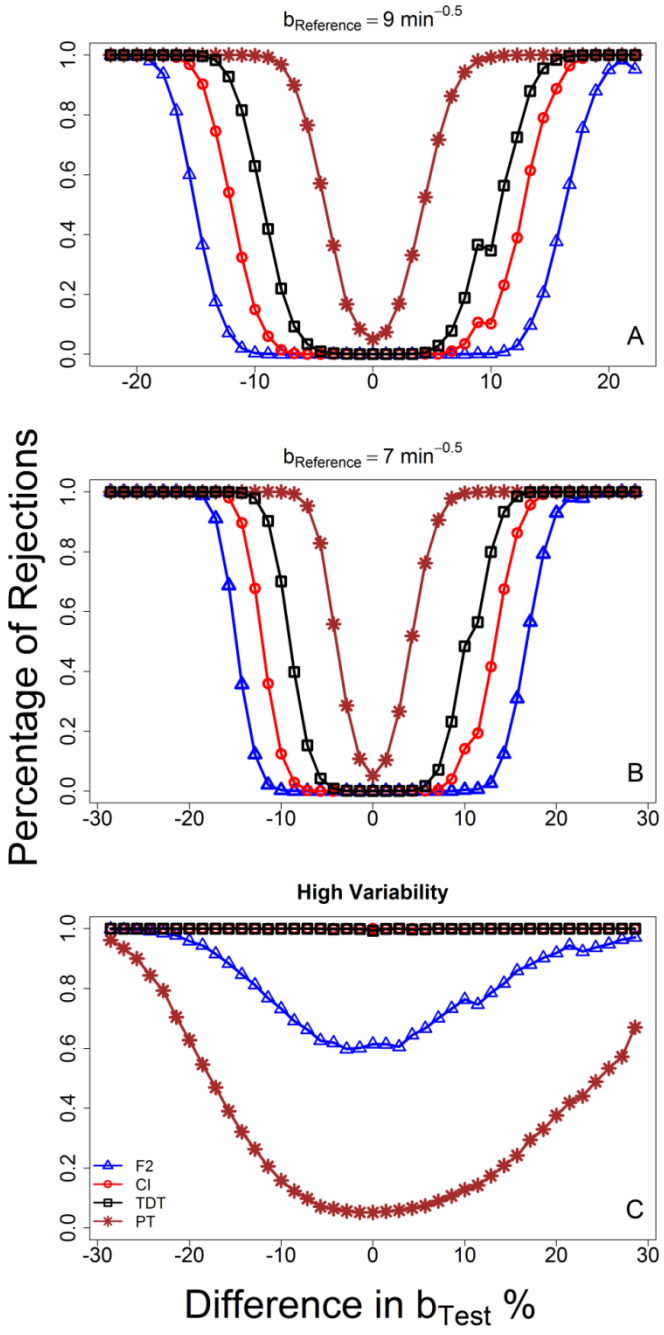


Figure 4.8. Power comparisson of the presented (Higuchi model). A and B present data from typical variability conditions (CV_{95} =0.1) form Reference formulations with different T_{85}. According to equation 3, in A $b_{Reference}$ was seted at 9 (T_{85} ≈ 90 mins) and b_{Test} varying from 7(T_{85} ≈ 150 mins) to 11 (T_{85} ≈ 60 mins). In B $b_{Reference}$ was seted at 7 (T_{85} ≈ 150 mins) and b_{Test} varying from 5 (T_{85} ≈ 300 mins) to 9 (T_{85} ≈ 90 mins). In C condtions are equal to B but in high variability conditions (CV_{95} =0.2).

Table 4.2. Detectable differences in single parameters with each test. For each paramater of each model, the minimum detectable difference (in percentage of the parameter) for each test (with power ≥ 0.8) is presented, the correspondent difference in t_{85} produced by the difference in the parameter is also displayed.

Model	Parameter	f2	CI	TDT	PT
Higuchi	b	16.6%	14.4%	12.22%	6.6%
	t_{85}	44%	35%	27.77%	10%
Korsmeyer	n	9%	6.75%	6.5%	2.75%
	t_{85}	33%	28.32%	27.53%	12.25%
	k	16.6%	14.4%	12.22%	6.6%
	t_{85}	44%	35%	27.77%	10%
Peppas	Kd	40.91%	29.55%	27.27%	15.90%
	t_{85}	23.08%	17.36%	16.15%	9.81%
	Kr	43.75%	34.37%	36.46%	16.6%
	t_{85}	25.47%	21.17%	22.17%	11.54%
Weibull	a	39.17%	30%	20.83%	14.17%
	t_{85}	35.64%	29.52%	22.3%	16.19%
	B	10%	7.67%	6%	3.67%
	t_{85}	39.51%	32.55%	26.87%	17.76%
Hilll	T_{50}	34.57%	29.90%	20.55%	7.13%
	t_{85}	38.33%	32.91%	25%	10%
	n	86.5%	81.1%	43.24%	10.8%
	t_{85}	33.5%	31.66%	22.08%	5%

Consistent with the results for robustness, the power of the CI and TDT tests under high variability conditions was rather poor, f2 performance was slightly better but still not acceptable, and PT showed the best performance in this scenario although its power was significantly decreased compared to low variability conditions (Figure 4.9).

The demand for a very powerful and robust statistical tool, able to detect small differences in dissolution profiles can be satisfied with the introduced PT. PT was able to detect with statistical power ≥0.8 the smallest differences in each model parameters,

normally more than two times smaller than the differences detected with the same power with f2 (Table 4.1). For example, in Korsmeyer model, PT was able to detect differences of 4% in the kinetic constant while f2 is able to detect just differences greater than 20%, this can represent a 10% detectable difference in T85 with PT against a 40% detectable difference in T85 with f2.

As we have shown, PT can be used to compare profiles even with high levels of variation, moreover, PT allows the user to choose the level of statistical uncertainty. Furthermore, this test is not especially sensitive to the sample size employed in the comparisons, provided that the sample size is greater than n = 3 (due to the permutation nature of PT, the sample size of n = 3 highly compromised the power of the test and should not be employed). A sample size of n = 6 could be used without significantly altering its good performance compared to a sample size of n = 12. PT appears ideal for situations in which high similarity should be proven, e.g., in cases of drugs with a narrow therapeutic window, or with low permeability and/or solubility or susceptible of intestinal transport or metabolism, and currently there is no test as powerful and robust that can do so with similar statistical consistency.

4.4.4. Flexibility of TDT

As previously mentioned, in some situations, however, detection of significant but small differences in dissolution profiles may not be the objective and a more tolerant and flexible test is needed. This flexibility to vary the tolerated δ in order to detect larger or smaller differences in dissolution profiles is precisely one of the designed properties of the TDT test.

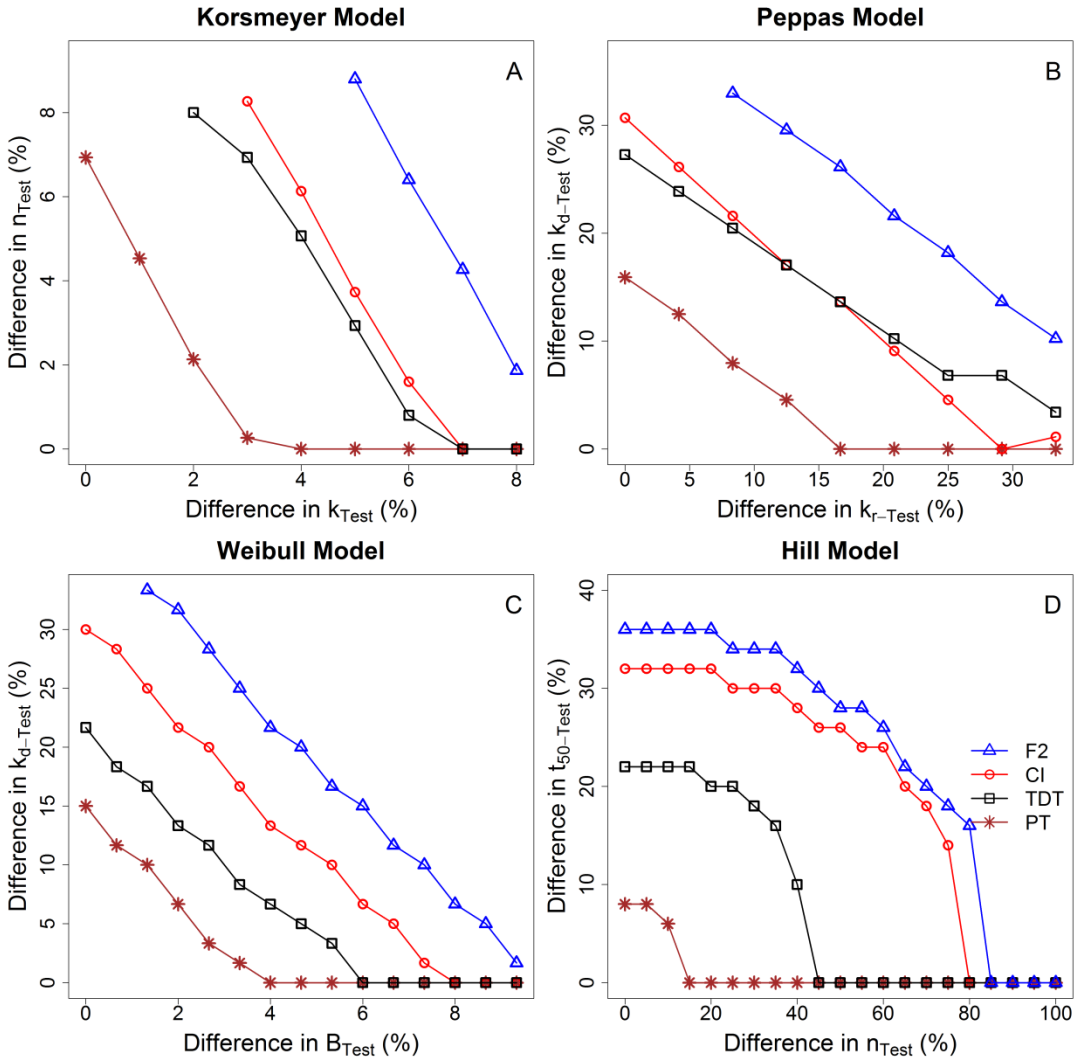


Figure 4.9. Bidirectional power exploration of the tests. Contour plots of power ≥ 0.8 for the tests. The combination of differences in two parameters required by each test to reach a power ≥ 0.8 is represented by a point in the contour plot. In A (Korsmayer Model), according to equation 4. $k_{Reference}$ was set at 7.5 and $n_{Reference}$ at 0.5 (T_{85} ≈ 130 mins) and k_{Test} and n_{Test} varying from 7.5 to 8.2 and 0.5 to 0.54 respectively (T_{85} ≈ 150 mins to T_{85} ≈ 65 mins). For Peppas Model (B), according to equation 5, $k_{r\text{-}Reference}$ was set at 0.6 and $k_{d\text{-}Reference}$ at 4.4 (T_{85} ≈ 130 mins) and $k_{r\text{-}Test}$ $k_{d\text{-}Test}$ varying from 0.6 to 0.8 and 4.4 to 6 respectively (T_{85} ≈ 130 mins to T_{85} ≈ 80 mins). For Weibull Model (C), according equation 6, $B_{Reference}$ was set at 0.75 and $k_{d\text{-}Reference}$ at 0.03 (T_{85} ≈ 250 mins) and $k_{d\text{-}Test}$ and B_{Test} varying from 0.03 to 0.045 and 0.75 to 0.82 respectively (T_{85} ≈ 250 mins to T_{85} ≈ 120 mins). In D (Hill Model), according to equation 7. $t_{50\text{-}Reference}$ was set at 1.605 h and $n_{Reference}$ at 1.85 (T_{85} ≈ 240

mins) and $t_{50\text{-Test}}$ varying from 1.605 to 3.405 h, and n_{Test} varying from 1.85 to 3 ($T_{85} \approx 160$ to $T_{85} \approx 520$ mins).

Flexibility of TDT is shown for TDT in Figure 4.10 in which TDT with $\delta = 5$ and $\delta = 10$ are compared to f2. It can be appreciated that increasing the value of δ decreases the power of the test, in this particular case TDT with $\delta = 5$ was more powerful than f2, while TDT with $\delta = 10$ was less powerful than f2.

In addition TDT takes into account information on every tablet at every single point and does not rely on measures of central tendency as do f2, CI and PT, therefore, the analysis it provides may be more comprehensive than those of the other tests.

4.4.5. Effect of Independence and Sample Size on Power

AS previously stated, the underlying principle of the TDT demands that the data from every time point of every tablet be independent and identically distributed (iid-conditions). Effects of iid-conditions were analyzed and compared with no-iid conditions to determine how necessary iid-conditions are to a proper performance of the test. The effect of iid was shown to be of no practical importance because, in all the cases and models studied, the differences in power between iid and no-iid conditions were typically less or equal to 5% (Figure 4.1 and Table 4.3). In principle, the TDT test will perform similarly under no-iid conditions or iid-conditions and the former may be preferred for convenience (a smaller number of tablets is needed).

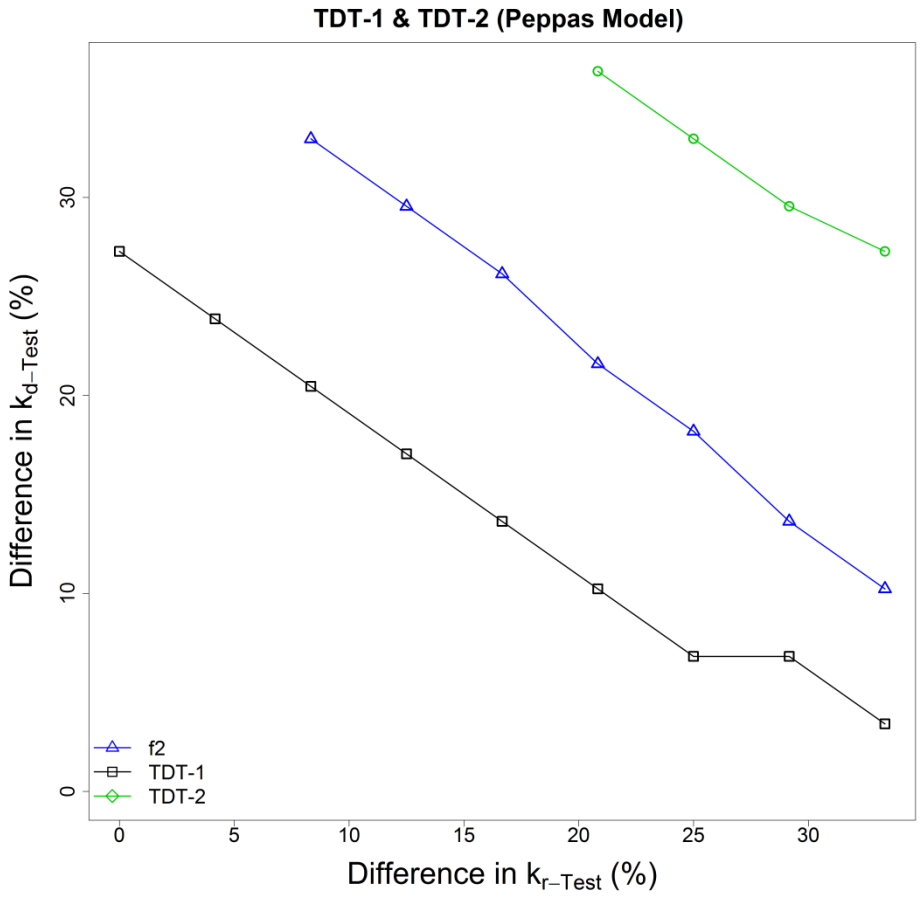


Figure 4.10. Flexibility of TDT. Power Comparison of f2 and TDT with two different values of δ. For Peppas Model, according to equation 5, $k_{r\text{-}Reference}$ was set at 0.6 and $k_{d\text{-}Reference}$ at 4.4 ($T_{85} \approx 130$ mins) and $k_{r\text{-}Test}$ $k_{d\text{-}Test}$ varying from 0.6 to 0.8 and 4.4 to 6 respectively ($T_{85} \approx 130$ mins to $T_{85} \approx 80$ mins.

Although iid-conditions had a minor effect on the power of the three tests, this did not alter the relative power of the tests (TDT-1 > f2 > TDT-2) in any of the studied models. The robustness of TDT for $\delta = 5$ was good and even better for higher values of δ.

The effect of sample size on the power of the tests is also summarized in table 4.3 for each test and each dissolution model; in general, smaller sample sizes reduced the power of PT and TDT and increase the power of CI and f2. According to standard statistical theory, the power of a test increase with sample size and should not be

increased by reduction in sample size as happened with f2 and CI in these simulations, it shows the limitations of the f2 similarity factor.

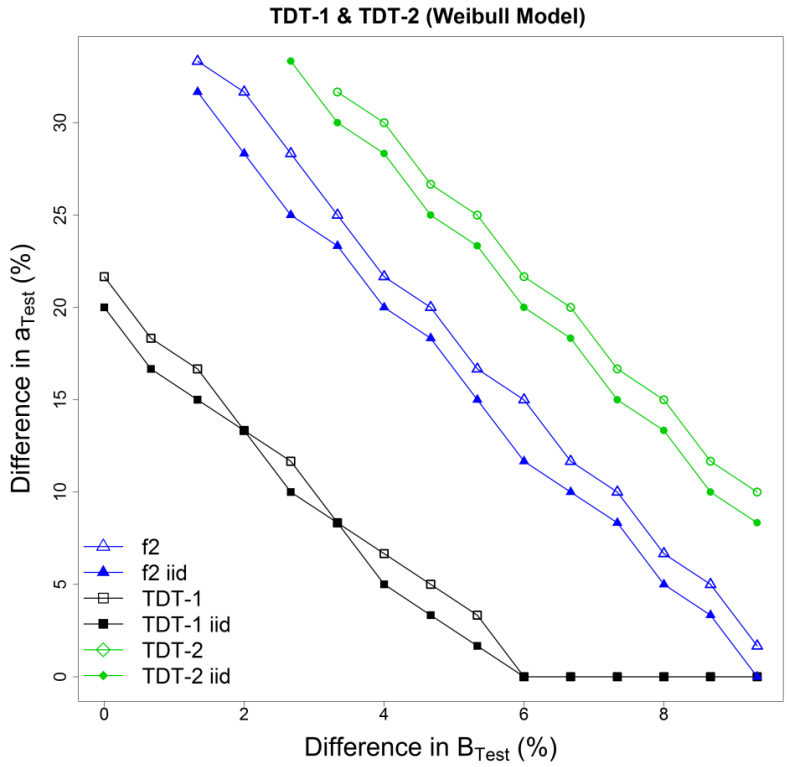


Figure 4.11. Effect of iid-conditions on DPC-tests Power. Power Comparison of f2 and TDT with two different values of δ under iid and no-iid conditions under Weibull model. according to equation 6, $B_{Reference}$ was set at 0.75 and $a_{Reference}$ at 0.03 ($T_{85} \approx 250$ mins) and a_{Test} and B_{Test} varying from 0.03 to 0.045 and 0.75 to 0.82 respectively ($T_{85} \approx 250$ mins to $T_{85} \approx 120$ mins)

Table 4.3. Effect of iid-conditions and sample size in statistical power for all the tests.

Test & Condition / Model	f2 iid	CI iid	TDT iid	PT iid	F2 S.S.S.	CI S.S.S	TDT S.S.S.	PT S.S.S.
Higuchi	( )	-	-	( )*	+	+ +	- -	-**
Korsmeyer	-	-	f	+	+	+ +	- -	-**
Peppas	( )	-	f	+	f	( )	-	-**
Weibull	( )	( )	( )	( )	( )	+	-	-**
Hill	( )	( )	( )	( )	( )	+	-	-**

iid : Independent identically distributed.
S.S.S. : Smaller Sample Size
+ : Slight increase in Power
+ + : Evident increase in Power
- : Slight decrease in Power
- - : Evident decrease in Power
( ) : No apparent effect
f : Fluctuating. A different effect (slightly increase or decrease) at different zones of the diagrams
-*For batch size = 3, level of rejections of PT remain at 0.03 at all values of the test parameters, it is not and effect of batch size in general but of this very small batch size in particular.

4.4.6. Time Sample Strategies

Figure 4.7 B and C show some apparent discontinuities in the power curves of f2, CI and TDT tests. For example, in Figure 4.7 B for $n_{Reference}$ = 0.5, the power of f2, CI and TDT first increases continuously at increasing values of n_{Test} (Korsmeyer model), but at n_{Test} = 0.5275 (difference of 5.25%) the power of the three tests is reduced. This unexpected phenomenon was identified as an artifact due to sampling times. The time sampling scheme was designed to be as realistic as possible (intervals of 5, 10, 15, 20, or 30 min. see section 4.3.2.3). According to this rules, solving equation 5 for n_{Test} = 0.52625, t_{85}=100.8161, samples must be collected at 6 time points (20,40,60,80,100

and 120 min), in contrast, when n_{Test} = 0.5275, t_{85} is 99.72 and just 5 time points need to be sampled (20,40,60,80 and 100 min). This reduction in the number of sampling time points can produce a 50% decrease in power in the f2, CI and TDT tests. To counteract this effect, an optimized sampling scheme was developed. In optimized sampling, the number of time points is fixed, and t_{85} (the smaller between Reference and Test) is divided into equidistant time points with t_{85} as the last time point, for example, fixing 6 time points for a t_{85} = 95 min, the time points are: 15.833, 31.667, 47.5, 63.333, 79.167 and 95 min. Optimized sampling with 6 and 5 points, respectively, was employed in the analysis (Figure 4.12). In either case of such optimized sampling the discontinuity in power was no longer present.

No significant difference was found between results obtained using 5 or 6 time points, confirming that the apparent discontinuity is due to the sampling strategy and not to the number of time points sampled. These finding suggest that optimized sampling should be employed as a first option for dissolution profile comparisons.

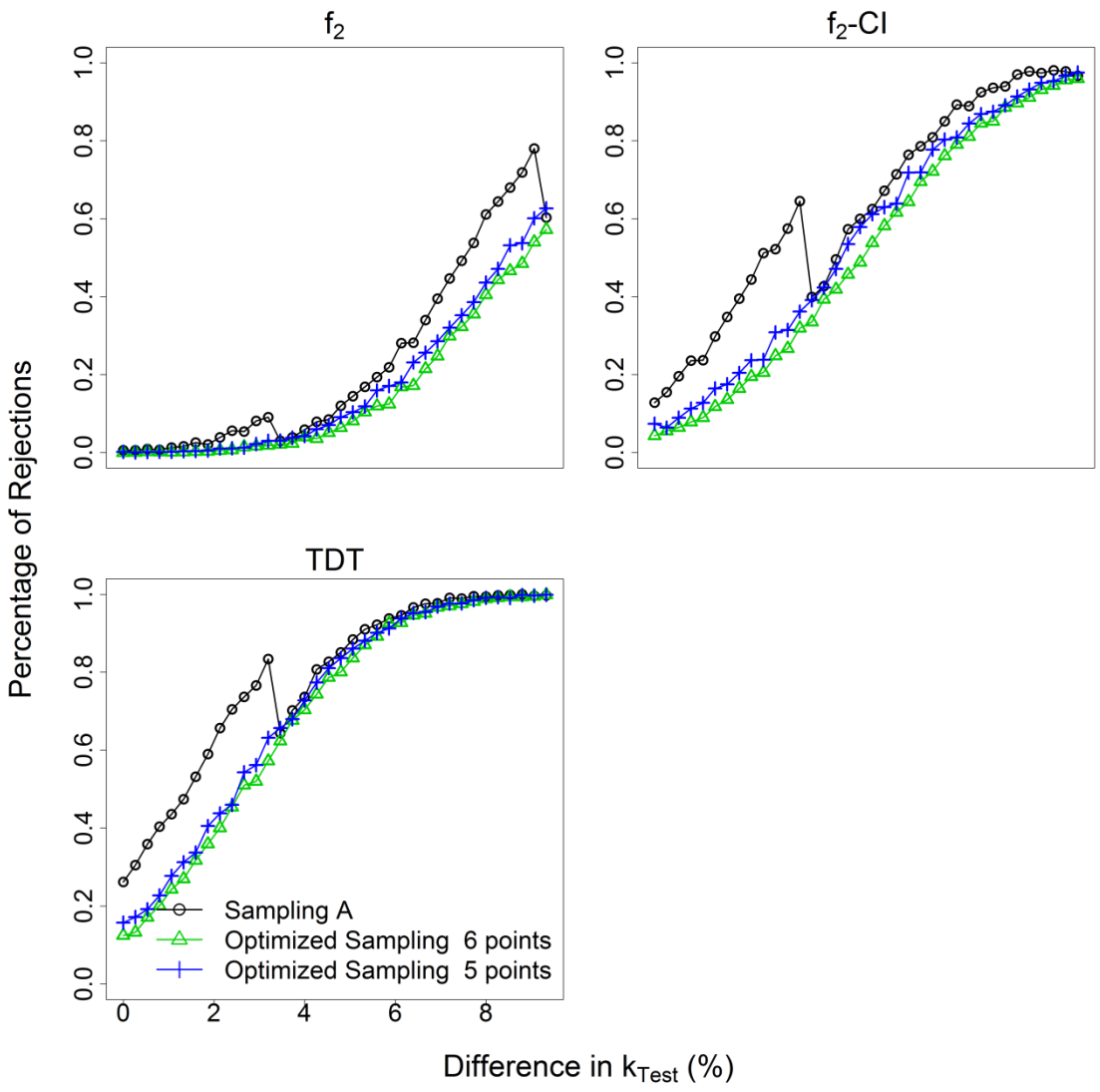


Figure 4.12. Effect of time sampling strategy on statistical power (Korsmeyer model). Power Comparison of f2, CI and TDT with three different sampling schemes at typical variability conditions (CV_{95} =0.1). According to equation 3, $k_{Reference}$ was set at 7 and $n_{Reference}$ at 0.5 (T_{85} ≈ 150 mins) and k_{Test} varying from 7.5 to 8.2 and n_{Test} fixed at 0.5.

4.4.7. Experimental Design Driven Strategy for Performing Optimal Dissolution Profile Comparisons

Combining the information presented here with basic principles of experimental design, we propose an experimental design driven strategy for performing optimal dissolution profile comparisons; this strategy is illustrated in Figure 4.13.

- If the goal is to detect small differences (Table 4.2) in dissolution profiles, PT must be employed with a sample size greater or equal to n = 6 and a standard time sampling can be employed.
- If a less strict comparison is needed or if there is no certainty about the degree of similarity that can be accepted, the following procedure should be followed:
    - Preliminary experiments must be conducted to determine the t_{85} of the Reference and Test formulations and to fit the dissolution data to one or several models, (including models not presented in this work).
    - The minimum detectable difference (the difference to be considered as not similarity), must be determined either, by finding the adequate difference at t_{85} or t_{50} (time at which 50% of the labeled drug is dissolved) or a combination of both, or ideally, through either an IVIVC model [72] indicating the differences in dissolution that may lead to differences in bioavailability, [73] or by fitting the dissolution data for preliminary experiments to available dissolution models [30], [74-76] and estimating the difference in parameters acceptable as similar. This step is the most complicated and the most susceptible to produce under- or over-estimation of the detectable difference due to personal interpretation.

- After the acceptable difference is determined and the dissolution model selected, simulations with TDT must be done to determine the value of δ and sample size at which the determined minimal difference in parameters or t_{85} or possible combinations is detected with an acceptable statistical power (power of 0.8 or higher is recommended) always using an optimized time sampling strategy.
- Preferably, effects of iid-conditions on variability, robustness and power of TDT should be addressed in simulation or laboratory experiments.
- Finally, the dissolution assays must be performed under the conditions (iid/no-iid-conditions, δ and sample size) found and the statistical comparison must be done using TDT.

In this way the flexibility of TDT is used to customize a comparison test (setting a specific δ value) able to detect the differences in dissolution that can produce a difference in the bioavailability/bioequivalence of the formulations. The procedure described may seem arduous compared to the current f2 standard, however, it follows the typical procedure employed in any experimental design aimed at detecting significant differences with a quantified statistical uncertainty and known type-I and type-II errors. The procedure involves the following steps: i)preliminary data analysis, ii)determination of minimum detectable acceptable difference, iii)determination of sample size according to a desired level of power and robustness and iv)experimentation and statistical computation.

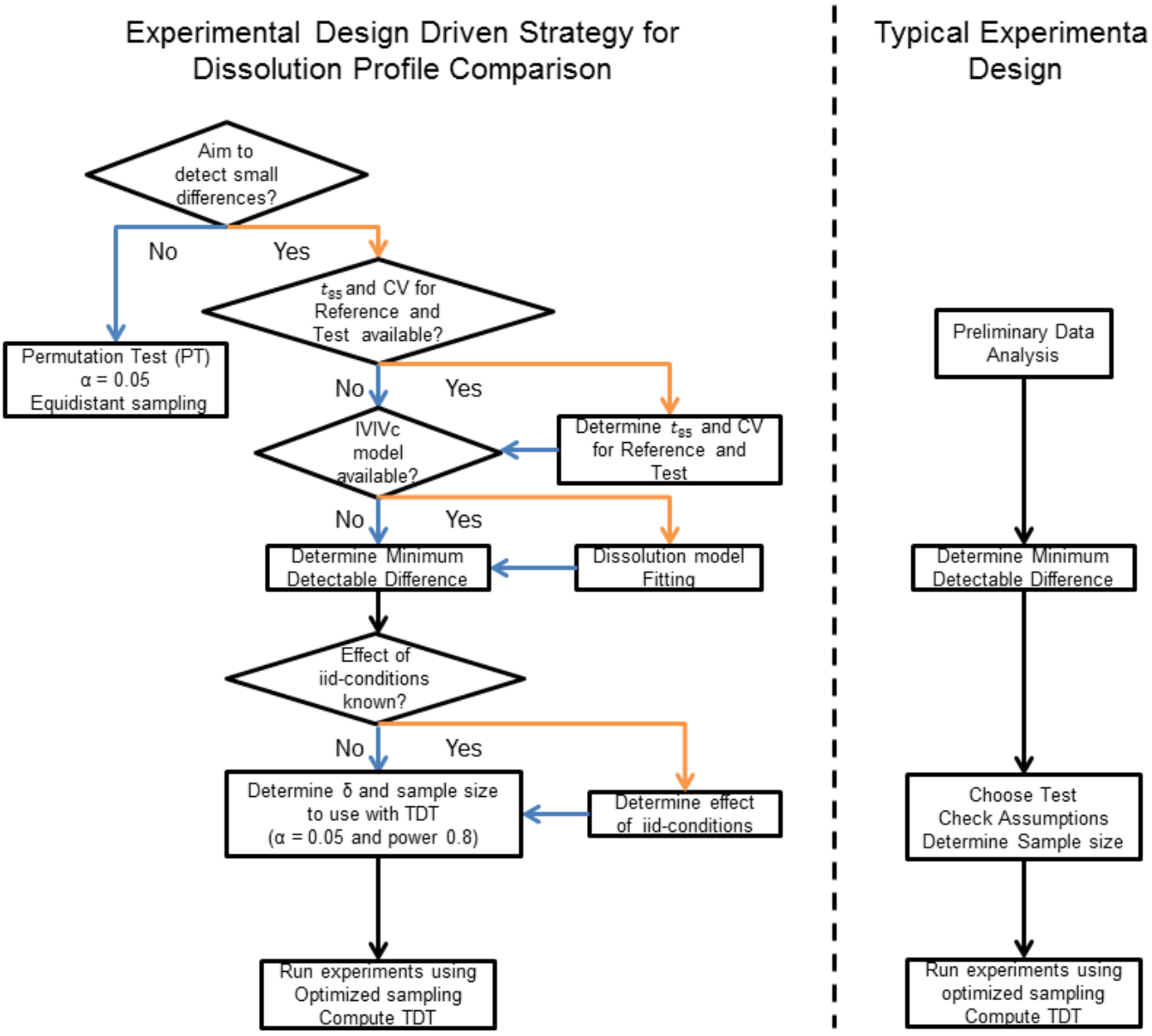


Figure 4.13. Experimental Design Driven Strategy to Perform Optimal Dissolution Profile Comparisons. Diagram flow of the proposed strategy to Perform Optimal Dissolution Profile Comparisons, Each stage of the presented strategy corresponds to a stage of a typical experimental design lustrated in the right.

Due to the simulated nature of the data presented here, experimental verification of the lack of effect for iid-conditions and examples of how to customize TDT with specific formulations are recommendable. Also, evaluation of additional available expressions

for modeling drug dissolution [74], including models for controlled released mechanisms [75, 76] might be an obvious subject for future studies.

4.5. Conclusions

Two new statistical tests, the permutation test (PT) and the tolerated difference test (TDT), are presented for dissolution profile comparison in which type-I and type-II errors can be quantified, and have a stronger statistical basis than the current alternatives (e.g., the f2 similarity factor). The two new tests showed acceptable robustness at standard conditions of variation ($CV_{95} \leq 0.1$). PT was the most robust and powerful test in all the conditions studied (even in conditions of high variability $CV_{95} \leq 0.2$ and reduced sample sizes). This test is strongly recommended for identifying small differences in dissolution. For $\delta = 5$, TDT showed good robustness and very good power in all the conditions studied.

The impact of iid-conditions in TDT was not particularly large, therefore the more usual no-iid conditions could be employed (experimental confirmation of this is still pending). The possibility to modify the value of δ confers great versatility on TDT and allows it to be customized for any specific formulation. To make the best use of the two new tests, a strategy to design and perform a dissolution profile comparison is presented under typical premises of statistical experimental design. Finally, it was shown that optimized time sampling should be employed when possible to avoid artificial discontinuities in the statistical power of the tests, except for PT which is not susceptible to this effect.

5. Tailor-Made DPC-tests for comparing ER Formulations Using IVIVC Models

Parts of this chapter were submitted for publication in a peer-reviewed journal:

Gomez-Mantilla JD, Casabo VG, Lehr T, Schaefer UF, Lehr CM. Identification of Nonbioequivalent Extended Release Formulations by Tailor-Made Dissolution Profile Comparisons Using *In Vitro-In Vivo* Correlation Models.

The author of the thesis made the following contribution to the publication:

- Design, Performance and interpretation of simulations.
- Writing the Manuscript.

5.1. Abstract

Current procedures for performing dissolution profile comparisons are restricted to mathematical distances (such as the f2 similarity factor) in which limits for declaring similarity or non-similarity are fixed, drug-unspecific and not based on any biopharmaceutical criteria. This problem and the lack of strong statistical basis, hinder the application of DPC-tests for evaluating similarity of ER formulations. This study aimed to develop drug-specific DPC-tests, able to detect differences in release profiles between ER formulations that represent a lack of BE. Dissolution profiles of Test formulations were simulated using the Weibull and Hill models. Differential equations based *in vivo-in vitro* correlation (IVIVC) models were used to simulate plasma concentrations. BE trial simulations were employed to find the formulations likely to be declared bioequivalent and nonbioequivalent (BE-space). Customization of DPC-tests was made by adjusting the delta of the tolerated difference test (TDT) described in the previous chapter. This delta value was tailored for three ER formulations (3.6 for metformin, 5.95 for diltiazem and 3.45 for pramipexole) to detect with a statistical power ≥ 80%, differences in release profiles identified as biorelevant limits (nonbioequivalence). The Impact of the dissolution profile comparisons conditions, BE-trial conditions, and drug properties in the determination of biorelevant limits were investigated. The other described DPC-test, the permutation test (PT), showed excellent statistical power. All the formulations declared as similar with PT were also bioequivalent. Similar case-specific studies may support the biowaiving of ER drug formulations based on customized DPC-tests.

5.2. Introduction

The pharmaceutical industry is constantly searching for effective surrogates for judging therapeutic equivalence of pharmaceutically equivalent drug products. One widely accepted and official procedure to ensure efficacy and safety of new formulations is the assessment of BE, in which it an absence of significant differences in the rate and extent to which the active ingredient become available at the site of drug action must be demonstrated [4-9].

In cases where the excipients of a formulation do not affect the absorption of the active pharmaceutical ingredient (API), the API is not a prodrug, does not have a narrow therapeutic index and is not intended to be absorbed in the oral cavity, *in vitro* testing has been accepted as a sufficiently reliable surrogate for an *in vivo* BE study [10, 11]. The regulatory acceptance of *in vitro* testing as a reliable surrogate for an *in vivo* BE is referred as “biowaiver” [10, 11].

Requirements for granting a biowaiver of an *in vivo* BE study depend on the type of drug, type of formulation, the type of post-approval change and the information available. For IR solid oral dosage forms of BCS class I drugs (highly permeable and soluble), demonstration of ≥ 85% dissolution in one or several media in 15 min is normally enough for conceding a biowaiving of BE studies in the case of post-approval changes of minimal impact [11]. Debate is still open as to whether biowaiving could be accepted for IR formulations of other BCS class drugs and guidelines differ in this point [10].

The therapeutic equivalence of drug formulations is assured by *in vitro* comparison of dissolution profiles. The term similarity has been employed to describe the lack of

difference between dissolution profiles from two different sources (formulations) and it is normally established by using the f2 similarity factor [2, 19, 41, 42]. In order to grant a biowaiver for ER formulations or for higher impact changes [1] in IR formulations, guidelines normally demand that similarity of profiles is demonstrated through the f2 similarity factor (equation 8). Additionally, in the case of ER, a validated IVIVC model must be available. However, the limits of rejection of f2 (≤ 50) are not justified by any mechanistic or biopharmaceutical reasons. As it is an empirical and fixed limit, it is unlikely to exhibit the specific discriminatory power required in all scenarios in which it is currently used. Moreover, recent publications have stated that on the one hand, f2 may classify formulations that are nonbioequivalent as similar [30, 44], while on the other hand f2 can also be over-discriminative in some cases [32, 70, 77]. Additionally, several publications have recognized some major statistical and conceptual limitations of f2, including, uncertain level of confidence, low statistical power, lack of biopharmaceutical or statistical reasons, lack of flexibility to perform in different scenarios and poor statistical consistency [30, 43, 44, 77, 78].

This rigidity of f2, being too restrictive in some cases and too liberal in others, ratifies that dissolution tests are only physical tests until they are linked to *in vivo* performance of the formulations tested [28, 79], and further highlights the need to identify dissolution limits which ensure clinical quality for each particular case [32]. Advances in developing more biorelevant dissolution methodologies are continuously being made [21-27], and have been identified as a major priority [28, 29]. However, statistical tools to compare dissolution profiles have not been improved in the last years, despite the fact that a more rigorous application of statistics to understand and incorporate variability and

uncertainty has also been identified as a priority in order to achieve a better integration of biopharmaceutics and quality for patient benefit. [80].

It was described in the previous chapter a new strategy to perform case-by-case dissolution profile comparisons [78], including two new statistical tests for comparing drug dissolution profiles; the PT, a very powerful and strict test to confer similarity, and the TDT, a flexible test in which the limits of rejection can be varied according to a desired level of tolerance without affecting its statistical properties. These two tests have the advantage of a better uncertainty quantification (Type I and Type II errors) and better statistical consistency of their estimators.

Using validated IVIVC models, plasma concentrations achieved by different formulations can be simulated from their dissolution profiles [72, 81, 82]. It is further possible using such models, to find what difference in dissolution, or in other words which formulations are likely to produce differences in-vivo large enough to be considered as nonbioequivalent [83]. We can then customize DPC-tests to declare non-similarity at levels of dissolution differences at which nonbioequivalence is expected.

This study aimed first, to develop drug-specific DPC-tests for three ER formulations (metformin, diltiazem and pramipexole) using IVIVC models, computer simulated BE trials and permutation tests. These customized tests should be able to detect, at a known level of certainty, differences in release profiles between ER formulations that represent a lack of BE. Secondly, we aimed to investigate the effect of Dissolution Profile comparisons conditions, BE-trial conditions, and drug/formulation properties in the determination of biorelevant limits.

5.3. Methods

5.3.1. General Strategy

The strategy used to identify bioequivalent and nonbioequivalent formulations is illustrated in Figure 5.1 taking pramipexole as example. In-vitro dissolution profiles were simulated for several formulations by modifying the Weibull model parameters and PK profiles of the formulations were generated using IVIVC models. Through BE simulation studies, nonbioequivalent formulations were detected. Once the BE-spaces were delimited, TDT a DPC-test, was customized to declare as non-similar, the formulations that were likely to be nonbioequivalent. The strategy used to investigate the effect of drug/formulation properties was similar (Figure 5.2). Starting with the same dissolution profile different PK profiles can be generated by varying the IVIVC model input parameters. Investigation of the effect of such variation on the BE-space for the theoretical drug is then possible.

5.3.2. *In Vitro-In Vivo* Correlation Models (IVIVC models)

Two published differential-equation-based IVIVC models were used for analysis. For diltiazem and metformin (Figure 5.3.a), a one compartment pharmacokinetic model with a first order rate elimination was employed for describing plasma concentrations in which the rate of in-vivo input is connected to the rate of in-vitro dissolution through a functional dependency that allows inclusion of time scaling, time shifting and absorption window [52].

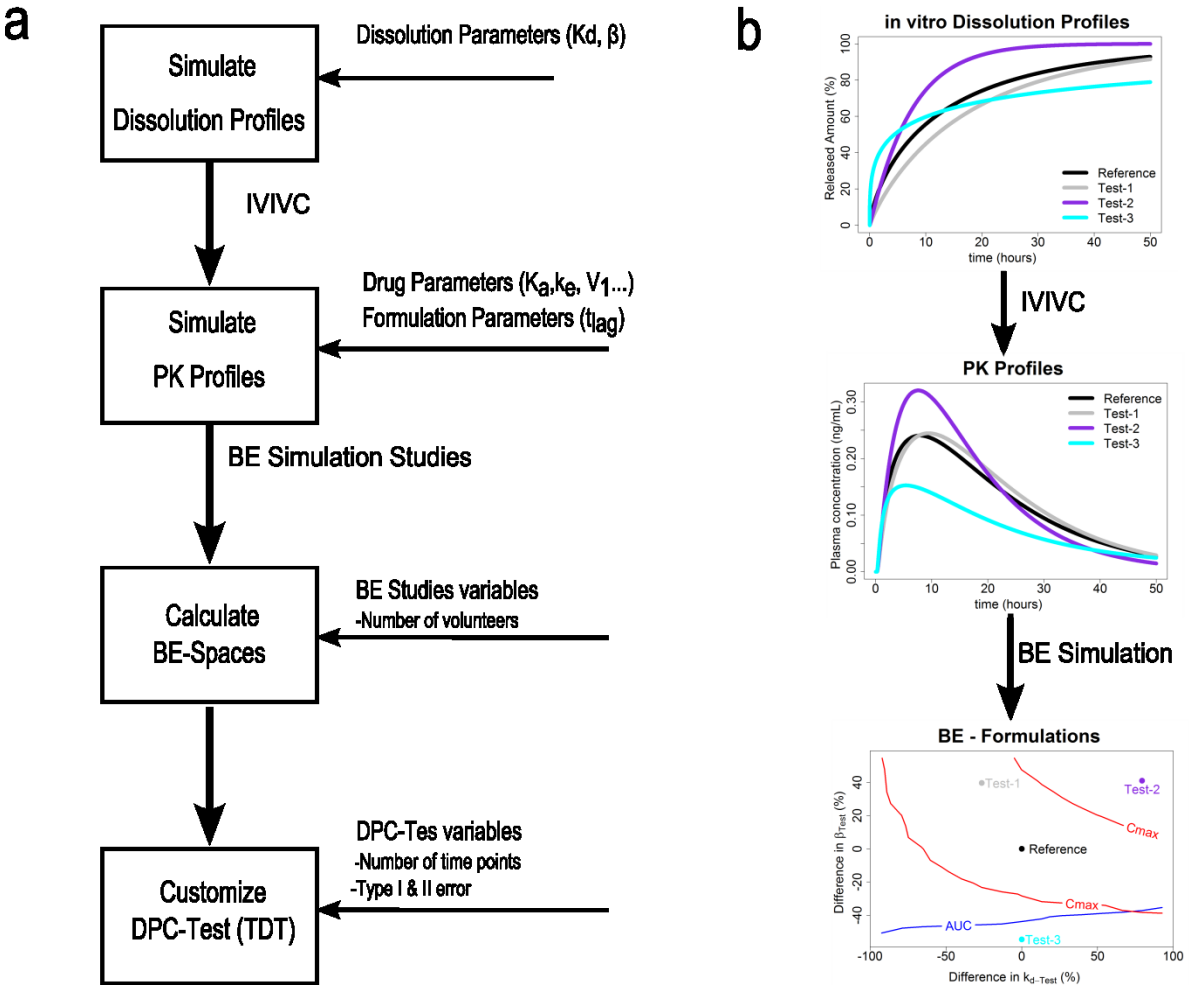


Figure 5.1. General strategy used to build the BE-spaces.

$$r(t) = \varphi_{abs}(t) S_r \, r_{dis} \, (t_0 + S_1 t) \qquad \text{equation 12}$$

Where S_1 is the time-scaling factor, S_r is the scaling factor, r_{dis} is the dissolution rate and $\varphi_{abs}(t)$ accounts for the variability of the in-vivo absorption as the drug moves along the gastrointestinal tract, including a truncated absorption at time t_{cut} :

$$\varphi_{abs}(t) = \frac{e^{-\eta(t-t_{cut})}}{1+e^{-\eta(t-t_{cut})}} \qquad \text{equation 13}$$

The dissolution rate (r_{dis}) was described by the Hill function [39] described in equation 7 Of which the differential expression is:

$$r_{dis}(t) = \frac{df_{dis}}{dt} = \frac{nF_{max}\, t_{50}^{n} t^{n-1}}{(t_{50}^{n} + t^{n})^{2}} \qquad \text{equation 14}$$

Where $f_{dis}(\mathrm{t})$ is the fraction (%) of drug released at time t, t_{50} is the time at which 50% of the drug is released from the formulation and n is a shape parameter. Data were generated to reproduce the data of Gillespie [84] for diltiazem and the data of Balan and co-workers [85] for metformin.

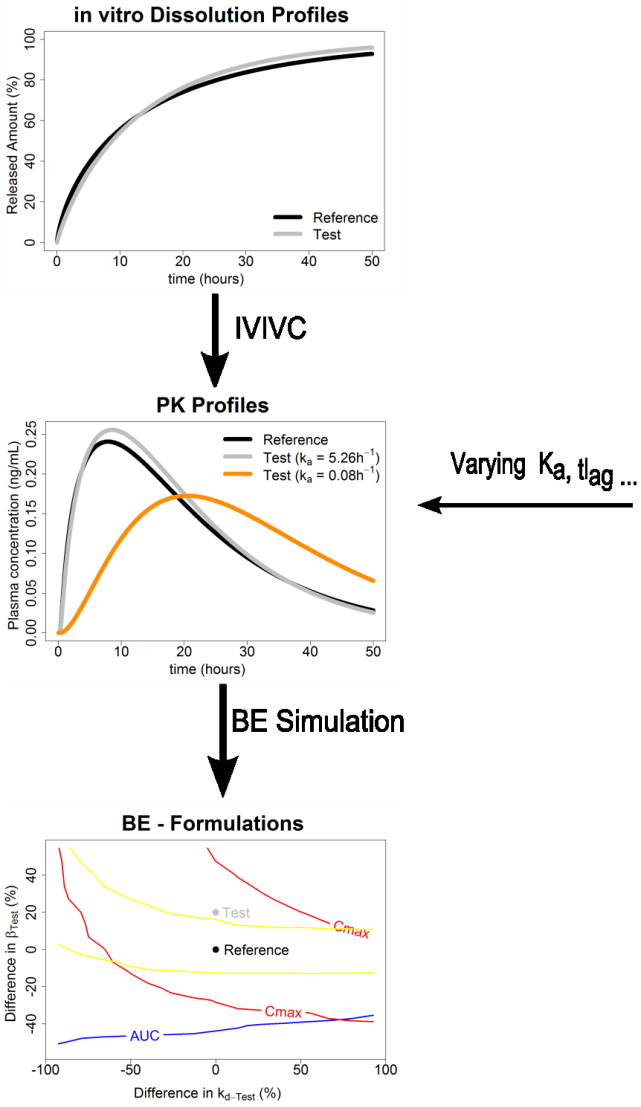


Figure 5.2. strategy used to investigate the effect of drug/formulation properties) on the determination of equivalent formulations. Starting with the same dissolution profile different PK profiles can be generated by varying the IVIVC model input parameters.

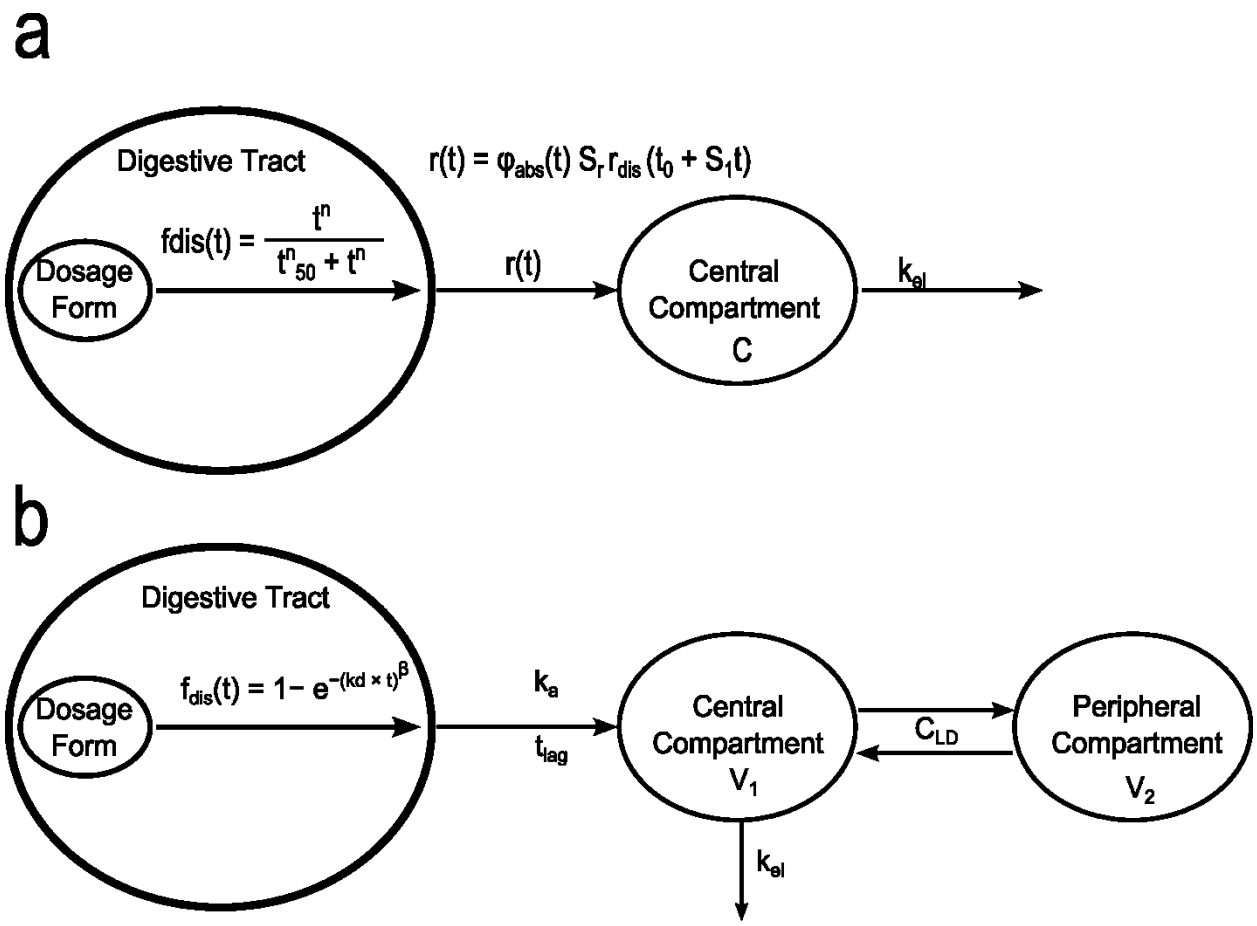


Figure 5.3. Schematic representation of the biopharmaceutic/pharmacokinetic models for the IVIVC of dialtiazem and metformin (a) and pramipexole (b).

For pramipexole (Figure 5.3.b), a two compartments model with first order absorption and elimination was used for describing plasma concentrations [53], in which the dissolution rate was described by the Weibull function described in equation 6 [36-38] of which differential expression is:

$$r_{dis}(t) = \frac{df_{dis}}{dt} = kd^{\beta} \times \beta \times t^{(\beta-1)} \times e^{-(kd \times t)^{\beta}} \qquad \text{equation 15}$$

Where $f_{dis}(\mathrm{t})$ is the fraction (%) of drug released at time t, kd is the dissolution constant and β is the shape parameter. The relationship between the $kd_{in-vivo}$ and $kd_{in-vitro}$ was modeled by $kd_{in-vivo} = kd_{in-vitro} + \theta_{SCL}$ where θ_{SCL} is a scale factor representing

the increment in the in-vivo dissolution. θ_{SCL} of $0.0581\ (13.75\%\ CV)$ was used in all simulations.

For the purpose of this study only differential equations-based IVIVC methods were included, because of the more mechanistic nature of these models [29, 86, 87]. All parameters employed are listed in Table 5.1.

Table 5.1. Population pharmacokinetic models parameters used in the IVIVC models.

	Pramipexole I	Diltiazem	Metformin
BCS	I	I	III
K_{el} (h^{-1})	0.087 (13)	0.138 (10)	0.23 (10)
t_{lag} (h)	0.22 (66.3)	0.57 (10)	0.86 (10)
t_{cut} (h)	NA	6.36 (20)	4.77 (20)
K_a (h^{-1})	5.26 (91.8)	NA	NA
V_1 (L)	351 (14.1)	NA	NA
V_2 (L)	60.9 (10)	NA	NA
C_{LD} (L/h)	33.2 (10)	NA	NA

Parameters are listed with the IIV in parenthesis. K_{el}, elimination constant; t_{lag}, lag time; t_{cut}, absorption window; K_a, absorption constant; V_1, V_2, volumes of distribution in the central and peripheral compartments respectively. CLD, Apparent distribution clearance, NA: Parameter not used in that model.

5.3.3. Test and Reference Formulations

Reference formulations were modeled as follows:

Metformin: $t_{50} = 1.77\ h$; $n = 2.6$ (Hill Model, equation 7)

Diltiazem: $t_{50} = 1.61\ h$; $n = 1.85$ (Hill Model, equation 7)

Pramipexole: $kd = 0.076\ h^{-1}$; $\beta = 0.732$ (Weibull Model, equation 6)

Test formulations were generated by varying simultaneously the two dissolution model parameters (t_{50} and n or kd and β) from -95% to 200% around those of the Reference formulation. Variability (CV 10%) was included at all dissolution points to mimic experimental data.

5.3.4. Simulations and Bioequivalent Studies

Simulations of plasma concentration were conducted in the R software environment (version 2.14.2. R Development core Team 2013) using the models detailed in the previous section. Inter individual variability (IIV) was include for each parameter (Table 5.1) to fit the reported experimental variability [52, 53] including an overall CV of area under the curve $(AUC)_{0-\infty}$ and C_{max} of 15%. In total, 1000 BE crossover simulated studies per scenario were conducted. In each study, 12 healthy volunteers were generated by Monte Carlo simulations. Each volunteer received an oral dose of the Test and Reference formulation with a wash-out period between the administrations. $AUC_{0-\infty}$ and maximum plasma concentration (C_{max}) were calculated from the generated plasma concentrations. BE between formulations was determined by calculating 90% confidence intervals (90%CI) of the ratio between Test and Reference means after log-transformation of $AUC_{0-\infty}$ and C_{max}. The formulations were considered bioequivalent if

the 90%CI of $AUC_{0-\infty}$ and C_{max} ratios were contained within the acceptance interval of 80.00 – 125.00%.

5.3.5. Dissolution Profile Comparisons Tests (DPC-tests)

Dissolution profiles from the Reference and Test formulations were compared using the f2 similarity factor (Equation 7) and two recently described tests, PT and TDT [78]. As described in section 4.3.3.

5.3.6. Statistical Power Explorations

Sets of 12 Reference formulation tablets and 12 Test formulations tablets were generated under conditions of non-similarity, when different model parameters were employed for the Reference and Test formulations. Each pair of batches was compared using f2, TDT and PT. At every condition, 5000 sets of Reference-Test were generated and the percentage of rejections (%detections of no similarity) was evaluated. More powerful tests are expected to detect smaller differences in the parameters used. In all cases equidistant sample points were sampled from which the last sample point was the smaller t_{85} (time to reach 85% release) of the two formulations.

5.4. Results

5.4.1. Illustrative Example

In Figure 5.1.b, results from pramipexole are outlined as an example. Test-1 is bioequivalent to the Reference formulation by AUC and C_{max}. Formulation Test-2 is bioequivalent to the Reference formulation by AUC but not by C_{max} and formulation

Test-3 is not bioequivalent to the Reference formulation by either AUC or C_{max}. The BE-space delimits the bioequivalent formulations from the nonbioequivalent. TDT was customized to declare as non-similar, formulations that are likely to be nonbioequivalent. To investigate the effect of drug/formulation properties the same dissolution profile (Test-1) was employed. Different PK profiles can be generated by varying the IVIVC model input parameters. In Figure 5.2, using the same Test formulation (equal k_d and β), two different PK profiles are generated by using different K_a values. The C_{max} BE-space for the theoretical drug with a smaller k_a was reduced (yellow BE-space) and the Test formulation is no longer bioequivalent to the Reference formulation by C_{max}. When the larger k_a is used the same Test formulation is bioequivalent to the Reference formulation (inside the red BE-space).

5.4.2. Bioequivalent and Similarity Spaces

For each drug, Test formulations were compared to the Reference formulation in their dissolution profiles and plasma levels. Figure 5.4 illustrates the effect of dissolution parameters on BE and similarity. The X and Y axes show the difference in each one of the dissolution parameters of the Test formulation compared to the Reference formulation. When the difference in the two parameters is zero (coordinates 0,0 in the contour plots) the Test and Reference formulations are the same. Response surfaces for AUC and C_{max} display the Test formulations (combination of dissolution parameters) with probability ≥ 80% of being nonbioequivalent by AUC or C_{max} respectively. Density contour plots displaying probability of rejection from 0 to 100% for AUC and C_{max} are shown in the annexes (A.2-A.7). Response surfaces for f2 and PT delimit the

combination of dissolution parameters at which the probability of declaring non-similarity, using the corresponding test, is ≥ 80% (Sim-space).

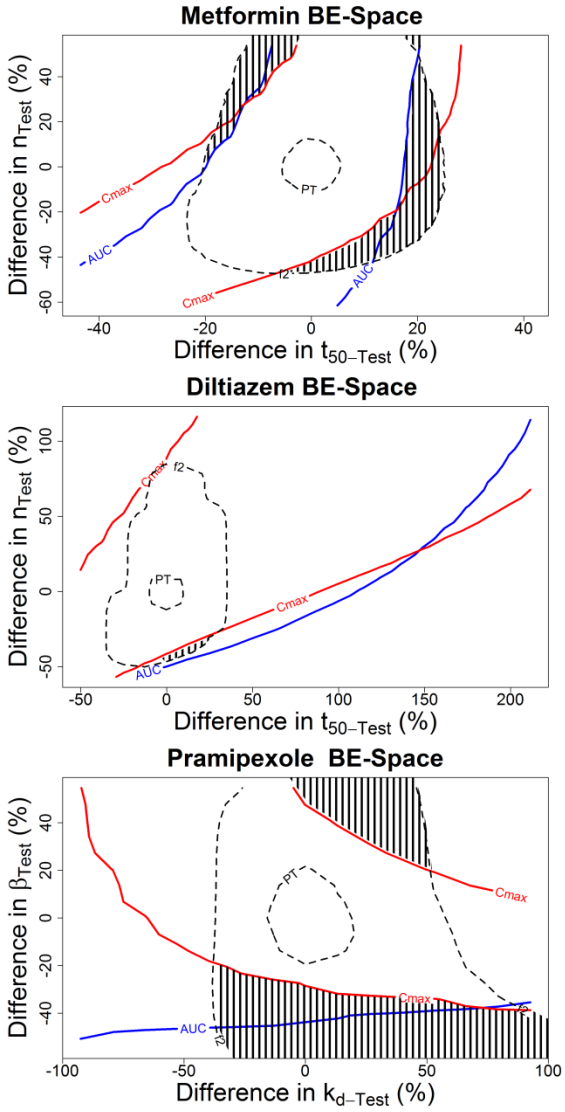


Figure 5.4. BE-Spaces of the three formulations. Contour lines for AUC and C_{max} display the Test formulations (combination of dissolution parameters) with probability ≥ 80% of being declaring nonbioequivalent by AUC or C_{max} respectively. Striped zones display the nonbioequivalent formulations declared as similar with f2. a) Metformin, b) Diltiazem and c) Pramipexole

PT was the most powerful test to declare as non-similar, Test formulations with changes in the dissolution model parameters compared to those of the Reference formulation. Consequently, formulations declared as similar with PT were always also bioequivalent and differences in C_{max} and AUC from the Test and Reference formulations were less than 1.5%. For all three drugs, f2 showed less power than PT, and several formulations declared as similar with f2 were not bioequivalent.

5.4.3. Customization of TDT

Dissolution profiles of the generated formulations were compared using TDT at different values of δ. Figure 5.5 shows the different similarity space (Sim-space) of TDT at increasing values of δ for the pramipexole formulation. A δ of 3.45 is the maximum δ at which all the formulations declared as similar with this test are also bioequivalent. A δ of 3.6 for metformin and 5.95 for diltiazem were found following the same procedure (A.8-A.9). This δ value represents the average tolerated difference (in %) between two formulations at any time point to produce bio-equivalent formulations under both criteria, AUC and C_{max}.

5.4.4. Effect of Drug & Formulation

The effect of changes in k_a, k_{el} (CL), t-lag, V_1, V_2 and CLD (model II in Figure 5.3) on the BE-space of the pramipexole formulation was studied. Changes in V_1, V_2 and CLD of ten-fold had no effect on the BE-space (A.10). Changes in k_a of 10-fold had no apparent effect on the BE-space of AUC or C_{max}.

Reductions in k_a of 50 fold or bigger showed an increasing reduction in the BE-space of C_{max} (Figure 5.6.a). Changes in t-lag had a direct impact in the BE-space of both AUC and C_{max} (Figure 5.6.b-c), when t-lag was not considered (t-lag =0) the BE-space was increased. For a t-lag of 2 hours, the BE-space was reduced to 25% of the original area. Changes in K_{el} had no effect on the BE-space of AUC. A slight effect in the BE-space of C_{max} by changes in k_{el} was observed, however, there was no change in the total area of the BE-space (less than 5%), but a small modification in the shape, regardless if the k_{el} was increased or decreased. The same effect was observed for metformin and diltiazem (A.11-A.12). Studied effects are summarized in Table 5.2.

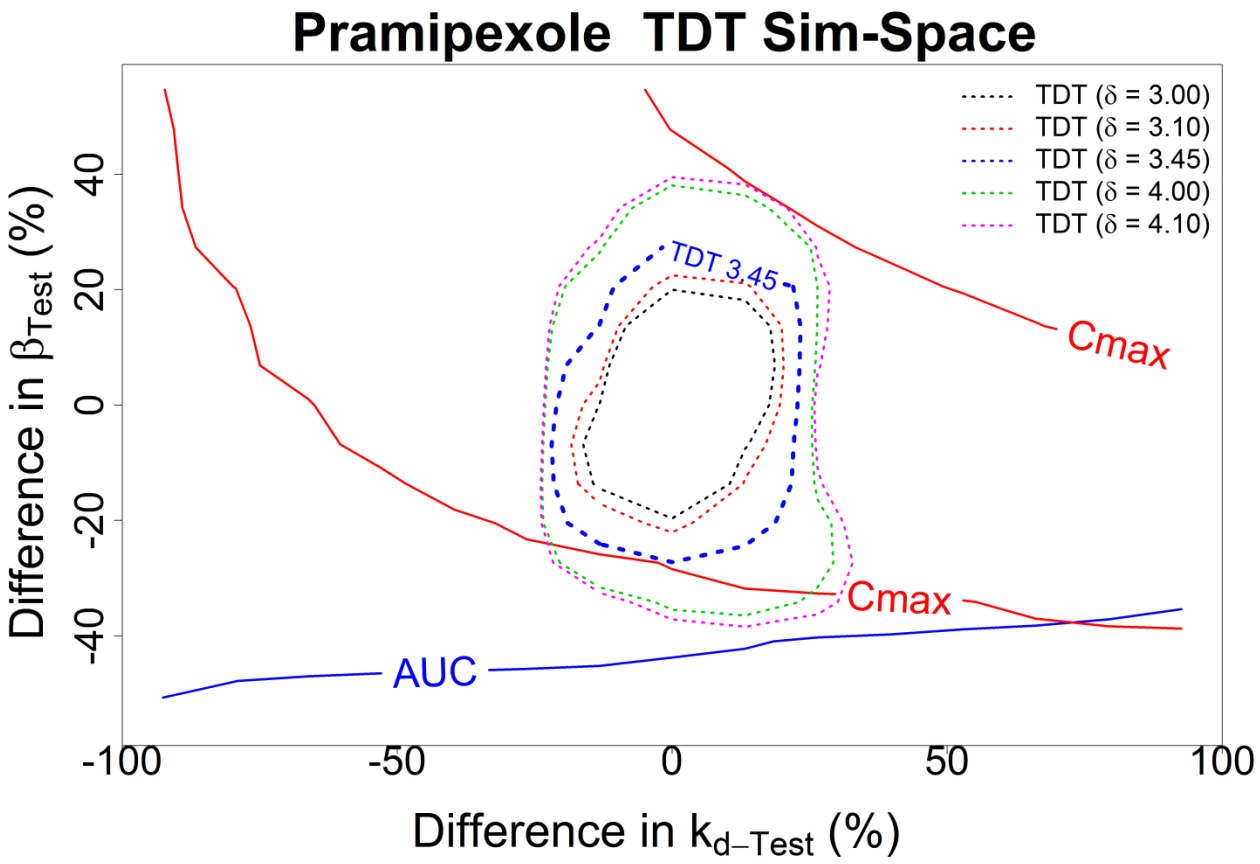


Figure 5.5. Customization of TDT. Sim-Spaces of TDT at different values of δ. for pramipexole formulations.

Table 5.2. Effect of volunteers sample size and drug parameters on the BE-space

	Pramipexole		Diltiazem		Metformin	
	AUC	Cmax	AUC	Cmax	AUC	Cmax
Increase sample size (volunteers)	↑	↑	↑	↑	↑	↑
Change Kel	↔	↑↓	↔	↑↓	↔	↑↓
Increase t-lag	↓	↓	NA	NA	NA	NA
Reduce ka	↔	↓	NA	NA	NA	NA
Change V1	↔	↔	NA	NA	NA	NA
Change V2	↔	↔	NA	NA	NA	NA
Change CLD	↔	↔	NA	NA	NA	NA

↑: Increase BE space; ↓: Reduce BE Space; ↑↓: Change BE space, some zones are augmented and some are reduced but no total increase or decrease; ↔ : No apparent effect; NA: Parameter not used in that model.

and c) effect of t_{lag} on C_{max} BE-Space.

5.4.5. Effect of BE trials Conditions and DPC-tests Conditions on BE-space and Sim-space

BE trials simulations were performed with different numbers of patients to study the effect of sample size in the BE-space, Figure 5.7.a shows the increment in the BE-space, in both AUC and C_{max}, at increasing numbers of patients. Nevertheless, the increment in the area from 12 to 18 patients was less than 3%, and the reduction in the BE-space from 12 to 6 patients was less than 5%.

The Sim-space was sensitive to the number of points used in the dissolution profile comparison, a reduction in Sim-space (increasing statistical power) at higher number of

time points was detected (Figure 5.7.b). These effects of number of patients and of time points were also observed for the metformin and diltiazem formulations (A.13-A.14).

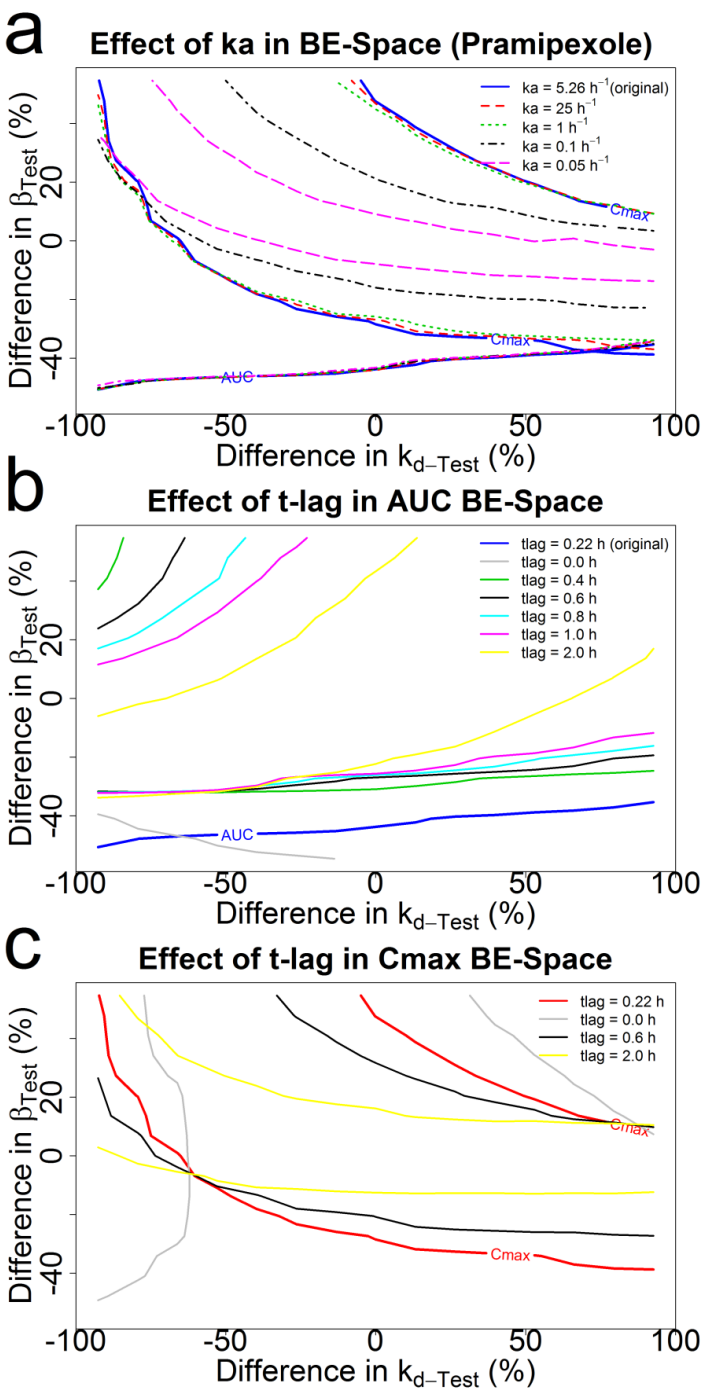


Figure 5.6. Effect of drug/formulation parameters in the determination of BE-Space. a) effect of k_a on BE-Spaces, b) effect of t_{lag} on AUC BE-Space, c) effect of t_{lag}.

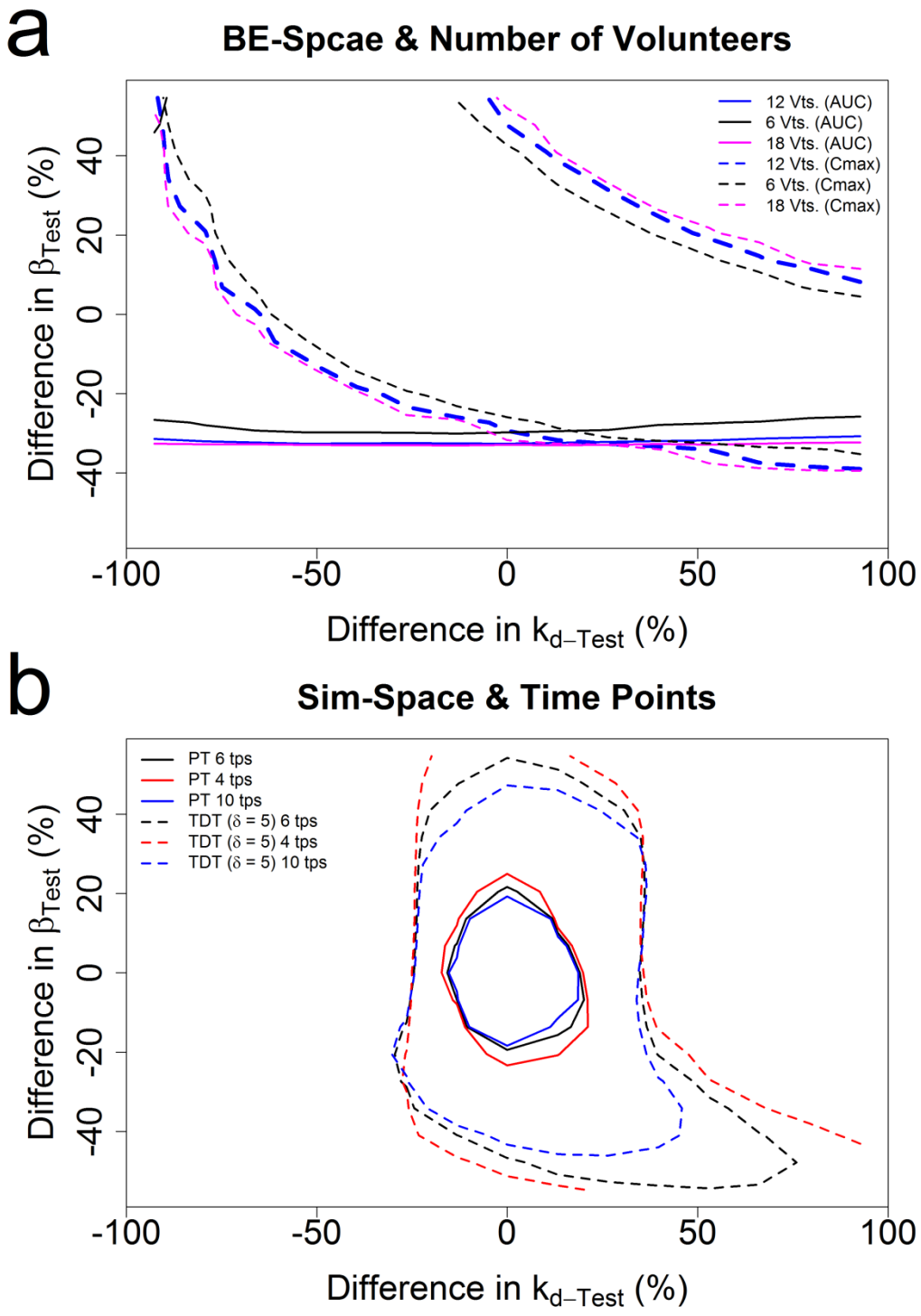


Figure 5.7. Effect of BE trials conditions and DPC-tests conditions on BE-Space and Sim-Space. a) effect of number of volunteers included in BE-trials on BE-Spaces, b) effect dissolution sampled time points on Sim-Space.

5.4.6. BE-space Compared to TDT to MDT and MRT

Mean dissolution time (MDT) and mean residence time (MRT) were calculated for all the reference formulations and compared to the BE-space. Surface responses of MDT and MRT are depicted in Figure 5.8 for pramipexole formulations. There is no overlap between the MDT or MRT response surfaces with the BE-space. Test formulations with the same MRT or MDT of the Reference formulation could be nonbioequivalent. Likewise, formulations with very different values of MDT and MRT are included in the BE-space. Similarly, BE-space did not match with the surface spaces of MDT or MRT for metformin or diltiazem (A.15-A.16).

5.5. Discussion

In this study, using IVIVC models, we aimed to design case-specific DPC-tests which were able to identify formulations likely to be bioequivalent in-vivo as in-vitro similar. As expected, the differences in dissolution necessary to produce nonbioequivalent formulations vary from one drug-formulation to another, depending on the drug and formulation. f2 failed to associate the *in vitro* similarity of two formulations with their comparative in-vivo performance in the three cases investigated. Moreover, f2 declared as similar not only formulations that are likely to be bioequivalent *in vivo*, but also formulations that are likely to be nonbioequivalent *in vivo* (Figure 5.4). This observation is in agreement with one of the criticisms of f2 [30, 32] of lack of flexibility to perform in a wider range of cases. We propose the need to analyze individually each formulation, and customize case-specifically, the limits of rejection of DPC-tests based on the type of formulation and the drug.

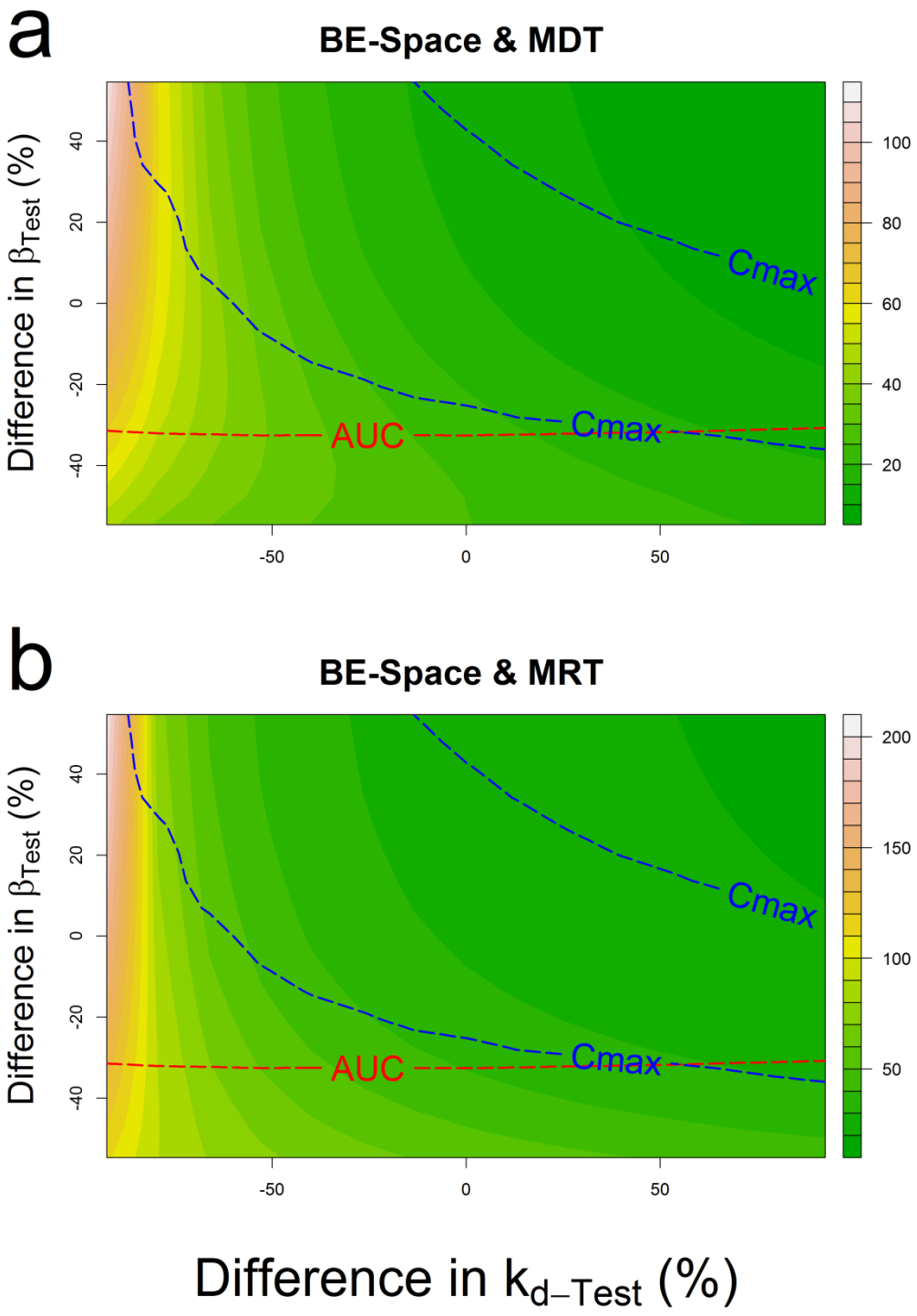


Figure 5.8. Comparison of BE-Spaces with a) MDT and b) MRT.

TDT is a DPC-test specifically designed to display this desired flexibility, by varying the δ value, without compromising its statistical properties (known type I and II errors). We were able to associate TDT limits of rejection with an in-vivo property (BE) of the tested formulations. Test formulations declared as similar to the Reference formulation using this customized DPC-test are expected to be also bioequivalent and consequently to retain the same efficacy and safety profile.

Having a customized DPC-test offers some technological and regulatory advantages; From the point of view of the manufacturer, once a DPC-test is customized, dissolution profile similarity can be used as a critical quality attribute (CQA) [88], for routine quality control, as a tool for post-approval changes, or to assure quality consistence of the same formulation manufactured in a new facility [88, 89].

From the regulatory point of view, an established DPC-test of an innovator formulation represents a fast, cheap and reliable protocol with clear acceptance criteria to test new generic formulations, reducing the costs and time of these submissions and potentially avoiding unnecessary in-vivo BE studies.

PT was the most restrictive test to confer similarity of dissolution profiles, typically, formulations declared as similar with this test did not differ in AUC and C_{max} more than 1.5% assuming the same patient with no intra-occasion variability. PT should be a more suitable test for monitoring similarity as a CQA because it would be more sensitive to detect CQA changes.

Ideally, all the bioequivalent formulations should be declared as similar, however, a total overlap of the BE-space and the Sim-space was not achieved with the investigated tests. Nevertheless, according to risk management principles [80, 90], the reduction of

risk caused to patients by declaring as similar formulations that are not bioequivalent, must be considered as a higher priority than reducing manufacturer risk of declaring as non-similar formulations that are bioequivalent. TDT allows maximization (Figure 5.5) of the overlap between BE-space and Sim-space without compromising the patient risk.

It has been stated that ER formulations can produce flip flop-like kinetics when the apparent $k_a < k_{el}$ (k_{el}/k_a ratio > 1), and could lead to miscalculations of the PK parameters [91], For pramipexole we observed reductions in the C_{max} BE-space for values of $k_a \leq 0.4\ h^{-1}$ (k_{el}/k_a ratio = 0.2). Calculating the BE-space for theoretical drugs with different k_{el} and k_a, the reduction in the C_{max} BE-space due to reduction in k_a was produced only at k_{el}/k_a ratios of 0.2 or higher (A.17). This seems to be the limit at which, the k_a is small enough, in comparison with the kel, to reduce the C_{max} in the PK profile. This aspect should be accounted for in the design of ER formulations, since a slow enough release can have in practical terms the same effect as reducing the k_a of the drug. We suggest that this flip flop-like phenomenon could be present at k_{el}/k_a ratios smaller than 1 (i.e. 0.2).

The BE-spaces of C_{max} from the diltiazem and pramipexole (Figure 5.4 and A.2-A.7) formulations were more affected for changes in the Y axis (shape parameter) than for changes in the X axis (speed parameter). It is appropriate to set DPC-tests sensitive to changes in the shape parameter because it is related to the release mechanism [28, 87], and small changes in this in-vitro mechanism may have a larger in-vivo impact.

For the metformin formulation, the AUC BE-Space and the C_{max} BE-space were affected by changes in both, the speed and shape parameters, mainly because of the narrow absorption window (t_{cut} = 4.77h). The δ value of TDT for this formulation was smaller

compared to the one for Diltiazem (Both using the same IVIVC model) supporting the concept that DPC-tests to compare formulations of drugs with small absorption windows must be very restrictive to declare similarity.

The δ value of TDT for the metformin formulation (BCS Class III drug with narrow absorption window, t_{cut} = 4.77h) was smaller compared to the one for Diltiazem (Both using the same IVIVC model), supporting the concept that DPC-tests to compare formulations of drugs with small absorption windows must be very restrictive to declare similarity. Metformin is a BCS class III drug, experiencing poor bioavailability (40-60%) [92, 93] and low permeability in-vivo (2.96-4.5 5 cm^{-7}/s) [92] and in-vitro (1.4 – 5 cm^{-7}/s) [94], this low permeability was included in the model (as smaller S1) and manifested as a slower progression (compared to diltiazem a BCS class I) in the plasmatic concentration versus percentage of dissolved drug relationship (A.18), reflected not only in a larger t_{max} (4.7 h vs 3.6 h for diltiazem) but also in the fact that the 50% of C_{max} in plasma is reached only after the 80% of the drug its released (Compared to 45% for diltiazem) .

Absorption is recognized as the rate-limiting step for BCS class III drug formulations [95, 96], however, for the metformin formulation, test formulations with faster release (smaller t_{50} and larger n) than the Reference formulation, generated plasma profiles with higher C_{max} and AUC, resulting in declaration of nonbioequivalence. Formulations with slower release (larger t_{50} and smaller n) were also declared as nonbioequivalent, generating plasma profiles with smaller C_{max} and AUC. These results indicate that in the case of ER products, dissolution may also play a substantial role in the in-vivo performance of BCS class III formulations.

The input function (equation 12) used in the metformin and diltiazem model, limits the potential processes that can be included in the model. For example, differential absorption across the GI tract, and concentration-dependent permeability mediated by a saturable process have been reported for metformin [92], and cannot be completely included in this particular model. However, these effects are not expected to be of high relevance in formulations of the same high dose (250 mg), unless the release is radically slow, in such case, the PK profile would be restricted more for the short absorption window, which is included in the model, than for the effect of saturable transport. The customized DPC-test should still be able to declare as similar only bioequivalent formulations. Notwithstanding, more mechanistic models, as the one used for pramipexole are preferable and necessary to develop in order to increase the knowledge of these types of characterizations and formulation comparisons.

MDT and MRT are statistical moments of the dissolution and PK-profile respectively, which have been proposed as predictors of the in-vivo performance of a formulation [72, 97]. When we compared the surface responses of these moments with the BE-spaces (Figure 5.8 and A.15-A.16) we found that MDT and MRT are not useful to discriminate between nonbioequivalent and bioequivalent formulations. Nonbioequivalent formulations can yield values of MDT and MRT identical or very similar to the MDT and MRT of the Reference formulation, and bioequivalent and nonbioequivalent formulations can yield the same values of MDT and MRT. We also observed that formulations with different MRT yield the same MDT and vice versa (A.19), showing that a correlation of these two moments is not possible in the investigated cases.

The observed increment in the BE-spaces when the volunteer sample size is incremented is in agreement with the fact that due to inter-individual variability, it is more likely to declare BE of two formulations when more volunteers are included in a BE trial. Likewise, the Sim-space was reduced when the number of time points sampled in the dissolutions profiles was increased. These results manifest that conclusions about BE or similarity can be affected by the specific setup of each particular comparison. Precise limits must be fixed in order to standardize comparisons and rejections criteria. Based on these results we propose that a number between 6 and 10 equidistant time points must be sampled in the dissolution comparisons, as a good predictor of BE studies with 12 individuals.

The biggest limitation of the introduced case-by-case DCP-tests customizations is that it requires a validated IVIVC model for each formulation, and in the case of generic drugs, serious harmonization efforts should be made to share these IVIVC models between agencies and manufacturers. The computational effort and knowledge required also currently impose real constraints on the wide diffusion and implementation of the strategy presented here. Besides, it is still questionable which is the most suitable procedure to declare BE [13, 98-101] and which other mathematical expressions could model drug release more mechanistically [40, 74] .

The strategy presented in this study of setting limits of rejection of dissolution similarity according to probabilities of presenting nonbioequivalence, can be refined, improved and applied to other drug-formulations when more IVIVC mechanistic models become available. For BCS class III drugs, further studies are required before analyzing the possibility of biowaving ER formulations of these drugs [102]. However, we propose that

under a deep case-specific analysis, biowaiving of ER of BCS class I drug formulations may be possible through customized DPC-tests.

5.6. Conclusions

In the present study, we have customized a DPC-test for three different ER formulations, linking limits of rejection with an *in vivo* attribute: the high probability of being nonbioequivalent. According to these simulations, formulations that can prove to be similar with the Reference formulation under the established conditions are likely to be bioequivalent. Established conditions were TDT with δ of 3.6, 5.95, and 3.45 for metformin, diltiazem and pramipexole respectively and sampling at least six time points in the release profiles. Once a specific test is developed for a particular formulation, this DPC-test can be used to explore post-approval changes by the manufacturer or to evaluate BE between products from different manufacturers, decreasing the need for future human BE studies and reducing costs of production. PT was the most powerful DPC-test and differences in C_{max} and AUC produced by formulations declared as similar with PT were less than 1.5% in all cases. T_{lag} and k_a were the drug/formulation parameters that influenced BE-space to the greatest degree. For the investigated cases, MDT or MRT were not suitable to detect bioequivalent formulations. Similar case-specific studies may support the biowaiving of ER drug formulations based on customized DPC-test.

6. Prediction of Equivalence in a Combined Dissolution and Permeation System using customized DPC-tests

The following author contributed to this chapter: José David Gómez-Mantilla, Sandra P. Gantzsch, Dominik Selzer, Thorsten Lehr, Ulrich F. Schaefer, Claus- Michael Lehr.

The author of the thesis made the following contribution to this chapter:

- Developing the mathematical model and fitting. (With contribution of D. Selzer).
- Design, Performance and interpretation of simulations.
- Construction of the equivalent and similar spaces and customization of the DCP-test.
- Design, performance and interpretation of the formulation optimization part.
- Writing the chapter.

Sandra P. Gantzsch manufactured the tablets and performed the experiments in the d/p-sytem.

6.1. Abstract

An apparatus for combined determination of dissolution and permeability (d/p-system) was previously described by our group and tested with propranolol IR and ER formulations. In this study we developed a mathematical model able to predict the permeated amount of propranolol from an ER formulation in the d/p-system. After considering this propranolol ER formulation as a Reference formulation, we were able to predict which dissolution profiles would lead to significant differences in permeability compared to the Reference formulation. Formulations that did not lead to significant differences in the calculated permeated amount were considered equivalent to the Reference formulation. Subsequently a DPC-test was customized to identify only equivalent formulations as *in vitro* similar. It was possible to group all the equivalent formulations in a set called the equivalent space. After customization, it was shown that a TDT with $\delta = 8$ will classify as *in vitro* similar to the Reference formulation only formulations that were also equivalent. Additionally two examples of computer-assisted formulation optimization were introduced. However, the need of further improvement in the model and the limitations of the conclusions are highlighted.

6.2. Introduction

The bioavailability of an orally administered drug is largely determined by the speed at which the drug crosses the gastrointestinal mucosa and reaches the blood stream. The speed at which a drug crosses a mucosa or a membrane is known as permeability and can be experimentally measured *in viv*o with humans and animals [103-106], or *in vitro* using cell lines and artificial membranes [107-112]. Due to its high morphological and physiological resemblance to intestinal cells and its spontaneous polarization into monolayers, the Caco-2 cell line model [113] is a very common procedure to study *in vitro* permeability. This model is useful to study transcellular and paracellular transport of drugs as well as transport events mediated by transporters [114]. Altogether, experiments performed with Caco-2 cells are considered the golden standard for *in vitro* prediction of intestinal drug permeability and absorption.[107, 115].

However, permeability experiments based on Caco-2 cells are normally performed with totally dissolved substances (drug and additives if used), which restricts the possibility of studying the effects of complete drug formulations on absorption. Furthermore, donor concentrations used in these experiments are normally constant, arbitrarily chosen and do not represent appropriately the *in vivo* situation, in which the drug concentration at the apical side of the enterocytes is variable and depends on the release out of the drug formulation.

As it has been stated in sections 3, 4.2 and 5.2, drug release from a formulation can also play an important role in the absorption and bioavailability of drugs administered as oral solid forms. Therefore, a tool to simultaneously study *in vitro* dissolution and permeability can be of great utility during the drug formulation development process to

study the effect of drug release and excipients on oral absorption in an easy and inexpensive way, which ultimately can lead to an optimization of the drug formulation.

A d/p-system that allows the evaluation of complete solid oral dosage forms in an open system using dynamic flow conditions was presented by Motz et al [60]. This d/p system has been improved to allow continuous measurements of the drug concentrations in the different compartments of the device [61] and continuous monitoring of the Caco-2 cells monolayer integrity by measuring the transepithelial electrical resistance (TEER) [62]. The d/p-system has also been recently adjusted to perform experiments with artificial membranes [63].

The d/p-system described by Motz et al [60] is schematically shown in Figure 6.1 and consists of two main parts. The dissolution module part is a flow through dissolution cell (USP apparatus 4). It is connected in line with the second part, the permeation module which enables the setup of a Transwell® with a Caco-2 cell monolayer. The apparatus includes an automated sampling and detection devices using a sequential injection analysis (SIA). As dissolution and permeation require different flow rates the dissolution and permeation modules are connected with each other by a stream splitter.

As it was stated in sections 3 and 5.2, declaring *in vitro* similarity of ER formulations is still an unresolved problem and methodologies able to link *in vitro* dissolution to an *in vivo* performance variable are highly desirable. Therefore, having two solid oral dosage forms of the same drug and strength, this d/p-system can help to predict whether a similar *in vivo* performance from the two formulations is expected or not. Moreover, after applying an adequate mathematical model of the d/p-system, a computational tool can be generated to predict the permeated amounts in the basolateral compartment in this

device from any dissolution profile obtained with the USP 4 apparatus. Therefore, when comparing the dissolution profiles between two formulations, it could be possible to predict in which cases the differences in release kinetics are large enough to produce significant differences in the permeability compartment of the d/p-system.

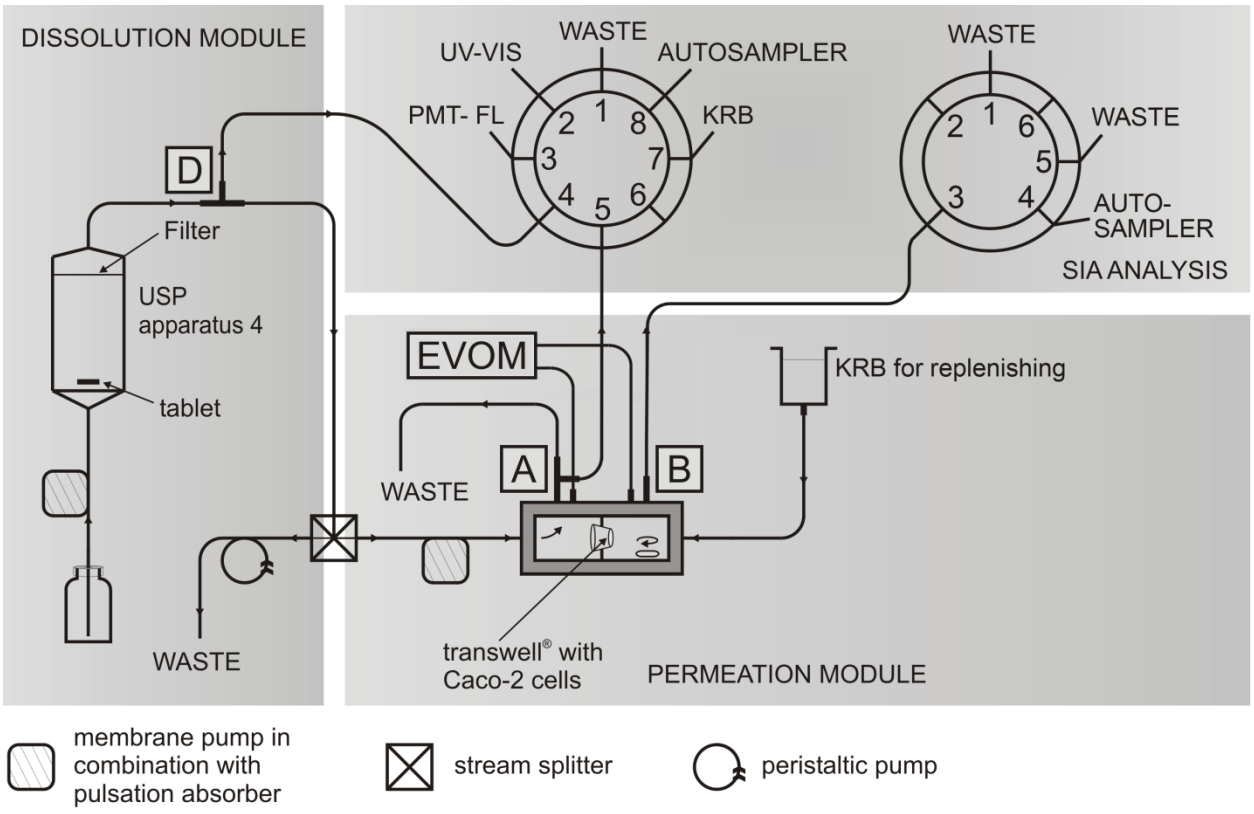


Figure 6.1 Schematic illustration of the d/p-system according to [60-63]. A, B and D are the sampling ports for apical, dissolution and basolateral compartments. KRB is the working buffer (Krebs Ringer Buffer), UV-VIS and PMT-FL are detector for UV and fluorescence respectively. EVOM is an epithelial voltohmmeter for TEER monitoring.

An ER formulation is usually designed pursuing two main objectives: 1) achieving safer and more constant *in vivo* concentrations of the administered drug, or 2) decreasing the administration frequency to improve compliance of the patient [29]. In both cases a

release as slow as possible is desired. The d/p-system complemented with the strategy presented here to predict equivalent formulations can be used to establish how slow the released of an ER can be without compromising its *in vivo* bioavailability. This would be expected to be proportional to the permeated amount in the system. Additionally, formulation optimization can be performed by predicting what formulations would lead to the largest amount of permeated drug in the permeation compartment or would have a slow release without compromising bioavailability, represented in this device by the permeated amount in the permeation module.

The aim of this work was first to efficiently model the data from the ER propranolol tablets in the d/p-system and secondly to identify through simulations, what formulations would be equivalent (in terms of permeated amount in the basolateral compartment (B)) to the ER tablets analyzed experimentally (considered as the Reference formulation). This group of equivalent formulations was considered as the equivalent space (eq-space). Once the critical differences in dissolution were established, TDT, a DPC-test, was customized to identify as *in vitro* similar only dissolution profiles from formulations with high probabilities of being equivalent to the Reference formulation. Additionally, nonlinear optimization was employed to predict what formulation would lead to the largest amount of permeated drug in the B compartment. The same method was employed to predict what formulation from the eq-space could have the slowest release.

6.3. Materials and Methods

All the experimental data were taken from the work of Gantzsch et al [63], in summary, ER tablets with 10 mg propranolol were prepared according to Motz et al [60], Caco-2 cells following an established protocol [60, 61] Passages 61 – 70 within 21 – 25 days after seeding were used for experiments. Only Transwells® showing TEER values above 300 Ω*cm² were used.

Experiments were performed in an automated apparatus (d/p-system) [60-62]. Two propranolol tablets were inserted in the flow-through dissolution cell for each experiment. At certain defined time points, sampling took place at the apical (A) and basolateral (B) compartment of the FTPC. For a detailed description see [63] .

6.3.1. Mathematical Modeling

Concentration of the drug in the Dissolution module was described by the Weibull function (equation 6, section 3.3). Fitting of the Weibull function was performed using a nonlinear least square fit. The dose from the formulation Q_{inf} was considered as an unknown due to the variability of propranolol content in the tablets (CV = 13%).

The transit through the d/p-system was modeled through a two-compartment pharmacokinetic model according to

Figure 6.2.

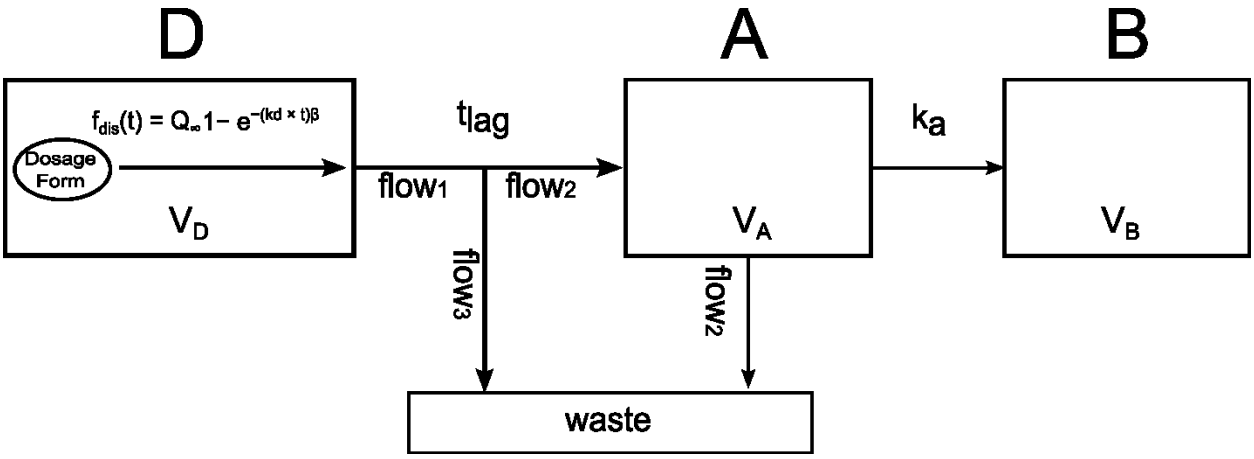


Figure 6.2 Illustration of the pharmacokinetic two compartments model for d/p system.

Change in D (mass in the dissolution module) was described by equation 16:

$$\frac{dD}{dt} = Q_\infty \times kd^\beta \times \beta \times t^{(\beta-1)} \times e^{-(kd \times t)^\beta} - D * \frac{flow_1}{V_D} \qquad \text{equation 16}$$

In which kd, β and Q_∞ are defined as in equation 6, $flow_1$ is the flow rate leaving the dissolution port, and V_D is the volume of the dissolution module $(13.18\ mL)$.

Change in A (mass in the apical Compartment) was described by equation 17:

$$\frac{dA}{dt} = \frac{1}{6.5} * D * \frac{flow_1}{V_D} - A * k_a - A * \frac{flow_2}{V_A} \qquad \text{equation 17}$$

k_a is the absorption constant of the drug through the basolateral compartment, $flow_2$ is the flow rate leaving the apical compartment, and V_A is the volume of the apical compartment. $(5.3\ mL)$. The factor $\frac{1}{6.5}$ describes the split of $flow_1$ into $flow_2$ and $flow_3$ shown in

Figure 6.2. A t_{lag} of 4 minutes was considered between compartments D and A according to times measured experimentally.

Change in B (mass in the basolateral compartment) was described by equation 18:

$$\frac{dB}{dt} = A * k_a \qquad \text{equation 18}$$

6.3.2. Construction of Equivalent Space (Eq-space)

The strategy used to identify equivalent formulations was similar to the one described in section 5.3.1 and illustrated in Figure 6.3. *In vitro* dissolution profiles were simulated for several formulations by modifying the Weibull model parameters and the permeated amounts were generated using the model described in the previous section.

Inter-individual variability (IIV) was include for each parameter in the model to fit the reported experimental variability. In total, 1000 equivalence simulated studies per scenario were conducted. In each study, 12 tablets of the Reference formulation and 12 tablets of the Test formulations were generated by Monte Carlo simulations. Equivalence between formulations was determined by calculating 90% confidence intervals (90%CI) of the ratio between Test and Reference means (n=12) after log-transformation of the permeated amount. The formulations were considered equivalent if the 90%CI of the ratios were contained within the acceptance interval of 80.00 – 125.00%. Eq-space was delimited by bounding the Reference formulations with high chances of being non-equivalent ($p \geq 0.8$). Once the Eq-space was delimited, TDT, a DPC-test, was customized to declare the formulations that are likely to be non-equivalent as non-similar, using the same procedure as in section 5.4.3.

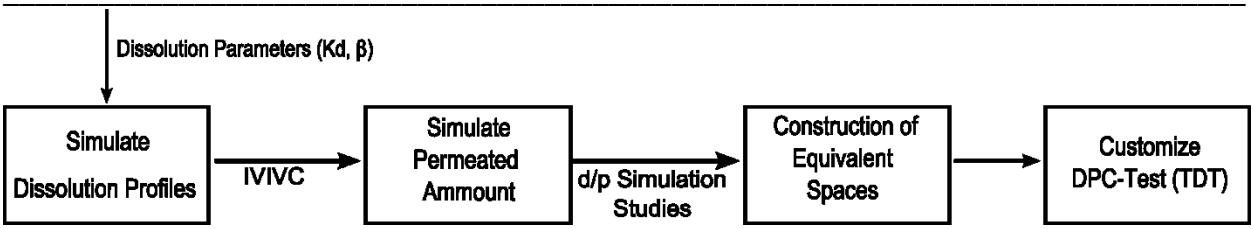


Figure 6.3 Strategy for building the equivalent spaces and customize a DPC-test.

6.3.3. Dissolution Profile Comparisons

Dissolution profiles from the Reference and Test formulations were compared using the DPC-tests described in section 4.3.4.

6.3.4. Customization of TDT

As described in section 5.4.3 Sim-spaces of TDT at increasing values of δ were constructed in order to find the maximum δ at which all the formulations declared as similar uisng this test are also equivalent.

6.3.5. Formulation Optimization

Using the same simulated dissolution profiles from Test formulations as in the previous section, the cumulative permeated amount was calculated for each Test formulation. The formulations with larger calculated permeated drug amounts were considered as the optimized formulations. Similarly the t_{85} of all the equivalent formulations was calculated to identify the equivalent formulations with the longest t_{85}.

6.4. Results

6.4.1. Model Fitting of the Combined Dissolution and Permeation Data

Dissolution data was modeled by the Weibull function and after fitting the average profile was described by equation 6 with $kd = 2.12 \pm 0.02 \times 10^{-2}\, h^{-1}$, $\beta = 1.14 \pm 0.01$ and $Q_{inf} = 17.04 \pm 0.06 \times 10^{4}\, \mu g$. Data presented as a mean $\pm\, S.D.$. The corresponding fitting plot is shown in Figure 6.4. For the permeation into the basolateral compartment, a permeation constant $k_a = 3.87 \pm 0.31 \times 10^{-4}\, h^{-1}$ was calculated. The fitting plot is shown in Figure 6.5.

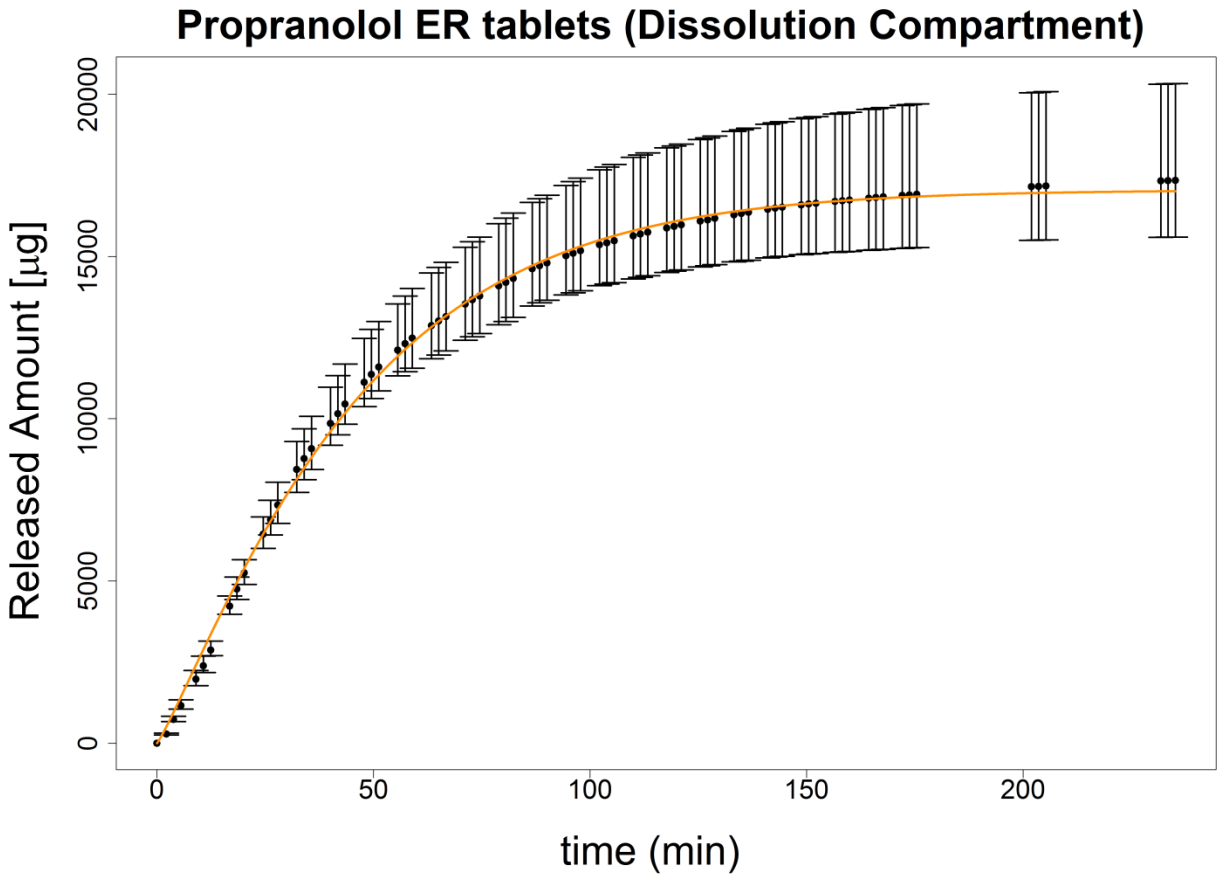


Figure 6.4 Fitting of the data from dissolution module. Experimental data (•) and predicted values (solid line), data presented as mean and range $n \geq 3$.

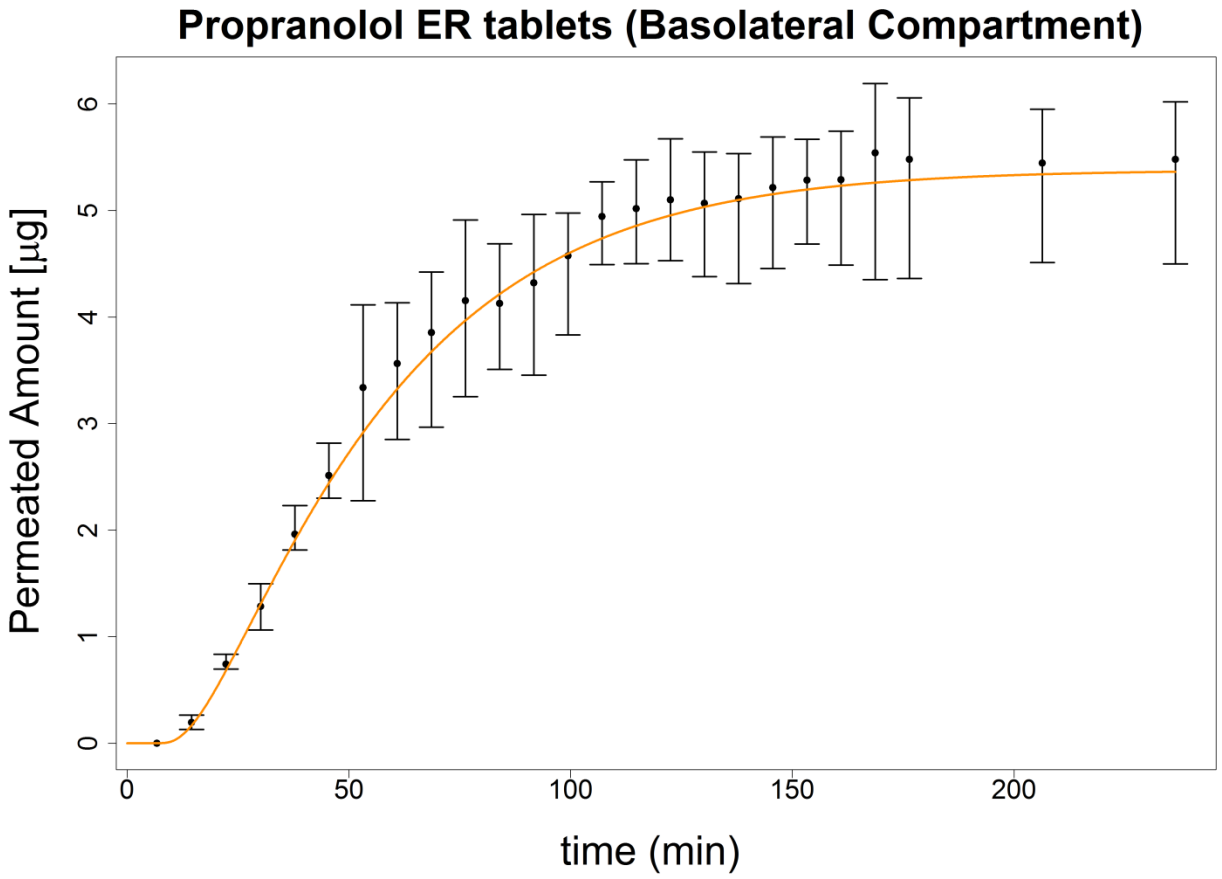


Figure 6.5 Fitting of the data from the basolateral module. Experimental data (•) and predicted values (solid line), data presented as mean and range $n \geq 3$.

6.4.2. Equivalent Space

Figure 6.6.a shows the *in vitro* dissolution profiles of the experimental formulation, for this purpose considered the Reference formulation and the dissolution simulated profiles for several Test formulations. On Figure 6.6.b, the contour plot displays the probability of a Test formulation for being non-equivalent (in terms of permeated amount) to the Reference formulation. It can be observed that Test formulations 1-3 have high probabilities of being non-equivalent, while Test formulation 4 has a low probability of being non-equivalent. Similar to section 5.4.2, the Eq-space is delimited by

separating the formulations based on a probability ≥ 80% of them being non-equivalent. All the formulations declared as similar with PT presented differences in the permeated amount compared to the Reference formulation of less than 0.8%.

6.4.3. Customization of TDT

Figure 6.7 shows two different similarity spaces (Sim-space) for TDT at different values of δ. A δ of 8 is the maximum δ at which all the formulations declared as similar with this test are also equivalent in terms of permeated amount of drug in the d-p system. This δ value represents the average tolerated difference (in %) between two formulations at any time point to produce equivalent formulations for the criteria of permeated amount.

6.4.4. Formulation Optimization

Figure 6.8 shows the calculated permeated amounts in the d/p-system for the formulations used as test formulations in previous experiments (Figure 6.6). The formulation with release kinetics described by a Weibull function with $kd = 3.52 \times 10^{-2}$ and $\beta = 1.3$ showed the largest calculated permeated amount ($6.37\ \mu g$) and is represented in the contour plot as Max PA. Its dissolution profile can be observed in Figure 6.10 in comparison to the Reference formulation. Similarly, Figure 6.9 displays the difference in release kinetics of the equivalent and non-equivalent formulations expressed as t_{85}. The formulation with a release kinetics described by $kd = 9.17 \times 10^{-3}$ and $\beta = 0.9$ was the equivalent formulation with the longest t85 ($223\ min$) and is denoted in the contour plot as Max t_{85}.

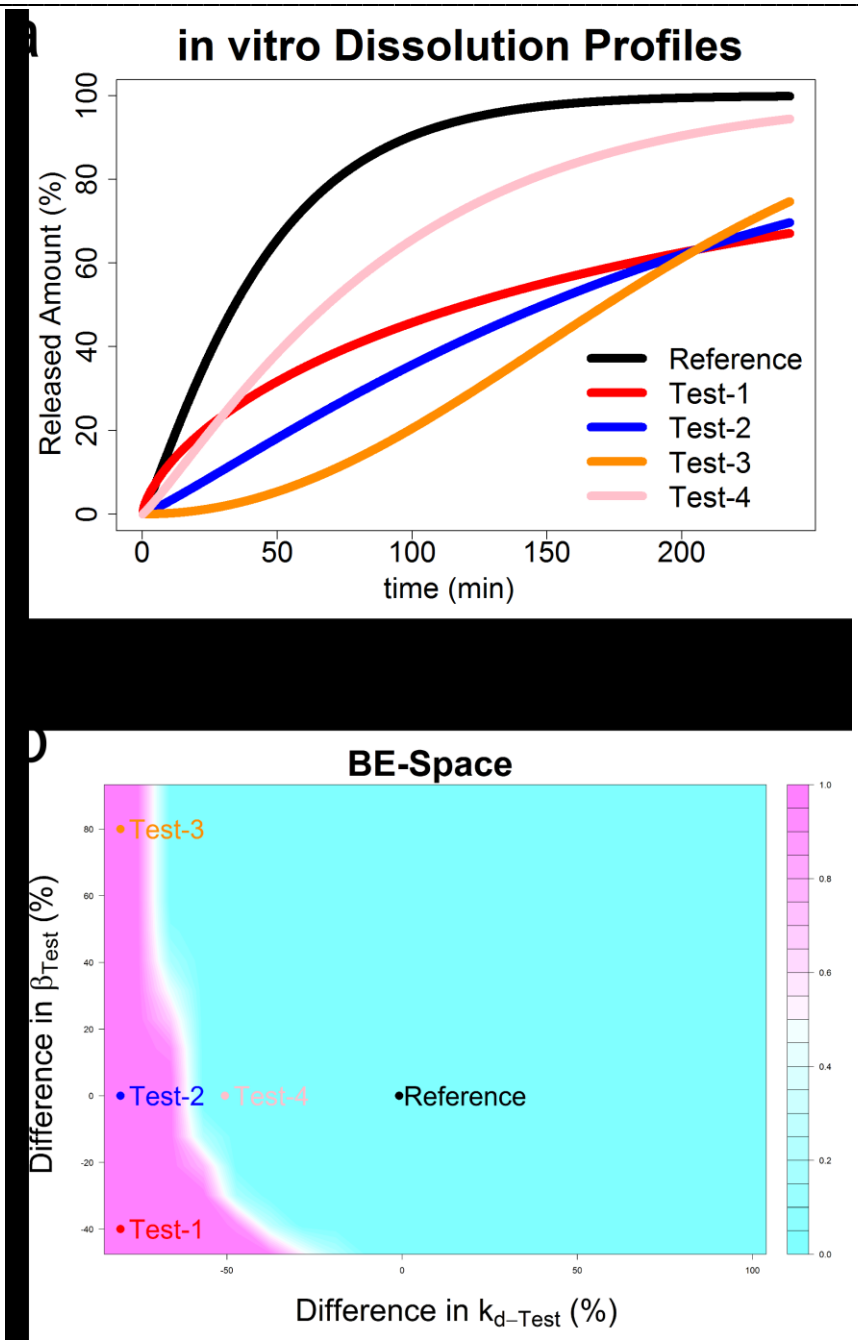


Figure 6.6 Construction of the Eq-space and identification of non-equivalent formulations. On the left the color scale of the probability of being nonequivalent to the Reference formulation. Formulations with high probabilities of being nonequivalent are located in the purple area.

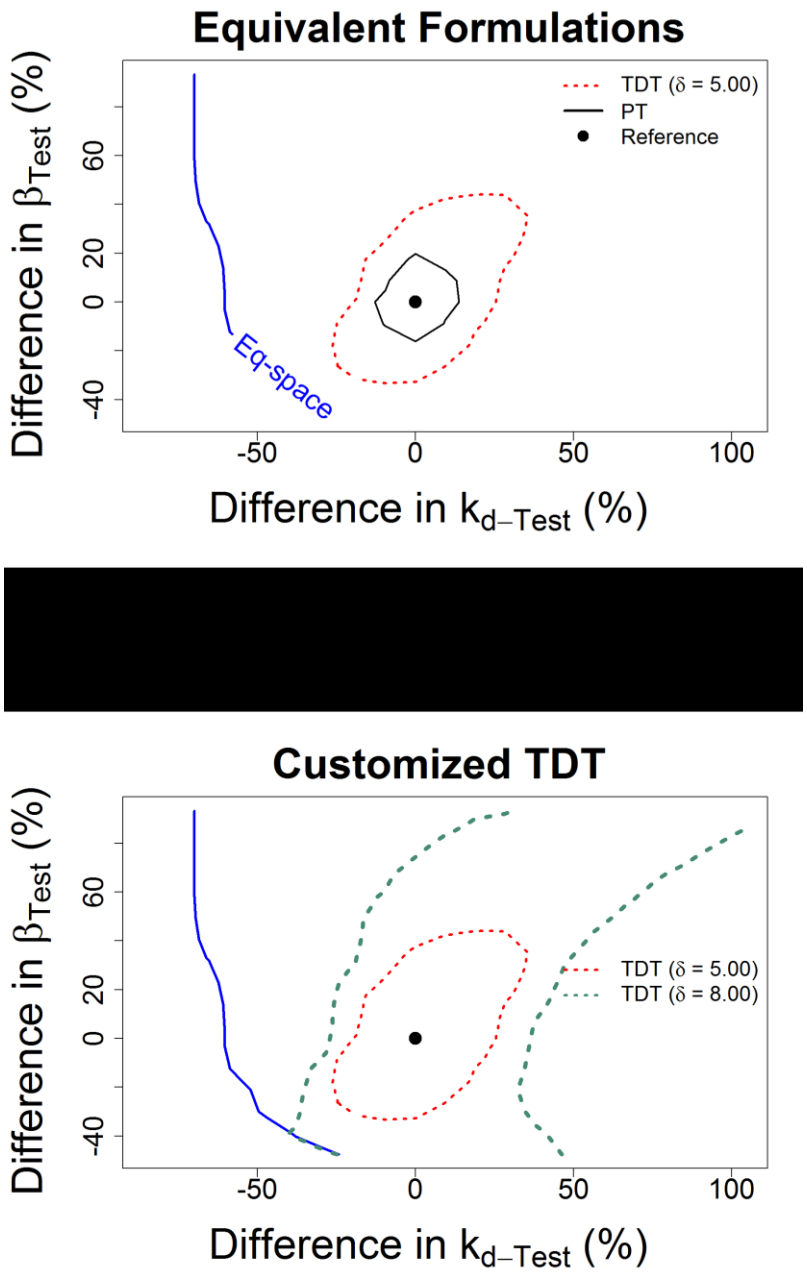


Figure 6.7. Sim-space and Customization of TDT Sim-Spaces of TDT at different values of δ. for propranolol formulations.

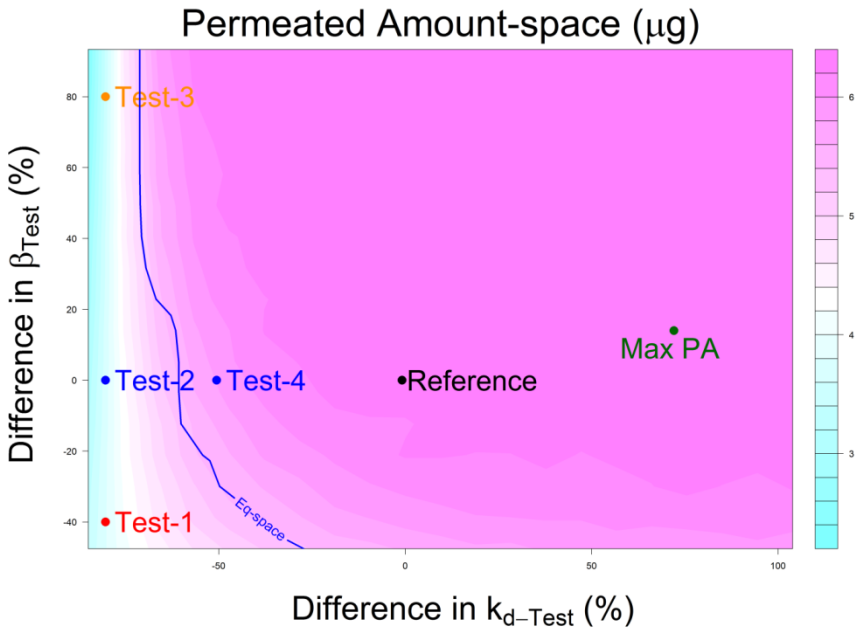


Figure 6.8. Counter plots of the calculated permeated amounts in the d/p-sytem. Max Pa, points to the formulation of largest calculated permeated amount ($6.37\ \mu g$).

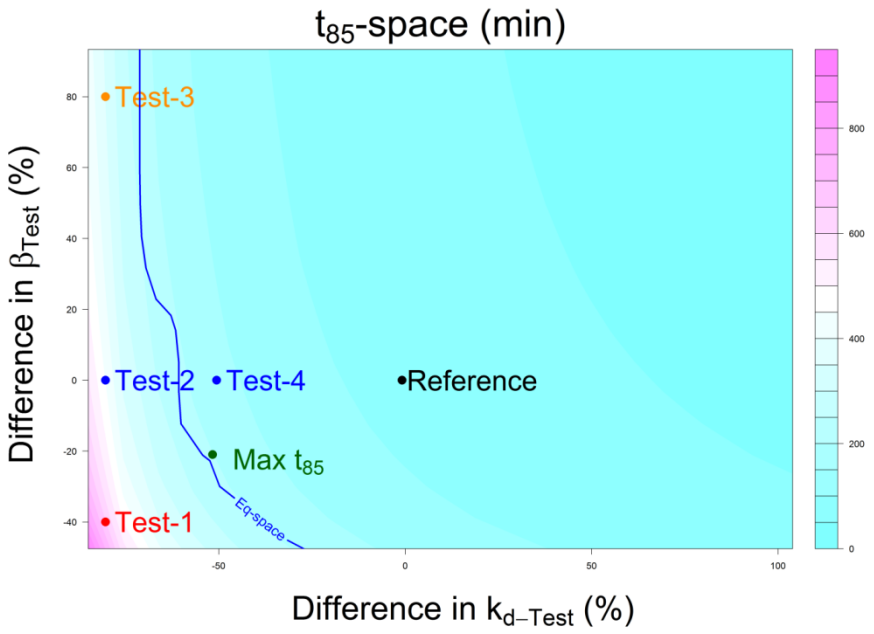


Figure 6.9. Counter plots of the t_{85} of the formulations in combination with the equivalent space of the d/p-sytem. Max t_{85} points to the formulation with the maximum possible t_{85} (223 min) that is inside the equivalent space.

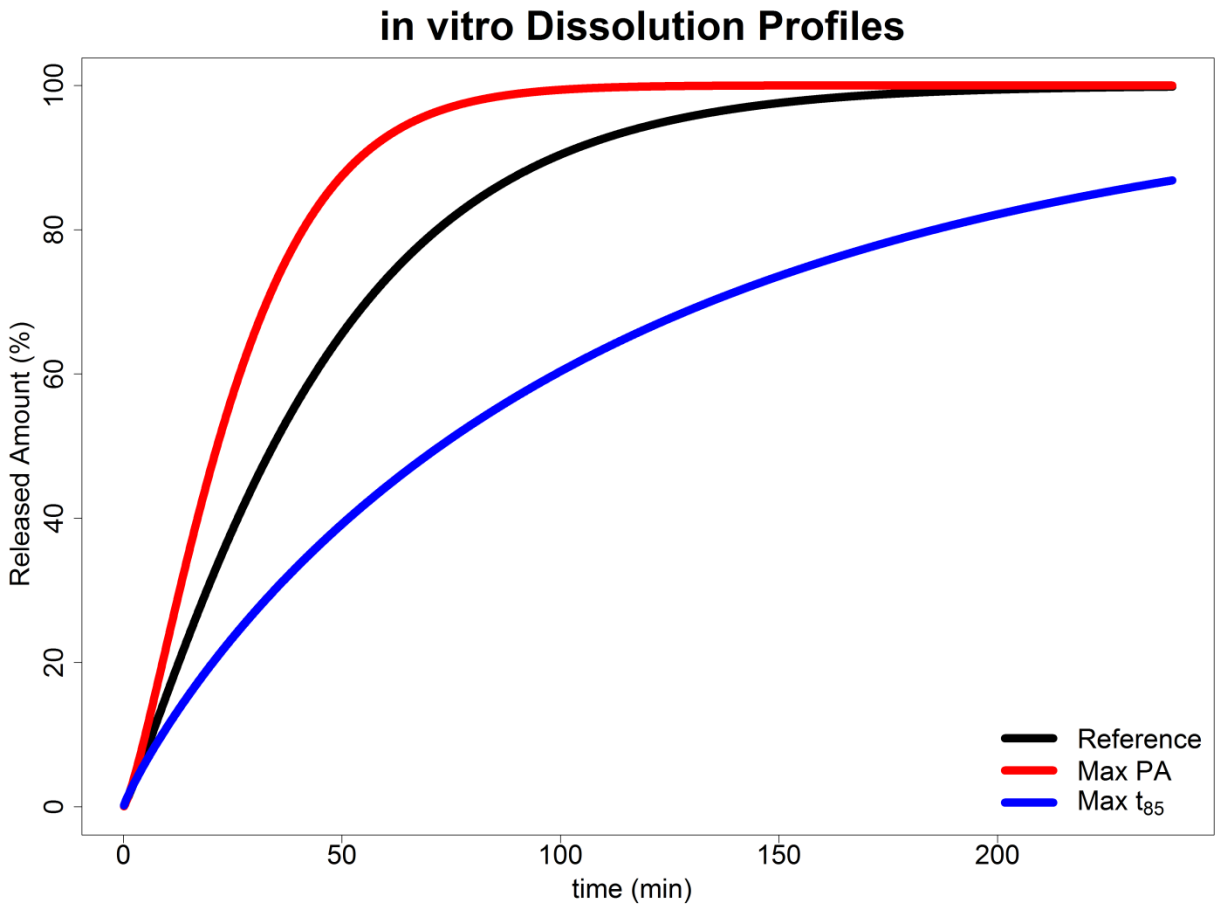


Figure 6.10. Dissolution profiles of the optimized formulations.

6.5. Discussion

It is highly desirable to link dissolution tests to *in vivo* performance of the formulation tested [28, 79]. In section 5 it was presented how to do make that link when and IVIVC model is available. However in early stages of drug development an IVIVC may not be always available and a fast and inexpensive system to study the effect of formulation on permeability can be of great use. In this study we also evaluated the potential of this d/p-system as a tool to predict equivalency of different formulations with a given Reference formulation as well as for computer-assisted formulation optimization.

It was possible to predict what formulations would be equivalent to the Reference formulation in terms of permeated amount in compartment B. In general, only formulations with very slow release kinetics lead to a calculated permeated amount significantly lower than the reference formulation. In agreement with the results in sections 4.4.3 and 5.4.2 and , PT showed very good statistical power and all the formulations declared as similar with this test differ less than 0.8% in the calculated permeated amount for the basolateral compartment. That reinforces the potential of this test in the sphere of quality by design where tools which detect small changes in a critical quality attribute (in this case dissolution) are highly needed.

In the sphere of generic drugs, the regulatory agencies could use the strategy presented here to detect if an ER Test formulation is equivalent to an ER Reference formulation in the market. Moreover, by customizing a TDT DPC-test, a simple *in vitro* dissolution test, without the permeability module may be sufficient to prove equivalency between two ER formulations. Previously, it must be proven that, for one, the excipients of the formulation do not affect the absorption of the drug, for two, the drug should not be a prodrug and it should not have a narrow therapeutic index or be intended to be absorbed in the oral cavity. Nevertheless, in order to achieve this level of reliance on the information generated by this strategy, it must be first verified that the d/p-system is a good representation of the *in vivo* case, which only can be concluded with further experiments. Likewise, the mathematical model can be improved to include the whole geometry of the GI tract and the inferences about the *in vivo* case would be more reliable. More generally, at least for ER formulations of BCS class I drugs like propranolol, in which absorption is expected to be driven by the release kinetics, the

strategy presented here is a reasonable approximation that can be improved step by step to identify equivalent formulations.

If the objective of the formulation development is to design an oral solid dosage form with the highest possible bioavailability, it is of advantage to predict what formulation would exhibit the largest permeated amount in a d/p-system. Likewise, if the objective of the formulation development is to design a formulation with the slowest possible release to achieve safer plasma concentration or decrease administration frequency without sacrificing bioavailability, it is of advantage to predict which of the formulations of the eq-space has the slowest release. We presented in this work a computer-assisted tool to help in the formulation optimization process. This tool can be employed to maximize the permeated amount of drug in the d/p-system which ultimately could represent a maximized absorbed drug *in vivo*. Similarly it can be used to find the equivalent formulation with the slowest possible release kinetics or in general an equivalent formulation with a specific desired kinetics. Nonetheless, this method is constrained by the same limitations as in the case of identifying equivalent formulations (the uncertainty of how good the d/p-system mimics the *in vivo* case), but also retains the same improve opportunities.

All the simulations were performed mimicking the typical experiment in the d/p-system which is performed for no more than 4 hours to assure the integrity of the Caco-2 cell monolayer. This time restriction is mathematically equivalent to an absorption window of 4 hours and may not mimic properly the *in vivo* case. Using the mathematical model it would be possible to explore longer absorption windows that are not possible experimentally. Nevertheless, It must be considered that the results obtained from eq-

space, customization of TDT as well as formulation optimization are valid only for an absorption window of four hours and that different absorption windows may lead to different results.

The mathematical model presented here can also be used to detect which setup variables (flow rates and volumes) have a stronger effect on the concentrations in the apical and basolateral compartments, therefore it can be determined whether the discrimination power of the d/p-system, i.e. the capacity to transform differences in dissolution into differences in the permeated amounts can be increased by a different setup. Also, deeper explorations can be performed to check, by simulations, if the system can be improved by mounting more permeability units after the port D to simulate a segment of the intestine exposed to decreasing concentrations of the drug. In general this mathematical model can be used to explore different alternatives of the apparatus setup to optimize it or to mimic better the *in vivo* case.

The presented mathematical model described reasonably well the experimental data. However, it is based on limited data (only one formulation) with high variability in all the ports sampled. Therefore conclusion must be drawn carefully and considering that the model must be improved considerably by including more experimental data. Additionally, the step in the splitter pump can be modeled differently by including expressions that describe the diffusion in the transit compartments, since the transit volume in the connecting tubes is almost 10 mL and the transit time is between 4 and 7 minutes.

6.6. Conclusions

A mathematical model was successfully developed to describe the experimental data of a d/p-system. Taking the tablets evaluated experimentally as a Reference formulation, the mathematical model was used to predict which formulations would be equivalent in terms of the permeated amount in the basolateral compartment to the Reference formulation. The group of equivalent formulations was called the eq-space, and it was possible to customize the DPC-test TDT in such a way that when the customized test (a TDT with $\delta = 8$) declares a formulation as *in vitro* similar to the Reference formulation, these formulations will most likely be equivalent regarding their permeated amount in the basolateral compartment. Through linear optimization it was possible to identify the equivalent formulations which would exhibit the longest possible t_{85} or the largest amount of permeated amount in the basolateral compartment. The mathematical model can also be used to evaluate modifications in the apparatus setup and configuration. However, the model must be further improved, mainly by the inclusion of more experimental data and better description of the transit between the compartments.

7. Summary and Outlook

Dissolution testing has been widely used as a global indicator of the ability of a dosage form to release API, and in doing so enable it to reach its site of action. Furthermore, comparative dissolution testing has been explored as a tool to evaluate equivalency between formulations. However, the current official procedure to compare dissolution profiles, the f2 similarity factor, lacks solid statistical foundation and the level of statistical uncertainty is unknown. Moreover, the limits of the f2 similarity factor to declare *in vitro* similarity ($f2 \geq 50$) not derived from any specific biopharmaceutical property. Such a test therefore cannot be considered as a good predictor of *in vivo* performance, especially in the case of ER formulations. The aim of this work was to design, develop and explore new DPC-tests with stronger statistical basis than the currently employed methodologies f2 similarity factor, and to link the limits of rejections of these new DPC-tests with significant differences in important biopharmaceutical properties with more predictive power of *in vivo* formulation performance. Examples of DPC-test customization have been presented for three ER formulations (metformin, diltiazem and pramipexole) using IVIVC models; one example of DPC-test customization for a propranolol ER formulation using a d/p-system has been also illustrated. In summary:

Exploration of the two new DPC-Tests

- Two new statistical tests, the PT and the TDT, have been presented for dissolution profile comparison. In these tests both type-I and type-II errors can be quantified; both tests also have stronger statistical basis than the current

alternative (e.g., the f2 similarity factor). The two new tests showed acceptable robustness at standard conditions of variation ($CV_{95} \leq 0.1$).

- A strategy to design and perform a dissolution profile comparison under typical principles of statistical experimental design has been presented.
- An optimized time sampling strategy was introduced in the current work. This strategy should be employed when possible in DPC-tests to avoid artificial discontinuities in the statistical power. The exception to the recommended use of such a strategy is in the case of PT which is not susceptible to this effect.

Customization of DPC-Tests

- A DPC-test, TDT, was customized for three different ER formulations and its limits of rejection linked with an *in vivo* attribute: the high probability of being nonbioequivalent. Formulations that proved to be similar to the Reference formulation using a customized DPC-test (TDT with δ of 3.6, 5.95, and 3.45 for metformin, diltiazem and pramipexole respectively) are likely to be bioequivalent. Such a result demonstrates a clear application of formulation-specific DPC-test in the exploration of post-approval changes by a manufacturer, or the evaluation of BE between products from different manufacturers. The use of such DPC-tests could decrease the need for future human BE studies and reduce costs of production.
- T_{lag} and k_a were found to be the drug/formulation parameters that influenced BE-space to the greatest degree for the pramipexole ER formulation.

- MDT or MRT were not suitable for bioequivalence prediction in the case of the ER formulations investigated (metformin, diltiazem and pramipexole).
- The presented work leads to the suggestion that case-specific studies, using IVIVC or d/p-systems may support the biowaiving of ER drug formulations, based on customized DPC-tests.

Application of DPC-test customization in a d/p-system

- A mathematical model was successfully developed to describe the experimental data of a d/p-system. This mathematical model could be used to predict which formulations would be equivalent to a Reference formulation in terms of the permeated amount in the basolateral.
- By establishing *in vitro* similarities between Test and Reference formulation, a customized TDT ($\delta = 8$) could be used to identify formulation likely to be equivalent with respect to the permeated amount in the basolateral compartment of the d/p-system.
- A computer assisted tool for formulation optimization has been presented which allow for the design of formulations with larger permeated amounts in the d/p-system and longer t_{85}.

Properties of PT

- PT was the most robust and powerful test in all the conditions studied (even in conditions of high variability $CV_{95} \leq 0.2$ and reduced sample sizes). Differences in C_{max} and AUC produced by formulations declared as similar with PT were less

than 1.5% for the metformin, diltiazem and pramipexole ER formulations. This test is therefore strongly recommended for identifying small differences in dissolution.

- In the case of the propranolol ER formulation, PT also showed excellent statistical power. All the formulations declared as similar with this test in the d/p-system differed by less than 0.8% in the calculated permeated amount in the basolateral compartment of the system.

Outlook

While the work presented in this thesis shows a promising application of both PT and TDT, it must be considered that more experimental confirmation is needed to prove the suitability of the new DPC-tests as alternatives for performing dissolution profile comparison. In particular, experimental verification of the lack of effect for iid-conditions (section 4.3.7) is necessary to ensure an adequate performance of the TDT under the standard (no iid-conditions).

Another step that should be explored is the linking of customized DPC-tests with clinical outputs or pharmacodynamics biomarkers instead of BE probabilities. This could be achieved using pharmacokinetic pharmacodynamic (PK/PD) modeling which, of course demands a deeper understanding of the pharmacology of the drug as well as a higher level of detail in the mathematical modeling.

The mathematical model employed to describe the data from the d/p-system has many opportunities for improvement. Specifically the inclusion of more experimental data and a better description of the transit between the compartments should be considered.

Similarly, attractive opportunities such as the exploration of formulation and apparatus optimization, and inclusion of data from drugs which are substrates of active transporters remain open for further development.

It should be emphasized that the excellent statistical power exhibited by the PT is a very promising outcome of this thesis. It is worth exploring the potential of PT as a quality by design tool due to its ability to detect even small differences in dissolution profiles. When dissolution profiles are used as a critical quality attribute (CQA), the performance of the formulation will not be compromised as long as similarity is declared by PT. Also knowledge of a manufacturing process can be improved by checking the influence of different process variables (compression pressure, granulation time, percentage of polymer etc.) on this CQA, and identifying the changes in these variables required to produce a non-similar dissolution profile.

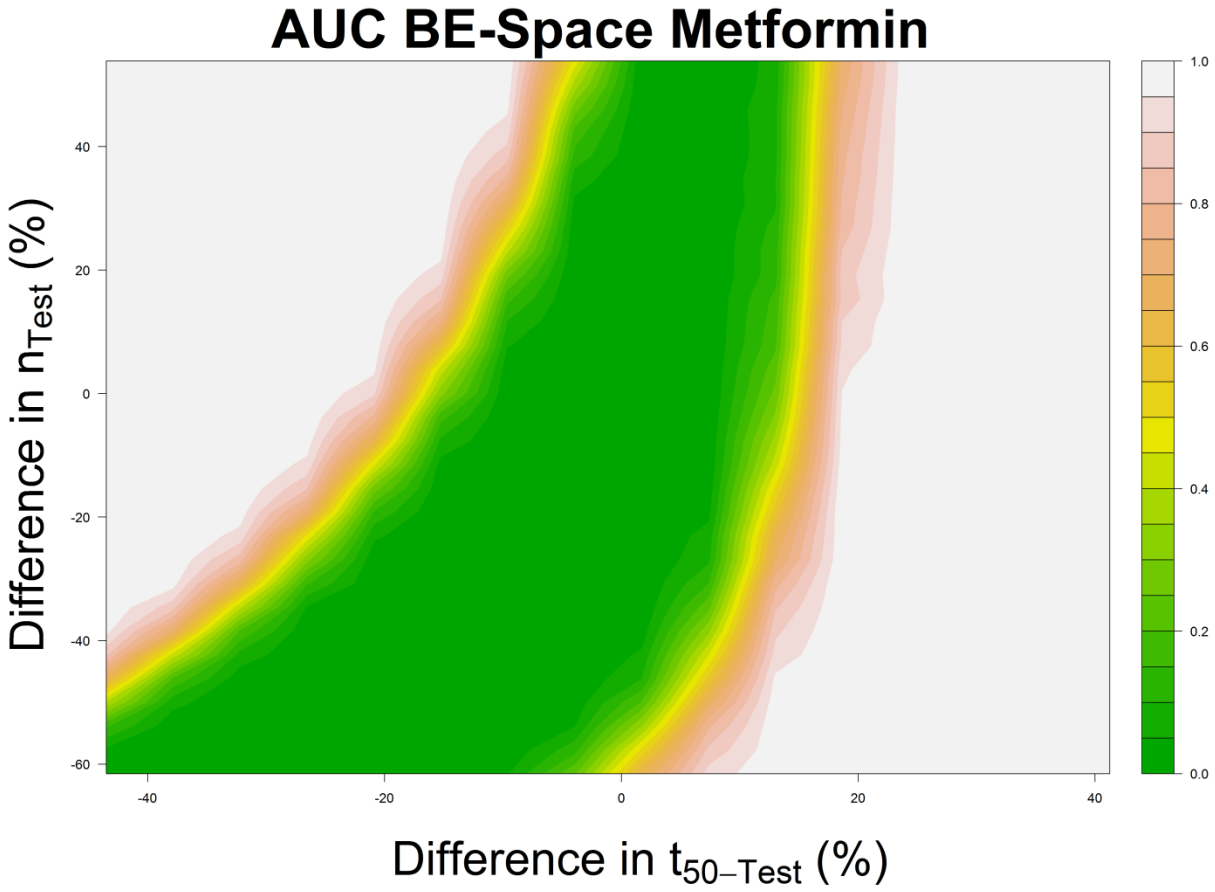
8. Abbreviations

90% CI	90% confidence intervals
A	Apical compartment
API	Active pharmaceutical ingredient (s)
AUC	Area Under the Curve
B	Basolateral compartment
BCS	Biopharmaceutical classification system
BE	Bioequivalence
BE-space	Bioequivalent space
Cmax	Maximum plasma concentration
CQA	critical quality attribute
D	Dissolution port in the d/p-system
d/p-system	Dissolution permeability system
DPC-test	Dissolution profile comparison test(s)
Eq-space	Equivalent space
EVOM	Epithelial volt ohm meter
FTPC	Flow-through permeation cell
GI	Gastro-intestinal
GIT	gastro-intestinal tract
iid-conditions	Independent identically distributed
IR	immediate release
KRB	Krebs ringer buffer
MDT	Mean Dissolution Time
MRT	Mean Residence Time
PK	Pharmacokinetic (s)
PK/PD	pharmacokinetic pharmacodynamic
PT	Permutation test
Sim-space	Similarity space
t_{85}	Time required to release 85% of the drug from an oral solid dosage form
TDT	Tolerated difference test
TEER	Transepithelial electrical resistance

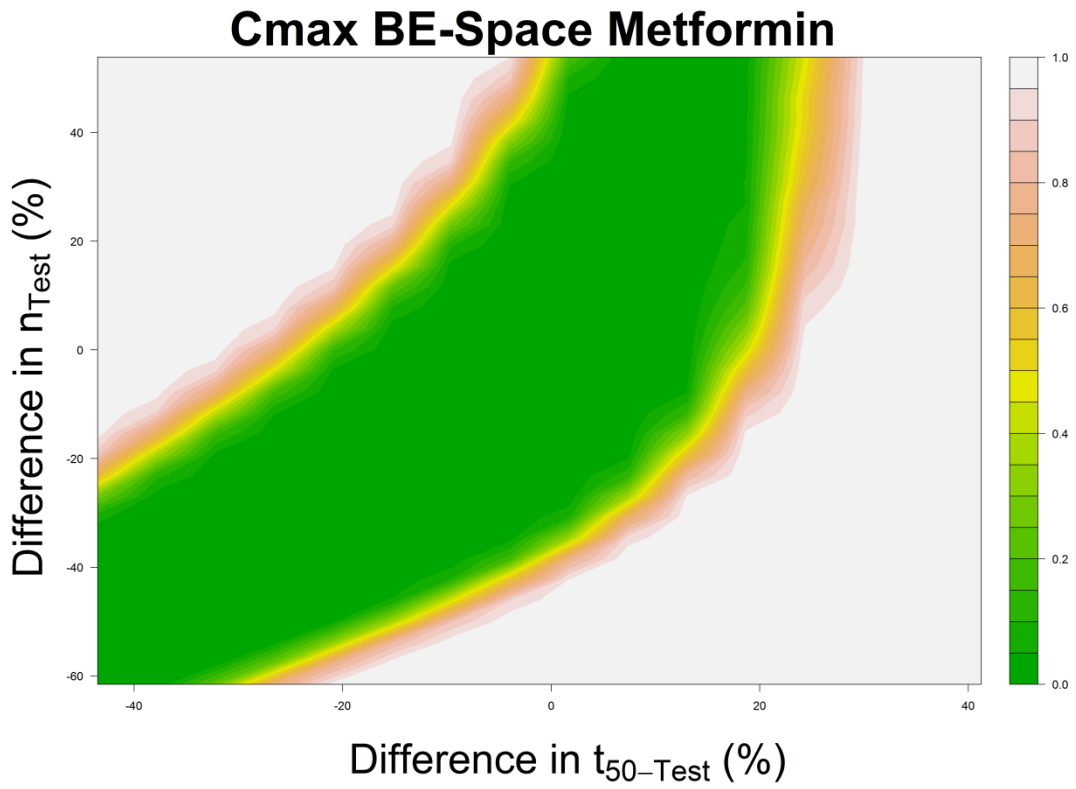
9. Annexes

Time Points / (1-α)	4	5	6	7	8	9	10
0.9	83.00	82.00	81.00	80.43	79.88	79.44	79.00
0.95	86.25	84.80	83.67	82.71	82.13	81.44	81.00
0.975	89.00	87.20	85.83	84.86	84.00	83.33	82.70
0.99	92.00	90.00	88.33	87.14	86.25	85.44	84.70
0.995	94.25	91.80	90.17	88.86	87.75	86.78	86.10
0.999	98.50	95.80	93.67	92.14	90.88	89.78	88.90
0.9995	100.25	97.20	95.00	93.43	92.00	90.89	90.00

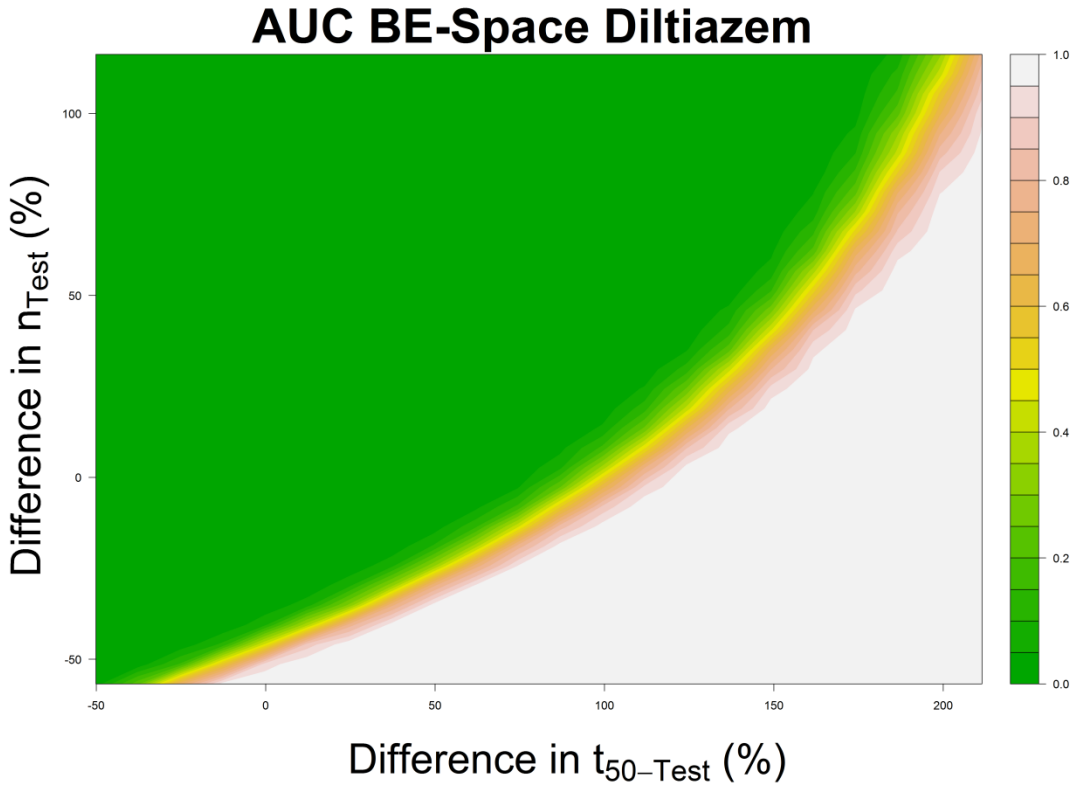
A.1. Rejection Values for TDT for several time points and uncertainty.



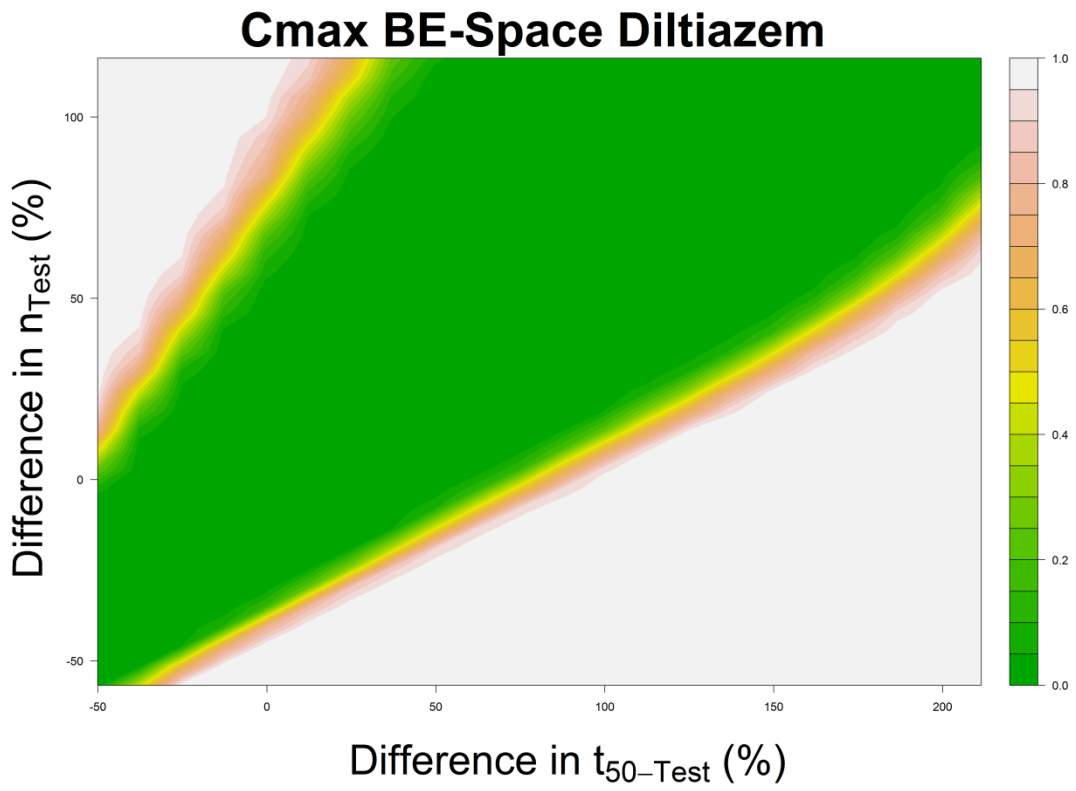
A.2. AUC BE-Space Metformin.



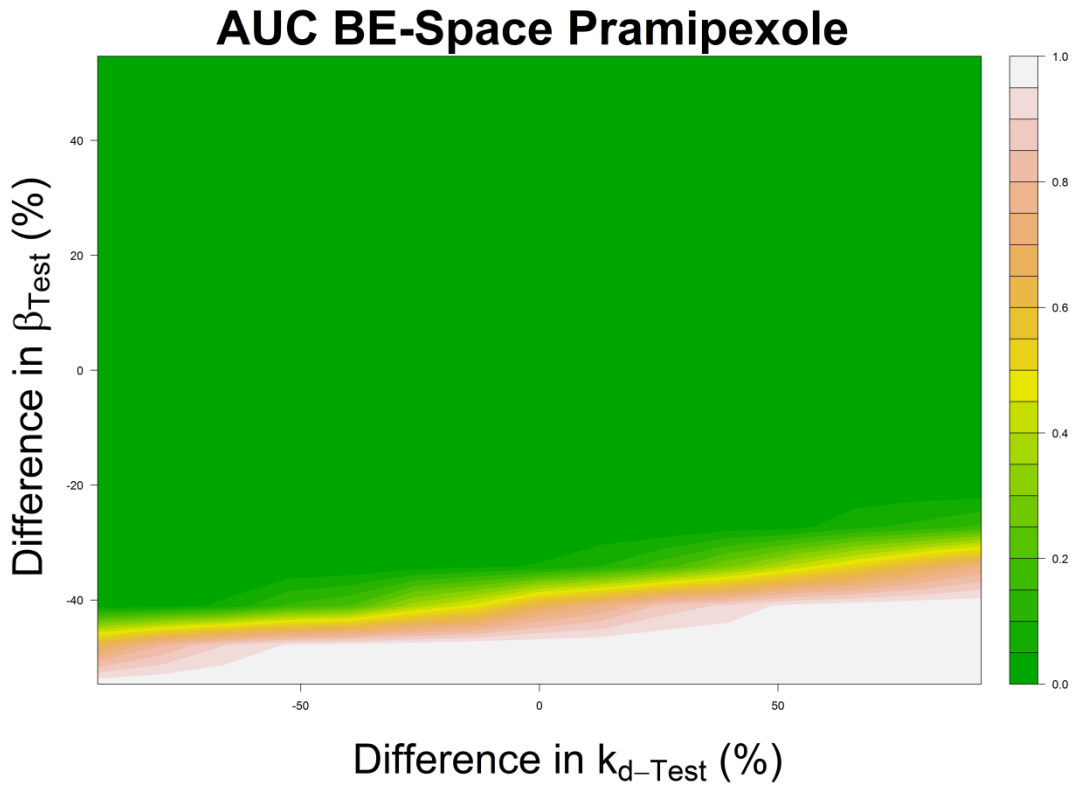
A.3. Cmax BE-Space Metformin.



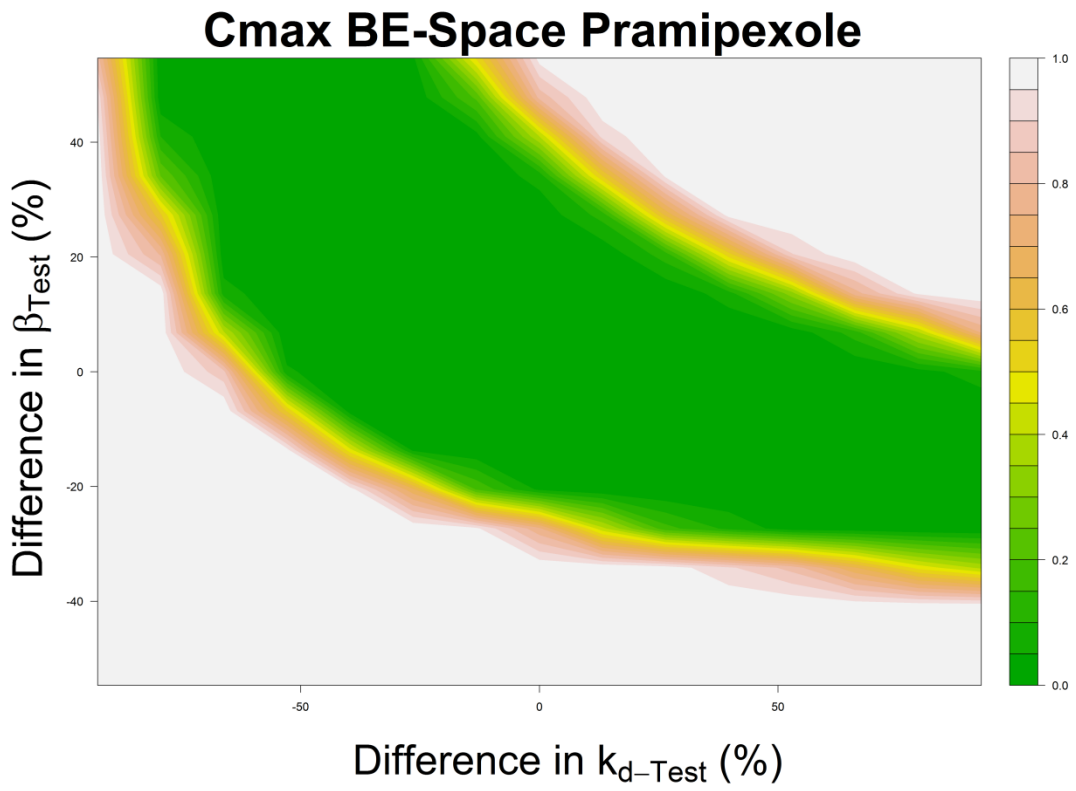
A.4. AUC BE-Space Diltiazem



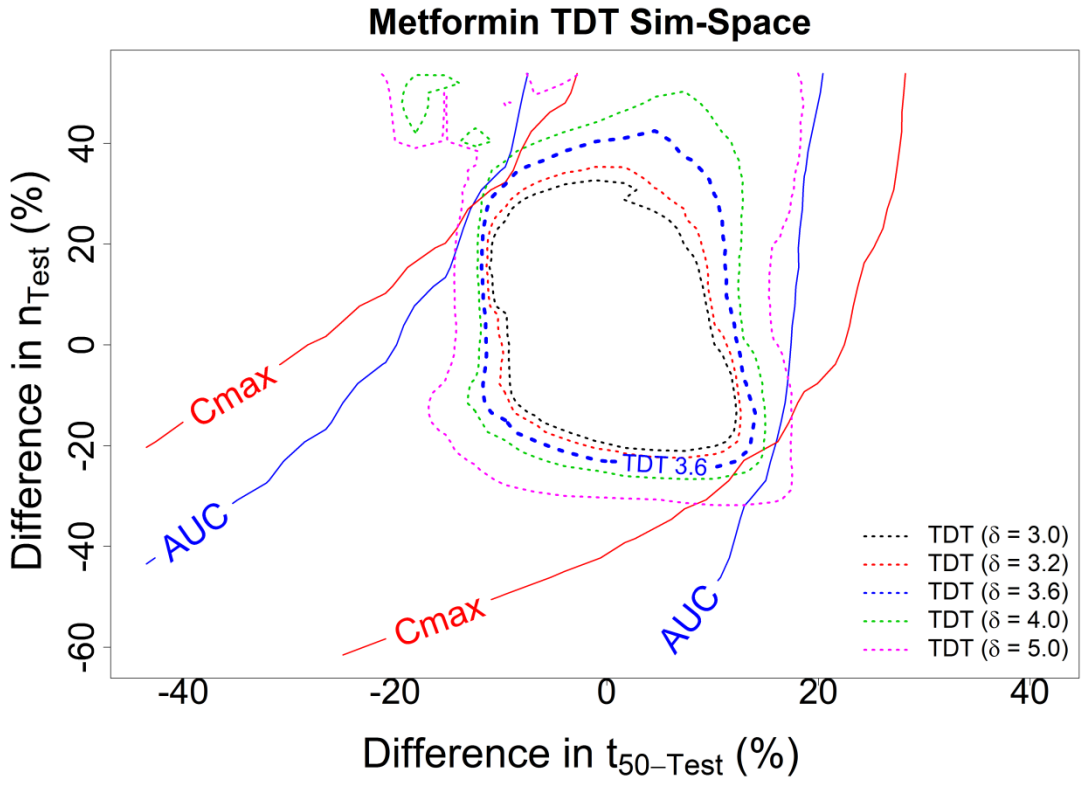
A.5. Cmax BE-Space Diltiazem



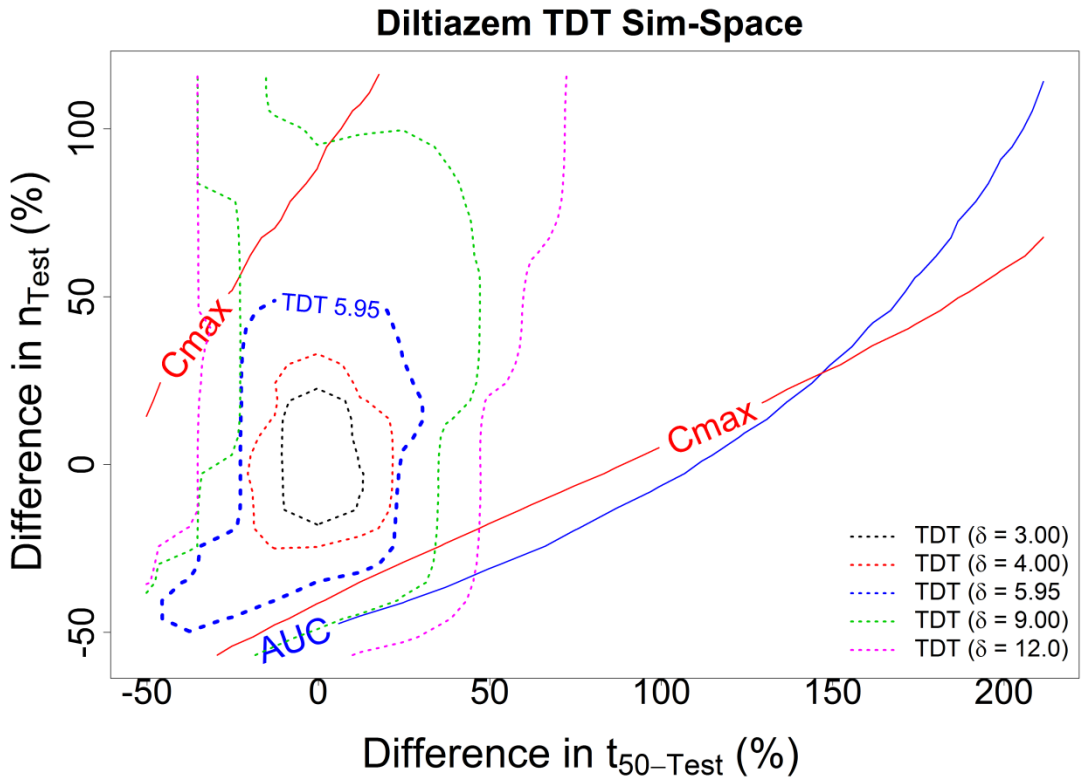
A.6. AUC BE-Space Pramipexole



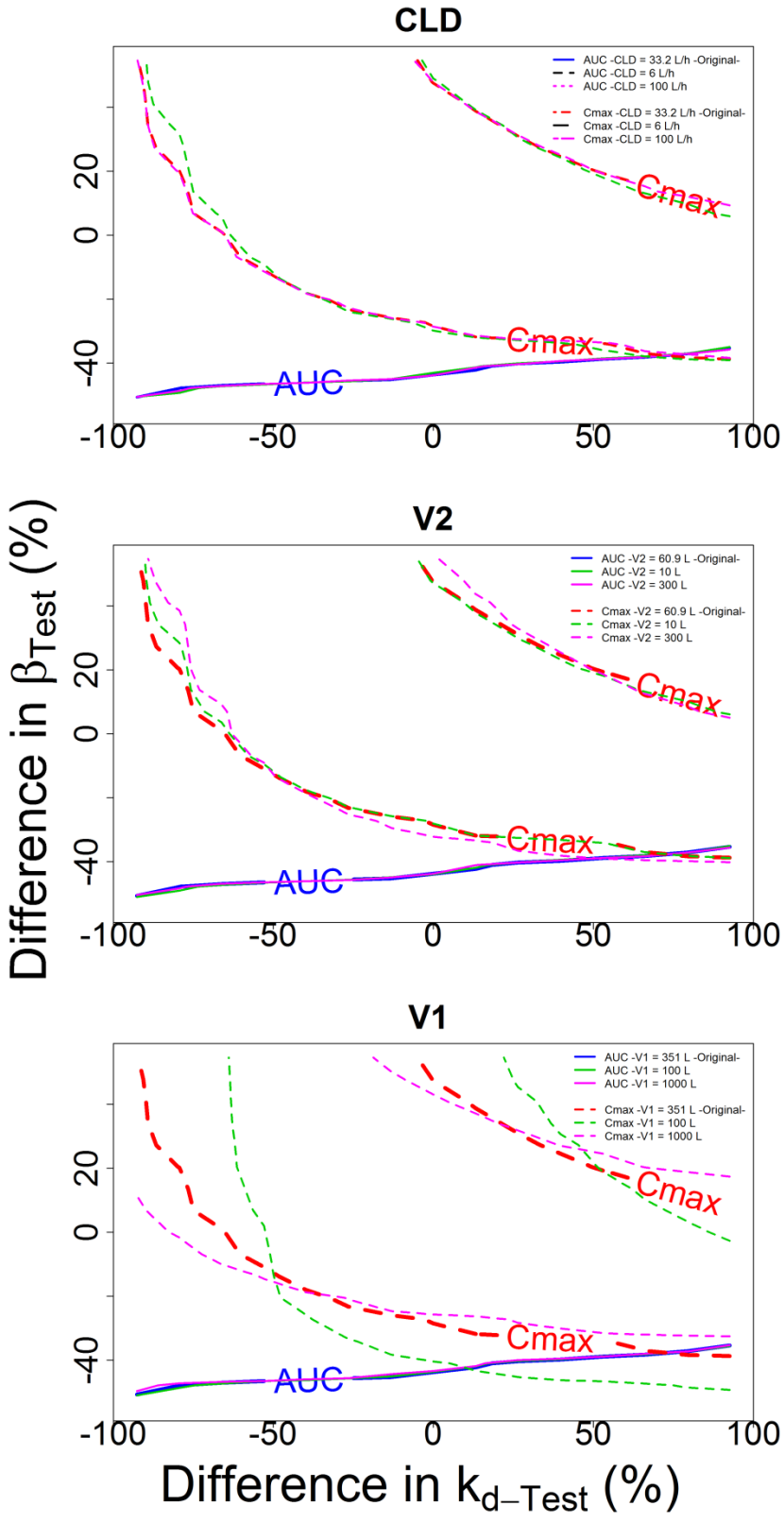
A.7. Cmax BE-Space Pramipexole



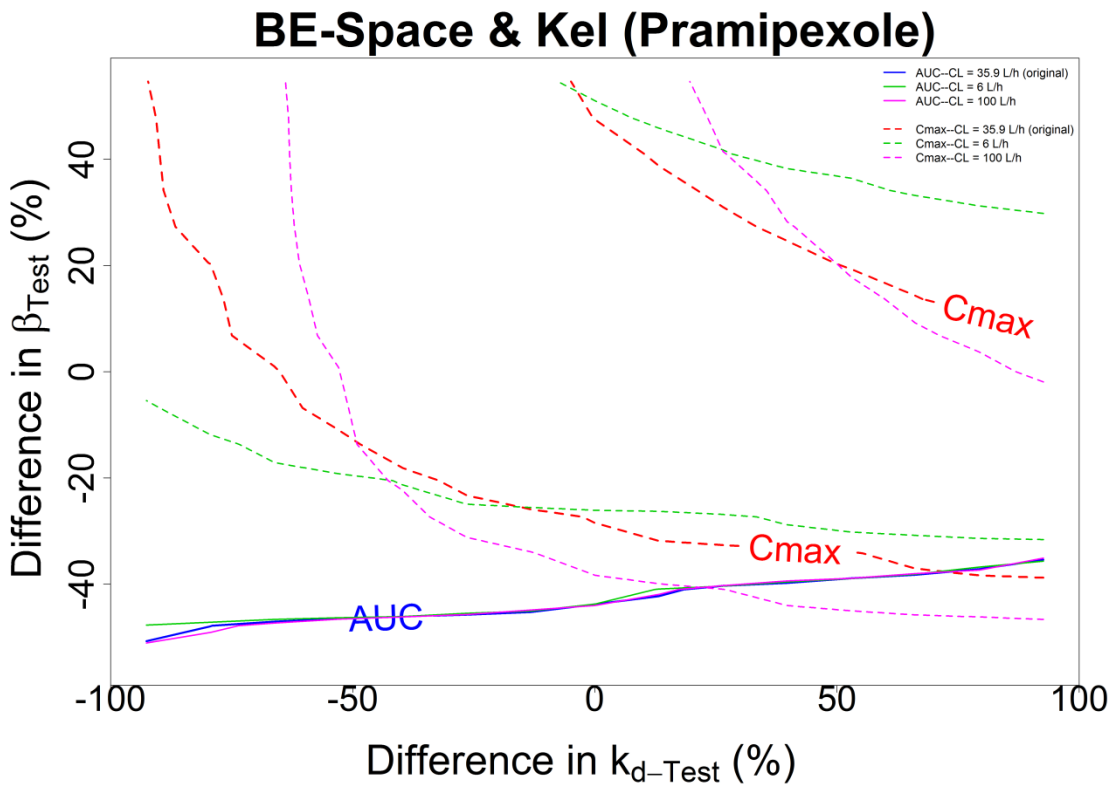
A.8. Customization of TDT (Metformin)



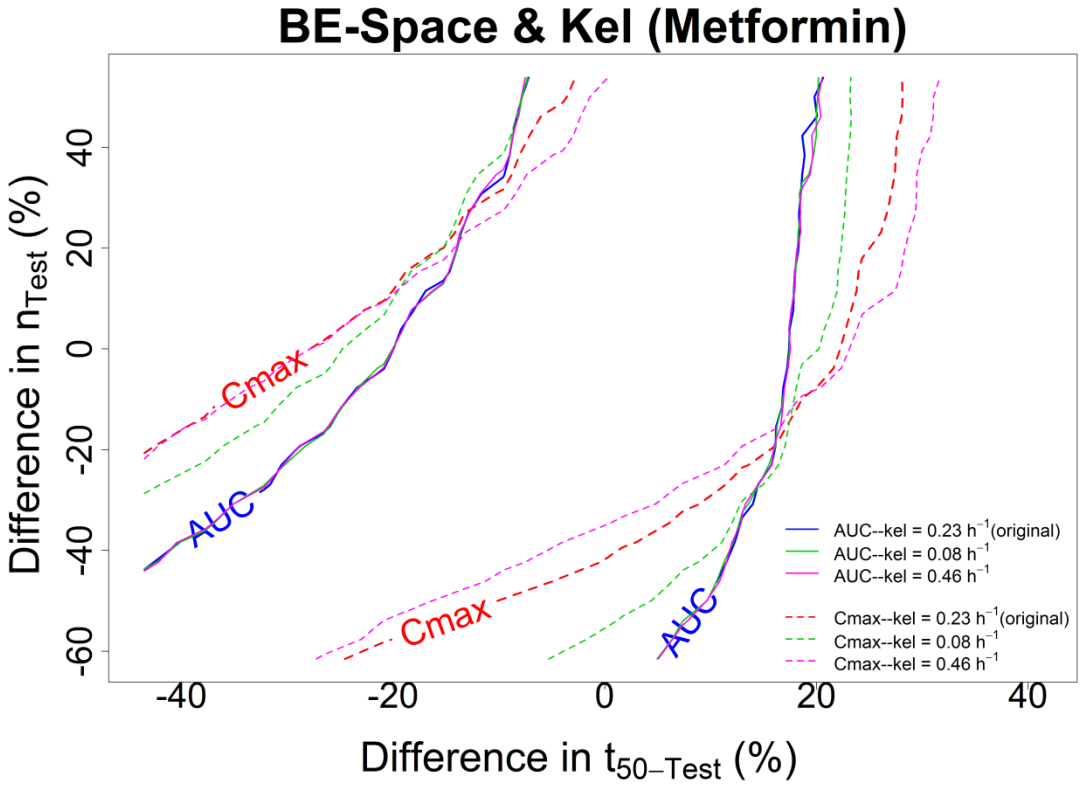
A.9. Customization of TDT (Diltiazem)



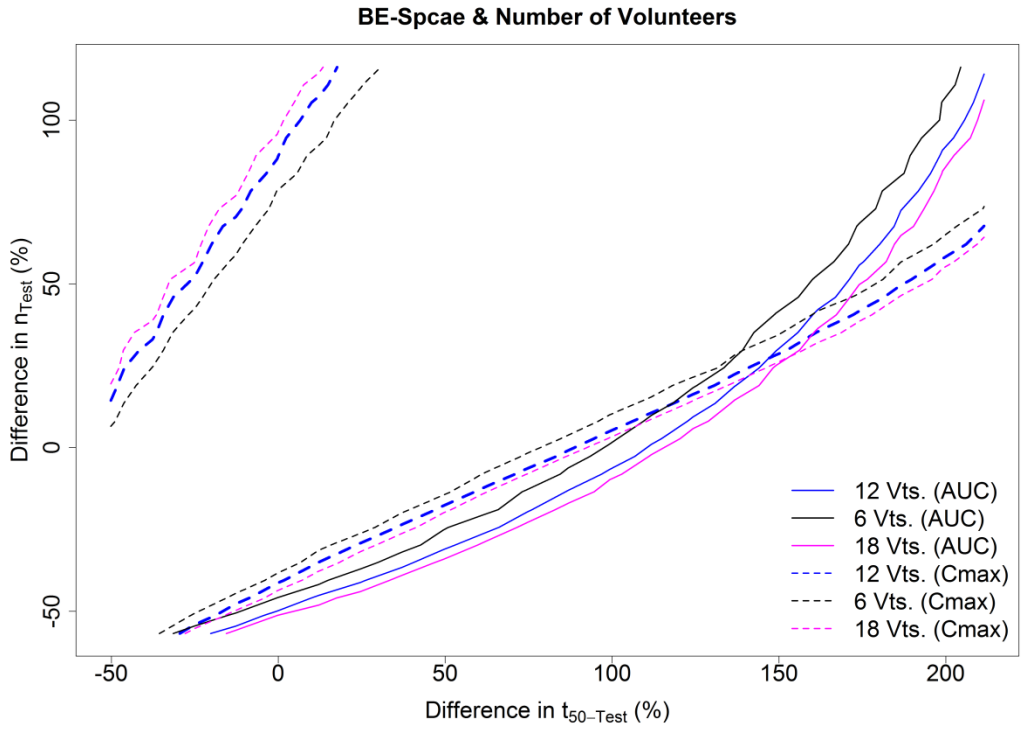
A.10. BE-Spaces under different values of V_1, V_2, C_{LD}.



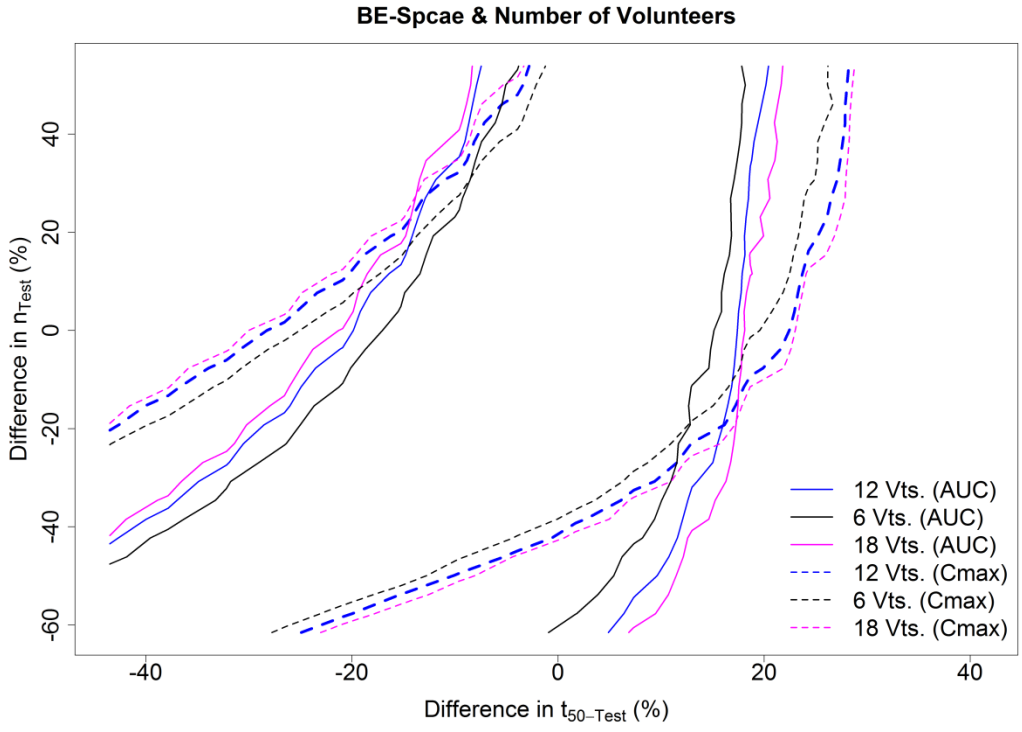
A.11. BE- Spaces at different values of k_{el}. (Pramipexole).



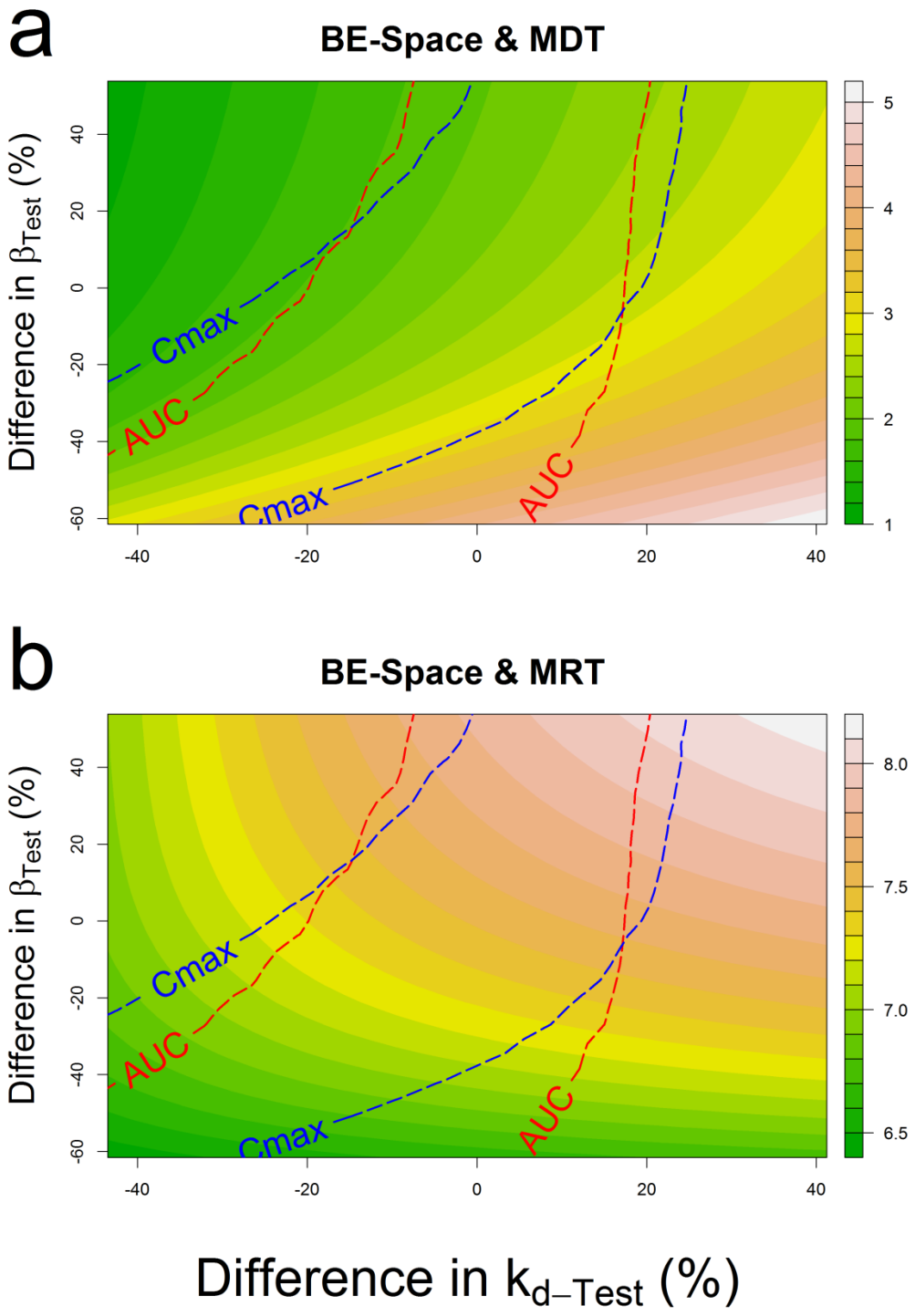
A.12. BE-Spaces at different values of k_{el}. (Metformin).



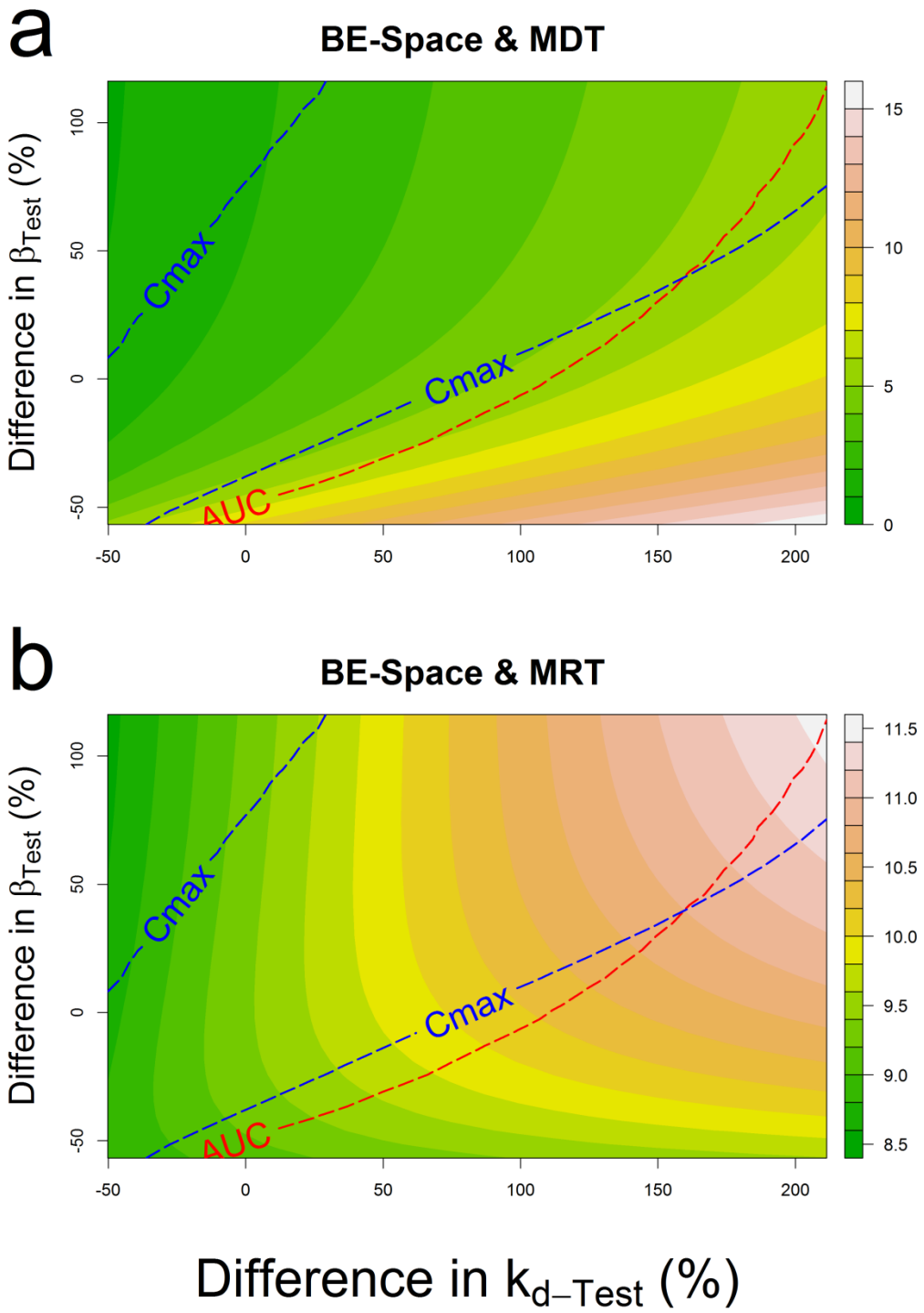
A.13. Effect of Number of volunteers on BE-Space (Metformin)



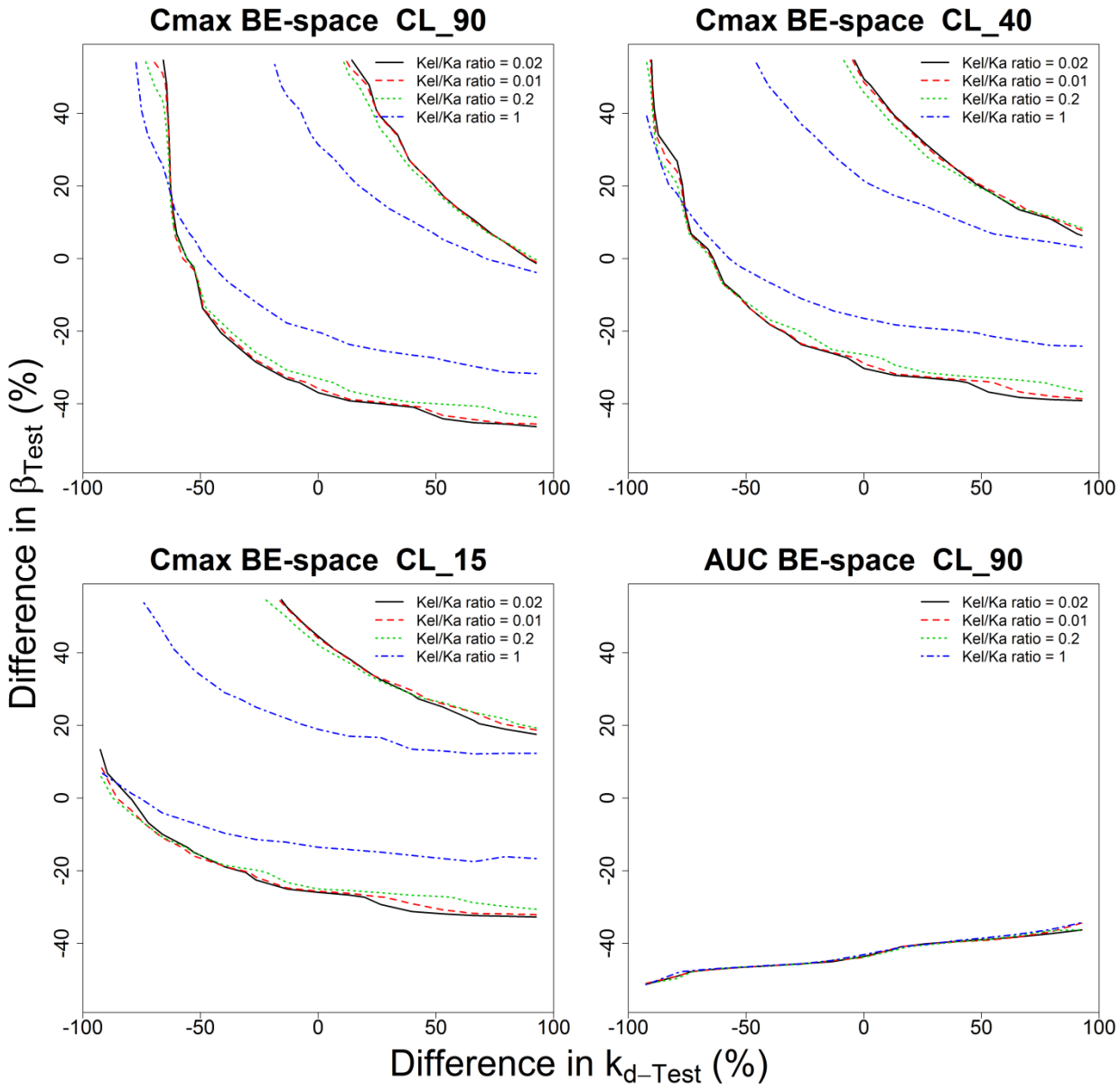
A.14. Effect of Number of volunteers on BE-Space (Diltiazem)



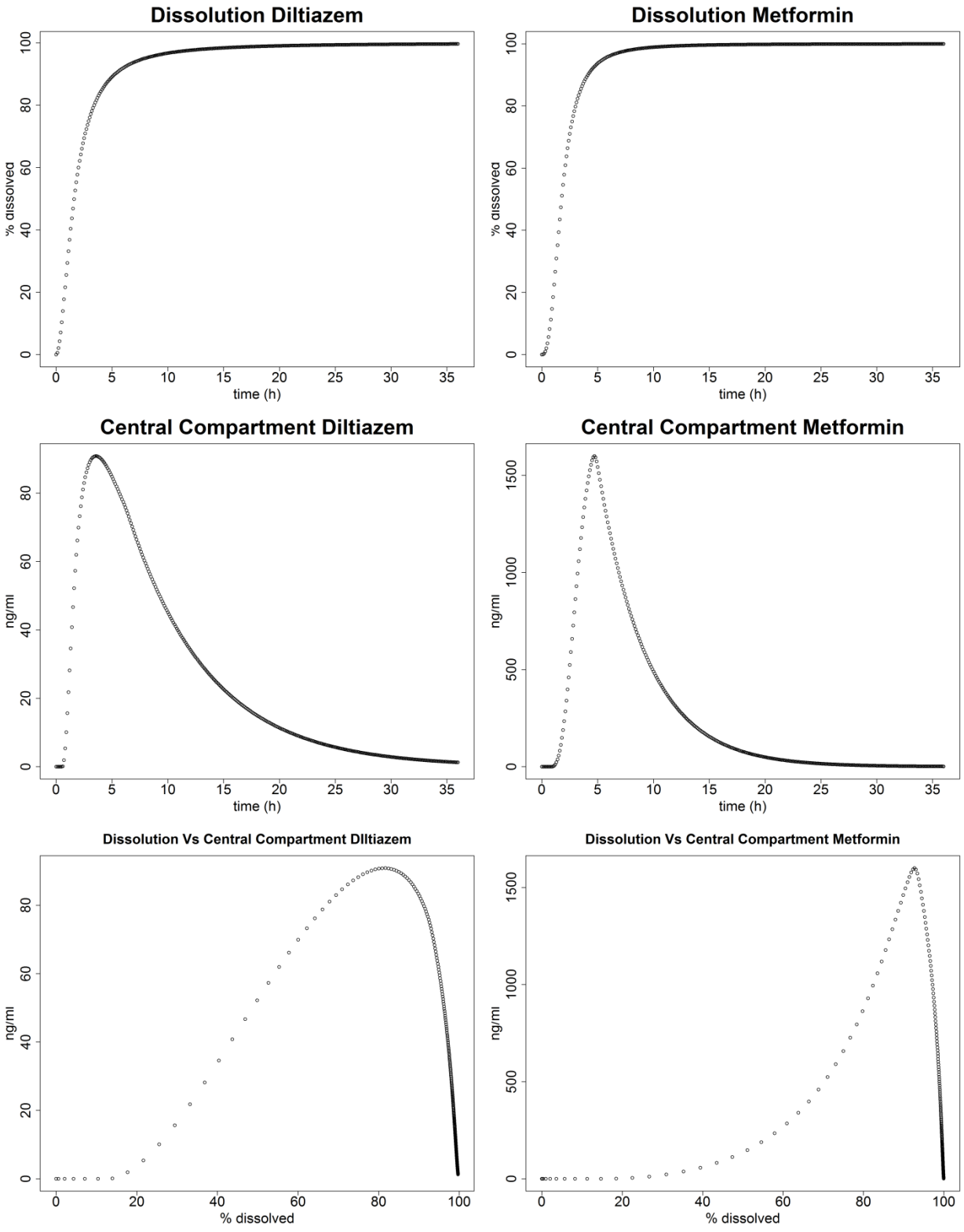
A.15. Comparison of BE-Spaces to a)MDT and b)MRT (Metformin).



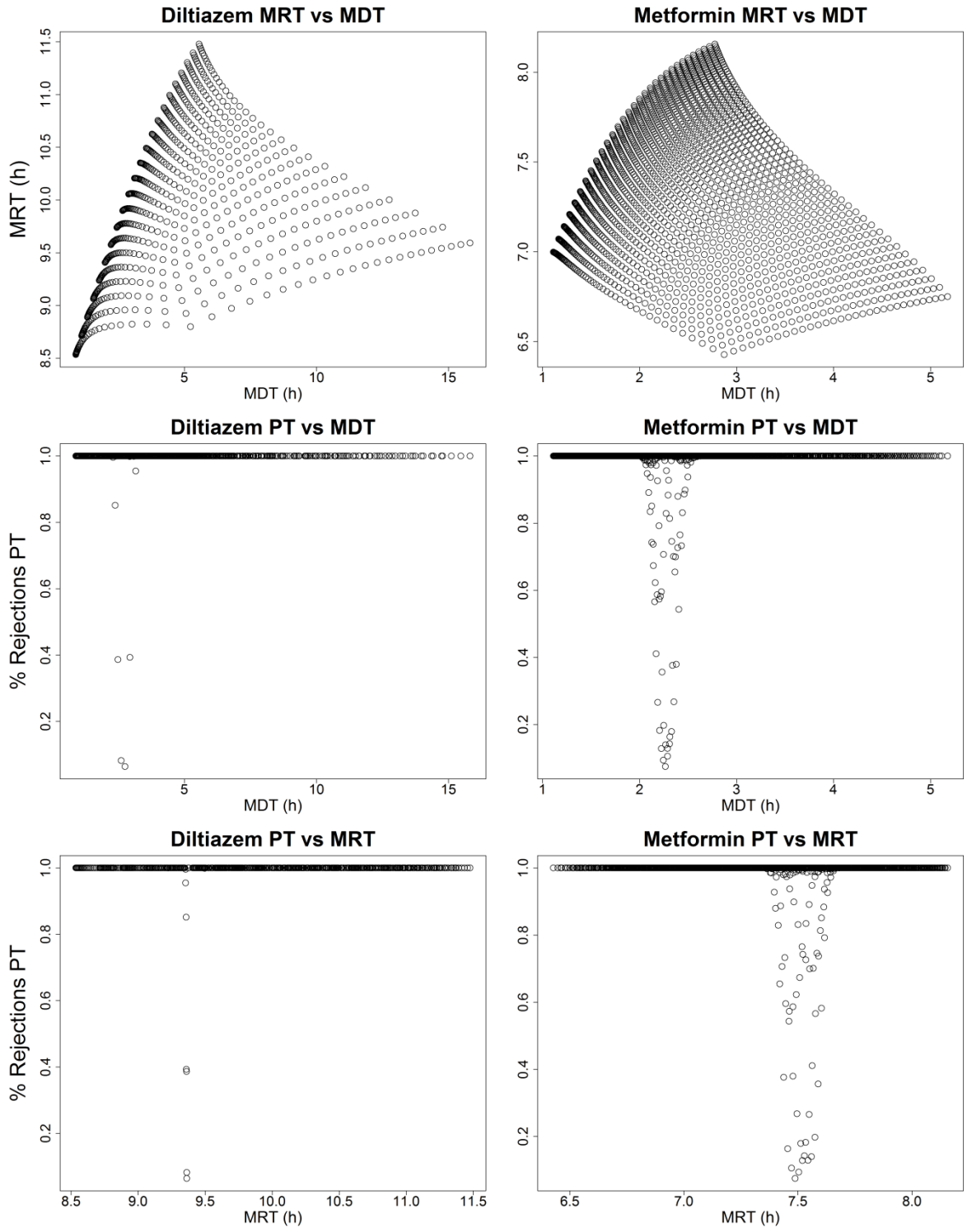
A.16. Comparison of BE-Spaces to a)MDT and b)MRT (Diltiazem).



A.17. Reduction in the Cmax BE-Space at different values of Clearance. The reduction is significant when the k_{el}/k_a ratio is 0.2 or higher. The AUC BE-Space is not affected (lower right).



A.18. Comparison of PK models of Metformin and Diltiazem. Dissolution, PK profile, and relationship between dissolution and concentration in the central compartment.



A.19. Relationships between Sim-Space and MDT and MRT. Several formulations can have the same MDT but different MRT for both metformin and diltiazem, no relationship was observed bwtween the BE-Space or the Sim-space and MDT or MRT.

10. Scientific Output

Original Papers

J.D. Gomez-Mantilla, V.G. Casabo, U.F. Schaefer, C.M. Lehr, Permutation Test (PT) and Tolerated Difference Test (TDT): Two new, robust and powerful nonparametric tests for statistical comparison of dissolution profiles, Int J Pharm, (2012).

J.D. Gomez-Mantilla, V.G. Casabo, U.F. Schaefer, T. Lehr, C.M. Lehr, Identification of Non-Bioequivalent Extended Release Formulations by Tailor-Made Dissolution Profile Comparisons Using *In Vitro-In Vivo* Correlation Models, (Submited).

Conference Abstracts

J.D. Gomez-Mantilla, V.G. Casabo, U.F. Schaefer, C.M. Lehr, Permutation Test (PT) and Tolerated Difference Test (TDT): Two new, robust and powerful nonparametric tests for statistical comparison of dissolution profiles
8th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Istanbul, Turkey, March 2012

J.D. Gomez-Mantilla, V.G. Casabo, U.F. Schaefer, T. Lehr, C.M. Lehr, Tailor-made dissolution profile comparisons using In Vivo-In Vitro Correlation Models
22nd Population Approach Group Europe (PAGE), June 2013, Glasgow, Scotland

11. Curriculum vitae

José David Gómez Mantilla
18.06.1979
Bogotá, Colombia

Education

Doctoral Thesis
10/2010 – 12/2013
Department of Biopharmaceutics and Pharmaceutical Technology
Saarland University, Saarbrücken, Germany
Thesis Project: "statistical approaches to perform dissolution profile comparisons".

Research Internship
10/2009 - 05/2010
Universidad de Valencia, Valencia, Spain

Specialization in Statistics
07/2004 - 07/2006
Universidad Nacional de Colombia, Bogotá, Colombia
Thesis Project: "Robustness exploration of F-permutation, kruskal-Wallis and F-Anova tests on biomedical ranges of interest".

Degree in Pharmacy
02/1997 - 07/2003
Universidad Nacional de Colombia, Bogotá, Colombia
Thesis Project: "Immunological evaluation in Chronic Mucocutaneous Candidiasis patients".

Universidad de Antioquia, Medellín, Colombia
Young Researcher
01/2002-8/2002

Professional Experience

Universidad Nacional de Colombia, Bogotá, Colombia
Teaching Assistant
01/2007-09/2009

Sun Pharmaceutical Service LTD.
Pharmaceutical Care Director
03/2004-12/2004

Schering-Plough S.A.
In-Process Control Inspector
05/2003-12/2003

Cooperativa de Medicamentos de Cundinamarca
Pharmaceutical Services Coordinator
01/2003-05/2003

National Army of Colombia
12/1995-12/1996 (Mandatory Military Service)

Awards and Recognitions

Colciencias Scholarship 01/2013-Present
Holder of a Colombian Government Scholarship to pursue a Doctoral Degree Abroad.

DAAD Scholarship 05/2010-Present
Holder of a DAAD Scholarship to pursue a Doctoral Degree in Germany.

Free Tuition for Outstanding Records 2005
Universidad Nacional de Colombia, Department of Statistics.

First Class Honours for Outstanding Records 1997
Universidad Nacional de Colombia, Department of Pharmacy.

Best National Exam Score 1995
Antonio Nariño High School, Bogotá.

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13. Acknowledgments

Since the first moment I understood what achieving a PhD means it became my dream to pursuit one. I feel extremely lucky for the chance I had during these years to focus only on my research question and the freedom, guide, encouragement and support and fun that I found on the way.

I can only thank Prof. Claus-Michael Lehr, not only for inviting me to his group, but also for encouraging me constantly to follow my academic appetites and keep trying harder. Also for all the fruitful scientific discussions in our project meetings and all his personal dedication to find collaborators for our projects and solutions to my problems. I can only condense my sincere gratitude by saying that every time after a meeting with him I was happy to have more work and that extra motivation was constantly helpful.

I am more than thankful to apl. Prof. Ulrich Schäfer for his personal concern in my career and for all his contribution to my work. I fairly admire his passion for detail and for digging deeper to find plausible answers. It was a luxury to count on someone with such enthusiasm to acquire and transmit knowledge and such extraordinary expertise in the laboratory and in the discussion of theoretical principles.

I thank also Jr. Prof. Thorsten Lehr for his great disposition to collaborate in my work and all the numerical and non-numerical fun that we had together in the last time. It was one a very happy coincidence to find someone in the middle of my PhD experience with such involvement in the field I was craving to explore and that I definitely want to keep discovering.

I also thank Prof. Vicente Casabó for his collaboration in my project and his unforgettable lessons. I was very motivated to meet for the first time another pharmacist with such a level of passion for mathematics and statistics. Undoubtedly his loss was a sad and bitter moment of the last year.

To Domink Selzer and Sandra Gantztsch for the nice collaboration in the work on the final chapter of this thesis.

To DAAD and Colciencias (Colombia) for the financial support during these years. In particular to Silke Hamacher from DAAD for her great help and personal attention to my case.

My open thankfulness to Prof. Marta Fontanilla of my University in Colombia for the help, encouragment and understanding.

Thanks to the Secretaries of the group Karin, Sarah and Isabelle for their help in the practical matters that surrounded this experience.

To Sarah Gordon, Christiane Mathes and Nico Mell for the language assitance in this thesis.

These years in Germany have been without a doubt a huge cultural experience and I consider myself very privileged for all the things I have had the chance to learn from so many people. I am very thankful to my colleagues from the group who gave me the opportunity to integrate my culture into theirs and were very empathetic to invite me to a long list of interesting activities and were always happy to join me in any social suggestion. Particularly I thank Clemens Tscheka for all the kilometers of sports, all the skiing, and his unconditional friendship.

It was never in my plans to do theater in Spanish in Germany, but it turned out to be a great chance to meet nice people and get to know myself better. I thank sincerely all the members of the theater group “Los Mutantes” for all the nice moments of disconnecting from everything and recharging energy, specially to Lina Ruiz, who also helped me to get installed in this city since the first week of my stay.

To Augusto Salazar and Anna Kühn for bringing more happiness to my German adventure and exciting plans for the future.

To my Montagpola friends Miguel Granados, Fidel Ramírez and José Manuel Brito, for letting me grow with them and acquire together a new vision of life and meaningfulness. Thank you for pushing me to widen my reading tastes and for being a guarantee of a nice and interesting conversations and help with my problems during these years. Muchas Gracias!

To Champetica for giving me new reasons for daydreaming.

Finally I want to say that I have the luck to come from a country where my mother´s last name stays into mine during my whole life and this long name that sounds strange to many people is for me a little tribute to her efforts and sacrifices. I will never be able to return all the things she has done for me. Because of her I had access to a very good middle education and she was also the person who encouraged me the most to be curious about science, nature, mathematics and foreign languages and to keep searching and demanding more from myself. She was also the person who gave me the discipline to work intensively on unresolved problems and woke me up early in the morning when necessary to keep on trying. Definitely, I would have never dreamed about a PhD without her. Gracias Mamá!